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Entomopathogenic nematodes for control of codling moth (Lepidoptera: Tortricidae) in apple and pear orchards: Effect of nematode species and seasonal temperatures, adjuvants, application equipment, and post-application irrigation

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Abstract

Codling moth (CM), a serious pest of apple and pear in most countries where these fruits are grown, overwinters in cryptic habitats as cocooned diapausing larvae. Control of diapausing CM larvae would reduce or eliminate damage to fruit early in the following growing season. Entomopathogenic nematodes (EPNs) have shown promise as biological control agents of cocooned CM larvae in the Pacific Northwest and elsewhere, but several factors, such as choice of EPN species and other operational factors warrant investigation to provide growers with practical control options. Field trials with *Steinernema carpocapsae* and *S. feltiae* were conducted in apple and pear orchards to determine the effects of seasonal temperatures, adjuvants, post-application irrigation, and method of application on control of cocooned CM larvae. In studies conducted in late summer, fall and early spring (1999–2000), EPNs were applied to apple trees (Golden Delicious) with a backpack sprayer at a rate of 10^6 infective juveniles (IJs)/tree plus supplemental wetting to aid survival of IJs. Good control by both EPN species was observed in September (94–95% mortality in sentinel CM larvae). In October, control by *S. feltiae* was also effective (90% mortality), but *S. carpocapsae* was less effective (58% mortality), ostensibly due to the cooler conditions. In identical applications the following spring, the efficacy of *S. carpocapsae* and *S. feltiae* was reduced during cool windy conditions in March 30 tests, providing 26 and 65% control of sentinel larvae, respectively, but improved during warmer conditions in April 12 tests (71 and 86% control, respectively). In further tests in the same location in mid-October 2001, *S. feltiae* (10^6 IJs/tree) were most effective for control of sentinel CM larvae cocooned in cardboard strips (≈ 80% mortality) and logs (34–47%) when combined with a wetting agent (Silwet L77) or a humectant (Stockosorb) and the trees were misted for 4 h post-treatment. In the absence of post-application wetting, the addition of either adjuvant (Silwet and Stockosorb) to IJs also increased larval mortality in strips, although it did not significantly improve nematode efficacy on logs. In another test in late summer 1999, the use of a lance applicator (applying 2.0×10^6 IJs/tree) did not significantly improve control of cocooned larvae for either EPN species, when compared with a tractor-mounted airblast sprayer. Two further tests in the fall of 2003 with *S. carpocapsae* and *S. feltiae* compared post-application wetting with existing and modified irrigation in 4-year-old trellised apple (Gala) and established Bartlett pear orchards. No significant improvements in sentinel larval mortality were observed following application of both EPN species with an airblast sprayer ($1–2.5 \times 10^9$ IJs/ha) when conventional overhead rotator sprinklers were replaced with lower volume microsprinklers.

Keywords: Entomopathogenic nematodes; Codling moth; *Cydia pomonella*; *Steinernema feltiae*; *Steinernema carpocapsae*; Temperature effect; Irrigation effect; Application effect

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1. Introduction

Codling moth (CM), *Cydia pomonella* (L.), is a global pest of apple, pear, and walnut (Barnes, 1991), and the principal pest of apple in the Pacific Northwest of the United States (Beers et al., 1993). In late spring, adults emerge and begin laying eggs when fruit is available. Neonate larvae enter the fruit and feed until full grown, then exit as fifth instars in search of cryptic habitats in which to spin their cocoons and pupate. In the Pacific Northwest there may be two to three generations per growing season depending upon weather (Beers et al., 1993).

A variety of broad spectrum insecticides are employed for CM control in conventional orchards during the growing season (Beers et al., 1993). Softer approaches that include the use of mating disruption (Calkins and Faust, 2003) and the CM granulovirus (Arthurs and Lacey, 2004) are also employed. In temperate climates, CM overwinters in cryptic habitats as cocooned diapausing larvae. Thus, overwintering larvae represent the entire CM population in the fall, winter, and early spring. Their elimination or significant reduction at this stage would provide complete or substantial protection to fruit early in the following growing season. However, few interventions are employed for control of overwintering larvae. Cryptic habitats, such as those used by CM for their overwintering sites (under loose bark, in litter at the base of trees, in nearby woodpiles, fruit bins and the like) may also provide favorable environmental conditions for entomopathogenic nematodes (EPNs) (Begley, 1990; Koppenhöfer, 2000). Used under optimal conditions of warm temperatures and available free water, EPNs can be effective control agents of cocooned CM larvae in orchards (Kaya et al., 1984; Lacey et al., 2000; Nachtigall and Dickler, 1992; Sledzevskaya, 1987; Unruh and Lacey, 2001) and fruit bins (Cossentine et al., 2002; Lacey and Chauvin, 1999; Lacey et al., 2005). Studies by Kaya et al. (1984), and Unruh and Lacey (2001) elucidated the importance of moisture for control of CM by *Steinernema carpocapsae* in California and Washington, respectively. The habitat of overwintering CM and environmental conditions may vary from orchard to orchard and within orchards depending on the season and other factors. Further improvements of application methods and how irrigation systems and adjuvants can be used to enhance persistence and activity of EPNs will be necessary to provide growers with practical control options. Other factors such as the choice of nematode species that could influence the success or failure of EPNs in orchards have yet to be studied in detail. It was the objective of our research to investigate the effects of EPN species and seasonal temperature, addition of adjuvants, application method, and type of post-application irrigation on the efficacy of EPNs for control of cocooned CM larvae. Field trials were conducted over a 4-year period in orchards in eastern Washington State to elucidate the effect of these factors.

2. Materials and methods

Table 1 summarizes experimental information for studies conducted from September 1999 through October 2003 including application rates, type of orchard, EPN strains, and factors addressed in each experiment. Two procedures that are common in the experiments reported below are method of assessment of mortality using sentinel CM larvae and quality control bioassays of the EPNs tested.

