Factors Affecting Entomopathogenic Nematodes (Steinernematidae) for Control of Overwintering Codling Moth (Lepidoptera: Tortricidae) in Fruit Bins

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ABSTRACT Fruit bins infested with diapausing codling moth larvae, *Cydia pomonella* (L.), are a potential source of reinfestation of orchards and may jeopardize the success of mating disruption programs and other control strategies. Entomopathogenic nematodes (EPNs) were tested as a potential means of control that could be applied at the time bins are submerged in dump tanks. Diapausing cocooned codling moth larvae in miniature fruit bins were highly susceptible to infective juveniles (IJs) of *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) in a series of experiments. Cocooned larvae are significantly more susceptible to infection than are pupae. Experimental treatment of bins in suspensions of laboratory produced *S. feltiae* ranging from 10 to 100 IJs/ml of water with wetting agent (Silwet L77) resulted in 51–92% mortality. The use of adjuvants to increase penetration of hibernacula and retard desiccation of *S. feltiae* in fruit bins resulted in improved efficacy. The combination of a wetting agent (Silwet L77) and humectant (Stockosorb) with 10 *S. feltiae* IJs/ml in low and high humidity resulted in 92–95% mortality of cocooned codling moth larvae versus 46–57% mortality at the same IJ concentration without adjuvants. Immersion of infested bins in suspensions of commercially produced nematodes ranging from 10 to 50 IJs/ml water with wetting agent in an experimental packing line resulted in mortality in cocooned codling moth larvae of 45–87 and 56–85% for *S. feltiae* and *S. carpocapsae*, respectively. Our results indicate that EPNs provide an alternative nonchemical means of control that could be applied at the time bins are submerged in dump tanks at the packing house for flotation of fruit.

KEY WORDS entomopathogenic nematodes, *Cydia pomonella*, *Steinernema*, formulation, fruit bins

CODLING MOTH, *Cydia pomonella* (L.), is a global pest of pome fruit and the most serious pest of apple in the Pacific Northwest (Beers et al. 1993). Control of this and other insect pests in conventional orchards in the region has been predominantly through the use of broad-spectrum chemical insecticides, such as azinphos methyl (Beers et al. 1993). An alternative/supplement to conventional chemical insecticides for codling moth control is the use of female sex pheromone for disruption of mating. This method is most effective when moth densities are low (Vickers and Rothschild 1991, Gut and Brunner 1998, Calkins and Faust 2003). In certain situations continuous control using mating disruption has been interrupted due to invasion of orchards by moths that have emerged from fruit bins that were infested the previous year and placed in orchards before harvest, often as early as midsummer (Higbee et al. 2001). Proverbs and Newton (1975) also report the reinfestation of orchards via contaminated bins where codling moth was under control by using sterile insect release. Control of diapausing cocooned codling moth larvae in infested fruit bins would contribute to the successful use of the mating disruption method and other control methods and enable a reduction in insecticide use.

Various methods have been demonstrated for eliminating cocooned codling moth larvae from fruit bins. One of the most effective methods is fumigation with methyl bromide (Moffitt 1971, Tebbets et al. 1986, Dentener et al. 1998), but a variety of disadvantages, including phytotoxicity to fruit and environmental and human safety issues, have necessitated development of alternatives to this agent. Waiting to place bins in the orchard until just before harvest and the use of plastic bins also can reduce the level of infestation (Higbee et al. 2001). Other methods include fumigation with carbon dioxide (Cossentine et al. 2004), heat treatment (Higbee et al. 2001), and the use of entomopathogenic nematodes (EPNs; Steinernematidae and Heterorhabditidae) (Lacey and Chauvin 1999, Cossentine et al. 2002), but none of these interventions are routinely used.

The efficacy of several EPN species has been demonstrated against a variety of insect pests (Kaya and Gaugler 1993, Gaugler 2002), including codling moth in orchards (Kaya et al. 1984, Unruh and Lacey 2001)

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and fruit bins. Research conducted on EPNs for codling moth in fruit bins by Lacey and Chauvin (1999) and Cossentine et al. (2002) addressed treatment of bins with suspensions of *Steinernema carpocapsae* (Weiser) infective juveniles (IJs) by submersion or drenching, respectively. This article presents new information on the use of EPNs for control of cocooned codling moth larvae in fruit bins and expands upon earlier studies conducted at our laboratory. It includes evaluation of *Steinernema feltiae* (Filipjev) IJ concentration on level of control, effects of adjuvants on efficacy, examination of host stage effects, and evaluation of *S. carpocapsae* and *S. feltiae* in an experimental packing line.

