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Laboratory and Field Assays with Entomopathogenic Nematodes for the Management of Oblique Banded Leafroller *Choristoneura rosaceana* (Harris) (Tortricidae)[1]

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Abstract: The activity of steinernematid entomopathogenic nematodes against *Choristoneura rosaceana* was evaluated under laboratory and field conditions. In petri dish trials, all instars were susceptible to *Steinernema carpocapsae* AII strain with LD_{50} values of 13, 5, 3, and 2 infective juveniles for the third-, fourth-, fifth-, and sixth-stage larvae, respectively. *Steinernema riobrave* 335, *S. feltiae* UK, *S. carpocapsae* AII, and *S. glaseri* 326 caused 85, 55, 45, and 8% mortality of third instars when exposed to the concentration of 25 infective juveniles per dish. When third instars were exposed to *S. carpocapsae* AII for 0, 1, 4, 8, 12, and 24 hours, larval mortality was 12, 13, 21, 47, 64, and 87%, respectively. At least 8 hours' exposure was required to cause a significant increase in mortality when compared with the control (water) and the 1 and 4-hour exposures. None of the tested adjuvants provided a significant improvement in the average total number and the average number of living *S. carpocapsae* AII per unit leaf area when compared to the water control. Under field conditions, foliar applications of *S. carpocapsae* AII at the rate of 2×10^9 infective juveniles/ha provided 37, 19, and 13% larval control. At present, efficacy level and treatment cost preclude nematode applications as a sole treatment against this pest.

Key words: apple, biological control, *Choristoneura rosaceana,* entomopathogenic nematode, field, foliar application, Lepidoptera, oblique banded leafroller, *Steinernema carpocapsae, S. feltiae, S. glaseri, S. riobrave,* Tortricidae.

The oblique banded leafroller (OBLR), *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae), is a pest of apple orchards of eastern North America. This pest has become resistant to several synthetic insecticides in New York (Reissig, 1978; Reissig et al., 1986) and Quebec (Carrière et al., 1994; Smirle et al., 1998). Alternative control methods have been investigated to address this problem and also to try to extend the effectiveness of currently recommended insecticides.

Entomopathogenic nematodes in the family Steinernematidae are soil-borne insect parasites associated with a mutualistic bacterium that kills the insect host in 24 to 48 hours (Kaya and Gaugler, 1993). Recent advances in the development of methods for producing, storing, and applying entomopathogenic nematodes have increased the probability of their use as a biopesticide (Kaya and Gaugler, 1993). They have been field-tested as potential control agents for numerous foliar insects with some successes but also with many failures (Begley, 1990; Bélair et al., 1998; Jaques, 1967; Kaya et al., 1981; Morris, 1985; Sledzevskaya, 1987; Vincent and Bélair, 1992). In apple orchards, foliar applications of steinernematid nematodes for control of the apple sawfly *Hoplocampa testudinea* Klug were promising in experimental plots (Vincent and Bélair, 1992) but failed to reduce damage under commercial field conditions (Bélair et al., 1998). *Steinernema carpocapsae* was effective against the larvae and adults of the plum curculio under controlled conditions (Brossard et al., 1989; Olthof and Hagley, 1993). Persistence of *S. carpocapsae* in the apple canopy was shown to reach more than 90 hours (Bélair et al., 1998). As several tortricid species, larvae of OBLR roll themselves in leaves, which creates a micro-habitat that may be favorable for the protection of nematodes from UV light and desiccation.

In the present work, we assessed the susceptibility of OBLR larvae to entomopatho-

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genic nematodes and evaluated the efficacy of foliar applications under natural field infestation in a commercial apple orchard.

Materials and Methods

Nematode supply: The entomopathogenic nematode strains *Steinernema carpocapsae* AII, *S. feltiae* UK, *S. glaseri* 326, and *S. riobrave* 335 (Thermo Trilogy, Columbia, MD) were obtained and stored at 5 °C. These nematodes were used within 2 weeks of receipt, or were cycled on *Galleria mellonella* larvae by the method of Dutky et al. (1964). In the latter case, infective juveniles (IJ) were stored at 5 ± 1 °C up to 1 month before use. Percent viability, based on movement, was determined with a dissecting microscope. A lot was not used if its viability was lower than 75%. Nematode dosages were adjusted according to their viability level.

Insect colony: Overwintered OBLR larvae were collected from a commercial apple orchard at Deux-Montagnes (N 45°29′, W 74°03′), Quebec, Canada. The larvae were reared in a growth chamber (21 °C, 16L:8D photoperiod, 65% relative humidity [RH]) on a pinto bean-based artificial diet modified from Shorey and Hale (1965).

