THE ENTOMOPATHOGENIC NEMATODE, *STEINERNEMA CARPOCAPSAE* (ALL STRAIN), FOR CONTROL OF BLACK VINE WEEVIL IN DRY-PICK CRANBERRIES IN BRITISH COLUMBIA

by

Anne E. Hayes

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Name: **ANNE ELIZABETH HAYES**

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Examining Committee:

Chair: Dr. Z. Punja, Associate Professor

Dr. John M. Webster, Senior Supervisor,
Department of Biological Sciences, SFU

Dr. J. Borden, Professor,
Department of Biological Sciences, SFU

Dr. S. M. Fitzpatrick, Research Scientist
Agriculture Canada

Dr. F. D. McElroy, President, Peninsu-Lab
Poulsbo, Washington
Public Examiner

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Title of Thesis/Project/Extended Essay

The entomopathogenic nematode, <u>Steinernema carpocapsae</u> (All strain),

for control of black vine weevil in dry-pick cranberries in British

Columbia

Author:

(signature)

Anne Elizabeth Hayes

(name)

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(date)

Abstract

The dry-pick cranberry industry in British Columbia suffers substantial losses from feeding damage of the black vine weevil (BVW), *Otiorhynchus sulcatus* Fabricius. Currently, aqueous applications of the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) (All strain), is the principal method of control. A survey of dry-pick growers revealed that the nematode does not always provide satisfactory control under local field conditions. This study, conducted on two farms on the Lower Mainland, examined various stages of the nematode application process as used by the growers. The results showed that most of the stages in the process did not inhibit the success of the nematodes but that their effectiveness declined once they were in the soil.

Nematodes from commercial packages were sampled immediately prior to the 1993 spring and fall applications. They were also sampled after passage through a sprinkler system on one farm and a boom sprayer on the other. Petri dish bioassays using *Galleria mellonella* L., a lepidopteran highly susceptible to nematodes, resulted in infectivity levels between 95-100%, which showed that almost all the nematodes in the suspensions were active and infective. Analysis of the distributions of the nematodes across the test plots showed that both application systems successfully delivered nematodes to all areas of the plots.

Soil samples were taken from both farms at various intervals following the applications. Soil bioassays with *G. mellonella* showed that nematodes were present in the top 10 cm of soil, the feeding zone of the BVW. The nematodes persisted in the field for up to 8 months but their infectivity level as determined from soil samples was low, ranging from 0-58%. Soil type, which may influence nematode effectiveness, was examined using the normal peat in which cranberries grow, loam, muck, and a silty clay loam. Only the loam inhibited nematode effectiveness. The low nematode infectivity in the soil samples from the field may have been caused by the dense canopy of the cranberry plants preventing the nematodes from reaching the soil, factors influencing the effectiveness of the soil bioassays, or the species of nematode being used.

Acknowledgements

Table of Contents

List of Figures

Introduction

The cranberry industry is an important component of agriculture in British Columbia. After apples and mushrooms, cranberries are the third most valuable, edible, horticultural crop in the province. The industry has enjoyed its most substantial growth in the last decade and at present, in terms of value and production, cranberries are the leading crop among small fruits (Bains, 1991). This is an impressive record for an industry that started in the early 1920's with just 4 ha.

The cranberry, *Vaccinium macrocarpon* Ait, is native to North America and is cultivated in only Canada and the United States (Bailey and Bailey, 1976) and, recently, in Chile (Stang and Joy, 1993). In the early 1850's, the Hudson's Bay Company shipped fresh, wild cranberries collected in the Fraser Delta of British Columbia to San Francisco. This was a short-lived business endeavour because by the end of the 1850's, there was more interest in gold than in wild cranberries. In the early 1920's, the first cultivated planting was established on the Queen Charlotte Islands and in 1924, a few hectares were planted in Richmond. Richmond was to become the centre of cranberry production in British Columbia, growing from 40 ha in the late 1950's to more than 1040 ha in 1990 (Bains, 1991). In the early stages of the industry, all cranberries were sold fresh to retail stores.

1

Ocean Spray Cranberries Limited is a grower-owned co-operative with headquarters in Massachusetts, and in 1958 several growers from British Columbia joined Ocean Spray, which markets both fresh and processed cranberries. In the early 1980's, to meet Ocean Spray's increasing demands, 480 new ha were planted in different areas on the Lower Mainland including Richmond, Pitt Meadows, and Delta. By 1990, British Columbia growers were producing 15.7 million kilograms of cranberries on 1060 ha, making British Columbia the third largest cranberry growing region in North America after Massachusetts and Wisconsin (Bains, 1991).

The industry continues to expand as the plantings from the early 1980's slowly reach full maturity. Preparing and planting a cranberry bog is an expensive and time-consuming operation (Hall, 1969). Cranberries need well-aerated, acidic soils, and large amounts of irrigation water for optimal survival and production. In general, cranberries in British Columbia are grown in peat bogs. The cranberry plant is a low-growing vine that forms a thick mat over the entire surface of a cultivated bog. The persistent leaves are a dark, glossy green during the growing season and turn reddish brown during the dormant season. The root system consists of very fine, fibrous roots that develop in the upper 10 cm of the soil. Cranberry roots have a symbiotic relationship with mycorrhizal fungi for the uptake of nutrients and, therefore, do not produce root hairs (Shawa et al., 1984). In late

2

summer, flower buds appear near the terminal ends of vertical shoots that arise from the main runners and the fruits are borne the following year.

Cranberries can be harvested in one of two ways. Most of those in British Columbia are water harvested: the bog is flooded and a harvesting machine called a water reel then beats the cranberries from the vines. After beating, the berries float to the surface and are gathered. The other harvesting method is known as dry picking. About 60 ha of cranberries in British Columbia are dry picked. Usually, these bogs cannot be flooded for various reasons such as inadequate water supply. Cranberries from dry-pick bogs are used for the fresh market. Dry-picking is somewhat less efficient than water harvesting, since about 15% of the crop is left in the bog, whereas only about 5% is left behind with water harvesting. However, Ocean Spray gives dry-pick growers a slightly higher price per kilogram for their cranberries because they are used for the fresh market.

Although it is costly to grow cranberries, the profits from this valuable crop can be quite high. For example, in 1990, the total value of the crop in British Columbia, including both processed and fresh, was $22.1 million (Bains, 1991). Fortunately, compared with many crops, cranberries are not subject to many important pests or diseases. The industry does, however, suffer substantial losses from damage to the roots caused by feeding of the larvae of the black vine weevil

(BVW), *Otiorhynchus sulcatus* Fabricius. The losses have not been clearly quantified in British Columbia. However, it is known that once the crop has sustained significant damage from BVW, the vines often have to be replanted and full yields are not expected for 7 yr thereafter.

BVW originated in Europe and is most common there and in North America, although it can be found on many horticultural crops throughout the temperate regions of the world (Moorehouse et al., 1992). In Canada, BVW is most often found in the eastern and western parts of the country (Warner and Negley, 1976). BVW is parthenogenetic, and although males have been described in Europe, only females have been found in North America. Parthenogenesis contributes significantly to the status of BVW as a pest (Neilson and Dunlap, 1981).

BVW adults have a wide host range consisting of over 140 plant species (Warner and Negley, 1976), including greenhouse and nursery crops (Maier, 1983), as well as small, berry fruit crops such as blueberries and cranberries. BVW is also able to survive on blackberry and salal which often border cranberry bogs. The adults are flightless because their elytra are fused together. Consequently, infestations of BVW in the field tend to be patchy but severe (Parrella and Keil, 1984). BVW larvae have a smaller host range than the adults and, because the larvae are even less mobile, they rely on the adults for host location (Hanula, 1988).

BVW generally has one generation per year (Cram, 1965),

although some adults are able to overwinter in plant debris and survive for two summers (Breakey, 1959). In June, adults emerge from the soil and begin feeding the following night. During the day, the adults hide in plant debris (Parrella and Keil, 1984). The heaviest period of adult feeding occurs in the first 10 wk after emergence from winter dormancy (Doss and Shanks, 1985). During this time, most of the feeding takes place in the pre-oviposition period which lasts for about 28-50 d (Smith, 1932), depending on temperature (Stenseth, 1979). A shorter pre-oviposition period allows for more eggs to be deposited (Maier, 1981) and for the larvae to develop more before the onset of winter (Neilson and Dunlap, 1981). Females oviposit beginning in July and are able to lay approximately 500 eggs. BVW prefers moist conditions for oviposition and peak egg-laying in British Columbia occurs in August (Cram, 1965). Eggs deposited in soil cracks have a higher survival rate than those laid on the soil surface. Oviposition ends by late August or early September, probably due to a photoperiod reduction (Garth and Shanks, 1978). Egg development may take from 8-56 d depending on temperature (Stenseth, 1979). There are six or seven larval instars whose development times range from 74-230 d, depending on temperature (Smith, 1932). The larvae feed on roots throughout the fall and stop feeding in the winter. They overwinter in cells made from soil, at a depth of 15-25 cm. As the temperature increases in spring, the larvae break out

of the overwintering cells and resume feeding. Pupation normally occurs between mid-May and mid-June (Garth and Shanks, 1978). Of the pre-adult stages, pupae are the most sensitive to variations in temperature (Stenseth, 1979). On average, the pupal period is 18 d at 14-20°C (Smith, 1932).

BVW adults feed on foliage causing characteristic notches on the leaves. This damage is economically insignificant compared with the damage caused by larvae feeding on the roots (Parrella and Keil, 1984). The underground nature of the critical root feeding results in the damage occurring before the infestation is detected (Neilson and Dunlap, 1981).

Adequate control of BVW in bogs that are suitable for flooding can be achieved by flooding for 2-3 wk in the fall after harvest (approximately September to November), a practice that suffocates the larvae (Shawa et al., 1984). Growers with dry-pick bogs, however, cannot use this management strategy. Consequently, dry pick bogs incur the most damage from BVW. Currently, growers with dry-pick bogs use a commercial product containing the entomopathogenic nematode, *Steinernema carpocapsae* Weiser (All strain), as one of the principal methods of controlling BVW.

Nematodes in the families Steinernematidae, such as *S. carpocapsae*, and Heterorhabditidae are being studied extensively and are becoming accepted as alternatives to chemical pesticides for controlling some insect pests. The first steinernematid was reported in Germany by Gotthold

Steiner in 1923, and the family Steinernematidae was erected in 1937 (Poinar, 1990). At that time, two genera were recognized in the family, *Neoaplectana* and *Steinernema*. It has since been determined that there is no difference between them, and *Neoaplectana* has been synonymized under *Steinernema* (Wouts *et al.*, 1982). The genus *Steinernema* consists of ten species (de Doucet and Doucet, 1990). The family Heterorhabditidae, erected in 1976, is also monogeneric and has three species. Both genera have been found on all continents except the Antarctic.

Interest in these entomopathogenic nematodes as potential biological control agents was sparked in 1929 when William Rudolph Glaser discovered dead Japanese beetle grubs, *Popilla japonica* Newm, infected with nematodes (Poinar, 1990). The nematode was described as *Neoaplectana glaseri* and is now known as *Steinernema glaseri*. In 1931, Glaser mass produced *S. glaseri* on artificial medium and, in 1935, made a successful field application for the control of Japanese beetle (Poinar, 1992). These nematodes are attractive as biological control agents because they have a host range that includes the majority of insect orders and families (Poinar, 1990). This is probably because they are able to kill their hosts so quickly that they do not form intimate, highly adapted, host-parasite relationships (Kaya and Gaugler, 1993). In addition, they can be mass produced, stored for long periods, and applied by conventional methods. They can

persist in natural environments and, because they are adaptable organisms, it is not likely that insect hosts would become immune to them (Poinar, 1990).