Materials and Methods

Test Insects. Codling moth larvae used in these studies were obtained from the colony maintained at the Yakima Agricultural Research Laboratory (YARL) on soya-wheat germ-starch artificial diet (Toba and Howell 1991) and reared under diapausing conditions (photoperiod of 10:14 [L:D] h, 20°C, and 40–50% RH).

Test Nematodes. The *S. feltiae* (Umea strain) and *S. carpocapsae* (Sal strain) IJs used in our studies at YARL were produced in wax moth, *Galleria mellonella* (L.), according to procedures described by Kaya and Stock (1997) and used within 2 wk of production. *S. carpocapsae* (All strain) and *S. feltiae* (UK76 strain) used in packing line tests 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 codling moth larvae in 15.2-cm^2 perforated cardboard strips (double faced, B flute, Weyerhauser, Tacoma, WA) by using 152 IJs in 1 ml of water (10 IJs/cm^2, four strips per treatment and control) and methods prescribed by Lacey and Unruh (1998). The treated strips were placed in filter paper lined petri dishes, incubated for 6 d at 25 ± 1.7°C, and then assessed for mortality.

Effect of Codling Moth Stage on Susceptibility. *S. carpocapsae* IJs were used to treat cocooned larvae or pupae in 15.2-cm^2 perforated cardboard strips using the procedures described by Lacey and Unruh (1998). Five strips for each stage were used for nematode treatments and controls. The strips were treated with 1 ml of water or 1 ml of water containing 152 IJs (10 IJs/cm^2) placed in filter paper lined petri dishes, incubated for 6 d at 25 ± 1.7°C, and then assessed for mortality. The experiment was replicated on four separate dates.

Codling Moth Infestation and Nematode Applications in Miniature Fruit Bins. One-eighth scale wooden fruit bins described by Lacey and Chauvin (1999) were used for all experiments, except the one to determine the effect of codling moth stage on susceptibility. The same materials used to construct commercial fruit bins (1.6-cm-thick CDX plywood and fir corner supports) were used to construct the miniature fruit bins. The bins were modified to include one corner support per bin that had been grooved (13 horizontal grooves per side, 2 to 3 mm in width, 3–5 mm in depth) on the sides facing the bin wall to facilitate infestation with diapausing larvae. Four round head screws were embedded into the surface of the corner supports that faced the sides of the bins to provide a 2- to 3-mm gap between the corner support and side of the bin. Diapausing larvae were placed in plastic bags on corner supports that had been removed from the bins and allowed to spin cocoons several days before testing. At least 20 successfully cocooned larvae were used for each support. After formation of cocoons the corner supports were stored in a 12 ± 0.5°C incubator until they were used for testing. On the day of the test, they were inserted into the corners of the fruit bins with wood screws and an electric drill.

In the majority of the tests, except those conducted in an experimental packing line, the miniature bins were treated by immersing them in a deep straight-sided wheel barrow (Rubbermaid farm cart, Fairlawn, OH) filled with 170.3 liters of water to within 2.8 cm of the top. In all tests using the wheel barrow, immersion time in the tank was 1 min. Water used in the experiments was nonchlorinated well water (20.6 ± 0.5°C, pH 7.5). In all experiments except those comparing the effect of adjuvants, 0.063% Silwet L77 (Silicone-polyether copolymer, Loveland Industries, Inc., Greeley, CO) was added to the water as a wetting agent. Between tests, bins were stored outdoors, as is the practice with commercial bins, to allow natural weathering.

Effect of *S. feltiae* IJ Concentration. Fruit bins with codling moth-infested corner supports were treated with four concentrations (10, 25, 50, or 100 IJs/ml) of *S. feltiae* IJs plus Silwet by using the methods described above. Controls were treated with water and Silwet (0.063%). Five bins were used for each concentration and control on each of three separate dates. After treating, the bins were stacked in the greenhouse at 20–25°C for 24 h after which time the corner supports were removed and stored in a walk-in incubator at 25 ± 1.7°C. Mortality of larvae in the supports was determined after 6 d of incubation.

