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# Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in Western Canada[1]

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*Abstract:* A survey was done in the summer months along the Alaska Highway, in other parts of British Columbia, in northern Alberta, and in the Yukon Territory for steinernematid and heterorhabditid nematodes occurring in the top 10 cm of soil. *Steinernema feltiae* and *Steinernema* spp. were found at 18 and *Heterorhabditis megidis* at 7 sites of 125 sampled. Most nematodes were found where visible insect infestation occurred and where human influence on the habitat was substantial (e.g., agricultural, forested and bush-hedgerow habitats); none was found in grassland or virgin forests. *Heterorhabditis megidis* occurred in only the southern, warmer, drier region of British Columbia. In the laboratory some steinernematid isolates and *H. megidis* killed *Galleria mellonella* larvae at 13 and 22 C, whereas some isolates of *Steinernema* killed the larvae at only 13 C. *Steinernema* spp. from three high altitude sites with low, average July temperatures (13–14 C) are cold-active in that they produced infective juveniles at 13 C and killed *G. mellonella* at 6 C.

*Key words:* biological control, entomopathogenic nematode, Heterorhabditidae, low-temperature activity, nematode, Steinernematidae, survey, temperature.

Nematodes belonging to the families Heterorhabditidae and Steinernematidae kill insects by releasing symbiotic bacteria, *Xenorhabdus* spp., into the insect's hemocoel. The free-living, infective juvenile stages of these nematodes persist for relatively long periods in the soil, and their ability to survive and to infect insects is influenced by temperature. As these organisms are used as biological control agents of insect pests in temperate climates, there is great interest in finding wild strains that are virulent against insects at low temperatures and in different soil types.

By using the *Galleria* trap method (3,14) it is possible to determine the occurrence of many of these nematode species in the soil. The distribution of *Steinernema* spp. and *Heterorhabditis* spp. is world-wide, e.g., Czechoslovakia (14,15), Poland (4), Sweden (6), Northern Ireland (5), Republic of Ireland (9), Great Britain (11,12), Australia (1), North Carolina (2), and the Hawaiian Islands (10). There is no detailed information on the occurrence of these entomogenous nematodes in Canada, but there are the brief reports on Steinernematids in British Columbia (16) and Newfoundland (7). The objective of this study was to survey part of Western Canada to determine the occurrence of *Steinernema* spp. and *Heterorhabditis* spp. and to identify the insects and temperature regimens associated with these nematodes.

## MATERIALS AND METHODS

Soil samples were taken in 1991 from British Columbia, Alberta, and the Yukon Territory, and nematodes were extracted using the *Galleria* trap method (14). Five soil samples, each of about 200 ml, were taken to a depth of 10 cm at each sampling site, an area of approximately 100 $m^2$. The samples from each site were mixed and transported in a closed plastic bag in cool conditions to the laboratory. After being held in the laboratory at 13 C for 1 hour, the soil from each site was divided and placed into each of two 200-ml containers on the bottom of which was one late instar larva of *Galleria mellonella*. The *Galleria* trap bioassays were incubated at 13 C or 22 C for 2 weeks and checked for larval mortality after 6, 10, and 14 days. Dead larvae were collected and replaced by living ones if mortality occurred at days 6 or 10. If the larvae did not die after 14 days, those soil samples were discarded. Each dead *G. mellonella* larva from the soil bioassays was placed in a separate petri dish with moist-

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ened filter paper and kept at room temperature (ca. 20 C). After 2 days, the cadavers were dissected to ascertain the number and the sex ratio of adult nematodes. Soil from nematode-positive samples was placed into petri dishes (15-cm-d) together with 10 late instar *G. mellonella* in order to build up a nematode population in the laboratory. The resulting nematodes were stored at 4 C.

Nematodes isolated from soil from subboreal and high mountain biopsies were tested in the laboratory for low-temperature activity at 6 C in the incubator. Ten *G. mellonella* larvae in each of three Petri dishes (9 cm diameter) for each isolate were exposed to 1,000 infective juveniles per dish for 11 days on moistened filter paper.

