Use of Entomopathogenic Nematodes (Steinernematidae) in Conjunction with Mulches for Control of Overwintering Codling Moth (Lepidoptera: Tortricidae)[1]

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Abstract Mulches were compared to test their utility for enhancing efficacy of entomopathogenic nematodes (EPNs) applied against over-wintering codling moth, *Cydia pomonella* (L.), larvae. Compared with bare ground, mulches may enhance control by providing cocooning sites for codling moth larvae and a substrate that is easy to treat, maintains moisture, and enhances nematode activity. *Steinernema carpocapsae* (Weiser) or *S. feltiae* (Filipjev) were applied at a rate of 2.5×10^9 infective juveniles (IJs)/ha against cocooned sentinel codling moth larvae in cardboard strips followed by 2 h of irrigation in plots that were covered with one of four mulches (clover, shredded paper, grass hay or wood chips) or to bare plots on 29 September 2003. Average mortalities of 97 and 98% were observed in paper-mulched plots treated with *S. carpocapsae* or *S. feltiae* IJs, respectively, compared to 80 and 76% mortality in bare plots. Larvicidal activity for *S. feltiae* against sentinel larvae in cardboard strips that were placed in crevices in the soil was nearly identical in all mulched and bare plots (97-100% mortality), but reduced for *S. carpocapsae* in wood chip and clover plots (76-79% mortality) relative to paper, grass hay and bare plots (93-97% mortality). A significant portion of sentinel larvae (25 and 14%) that were placed in crevices in the soil in hay- and paper-mulched control plots revealed the natural presence of EPNs (*Heterorhabditis* sp.). Applications of *S. carpocapsae* and *S. feltiae* at a reduced rate of 10^9 IJs/ha on 20 April 2004, followed by 1 h of irrigation resulted in 13.1 and 7.4% reduction in sentinel larvae in bare plots compared to 36 and 62% in mulched plots, respectively. Applications of *S. carpocapsae* and *S. feltiae* at a rate of 2.5×10^9 IJs/ha on 21 September 2004 to bare and wood chip-mulched plots followed by 1 h of irrigation resulted in 21 and 65% reduction in sentinel larvae in bare plots compared to 93 and 85% in mulched plots, respectively. Residual larvicidal activity of EPNs 3 d after applications in treated plots was low, but significant in the nonmulched plots (12-17% mortality) relative to untreated controls (1-2% mortality).

Key Words Entomopathogenic nematodes, codling moth, *Cydia pomonella, Steinernema feltiae, Steinernema carpocapsae,* mulch

Codling moth, *Cydia pomonella* (L.), has been traditionally controlled in apple and pear orchards with the routine application of broad spectrum insecticides (Beers et al. 1993). Softer interventions, notably the use of mating disruption, have been increas-

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ingly used in North America and Europe (Vickers and Rothschild 1991, Calkins and Faust 2003) for a variety of environmental and safety concerns. In addition to mating disruption, the options used by organic orchardists in North America for codling moth control during the growing season are limited to ovicidial oils, trapping, manual removal of infested fruit and recently the application of spinosad and codling moth granulovirus (Arthurs and Lacey 2004, Arthurs et al. 2005, Lacey et al. 2004). The overwintering stage of codling moth, cocooned larvae within hibernacula, is a difficult stage to kill using conventional approaches because of the sheltered nature of cocooning sites. Throughout the fall and winter, virtually the entire codling moth population is in this stage as a captive audience. The elimination or substantial reduction of codling moth at this time would provide complete or significant protection to fruit early in the following growing season. Currently very little is done to control the overwintering codling moth population. Cardboard bands that are applied to the trunks of trees to provide sites in which overwintering larvae spin cocoons are collected and destroyed in the fall by some growers, but this method catches only a portion of the population and is very labor intensive (Judd et al. 1997).

Entomopathogenic nematodes (EPNs) offer an option for controlling a large portion of overwintering codling moth populations (Kaya et al.1984, Nachtigall and Dickler 1992, Unruh and Lacey 2001). EPNs are capable of controlling cocooned larvae when moisture is maintained for 6-8 h post application and temperatures permit the infective activity of the species that is applied (Lacey and Unruh 1998). Maintenance of moisture long enough for EPN infective juveniles (IJs) to find the host and penetrate its cocoon is critical to the success of EPN for control of overwintering larvae (Lacey and Unruh, 1998; Unruh and Lacey 2001, Lacey et al. 2006).