Steinernematids and heterorhabditids, both of whose natural habitat is soil, possess similar life cycles which include an egg, four juvenile stages, and an adult. The third stage infective juvenile (IJ) is non-feeding and is the only stage capable of surviving outside the insect host. Morphological adaptations, such as closure of the mouth and anus and collapse of the intestinal walls, allow IJs to survive outside an insect host for prolonged periods without nourishment until a suitable host is found (Poinar, 1990). IJs are ensheathed by the second-stage cuticle and, although *Steinernema* spp. tend to lose it after movement through soil, it helps to provide protection from antagonists and desiccation (Timper and Kaya, 1989).

Host searching strategies of IJs differ among entomopathogenic nematode species. *S. glaseri* and heterorhabdidtids are cruisers which means they move through the soil in search of hosts (Poinar, 1990). Cruisers probably use chemical cues to aid in locating their hosts (Lewis et al., 1993). *S. carpocapsae*, a smaller nematode, is an ambusher that tends to search for hosts at or near the surface of the soil (Gaugler, 1988). Ambushers tend to remain sedentary and wait for a potential host to pass. Chemical cues are probably only minimally important to ambushers (Lewis

8

et al., 1993). *S. carpocapsae* actively nictates in that all but the posterior portion of the nematode's body is lifted from the substrate and waved from side to side. This behaviour is not fully understood but may be associated with host-seeking (Ishibashi and Kondo, 1990).

Soil type can affect the host-seeking abilities of nematodes by influencing their dispersal and survival. Georgis and Poinar (1983a) showed that nematode movement is restricted in soils with high clay or silt content. To move in the soil, nematodes require a film of water and clay soils have small pores that probably do not provide adequate moisture or aeration (Kaya and Gaugler, 1993). Nematodes were shown to survive better in sandy loam and sand than in clay loam and clay (Kung *et al.*, 1990a). The survival and host-seeking abilities of *S. carpocapsae* in the peat soil in which cranberries are grown in British Columbia have not been well studied.

Once a suitable insect host is found, IJs enter the host's haemocoel via natural openings including the mouth, anus, and spiracles. Heterorhabditids have a dorsal tooth that enables them also to enter directly into the haemocoel by penetrating the insect's cuticle (Bedding and Molyneux, 1982). In their intestinal tracts, the IJs of *Steinernema* and *Heterorhabditis* carry the bacterial cells of *Xenorhabdus* spp. Once in the haemocoel of the host, the IJs release bacteria through the anus. The bacterial cells multiply in the

haemocoel causing septicemia and kill the host within 48 h. The nematodes feed on the bacterial cells and host tissues, produce two or three generations, culminating in IJs being formed that emerge from the host and search for a new one in the surrounding soil. One difference between *Steinernema* and *Heterorhabditis* is in their development following entry into the insect (Figure 1). The IJs of *Steinernema* develop into an amphimictic first generation with females or males. The IJs of *Heterorhabditis*, however, develop into hermaphroditic females. The second generation of both genera is amphimictic (Poinar, 1990).

The associations between steinernematids with *Xenorhabdus* spp. and heterorhabditids with *Photorhabdus* sp. are mutualistic, a trait unique to these nematodes. The nematodes rely on the bacteria to kill the insect host, serve as a food source, break down host tissues, and to produce antibiotics that inhibit the growth of other microorganisms. The bacteria rely on the nematodes for protection and transport to the insect host's haemocoel. Each species of nematode is associated with just one species of *Xenorhabdus* but a *Xenorhabdus* species can be associated with more than one species of nematode (Kaya and Gaugler, 1993). There are four species of *Xenorhabdus* associated with *Steinernema* spp. and *X. nematophilus* is the species associated with *S. carpocapsae* (Akhurst and Boemare, 1990). *Photorhabdus luminescens* is the only species associated with *Heterorhabditis* sp. (Boemare *et*

10
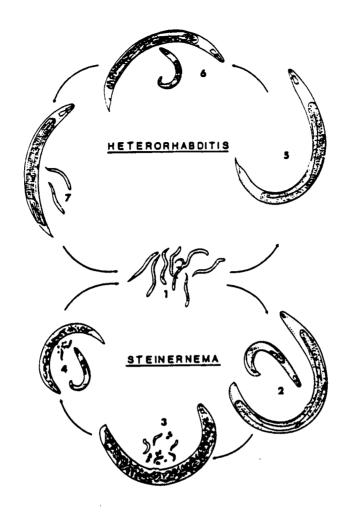
Figure 1. Generalized life cycle of species *Steinernema* and
Heterorhabditis (from Poinar, 1990).

1. Steinernematid and heterorhabditid infective
   juveniles.
2. Steinernematid, amphimictic female and male.
3. Steinernematid female, eggs, and juveniles.
4. Steinernematid female, male, eggs, and juveniles of
   second generation.
5. Heterorhabditid, hermaphroditic female.
6. Heterorhabditid, amphimictic female and male.
7. Heterorhabditid juveniles inside and outside the
   female.
   (from Chen, 1992).

11

al., 1993).

There is great interest in *Steinernema* spp. and *Heterorhabditis* spp. as biological control agents of insect pests, particularly soil-borne ones, because they are naturally adapted to soil, kill their hosts quickly, and have a wide host range. This interest has led to great advances in the development of nematode products for commercial use. Commercial development of these nematodes requires strategies for mass production, formulation, storage, and application.

Entomopathogenic nematodes can be reared on insect hosts. *Galleria mellonella* L., a lepidopteran highly susceptible to nematode infection, is most commonly used (Friedman, 1990). Dutky *et al.* (1964) and Woodring and Kaya (1988) describe methods for rearing the insect host, nematode infection and incubation, and harvesting of the IJs. Using insects to produce nematodes is costly, however, and not very practical for large-scale production. It is also undesirable to rely on a living host that would be sensitive to biological variations. Currently, nematodes are produced commercially on solid media (Bedding, 1984) or using liquid fermentation (Buecher and Popiel, 1989).

Reliable and stable formulations of entomopathogenic nematodes are essential for successful applications. To prevent depletion of their energy reserves, nematodes must be immobilized which can done by maintaining aqueous suspensions at low temperatures (5-15°C). However, this is not

12

commercially desirable (Kaya and Gaugler, 1993). More desirable methods include immobilizing the nematodes in carriers such as alginate or polyacrylamides. These formulations can be used to control soil-borne insects by applying them as aqueous sprays of the extracted nematodes. Bait formulations containing a carrier and a feeding stimulant also have been developed. Baits are useful against insect pests at the soil surface and in fields where irrigation is not practical (Georgis, 1990).

Several different methods can be employed to apply nematodes in the field including conventional hand and ground sprayers, sprinkler systems, boom sprayers, drip irrigation, soil injecting sprayers, and aircraft (Georgis, 1990). Nematode applications are usually done in the early morning or evening to minimize the adverse effects of solar radiation (Gaugler and Boush, 1978). Soil temperature is also an important consideration in the timing of applications. Low soil temperature (10-16°C) can cause entomopathogenic nematodes to give inconsistent control (Georgis, 1990).

Since entomopathogenic nematodes are being widely applied in the field, it is important to consider the safety of organisms, other than the target pest, with which they come in contact. As stated, *Steinernema* and *Heterorhabditis* have been shown to have a broad host range. While this is true, these nematodes attack a larger number of insects in the laboratory where there are no ecological barriers than they do in the

13

field. Their host range is more restricted in natural systems (Kaya and Gaugler, 1993). *Steinernema* and *Heterorhabditis* do not harm plants or mammals (Poinar, 1979), but there is some concern about some vertebrates and non-insect invertebrates. Although rats (Gaugler and Boush, 1979) and mice (Poinar *et al.*, 1982) show no susceptibility to these nematodes, young tadpoles have been shown to be susceptible. Nematodes generally do not mature in tadpoles but the tadpoles are killed by other bacteria introduced with the nematodes. *Steinernema* and *Heterorhabditis* can enter and kill non-insect invertebrates in the laboratory but they generally do not complete their life cycles in these hosts (Poinar, 1990).

Entomopathogenic nematodes are effective in controlling BVW on several plant species normally sold in pots, such as yew, cyclamen, grapes (Bedding and Miller, 1981), laurel (Klingler, 1988), and on strawberries grown in pots for experimental use (Rutherford *et al.*, 1987; Kakouli *et al.*, 1993). BVW has been controlled by nematodes in field crops such as hops (Dorschner *et al.*, 1989). Shanks and Agudelo - Silva (1990) reported the susceptibility of BVW to *Heterorhabditis* spp. and to *S. carpocapsae* (All strain) on cranberries in Washington. Dry-pick cranberry growers in British Columbia apply nematodes in the spring to control overwintering BVW larvae and again in the fall to control the new generation of insects. Spring applications are made when soil temperatures have reached 13°C. Although steinernematids

14

tolerate lower temperatures better than heterorhabditids (Molyneux, 1986), this low temperature may cause the nematodes to be ineffective. If this temperature is not reached until late spring, the BVW life cycle may be too advanced for the nematodes to be effective. Work by Ingraham (1991), and the repeated comments of growers, have suggested that *S. carpocapsae* does not always provide satisfactory control under local field conditions in British Columbia. Besides the damage that results, this is also a concern because nematodes are an expensive means of control ($1000/ha).

The present study was designed to determine the cause of the apparent, low effectiveness of *S. carpocapsae* (All strain) controlling BVW on dry-pick cranberries in British Columbia. A survey of local growers was conducted to identify the nature of their concerns with the effectiveness of this nematode. Bioassays using *G. mellonella* and BVW were conducted to test the infectivity of the nematodes used by the growers. Infectivity was checked before the nematodes were applied in the field and after they had passed through the application systems. Nematode presence, infectivity, and survival in the soil was checked using *G. mellonella* in soil bioassays. Activity of the nematodes in the peat soil in which local cranberries are grown was evaluated and compared with their activity in other soil types.

2. Materials and Methods

2.1 Survey of growers

To understand how nematodes are used to control BVW in cranberries in the Lower Mainland, a survey of local dry-pick cranberries growers was conducted in March, 1993 (Appendix I). This provided information on the nematode application process, as well as on the potential problems, as seen by the growers, of using nematodes.

The survey was divided into six major sections. The first was the history of the grower's bog. This included how long the grower had been growing cranberries, the age of the vines, and the trends in BVW damage. The second section addressed the grower's experience with using nematodes to control BVW. This included information on the number of years nematodes had been used, the company from which they were obtained, and the company that distributed them. This section also examined the procedure for nematode application (e.g. when they were obtained and when they were applied) and the time of application each year. The third section determined the use of chemical pesticides on the farms.

