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Foliar Sprays with *Steinernema carpocapsae* against Early-season Apple Pests[1]

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Abstract: Persistence and field efficacy of the entomopathogenic nematode *Steinernema carpocapsae* All strain applied by foliar sprays were evaluated against the apple sawfly *Hoplocampa testudinea* and the plum curculio *Conotrachelus nenuphar,* two early-season pests in Quebec apple orchards. From 1992 to 1995, bioassays with *Galleria mellonella* larvae were conducted to assess the persistence of *S. carpocapsae* on leaves, flower clusters, and twigs up to 4 days after evening application. *S. carpocapsae* juveniles remained infective on apple leaves 24, 42, 98, and 24 hours after application in 1992, 1993, 1994, and 1995, respectively. In bioassays, the percentage of *G. mellonella* mortality was consistently higher on leaves (average = 84%), intermediate on flower clusters (73%), and lower on twigs (43%) for all application dates. In 1992 and 1993, single nematode sprays applied every 2 to 3 days from early May to mid-June on apple tree limbs reduced primary damage caused by *H. testudinea* by 98% and 100%, respectively, but none of the treatments was effective in 1994. In 1993 and 1994, multiple border-row sprays were applied against *C. nenuphar* adults with a commercial hand-gun applicator in an insecticide-free orchard. At harvest, plum curculio damage in the nematode-treated orchard reached 5% and 55% in 1993 and 1994, respectively, as compared to 80% and 85% in an adjacent insecticide-free orchard. In a second experiment performed in 1994, multiple broadcast sprays with a commercial orchard sprayer caused no significant effect on plum curculio damage (nematode = 28%; control = 31%). Although some efficacy of canopy sprays of nematodes was detected against early-season apple pests, the inconsistent results and high application costs preclude their use as a sole control tactic against these pests in commercial apple orchards.

Key words: apple, biological control, Coleoptera, *Conotrachelus nenuphar,* Curculionidae, European apple sawfly, field persistence, foliar application, *Hoplocampa testudinea,* Hymenoptera, nematode, plum curculio, *Steinernema carpocapsae,* Tenthredinidae.

The apple sawfly, *Hoplocampa testudinea* Klug (Hymenoptera: Tenthredinidae), and the plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae), are two early-season pests in apple orchards of eastern North America. The apple sawfly is a direct pest of apples in southwestern Quebec where it may cause up to 15% damage in commercial orchards (Vincent and Mailloux, 1988). Adults emerge in spring slightly before the pink stage. First-instar larvae feed on the surface of young apple tissue, causing a ribbon-like scar known as primary damage. Occasionally, second- or third-instar larvae leave a fruit to enter another fruit. In burrowing their way to the center of the fruit, they leave frass pellets at the entry point. This is secondary damage (Miles, 1932).

The plum curculio is an important pest of apple fruit in North America (Croft and Hull, 1983). In the absence of insecticide treatments, adults may cause up to 85% damage to fruit (Racette et al., 1993; Vincent and Bostanian, 1988). The insect is univoltine, and most adults overwinter in woodlots or hedgerows at the periphery of orchards. In spring, adults migrate from overwintering sites into apple orchards. Depending on the temperature, they are active when apple buds have reached the pink to full-pink stage of phenological development (Chouinard et al., 1993; Chouinard et al., 1994; Racette et al., 1991). At this time, they feed on leaves and blossoms and later they attack the fruits (Chouinard et al., 1993; Lafleur and Hill, 1987; Racette et al., 1993). Insecticide sprays usually are applied either throughout the entire orchard when at least 80% of the petals have fallen or to the outer four to six rows of trees bordering overwintering sites (Chouinard et al., 1992; Choui-

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nard 1997; Vincent et al., 1997). Additional applications may be needed if fruit injury is found 7 days after the first spray.