Mulching and crop residue have facilitated prolonged survival of EPNs in other cropping systems and enhanced their insect parasitic activity (Stewart et al. 1998, Sweeney et al. 1998, Shapiro et al. 1999, Wilson et al. 1999, Acevedo and Lopez-Nunez 2003). In orchard agroecosystems, surface mulches have been used for weed control, improvement of soil nutrient status and biological activity, and have buffered trees against moisture stress resulting from inadequate irrigation (Forge et al. 2003, Neilsen et al. 2003a, b, 2004). Mulches have also resulted in enhanced biodiversity in orchards including an increase in the numbers of ground-dwelling predators (Kienzle and Zebitz 1997, Forge et al. 2003, Miñarro and Dapena 2003, Mathews et al. 2004). Codling moth larvae have been reported to use the leaf litter at the base of trees as overwintering sites (Beers et al. 1993). Mulches could also provide a habitat in which larvae overwinter.

During the past decade, new apple orchards in the Pacific Northwest have been planted with trellised, high density trees with smooth bark characteristic of modern varieties on dwarf rootstocks. These trees provide few if any cocooning sites for overwintering larvae. Mulch beneath smooth-barked trees in which there is a paucity on-tree cocooning sites, could provide an attractive overwintering habitat for codling moth larvae that subsequently can be treated with EPN suspensions with minimal postapplication watering. Our objectives in this study were to evaluate the effect of different mulch types on the larvicidal activity of EPNs against cocooned codling moth larvae and to assess the persistence and recycling potential of candidate EPNs in apple agroecosystems with wood chip mulch.

Materials and Methods

Production and preparation of diapausing codling moth larvae. Larvae used in field trials and quality control assessments were produced on soya-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). Cocooned diapausing larvae were used in laboratory bioassays and in the field in perforated cardboard strips (8 cm × 1.9 cm [15.2 cm^2], double faced, B flute, Weyerhauser, Tacoma, WA). The cardboard strips were perforated using a sewing machine with 3 rows of holes (75 holes, 0.5 mm diam) on each side to facilitate passage of infective juveniles. The strips were infested by placing them individually 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. The infested strips were stored at 12°C until use. Diapausing larvae, not in perforated strips, were also released into enclosures in one field experiment described below.

Production of entomopathogenic nematode infective juveniles. The infective juveniles (IJs) of *Steinernema feltiae* (Filipjev) (Umea strain) and *S. carpocapsae* (Weiser) (Sal strain), used in the Fall 2003 studies and the *S. feltiae* IJs used in the April 2004 tests were produced at YARL in wax moth larvae, *Galleria mellonella* (L.), according to procedures described by Kaya and Stock (1997) and used within 2 wks of production. *Steinernema carpocapsae* (All strain) IJs used in the April and September 2004 tests were supplied by Certis USA (Columbia, MD). The *S. feltiae* (UK76 strain) IJs used in the September 2004 tests were supplied by Becker Underwood (Ames, IA).

Quality control of IJ infectivity was conducted on the morning of each experiment against diapausing cocooned codling moth larvae in 15.2 cm^2 perforated cardboard strips in the laboratory. For this, 152 IJs suspended in 1 ml water (10 IJs/cm^2) were spread over the surface of each of the strips (4 strips per treatment and control) using methods described by Lacey and Unruh (1998). Treated strips were placed in moistened filter paper-lined Petri dishes, incubated for 6 d at 25 ± 1.7°C and then assessed for larval mortality.

Evaluation of different types of mulch for effect on EPN larvicidal activity (Fall 2003). A stratified randomized block design was used to assign mulch types in rows of trellised 'Delicious' apple trees (2759 trees/ha) within the organically managed section of the Wenatchee Valley College Auvil Orchard in East Wenatchee, WA. Five 6.4-m row sections (containing 7 trees) for each of five mulch types (living dwarf New Zealand white clover, grass hay, shredded paper, wood chips [from local arborist companies, mixed species, composition unknown] or conventionally maintained bare ground used as a nonmulched control), were used for evaluation of two EPN species (*S. carpocapsae*, *S. feltiae*) or for water controls. Within each row section for each mulch type or bare ground, three randomly assigned 1-m^2 subplots were designated for treatment with either 2.5×10^5 IJs of *S. feltiae* or *S. carpocapsae*, or water + wetting agent only. Prior to treatment, two 15.2 cm^2 perforated cardboard strips each containing ≈ 20 cocooned diapausing codling moth larvae were placed in each plot, one flat on the surface of the ground, the other in a vertical crevice in the ground made with a spade. The strips were then covered with 2-3 cm of mulch (exception of living clover). Sentinel larvae were similarly placed in the bare ground plots. Location of sentinel strips was marked with stake flags (L. S. Starrett Company, Athol, MA).