Nematode activity against OBLR larvae: All nematodes were suspended in deionized water. A 0.5-ml aliquot of the nematode suspension was deposited in a 5-cm-diam. petri dish lined with a filter paper (Whatman No. 1). OBLR larvae were transferred singly into each of the dishes. All assays were carried out in the dark at 23 ± 1 °C unless otherwise specified. Insect mortality was recorded after 72 hours. In these experiments, 60 insects were used for each treatment. The experiments were replicated three times. The *S. carpocapsae* AII strain was used at concentrations of 0, 10, 25, and 100 IJ/dish. Third, fourth, fifth, and sixth instars were tested. LD_{50} values for each insect stage were computed with a probit analysis (Polo-PC, LeOra Software, Berkeley, CA). The other strains listed above were applied at the concentration of 0 and 25 IJ/dish against third and fourth instars.

Third instar larvae were exposed to *S. carpocapsae* AII strain at the concentrations of 0 or 100 IJ/dish. After 0, 1, 4, 8, 12, and 24 hours, insects were transferred to nematode-free dishes; mortality was recorded after an additional 72 hours.

Screening adjuvants for foliar application of nematodes: This assay was done to assess the effect of various commercially available adjuvants (Table 1) on nematode density and survival on apple leaves. *Steinernema carpocapsae* AII IJ were suspended in deionized water at a concentration of 4,000 IJ/ml. Aqueous solutions of the various adjuvants were mixed with the nematode suspensions (1:1 ratio) to provide test concentrations and a nematode concentration of 2,000 IJ/ml. Nematodes were sprayed on the foliage of 2-year-old apple seedlings cv. Liberty grown in 6-liter pots. Each plant was sprayed until runoff with a manual 1-liter plastic sprayer. This test was carried out twice in a growth chamber at 20 ± 1 °C in the dark, once at 60% RH and once at 70% RH. After 24 hours, six leaves were randomly sampled in three treated trees. Each leaf was washed on

Table 1. Adjuvants tested for activity with *Steinernema carpocapsae* on apple leaves.

Commercial name	Main ingredients (conc. [a.i.])	Action	Manufacturer
Agral 90	Phenolethylene condensate (92%)	Spreader/wetting agent	Zeneca Agrochemicals, England
Citowett Plus	Polyethylene (50%)	Spreader-sticker	duPont, Mississauga, Ontario
Corn Oil	Paraffin (98.9%)	Surfactant	United Agri Products, Dorchester, Ontario
Folicote/Leafshield	Paraffin emulsion (30%)	Anti-transpirant	Aquatrols Corporation of America, Cherry Hill, NJ
Super Spread	Propylene glycol + ethanol (12%)	Spreader/wetting agent	United Agri Products, Dorchester, Ontario

both sides by squirting approx. 60 ml of water with a washing bottle. The water containing nematodes was recovered in a 75-ml 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 was counted after 24 hours. Leaf area measurements were made with a portable area meter (LI-COR model LI-3000, LI-COR Inc., Lincoln, NE).

Field applications: Foliar applications of nematodes on apple were assessed on a farm in Deux-Montagnes. The 0.5-ha orchard (cultivars Liberty and Red Fry) on dwarf rootstock was bordered by an insecticide-sprayed apple orchard. Field trials 1, 2, and 3 were performed on 3, 9, and 16 August 1994, respectively. *S. carpocapsae* AII strain was applied as a water suspension with an adjuvant (Agral 0.05%) with a backpack sprayer (70 kPa) at the rate of 2×10^9 IJ/ha. Each experimental unit consisted of three adjacent apple trees. One unsprayed apple tree was left between each treatment. The check plots were sprayed with water and adjuvant only. The experiment was arranged in a randomized complete block design with 6 replications/treatment. Treatments were applied at sunset. Fungicides were applied for apple scab control at commercially recommended rates (Chouinard, 1997).

Statistical Analysis: Percentage values were normalized with arcsin transformation. LD_{50} values for each insect instar were computed with probit analysis (Polo-PC, LeOra Software, Berkeley, CA). The Waller-Duncan k-ratio *t*-test was used to compare treatments when analysis of variance and general linear models (SAS Institute, Cary, NC) showed significant differences among means.

Results and Discussion

Nematode activity against OBLR larvae: The susceptibility of OBLR larvae to nematode infection increased with larval development (Table 2). LD_{50} values of third, fourth, fifth, and sixth instars were 13, 5, 3, and 2 IJ/larva, respectively. The slopes were estimated at 1.6, 1.9, 1.6, and 0.7 for third, fourth, fifth, and sixth instars, respectively. Pathogenicity to OBLR larvae varied with nematode species (Table 3). At a concentration of 25 IJ/dish, *S. riobrave* was the most pathogenic to third or fourth instars with 88.8% mortality, followed by *S. feltiae* UK strain and *S. carpocapsae* AII strain with 79.2 and 74.7%, respectively. *S. glaseri* was the least pathogenic with 28.3% mortality.