The fourth, fifth, and sixth sections focused on the overall nematode application process. The fourth examined the handling of the nematodes prior to use. This included the conditions under which the nematodes were transported to the

16

farms, how soon they were used after delivery, and the storage practices. The growers were asked also if they checked to see if the nematodes were alive immediately upon delivery and/or just before they were applied. The fifth section addressed the actual application. Topics covered were the conditions under which the nematodes were applied including time of day, weather conditions, and soil temperatures. As well, this section examined pre-application practices such as irrigation and whether the nematodes had been agitated during application. The last section addressed post-application practices, such as irrigation, whether or not the growers were satisfied with the control the nematodes provided, and how the level of satisfaction was measured.

2.2 Source and maintenance of experimental nematodes

Steinernema carpocapsae (All strain), marketed as the insecticide, Biosafe[R], was obtained from Biosys, Inc. (Palo Alto, CA 94303), for use in experiments testing the effect of time on the outcome of soil bioassays and nematode effectiveness in different soil types. The nematodes were packaged for transportation and distribution in a sealed plastic container containing a gel matrix suspended in a synthetic net. Biosafe, which is packaged for garden use, was stored at 10°C as recommended by Biosys, Inc. until required. After dissolving the gel in water to release the nematodes,

17

the aqueous suspension was applied with a watering can.

Nematodes used in experiments to test their infectivity before and after field application were obtained from Biosys by the two growers cooperating in the field trials. This product, Biosafe-N[R], consists of the same nematode species as Biosafe, but is packaged for farm use and can be applied through a sprinkler or a boom sprayer. Each box of Biosafe-N contained three mesh packages of nematodes immobilized with a gel matrix. The product is distributed for Biosys by a local company, Agrico Sales, Ltd. (Delta, B.C. V4G 1E7), that generally receives the nematodes by air 1 d before delivering them to the growers and stores them at 9-10°C (T. Ghan, pers. comm. Agrico Sales, Ltd. Delta, B.C.). The nematodes were used by the growers either immediately upon delivery, or were held overnight in cool conditions (2-9°C) and used the following morning.

2.3 Source and maintenance of experimental insects

Late-instar larvae of *G. mellonella* were used as a substitute for the BVW larvae because they are easier to produce and handle and have a higher susceptibility to nematodes. They were obtained from the insectary at Simon Fraser University, Burnaby, B.C., where they are reared on an artificial medium as described by Dutky *et al.* (1962).

BVW larvae were collected from two sites on the Lower

Mainland of British Columbia in the fall of 1992. Early
instars were collected from an abandoned cranberry farm in
Richmond during September and October. To collect the larvae
from this site, the cranberry vines were pulled back and the
soil was loosened and turned to a depth of about 8 cm using a
trowel. The larvae were removed from the soil and placed in
500 ml plastic containers with peat soil (>40% organic matter)
and cranberry roots. Early and late instars were collected
from Husband's Currant Farm on Westham Island, in October and
November. To collect larvae from this site, a screw driver
was used to dig and loosen the soil around the base of the
currant plants, to a depth of about 8 cm. The larvae
collected were placed in 500 ml plastic containers with peat
soil and cranberry roots. Nematode treatments had not
previously been used at these two sites. The larvae were
transported in a cooler to the Pacific Agriculture Research
Centre (Agriculture and Agri-Food Canada) on the University of
British Columbia campus in Vancouver. They were stored one
per cup in 30 ml plastic cups containing either peat soil
(>40% organic matter) with cranberry roots or an artificial
medium (Shanks and Finnigan, 1973) at $9^{\circ}C$ until required.

2.4 Location and preparation of field sites

Field experiments were conducted in 1993 during the
routine spring and fall nematode applications on two cranberry

19

farms in the Lower Mainland. One field site was on the Van

Kempen Farm, Pitt Meadows (Figure 2), where the Bergman

variety of cranberry is grown and is harvested in September.

The other site was on the Raine Farm, Richmond, where Bergmans

and McFarlins, which are harvested in October, are grown.

For the spring trial on the Van Kempen Farm, 20 flags

were placed in a plot of about 0.2 ha that was set up to

include two adjacent bogs. The plot was at the east end of

the bogs (Figure 3). A separate plot was set up for the fall

trial to include the same two bogs, but this plot was 43 m

from the east end of the bogs (Figure 4).

For the spring trial on the Raine Farm, 20 flags were

placed in a plot of about 0.2 ha that was also set up to

include two adjacent bogs. The plot was at the east end of

the bogs (Figure 5). The fall trial was set up at the west

end of a bog that had not been treated in the spring trial,

and 20 flags were placed in the plot that was about 0.2 ha

(Figure 6).

2.5 Application of nematodes

On the Van Kempen Farm, nematodes (Biosafe-N) were

applied from two boxes, each containing three packages, in the

spring and from three boxes (nine packages) in the fall.

The directions to the users from Biosys for soaking the

packages to remove the nematodes, instruct the user to fill
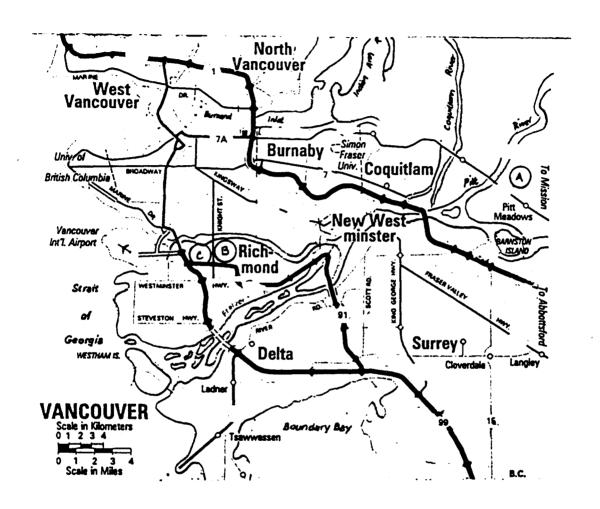
Figure 2. Map showing the Lower Mainland of British Columbia and the general location of the (A) Van Kempen Farm (Pitt Meadows) and (B) Raine Farm (Richmond) experimental sites, and (C) the abandoned cranberry farm.

21
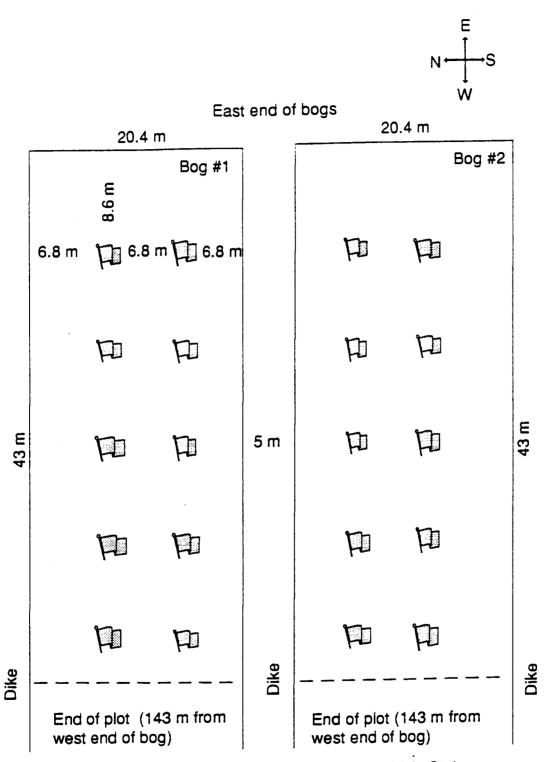
Figure 3. Diagram of the Van Kempen field plot to which *Steinernema carpocapsae* was applied in spring using a sprinkler system. The flags mark the location of collection cups and soil sampling sites in the two adjacent bogs.

E

N ←—→ S

W

20.4 m

20.4 m

End of plot (43 m from east end of bog)

End of plot (43 m from east end of bog)

8.6 m

Bog #1

Bog #2

6.8 m 6.8 m 6.8 m

5 m

43 m

43 m

Dike

Dike

Dike

End of plot (100 m from west end of bog)

End of plot(100 m from west end of bog)
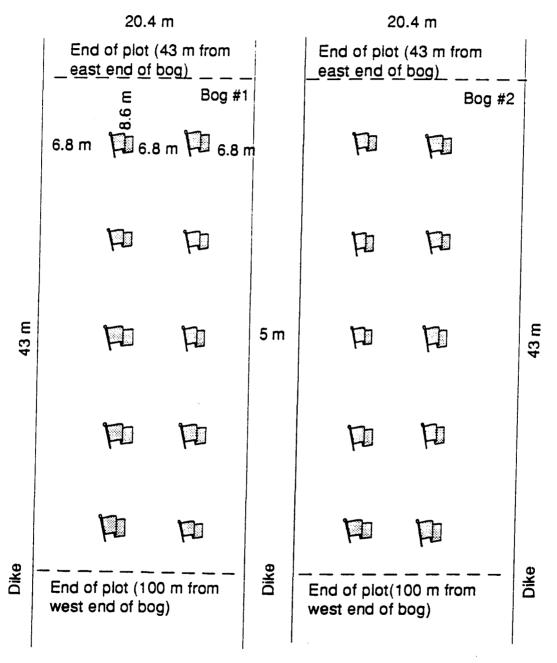
Figure 4. Diagram of the Van Kempen field plot to which *Steinernema carpocapsae* was applied in fall using a sprinkler system. The flags mark the location of collection cups and soil sampling sites in the two adjacent bogs.

East end of bogs

E

N — S

W

18.6 m   18.6 m

Bog #1   Bog #2

7.8 m

6.2 m ▢ 6.2 m ▢ 6.2 m

39 m   39 m

5 m

Dike   Dike   Dike

End of plot (116 m from   End of plot (116 m from
west end of bog)   west end of bog)
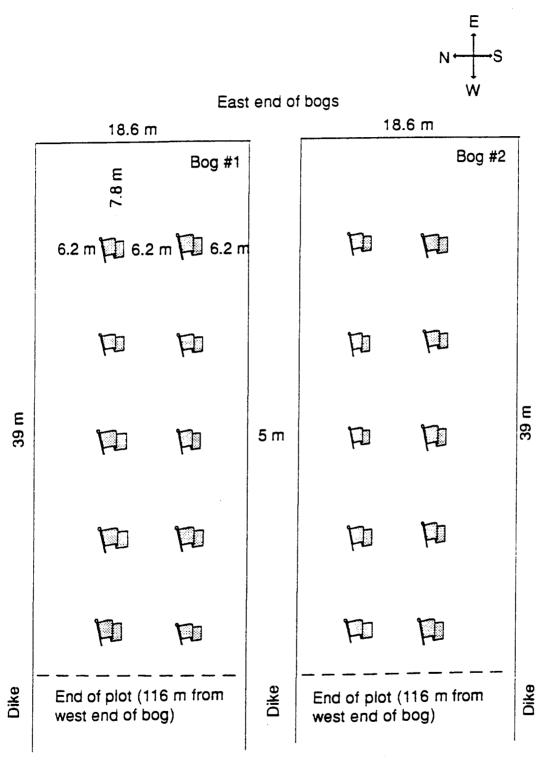
Figure 5. Diagram of the Raine field plot to which *Steinernema carpocapsae* was applied in spring using a boom sprayer. The flags mark the location of collection cups and soil sampling sites in the two adjacent bogs.

