

Efficacy of Steinernematid Nematodes Against Three Insect Pests of Crucifers in Quebec¹

G. BÉLAIR,² Y. FOURNIER,² AND N. DAUPHINAIS²

Abstract: Steinernematid nematodes were evaluated against the three major cruciferous insect pests: the imported cabbageworm *Artogeia rapae*, the diamondback moth *Plutella xylostella*, and the cabbage looper *Trichoplusia ni*. LC₅₀ values of *S. carpocapsae* All, *S. feltiae* UK, *S. feltiae* 27, and *S. riobrave* 335 were 18.2, 3.6, 5.7, and 8.3 on *A. rapae* L₂; 24.5, 2.3, 6.0, and 15.5 on *P. xylostella* L₃; and 10.1, 4.7, 9.5, and 7.8 on *T. ni* L₂, respectively. Insect mortality from the nematode species and isolates was modulated by temperature. Maximum mortality (100%) was recorded for *A. rapae* L₂ from *S. riobrave* at 30 °C, 95.8% from *S. feltiae*, and 91.7% from *S. feltiae* 27 at 25 °C and 75.7% from *S. carpocapsae* at 30 °C. Mortality of *A. rapae* L₂ increased with contact time to nematode. Mortality of 76% and 78% was achieved for *S. carpocapsae* and *S. feltiae*, respectively, after 12-hour exposure. Susceptibility of *A. rapae*, *P. xylostella*, and *T. ni* larvae to entomopathogenic nematodes increased with larval age development. The addition of adjuvants — Corn Oil (0.9%, 1.8%, 3.6%), Leafshield (3.0%, 6.0%, 12.0%), Seaweed (0.1%) and Agral (0.05%) — significantly increased the density and survival rate of *S. carpocapsae* on cabbage leaves compared to water only. At 20 °C and 70% relative humidity (RH), survival rates of *S. carpocapsae* All, *S. feltiae* UK, and *S. riobrave* 335 on cabbage leaves were 43%, 2%, and 0% after 4 hours following application. Under field conditions, foliar applications of *S. carpocapsae* provided 35.3% and 33.0% control of *A. rapae* (L₃-L₅) on Brussels sprouts and broccoli in 1996 and 24.9%, 19.4% and 14.9% on Brussels sprouts, broccoli, and cauliflower, respectively, in 1999. Based on our field results, foliar applications of *S. carpocapsae* do not provide an acceptable level of *A. rapae* control under Quebec's environmental conditions.

Key words: *Artogeia rapae*, cabbage looper, diamondback moth, entomopathogenic nematodes, foliar application, imported cabbageworm, *Plutella xylostella*, *Trichoplusia ni*.

In North America, three lepidopterous species commonly occur on cruciferous crops: the imported cabbageworm, *Artogeia* (= *Pieris*) *rapae* (L.) (Lepidoptera: Pieridae); the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae); and the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) (Chagnon et al., 1990; Harcourt, 1963). These species are potential pests for many cruciferous crops in Quebec, which is the second most important production area of crucifers in Canada (Harcourt, 1963; Richard and Boivin, 1994). In Quebec, insecticide applications are the major control technique used against cruciferous pests (Chagnon et al., 1990). Alternative control measures such as biopesticides are needed to avoid insect resistance to pesticides and hazards to the environment.

Entomopathogenic nematodes (EPN) are symbiotically associated with the bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. When these bacteria are released into the insect hemocoel, they cause septicemia and death of the insect in 24 to 48 hours (Kaya and Gaugler, 1993). EPN, especially in the Steinernematidae, have a great potential as biological control agents against agricultural and horticultural insect pests because of their wide host range (Cabanillas et al., 1994; Poinar, 1990). Furthermore, they can be easily mass-produced, formu-

lated, and applied as biopesticides (Kaya and Gaugler, 1993).

Infectivity of steinernematid and heterorhabditid species has been documented against a broad range of insect pests in a variety of habitats with some successes but also with many failures (Begley, 1990; Bélaïr et al., 1999; Jaques, 1967; Kaya and Gaugler, 1993; Morris, 1985; Wang and Li, 1987). Foliar applications of EPN were generally not effective in reducing insect pest populations (Begley, 1990) because nematodes are adapted to the soil environment. The exposure of EPN on foliage to extreme temperature (Grewal et al., 1994; Kaya, 1990; Molyneux, 1984, 1985), ultraviolet (UV) light (Gaugler and Boush, 1978; Gaugler et al., 1992), and rapid fluctuation in moisture that causes desiccation (Baur et al., 1995; Simons and Poinar, 1973; Womersley, 1990) reduces their potential as biocontrol agents against foliage-feeding insects. Accordingly, EPN applied to foliage must be protected from these detrimental environmental effects by avoiding high temperature and UV radiation with evening applications (Gaugler and Boush, 1978; Wang and Li, 1987) and desiccation by using adjuvants or antidesiccants (Baur et al., 1997; Glazer and Navon, 1990). These additives are useful when nematodes are applied to waxy and glabrous leaves such as many cruciferous crops (Baur et al., 1997; Wang and Li, 1987).

In this study, we investigated the susceptibility of *A. rapae*, *P. xylostella*, and *T. ni* larvae to EPN in laboratory and greenhouse trials and evaluated the efficacy of foliar applications against *A. rapae* under natural field conditions in cruciferous crops.

MATERIALS AND METHODS

Nematodes: For laboratory and growth chamber experiments, the EPN strains *Steinernema carpocapsae* All, *S.*

Received for publication 9 October 2002.