Plum curculio management currently is achieved by targeting chemical insecticide sprays against the adults as they invade host trees after overwintering. Behavioral studies conducted in Quebec revealed that, in spring, most plum curculios return to orchards from their overwintering sites. They remain on the ground under the perimeter rows of apple trees for several weeks before they move toward the interior of the orchard around petal fall (Chouinard et al., 1994; Lafleur and Hill, 1987). Plum curculio could be efficiently controlled by restricting petal control sprays to perimeter rows or to the outer four to six rows of trees bordering overwintering sites (Chouinard et al., 1992; Vincent et al., 1997).

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae are soil-borne insect parasites associated with a mutualistic bacterium that kills the insect host in 24 to 48 hours (Kaya and Gaugler, 1993). They are of considerable value as biological control agents and serve as alternative measures to chemical control of insect pests. Recent advances in the development of methods for producing, storing, and applying entomopathogenic nematodes have decreased the cost and increased the use of these organisms for controlling these pests (Kaya and Gaugler, 1993). They have been field-tested as potential control agents for numerous foliar insects with some successes and also many failures (Begley, 1990; Georgis and Hague, 1988; Jaques, 1967; Kaya et al., 1981). In previous work, foliar applications of steinernematid nematodes were successful in reducing secondary damage caused by the apple sawfly (Vincent and Bélair, 1992). *Steinernema carpocapsae* was found effective against the larvae and adults of the plum curculio under controlled conditions (Brossard et al., 1989; Olthof and Hagley, 1993). In an integrated pest management strategy, nematode applications could also be timed to control plum curculio adults as they invade host trees after overwintering and/or during their egg-laying period.

In the present work, *S. carpocapsae* All strain applied by foliar sprays was evaluated for effectiveness and persistence in controlling *H. testudinea* and *C. nenuphar* under field conditions.

Materials and Methods

Nematode supply: Steinernema carpocapsae All strain (Biovector, Thermo Trilogy, Columbia, MD) was obtained annually and stored at 5 °C until used. Before use, nematode percent viability was determined by examining movement under a dissecting microscope. A lot was not used if its viability was lower than 75%. Nematode dosages were adjusted according to the viability level.

Persistence of S. carpocapsae on the apple canopy: From 1992 to 1995, the persistence of *S. carpocapsae* infective juveniles (IJ) on the apple canopy was assessed at the Agriculture and Agri-Food Canada Experimental Farm at Frelighsburg (45°03'N, 75°50'W), Quebec, Canada. The 0.5-ha orchard, planted to cultivars Empire and Vista Bella, was bordered by woodlots on the east and west sides and by insecticide-sprayed apple orchards on the north and south sides. Full bloom of apple trees occurred on 20 May 1992, 19 May 1993, 28 May 1994, and 23 May 1995. *Steinernema carpocapsae* was applied as a water suspension with a non-ionic wetting agent (Agral 0.1%) with an Oxford precision sprayer (MDM Engineering, Portsmouth, Hampshire, UK) at 70 kPa. This sprayer was modified so that the spray went through one Teejet nozzle of size Allman No. 3. Single applications were done at sunset every 2 to 3 days on seven, seven, and six occasions in 1992, 1993 and 1994, respectively. On each application day, 20 randomly choosen 50-cm-long branch segments were sprayed with a 75-ml water suspension containing ca. 100,000 IJ. Each branch harbored six to eight fruit clusters.