Suspensions of IJs of both EPN species were applied on 22 September 2003 with a CO_2 pressurized backpack sprayer (Model T, R & D Sprayers, Opelousas, LA) in 374 ml of water/m^2 (3,739.6 L/ha) with 0.05% Silwet L77® (Silicone-polyether copolymer, Loveland Industries, Inc., Greeley, CO) as a wetting agent. The sprayer was equipped with a hand-held spray wand and a single nozzle (8,008 VS TeeJet®, Wheaton, IL) and operated at 276 kPa (40 psi). All of the screens were removed from the wand to minimize shearing forces on the IJs. The accuracy of spray applications was facilitated by using a 1 m^2 frame made of 2-cm diam PVC pipe as a guide. Temperature and humidity were monitored every 15 min with a Hobo H8 Pro Series data logger (Onset Computer Corp., Pocasset, MA) mounted in the scaffold branches of one of the trees from just before and for 48 h following IJ application. The orchard was irrigated with microjet sprinklers with a flow rate of ≈ 45 L/h, suspended on wires within the rows ≈ 1 m above the ground. Prior to applications of IJs, the irrigation was run for 15 min. After application, the irrigation was run for 2 h. Sentinels were collected 48 h after spraying and incubated for 6 d at 25 ± 1.7°C and then assessed for mortality as described by Lacey and Unruh (1998). A second set of sentinel larvae in cardboard strips was placed in each of the plots 8 d after applications of nematodes in an identical manner to the first set. The sentinel larvae were retrieved 48 h later and incubated as described above.

Spring and fall applications, 2004. The second and third field trials were conducted in the spring (20 April) and early fall (21 September) of 2004 in a block of trellised organically managed 'Gala' apples (2070 trees/ha) in the Wenatchee Valley College Auvil orchard. Five randomly assigned 23-m row sections were covered with wood chip mulch and five 23 m sections were kept bare. Three 7.6 m-lengths of each row section (mulched and bare ground) were randomly assigned for treatment with aqueous suspensions of *S. feltiae*, *S. carpocapsae*, or water with wetting agent. Each area of the resulting 30 plots was 15.2 m^2. The 7.6 m^2 half of the plots on the east side of the rows was used for spring trials, and the west side of the rows was used for fall trials. Just prior to applications, 15.2 cm^2 perforated cardboard strips containing ≈ 20 cocooned sentinel larvae each were placed on the surface of the ground in 3 locations in each of the 15 mulch plots, marked with stake flags and covered with 2-3 cm of mulch. Sentinel larvae were similarly placed and marked in each of the 15 bare ground plots. Five mulched and bare ground plots were used for each EPN species and equivalent plots served as controls. Irrigation, as previously described, was run for approximately 15 min before nematode treatments. Applications of aqueous suspensions of IJs plus wetting agent or water plus wetting agent were made with the sprayer described above. Each plot was treated with 2.85 L of water (= 3,739.6 L/ha). After application of IJs, sentinel strips in bare ground plots were covered with open mesh plastic screen held in place with brads to reduce damage by birds. Irrigation was run for approximately 1 h following treatments. Temperature and humidity were recorded continuously with a Hobo data logger from just before IJ applications until sentinels were collected. For the fall 2004 applications, temperature and humidity were monitored for an additional 5 d after sentinels were collected.

In the spring trial, field plots were treated with aqueous suspensions of *S. feltiae* or *S. carpocapsae* IJs plus 0.05% Silwet L77 at a rate of 10^9 IJs/ha. Control plots were treated with water plus Silwet. Sentinel strips were placed in the plots as described above just prior to treatment and collected 48 h after applications and incubated for 6 d before assessment of larval mortality as described above.

In the fall 2004 trial, aqueous suspensions of *S. feltiae* or *S. carpocapsae* IJs were applied with an organically approved wetting agent, 0.05% Natural Wet® (J.H. Biotech, Inc., Ventura, CA) at a rate of 2.5 × 10^9 IJs/ha . Control plots were treated with 2.85 L of water with 0.05% Natural Wet. Sentinel strips were placed in the plots as described above just prior to treatment and collected 72 h after applications and incubated for 6 d before assessment of larval mortality.