*Highway and spot sampling:* From the 87th kilometer point on the Dempster Highway (Tombstone campsite, Yukon Territory), along the Alaska Highway and to Spences Bridge, British Columbia, in the upper part of the Thompson Canyon, samples were taken from adjacent roadside biotopes during the last week of June every 100 ± 10 km (Fig. 1). Sites in Southern British Columbia at localities of low average temperature (e.g., mountain elevation over 1,000 m) were sampled from mid-April to early September in a range of ecosystems.

*Sampling of insect outbreaks:* Samples were taken from localities where moderate or severe defoliation by insects had occurred in the forest ecosystems (e.g., western hemlock by the gray spruce looper, *Caripeta divisata;* Douglas fir by the western spruce budworm, *Choristoneura occidentialis*) and in the agricultural ecosystems (e.g., fruit orchards in the Okanagan Valley by the codling moth, *Cydia pomonella;* and blueberry and cranberry plantations in the Vancouver area by the black vine weevil, *Otiorhynchus sulcatus* and winter moth, *Operophthera brumata*). The presence of insects and insect defoliation at these sampling sites was noted and correlated with information obtained (pers. comm.) from Agriculture Canada and Forestry Canada on the nature, precise location, and relative density of insect pest outbreaks.

*Sequential sampling:* About every 2 weeks (at the beginning and middle of each month) from mid-April until early October (1991) three habitats were sampled in or close to Discovery Park, Simon Fraser University, Burnaby, British Columbia. These locations were beneath a canopy of i) western hemlock (*Tsuga heterophyla*), ii) western red cedar (*Thuja plicata*), and iii) broad leaf trees and bushes, red alder (*Alnus rubra*), vine maple (*Acer circinatum*), and salmonberry (*Rubus spectabilis*).

## Results

*Field observations:* Soil samples were examined from 125 sampling sites in 87 locations in Western Canada (Fig. 1). Entomogenous nematodes were recovered from 25 sites (20% of total) at 21 localities; 18 sites (14.4%) were positive for the occurrence of steinernematids and seven (5.6%) sites for heterorhabditids (Fig. 1, Table 1). Of the sites sampled, 106 are in British Columbia, 17 in the Yukon Territory, and 2 in Alberta. Both steinernematids and heterorhabditids were recovered from one site (#89, Peachland). One site in Alberta (#99, Jasper) was positive for steinernematids; none was recorded from the Yukon Territory. The steinernematids from sites 8, 37, and 89 were identified as *Steinernema feltiae*. The other steinernematid isolates belong to at least two species, and their identification requires more detailed electron microscopy study and DNA analysis. All heterorhabditid isolates were identified as *Heterorhabditis megidis*.

The entomogenous nematodes occurred in forest and bush hedgerow (78 sites, 12 positive) and agricultural (36 sites, 13 positive) ecosystems (Table 2). No nematodes were recovered from 11 grassland (alpine meadows, native pastures, steppes, etc.) ecosystems. *Heterorhabditis megidis* was recorded only from sites in the fruit orchards in the Okanagan Valley and Thompson Canyon. Steinernematids were recorded from coniferous forests (sites 18 and 102), broad-leaf, tree forests and