From 1992 to 1995, the persistence of *S. carpocapsae* on leaves, flowers (or fruitlets), and twigs was monitored up to 4 days after each application using a modified *Galleria* *mellonella* biotest (Bedding and Akhurst, 1975). On each sampling occasion, 10 samples made of three leaves, three flower clusters, or three 5-cm pieces of twigs per branch were randomly picked from the 20 treated branches and deposited in a 25-ml cup containing one late instar *G. mellonella* larvae. After 1 week at 22 °C, *G. mellonella* mortality was recorded and their cadavers checked for nematode infection. Percent mortalities obtained from leaves, flowers, and twigs were averaged for all application dates on each year. In 1994 and 1995, the total numbers of living IJ per leaf surface were determined by sampling apple leaves from nematode-sprayed branches. Ten more 50-cm-long branch segments were sprayed with a 75-ml water suspension containing ca. 100,000 IJ. The nematode survival rates on apple leaves were determined 0^+ (i.e., immediately after application), 12, 18, 24, and 48 hours after spraying in 1994 and 0^+, 12, 18, 24, and 36 hours in 1995. On each sampling occasion, six leaves were randomly taken. Each leaf was individually dipped into 20 ml water in a 25-ml plastic cup and shaken thoroughly in the water suspension to ensure that all the nematodes were removed. After 24 hours, the number of dead and live nematodes in each dish was counted. Total leaf surfaces were recorded on a portable area meter (LI-COR Model LI-3000, Lambda Instruments, NE). Temperature, relative humidity, precipitations, and sunlight intensity during the experiments were recorded continuously by the weather station located in the experimental orchard.

Foliar applications against apple sawfly: From 1992 to 1994, the efficacy of foliar nematode treatments to reduce apple sawfly damage was assessed under field conditions. The experiments were carried out in the same orchard as described above. Nematodes were applied in a 75-ml water suspension containing ca. 100,000 IJ on a 50-cm-long branch segment that harbored six to eight fruit clusters. Each year, single nematode applications were performed on separate branch segments from early May to mid-June, and directed against apple sawfly larvae to reduce primary damage on apple fruit. Applications were done at sunset every 2 to 3 days on seven, seven, and six occasions in 1992, 1993 and 1994, respectively. Control branches were treated with water and Agral 0.1% on the last treatment day of each year only. To exclude adult sawflies from treated segments, the branches were covered with a sleeve cage immediately after applications. The experiment was conducted as a randomized complete-block design with 10 to 20 replications per treatment, where all treatments were randomized on 10 to 20 tree rows (i.e. blocks). In late June and early August, all fruits were examined for primary (ribbon-like scar) and secondary (frass pellets at entry point) types of damage. Apple sawfly activity was determined by positioning four white traps (17.8 × 14 cm) on trees in the Frelighsburg orchard. The traps, positioned on trees at ca. 1.5 m from ground level, were covered with Tangletrap (Tanglefoot, Grand Rapids, MI). The traps were set on 5 May 1992, 4 May 1993, 26 April 1994, and 25 April 1995 before the pink stage of McIntosh trees. Adult captures were recorded weekly. Apple sawfly damage was evaluated by examining 500 fruits randomly collected at harvest. Fungicides were applied against apple scab at commercially recommended rates (Chouinard, 1997).

Foliar applications against plum curculio-border row treatment: This trial was conducted in 1993 and 1994 at the Agriculture and Agri-Food Canada Experimental Farm at Frelighsburg, Quebec. The 0.6-ha apple orchard, consisting of cultivars Cortland and McIntosh, was bordered by woodlots on the west and south sides and sprayed apple orchards on the north and east sides. The first nematode application was performed at fruit set, and additional applications were made when a threshold of 1% to 2% plum curculio fruit damage was reached (biweekly monitoring of fresh oviposition scars on fruits) (Vincent et al., 1997). Nematodes were applied on 7, 22, and 30 June 1993 and on 7, 13, and 18 June 1994. On each treatment date, nematodes were applied with a hand gun (2,000 kPa) mounted on a commercial sprayer at the rate of 8 billion IJ/ha in 4,000 liters of suspension/ha with Agral 0.1%. Half of the suspension was applied on the foliage and the other half on the ground underneath a 20-m band of apple trees at the periphery of the orchard. Fungicides were applied against apple scab at commercially recommended rates (Chouinard, 1997). On 19 August 1993 and 15 September 1994, 200 apples were picked at random from each of five sectors (N, S, E, W, and center) defined according to Chouinard et al. (1992) and Vincent et al. (1997) and examined for presence of plum curculio damage. Peripheral sectors N, S, E, W comprised the first 20 m of trees facing each cardinal point; the center contained all the other trees. Damage at harvest was compared with damage from an adjacent insecticide-free orchard (100 m away) and a commercially managed orchard (30 m away).