24

End of plot (108 m from east end of bog)

E

N ← → S

W

47 m

Dike

Dike

6.8 m   6.8 m   6.8 m

4.7 m

18.6 m

West end of bog
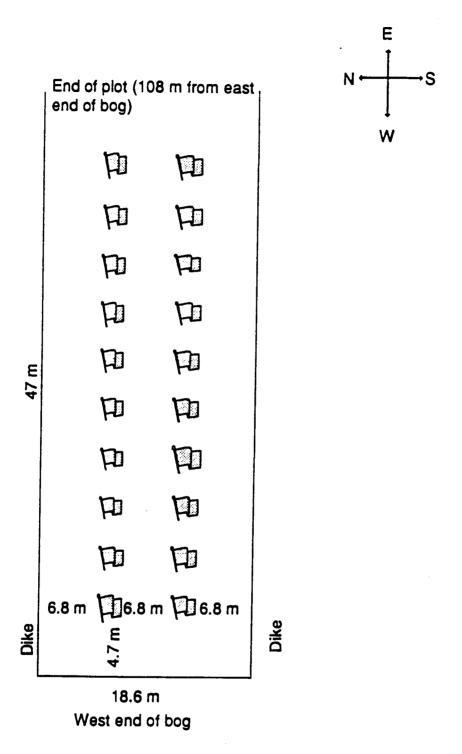
Figure 6. Diagram of the Raine field plot to which *Steinernema carpocapsae* was applied in fall using a boom sprayer. The flags mark the location of collection cups and soil sampling sites in the bog.

three buckets with tap water. The first package is rinsed in all three buckets and the water from each bucket is poured into the spray tank. This process is repeated for each package. It has been shown that 93.1% of the nematodes in each package are extracted in the first rinse, 6.1% in the second, and 0.8% in the third (Dapsis, 1992). Given this information, the grower modified the directions from Biosys in order to decrease the amount of excess water put in the tank. The directions were modified as follows: three buckets were filled with tap water. The first package was rinsed in buckets #1, 2, and 3, respectively. The water in bucket #1 was poured into the spray tank and the bucket was refilled with water. The second package was rinsed in buckets #2, 3, and 1, respectively. The water in bucket #2 was poured into the spray tank and the bucket was refilled with water. The third package was rinsed in buckets #3, 1, and 2 respectively. The water in bucket #3 was added to the spray tank and the bucket was refilled with water. This procedure was repeated for each box of three packages. Three different buckets were used for each box of three packages. Nematodes were applied through a sprinkler system with a nozzle pressure of 310.5 kPa. For both the spring and fall applications, the nematodes were applied between 7:00 and 8:00 am. The bogs were irrigated for 2 h prior to the nematode applications and for 2-3 h following. It took 15 min to apply the nematodes through the sprinkler system. During the applications, the

nematode suspension in the spray tank was stirred by the grower with a wooden paddle, to ensure that the nematodes stayed in suspension.

On the Raine Farm, two packages of nematodes (Biosafe-N) were applied in the spring and one package was applied in the fall using a boom sprayer with a nozzle pressure of 414.0 kPa. The packages were soaked, to remove the nematodes, according to the directions provided by Biosys. The spring application was made at 6:00 pm; the bogs were irrigated by a sprinkler for 4 h prior to the application and for 2 h following. The fall application was made at 7:00 am; the bog was irrigated for 2 h prior to the application and for 1 h following. It took 15 min to apply the nematodes by driving the spray tractor on a dike that runs between the bogs (Figures 5 and 6). The sprayer boom extended half-way across one bog (9 m). The nematode suspensions were applied to one side of the bog and then the other side and, in the spring, the process was repeated for the second bog that was treated. The nematode suspensions in the spray tank were agitated mechanically during spraying.

2.6 Bioassay techniques

The three bioassay techniques used in the experiments are described here. In the descriptions of the experiments, the bioassays are referred to by name.

2.6.1 Petri dish bioassay

The Petri dish bioassay method as described by Woodring and Kaya (1988) was modified as follows: 60 X 15 mm Petri dishes were used, each of which contained five paper towel disks of the same size under one piece of filter paper (Whatman #1) to maintain humidity. Using pipettes, 1 ml of distilled water and 1 ml of nematode suspension from the sample being tested were placed onto the filter paper in each treatment dish. Control dishes received 2 ml distilled water. One late-instar *G. mellonella* larva was placed in each Petri dish and the dishes were stored in plastic bags at 25°C. After 96 h, the larvae were dissected and the presence or absence of nematodes in the larvae was determined by examination under a Wild Heerbrugg dissecting microscope (12X).

2.6.2 BVW bioassay

Plastic collection cups (100 ml) were filled half-full with autoclaved (20 min, 120°C) peat soil collected from the abandoned cranberry farm. One BVW larva was buried in the middle of the soil in each cup. Each treatment received 1 ml of nematode suspension from the sample being tested and 6 ml of distilled water, all of which was applied to the surface using a pipette. Controls received 7 ml distilled water.

Lids with screened holes in the centre were placed on the cups which were held on a flat in a plastic bag at 25°C. The larvae were dissected after 15 d and the presence or absence of nematodes in the larvae was determined under the microscope.

2.6.3 Soil bioassay

Soil bioassay techniques, as described by Bedding and Akhurst (1975), were modified and used in the following manner: two, late instar *G. mellonella* larvae were placed in a Petri dish (100 X 15mm). Soil samples from the plots were placed on top of the larvae, filling the dish. The Petri dish was covered and Parafilm[R] was used to attach the lid to the dish. The samples were held at 25°C for various times ranging from 4-12 d. The larvae were then dissected, and the presence and infectivity of nematodes was determined under the dissecting microscope.

2.7 Experiments

2.7.1 Effect of time on outcome of soil bioassay

On the Raine Farm, twenty 50 X 50 cm treatment plots were set up on one bog and twenty control plots on a second bog. Using a watering can, 2500 ml of water (equivalent to 1 cm of

irrigation) were applied to each treatment plot. A nematode suspension (220 nematodes/ml water) was prepared using Biosafe. Using a watering can, 500 ml of the suspension (5 bil/ha) was applied to each treatment plot. Each treatment plot then received 3750 ml of water which is equivalent to 1.5 cm of irrigation. Each control plot received 6750 ml of water.

After 7 d, five soil samples were taken at random from each plot. Each soil sample consisted of four cores that were 2.5 cm in diameter and 5 cm deep. To test the effect of the duration of the bioassays on their outcome, each of the five soil samples taken from each plot was tested, using the soil bioassay, in an incubator (25 $^{\circ}$C) for different periods of time. One soil sample from each plot was examined at each of 2, 4, 8, 10, and 12 d after being placed in the incubator.

2.7.2 Infectivity of nematodes at acquisition

Prior to both the spring and fall applications, the nematodes were sampled from each box of three packages used on the Van Kempen Farm and from each package used on the Raine Farm. Using 100 ml collection cups, samples of the nematode suspensions were taken from the buckets in which the packages had been soaked. Two 100 ml samples, which were combined to make one sample, were taken from each box or package. As well, before each application, four 100 ml samples, which were

30

combined to make one sample, were taken from the spray tanks where the nematodes from either all the boxes or all the packages had been mixed together.

In the laboratory, visual observations were made of the nematode suspensions and the Petri dish bioassay was used for each sample, to determine the infectivity of the nematodes. To test each sample, 20 *G. mellonella* were used.

2.7.3 Infectivity of nematodes after application

For each spring and fall application, two 100 ml collection cups were placed at each of the 20 flags in the field plots. The cups caught the nematode suspension being applied through either the sprinkler system on the Van Kempen Farm or the boom sprayer on the Raine Farm. Immediately following the applications, the cups were retrieved from the field and the contents of the two cups at each flag were combined. In the fall, each of the combined suspensions was diluted 10X, in the field. This was done so the sides of the collection cups could be rinsed. This also allowed for a nematode counting dish, which requires more water than was caught in the cups, to be used in the lab to determine the concentration of nematodes in each cup.

In the laboratory, visual observations of the nematode suspensions were made and the Petri dish bioassay was used to test for nematode infectivity in each of the 20 collection

31

cups retrieved after each nematode application. One *G. mellonella* was used for each collection cup sample.

2.7.4 Infectivity of nematodes against BVW before and after application

In the spring, the BVW bioassay was used to test infectivity of the nematodes sampled from the spray tank before application and from the collection cups after application on the Van Kempen Farm. To test the infectivity of the nematodes sampled from the spray tank, 20 BVW larvae were treated with suspension and 20 were treated with water (controls). To test the infectivity of the nematodes caught in the collection cups, 12 BVW larvae were treated with suspension and 12 were treated with water (controls).

2.7.5 Distribution of nematodes after application

To determine the distribution of the nematodes across the bogs after sprinkler or boom application, the concentration of the nematodes in each collection cup was determined. In the spring, this was done by counting the number of nematodes in a 0.01 ml aliquot of nematode suspension taken from each collection cup. Fifty 0.01 ml aliquots were taken from each collection cup, and the number of nematodes/ml water collected was determined. In the fall, the concentration was determined using a nematode counting dish borrowed from Dr. Thierry Vrain

at the Pacific Agriculture Research Centre, Vancouver, British
Columbia. The dish was 5 X 5 cm with a 100 square grid on the
bottom. Using a pipette, 4 ml of nematode suspension from a
collection cup were placed in the dish and allowed to settle.
The total number of nematodes in the dish was determined by
adding together the number of nematodes in each square of the
grid. This number was multiplied by ten to account for the
dilution of the sample. The resulting number was multiplied
by 1.56, which is a conversion factor determined in Dr.
Vrain's lab, to account for the edges of the dish. The
resulting number was divided by four to determine the number
of nematodes/ml of water collected in the cup. This procedure
was repeated for each collection cup. Where the field plots
were comprised of two adjacent bogs, the distributions on the
bogs were compared with each other. On the field plot that
had just one bog, the distributions on the two sides of the
bog were compared.

2.7.6 Effectiveness of nematodes applied in the field

To determine if the nematodes remained infective in the
soil and reached the location of the BVW, soil samples were
taken at each of the 20 flags in the field plots. Samples
were taken before and after each spring and fall application.
Pre-application samples were taken the day before the
applications.   On the Van Kempen Farm, post-application

33

samples in the spring were taken 3, 7, and 25 d after the application. Post-application samples in the fall were taken 3, 7, 35, 60, 135, and 250 d after the application. On the Raine Farm, post-application samples in both the spring and fall were taken 3, 7, and 25 d after the applications.

Each soil sample, taken at each of the 20 flags in the field plots, consisted of four cores that were 2.5 cm in diameter and 10 cm deep. Each core was separated into the top 1-5 cm and the bottom 5-10 cm, for a total of 20 top and 20 bottom samples from each field plot on each sampling day. In the laboratory, each soil sample was tested for the presence and infectivity of nematodes using the soil bioassay. For the soil bioassays done in the spring, the *G. mellonella* larvae were exposed to the soil samples for 4 d, and in the fall, they were exposed for 10 d.