To assess residual activity of IJs, 3 days following the application of IJs in the fall 2004 field trial, 30 diapausing larvae were released into each of 3 enclosures in each plot. The enclosures were constructed of sections of 30 gauge furnace pipe (25 cm diam., 20 cm high, Standex ADP, Philadelphia, PA) and sunk 3-4 cm into the soil of each plot (treated and controls, mulched and bare) prior to application of nematodes, to minimize disturbance of the treated mulch. Duct tape was used to cover the exposed sharp edges of the enclosures to prevent injury. The enclosures were monitored regularly for several hours after addition of larvae to prevent escape. Five empty 15.2 cm^2 perforated cardboard strips were placed within the enclosures in the bare soil plots to provide locations in which larvae could spin cocoons. Larvae readily entered the strips in the bare plots or mulch soon after introduction into the enclosures. No additional irrigation was provided. The cardboard strips in the bare plots and all of the mulch within the arenas of the mulched plots were retrieved 5 d after releasing larvae. The arenas in the bare plots were searched for larvae that might have pupated outside of the strips. All samples were individually stored in large Ziploc® plastic bags (S. C. Johnson & Son, Inc., Racine, WI) at 12°C until they were processed and larval mortality was assessed. Cardboard strips were similarly stored in small Ziploc bags until larval mortality was assessed.

Analysis. All analyses were performed using SPSS 12.0.1 for Windows. Treatment effects were compared using univariate ANOVA or independent samples T-tests. Significant F-ratio means in ANOVA were further separated with Fisher's LSD for multiple comparisons, at $P < 0.05$. All proportional data were normalized via arcsine prior to analysis. In these studies, each separate mulch plot acted as a replicate for a sample size of five.

Results

Effect of mulch type on EPN activity. *Steinernema carpocapsae* and *S. feltiae* IJs in all types of mulched plots and bare ground plots in the September 2003 trials were highly significant factors for larval mortality ($F_{2,120} = 222.8$, $P < 0.0001$). Compared with untreated plots, in which larval mortality did not exceed 25%, infection of larvae in EPN-treated plots resulted in 60.8-100% mortality in sentinel larvae in situ at the time of application (Fig. 1A). There also were significant differences between the EPN-treated plots. EPN species ($F_{1,80} = 10.26$, $P < 0.005$) and location of sentinel larvae ($F_{1,80} = 6.53$, $P = 0.013$) were significant factors in two-way ANOVA. Overall, *S. feltiae* was more effective than *S. carpocapsae*, and sentinel larvae in the ground were infected at a higher rate compared with those on ground (Fig. 1A). There were no statistical interaction terms between these independent variables.

Because of the variability and relatively high level of sentinel mortality observed in EPN-treated bare ground plots, mulch substrate was not a significant factor for larval mortality in EPN-treated plots in two-way ANOVA ($F_{4,80} = 1.93$, $P = 0.114$) and also when compared separately for each EPN species and sentinel location ($P > 0.05$ following one-way ANOVA). Nevertheless, when mulch treatments were compared side-by-side, there were some interesting trends. Higher larvicidal activity of both