Table 2. Pathogenicity of *Steinernema carpocapsae* AII strain to various OBLR instars (180 insects/treatment).

Instar	LD_{50}[a]	95% confidence limits
Third	13	6–27
Fourth	5	2–10
Fifth	3	2–5
Sixth	2	1–4

[a] LD_{50} values = number of IJ per insect needed to give 50% mortality.

Mortality of third instar OBLR was increased with exposure time to IJ (Fig. 1). After 24 hours of exposure, larval mortality was 93.7%. A minimum 8-hour exposure was needed to achieve a significant increase in mortality.

These results confirm the susceptibility of OBLR larvae to nematode infection. Similar results have been obtained for several lepidopteran insect pests under controlled conditions (Jaques, 1967; Morris, 1985), confirming that an exposure of at least 8–12 hours to the nematodes was necessary to reach a significant level of larval mortality. Similar effective exposure times have been reported for other foliage-feeding insects (Glazer and Navon, 1990; Sledzevskaya, 1987).

Even though *S. riobrave* and *S. feltiae* were

Table 3. Pathogenicity of *Steinernema* spp. to OBLR third and fourth instars.

Nematode species	Strain	Percent mortality	Standard error
S. riobrave	335	88.8 a[a]	2.1
S. feltiae	UK	79.2 b	5.7
S. carpocapsae	AII	74.7 b	6.5
S. glaseri	326	28.3 c	7.4
Control (water)	–	0.7 d	0.6

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

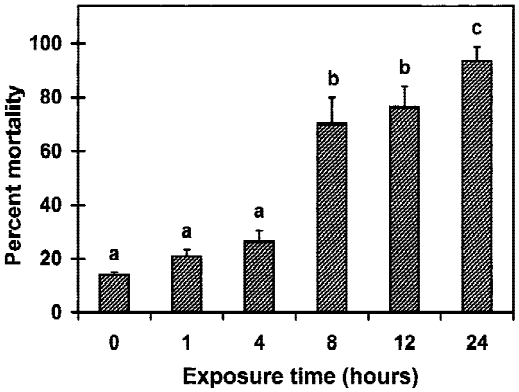

FIG. 1. Mortality (± standard error bar) of OBLR third instars as a function of time of exposure to *Steinernema carpocapsae* AII strain. Bars with the same letter are not significantly different according to the Waller-Duncan k-ratio *t*-test ($P \leq 0.05$).

more pathogenic under controlled conditions, *S. carpocapsae* AII strain was chosen for subsequent assays because of the availability of this species for field trials and due to the higher survival rate of this nematode when exposed to desiccation (Baur et al., 1995; Simons and Poinar, 1973).

Screening adjuvants for foliar application of nematodes: At 60% RH, all adjuvants, with the exception of Citowett Plus (0.1 and 0.2%) and Superspread (0.2 and 0.4%), had an adverse effect on the survival of the nematodes (Table 4). At 70% RH, no significant effect of adjuvants was detected. Based on these two experiments, the addition of an adjuvant in the aqueous solution did not significantly improve the average total number and the average number of living nematodes per unit leaf area when compared to the water control.

Earlier attempts to increase survival on foliage by the addition of adjuvants and antidesiccant formulations were only partly successful (Glazer, 1992; Kaya et al., 1981; MacVean et al., 1982; Shapiro et al., 1985). Based on our results, it was decided to use Agral 0.05% as a standard adjuvant for our field applications.

Field applications: Foliar applications of *S. carpocapsae* AII at the rate of 2×10^9 infective juveniles/ha provided 37, 19, and 13% larval control of OBLR in the first, second, and third trials, respectively (Table 5). Weather conditions following trial 1 (high relative humidity and overcast conditions for 48 hours after treatment) could explain its better performance when compared to the two other nematode applications.

The DD-136 strain of *S. carpocapsae* was tested against five different foliage-feeding pests of apple in the laboratory and field. This nematode caused high mortality in the

TABLE 4. Effects of various adjuvants on density and survival of *Steinernema carpocapsae* AII strain in aqueous solutions following a 24-hour exposure to 60% or 70% relative humidity (RH) at 20 °C on apple leaves.