2.1. Source of CM larvae and use as sentinels

Sentinel larvae used in field trials were produced on soy-wheat germ-starch artificial diet (Toba and Howell, 1991) and reared under diapausing conditions [photoperiod of 10:14 (L:D), 20 °C, 40–50% RH] in the colony maintained at the Yakima Agricultural Research Laboratory (YARL). Based on two reliable methods for using CM sentinel larvae that were developed in an earlier study (Unruh and Lacey, 2001), cocooned diapausing larvae were used in the field in perforated cardboard strips [8×1.9 cm ($15.2\ cm^2$), double faced, B flute, Weyerhauser, Tacoma, WA] and in apple and

Table 1
Summary of field studies evaluating *S. carpocapsae* and *S. feltiae* for codling moth control, 1999–2003

Factor(s) addressed	Orchard (trees/ha)	Nematode species (strain)	Rate (IJ)	Date(s) applied	Quality control[a]	Post wet[b]
Seasonal temperature	Golden Delicious; (500)	*S. feltiae* (Umea)/ *S. carpocapsae* (Sal)	10^6/tree	September 16, 1999	0, 54.4, 62.8	Y
				October 14, 1999	0, 67.1, 88.9	Y
				March 30, 2000	0, 93.6, 80	Y
				April 12, 2000	0, 72.9, 75	Y
Adjuvants and post-application wetting		*S. feltiae* (Umea)	10^6/tree	October 19, 2001	0, 73, na	Y/N
Sprayer type	Mixed apple varieties; (840)	*S. feltiae* (Umea)/ *S. carpocapsae* (Sal)	2.0×10^6/tree	August 25, 1999	1.7, 81.3, 81.5	Y
Sprinkler type (post-application wetting)	Trellised Gala; (3088)	*S. carpocapsae* (All)/ *S. feltiae* (UK76)	10^9/ha	October 1, 2003	0, 36.4, 92.7	Y
	Bartlett pear; (538)		2.5×10^9/ha	September 29, 2003	0, 42.9, 71.4	Y

[a] Percentage codling moth sentinel larval mortality in cardboard strips treated with water, or 10 IJs/cm^2 of *S. feltiae* or *S. carpocapsae*, respectively.
[b] Additional irrigation applied post nematode application (yes/no). All plots were irrigated pre-treatment.

pear logs. The cardboard strips were perforated using a sewing machine with approximately 75 holes (0.5 mm diam) on each side to facilitate passage of infective juveniles. The strips were individually infested by placing them in 9 cm diam petri dishes with 20 laboratory-reared diapausing larvae which were allowed to spin cocoons in the cells of the cardboard over a 24-h period. Apple logs (7–9 cm diam × 30–35 cm length) were prepared for sentinel larvae by sawing grooves (6–8 cm long, 2–3 mm wide, 5–10 mm deep) in the logs with a table saw. Twenty diapausing larvae were placed on the logs and confined to the space over the grooves with a piece of clear plastic held in place with tape. The larvae utilized the grooves as cocooning sites over the following 24-h period. On pear logs, larvae utilized natural crevices under rough bark in which to spin cocoons and no sawing was required. Infested logs and strips were stored at 12 °C until used.

2.2. Test nematodes

The infective juveniles (IJs) of *S. carpocapsae* (Weiser) (Sal strain) and *Steinernema feltiae* (Filipjev) (Umea strain) were produced in wax moth according to procedures described by Kaya and Stock (1997) and used within 2 weeks of production. The *S. carpocapsae* Sal strain and *S. feltiae* Umea strain used in our studies conducted between 1999 and 2001 were originally obtained from Integrated BioControl Systems (Aurora, IN) and BioLogic (Willow Hill, PA), respectively. The *S. carpocapsae* (All strain) and *S. feltiae* (UK76 strain) used in large field trials in commercial orchards were provided by Certis USA (Columbia, MD) and MicroBio Ltd (now Becker Underwood, Ames, IA), respectively.

Quality control of test nematode infectivity was conducted for each experiment against diapausing cocooned CM larvae in 15.2 cm^2 perforated cardboard strips using 152 IJs in 1 ml water spread over the surface of the strips (10 IJs/cm^2, 4 strips per treatment and control) and methods described by Lacey and Unruh (1998). The treated strips were placed in moistened filter paper-lined petri dishes, incubated for 6 days at 25 ± 1.7 °C and then assessed for larval mortality. The results of these quality control tests are reported in Table 1.

2.3. Experiment 1. Effect of seasonal temperatures on nematode species

These tests were designed to evaluate the two nematode species under operationally appropriate times of the season, in late summer to fall after harvest, and in early to mid spring before CM pupation. A block of 43-year-old Golden Delicious apple trees located next to YARL was used for four studies conducted in the late summer, early fall 1999 and early spring 2000 to determine the effect of temperature on infectivity of *S. carpocapsae* and *S. feltiae* for CM larvae (Table 1). Six single tree plots were used for each treatment and controls on each test date, using a completely randomized block design. When the tests were conducted, an apple log infested with cocooned CM larvae was attached to the trunk of each tree with wire ≈1.5 m above the ground. Temperature and humidity were monitored every 15 min just before and for 8 h following application of nematodes with a Hobo H8 Pro Series data logger (Onset Computer, Pocasset, MA) mounted in the scaffold branches of one of the treated trees. Prior to nematode applications, the trees were wetted in the zone from the scaffold branches (approximately 2 m high) to the ground using misting spray nozzles connected to garden hoses.