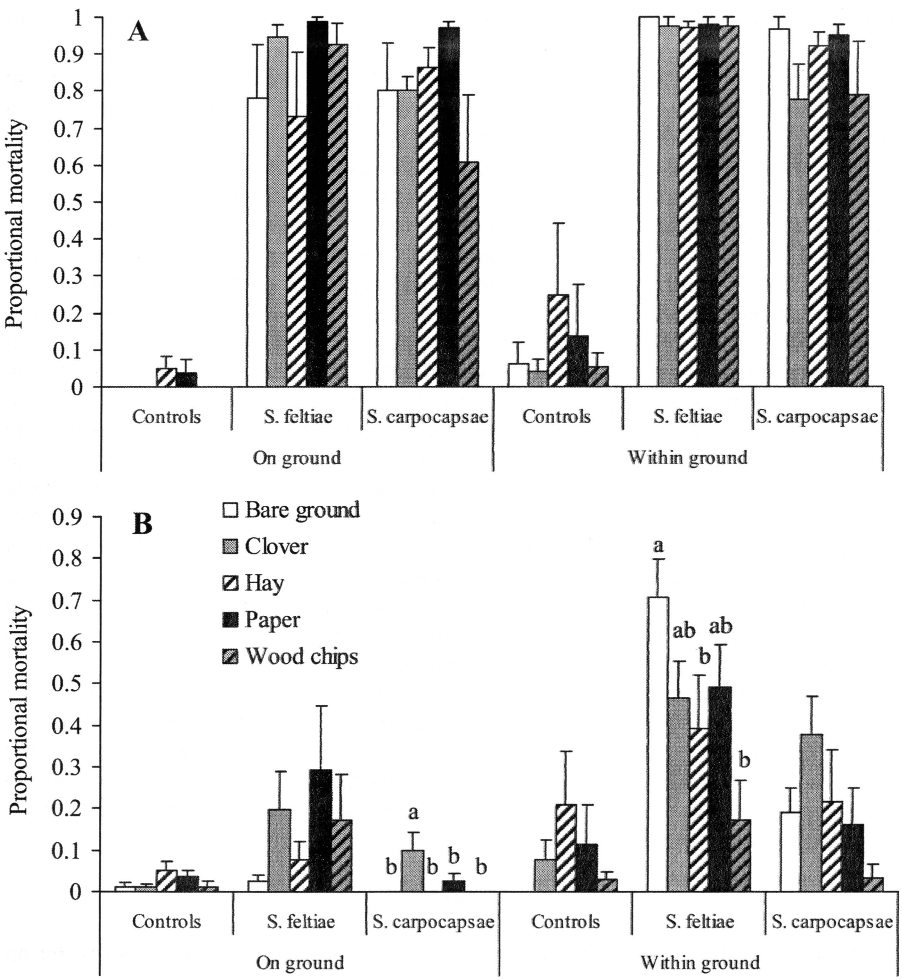

Fig. 1. Evaluation of *Steinernema carpocapsae* and *S. feltiae* (2.5 billion IJs/ha) against sentinel codling moth larvae in plots with four types of mulch. September 2003. Data show average mortality ± SEM for sentinels (A) placed in plots immediately before spraying and (B) placed in plots 8 days after spraying. The effect of mulch type was analyzed independently for each nematode species and sentinel location; letters indicate statistical differences between mulches following significant 1-way ANOVA; Fishers LSD at $P < 0.05$. The use of mulches in combinations without letters was not statistically significant. 'On ground' and 'within ground' refers to the location of cardboard strips containing codling moth sentinel larvae.

EPN species was observed in paper- mulched plots relative to nonmulched plots (Fig. 1A). Larvicidal activity of *S. feltiae* was also higher in clover and wood chip plots, whereas *S. carpocapsae* activity in wood chip plots was lower than that of nonmulched plots. Larvicidal activity for *S. feltiae* against sentinel larvae that were placed in crevices in the soil was identical in all mulched plots and the nonmulched plot (97-100% mortality), but reduced for *S. carpocapsae* in clover and wood chip plots relative to paper, grass hay and nonmulched plots (Fig. 1A). Infection by EPNs and larval mortality (25 and 14% in grass hay and paper plots, respectively) in sentinels in grooves in untreated control plots revealed the presence of native entomopathogenic nematodes (*Heterorhabditis* sp.).

Significant larval mortality also was observed in sentinel strips that were placed in treated plots 8 days post application, suggesting residual activity of IJs (Fig. 1B). In this case, in addition to nematode species ($F_{1,80}$ = 24.7, P < 0.001) and location of sentinel strips ($F_{1,80}$ = 34.7, P < 0.001), univariate ANOVA revealed that mulch substrate was also a significant factor for larval mortality in nematode-treated plots ($F_{4,80}$ = 2.9, P = 0.028). Mulch treatments also were compared independently for each nematode species and sentinel location with one-way ANOVA. These tests revealed significantly higher larval mortality in sentinel strips 'on ground' in the clover mulch treated with *S. carpocapsae.* Mortality in sentinel larvae placed in crevices in the ground was pronounced in the *S. feltiae* treated plots, but also apparent in most of the *S. carpocapsae* treated plots. The notable exception was the *S. carpocapsae* treated wood chip plots. For 'within ground' sentinels, larval mortality in the bare ground plot treated with *S. feltiae* was higher compared with the wood chip mulch, which may have acted as a barrier (Fig. 1B). As in the first set of sentinels, mortality due to native *Heterorhabditis* sp. was detected in 'within ground' sentinel larvae in control plots covered with grass hay and paper. No differentiation between *Steinernema*-infected and *Heterorhabditis*-infected sentinel larvae was made in 'within ground' sentinel larvae in the treated plots due to the possibility of dual infections.

The quality control strips treated with water, 10 IJs/cm^2 of *S. feltiae* or *S. carpocapsae* that were used for the September 2003 field experiment resulted in average mortalities of 0, 90.0 and 81.3%, respectively. A mean temperature of 19.2°C (min 9.0, max 34.4°C) was recorded during the 48 h interval from just before treatment until the first set of sentinel larvae were retrieved. A mean temperature of 19.7°C (min 7.4, max 38.3°C) was recorded during the 48 h interval in which the second set of sentinel larvae were in the plots.