¹ This is contribution No. 335/2003.07.01R of the Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada J3B 3E6.

² Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 Gouin Blvd., Saint-Jean-sur-Richelieu, Quebec, Canada J3B 3E6.

The authors thank Nicole Simard for dedicated technical assistance.

E-mail: belairg@agr.gc.ca

This paper was edited by S. Patricia Stock.

feltiae 27, and *S. riobrave* 335 were obtained from Biosys (Palo Alto, CA), and *S. feltiae* UK was obtained from MicroBio Limited (Hertfordshire, UK). The nematodes were cycled on *Galleria mellonella* larvae by the method of Dutky et al. (1964), and infective juveniles (IJ) were stored at 5 ± 1 °C up to 1 month before use. Percentage viability, based on movement in water, was determined with a dissecting microscope. The J2 were not used if their viability was lower than 75%. Nematode concentrations were adjusted according to their viability level. For field experiments, the nematode supply of *S. carpocapsae* was obtained from Biosys (BioVector) and MicroBio Limited in 1996 and 1999, respectively.

Insects: Three insect pests of crucifers were used in this study: the imported cabbageworm (*Artogeia rapae* (L.)), the diamondback moth (*Plutella xylostella* (L.)), and the cabbage looper (*Trichoplusia ni* (Hübner)). *Artogeia rapae* was reared on a wheat-germ diet (Webb and Shelton, 1988), *P. xylostella* was cultured on a wheat-germ-based artificial diet (Biever and Boldt, 1971), and *T. ni* was maintained on a modified pinto bean diet where myacin was replaced by aureomycin (Glass and Roelofs, 1985). Insects were reared between 21 to 27 °C depending on the species, with a 16-hour light/8-hour dark photoperiod and at about 65% relative humidity (RH).

Plants: Broccoli (*Brassica oleracea* L. var. *italica* cv. Emperor), Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* cv. Jade Cross), cabbage (*Brassica oleracea* L. var. *capitata* cv. Bartolo), and cauliflower (*Brassica oleracea* L. var. *botrytis* cv. White-Rock) were grown in individual 4-liter pots containing a 50:50 mixture of sand and organic soil. The greenhouse temperature was maintained at 20 °C, with a light regime of 16 hours light/8 hours dark. Broccoli, Brussels sprouts, cabbage, and cauliflower were grown until they had 10, 20 to 22, 18, and 10 leaves, respectively.

Laboratory conditions: Petri dishes (5-cm-diam.) lined with Whatman No. 1 filter paper were used for all laboratory experiments. Nematodes were always deposited on the filter paper in 0.5 or 0.6 ml water. Insect larvae were transferred individually into each petri dish with a cabbage leaf disk (1-cm-diam.) as a food source. Cabbage leaf disks were cut from cabbage grown in the greenhouse. All experiments were carried out in the dark at 20 ± 1 °C, 70% RH, unless otherwise specified.

Pathogenicity of four EPN: *Steinernema carpocapsae* All, *S. feltiae* 27, *S. feltiae* UK and *S. riobrave* 335 were tested against *A. rapae* second instar (L₂), *P. xylostella* third instar (L₃), and *T. ni* second instar (L₂). Forty larvae for each insect species and nematode species combination were used. *Steinernema riobrave* 335 and *S. carpocapsae* All were tested at the rate of 0, 5, 25, 50, 100, 200 IJ per larva. An additional rate of one IJ per larva was applied for *S. feltiae* 27 and UK to the listed insects. This experiment was conducted three times ($n = 120$). Mortality data were recorded after 72 hours.

Effect of temperature on efficacy of EPN: The effect of temperatures (15, 20, 25, and 30 ± 1 °C) on EPN against *A. rapae* L₂ was assessed. Nematodes were inoculated at the rate of 100 IJ per larva in 0.6 ml water. Thirty larvae per nematode species and temperature were used. This experiment was carried out four times for each combination of nematode and temperature for a total of 120 larvae per treatment. Petri dishes were incubated in growth chambers in the dark with 70% RH. Mortality was noted at 36 hours after contact between the insect and the nematodes.

Effect of contact time: The effect of contact time of *A. rapae* L₂ with *S. carpocapsae* All and *S. feltiae* UK was evaluated. Nematodes were inoculated at the rate of 100 IJ per larva in 0.6 ml water. Thirty insects were used for each nematode species and exposure time. The experiment was performed four times. After 0, 1, 2, 4, 6, 8, 10, and 12 hours of exposure to nematodes, all insects were transferred to nematode-free dishes with a cabbage leaf disk, and mortality was recorded after an additional 72 hours.

Insect stage: The efficacy of *S. carpocapsae* All against different larval stages of *A. rapae* L₂-L₃-L₅, *P. xylostella* L₃-L₄, and *T. ni* L₂-L₃ was tested. All larvae were exposed to *S. carpocapsae* All at the concentrations of 0, 5, 25, 50, 100, 200 IJ per larva in 0.5 ml of water except for *A. rapae*, where the rate of 200 IJ was not used. For *A. rapae* the experiment was performed three times for a total of 120 larvae per treatment, and in the case of *P. xylostella* and *T. ni* only one experiment was carried out for a total of 40 larvae per treatment. Percentage mortality data were recorded after 72 hours.

Growth chamber experiments: All growth chamber experiments were conducted in the dark at 20 ± 1 °C, 70% RH.