Adjuvant	Percent concentration (v/v)	60% RH		70% RH	
		IJ/cm^2	Living IJ/cm^2	IJ/cm^2	Living IJ/cm^2
Control (water)	—	49 ± 6.2 ab[a]	38 ± 5.2 a	93 ± 21.7 a	61 ± 14.0 a
Citowett Plus	0.2	53 ± 5.8 a	36 ± 4.2 ab	52 ± 14.5 a	35 ± 9.8 a
Citowett Plus	0.1	49 ± 6.4 ab	33 ± 4.7 abc	59 ± 9.6 a	44 ± 7.7 a
Super Spread	0.4	49 ± 3.7 ab	31 ± 3.1 abcd	76 ± 8.4 a	60 ± 7.5 a
Leafsheald	6.0	46 ± 7.7 abc	22 ± 3.0 def	69 ± 11.8 a	45 ± 7.2 a
Super Spread	0.2	44 ± 3.3 abc	31 ± 2.9 abcd	68 ± 12.9 a	57 ± 12.0 a
Leafsheald	3.0	44 ± 4.8 abc	25 ± 3.1 cde	69 ± 14.9 a	45 ± 1.0 a
Citowett Plus	0.4	43 ± 4.2 abcd	27 ± 3.4 bcde	80 ± 12.5 a	35 ± 8.4 a
Corn Oil	4.4	42 ± 4.1 abcd	24 ± 1.8 cde	46 ± 7.0 a	43 ± 5.6 a
Corn Oil	1.1	40 ± 4.3 abcd	26 ± 3.2 bcde	72 ± 11.9 a	58 ± 10.0 a
Super Spread	0.1	39 ± 2.6 abcd	27 ± 1.9 bcde	64 ± 14.9 a	54 ± 13.6 a
Agral 90	0.05	38 ± 2.4 abcd	23 ± 1.5 def	68 ± 24.9 a	43 ± 15.7 a
Leafsheald	12.0	36 ± 7.2 bcd	23 ± 5.1 def	50 ± 11.7 a	31 ± 6.6 a
Corn Oil	2.2	35 ± 4.3 bcd	24 ± 3.2 cde	51 ± 6.8 a	43 ± 5.4 a
Agral 90	0.02	30 ± 4.4 cd	18 ± 2.9 ef	73 ± 18.2 a	44 ± 11.2 a
Agral 90	0.1	26 ± 4.5 d	14 ± 1.9 f	—	—

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

TABLE 5. Mortality of OBLR larvae on apple trees sprayed with *Steinernema carpocapsae* AII strain in three field trials.

	Nematode		Control (water)	
Trial	Percent mortality	Number of insects collected	Percent mortality	Number of insects collected
#1	37.3	74	0	32
#2	19.2	107	0	101
#3	13.0	108	0	68

laboratory, but field application against a dense population of the winter moth *Operophtera brumata* did not result in larval control (Jaques, 1967). The discrepancy between laboratory and field performance was attributed to rapid nematode desiccation and to the application method. Jaques (1967) also showed that applications of nematodes to leaves by dipping in a suspension was more effective than application by spraying, primarily because more nematodes were deposited on dipped apple leaves than on sprayed leaves. The difference was thought to be due largely to the numbers of nematodes that adhered to the hairy undersurface of dipped leaves. Sledzevskaya (1987) reported that the gooseberry fruit worm, *Zophodia grossulariata*, was highly susceptible to *S. carpocapsae* Agriotos strain in petri dishes but that mortality was low under field conditions. By covering the plants with polyethylene sheets after nematode applications for an 8 to 12-hour period, high relative humidity on the branches was maintained and yielded a 50% larval mortality of *Z. grossulariata*. Mortality dropped to 30% when tarping, and high moisture levels were maintained for only 2 to 4 hours. Our field results are in line with their findings.

The possibility of nematodes reaching OBLR larvae inside the rolled-up leaves was first thought to increase nematode survival rate by protecting them from UV light and rapid desiccation. Our results suggest that nematodes hardly reached larvae because the suspension could not penetrate easily the tightly woven larval silk. This point was illustrated by comparing dipping or spraying rolled leaves with the nematode solutions. Spraying gave less than 30% larval mortality, while dipping leafrolls gave 100% mortality (G. Bélair, unpubl. data).

The level of control with *S. carpocapsae* in an open environment such as the foliage is rate-dependent (Begley, 1990; Glazer, 1992; Sledzevskaya, 1987). On apple leaves, *S. carpocapsae* survived up to 90 hours after application (Bélair et al., 1998). However, the survival rate was too low to attain an effective control level. Based on our field results, the nematode does not provide an acceptable level of control for this pest. Better nematode formulations, including genetically improved isolates and a good antidesiccant, are needed to make the use of entomopathogenic nematodes acceptable in commercial apple orchards.

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