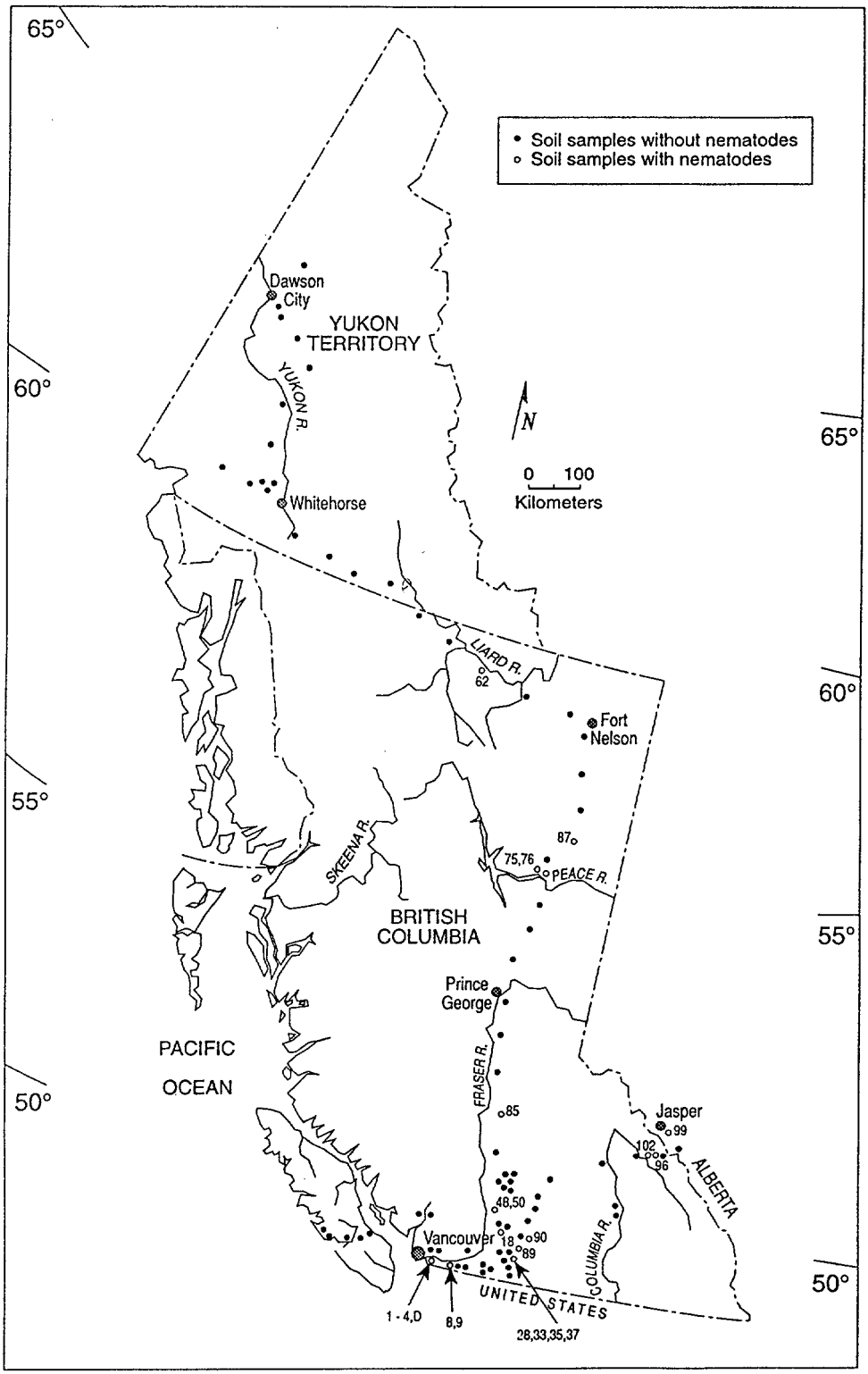


FIG. 1. Map of sampling areas in Western Canada for *Steinernema* spp. and *Heterorhabditis* spp. showing the location of numbered sites where nematodes were found.