Persistence on leaves: *Steinernema carpocapsae* All, *S. feltiae* UK, and *S. riobrave* 335 were sprayed on the foliage of cabbage plants (10 leaf stage) cv. Bartolo at the rate of 2,000 IJ/ml with Agral 0.05%. For each nematode species, six plants were used. Each plant was sprayed until runoff with a manual 1-liter plastic sprayer. The survival rate was estimated after 0, 1, 2, 4, 8, and 12 hours following application time. At these time points, three leaves were randomly sampled on the same seedling. Each leaf was punched once to obtain a leaf disk of 5.5-cm diam. Three leaf disks from the same plant were washed on both sides by applying approximately 60 ml of water from a wash bottle. The water containing nematodes was recovered in a glass tube topped with a glass funnel. Following a 2-hour settling period, the nematodes were concentrated by removing the supernatant. The number of living and dead nematodes were recorded after 24 hours.

Adjuvants: The effect of six adjuvants on the survival of *S. carpocapsae* All on cabbage leaves was tested. These were: Agral 90 (Syngenta International AG, Basel, Switzerland), Seaweed "Acadie" (Distival Canada, Fortier-

ville, Quebec), Citowett Plus (DuPont, Mississauga, Ontario), Corn Oil (United Agri Products, Dorchester, Ontario), Leafshield (Aquatrols Corp. of America, Cherry Hill, NJ), and Super Spread (Wilbur-Ellis, San Francisco, CA). Each adjuvant was tested at three concentrations. Aqueous solutions of the various adjuvants were mixed with the nematode suspension to provide a nematode concentration of 2,000 IJ/ml. The solutions were sprayed on the leaves with a 1-liter plastic hand sprayer until runoff on the foliage of cabbage plants (10-12 leaf stage) cv. Bartolo. Six plants were used for each treatment (adjuvant x concentration). A water control was included. After 12 hours, three leaves were randomly sampled on each plant. The nematode recovery and survival estimate methods follow the same procedures as described in the previous experiment.

Field experiments: The efficacy of foliar applications of *S. carpocapsae* against *A. rapae* in the field was assessed on Brussels sprouts, broccoli, and cauliflower at the experimental farm of Agriculture and Agri-Food Canada at l'Acadie (45°18'N, 73°21'W). Two field trials were conducted—one in 1996 (trial 1) on Brussels sprouts and broccoli and one in 1999 (trial 2) on the same crops plus cauliflower. Brussels sprouts, broccoli, and cauliflower plots were 30 m wide and 4 m long. Each plot contained 40 rows and 9 plants per row. For trial 1, Brussels sprouts and broccoli were transplanted on 21 June 1996. For trial 2, Brussels sprouts and broccoli seedlings were transplanted on 15 June 1999 and cauliflower on 9 July 1999. Plants were planted 45 cm apart within a row and 75 cm between rows.

Two treatments were made: (i) nematode + Agral 0.05% and (ii) Agral 0.05% alone. The nematode suspension was stirred to prevent nematodes from settling during spray-tank application. Nematodes were applied at sunset on 2 September 1996 (trial 1) and on 24 August 1999 (trial 2) with a Comet MC 25 Portotata equipped with a handgun sprayer (between 483 and 1,379 kPa) until runoff at the rate of 4 billion/ha.

The nematodes were sprayed on six random 2-m double rows for each treatment in each crop. Eighteen plants for each treatment and each crop were randomly chosen. The first and last plant on each treated row served as a buffer and were not sampled.

Insect mortality caused by nematodes was evaluated

48 hours after nematode application by determining the number of living and dead *A. rapae* larvae per plant. A larva was scored dead if it failed to respond to mechanical stimulation. All recovered larvae were deposited in multicell plates and returned to the laboratory for observation. One week later, larvae were dissected to observe the presence or absence of nematodes inside the cadavers.

Air temperature and relative humidity data were monitored at 2 m from the soil surface and provided from a weather station located on the experimental farm, approximately 0.5 km from the study site.

Statistical analysis: LC₅₀ values for each insect species were computed with a Probit analysis (Polo-PC, LeOra Software, Berkeley, CA). Mortality or survival data were transformed with arcsin (\sqrt{x}) and were analyzed by analysis of variance (ANOVA) followed by the Waller-Duncan k-ratio *t*-test (Proc GLM, SAS Institute, Cary, NC). Data are expressed as percentage mortality or survival means with standard errors. Only untransformed data are presented.

RESULTS AND DISCUSSION

Laboratory experiments: The four EPN studied were highly pathogenic to *A. rapae*, *P. xylostella*, and *T. ni*. *Steinernema feltiae* UK was the most virulent, closely followed by *S. feltiae* 27, *S. riobrave*, and *S. carpocapsae* (Table 1). LC₅₀ values ranged from 3.6 to 18.2 for *A. rapae* L₂, from 2.3 to 24.5 for *P. xylostella* L₃, and from 4.7 to 10.1 for *T. ni* L₂ (Table 1). These results are similar to previous reports by Baur et al (1995), Morris (1985), and Ratnasinghe and Hague (1995) for all three species.