Spring 2004 application of EPNs. Univariate ANOVA revealed that mortality in mulch plots treated with EPNs was significantly higher compared with bare ground plots ($F_{1,16}$ = 41.12, P < 0.0001). As in the September 2003 trials, *S. carpocapsae* produced significantly lower mortality in codling moth larvae in wood chip mulch plots than *S. feltiae*, (Fig. 2), indicated by a species/mulch interaction term ($F_{1,16}$ = 7.77, P = 0.013). Control mortality in the sentinel strips in the bare ground plots was high (mean 19.2% mortality) due to predation by ants. The quality control strips treated with water, 10 IJs/cm^2 of *S. feltiae* or *S. carpocapsae* that were used for the 20 April 2004 field experiment resulted in average mortalities of 1.3, 89.7 and 81.7% respectively. A mean temperature of 12.6°C (min 4.3, max 22.9°C) was recorded during the interval from just before treatment until the sentinel larvae were retrieved 48 h later.

Fall 2004 application of EPNs and assessment of short-term persistence. Application of the higher concentrations of IJs in the fall of 2004 resulted in fair control of codling moth larvae by *S. feltiae* in the bare ground plots (Fig. 3). However, as in the spring 2004 trial, mortality in mulch plots treated with EPNs was significantly higher compared with the bare ground plots ($F_{1,16}$ = 40.97, P < 0.0001). Control by *S. carpocapsae*, however, was considerably reduced in the bare ground plots (Fig. 3), indicated by a species/mulch interaction ($F_{1,16}$ = 10.57, P < 0.01). The quality

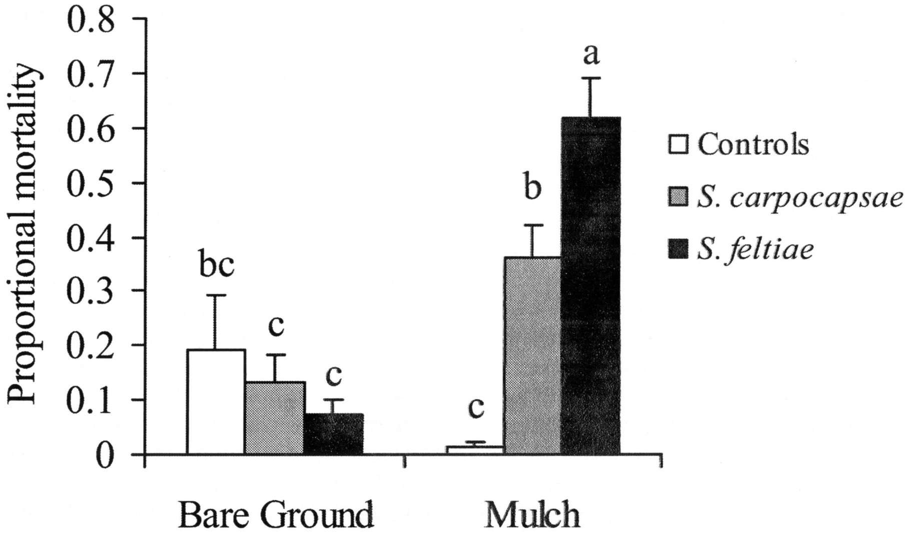

Fig. 2. Evaluation of *Steinernema carpocapsae* and *S. feltiae* (1 billion IJs/ha) on bare ground and in plots with wood chip mulch, Spring 2004. Letters indicate differences between treatments; Fishers LSD at $P < 0.05$.

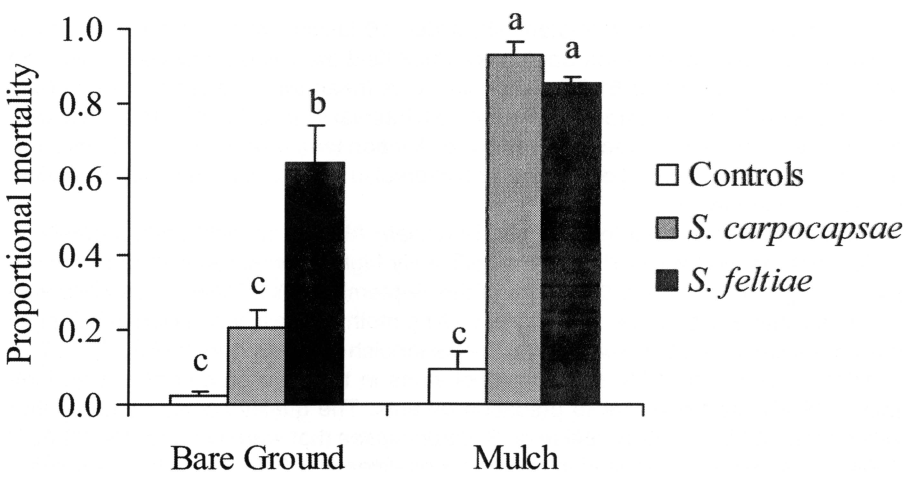

Fig. 3. Evaluation of *Steinernema carpocapsae* and *S. feltiae* (2.5 billion IJs/ha) on bare ground and in plots with wood chip mulch, September 2004. Letters indicate differences between treatments; Fishers LSD at $P < 0.05$.