Treatments were applied with a CO_2 pressurized backpack sprayer (Model T, R&D Sprayers, Opelousas, LA), between 9 and 10 a.m. The sprayer was equipped with a hand-held spray wand with a single nozzle (8008 VS TeeJet) and operated at 276 kPa (40 psi). All of the screens were removed from the wand to minimize shearing forces. Applications of *S. carpocapsae* or *S. feltiae* were made in 2 L of deionized water plus wetting agent (Silwet L77 at 0.05%, Silicone-polyether copolymer, Loveland Industries, Greeley, CO). Following application of nematodes, the trees were briefly misted every 30 min for the next 6 h. The sentinel logs were removed from the orchard 24 h after treatment and incubated for 6 days at 25 ± 1.7 °C. Removal of the logs 24 h after application of nematodes prevented excessive mortality due to predators. Since nematodes die very soon after sprayed surfaces become dry, exposure of sentinel larvae to IJs ended before logs were removed. After incubation, larvae were removed from the logs and assessed for mortality and nematode infection. Data were analyzed as independent sample t tests for each date with nematode species as the effect variable.

2.4. Experiment 2. Effect of wetting agent, humectant, and post-application wetting

Experiment 2 was conducted in the same orchard as experiment 1 on October 19, 2001 to determine the effect of a wetting agent (Silwet L77 at 0.05%) or a humectant (Stockosorb at 0.2%, also known as Sta-Moist, a cross-linked potassium polyacrylate/polyacrylamide copolymer, Stockhausen, Greensboro, NC), on the infectivity of *S. feltiae* applied with and without post-application wetting (Table 1).

Prior to application of *S. feltiae*, sentinel larvae in two types of substrates were placed on each of 35 trees. In addition to sentinels in apple logs mounted on trees (as described above), diapausing larvae in perforated cardboard strips were secured onto the tree trunks with staples 1 m above the ground. Pre-wetting of trees followed that used in Experiment 1. Ten replicate trees were used for each of the three treatments arranged as a complete randomized block (*S. feltiae* in water only, *S. feltiae* with water and Silwet, and *S. feltiae* with water and Stockosorb). Five randomly selected trees for each of the treatments received post-application wetting using misting spray nozzles every 30 min for 4 h. The other 5 trees for each of the treatments were not wetted after treatment. Five control trees received pre- and post-application wetting without adjuvants. Results of studies reported by Lacey et al. (2005) indicated that neither Silwet L77 nor Stockosorb at these concentrations killed cocooned CM larvae. Application methods and assessment of mortality in sentinel larvae employed the same methods as described above. The mean temperature and humidity for the 8 h following applications was 15.4 °C (range 8.6–19.4 °C) and 75.5% (range 47.5–100% r.h.). Data were analyzed as a three-way ANOVA with substrate, adjuvants and post-wetting regime as crossed effects.

2.5. Experiment 3. Comparison of airblast sprayer with hand-held lance applicator

A portion of a 0.57 ha block of mixed apple varieties (Gala, Red Delicious, Golden Delicious, Fuji), at the USDA-ARS experimental farm near Moxee, Washington was used for tests to compare the difference in EPN efficacy using two types of sprayers (Table 1). Spacing between trees within each row was 2.4 m and between rows was 4.9 m. Apple trees were treated in late summer (August 25, 1999) at the rate of 2×10^6 IJs/tree with either *S. feltiae* or *S. carpocapsae* using an airblast sprayer or a lance applicator. The mean temperature and humidity during the 8 h post-application interval were 19.2 °C (range 13.7–29.1 °C) and 63.4% (range 27.1–98.5% r.h.).

Nine 10 tree plots were used for the experiment. Separate portions of each plot were used for either airblast or lance applicator treatments. The plots were arranged in three separate rows of the orchard and randomly assigned EPN species or control. The rows used for plots were separated from each other by five rows. Plots within rows were separated from one another by 2–3 trees. The central six trees (3–8) of each plot were treated with the airblast sprayer for EPN species and controls (water only). The airblast sprayer (Orchard Master) was configured with D-8 nozzles (TeeJet) on the lower three nozzle ports of one side of the sprayer. The other ports were turned off. The nozzles were angled to provide coverage of the trees from the base of the trunk into the scaffold branches. Screens and swirl plates were removed from the sprayer to minimize shearing forces on IJs. The pressure used was 276 kPa (40 psi) and the fan was turned off. The speed of the tractor was adjusted to provide an application rate of 3740 L/ha (46 L/plot). The six airblast-treated trees in each plot were sprayed using a separate pass of the tractor for each side of the row. The 2nd and 9th trees in each plot were unsprayed and used as buffer trees between airblast and lance applicator-treated trees. With the low pressure, large nozzles, and no fan, little or no spray drift reached the adjacent rows or beyond buffer trees in the same row. Sentinel larvae were used to assess efficacy of treatments. Two infested apple logs were secured to the opposite sides (east and west facing) of each of two trees (the 4th and 6th tree) in each plot ≈1.2 m high facing the drive rows. Prior to applications, trees were wetted from the scaffold branches (approximately 2 m high) down using misting spray nozzles connected to garden hoses. Trees 1 and 10 of each block were used for applications of EPNs or water with a Rears Pull-Tank hand-held lance applicator (Rears Manufacturing, Eugene, OR). Logs infested with sentinel larvae were secured to each tree and pre-application wetting of trees was done as above. Application of IJs in 2.3 L of water or water alone to the trunk and scaffold branches of each tree was made with the lance-applicator equipped with a 8002VS nozzle. Post-wetting of all sentinel trees and mortality assessments exactly followed Experiment 1. Data were analyzed by two-way ANOVA with the two sprayer treatments and the two EPN species (and controls) representing the treatment levels of crossed effects (ANOVAs with and without controls were compared).

2.6. Experiment 4. Effect of post-application irrigation type on nematode efficacy

The effect of different types of irrigation for post-application wetting on the efficacy of EPNs was studied in two commercial orchards in the fall of 2003 (Table 1). Each orchard provided different habitats for overwintering CM and sentinel larvae were placed in locations which were most similar to habitats where CM hibernacula are found.