The efficacy of EPN was modulated by temperature. Maximum mortality was recorded for *A. rapae* L₂ (100%) from *S. riobrave* at 30 °C, 95.8% from *S. feltiae* and 91.7% from *S. feltiae* 27 at 25 °C, and 75.7% from *S. carpocapsae* at 30 °C (Table 2). At 15 °C, average *A. rapae* mortality rates by EPN ranged from 1.7% to 19.2%. Although no significant difference was detected between isolates, both *S. feltiae* strains were more effective than *S. carpocapsae* and *S. riobrave*. At 20 °C, EPN were significantly more effective than at 15 °C except *S. riobrave*. Again, *S. feltiae* strains performed similarly and

TABLE 1. LC₅₀ values and 95% confidence limits (CL) of four entomopathogenic nematodes against *Artogeia rapae*, *Plutella xylostella*, and *Trichoplusia ni*.

| Steinernema species | Strain | <i>A. rapae</i> L ₂ | | <i>P. xylostella</i> L ₃ | | <i>T. ni</i> L ₂ | |
|-----------------------|--------|--------------------------------|-----------|-------------------------------------|-----------|-----------------------------|----------|
| | | LC ₅₀ ^a | 95% CL | LC ₅₀ | 95% CL | LC ₅₀ | 95% CL |
| <i>S. carpocapsae</i> | All | 18.2 | 11.8–27.3 | 24.5 | 18.3–32.4 | 10.1 | 7.3–13.8 |
| <i>S. feltiae</i> | UK | 3.6 | 2.4–5.3 | 2.3 | 1.4–3.4 | 4.7 | 3.4–6.4 |
| <i>S. feltiae</i> | 27 | 5.7 | 3.7–8.4 | 6.0 | 4.4–8.0 | 9.5 | 6.9–12.8 |
| <i>S. riobrave</i> | 335 | 8.3 | 5.3–12.3 | 15.5 | 11.5–20.5 | 7.8 | 5.5–10.7 |

^aLC₅₀ values = number of IJ per insect needed to reach 50% mortality.

TABLE 2. Mortality of *Artogeia rapae* L₂ (after 36 hour time exposure) as affected by nematode species and temperature.

| Temperature (°C) | <i>Steinernema</i> species | | | |
|---------------------|----------------------------|----------------------|----------------------|------------------------|
| | <i>S. carpocapsae</i> All | <i>S. feltiae</i> UK | <i>S. feltiae</i> 27 | <i>S. riobrave</i> 335 |
| 15 | 1.7 cA ^a | 19.2 cA | 10.0 cA | 4.2 cA |
| 20 | 36.7 bAB | 70.8 bA | 57.9 bA | 10.0 cB |
| 25 | 65.8 abB | 95.8 aA | 91.7 aA | 89.2 bA |
| 30 | 68.2 aB | 61.7 bB | 48.3 bB | 92.5 aA |

^a Values in the same column followed by the same lowercase letter and in the same row followed by the same uppercase letter are not significantly different from ($P \leq 0.05$) one another as determined by Waller-Duncan k-ratio *t*-test.

were significantly more effective than *S. carpocapsae* and *S. riobrave*. At 25 °C, all EPN were significantly more effective than at 20 °C, where *S. feltiae* strains and *S. riobrave* were significantly more pathogenic than *S. carpocapsae*. At 30 °C, the infectivity of *S. riobrave* increased, *S. feltiae* strains decreased, and *S. carpocapsae* remained unchanged when compared to 25 °C (Table 2). Many other studies have shown that temperature influences the nematode's location as well as infection and killing of insect (Grewal et al., 1994; Kaya, 1990; Molyneux, 1984, 1985). Molyneux (1984) noted a correlation between the temperature optima of various species and strains of *Steinernema* and *Heterorhabditidis* and their geographic origins, where nematodes from the tropics had higher temperature optima than those from temperate regions. *Steinernema riobrave*, which is native to the Lower Rio Grande Valley of Texas (Cabanillas et al., 1994), was the most pathogenic nematode under high-temperature conditions in our experiment. Molyneux (1984) also has demonstrated that *S. feltiae* is a poor survivor at high temperature, probably because of its high motility and respiration, which rapidly exhausts food reserves. This could explain why *S. feltiae* 27 and UK were more pathogenic than *S. carpocapsae* to *A. rapae*, except at 30 °C. Our results regarding the pathogenicity of *S. carpocapsae* to *A. rapae* under different temperatures are in accordance with those observed by Ratnasinghe and Hague (1998), who demonstrated that the optimal temperature range for the infectivity of *S. carpocapsae* against *Plutella xylostella* was between 20 and 30 °C with an optimum at 25 °C. *Steinernema carpocapsae* is known to be tolerant to high temperature and desiccation stress. This is related to its specific habitat, which is near the soil surface (Campbell and Gaugler, 1993; Glazer and Navon, 1990; Kaya, 1990; Simons and Poinar, 1973; Womersley, 1990). Nematodes that live near the soil surface are generally more tolerant to high-temperature stress and desiccation than nematode species that are present deeper in the soil (Kaya, 1990).

Mortality of *A. rapae* L₂ significantly increased with exposure time to *S. carpocapsae* ($P < 0.0001$) and *S. feltiae* UK ($P < 0.0001$) (Fig. 1). These nematodes killed 50% of the larvae with a 6-hour exposure. After 12 hours, *S.*

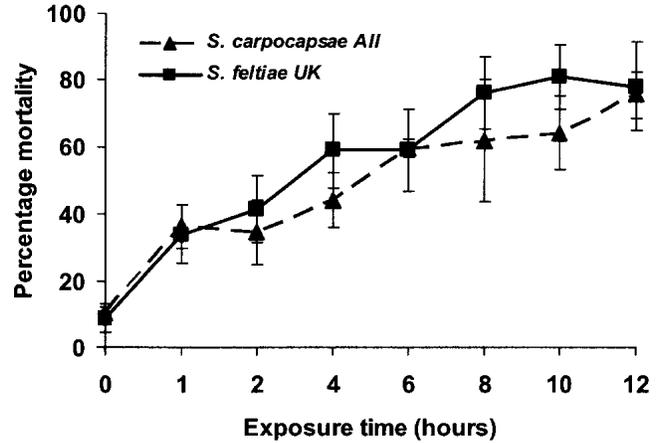


FIG. 1. Mortality of *Artogeia rapae* L₂ as affected by contact time to *Steinernema carpocapsae* All and *S. feltiae* UK (100 IJ/larvae at 20 °C).

carpocapsae and *S. feltiae* caused 76% and 78% mortality, respectively, and no significance difference between the two species was detected (Fig. 1). Similar results have been observed for many lepidopteran pests under controlled conditions (Baur et al., 1995; Jaques, 1967; Morris, 1985), confirming that an exposure time of at least 6 to 8 hours to the nematodes is necessary to reach a significant level of larval mortality.