2.7.7 Influence of soil type on infection of *Galleria mellonella* by *Steinernema carpocapsae*

Four types of soil were collected, with as little disturbance to their structure as possible, from four different field sites in the Lower Mainland of British Columbia: a loamy soil from Abbotsford (soil A), a peat soil from Richmond (soil B), a muck soil from Cloverdale (soil C), and a silty clay loam from the UBC Field Station in Vancouver (soil D). The soil samples were 30 X 35 cm and 9 cm deep, had

no crop canopies, and were held separately in 10 cm deep, plastic containers. Two containers, each with solid bottoms, were used for each soil type. Each container of soil, except those containing soil C because it was already moist, received 1050 ml of water which is equivalent to 1 cm of irrigation. A nematode suspension (300 ml of suspension; 150 nematodes/ml) which is equivalent to 7.5 billion nematodes/ha, using Biosafe, was applied with a watering can to one of the containers of each soil type. The remaining containers served as controls and received 300 ml of water. Soils A and B received an additional 525 ml of water immediately following the treatment. Soils C and D received no additional water in order to avoid having the nematodes caught in standing water. The uncovered containers were held in the laboratory for 7 d at 16°C after which, 20 soil samples were taken from each container. The soil samples consisted of three soil cores each of which was 2.5 cm in diameter and 5 cm deep. Nematode effectiveness in each of the soil types was determined by testing each soil sample (i.e the mixed soil from three cores) using the soil bioassay. Control samples were tested in the same way. For the bioassay, the samples were held in an incubator (25 ^{o}C) for 10 d.

2.8 Statistical analysis

Data on the distribution of nematodes across the bogs

35

following the spring and fall applications of *S. carpocapsae* through a sprinkler system on the Van Kempen Farm and a boom sprayer on the Raine Farm, are expressed as the mean ± standard error of the mean, and the data were analyzed using Student's *t*-tests (SAS Institute, 1988). Data from experiments on the presence and activity of nematodes in field locations and in the soil experiments, were corrected for natural infectivity using Abbott's Formula (Abbott, 1925).

Nematode infectivity in the various soil types was compared using Fisher's Exact Probability test (SAS Institute, 1988), and Bonferroni Adjusted Values test (Weimer, 1993) were calculated. The cumulative percentage of infected larvae of *G. mellonella* in relation to the time (days) for which larvae were exposed to Petri dishes containing soil treated with *S. carpocapsae*, was analyzed by a simple linear regression (Harvard Graphics, 1993).

3.0 Results

3.1 Survey of growers

Three of the five dry-pick cranberry growers in the Lower Mainland participated in the growers' survey conducted in March, 1993. The participants were Tony Van Kempen, David Raine, and Don March.

Tony Van Kempen has been growing cranberries since 1979. Serious damage from BVW first occurred on his farm in 1988. In 1989, he began monitoring the adult population and used Guthion as a method of control. This worked well, but he stopped using Guthion the following year because it was killing the pollinating bees. He first applied nematodes in July, 1989. The nematodes were not very effective that first year, and he believes that this was due to the inappropriate timing of the application. In subsequent years, he applied nematodes in May when the soil temperatures had reached 13°C and/or in September. He applies them through a sprinkler system early in the morning under cloudy or wet conditions. He irrigates for about 1 h before and about 3 h after nematode application. The nematodes have always originated from Biosys but have been distributed by Agrico only since 1990. The nematodes are delivered to Tony in unrefrigerated trucks the evening before the application. He stores them in a cool basement (2°C) overnight. Prior to 1993, Tony did not check

to see if the nematodes were alive when they were delivered. However, he has now purchased a monocular microscope for this purpose because the shipment he got in the fall of 1992 was dead. In general, Tony has been quite satisfied with the BVW control provided by the nematodes. He measures this by monitoring the adult BVW populations and by the level of damage that appears in the years following the applications. For example, in 1992, he collected no BVW adults.

David Raine has been growing cranberries for 20 years. On his farm, there are pockets where BVW persists and the damage is quite severe. He used Malathion in the past to control the adults, but stopped because it can affect the blossoms of the cranberry plants. David has been using nematodes since 1989. He treats in May when the soil temperatures have reached 13°C and again in August. The application in the spring is a small one; only a 0.2 ha area is treated because BVW is consistently a problem in that area. In the fall, most of the farm (6 ha) is treated. David uses a boom sprayer to apply the nematodes in the evening, or in the morning, or during the day under cloudy conditions. When the whole farm is being treated, it is irrigated for 6 h before and 2 h after the application. Less irrigation is required for the smaller spring treatment. The nematodes have always originated from Biosys and have been distributed by Agrico since 1990. David usually receives the nematodes the day of the application. They are delivered in an

unrefrigerated truck and have occasionally been left in the sun by the distributor. David also received dead nematodes in 1992 and, although he does not examine them under the microscope, he could tell they were dead because they smelled putrid. In general, David believes the nematodes have worked. He is concerned about the occurrence of pockets of the bog that consistently show BVW damage, but thinks that the nematodes prevent the damage from spreading. He is concerned about the procedures the distributor uses for storage and delivery of the nematodes.

Don March has been growing cranberries since 1968. BVW has been a serious problem since 1988. He has used Guthion and Malathion to control the adults. Don has been using nematodes since 1990 and does only fall applications. He treats in the morning, or in the evening, or during the day under cloudy conditions using a sprinkler system. He treats the whole farm and irrigates for 4 h before and 2 h after the application. The nematodes have always originated from Biosys and been distributed through Agrico. Don gets the nematodes on the day of the application and checks to see if the bags smell putrid before he uses them. He is familiar with the condition of mouldy bags because the bags usually become mouldy once they are used. One of Don's major concerns is whether or not the nematodes are penetrating the canopy of the cranberries into the soil. He is concerned also about the cost of the nematodes. He considers the cost to be quite high

and, if they were less expensive, he might also treat in the
spring.
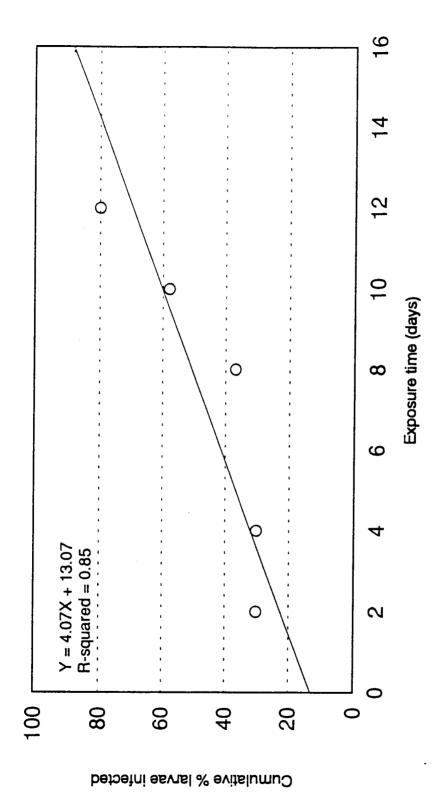
3.2 Experiments

3.2.1 Effect of time on outcome of soil bioassay

Results of the soil bioassay using *G. mellonella* showed
that nematode infectivity, i.e. the percentage of larvae
infected with nematodes, in the soil samples increased as the
duration of the bioassay increased from 2 to 12 d. The
regression run on infectivity data collected 2, 4, 8, 10, and
12 d after initiating the soil bioassay showed a significant
positive relationship between infectivity and the number of
days that the *G. mellonella* larvae were exposed to the soil
samples (Figure 7). At 2 d, there was a 30% infectivity and
at 12 d, 80%.

3.2.2 Infectivity of nematodes at acquisition

Visual observations of the nematode suspensions collected
from both farms revealed that the nematodes were active. The
Petri dish bioassays of samples of an aqueous solution of *S.
carpocapsae* taken from commercial packages and from the spray
tank prior to the spring and fall applications at the Van
Kempen Farm showed infectivity levels between 95 and 100%,
proving that almost all the nematodes in the suspensions were

40

Figure 7. Percentage of *Galleria mellonella* larvae infected with *Steinernema carpocapae* after exposure for 2, 4, 8, 10, and 12 days to soil treated with the nematodes. Each data point represents a mean of 20 samples.

active and infective (Table 1). Larvae used in the untreated controls showed no nematode parasitism, which proved that the *G. mellonella* used in the experiment were free of nematodes before treatment.

Similarly, Petri dish bioassays of samples of an aqueous solution of *S. carpocapsae* taken from commercial packages and from the spray tank prior to the spring and fall applications at the Raine Farm showed infectivity levels between 95 and 100%, again proving that the nematodes in the suspensions were infective (Table 2). Larvae used in the controls again showed no nematode parasitism, which proved that the *G. mellonella* used in this experiment were free of nematodes before treatment.

3.2.3 Infectivity of nematodes after application

Visual observations of the nematode suspensions collected from both farms revealed that the nematodes were active. Petri dish bioassays testing the infectivity of *S. carpocapsae* after application through either a sprinkler or a boom sprayer showed infectivity levels between 95 and 100%, showing that the nematodes used remained viable after passing through either of the application systems (Table 3). Larvae used in the controls showed no nematode parasitism, proving that these *G. mellonella* larvae used for the bioassay were free of nematodes prior to treatment.

42

Table 1. Infectivity of *Steinernema carpocapsae* prior to spring and fall applications on the Van Kempen Farm. Results based on a Petri dish bioassay, using *Galleria mellonella* larvae, of samples of an aqueous suspension of the nematodes taken from commercial packages (boxes A-E) and from the spray tank prior to sprinkling.

Sample	Application Time	Percentage of *G. mellonella* infected (n=20)
Box A	Spring	95
Control		0
Box B	Spring	95
Control		0
Box C	Fall	100
Control		0
Box D	Fall	100
Control		0
Box E	Fall	100
Control		0
Tank (A+B)	Spring	100
Control		0
Tank (C+D+E)	Fall	100
Control		0

Table 2. Infectivity of *Steinernema carpocapsae* prior to spring and fall applications on the Raine Farm. Results based on a Petri dish bioassay, using *Galleria mellonella* larvae, of samples of an aqueous suspension of the nematodes taken from commercial packages (packages A-C) and from the spray tank prior to spraying with a boom.

Sample	Application time	Percentage of *G. mellonella* infected (n=20)
Package A	Spring	100
Control		0
Package B	Spring	100
Control		0
Package C	Fall	100
Control		0
Tank (A+B)	Spring	100
Control		0
Tank (C)	Fall	95
Control		0

44

Table 3. Infectivity of *Steinernema carpocapsae* after application through either a sprinkler or a boom sprayer. Results based on a Petri dish bioassay, using *Galleria mellonella* larvae, of samples collected in cups placed in grids on the bogs before spring and fall applications.

Sample	Application system	Application time	Percentage of *G. mellonella* infected (n=20)
Collection cup	Sprinkler	Spring	95
Control			0
Collection cup	Sprinkler	Fall	95
Control			0
Collection cup	Boom sprayer	Spring	100
Control			0
Collection cup	Boom sprayer	Fall	100
Control			0

3.2.4   Infectivity of nematodes against BVW before and after application

Results of BVW bioassays using aqueous suspensions of *S. carpocapsae* sampled before and after a spring application from a sprinkler system at the Van Kempen Farm showed 92.9 and 66.7% of the BVW larvae becoming infected respectively (Table 4).   Larvae used in the controls showed no infectivity, indicating that the BVW larvae used in the bioassays were free of nematodes.   In the experiment that tested the infectivity of the nematodes sampled before the application, six BVW larvae that were treated with suspension and one control larva escaped.   Therefore, percentages for this experiment were based on 14 larvae treated with suspension and 19 controls.