Foliar applications against plum curculio-broadcast treatment: This trial was conducted in 1994 in a commercial organically managed orchard at Henryville, Quebec. The 0.2-ha orchard (cultivars Cortland and McIntosh) was bordered by annual crops on all sides. The first nematode application was conducted at fruit set; additional applications were made as in the above section when a threshold of 1% to 2% curculio fruit damage was reached (bi-weekly monitoring of fresh oviposition scars on fruits) (Vincent et al., 1997). Nematodes were applied on 7, 13, 20, and 30 June 1994 with an airblast orchard sprayer (1,000–2,000 kPa) at 5 billion IJ/ha in 5,000 liters of suspension/ha and Agral 0.1%. Half of the suspension was applied on the foliage, and the other half was applied on the ground underneath each tree block (250 m^2 each). Control plots received water and Agral only. The experiment was arranged in a completely randomized design with four replications. Beginning 3 days after the first nematode application, monitoring of plum curculio oviposition scars was performed as described above. At each sampling occasion, 300 fruits were chosen at random in each block (20 fruits in each of the 15 central trees of each block). Damage at harvest was assessed in a similar manner on 15 September 1994.

Results and Discussion

Persistence of S. carpocapsae in apple canopy: IJ retained infectivity up to 24, 42, 98, and 24 hours after application on sprayed apple leaves in 1992, 1993, 1994, and 1995, respectively (Fig. 1). In *G. mellonella* assays, insect mortality was consistently higher on leaves, intermediate on flower clusters, and lower on twigs for all 4 years of trials. In 1992, 1993, and 1994, insect mortality on sampled leaves and flower clusters was consistently 100% 15 hours following application, and then a gradual decline was observed. On twigs, nematodes consistently caused less larval mortality when compared to leaves and flower clusters. Immediately after application, insect mortality on twigs ranged from 60% to 95% and then followed a decline that parallelled that observed on leaves and flowers. In 1995, insect mortality on sampled leaves and flower clusters was 90% and 65%, respectively, 15 hours following application, after which there was a sharp drop to less than 5% mortality 18 hours after application (Fig. 1). Significant differences in the number of living IJ on apple leaves were recorded over a 48-hour period (Table 1). In 1994, the number of living IJ significantly decreased 48, 12, and 12 hours after applications for treatments made on 24 May, 30 May, and 13 June, respectively. In 1995, the number of living IJ remained unchanged 12 hours after application, but, after 18 hours, none were detected on all three application dates. This persistence of *S. carpocapsae* on apple foliage was longer than what has been reported elsewhere. Jaques (1967) found that the percentage of *S. carpocapsae* survival on apple foliage was 20% after 2 hours at 45% relative humidity and 21 °C. Apple leaves are pubescent and thought to retain a water film at their surface for an extended period as compared to a smooth surface (Baur et al., 1995; Glazer, 1992). In our field trials, the persistence of *S. carpocapsae* on apple foliage was variable from one application to another, and even more so from one year to another. Climatological conditions prevailing at time of application and also those following the application likely af-