The susceptibility of *A. rapae* larvae to *S. carpocapsae* All increased with larval development ($P < 0.0007$) (Fig. 2A). Maximum mortality rates were: 93.3% with 200 IJ per L₂ larva, 95.5% with 200 IJ per L₃ larva, and 100% with 50 IJ per L₅ larva (Fig. 2A). Similarly, susceptibility of *P. xylostella* increased with larval development ($P < 0.01$) (Fig. 2B). Maximum mortality rates were 82.4% with 200 IJ per L₃ larva and 90% with 100 IJ per L₄ larva (Fig. 2B). *Trichoplusia ni* L₂-L₃ were very susceptible to nematode infection (Fig. 2C). The highest mortality rates were 98.3% with 100 IJ per L₂ larva and 100% with 100 IJ per L₂-L₃ larva (Fig. 2C). Our results on the efficacy of *S. carpocapsae* against different larval stages are in accordance with those observed by Kaya (1985), who demonstrated that the susceptibility of insect to EPN increased with larval development.

Growth chamber experiments: Survival rate of *S. carpocapsae* All on cabbage leaves was higher than *S. feltiae* UK and *S. riobrave* 335 (Fig. 3). After 1 hour, the survival of *S. feltiae* UK and *S. riobrave* was significantly reduced ($P < 0.0001$) when compared to *S. carpocapsae* (Fig. 3). Furthermore, after 4-hour exposure, the survival of *S. feltiae* UK and *S. riobrave* were 2% and 0%, respectively, in comparison with 42.8% for *S. carpocapsae* All ($P < 0.0001$) (Fig. 3).

The addition of adjuvants except for Seaweed (0.05%, 0.1%) significantly increased the average total number ($P < 0.0001$) and the average number of living nematodes per unit leaf area ($P < 0.0001$) when compared to the water control (Table 3). Corn Oil (0.9%, 1.8%, 3.6%), Leafshield (3.0%, 6.0%, 12.0%), Seaweed

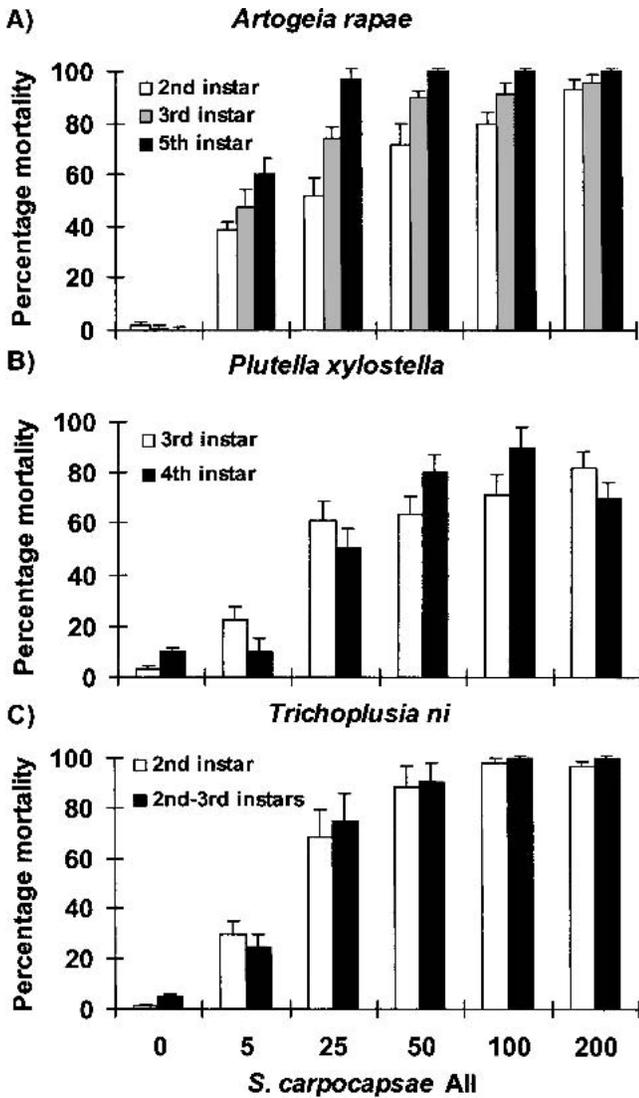


FIG. 2. Mortality of early instars of A) *Artogeia rapae*, B) *Plutella xylostella*, and C) *Trichoplusia ni*, as affected by *S. carpocapsae* All concentrations.