A 4.9 ha 4-year-old block of trellised Gala apple trees near Quincy, Washington was used to compare the effect of three types of sprinkler on EPN treatments applied against cocooned sentinel larvae on the ground. Trees were <2 m tall and planted at a high density with smooth bark characteristic of modern varieties on dwarf rootstocks. A rectangular section of the orchard was divided into three adjacent blocks (each 0.34 ha comprising three rows of ≈350 trees). Each block was separated from the next by four buffer rows and treated with either *S. feltiae*, *S. carpocapsae* or water plus NuFilm 17 (Miller Chemicals and Fertilizer, Hanover, PA) (control). Thus each block represents separate experiments wherein post-wetting irrigation configurations were compared for effects on EPN efficacy using three sentinel types as crossed effects.

Three types of irrigation (existing over-tree sprinklers and two configurations of under-tree sprinklers) were each assigned to five plots within each of the three blocks for a total of 45 plots. Fifteen plots were arranged along the central row in each block such that no two adjacent plots had the same type of sprinkler. Existing irrigation (standard) used in 15 of the plots comprised over-tree rotator spray heads (R2000 model, Nelson Irrigation, Walla Walla, WA) fitted on vertical 3.5 m PVC risers (61 risers/ha) each delivering ≈8.4 L/min with a 15 m throw diameter at 380 kPa (55 psi). Two configurations of under-tree sprinkler attachments (fitted near the base of the vertical risers) were used for modified irrigation treatments. Fifteen Rotor Rain Microsprinklers (Antelco, Longwood, FL) were fitted with blue restrictors (delivering ≈3 L/min and 10 m throw diameter) and the remaining 15 were fitted with olive restrictors (≈5 L/min and 11 m throw). The over-tree sprinklers were turned off in the 30 plots with under-tree microsprinklers.

On the day of applications, a set of sentinels each consisting of one CM-infested pear wood log and four infested perforated cardboard strips were placed in each of the 45 plots. Infested pear logs were placed horizontally on the ground along the tree row and a cardboard strip was placed on the ground either side of the log (on ground). Another two identical strips were placed in crevices made in the soil with a trowel close to the other sentinels (within ground). All sentinels were located approximately mid way from the throw of the nearest sprinkler head. Each set of sentinels were wetted by only one type sprinkler.

EPNs were applied in a spray volume of 3740 L/ha using a tractor-mounted Rears PowerBlast Sprayer air blast sprayer at 700 kPa (100 psi) with the fan on. NuFilm17 was used as a spreading agent at 2.38 L/ha. Applications were made using TeeJet D12 nozzles with swirl plates and screens removed. Only the lower four nozzles per side were used (the remainder turned off) and directed downwards to apply IJs along a swath (≈4 m wide) running along the tree line where sentinels were located. The orchard was irrigated for 2 h prior and 6 h after application of EPNs. Mean temperature and humidity during the 8 h following applications was 20.5 °C (range 14.9–24.4 °C) and 91.3% (range 53–100% r.h.). Mortality assessments followed those used in Experiments 1–3.

A 40-year-old Bartlett pear orchard in Monitor, Washington was used to compare EPN treatments applied against cocooning larvae above ground, on the trunk and main branches of trees (Table 1). A rectangular 1.2 ha section of the orchard was divided into three adjacent blocks (each ≈0.4 ha comprising five rows of ≈40 trees) which were treated with either *S. feltiae*, *S. carpocapsae* or water (control) as at Quincy. Sprinkler type was the independent variable tested in each block.

Existing orchard irrigation was similar to Quincy with overhead rotator (H5 model, Toro, Bloomington, MN) on vertical 4.3 m risers every fifth tree on alternate rows (54 risers/ha). In modified post-wetting treatments rotator spray heads were replaced with Rotor Rain Microsprinklers fitted with yellow restrictors delivering ≈4 L/min and 11 m throw diameter. The day prior to nematode applications, 30 sentinel pear logs were secured to trees in each treatment block. Logs were tied with garden wire ≈1.5 m above the ground on the trunk or main branches of trees and positioned to receive wetting from either the standard or modified irrigation treatments. Four replicates for each irrigation treatment for each EPN species and control were established, based on the proximity of sentinels to sprinklers (four sprinklers of each type arranged alternately within the block).

The orchard was irrigated for 2 h prior to and during application. Nematodes were applied at 3740 L/ha using a 1600-L tractor-mounted airblast sprayer at 700 kPa (100 psi) with the fan on. A wetting agent was included at 0.25% v/v (BioLink, organically approved surfactant and penetrant, Westbridge, Vista, CA). Applications were made using TeeJet D12 nozzles with screens and swirl plates removed to protect IJs. Four nozzles per side were used (top ones turned off) and directed from the base of the trunk up to the lower part of the canopy. The irrigation was run for an additional 24 h following applications. Mean temperature and humidity during the 8 h following applications was 17.1 °C (12.2–21.0 °C) and 100% r.h. Mortality assessments followed those used in Experiments 1–3.

2.7. Data analysis

All analysis was performed using SPSS (SPSS, 2003). Treatment effects were compared using independent sample *t* tests or univariate ANOVA (percent mortality) as specified in each experiment. Mortality data were normalized using the arcsine transformation prior to statistical analysis. Significant *F*-ratio means were further separated with Fisher's LSD for multiple comparisons, at $P < 0.05$.

3. Results

3.1. Experiment 1. Nematode species and seasonal temperature

The efficacy of nematode applications (10^6 IJs/tree applied with a back pack sprayer) varied between species on two of the four treatment dates ($P < 0.0001$ in each case) (Fig. 1). In all cases control mortality remained ⩽5.3%. Late summer applications (September) were the most effective, resulting in 94.4% (*S. carpocapsae*) and 94.7% (*S. feltiae*) mortality in CM larvae (Fig. 1B). *S. feltiae* was also effective in the mid-October application, although there was a significant reduction of control for *S. carpocapsae* to 58% mortality. Despite the somewhat low mortality in the quality control bioassays for both species in September (Table 1), they were very effective in controlling the sentinel larvae in the field test. However, despite high mortality for *S. carpocapsae* in the October quality control bioassay, cooler temperatures were a limiting factor for this species in the field (Fig. 1B).