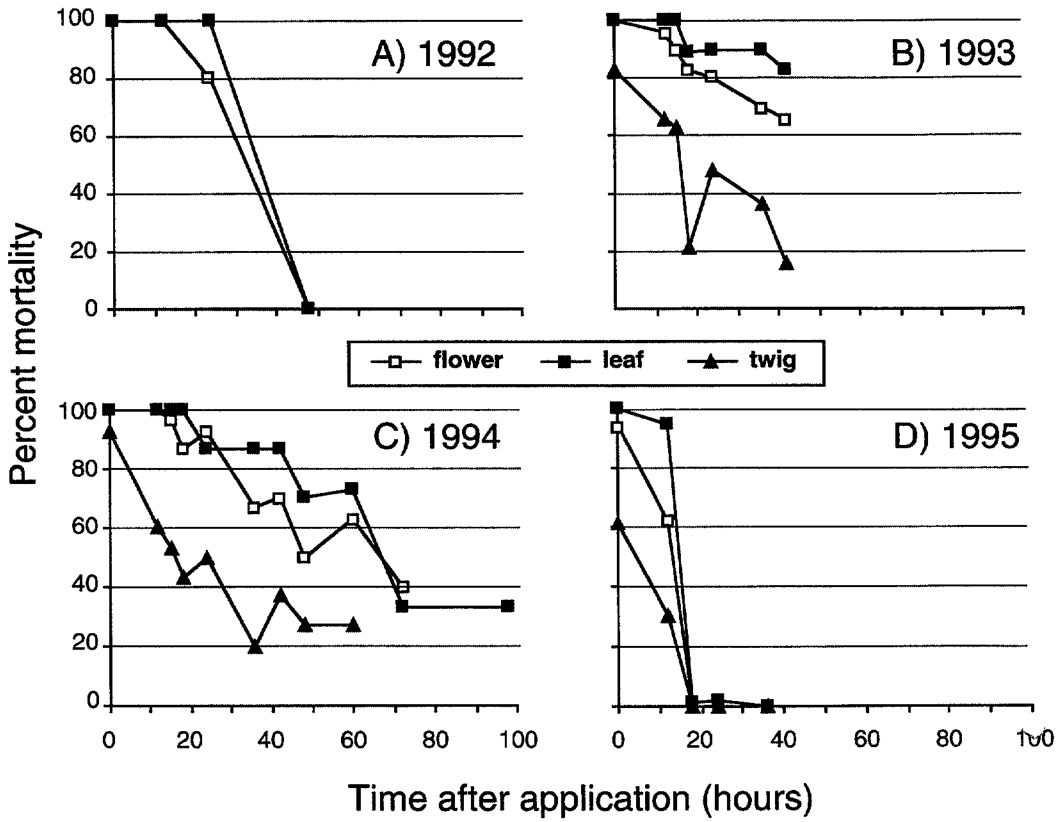

FIG. 1. Persistence of *S. carpocapsae* in the apple canopy as revealed by *G. mellonella* bioassays. A) 1992. B) 1993. C) 1994. D) 1995.

fected nematode survival. Entomopathogenic nematodes are sensitive to dehydration and UV light. Foliage constitutes a hostile environment for these organisms (Kaya and Gaugler, 1993). Weather conditions prevailing in our plots in 1995 were much drier and sunnier than those prevailing in 1994, and these conditions might have de-

TABLE 1. Living *Steinernema carpocapsae* infective juveniles on apple leaves in 1994 and 1995.

Time after application (hours)	Living *S. carpocapsae* IJ/cm² of apple leaves[a]					
	1994			1995		
	24 May	30 May	13 June	30 May	5 June	13 June
0+	32 a	17 a	20 a	22 a	20 a	21 a
12	19 a	6 b	3 b	17 a	18 a	20 a
18	25 a	1 c	2 b	0 b	0 b	0 b
24	13 a	0 c	0 c	0 b	0 b	0 b
48	9 b	0 c	0 c	0 b	0 b	0 b
Temperature (average)[b]	14.6	23.7	21.6	21.0	21.8	17.1
Temperature (min–max)	11.5–16.7	20.4–27.0	19.6–24.0	16.9–25.5	16.6–26.8	11.5–21.7
Relative humidity (min–max)	45–100	46–100	74–100	42–71	44–76	42–88
Precipitation (mm)	0.8	0	21.2	0.8	0	0
Sunlight (MJ/m²/day)	2.3	21.2	29.2	26.5	26.7	22.3

[a] Numbers are the means of six replications. Values in columns followed by the same letters are not significantly different according to the median χ^2 test ($P \leq 0.05$) (Steel and Torrie, 1980).

[b] Meteorological conditions prevailing 24 hours after application time.

creased the survival of *S. carpocapsae* on the foliage (Table 1).