(0.1%), and Agral (0.05%) increased the survival of nematodes ($P < 0.0001$) in comparison with the water control (Table 3). All other adjuvants were similar to

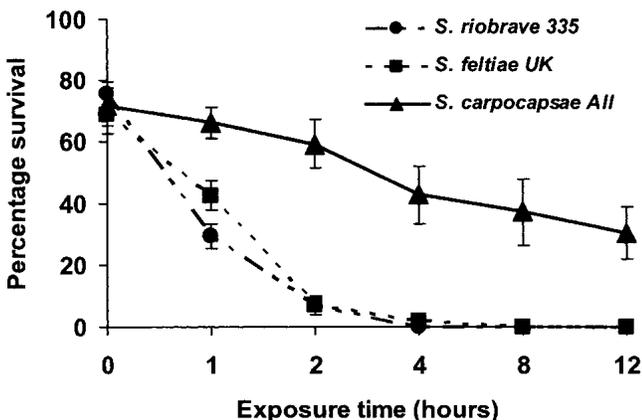


FIG. 3. Effects of time on survival of three entomopathogenic nematodes on cabbage leaves.

the water control with the exception of Seaweed at 0.05%, which reduced nematode survivorship on the leaves (Table 3).

Steinernema carpocapsae was chosen for subsequent field trials because of its availability for field trials and higher survival rate when exposed to desiccation on leaf surface (Baur et al., 1995; Simons and Poinar, 1973). The use of EPN against foliage pests is commonly perceived to be limited by their temperature range (Grewal et al., 1994; Molyneux, 1985), their ability to survive desiccation (Baur et al., 1995; Simons and Poinar, 1973; Womersley, 1990), and UV radiation (Gaugler and Boush, 1978). Thus, nematodes applied to foliage must be protected from these detrimental and often lethal effects. Adjuvants are known to indirectly enhance desiccation survival of IJ by reducing the evaporation rate of droplets and decreasing the number of nematodes lost during application. The majority of adjuvants in our study increased the number of IJ per unit area and their survival rate when compared to the water control, but the number of nematodes (total and living) recovered from cabbage foliage was lower than that observed on apple leaves in a previous study, where the same adjuvants were used (Bélaire et al., 1999). These differences could be related to the physical properties of cabbage vs. apple leaves. Glazer (1992) demonstrated that the leaf surface is an important factor affecting nematode survival. His study demonstrated that *S. carpocapsae* (Mexican strain) IJ survival was higher on the pubescent leaves of tomato (*Lycopersicon esculentum* Mill.) and soybean (*Glycine max* (L.) Merr.) than on the glabrous leaves of pepper (*Capsicum frutescens* L.) and bean (*Phaseolus vulgaris* L.) plants. Pubescence stabilizes the microclimate near the leaf surface by affecting relative humidity, temperature, and light (Baur et al., 1995; Glazer, 1992). Furthermore, the wax layer on a leaf surface can decrease the adherence of water droplets to leaves. This is probably the case for cabbage leaves that are glabrous and waxy. The adjuvant Agral (0.05%) was retained for further trials based on efficacy, cost, and availability.

Field experiments: Foliar applications of *S. carpocapsae* provided 35.3% and 33.0% control of *A. rapae* on Brussels sprouts and broccoli, respectively, in trial 1 (1996) and 24.9%, 19.4%, and 14.9% on Brussels sprouts, broccoli, and cauliflower, respectively, in trial 2 (1999). No mortality was observed on crucifer plants treated with Agral alone (Table 4). The number of *A. rapae* larvae observed on each crop was not significantly different from the control (Table 4). Crop type did not affect the efficacy of *S. carpocapsae* in either experiments. At application time, air temperature and relative humidity were 22.9 °C and 75% in 1996 and 24.4 °C and 65% in 1999. From application time (7h00 PM) to sunrise (6h00 AM), average air temperature was 16.3 °C and 18.9 °C in 1996 and 1999, respectively.

TABLE 3. Effect of adjuvants on density and survival of *Steinernema carpocapsae* All on cabbage leaves after a 12-hour exposure time (20 °C and 70% relative humidity).

| Adjuvant | Percent concentration (v/v) | Total IJ/cm ² | Living IJ/cm ² | % Survival |
|-----------------|-----------------------------|---------------------------|---------------------------|------------|
| Control (water) | — | 2.9 ± 0.5 fg ^a | 2.1 ± 0.5 i | 64.9 fg |
| Corn Oil | 1.8 | 9.9 ± 0.5 abc | 8.8 ± 0.4 ab | 89.2 a |
| Leafshield | 12.0 | 9.6 ± 1.5 bcd | 8.3 ± 1.3 abc | 85.4 ab |
| Corn Oil | 0.9 | 9.7 ± 1.2 bcd | 7.9 ± 1.2 abcd | 80.4 bc |
| Leafshield | 3.0 | 10.8 ± 2.4 bc | 8.4 ± 1.9 abcd | 78.4 bcd |
| Corn Oil | 3.6 | 10.5 ± 1.5 ab | 8.3 ± 1.2 ab | 78.2 bcd |
| Seaweed | 0.1 | 3.1 ± 0.6 f | 2.4 ± 0.5 i | 77.0 cde |
| Leafshield | 6.0 | 13.6 ± 1.6 a | 10.3 ± 1.0 a | 76.3 cde |
| Agral | 0.05 | 7.3 ± 0.6 d | 5.5 ± 0.6 efg | 75.4 cde |
| Citowett Plus | 0.4 | 9.4 ± 0.6 bcd | 7.1 ± 0.6 bcdef | 74.5 cdef |
| Agral | 0.1 | 7.6 ± 0.6 bcd | 5.6 ± 0.5 defg | 73.7 cdef |
| Super Spread | 0.1 | 10.2 ± 0.7 ab | 7.5 ± 0.7 abcde | 73.5 cdef |
| Super Spread | 0.2 | 7.8 ± 0.6 bcd | 5.8 ± 0.6 cdefg | 73.3 cdef |
| Seaweed | 0.2 | 5.6 ± 1.7 e | 4.1 ± 1.2 h | 72.9 cdef |
| Agral | 0.025 | 9.1 ± 0.2 bcd | 6.5 ± 0.4 bcdef | 71.1 defg |
| Citowett Plus | 0.1 | 8.4 ± 1.2 bcd | 5.8 ± 0.8 cdefg | 69.1 defg |
| Super Spread | 0.4 | 7.4 ± 0.5 cd | 5.1 ± 0.4 fgh | 68.8 efg |
| Citowett Plus | 0.2 | 7.3 ± 0.3 cd | 4.6 ± 0.2 gh | 62.4 g |
| Seaweed | 0.05 | 2.2 ± 0.7 g | 1.0 ± 0.6 j | 32.0 h |