TABLE 1. Survey and characteristics of sampling sites positive for *Heterorhabditis* (H) and *Steinernema* (S) and an indication (+/−) as to whether these nematodes were reared successfully in the laboratory.

| Sampling site # | Locality | Habitat | Probable target host | Altitude (m) | Sampling time (month) | Nematode genus | Laboratory rearing |
|---|---|---|---|---|---|---|---|
| 1 | Richmond | Blueberry plantation | Black vine weevil Winter moth | 50 | April | S | − |
| 2 | Richmond | Cranberry plantation | Black vine weevil | 50 | April | S | − |
| 3 | Richmond | Blueberry plantation | Black vine weevil Winter moth | 50 | April | S | − |
| 4 | Richmond | Birch forest | Winter moth | 50 | April | S | + |
| 8 | Langley | Apple orchard | Various moths | 50–100 | April | S | + |
| 9 | Langley | Douglas fir forest-nursery | Root weevil | 50–100 | April | S | + |
| 18 | Merritt (Prospect Creek) | Coniferous forest | Unknown | 1,100 | May | S | − |
| 28 | Summerland | Cherry orchard | Various moths | 450 | May | H | + |
| 33 | Oliver | Peach orchard | Various moths | 550 | May | H | − |
| 35 | Cawston | Apple orchard | Various moths | 550 | May | H | + |
| 37 | Cawston | Apple orchard | Various moths | 550 | May | S | + |
| 48 | Spences Bridge | Plum orchard | Various moths | 400 | June | H | − |
| 50 | Spences Bridge | Apple orchard | Various moths | 400 | June | H | − |
| 69 | Liard Hot Springs | Coniferous forest, bushes | Unknown Budworm & insects | 850 | June | S | + |
| 75 | Hudson Hope 60 km east | Grain field | Wireworm | 650 | June | S | − |
| 76 | Hudson Hope 40 km east | Grain field | Wireworm | 650 | June | S | + |
| 85 | Lac la Hache | Roadside aspen | Unknown | 800 | June | S | − |
| 87 | Wonovon | Roadside Birch, aspen | Unknown | 1,000 | June | S | − |
| 89 | Peachland | Campground var. vegetation | Various moths | 450 | July | S + H | ++ |
| 90 | Oyama | Apple orchard | Various moths | 500 | July | H | + |
| 96 | Golden 20 km east | Roadside Aspen | Various moths | 1,000 | July | S | − |
| 99 | Jasper | Campground Aspen | Various moths | 1,000 | July | S | + |
| 102 | Rodgers Pass | Conferous forest, blueberry | Unknown | 1,300 | July | S | + |
| D | Discovery Park | Deciduous trees and bushes | Various moths | 100 | May–September | S | + |

TABLE 2. Occurrence of steinernematids and heterorhabditids in ecosystems in Western Canada, categorized according to the possible influence of people and insects.

| Category | No. of sampling sites | No. of sites with nematodes | % of sites with nematodes |
|---|---|---|---|
| | Primary location | | |
| Agricultural | 36 | 13 | 36.1 |
| Forest and hedgerows | 78 | 12 | 15.4 |
| Grasslands | 11 | 0 | 0 |
| Chi-square value | $\chi^2_2 = 7.703$, $P < 0.025$ | | |
| | Impact of people | | |
| None or slight (virgin or subvirgin biotopes) | 24 | 0 | 0 |
| Moderate (economic woodlands) | 59 | 10 | 16.9 |
| Severe (agricultural land) | 42 | 15 | 35.7 |
| Chi-square value | $\chi^2_2 = 10.26$, $P < 0.01$ | | |
| | Impact of insect host | | |
| No signs of insect damage | 71 | 2 | 2.8 |
| Slight to moderate insect damage | 27 | 12 | 44.4 |
| Severe insect damage | 27 | 11 | 40.7 |
| Insect infestation (insects with part of life cycle in soil) | 32 | 23 | 71.8 |
| Chi-square value | $\chi^2_3 = 38.35$, $P < 0.01$ | | |

bushes (sites 4, 96, and 99), orchards (site 37) and grain fields (sites 75 and 76).

Nematodes occurred at sites where human impact on nature has been substantial (e.g., agricultural land, roadsides; sites 1–3, 8, 33, 35, 37, 90) or moderate (e.g., forest logged areas or regions adjacent to those with severe impact; sites 4, 9, 99) (Table 2). No nematode-positive soil samples were recorded at sites where the impact of humans has been slight (alpine or steppe meadows, unlogged mountain forests, natural parks, and virgin rain forest).