Effect of Substrate Containing Cocooned Larvae. An experiment to test the difference between methods used by Lacey and Chauvin (1999) and those used in the current study was conducted. The grooved corner supports, used in the current study, and the perforated cardboard strips (41.0 cm in length by 1.9 cm in width, double faced, C flute) used by Lacey and Chauvin (1999) were infested with diapausing codling moth larvae (20 or more larvae per strip or corner support) and placed in each of 10 bins. The infested cardboard strips were placed in spaces in one corner of each of the 10 bins. The space was made by placing a strip of Plexiglas (24.1 cm in length by 2.5 cm in width by 6.4 mm in thickness) between one of the corner supports and adjacent walls 2 cm from the corner. The Plexiglas was held in place with two 6.4-cm wood screws drilled through the outer wall, Plexiglas, and corner support. Infested grooved corner supports were installed in each of the bins as described above. Five of the bins were immersed in suspensions of *S. feltiae* (10 IJs/ml + Silwet), and five bins were immersed in water and Silwet only. The treated bins were stacked in a heated greenhouse (20–25°C). After 24 h in the greenhouse, the infested strips and corner supports were removed and incubated at 25 ± 1.7°C for 6 d after which time mortality was assessed. The experiment was repeated on three separate dates.

Effect of Adjuvants on Activity of *S. feltiae*. The effect of IJ suspensions in water alone, water with wetting agent (Silwet L77 at 0.063%), water with a humectant (Stockosorb at 0.2%, also known as Sta-Moist, cross-linked potassium polyacrylate/polyacrylamide copolymer, Stockhausen, Inc., Greensboro, NC), and water with humectant and wetting agent was determined using *S. feltiae* at 10 IJs/ml and the submersion methods described above. Two sets of controls consisted of treatment of infested bins by immersion in water only and water with wetting agent and humectant for 1 min and then storing them for 24 h in greenhouse rooms that were heated and humidified (mean, 23.4°C; 77.2% RH, range 58.4–100%) or heated and not humidified (mean, 23.7°C; 45.6% RH, range 36.2–72.5%). In this experiment, treated bins were not stacked. Five bins were used for each treatment and control for each humidity regimen on each of three separate dates. After 24 h in the greenhouse, the corner supports were removed from the bins and incubated at 25 ± 1.7°C for 6 d after which time mortality was assessed.

Entomopathogenic Nematode Treatments Using an Experimental Packing Line. One corner support in each of 70 miniature bins was infested at YARL with diapausing codling moth larvae as described above and transported to an experimental packing line at the USDA–ARS Physiology and Pathology of Tree Fruit Laboratory, in Wenatchee, WA. Ten bins were used for each of three concentrations (10, 25, and 50 IJs/ml) of either *S. feltiae* or *S. carpocapsae* and controls. The drop tank of the packing line was filled with 2,839 liters of water to which Silwet (0.05%) was added. The miniature bins were treated two at a time by placing them in a regular-sized plastic commercial bin, and loading them into the dunking apparatus of the drop tank with a forklift. The bins were immersed for 1 min., removed from the drop tank, stacked indoors, and partially covered with a tarp.

Water, Silwet, and nematodes were added after treatment of every four to six bins to compensate for loss due to absorption by the bins and spillage during the treatment process. Counts of the nematode concentration were performed on drop tank water samples with a dissecting scope (40×) before treatments began with a particular species and concentration and again after addition of water and nematodes to ensure appropriate concentration. Agitation of the water due to immersion and removal of bins and use of the circulation pump in the packing line ensured even distribution of IJs in the drop tank water. Increase in IJ concentration for the same EPN species were made by adding IJs to the previous concentration. Between treatments with *S. feltiae* and *S. carpocapsae* the drop tank was emptied, flushed with water, and refilled. Water temperature in the drop tank was 14°C during the treatments. Temperatures in the treated bins monitored with a Hobo H8 Pro Series data logger (Onset Computer Corp., Pocasset, MA) ranged from 14.1 to 22.1°C during the 24 h the bins were stored in the packing house. Infested corner supports were removed from the bins 24 h after treatments were made, returned to YARL, and incubated for 6 d at 25 ± 1.7°C after which time mortality was determined.