^a Values in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to the Waller-Duncan k-ratio *t*-test.

In our study, laboratory trials were not good predictors of field efficacy. Even though nematodes were applied in both experiments at sunset to protect nematodes from radiation and desiccation (Gaugler and Boush, 1978; Gaugler et al., 1992), *S. carpocapsae* provided low efficacy levels against *A. rapae*. This low nematode activity can be related to many factors, including desiccation caused by unfavorable moisture conditions on the leaf surface, short contact period with the insect pest, and low night temperature. In most cases foliage-feeding lepidopteran larvae are highly susceptible to infection by EPN in petri dishes but are rarely effective in the field (Kaya and Gaugler, 1993). For example, Jaques (1967) evaluated the DD-136 strain of *S. carpocapsae* against five different foliage-feeding pests of apple in the laboratory and field. He showed that *S. carpocapsae* DD-136 was very effective in killing the winter moth *Operophtera brunata* in the laboratory, but field application did not result in larval suppression. In this

study, the poor field efficacy was attributed to rapid nematode desiccation and, to a lesser extent, to the application method. Alternatively, Wang and Li (1987) reported that *S. carpocapsae* DD-136 caused 89.4% mortality of *Pieris rapae* in 72 hours in field trials when humidity was nearly 100% for the 15 hours following evening applications. The discrepancy between our field results and those reported by Wang and Li (1987) could be attributed to rapid desiccation of EPN caused by the lower relative humidity, which was between 65% and 75% at application time. Nematode activity on the leaf surface also may have a significant effect on their efficacy against insect pests. Search strategies of EPN should be considered when nematodes are used as biological insecticides against foliage-feeding insects. *Steinernema carpocapsae* is known to be one of the least motile EPN because of its sit-and-wait searching strategy (Ishibashi and Kondo, 1990). The sit-and-wait strategy of *S. carpocapsae* consists of remaining stationary in the absence of stimulus (Lewis et al., 1992). This behavior increases the time taken for the nematode to contact a potential host and thus reduces its efficacy in unfavorable habitats such as foliage. Finally, low night temperature also could have played an even more significant role in reducing field efficacy. As mentioned previously, average night temperature was below 20 °C in both trials, and the minimum temperature recorded was near 15 °C. In this study, these low temperatures reduced drastically the efficacy of all EPN, including *S. carpocapsae*.

Based on our field results, *S. carpocapsae* does not provide an acceptable level of control of *A. rapae* under Quebec's environmental conditions. Although great potential exists for large-scale use of EPN, further stud-

TABLE 4. Mortality of *Artogeia rapae* following foliar applications of *Steinernema carpocapsae* on cruciferous crops under field conditions.

| Crop | No. larvae/plant | Treatment | |
|------------------|------------------|-----------------------------|--------------|
| | | Nematode + Agral | Agral alone |
| <i>Trial 1</i> | | | |
| Broccoli | 16 a | 33.0 ± 13.5 aA ^a | 0.0 ± 0.0 aB |
| Brussels sprouts | 15 a | 35.3 ± 3.1 aA | 0.0 ± 0.0 aB |
| <i>Trial 2</i> | | | |
| Broccoli | 20 a | 19.4 ± 3.5 aA | 0.0 ± 0.0 aB |
| Brussels sprouts | 22 a | 24.9 ± 2.7 aA | 0.4 ± 0.4 aB |
| Cauliflower | 19 a | 14.9 ± 4.1 aA | 0.0 ± 0.0 aB |

^a For both experiments, values in the same column followed by the same lowercase letter and in the same row followed by the same uppercase letter are not significantly different from ($P \leq 0.05$) according to the Waller-Duncan k-ratio *t*-test.

ies concerning nematode formulation, genetically improved isolates (desiccation, low-temperature activity), and adjuvants are needed to increase the feasibility of foliar applications in cruciferous crops.