In spring applications, the efficacy of both species was reduced in March, although *S. feltiae* was again significantly more effective compared with *S. carpocapsae*, recapitulating the pattern seen in October. Mild but constant breezes following applications and a reduced daytime high temperature in March compared to September may account for the reduced efficacy. The mortalities in sentinel CM larvae following the April application during warmer temperature conditions were greater for both nematode species compared with March treatments, and there were no significant differences between the nematode species, similar to the pattern seen in the warm weather of September. Under the range of realistic seasonal temperatures, *S. feltiae* was markedly more effective when mean temperature dipped below 15 °C.

3.2. Experiment 2. Effect of wetting agent, humectant, and post-application wetting

For nematode treatments, univariate ANOVA revealed significant main effects for post-treatment wetting ($F_{1,48} = 29.5$, $P < 0.0001$), the use of adjuvants, i.e., none, Sil-

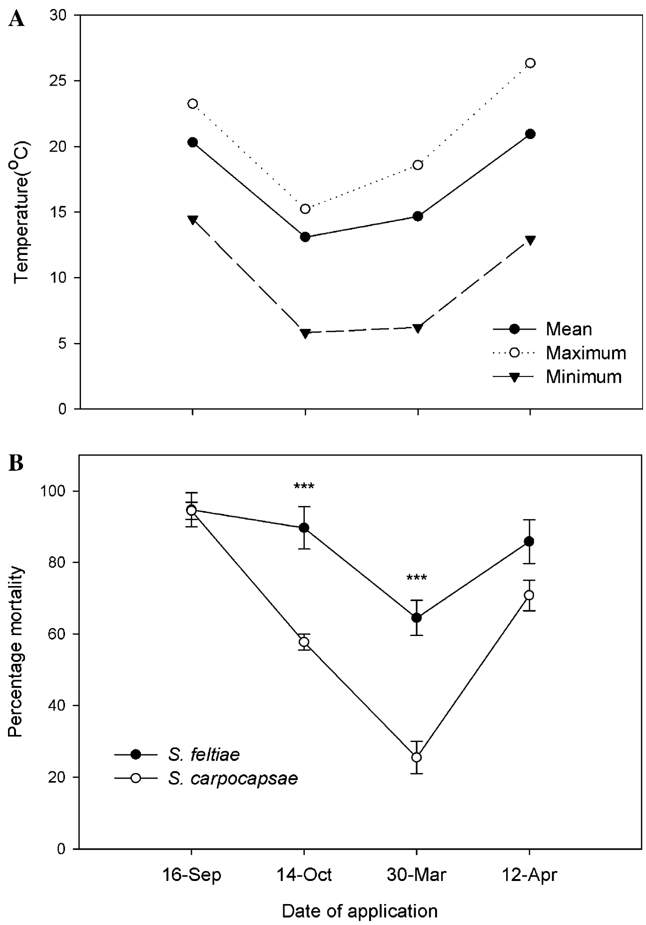

Fig. 1. The effect of seasonal temperature on mortality of cocooned codling moth larvae on Golden Delicious trees treated with two nematode species at 1 million IJs per tree. Data show (A) temperature range for the 8 h post-application and (B) average mortality ± SEM for six tree plots. Symbols denote differences between nematode species following independent samples *t* tests, (***$P < 0.0001$). Mortality of untreated control larvae did not exceed 5.3% on any date.

wet or Stockosorb ($F_{2,48} = 17.8$, $P < 0.0001$) and sentinel substrate, i.e., logs or strips ($F_{1,48} = 41.8$, $P < 0.0001$). There were no significant two- or three-way interaction terms. Means for each treatment including untreated controls were further compared with one-way ANOVA and post hoc comparisons (Fig. 2).

Steinernema feltiae IJs were most effective for control of sentinel CM larvae in logs and cardboard strips when combined with either adjuvant (Silwet or Stockosorb), and the trees were misted for 4 h post-treatment. We expected a reduced effect of post-wetting when the humectant was used, but this was not observed. In combination treatments (adjuvant + post-application wetting) the highest rates of control (≈80% mortality) were observed in sentinel larvae in cardboard strips (Fig. 2A), although mortality in equivalent treatments was lower on logs (34–47%) (Fig. 2B). In the absence of post-wetting, the addition of either adjuvant alone also increased larval mortality in strips, although did not significantly improve nematode efficacy on logs. Cool temperatures (mean 15.4 °C) may have contributed to the lower activity of *S. feltiae* compared to tests reported in Fig. 1.

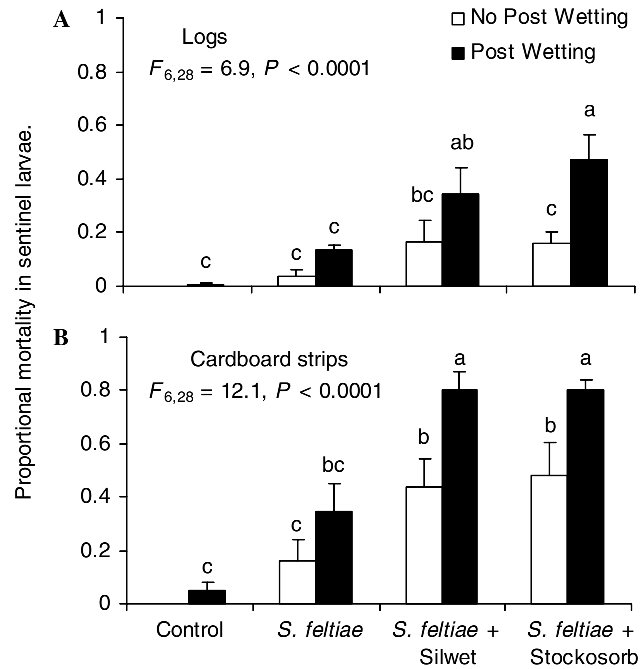

Fig. 2. The effect of two adjuvants, a wetting agent (Silwet L77) or humectant (Stockosorb), and post-application wetting on larvicidal activity of *S. feltiae* infective juveniles (IJs) against cocooned codling moth sentinel larvae on Golden Delicious trees treated at the rate of 1 million IJs per tree. Data show average ± SEM for sentinels in (A) grooved logs and (B) cardboard substrates. Different letters indicate differences between treatments following one-way ANOVA and Fisher's LSD at $P < 0.05$.