3.2.5   Distribution of nematodes after application

Analysis of the distribution of a spring application of nematodes applied through a sprinkler system at the Van Kempen Farm showed that the nematodes were successfully spread, although somewhat unevenly (mean = 245 ± 28), across the field plot (Figure 8).   Means of 250 ± 44 and 241 ± 38 nematodes/ml water were collected from bog #1 and bog #2, respectively. The variances of nematodes/ml water collected were equal between the two adjacent bogs (F' = 1.20; d.f. = 6,7; P = 0.8044).   Student's *t*-test showed no significant

46

Table 4. Infectivity of *Steinernema carpocapsae* sampled before and after a spring field application through a sprinkler system on the Van Kempen Farm. Results based on BVW bioassays.

Source	Number of samples examined	Percent samples with infected BVW larvae
Before		
Tank	14	92.9
Control	19	0
After		
Collection cups	12	66.7
Control	12	0
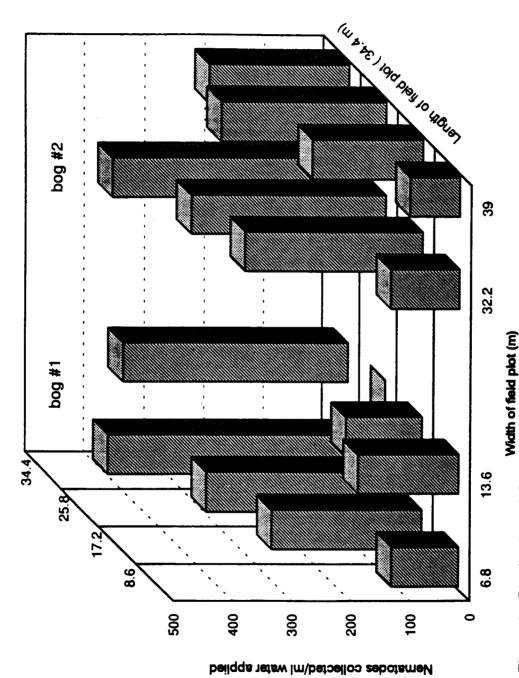
Figure 8. Distribution of *Steinernema carpocapsae* following the spring application of nematodes through the sprinkler system to two adjacent bogs on the Van Kempen Farm.

48

difference between the numbers of nematodes/ml of water collected on the two adjacent bogs (t = 0.1619; d.f. = 13; P = 0.8739).

Analysis of the distribution of a fall application of nematodes applied through a sprinkler system at the Van Kempen Farm showed that the nematodes were successfully spread, and relatively evenly (mean = 583 ± 28), across the field plot (Figure 9). Means of 563 ± 40 and 604 ± 40 nematodes/ml water were collected from bog #1 and bog #2, respectively. The variances of nematodes/ml water collected were equal between the two adjacent bogs (F' = 1.04; d.f. = 9,9; P = 0.9591). No significant difference occurred between the numbers of nematodes/ml of water collected on the two adjacent bogs (t = 0.1619; d.f. = 13; P = 0.8739).

Analysis of the distribution of a spring application of nematodes applied through a boom sprayer at the Raine Farm showed that the nematodes were successfully spread, and relatively evenly (mean = 519 ± 61), across the field plot (Figure 10). Means of 598 ± 95 and 430 ± 65 nematodes/ml water were collected from bog #1 and bog #2, respectively. The variances of nematodes/ml water collected were equal between the two adjacent bogs (F' = 2.34; d.f. = 9,8; P = 0.2458). There was no significant difference between the numbers of nematodes/ml of water collected on the two adjacent bogs (t = 1.4234; d.f. = 17; P = 0.1727).

Analysis of the distribution of a fall application of
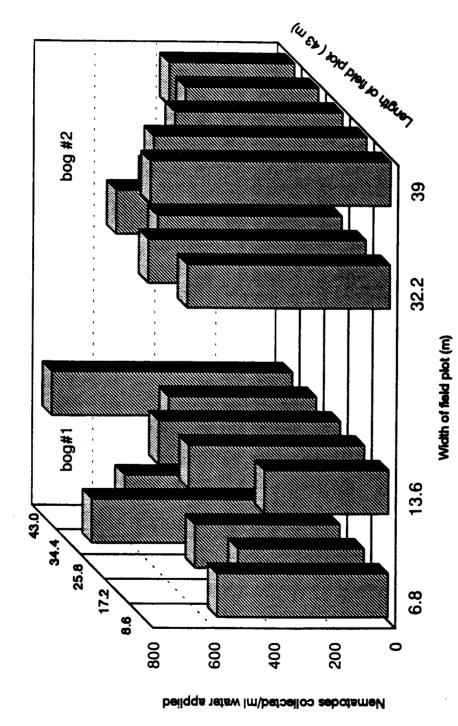
Figure 9. Distribution of *Steinernema carpocapsae* following the fall application of nematodes through a sprinkler system to two adjacent bogs on the Van Kempen Farm.
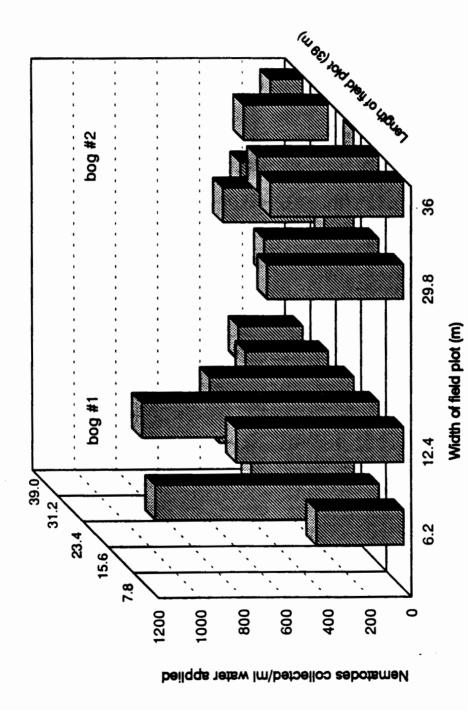
Figure 10. Distribution of *Steinernema carpocapsae* following the spring application of nematodes through a boom sprayer to two adjacent bogs on the Raine Farm.

nematodes applied through a boom sprayer at the Raine Farm showed that the nematodes were successfully spread, and relatively evenly (mean = 518 ± 35), across the field plot (Figure 11). Means of 539 ± 53 and 496 ± 46 nematodes/ml water were collected from side #1 and side #2, respectively. The variances of nematodes/ml water collected were equal between the two sides (F' = 1.32; d.f = 9,9; P = 0.6850). There was no significant differences between the numbers of nematodes/ml of water collected on the two sides of the bog (t = 0.6053; d.f. = 18; P = 0.5525).

The blanks that appear in the diagrams for both spring applications do not indicate that nematodes were not caught by the collection cups placed in those areas. Due to a sampling error, the concentrations in those cups could not be determined.

3.2.6 Effectiveness of nematodes applied in the field

Results of the 4 d soil bioassay using soil collected before and after the spring application of *S. carpocapsae* from the sprinkler system on the Van Kempen Farm showed that nematode infectivity was related to the time of sampling (Table 5). Infectivity decreased over time for both the 0-5 and 5-10 cm depths. At 25 d post-application, 0% infectivity was reported for both depths of soil. By contrast, the 10 d bioassay using soil collected before and after the fall
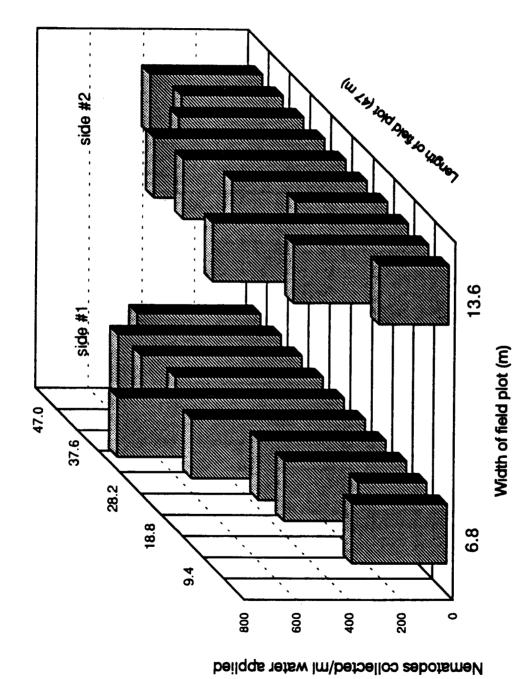
Figure 11. Distribution of *Steinernema carpocapsae* following the fall application of nematodes through a boom sprayer to a bog on the Raine Farm.

Table 5. Effectiveness of *Steinernema carpocapsae* applied through a sprinkler system on the Van Kempen Farm. Results based on a 4 d soil bioassay of *Galleria mellonella* larvae using soil from 0-5 and 5-10 cm depths collected before and after a spring application.

Days after application	Soil depth (cm)	Percent of soil samples containing nematodes (n=20)[a]	Predicted percent of soil samples containing nematodes for 10 d bioassay[b]
Pre-application	0-5	0	
	5-10	15.0	~30
3	0-5	15.0	~30
	5-10	11.8	~24
7	0-5	5.0	~10
	5-10	0	-
25	0-5	0	-
	5-10	0	-

[a] To calculate percent samples infected, the value of soil samples containing nematodes was corrected for the control (pre-application) response, using Abbott's formula (Abbott, 1925).

[b] Values based on regression equation obtained in section 3.2.1.

application on the Van Kempen Farm showed that the nematodes were in the soil and infective up to and including 250 d post-application. From 3 d to 7 d post-application, the level of infectivity ranged from 15.8-57.8% and 15.5-57.9% for the 0-5 and 5-10 cm depths, respectively (Table 6).

Results of the 4 d soil bioassay using soil collected before and after the spring application of *S. carpocapsae* from the boom sprayer on the Raine Farm showed that the nematodes were present and remained infective up to and including 25 d post-application (Table 7). Nematode infectivity declined somewhat over time. Results of the 10 d soil bioassay using soil collected before and after the fall application on the Raine Farm also showed that the nematodes remained infective up to and including 25 d post-application (Table 8). Infectivity levels in the 0-5 cm depth remained fairly constant over time but declined somewhat in the 5-10 cm depth at 25 d post-application.

Using the regression equation obtained from the results of the bioassays conducted to determine the effect of time on the outcome of the soil bioassay (section 3.2.1), a prediction was made of the infectivity that would have resulted if the spring bioassay had been 10 d. The predicted infectivities for the spring application on the Van Kempen Farm ranged from 10-30% (Table 5) and those on the Raine Farm were as high as 90% (Table 7).

Table 6. Effectiveness of *Steinernema carpocapsae* applied through a sprinkler system on the Van Kempen Farm. Results based on a 10 d soil bioassay of *Galleria mellonella* larvae using soil from 0-5 and 5-10 cm depths collected before and after a fall application.

Days after application	Soil depth (cm)	Number of soil samples	Percent of soil samples containing nematodes[a]
Pre-application	0-5	20	5.0
	5-10	19	5.3
3	0-5	20	15.8
	5-10	20	31.4
7	0-5	20	57.9
	5-10	19	50.0
35	0-5	20	42.1
	5-10	20	41.9
60	0-5	20	21.1
	5-10	20	36.6
135	0-5	20	52.6
	5-10	20	57.8
250	0-5	20	26.3
	5-10	20	15.5

[a]To calculate percent samples infected, the value of the soil samples containing nematodes was corrected for the control (pre-application) response, using Abbott's formula (Abbott, 1925).