control strips treated with water, 10 IJs/cm^2 of *S. feltiae* or *S. carpocapsae* that were used for the 21 September 2004 field experiment resulted in average mortalities of 9.9, 87.0 and 65.3%, respectively. A mean temperature of 15.1°C (min 8.3, max 24.3°C) was recorded during the interval from just before treatment until sentinel larvae were retrieved 72 h later. Rainfall between the time of application of IJs and collection of sentinel strips was reported by orchard personnel.

Low mortality (12.1-16.7%) due to both EPN species was observed in larvae that were added to both mulched and nonmulched plots 3 d after application of IJs (Table 1). Compared side by side, mortality in EPN-treated plots was not statistically different between the mulched and nonmulched plots. However, cocooned larvae in the enclosures in the mulched plots tended to be clumped in groups of 4-5 within the wood chips and may have become infected due to recycling had the assessments continued (see discussion). A mean temperature of 18.2°C (min 11.0, max 26.7°C) was recorded during the 120 h from the time the sentinel larvae were released into the enclosures until they were retrieved.

Discussion

Webster (1973) proposed manipulation of habitats where EPNs will be applied to favor IJ survival and infectivity. Environmental manipulation of the orchard agroecosystem by combining irrigation and mulches has the potential to extend the survival of IJs by maintaining the moisture necessary for their activity. Mulches such as wood chips also can provide an attractive habitat for overwintering larvae, especially in orchards where smooth-barked trees provide few alternative sites for hibernacula. Younger high density trellised orchards such as the East Wenatchee Auvil orchard will be ideal sites for using mulches and EPNs for control of overwintering codling moth. The combination of providing easily-treated sites that are attractive for codling moth hibernacula and irrigation to maintain adequate moisture will facilitate IJ contact with and infection of overwintering codling moth larvae.

The results of each of the field tests are markedly different from one another for a number of reasons. There were differences among the 3 test dates in terms of source of EPNs, dosage, temperature and moisture and presence of predators (ants, Spring 2004). The highest mortalities in sentinel larvae were obtained at the higher application rates, when more moisture was present and optimal temperatures prevailed

Table 1. Mortality of cocooned sentinel codling moth larvae added to enclosures in mulched and non-mulched plots. Plots were treated with *Steinernema carpocapsae* and *Steinernema feltiae* (2.5×10^9 IJ/ha) 3 days earlier. A mean temperature of 18.2°C (min 11.0, max 26.7°C) was recorded during the 120 h from the time the sentinel larvae were released into the enclosures until they were retrieved

Nematode treatment	% mortality ± SEM Non-mulched	Mulched
Control	1.3 ± 1.1 aA	2.4 ± 1.2 aA
Steinernema carpocapsae	16.7 ± 4.8 bA	14.4 ± 4.6 aA
Steinernema feltiae	16.4 ± 4.8 bA	12.1 ± 5.1 aA

Different lower case letters in the same column indicate differences between nematode treatments; Fishers LSD at $P < 0.05$. Different upper case letters in the same row indicate differences between mulched and non-mulched plots; independent samples T-test at $P < 0.05$.

(September 2003, 2004). We reduced the exposure of sentinels within the mulch and bare plots to 48 h in the September 2003 and April 2004 trials to minimize control mortality. Under operational conditions, the exposure of cocooned codling moth larvae to viable IJs in mulch would not only be longer, the potential for recycling of IJs produced in infected larvae and subsequent infection of adjacent larvae could enhance the level of control. The higher mortality produced by *S. carpocapsae* in the September 2004 trial compared with that in the wood chip mulch plots treated with the same species and rate in the 2003 trial may have been due in part to collection of sentinels 72 h after applications and rainfall on the evening following application.