3.3. Experiment 3. Comparison of airblast sprayer with lance applicator

Univariate two-way ANOVA revealed that EPN species had significant effects on mortality of sentinels both with ($F_{2,12} = 37.5$, $P < 0.0001$) and without ($F_{1,8} = 5.7$, $P = 0.04$) controls considered. In contrast, there were no significant effects of sprayer ($F_{1,12} = 2.2$, $P = 0.17$) ($F_{1,8} = 2.2$, $P = 0.18$). No significant interaction between sprayer and EPN treatments were observed ($P > 0.48$ in both cases). Means for each treatment including untreated controls were further compared with one-way ANOVA and post hoc comparisons (Fig. 3). Rates of control were ≈10% higher with hand-applied IJs, although the differences between sprayers were also not significant for either nematode species compared individually (Fig. 3). However, when nematode species were compared, *S. feltiae* was more effective compared with *S. carpocapsae* when applied with the lance applicator, but not when applied with the airblast sprayer.

3.4. Experiment 4. Effect of post-application irrigation on nematode efficacy

The application of 10^9 IJs/ha in the trellised Gala orchard (Quincy) provided good CM control using *S. carpocapsae* and moderate control for *S. feltiae* (Fig. 4).

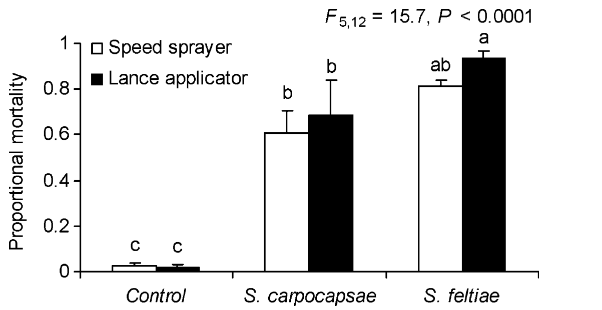

Fig. 3. Mortality in cocooned codling moth sentinel larvae after application of 2 million *S. feltiae* or *S. carpocapsae* infective juveniles per tree using an airblast sprayer or lance applicator. Data show average ± SEM for sentinels in three orchard plots and different letters indicate differences between proxy treatments following one-way ANOVA and Fisher's LSD at $P < 0.05$ where the proxy treatments represent all combinations of sprayer and sentinel types. See text for two-way ANOVA.

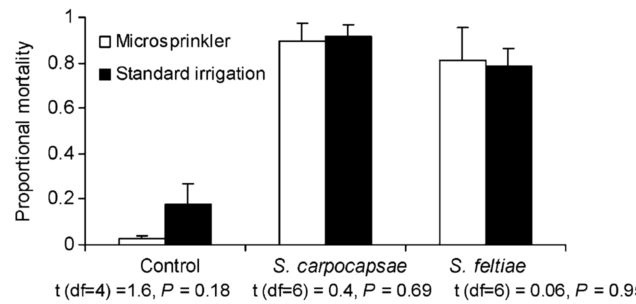

Fig. 5. Mortality in cocooned codling moth sentinel larvae on the trunk or main branches after treatment with *S. feltiae* and *S. carpocapsae* at 2.5×10^9 IJ/ha in a 1.2 ha pear orchard (Monitor). Applications were followed by supplemental wetting using standard or modified (microsprinklers) irrigation. Data show mean ± SEM for sentinels in four locations in 0.4 ha blocks. Individual blocks treated with *S. feltiae*, *S. carpocapsae* or left untreated were analyzed separately.

S. carpocapsae provided >85% control against sentinel larvae in all three methods of assessment. Neither sprinkler type ($F_{2,36} = 0.41$, $P = 0.66$), substrate ($F_{2,36} = 2.97$, $P = 0.06$), nor their interaction ($F_{4,36} = 0.1$, $P = 0.98$) were significant effects for *S. carpocapsae*. ANOVA indicated no difference between the two microsprinkler types and these were pooled for these analysis. *S. feltiae* was less effective on all substrates with the lowest mortality found on logs. Here, substrate ($F_{2,35} = 23.2$, $P < 0.0001$) represented a significant effect while sprinkler type ($F_{2,35} = 1.97$, $P = 0.16$) and the interaction ($F_{4,36} = 1.3$, $P = 0.29$) were not significant. In summary, no significant difference in sentinel mortality was observed between post-wetting treatments. Quality control strips revealed significantly less mortality produced by *S. feltiae* compared with *S. carpocapsae* (independent samples *t* test, $t = 4.4$, $df = 6$, $P < 0.01$) recapitulating the pattern we saw among blocks.

Highest mortality for both species was noted from sentinels in cardboard strips in the ground. Interestingly, the majority of the mortality observed in 'within ground' control sentinels was due to infection by a native *Heterorhabditis* sp. Determination of the genus was based on color of the insect cadaver and morphology of adult male nematodes observed in larval cadavers (i.e., presence of bursa). No mortality caused by *Heterorhabditis* sp. in the 'on ground' cardboard or log sentinels was observed. *Heterorhabditis*-infection could not be discounted as a contributing factor to the mortality of 'within ground' sentinels in our nematode-treated plots.

The results from the pear orchard (Monitor), where sentinels in logs were attached to the trees above ground and treated at 2.5×10^9 IJs/ha, are shown in Fig. 5. *S. carpocapsae* was effective, with >89% mortality. Mortality due to *S. feltiae* was higher compared with sentinels in the previous study (Quincy). Again, no benefits of the modified post-wetting treatments were demonstrated over that of the existing irrigation. Quality control strips again showed a decline in the mortality due to *S. feltiae* ($t = 3.6$, $df = 6$, $P < 0.05$).