Foliar applications against the apple sawfly: Nematode treatment significantly reduced apple sawfly primary damage in 1992 and 1993, but not in 1994 (Table 2). None of the treatments reduced secondary damage in all 3 years. In 1992 through 1994, nematode applications were performed during apple sawfly flight activity (Fig. 2). Based on fruit examination at harvest, apple sawfly primary damage was estimated at 1.0%, 2.6%, and 1.0% in 1992, 1993, and 1994, respectively. In an earlier work (Vincent and Bélair, 1992), foliar applications of *S. carpocapsae* significantly reduced secondary damage. The positive results against sawfly larvae from foliar applications of *S. carpocapsae* probably were obtained because of the use of cages that increased the relative humidity and, thus, nematode survival and infectivity. Working with the web-spinning larch sawfly, Georgis and Hague (1988) reported that foliar applications of *S. carpocapsae* resulted in a larval infection level ranging from 3.4% to 29.4% even though the nighttime relative humidity was favorable for nematode survival. They concluded that the lack of contact between the nematode and the sawfly was responsible for low efficiency.

TABLE 2. Effect of foliar applications of *Steinernema carpocapsae* All strain on primary and secondary fruit damage caused by *Hoplocampa testudinea* (apple sawfly) in apple orchard plots.

Application date	Apple sawfly damage (%)[a]		
	June		August
	Primary	Secondary	Primary
1992 (10 replications)			
22 May	0.3 cd	5.0 a	0.0 a
25 May	0.4 e	1.2 a	0.9 a
27 May	2.3 cd	0.9 a	5.4 a
29 May	3.8 b	1.5 a	2.7 a
1 June	0.8 bc	3.0 a	1.3 a
3 June	1.0 cd	1.2 a	1.3 a
5 June	5.5 a	1.0 a	1.6 a
Check (water)	5.0 a	6.4 a	2.0 a
1993 (10 replications)			
18 May	6.6 a	15.4 a	1.9 c
21 May	9.7 a	17.7 a	2.7 b
23 May	7.7 a	18.3 a	1.3 c
25 May	4.5 a	14.0 a	0.0 d
27 May	5.9 a	10.1 a	0.0 d
30 May	5.2 a	16.1 a	0.3 d
8 June	3.3 a	7.0 a	3.3 a
Check (water)	5.4 a	11.4 a	4.6 a
1994 (20 replications)			
24 May	0.0 a	5.1 a	0.0 a
30 May	0.5 a	3.6 a	0.0 a
2 June	1.8 a	14.9 a	0.0 a
7 June	4.2 a	8.9 a	2.0 a
9 June	2.4 a	12.9 a	0.0 a
13 June	2.3 a	9.5 a	0.0 a
Check (water)	1.3 a	15.4 a	0.0 a

[a] Values in columns followed by the same letters are not significantly different according to Friedman's test ($P \leq 0.05$) (Steel and Torrie, 1980).

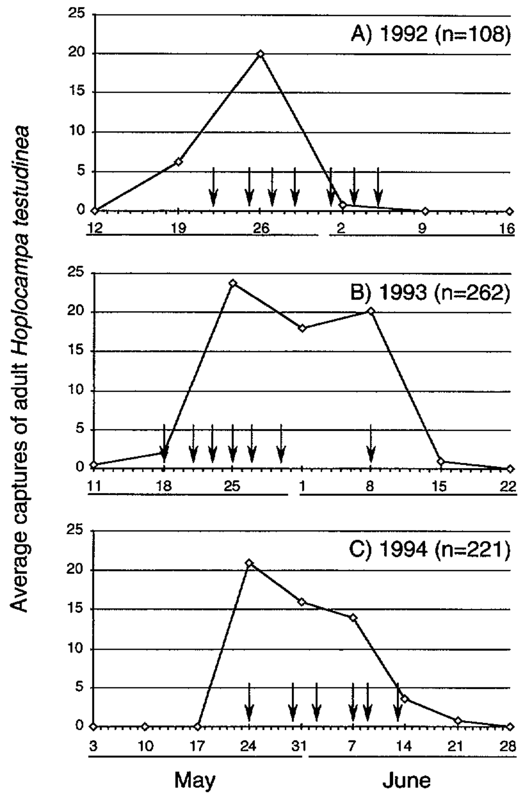

FIG. 2. Average (n = 4 traps) weekly captures of adult *Hoplocampa testudinea.* Numbers in parentheses indicate total seasonal captures. Arrows indicate treatments with entomopathogenic nematodes.