Statistical Analyses. Comparisons of substrate effect on the activity of *S. feltiae* and effect of codling moth stage on susceptibility were done using Students *t*-test. Mortality data were transformed using the arcsine of the square-root transformation before analysis of variance (ANOVA). ANOVA was performed using SAS (version 8.02) (SAS Institute 2004) for tests on the effect of IJ concentration and packing line treatments. The effect of EPN species in the packing line tests was analyzed by combining the two species and controls in the same ANOVA. The effects of IJ concentration in the packing line tests were determined by analyzing each EPN species separately. Means were separated using Duncan's new multiple range test (MRT). Data on the effects of adjuvants on larvicidal activity of *S. feltiae* were analyzed using a factorial ANOVA (SAS Institute 2004), and means were separated using Duncan's new MRT.

Results

Effect of Codling Moth Stage on Susceptibility. Cocooned larvae were significantly more susceptible to infection by *S. carpocapsae* than pupae in cardboard strips treated with 10 IJs/cm^2 (df = 6, $t = 6.03$, $P < 0.001$). Mortality of larvae was 87.6 ± 3.7% compared with 63.1 ± 1.7% for pupae. Control mortality was 2.4 ± 1.7 and 2.7 ± 1.6% for larvae and pupae, respectively.

Effect of *S. feltiae* IJ Concentration. There is a direct and positive relationship between the concentration of *S. feltiae* IJs and mortality of codling moth larvae in fruit bins that have been treated by submersion into suspensions ranging from 10 to 100 IJs/ml ($F_{4,\ 10} = 71.1$; $P < 0.0001$) (Fig. 1). The mortality in the controls and 10 and 25 IJ concentrations were significantly different from one another and from the two highest concentrations. The two highest concentrations were not significantly different from one another. The quality control strips that were treated with water or 10 IJs/cm^2 resulted in average mortalities of 1.7 and 76.3%, respectively, over three test dates.

Effect of Type of Substrate Containing Cocooned Larvae. The susceptibility of cocooned codling moth larvae to infection by *S. feltiae* at a concentration of 10 IJs/ml in cardboard strips (85.5 ± 5.7% mortality) was not significantly different from that of larvae in infested corner supports (80.4 ± 5.3% mortality) (df = 4, $t = -0.65$, $P = 0.28$). Control mortality of larvae in cardboard strips and corner supports treated with water and wetting agent was 1.6 ± 0.8 and 1.4 ± 0.03, respectively. The quality control strips that were

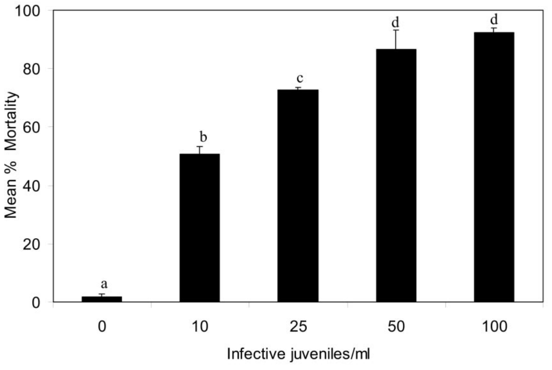

Fig. 1. Effect of *S. feltiae* infective juvenile concentration on mortality of cocooned codling moth larvae in fruit bins. Columns with the same letter are not significantly different at the 0.05 level.

treated with water or 10 IJs/cm^2 resulted in 5.8 and 86.8% mortality, respectively.

Effect of Adjuvants on Activity of *S. feltiae*. The effect of using a wetting agent and/or a humectant with *S. feltiae* IJ suspensions compared with IJs in water alone is presented in Fig. 2. The high humidity regimen produced the most clearcut differences between the various treatments ($F_{5,\ 12}$ = 157.44; $P <$ 0.0001). All of the treatments were significantly different from one another and the controls. The combination of *S. feltiae* IJs with Silwet and Stockosorb resulted in the highest level of mortality (95.36 ± 0.5%). The two sets of controls in the high humidity regimen were not significantly different from one another. The results of the low humidity tests were more variable and less conclusive. All treatments were significantly different from the two controls, but not from one another ($F_{5,\ 12}$ = 10.38; $P <$ 0.0005). The quality control strips that were treated with water or water with *S. feltiae* (10 IJs/cm^2) resulted in 2.2 and 87.5% mortality, respectively.