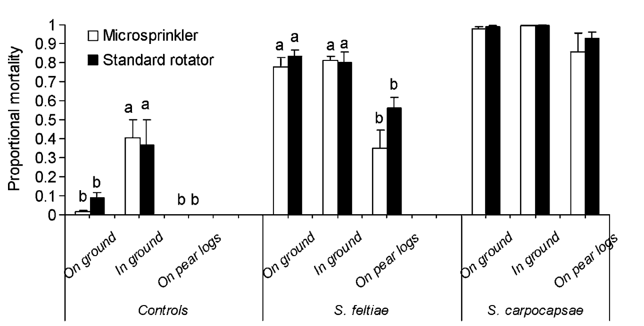

Fig. 4. Mortality in cocooned codling moth sentinel larvae in three different locations after treatment with *Steinernema feltiae* or *S. carpocapsae* infective juveniles (10^9/ha) in a 4-year-old trellised apple orchard (Quincy). Nematode treatments were followed by irrigation with standard overhead rotators or under tree microsprinklers. Different letters indicate proxy treatments differences based on one-way ANOVA; and Fisher's LSD at $P < 0.05$ where the proxy treatments represent all combinations of post-wetting and sentinel types. See text for two-way ANOVA. Individual blocks treated with *S. feltiae*, *S. carpocapsae* or left untreated were analyzed separately.

4. Discussion

The main obstacles for successful CM control with EPNs are low temperatures and desiccation of IJs before they have penetrated the host's cocoon. Applications that are made too late in the fall or too early in the spring when prevailing temperatures remain below the threshold of activity of the EPN species will be ineffective. CM pupae are also less susceptible to infection by EPNs than CM larvae (Lacey et al., 2005) emphasizing the need to target spring applications before larvae pupate. The results of our seasonal studies indicate that the optimum time to apply EPNs for CM control in the Pacific Northwest will be in the early fall after most or all of the larval population has entered diapause or after fruit has been harvested which ensures that all larvae are in this stage. Use of an EPN species that is both cold hardy and sufficiently efficacious for control of CM larvae will therefore improve the likelihood of success. *S. carpocapsae* is considerably less infective for CM below 15 °C and inactive at 10 °C (Lacey and Unruh, 1998; Vega et al., 2000). As demonstrated in the studies reported here (Fig. 1), *S. feltiae* IJs are significantly more effective than *S. carpocapsae* against CM larvae under the cooler conditions in October and March (Fig. 1B). Grewal et al. (1996) reported good infective activity in wax moth larvae for a cold-selected population of the SN strain of *S. feltiae* at 8 °C. Several studies on cold active EPN species have been conducted with other insect hosts (Griffin and Downes, 1991; Grewal et al., 1994b, 1996; Mráček et al., 1998; Wright, 1992). Similar investigations are warranted for other cold active EPN species for CM control.

The importance of maintaining moisture for IJ survival and activity against CM has been noted in several studies (Kaya et al., 1984; Lacey and Unruh, 1998; Nachtigall and Dickler, 1992; Sledzevskaya, 1987; Unruh and Lacey, 2001). The highest mortality of CM larvae due to *S. carpocapsae* applications made by Kaya et al. (1984) was observed when cardboard bands containing larvae remained moist from rainfall. Unruh and Lacey (2001) demonstrated the benefits of wetting (misting) trees both before and after application of *S. carpocapsae* IJs. From the time IJs are applied they must enter the cryptic microhabitat of the host, penetrate the cocoon, and successfully enter the host before drying of the habitat results in loss of movement and eventually death of the IJ. Selection of an EPN species with good activity for CM with an active host searching capability could reduce the amount of time the host habitat has to be wet. *S. carpocapsae* is regarded as an ambusher species, i.e., one with limited searching behavior (Campbell and Gaugler, 1997; Lewis et al., 1995). Nevertheless, this species is a good biological control agent of cocooned CM larvae under optimal temperature and moisture conditions. *S. feltiae* is regarded as an intermediate search strategist with greater search capacity than *S. carpocapsae* (Campbell and Gaugler, 1997; Grewal et al., 1994a; Lewis et al., 1995). An EPN species with greater host seeking capacity (i.e., cruiser) with good activity against CM larvae could facilitate host finding before treated habitats dry and permit shorter periods of post-application wetting.

Our study on the use of adjuvants during somewhat cool conditions demonstrated the potential of both wetting agent and humectant for improving IJ activity, especially if combined with post-application wetting (Fig. 2). In a separate study on the use of EPNs for control of cocooned CM larvae in fruit bins, both Silwet and Stockosorb individually improved activity of *S. feltiae* (Lacey et al., 2005). The combined use of wetting agent and humectant further improved efficacy of *S. feltiae* in fruit bins. Adjuvant combinations were not included in the study reported here solely due to logistic limitations. Foliar and other exposed habitats represent the most challenge to IJs in terms of rapid desiccation (Arthurs et al., 2004). Improvements in EPN efficacy with the use of adjuvants in exposed habitats has been reported by Glazer (1992), Broadbent and Olthof (1995), Baur et al. (1997), Mason et al. (1998), and Piggott et al. (2000). In the case of CM which uses cryptic habitats, adjuvants would be used to improve IJ penetration of habitats and host cocoons and to prolong moisture for IJ survival. Further research on some of the more promising adjuvants for CM control under a variety of orchard conditions and during different seasons is warranted.