Nematodes were more prevalent at sites with visible insect pest damage to foliage. For example, from 54 sites associated with a visible insect presence, 23 were nematode-positive, whereas in the sites with no apparent insect damage, only two were nematode-positive out of 71 sampled. Of the 54 sites associated with insect damage, 32 sites contained insects that had a part of their life cycle in the soil (e.g., weevil larvae, wireworms, moth larvae, etc.) and 23 (71.8%) of these site samples contained entomopathogenic nematodes (Table 2). All differences are significant ($P < 0.001$–$P < 0.025$) when tested by chi-square test (Table 2).

Nematodes were recovered from soil samples in the Burnaby area from April to September, and there was a tendency for more sites to be nematode-positive in the early summer (i.e., April–July) than in the late summer (i.e., August–September). Steinernematids, but not heterorhabditids, were recovered from the Burnaby soil samples beneath a canopy of red alder, vine maple, and salmonberry. Slight defoliation of these plants occurred, caused by various lepidopterous larvae. No nematodes were found in samples from beneath Douglas fir, western hemlock, or red cedar, and no insect defoliation of these coniferous trees was observed.

*Laboratory observations:* In the laboratory bioassays, some steinernematid and heterorhabditid isolates killed *G. mellonella* larvae at both 13 and 22 C (from sites 1–4, 8, 9, 18, 33, 35, 37, 48, 89, D), some at only 13 C (from sites 69, 75, 76, 85, 87, 96, 99, and 102), and four *H. megidis* isolates (from sites 28, 50, 89, and 90) killed at only 22 C. In these bioassays, nematodes from the soil samples of 18 sites infected the one larva/pot after 6 days, from the soil of 14 sites they infected the second sequentially placed larva after 10 days, and from the

soil of 3 sites they infected the third sequentially placed larva after 14 days.

Usually (14 times), 1–5 infective juveniles entered the hemocoel of a *Galleria* larvae and developed to adults. In five instances, no adult nematodes were found in dissected cadavers, despite the typical color of the larvae with septicaemia. On one occasion, the number of adult nematodes per larva was 6–10, on three occasions it was more than 10, and once there were 26 adult nematodes in the cadaver. The sex ratio for steinernematids was 31 males to 64 females (n = 18) at 13 C and 14 males to 43 females (n = 12) at 22 C.

*Sampling of cold sites:* Nematodes isolated from sites 69, 99, and 102 that were successfully established in laboratory culture originated from localities with extreme climatic conditions (e.g., sub-boreal biotopes, short growing seasons, high altitude, etc.) (Fig. 1, Table 1). Site #69 is almost on the British Columbia/Yukon Territory boundary (at about latitude 60°N) at the northern extent of the Rocky Mountains, where the average July air temperature is less than 15 C and the winter temperature drops to −40 C. Sites #99 (Jasper) and 102 (Rodger's Pass) are from the central range of the Rocky Mountains at an altitude of about 1,000 and 1,300 m, respectively. The Rodger's Pass locality is very close to glaciers and permanent snow and is near to where frosts occur from October to mid-June.

Preliminary laboratory bioassays showed that these three isolates (from sites 69, 99, and 102) killed *G. mellonella* larvae in 11 days at 6–7 C. At higher temperatures (20 C) the nematodes killed *Galleria* larvae and matured to the adult stage, but they ceased developing at the "giant generation" stage and died within 3 weeks. No offspring were observed at or above 20 C, but these isolates produced infective juveniles within 3 to 4 weeks at 13 C.

## Discussion

This survey provides a perspective on the distribution of steinernematids and heterorhabditids in Western Canada and some indication of the factors that may be influencing their distribution.