Entomopathogenic Nematode Treatments Using an Experimental Packing Line. Both commercial formulations of *S. carpocapsae* and *S. feltiae* produced significant mortality in codling moth larvae that were cocooned within the corner supports of bins (Fig. 3) ($F_{6,\ 63}$ = 7.11; P = 0.0016). The mortality of cocooned codling moth larvae due to treatment with *S. feltiae* IJs was, as expected, progressively higher at higher nematode concentrations ($F_{2,\ 27}$ = 8.83; P = 0.0011). An unexpectedly high mortality response was recorded for the low concentration (10 IJs/ml) *S. carpocapsae* treatment ($F_{2,\ 27}$ = 4.21; P = 0.0256). Mortality produced at this concentration for *S. carpocapsae* was significantly higher than that of *S. feltiae* at the same concentration ($F_{1,\ 18}$ = 16.79; P = 0.0007). Mortality for the two higher concentrations were not significantly different between the two species. The quality control strips that were treated with water or 10 IJs/cm^2 of *S. carpocapsae* or *S. feltiae* resulted in 3.3, 93.3, and 95.6% mortality, respectively.

Discussion

The results of our studies on *S. feltiae* and *S. carpocapsae* against cocooned codling moth larvae in wooden fruit bins indicate the potential of EPNs as an effective alternative means for control of this pest. The drop tank used to float apples from fruit bins in packing houses provides an excellent means of treatment. Our findings also corroborate earlier reports by Lacey and Chauvin (1999) and Cossentine et al. (2002) where different methodology (drencher application) was used. The results of tests with *S. feltiae* indicate a comparable level of activity to that reported previously for *S. carpocapsae.* The lower mortality for codling moth larvae in the 10 IJ/ml concentration in the *S. feltiae* concentration test compared with other tests reported in this article may have been due to the relative age of the bins. The bins were newer at the time the tests were conducted. Newer bins tend to be

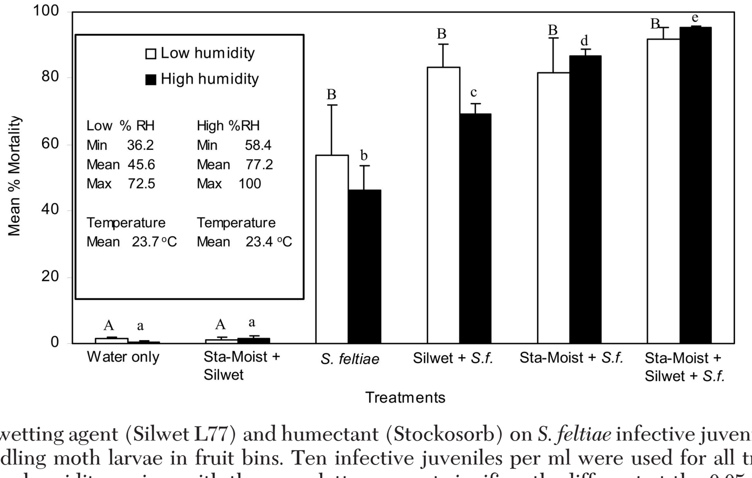

Fig. 2. Effect of wetting agent (Silwet L77) and humectant (Stockosorb) on *S. feltiae* infective juvenile larvicidal activity against cocooned codling moth larvae in fruit bins. Ten infective juveniles per ml were used for all treatments. Columns representing the same humidity regime with the same letter are not significantly different at the 0.05 level.