The difference in mortality observed between *S. carpocapsae* and *S. feltiae* treated with the 10^9 IJs/ha rate could be related to the search strategies and cold tolerances of the two species. *Steinernema carpocapsae* is regarded as an ambusher species with a limited searching strategy, whereas *S. feltiae* has a more active searching behavior (Grewal et al. 1994, Campbell and Gaugler 1997). The 2-3 cm of wood chips could thus provide a greater barrier to *S. carpocapsae* compared with *S. feltiae*. In addition, the infectivity of *S. carpocapsae* for codling moth larvae (and a variety of other insects) decreases considerably at temperatures below 15°C and ceases altogether at 10°C (Lacey and Unruh 1998, Vega et al. 2000). *Steinernema feltiae* on the other hand, is active at 10°C and lower temperatures (Grewal et al. 1996). The most favorable temperature conditions for *S. carpocapsae* prevailed during the fall 2003 trial. The temperatures in the spring application of 2004 would undoubtedly favor greater efficacy in *S. feltiae* than *S. carpocapsae*. This was the case in the mulched plots, but neither species performed well in the bare plots. Interestingly, the application of EPNs in these bare plots may have had a repellent effect on ant predation similar to that reported by Zhou et al. (2002) as evidenced by the higher mortality in untreated controls due to ants. The warmer temperatures in the fall 2004 application and higher application rate apparently enabled good control of sentinel larvae by both species in the mulched plots. The rainfall following applications in fall 2004 could have facilitated deeper penetration of *S. carpocapsae* into the wood chip mulch than was observed for the same dosage in the fall 2003 trial. The higher mortality in bare plots treated with *S. feltiae* in fall 2003 could reflect its searching behavior relative to that of *S. carpocasae*. *Steinernema feltiae* is classified as intermediate between ambusher and cruiser in its search strategy (Grewal et al. 1994, Lewis et al. 1995, Campbell and Gaugler 1997). Use of a cold hardy EPN with more active search behavior, such as exhibited by some of the *Heterorhabditis* species warrants further attention for control of cocooned codling moth larvae in mulch.

Evidence of persistence of EPN activity in September 2003 *vis-à-vis* mortality in the second set of sentinel larvae 8 d after IJ application indicates the potential for longer term control following a single treatment. Although the level of control of codling moth larvae that were released into enclosures that had been treated with EPNs 3 days earlier in September 2004 was not as high as hoped, the larvae that were infected could serve as highly virulent sources of IJs for continued persistence and recycling of the EPNs in the orchard, especially in cases of high density pest infestations. As mentioned above, a longer exposure of sentinels than 48 h would probably result in higher mortality. Duthie et al. (2003) showed that codling moth larvae cocoon in aggregations. Recycling from one infected larva in a cluster of cocooned larvae to adjacent hosts could be facilitated by proximity of other larvae, moisture retention in the mulch, and improved virulence of IJs emerging directly from infected cadavers compared with commercial preparations prepared in vitro. Shapiro and Glazer (1996) observed that the dispersal ability of *Heterorhabditis bacteriophora* Poinar and *S. carpocapsae* was significantly greater when nematodes were applied in *G. mellonella* cadavers relative to when they were applied in aqueous suspension. Shapiro and Lewis (1999) demonstrated that infectivity of *H. bacteriophora* was significantly greater when IJs emerged directly from *infected G. mellonella* larvae into sand compared with application of aqueous suspensions. Perez et al. (2003) showed that emergence of IJs of 3 EPN species from a host cadaver directly into a simulated habitat increased their survival and infectivity. The role of mulches in the long-term persistence of EPNs in the orchard agroecosystem warrants further attention, especially in terms of the potential for extended codling moth control. For example, if applications of EPNs are made when all diapause-destined larvae have not yet exited the fruit, irrigating treated mulches may extend the larvicidal activity of IJs. The benefits of supplementary irrigation on residual persistence and infectivity of IJs in mulches should, therefore, be investigated.

Control of the overwintering stage of codling moth will decrease the oviposition pressure the following spring. Moreover, EPNs used with mating disruption (Vickers and Rothschild 1991) and the codling moth virus against neonate larvae (Arthurs and Lacey 2004, Arthurs et al. 2005, Lacey et al. 2004) combine different tactics that together may eliminate the need for intervention with chemical pesticides. Because of their specificity for insects, entomopathogens are ideal candidates for incorporation into IPM where their effects on natural enemies will be minimal as compared with most presently used chemical pesticides. In addition to facilitating EPN contact with overwintering larvae, mulching can also enhance the abundance and activity of ground-dwelling codling moth predators in the orchard (Brown and Tworkiski 2004, Mathews et al. 2004). Kienzle and Zebitz (1997) reported an increase in a wide range of predators, parasitoids and other beneficial arthropods and a decrease in the fruit-peel tortricid, *Adoxophyes orana* (Fischer von Röslerstamm.), and other pests in extensively mulched orchards.

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