Table 7. Effectiveness of *Steinernema carpocapsae* applied through a boom sprayer on the Raine Farm. Results based on a 4 d soil bioassay of *Galleria mellonella* larvae using soil from 0-5 and 5-10 cm depths collected before and after a spring application.

Days after application	Soil depth (cm)	Number of soil samples	Percent of soil samples containing nematodes	Predicted percent of soil samples containing nematodes for 10 d bioassay[a]
Pre-application	0-5	20	0	‾
	5-10	19	0	‾
3	0-5	20	30.0	‾60
	5-10	20	45.0	‾90
7	0-5	20	10.0	‾20
	5-10	20	10.0	‾20
25	0-5	20	10.0	‾20
	5-10	20	15.0	‾30

[a]Values based on regression equation obtained in section 3.2.1.

Table 8. Effectiveness of *Steinernema carpocapsae* applied through a boom sprayer on the Raine Farm. Results based on a 10 d soil bioassay of *Galleria mellonella* larvae using soil from 0–5 and 5–10 cm depths collected before and after a fall application.

Days after application	Soil depth (cm)	Percent of soil samples containing nematodes (n=20)[a]
Pre-application	0-5	0
	5-10	5.0
3	0-5	40.0
	5-10	36.8
7	0-5	45.0
	5-10	36.8
25	0-5	40.0
	5-10	10.5

[a]To calculate percent samples infected, the value of soil samples containing nematodes was corrected for the control (pre-application) response, using Abbott's formula (Abbott, 1925).

58

3.2.7 Influence of soil type on infection of *Galleria mellonella* by *Steinernema carpocapsae*

The 10 d bioassay of *G. mellonella* larvae using four soil types inoculated with *S. carpocapsae* showed that with the peat, muck, and silty clay loam, > 90% of the insect larvae exposed to the inoculated soils in Petri dishes became infected (Table 9). In the treatment using loam as the medium, only 33.3% of the insect larvae exposed to the inoculated loam became infected. Fisher's Exact Probability test showed that the level of infectivity in the four soil types was significantly different (P=2.24E-05). A Bonferroni Adjusted *t*-test showed that the infectivity in the loam soil was significantly different from those in peat, muck, and sandy clay loam, but that the infectivities in the latter three were not significantly different from each other.

Table 9. Influence of four soil types on the infection of *Galleria mellonella* by *Steinernema carpocapsae* over 10 d soil bioassay.

Soil type	Number of treated soil samples containing nematodes (n=20)	Corrected value of percent soil samples containing nematodes[a]
Loam	10	33.3a
Peat	20	100b
Muck	19	94.7b
Silty clay loam	19	93.8b

[a]To calculate the corrected value of percent infected, the value of the soil samples containing nematodes was corrected for the control response, using Abbott's formula (Abbott, 1925). Percents followed by the same letter are not significantly different, as determined by Bonferroni Adjusted Values Test.

4.0 Discussion

A survey of dry-pick cranberry growers in British
Columbia showed that *S. carpocapsae* (All strain) does not
always provide satisfactory control of BVW. The present study
examined the various stages of the nematode application
process as used by the growers. The results show that most of
the stages of the process do not inhibit the success of the
nematodes but that their effectiveness seems to decline once
they are in the soil.

Petri dish bioassays with *G. mellonella* showed that the
nematodes, as delivered to the growers at the time of the
experiments were, virtually, 100% infective. The formulated
nematode product has a shelf life of 1-6 months when
refrigerated, but 1-3 months at room temperature (Georgis,
1990). The product must be kept out of direct sunlight
because of the intolerance of the nematodes to heat (Molyneux,
1986) and UV radiation (Gaugler *et al.*, 1992). Delivery of
dead nematodes in the past may have been due to the packages
of nematodes having been left in direct sunlight for an
extended period. The growers are careful to store the
nematodes out of direct sunlight and at cool temperatures (2-
10°C) if they do not use them immediately upon receiving them.
The growers generally do not store the nematodes longer than
24 h. It would be useful, and would help to increase customer
confidence, to have the date that the nematodes were produced

and an expiration date clearly marked on the box. Expiration dates appear on Biosafe, which is packaged for garden use, but not on Biosafe-N which is packaged for farm use. The growers are beginning to use equipment such as monocular microscopes to check the quality of the nematodes before they apply them. This is a good practice and one to be encouraged.

There are at least two reasons why it is essential that the growers are provided with active, infective nematodes when they order them. Firstly, the amount of money the growers are spending on the product is quite high so the quality also should be high. Secondly, if the grower has to return a shipment to the distributor and order a replacement, the timing of the application may no longer be optimal. Consequently, the cranberries may sustain BVW damage that could have been avoided.

Petri dish and BVW bioassays showed that the nematodes remained, virtually, 100% infective once mixed in the tanks prior to application. Keeping nematodes in suspension is important for an effective, uniform application (Kaya, 1985). Moreover, the growers recognize the importance of using clean spray tanks and equipment. Residues of pesticides could be harmful to the nematodes (Kaya, 1990a).

Entomopathogenic nematodes can be applied in the field using a variety of different types of spray equipment. In this study, nematodes survived and remained infective when applied through either a sprinkler system or a boom sprayer.

This was expected since the nematodes were applied at pressures considerably lower than the highest pressure they can tolerate. The nozzle pressure of the sprinkler system was 310.5 kPa and that of the boom sprayer was 414.0 kPa, and it is known that nematodes can withstand application pressures of 2070.0 kPa (Georgis, 1990).

The diagrams of nematode distribution across the treatment plots show that all the applications successfully delivered nematodes throughout the plots. On the Van Kempen Farm in the spring, the eastern ends of the bogs seemed to receive a slightly higher concentration of nematodes than the rest of the plot. This could have been due to a slight breeze that may have blown the spray toward the east end, or could have been caused by variation in the spray patterns of individual sprinkler heads. Spray patterns are difficult for a grower to control because the motion of each sprinkler head is slightly different and the mechanisms wear differently over time (T. Van Kempen, pers. comm. British Columbia Cranberry Growers Association, Pitt Meadows, B.C.). On both farms, the fall applications resulted in quite uniform distributions of the nematodes across the bogs.

For each application, the nematodes penetrated the upper 10 cm of the soil where they survived and remained infective. The nematodes were shown to persist for up to 250 d which confirms other studies that have shown *S. carpocapsae* to persist in soil for 28 d (Wright *et al.*, 1993) and 3 months

(Pye and Pye, 1985). Entomopathogenic nematodes have been shown to persist in the soil for up to 16 months (Bayers and Poinar, 1982). Persistence is a desirable attribute in a biological control agent, especially in one as costly as *S. carpocapsae.* Although the percent infectivity , i.e. the number of infected insects taken from the soil bioassay in the laboratory, differed somewhat between the sampling days, the infectivities in the 0-5 and 5-10 cm samples were quite similar for each sampling day, indicating that the vertical migration of the nematodes is adequate. Since the cranberry roots grow in the top 10 cm of the soil, it is beneficial to have infective nematodes in the top 10 cm, in order to control BVW through the entire growing zone.

On the Raine Farm, infectivity in the soil bioassays, after the spring results had been adjusted from a 4 to a 10 d soil bioassay, was higher after the spring application than after the fall application. The percentages from the spring ranged from 20-90% and those in the fall from 10.5-40%. There may have been a higher population of BVW in the soil in the spring since the area treated was one that consistently sustained BVW damage. This would allow the nematodes to reproduce more readily (Kaya, 1990a) and increase the soil population. Such an occurrence could account for the greater percent infectivity.

By contrast, on the Van Kempen Farm, the adjusted percent infectivities for the spring were virtually the same as those

for the fall. The BVW populations in the spring and fall field plots on the Van Kempen Farm may have been similar in size. This could result in similar rates of nematode reproduction which may explain the similarity in the percent infectivities shown by the soil bioassays for the spring and fall applications on the Van Kempen Farm.

Although one of the adjusted values for the spring soil bioassays was 90% infectivity, in general, the results of the soil bioassays using *G. mellonella*, a highly susceptible host, show that the percent infectivities are not sufficiently high for the satisfactory control of BVW, a less susceptible host. A low percent infectivity in a laboratory bioassay probably indicates low infectivity in the field. A high value crop with a low economic threshold, such as cranberries, requires a biological control agent that provides a percent infectivity higher than 60%, which was the next highest percent infectivity achieved per sampling day in the soil bioassays using *G. mellonella*. This amount of infectivity would be considered to provide only moderate control (Georgis and Poinar, 1989), but for cranberries, a more desirable percent infectivity achieved in the soil bioassays would have been >80%. Since such high percentages were obtained in the bioassays for the nematodes sampled before the application and just after passage through the application systems, the decline in effectiveness of the nematodes appears to occur once they are in the soil.

There are several possible reasons for this observed decrease in nematode effectiveness as a biological control agent of BVW in the soil. Firstly, the effectiveness of the bioassays themselves should be considered. The Petri dish bioassay used for testing the nematodes in water has been shown (Woodring and Kaya, 1988) to give more consistent results than other assay methods, and it is commonly used with *G. mellonella* as the host. There are, however, some limitations to the Petri dish bioassay. There can be a high nematode to host ratio that can mask the presence of less pathogenic nematodes (Georgis, 1990). The bioassay gives little information on the details of the interaction between the nematodes and the target insect, such as the speed of penetration (Glazer, 1992). Rather, the Petri dish bioassay simply measures the ability of the nematodes to enter and kill the target insect, which was an adequate measurement for the purposes of this study. However, *G. mellonella* is highly susceptible to nematodes and, therefore, may not be an ideal bioassay host (Woodring and Kaya, 1988), particularly if the infectivities of two or more species or strain of nematodes are being compared. A bioassay using a less susceptible host may be more sensitive to minor differences in infectiveness between different nematode species or strains and, consequently, may be preferable (Kaya, 1990b). *G. mellonella* is readily available, however, and well-suited to tests done on filter paper, so it continues to be widely used as a

66

standard. In the present study, just one nematode species was being examined for its infectivity, so *G. mellonella* was believed to be a suitable target insect.

Not all insect hosts are suited to the Petri dish bioassay. Ingraham (1991) suggested that BVW is unduly stressed on filter paper without soil and is, therefore, poorly suited for this bioassay. Tests done in the present study indicate that BVW may be better suited to a bioassay done in soil and work on this technique should be pursued.

There are many environmental factors that can influence the effectiveness of a soil bioassay. The present study evaluated the influence of four different soil types on the infection of *G. mellonella* by *S. carpocapsae*. The results indicate that soil type may influence the effectiveness of the bioassay. These findings are in agreement with studies that have shown that soil type can influence movement, infectivity, and persistence of steinernematids (Georgis and Poinar, 1983a, 1983b; Kung *et al.*, 1990a; Barbercheck and Kaya, 1991). In the present study, a high percentage of *G. mellonella* were infected in the peat (100%), muck (94.7%), and silty clay loam (93.8%). Loamy soil may inhibit the effectiveness of the soil bioassay which could explain the low percentage (33.3%) of *G. mellonella* infected in the loam soil. There may have been residual insecticide or nematicide in the loam, which would have contributed to the lower infectivity. Peat soil did not appear to inhibit the effectiveness of the soil bioassay.