The airblast sprayer pulled behind a tractor is the conventional and preferred means of application of pesticides by orchardists. In earlier studies on the use the airblast sprayer and lance applicator for CM control with *S. carpocapsae*, a direct comparison of the two methods of application of IJs was not made (Unruh and Lacey, 2001). Our experiment was designed for comparison of the two application methods and species of EPN. The efficacy of IJs applied with the airblast sprayer was numerically, but not statistically, less than with the lance applicator. A greater number of plots in future studies could help to determine if significant differences exist between the two types of sprayers. Although the lance application provides more concentrated coverage of IJs on the trunk and scaffold branches compared with the less directed airblast sprayer, there is a distinct labor saving advantage of the airblast sprayer. In addition to coarser droplets and less pressure, higher concentrations of IJs and the use of adjuvants that retard evaporation might compensate for a potential decrease in efficiency of application using the airblast sprayer. Although *S. feltiae* was evaluated for CM control in limited studies by Nachtigall and Dickler (1992), this and results of Experiment 4 are the first reports of airblast sprayer application of this species for CM control.

The decrease in efficacy of *S. carpocapsae* relative to *S. feltiae* could be a function of the difference between the search behavior of the two species; *S. feltiae* may seek out and enter hosts faster than *S. carpocapsae* before the habitat dries. The comparative tests were conducted under conditions that were the warmest and driest (daytime high 29.1 °C with concomitant 27.1% r.h.) of the four experiments reported herein which could have facilitated rapid drying.

Environmental modification to enhance or extend the activity of IJs in cryptic habitats has been proposed by Webster (1973) and others working with a variety of target insects. In orchards, for example, moisture can be provided with certain types of irrigation sprinklers that cover the area most likely to contain CM hibernacula. Depending on the type of orchard, the layout of irrigation, volume of water and droplet size will be important considerations. The orchard in Quincy is typical of new plantings of apple in the Pacific Northwest. The bark of the trees was smooth and the metal trellis provided few crevices in which CM could spin cocoons above ground level. The even coverage of the orchard with the existing and modified sprinklers enabled effective control at the relatively low application rate of 10^9 IJs/ha. By comparison, the nature of the bark in the older pear orchard in Monitor, provided a myriad of sites on the trees in which CM could spin cocoons. However, the highly fissured pear bark also retained moisture better than smooth apple bark.

Although we anticipated that the more even coverage and smaller droplet size of the microsprinklers might have provided better post-treatment wetting without washing IJs from treated surfaces, no improvement in nematode performance was observed (Figs. 4 and 5). Both systems presumably provided sufficient moisture (at least in the sites where sentinels were placed) both before spraying and for up to 8 h post-treatment. We emphasize that the sentinel larvae were positioned to be contacted by irrigation. In the Quincy orchard the ground was evenly wetted throughout the study area, whereas some portions of the pear trees in Monitor remained dry. This indicates the need to address the issue of post-application wetting using existing irrigation on an orchard by orchard basis. The reduced performance of *S. feltiae* on the pear log sentinels relative to that in cardboard strips in the Quincy experiment may have been due in part to the strips retaining greater moisture than the logs (SPA, personal observation).

Another approach to maintain adequate moisture for EPN activity could be the use of mulches. Under trellised smooth barked trees, mulch could provide an alternative overwintering habitat for CM larvae that can subsequently be treated with IJ suspensions. Mulches retain moisture longer than the surface of bare ground or trunks of trees and thus may facilitate host finding by IJs before they desiccate. Mulches and crop residue have facilitated prolonged survival of EPNs in other cropping systems and enhanced their parasitic activity (Shapiro et al., 1999; Sweeney et al., 1998; Wilson et al., 1999). Research on the use of mulches to enhance activity of EPNs in orchards is currently underway at YARL.

When compared with *S. carpocapsae*, the efficacy of *S. feltiae* in the Quincy and Monitor tests, mortality in the corresponding quality control strips was lower than that observed in the other tests reported here. The *S. feltiae* furnished by Becker Underwood were within 10 days of the expiration date printed on the label, whereas the *S. carpocapsae* were recently produced and unformulated. The field tests in Quincy and Monitor were not specifically designed to compare the two EPN species, but rather to assess the effects of type of irrigation on efficacy. Differences between two EPN species from separate sources that are evaluated under optimal conditions might not always provide a good measure of intrinsic virulence. Differences in mortality could also be due to formulation or production technology, time elapsed between production and use, and conditions during transportation. In this case, the age of the formulated *S. feltiae* could have contributed to the decreased efficacy. In other research on EPNs for CM control conducted at YARL (Lacey et al., 2005), different batches of *S. feltiae* from Becker Underwood have exhibited activity comparable to that reported for *S. feltiae* in Figs. 1–3.

Natural infections of cocooned CM larvae with *S. carpocapsae* have been reported in Europe and North America when larvae are located near the base of trees and close to the soil (Dutky and Hough, 1955; Poinar, 1991; Vega et al., 2000; Weiser, 1955). The natural infection of sentinel larvae in soil crevices by *Heterorhabditis* sp. in the Quincy orchard could provide another candidate EPN that is established in and adapted to the orchard environment in eastern Washington. Isolation and evaluation of native EPNs for CM control will be pursued in future studies.

5. Conclusions

EPNs can provide effective control of overwintering CM when temperatures are 10–15 °C and higher (depending on nematode species) and moisture is maintained for several hours post-application. The use of a cold-active species, such as *S. feltiae*, will provide more flexibility on when orchards can be treated relative to less cold-active species such as *S. carpocapsae*. In our studies, moisture was adequately maintained by wetting trees before and after application of IJs using existing irrigation systems. Further improvement in EPN activity in orchards is possible through the use of wetting agents and humectants. In orchards where the majority of overwintering sites will be on the ground and irrigation can adequately maintain moisture throughout the orchard, application rates as low as 10^9 IJs/ha could provide effective CM control. Control of overwintering CM will decrease or eliminate initial oviposition pressure the following spring. EPNs used in combination with the CM granulovirus and mating disruption have the potential for effective and conventional pesticide-free control of CM. EPNs also have an excellent safety record (Akhurst, 1990; Akhurst and Smith, 2002) and are commercially available as formulations approved for application in organic orchards.

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