Foliar applications against plum curculio-border row and broadcast treatment: In 1993 and 1994, border-row treatments reduced plum curculio damage by 75% and 30% when compared to the adjacent insecticide-free orchard. Damage at harvest (5%) was economically acceptable in 1993 but unacceptable (55%) in 1994 (Table 3). In 1994, broadcast treatments with *S. carpocapsae* did

TABLE 3. Effect of foliar applications of *Steinernema carpocapsae* All strain on fruit damage caused by *Conotrachelus nenuphar* in apple orchards.

	Average damage at harvest (%)[a]		
	Frelighsburg[b]		Henryville[c]
Treatment	1993	1994	1994
Nematode	3.3 b	57.2 b	27.1 a
Insecticides	0.0 a	0.1 a	—[d]
Check	81.0 c	96.5 c	31.1 a

[a] Means in a column followed by the same letter are not significantly different according to the median χ^2 test ($P \leq 0.05$) (Steel and Torrie, 1980).
[b] Border row treatments.
[c] Full block applications.
[d] No data.

not reduce plum curculio damage (Table 3). Under laboratory conditions, plum curculio adults were susceptible to *S. carpocapsae* (80% mortality in petri dishes) but not affected by *Heterorhabditis* spp. and *S. bibionis* (Brossard et al., 1989). Foliar applications of nematodes against coleopteran foliage-feeding insects generally do not give sufficient insect control to meet commercial standards (Begley, 1990). Adult elm leaf beetles were susceptible in petri dish bioassays, but the pathogenic effect decreased when the adults were allowed to move away from the nematodes (Kaya and Gaugler, 1993). From pink to after-fruit-set stages, spurs and twigs are the main apple structures occupied by adult plum curculio in southeastern Quebec (Chouinard et al., 1994). Plum curculio adults are in their period of highest dispersal activity between 21:00 and 06:00 hours. We found that nematodes survived less on apple spurs than on leaves or flowers (Fig. 1). There seemed to be a spatial gap between these two organisms, thereby reducing the chances of successful infection and control.

There has been some success in controlling foliage-feeding insects with nematodes, but desiccation has been a major limiting factor (Baur et al., 1995; Begley, 1990; Jaques, 1967; Kaya et al., 1981). In our study, relative humidities and temperatures at night were generally favorable for nematode survival, but apple sawfly and plum curculio control was low, suggesting a lack of contact between these organisms, as previously mentioned by Georgis and Hague (1988). The inconsistent results and high application costs preclude the use of nematode foliar sprays as the sole control tactic against these pests in commercial apple orchards. In Quebec, the extended persistence and infectivity of *S. carpocapsae* on apple trees in early season and particularly on apple leaves increase the potential of entomopathogenic nematodes as biological control agents for other orchard pests such as the oblique banded leafroller, *Choristoneura rosaceana* Harris. The larvae of *C. rosaceana* wrap themselves in leaves and such cryptic microhabitats are known to be more favorable for the survival and infectivity of entomopathogenic nematodes.

As for future research areas, nematode applications at or near the base of the trunk of apple trees should be investigated for the control of plum curculio. Just before entering the apple trees, adults of plum curculio are most abundant at the base of the trunk (Chouinard et al., 1994). Nematode applications in this moist less exposed environment are likely to be more suitable for the survival and infectivity of entomopathogenic nematodes than foliage and, thus, increase the chances of infecting this pest insect during its early migration to the tree canopy, leading to reduced insect damage to the fruit. This approach is currently being investigated.

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