Although the distribution of these nematodes may be worldwide, our observations in Western Canada indicate that it is sometimes difficult to find them in soil. Some 20% of the sampling sites were positive compared with 48.6% in Great Britain (12), 10.5% in Ireland (9), and 3.8% in Northern Ireland (5). Steinernematids were more prevalent than heterorhabditids, which were restricted to the warmer areas of the Okanagan Valley and Thompson Canyon. Heterorhabditids seem to occur more frequently in warmer and drier areas as shown also for North Carolina (2), Australia (1), and Hawaii (10), although there are some aberrant occurrences in Great Britain (12) and Ireland (9). There is no evidence of heterorhabditids occurring in Sweden (6) and Finland (13). The upper part of the Okanagan Valley is possibly the northern boundary for heterorhabditids, such as *H. megidis* in Western Canada.

Our data suggest that the most important factor influencing the occurrence of these entomogenous nematodes in sufficient numbers to be recovered by this sampling process is the presence of many insect hosts. Moreover, steinernematids and heterorhabditids appear to be more prevalent in ecosystems where human impact has been substantial (e.g., in agricultural areas) rather than in natural habitats. This supports similar observations made in North Carolina (2) and may be due to the outbreaks of insect pests associated with intensive crop monocultures. The greatest proportion (39%) of nematode-positive sample sites occurred in the Okanagan Valley, an extensive area of fruit orchards associated with a high density (as defined by Agriculture Canada) of insect pests. Similarly, in the insect-infested berry plantations near Vancouver in the spring, when black vine weevil larvae populations were at their most damaging, there was a greater frequency of nematode-positive samples. However, in the late summer when the number of insects diminished,

no nematodes were recovered. Steinernematids were not recorded from forest nursery tree-beds, despite their presence in an adjacent forest area. This may have been due to the use of chemical insecticides against root weevils in the tree-beds. Samples of a forest nursery site that had been treated with *Heterorhabditis heliothidis* 7 years ago (16) were negative. In general, it was difficult to find steinernematids or heterorhabditids in the soil in the absence of large numbers of susceptible hosts.

Western Canada is mostly wilderness, and insect population outbreaks in areas influenced by humans are often separated by great distances. No nematodes were found in samples from the cool, natural habitats of the Yukon Territory. The positive site (#69, Liard Hot Springs) was associated with defoliation of coniferous and deciduous trees by a budworm and other unknown insects. The absence of entomopathogenic nematodes from grassland samples may have been due to the paucity of susceptible insect hosts in such locations. Similarly, there was a lower diversity of insects recorded for heathlands of Great Britain (12) than on the more calcareous biotopes.

The apparent absence of nematodes from certain forest localities (e.g., in British Columbia, where western spruce budworm larvae and pupae were present on trees) may be significant. This may be because of the unsuitability of the forest litter for nematode survival. Prepupae and pupae of grey spruce looper were abundant in the litter in some Nelson forest localities, but no nematodes were recovered. These observations parallel those in Czechoslovakia (15), where *S. kraussei* was absent from leaf litter but present in organic and mineral soils together with diapusing larvae of a sawfly.

The steinernematids from southern British Columbia killed *G. mellonella* larvae at both 13 and 22 C, but those from the northern part of the province killed larvae at only 13 C. These results may have been influenced by the lower temperature adaptability of the northern strains. July temperature at the locations of sites 69 and 102, where the low temperature active isolates were obtained, averaged 14.9 (#69) and 13.3 C (#102). These isolates kill *Galleria* larvae in 2 weeks at temperatures as low as 6 C. In this regard, steinernematids seem to be better adapted than heterorhabditids to survive at low temperatures (8). The fact that these isolates could kill *G. mellonella* in the laboratory at temperatures as low as 6 C and produce infective juveniles at lower (i.e., 13 C) but not at higher (i.e., 20 C) temperatures suggests that these isolates have evolved toward temperature activity. Such cold-active isolates have the potential to be exploited in some temperate agricultural and forestry regions for the control of insect pests (16).

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