The present study determined the optimal duration of the soil bioassay under the prevailing experimental conditions. The results showed that the percent infectivity increased as the duration of the bioassay increased from 2 to 12 d. In particular, the data suggest that if nematodes are present in the samples, they are more likely to infect the target insect if exposed to it for 10 to 12 d. Inaccurate conclusions may be drawn regarding the presence of nematodes in soil samples if the duration of the bioassay is too short. Based on these results, the duration of the soil bioassays done to examine the influence of soil type on infection of *G. mellonella* by *S. carpocapsae* was extended to 10 d.

Hominick and Reid (1990) stress that one of the limitations of the soil bioassay is that negative results do not necessarily reflect an absence of nematodes in a soil sample. It has been suggested that the infective juveniles of some nematodes may enter a dormant stage after emerging from their host (Gaugler *et al.*, 1989). These inactive nematodes may not be detected in the soil bioassay and may not be effective biological control agents when applied to control BVW. An alternative method for determining the presence of nematodes in a soil sample is to extract them using a standard method, such as a Baermann funnel technique, which is commonly used for phytophagous nematodes (Saunders and All, 1982). Nevertheless, this method, which is labour intensive, would not separate the infective nematodes from the overall soil

nematode population and a bioassay would still have to be conducted (Fan and Hominick, 1991). Fan and Hominick (1991) reported that, after 12 d, about 80% of the infective nematodes in a soil sample could be extracted using *G. mellonella*. They suggested that this is more likely to occur if the *G. mellonella* larvae are replaced two or three times during the 12 d period. Replacement of the larvae appears to be particularly useful when determining the actual number of nematodes in a soil sample and perhaps not as crucial when determining merely their presence. Until other methods are discovered, such as biochemical ones, bioassays will remain the principal method for analyzing infectivity of entomopathogenic nematodes (Georgis, 1990).

The pH of the soil may be a factor that inhibits the effectiveness of these nematodes in the field. Kung *et al.* (1990b) showed that the survival of *S. carpocapsae* gradually declined as the soil pH decreased from pH 8 to pH 4. They showed also that there is an extreme decline in the infectivity of *S. carpocapsae* at pH 4 after 4 wk. The pH of peat soil in a cranberry bog can be as low as pH 3 (T. Van Kempen, pers. comm. British Columbia Cranberry Growers Association, Pitt Meadows, B.C.). This may be too harsh a condition for *S. carpocapsae*. Antagonists in such soils may also contribute to the decrease in nematode effectiveness by causing significant nematode mortality. Possible antagonists include nematode-trapping fungi, bacteria, and mites.

In addition to influencing the effectiveness of the soil bioassay, soil type may affect nematode performance in the field. Soil particle size can influence nematode persistence and movement. For example, because clay soils have small pore sizes, nematodes, especially the larger ones, are unable to move easily in them. By contrast, nematodes can move more easily in sandier soils, but survival may not be so good. Kung *et al.* (1990a) reported that survival and pathogenicity of *S. carpocapsae* were favoured in a sandy loam soil when compared with a sand, clay loam, and clay soil. In the present study, the survival and infectivity of *S. carpocapsae* (All strain) in a peat soil from a cranberry bog was compared with that in a muck, silty clay loam, and loam soil, all from different agricultural fields on the Lower Mainland of British Columbia. The results of the soil bioassays using *G. mellonella* showed over 90% infectivity in the silty clay loam, muck, and peat soils. This indicated that there is a potential for the use of nematodes against insect pests in these fields. By contrast, the results showed only 33.3% infectivity by the nematodes in the loam soil. This was a surprising result, but Simons (1981) reported poor control of BVW by nematodes in cyclamen and attributed it to the inability of the nematodes to move easily in the loam soil in which the cyclamen were planted. In the present study, this result may have been due to residual insecticide or nematicide in the loam soil.

The possibility that the nematodes get caught in the cranberry canopy cannot be overlooked. Irrigating before and particularly after an application is essential in order to prevent the nematodes from desiccating and dying on the foliage or soil surface (Georgis and Gaugler, 1991; Gaugler *et al.*, 1992). The irrigation water washes the nematodes off the foliage and into the soil, which is especially important for *S. carpocapsae* because, as an ambusher, it tends to remain at the point of application and wait for its host (Curran, 1992). Shetlar *et al.* (1988) found that a minimum of 2.54 cm of irrigation was required to promote infection at 2.5-5.0 cm for 11 d after application. Biosys suggests that 1.26 cm of irrigation be used. Perhaps this amount could be increased but probably not by very much. If there is too much water, the nematodes will become stranded in standing water and their survival will be low. Also, in water-saturated soils, the oxygen content is low and Kung *et al.* (1990b) showed that *S. carpocapsae* does not survive well under these conditions. Soils with high organic matter, such as peat soils, have low oxygen content (Kaya, 1990a) so special attention should be paid to the amount of water in the soil of the cranberry bogs.

Improved spray equipment may deliver the nematodes more effectively. In the experiment comparing soil types, the nematodes were effective in the soil in containers but there were no crop canopies above the soil as there are in the field. This indicates that some nematodes may be getting

71

caught in the canopy of the cranberries in the field. Since nematodes can withstand a nozzle pressure of 2070.0 kPa, and the growers use a much lower pressure, perhaps an increase in the nozzle pressure used could aid in driving the nematodes through the canopy. The overall application process used by the growers including the handling of the nematodes, the type of spray equipment used, and the irrigation practices are sound. Modification to the nozzle pressure of the spray equipment may increase the number of nematodes that reach the soil and improve the effectiveness of *S. carpocapsae*.

Curran (1992) found that nematodes were more effective at controlling BVW in field strawberries when applied with a surface sprayer or with soil injection techniques than when applied by trickle irrigation. The difference in the results was attributed to the differences in the initial placement of the nematodes. Perhaps the surface sprayer and the soil injection techniques better penetrate the crop canopy and more nematodes were able to come into contact with the soil. Application of higher concentrations of nematodes may also achieve this but this would be a costly approach.

The species of nematode being used is another factor to consider when examining nematode control of BVW in cranberry bogs. Kaya and Gaugler (1993) stress the importance of using a nematode species or strain that is suitable for the target insect and the environmental conditions. Unsatisfactory results in the field may be due to *S. carpocapsae* being poorly

adapted to controlling BVW in cranberry bogs.

Nematodes of the genus *Heterorhabditis* have been called the "nematodes of choice" for biological control against soil-dwelling insects (Kaya, 1990a) because of their high motility. Heterorhabditids have been shown to have a greater tendency than steinernematids, particularly ambushers, to move vertically (Georgis and Poinar, 1983c) and horizontally (Alatorre-Rosas and Kaya, 1990) in soil, including organic peat soils (Barbercheck and Kaya, 1991), making them good candidates for control of BVW in cranberry bogs. Shanks and Agudelo-Silva (1990) reported that heterorhabditids were more effective than *S. carpocapsae* for control of BVW in cranberry bogs in Washington. They attributed this, in part, to the variation in movement and host location by the two genera. The host-seeking abilities of heterorhabditids are superior to those of steinernematids (Choo *et al.*, 1989) and they persist in the field for up to 10 months (Shanks and Agudelo-Silva, 1990). However, the effectiveness of heterorhabditids at temperatures suitable for BVW infestations in coastal British Columbia needs to be examined, because there is evidence of heterorhabditids occurring most frequently in warmer soils (Hominick and Briscoe, 1990; Mracek and Webster, 1991). Although there is great potential for heterorhabditids as biological control agents, their production on a large scale has not been as successful as that of steinernematids (Hominick and Reid, 1990). Until a proper method for mass

production, storage, and transport is found, the use of heterorhabditids in the field appears to be limited.

Ingraham (1991) reported a low incidence of infection of BVW larvae by *S. carpocapsae* (All strain), *S. carpocapsae* (Umea), *S. feltiae* 252, and *S. feltiae* 27, and suggested that these strains may not be suitable for controlling BVW in cranberry bogs. Poinar (1978) considers *S. glaseri* to be more suitable for the control of soil-dwelling insects than any other steinernematid species, because of its ability to attack insects beneath the soil surface, and there have been successful tests conducted using *S. glaseri* to control BVW in glasshouses (Georgis and Poinar, 1984).

S. glaseri was shown to be more pathogenic than *S. carpocapsae* at pH 4 (Kung *et al.*, 1990b). This is a desirable characteristic for a nematode used to control a pest in an acidic soil such as peat. Also, whereas *S. carpocapsae*, an ambusher, tends to stay at the point of application and wait for its host, *S. glaseri*, a cruiser moves around in the soil. Using soil column tests, Georgis and Poinar (1983b) showed that *S. glaseri* had the ability to move throughout 32 cm columns when initially placed in the centre of the columns. Alatorre-Rosas and Kaya (1990) showed that, of the steinernematids they tested, *S. glaseri* had the greatest dispersal ability. Since *S. glaseri* has greater motility, it may be able to find and attack more BVW larvae, which themselves are rather slow moving, and provide better control

74

than *S. carpocapsae.* By using a more motile nematode that can find BVW more readily, it may be possible to apply fewer nematodes and still achieve adequate control. Therefore, provided production costs of *S. glaseri* were comparable with those of *S. carpocapsae*, application costs would be lower than they are presently. A more motile nematode also may help to compensate for uneven field applications. Although more research is required to determine the ability of *S. glaseri* to control BVW, particularly its ability to be effective at temperatures typical for coastal British Columbia (Bedding and Miller, 1981), it may be an effective alternative to *S. carpocapsae* for controlling BVW in dry-pick cranberries.

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Appendix I. Format of a questionnaire circulated among cranberry growers in British Columbia in March, 1993.

GROWERS' SURVEY

BOG HISTORY

1. How long have you been growing cranberries?

2. How old are your vines?

3. Do you have a feel for what the trends in BVW damage have been over the years?

NEMATODE HISTORY

1. How many years have you been using nematodes to control BVW?

2. Have they always come from BIOSYS?

3. Have they always been distributed by AGRICO?

4. Has your pattern of use always been the same (i.e. when you ordered your nematodes, when you applied them, etc...)?

NEMATODE HANDLING PRIOR TO USE

1. Transport: Do the nematodes arrive in refrigerated trucks?

2. Do you check to see if they are alive as soon as you get them? If you do, and they appear to be dead, do you send them back?

3. How soon before you use the nematodes do you receive them?

4. In as much detail as you can, describe exactly what you do with the nematodes between the time you receive them and the time you apply them (i.e. where do you store them? at what temperature do you store them? etc...).

5. Do you check to see if the nematodes are alive just before you apply them?

APPLICATION

1. When do you apply the nematodes? (Timing of application)

2. Under what weather conditions do you apply the nematodes?

3. Temperature: At what temperature do you apply the nematodes? Do you know the temperature of the bog? How do you measure it? If you use a probe, do you just take a reading at the damaged areas? How far down in the soil do you stick the probe?

4. What type of agitator do you use to mix the nematodes during application? Do you feel the nematodes are adequately mixed? Or do they seem to collect at the bottom of the mixer?

POST-APPLICATION

1. Do you irrigate after applying the nematodes?

2. Are the nematodes evenly spread across the bog?

3. Do you check to see if the nematodes are alive after having gone through the sprinkler system?

4. Are you satisfied with the control you get? How do you measure satisfaction?

PESTICIDES

1. Do you use any pesticides?

2. If yes: What kind? What are they used to control? When are they applied? How are they applied?