LITERATURE CITED

- Baur, M. E., H. K. Kaya, R. Gaugler, and B. Tabashnik. 1997. Effects of adjuvants on entomopathogenic nematode persistence and efficacy against *Plutella xylostella*. *Biocontrol Science and Technology* 7: 513–525.
- Baur, M. E., H. K. Kaya, and G. S. Thurston. 1995. Factors affecting entomopathogenic nematode infection of *Plutella xylostella* on a leaf surface. *Entomologia Experimentalis et Applicata* 77:239–250.
- Begley, J. W. 1990. Efficacy against insects in habitats other than soil. Pp. 215–231 in R. Gaugler and H. K. Kaya, eds. *Entomopathogenic nematodes in biological control*. Boca Raton, FL: CRC Press.
- Bélaire, G., C. Vincent, S. Lemire, and D. Coderre. 1999. Laboratory and field assays with entomopathogenic nematodes for the management of oblique banded leafroller *Choristoneura rosaceana* (Harris) (Tortricidae). Supplement to the *Journal of Nematology* 31:684–689.
- Biever, K. D., and P. E. Boldt. 1971. Continuous laboratory rearing of the diamondback moth and related biological data. *Annals of the Entomological Society of America* 64:651–655.
- Cabanillas, H. E., G. O. Poinar, Jr., and J. R. Raulston. 1994. *Steinernema riobravisi* n. sp. (Rhabditida: Steinernematidae) from Texas. *Fundamental and Applied Nematology* 17:123–131.
- Campbell, J. F., and R. Gaugler. 1993. Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* 126:155–169.
- Chagnon, M. A., A. Payette, C. Jean, and C. Cadieux. 1990. Modes alternatifs de répression des insectes dans les agroécosystèmes québécois, tome 2: Identification des insectes ravageurs et état de l'agriculture biologique au Québec. Québec, Canada: Ministère de l'Environnement et Centre Québécois de Valorisation de la Biomasse.
- Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology* 6:417–422.
- Gaugler, R., A. Bednarek, and J. F. Campbell. 1992. Ultraviolet inactivation of Heterorhabditid and Steinernematid nematodes. *Journal of Invertebrate Pathology* 59:155–160.
- Gaugler, R., and G. M. Boush. 1978. Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoplectana carpocapsae*. *Journal Invertebrate Pathology* 32:291–296.
- Glass, E. H., and W. L. Roelofs. 1985. *Argyrotaenia velutinana*. Pp. 197–205 in E. G. King and N. C. Leppla, eds. *Handbook of insect rearing*, vol. 2, New York, NY: Elsevier Science Publishers.
- Glazer, I. 1992. Survival and efficacy of *Steinernema carpocapsae* in an exposed environment. *Biocontrol Science and Technology* 2:101–107.
- Glazer, I., and A. Navon. 1990. Activity and persistence of entomoparasitic nematodes tested against *Heliothis armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 83:1795–1800.
- Grewal, P. S., S. Selvan, and R. Gaugler. 1994. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology* 19:245–253.
- Harcourt, D. G. 1963. Biology of cabbage caterpillars in eastern Ontario. *Proceedings of the Entomological Society of Ontario* 93:61–75.
- Ishibashi, N., and E. Kondo. 1990. Behaviour of infective juveniles. Pp. 139–150 in R. Gaugler and H. K. Kaya, eds. *Entomopathogenic nematodes in biological control*. Boca Raton, FL: CRC Press.
- Jaques, R. P. 1967. Mortality of five apple insects induced by the nematode DD136. *Journal of Economic Entomology* 60:741–743.
- Kaya, H. K. 1985. Susceptibility of early larval stages of *Pseudaletia unipuncta* and *Spodoptera exigua* (Lepidoptera: Noctuidae) to the entomogenous nematode *Steinernema feltiae* (Rhabditida: Steinernematidae). *Journal of Invertebrate Pathology* 46:58–62.
- Kaya, H. K. 1990. Soil ecology. Pp. 93–115 in R. Gaugler and H. K. Kaya, eds. *Entomopathogenic nematodes in biological control*. Boca Raton, FL: CRC Press.
- Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* 38:181–206.
- Lewis, E. E., R. Gaugler, and R. Harrison. 1992. Entomopathogenic nematode host finding: Response to contact cues by cruise and ambush foragers. *Parasitology* 105:309–315.
- Molyneux, A. S. 1984. The influence of temperature on the infectivity of heterorhabditid and steinernematid nematodes for larvae of the sheep blowfly *Lucilia cuprina*. *Proceedings of the Fourth Australian Applied Entomological Research Conference*, Adelaide, Australia. Pp. 344–351.
- Molyneux, A. S. 1985. Survival of infective juveniles of *Heterorhabditis* spp. and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and the subsequent infectivity for insects. *Revue de Nématologie* 8:165–170.
- Morris, O. N. 1985. Susceptibility of 31 species of agricultural insect pests to the entomogenous nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*. *Canadian Entomologist* 117:401–417.
- Poinar, G. O., Jr. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. Pp. 23–61 in R. Gaugler and H. K. Kaya, eds. *Entomopathogenic nematodes in biological control*. Boca Raton, FL: CRC Press.
- Ratnasinghe, G., and N. G. M. Hague. 1995. The susceptibility of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), with entomopathogenic nematodes. *Afro-Asian Journal of Nematology* 5:20–23.
- Ratnasinghe, G., and N. G. M. Hague. 1998. The invasion, development, and reproduction of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Nematropica* 28:1–6.
- Richard, C., and G. Boivin. 1994. Maladies et ravageurs des cultures légumières au Canada. Ottawa: Société Canadienne de Phytopathologie et Société d'Entomologie du Canada.
- Simons, W. R., and G. O. Poinar, Jr. 1973. The ability of *Neoplectana carpocapsae* (Steinernematidae: Nematodea) to survive extended periods of desiccation. *Journal of Invertebrate Pathology* 22:228–230.
- Wang, J. X., and L. Y. Li. 1987. Entomogenous nematode research in China. *Revue de Nématologie* 10:483–489.
- Webb, S. E., and A. M. Shelton. 1988. Laboratory rearing of the imported cabbageworm. *New York's Food and Life Sciences Bulletin* 122:1–6.
- Womersley, C. Z. 1990. Dehydration survival and anhydrobiotic potential. Pp. 117–137 in R. Gaugler and H. K. Kaya, eds. *Entomopathogenic nematodes in biological control*. Boca Raton, FL: CRC Press.