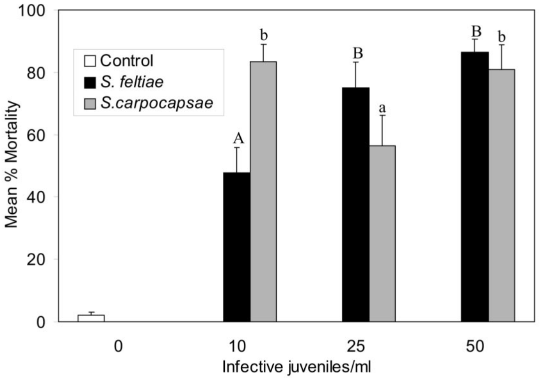

Fig. 3. Mortality in cocooned codling moth larvae treated by immersion in suspensions of three concentrations of *S. feltiae* and *S. carpocapsae* infective juveniles in a packing line drop tank. Columns representing the same nematode species with the same letter are not significantly different at the 0.05 level.

somewhat water repellent, whereas older weathered bins absorb and retain more liquid.

An advantage offered by *S. feltiae* is that it is active at lower temperatures (Grewal et al. 1996) and has exhibited greater search capacity than the ambusher strategist, *S. carpocapsae* (Grewal et al. 1994a, Campbell and Gaugler 1997). At 10°C, *S. carpocapsae* is virtually inactive against codling moth larvae (Lacey and Unruh 1998, Vega et al. 2000) and other species (Grewal et al. 1994b), whereas *S. feltiae* is infective at 8°C and lower (L.A.L., unpublished data; Grewal et al. 1996). *S. feltiae* is regarded as an intermediate host search strategist, exhibiting both ambusher and cruiser behavior. True cruiser species, such as *Heterorhabditis bacteriophora* Poinar and others warrant attention as potential candidates for codling moth control. Cold hardiness and low temperature activity also have been reported for *H. bacteriophora* and other *Heterorhabditis* species (Griffin and Downes 1991; Grewal et al. 1994b). It should be noted that differences between two EPN species from separate sources that are evaluated under optimal conditions may or may not be attributed directly to virulence. They also could be due to differences in formulation or production technology, time elapsed between production and use, and conditions during transportation.

Based on results of studies reported here and that of other research conducted in the Pacific Northwest (Lacey and Unruh 1998, Lacey and Chauvin 1999, Unruh and Lacey 2001, Cossentine et al. 2002), we conclude that it will be necessary to store bins at temperatures above 10–15°C (depending on EPN species used) in an environment that will help to maintain moisture in the bins for a minimum of 8 h after treating. It also will be important to treat bins before larvae pupate. It should be possible to provide the conditions that will enable effective IJ activity if treated bins are temporarily stacked indoors in available warehouse space. If stacked outdoors when temperatures are above 15°C, supplemental watering should be provided with sprinklers to prevent drying of the bins. Feasibility studies on these and other approaches are warranted and should be conducted under operational conditions at various times of the year.

The addition of adjuvants that would improve penetration of spaces within bins and codling moth hibernacula and retard desiccation also could play a role in improving EPN efficacy. The wood in new fruit bins can be water repellent as can the silk of codling moth cocoons. A wetting agent clearly improved the penetration of cocoon sites in our studies as evidenced by significantly higher mortality in treatments with wetting agent in contrast to nematode suspensions of *S. feltiae* IJs without wetting agent. Different surfactants may produce considerably different effects on EPN efficacy. Lacey and Chauvin (1999) reported no effect of the wetting agent Tween 80 on improvement of the larvicidal activity of *S. carpocapsae* for control of codling moth larvae in fruit bins. However, the differences in the concentrations used, 0.01% for Tween 80 by Lacey and Chauvin (1999) and 0.063% for Silwet L77 in the current study, do not permit an accurate comparison of the two. Schroeder and Sieburth (1997) reported enhanced efficacy for *S. riobrave* for control of Diaprepes root weevil, *Diaprepes abbreviatus* (L.), larvae in potted citrus plants when combined with various surfactants, including Silwet. EPN suspensions with humectants, such as Stockosorb, have the potential to retain water for longer periods, enabling penetration of codling moth larvae before the IJs desiccate.

A substantial amount of research has been devoted to the formulation of EPNs with the majority of efforts focused on IJ stabilization and improving shelf life (Georgis and Kaya 1998, Strauch et al. 2000). IJ persistence on foliage has been improved with certain adjuvants (Bauer et al. 1997, Mason et al. 1998, Piggott et al. 2000). Improvement of EPNs in foliar/cryptic habitats such as leaf mines has been reported with the addition of adjuvants such as glycerin (Broadbent and Olthof 1995) and certain polymers (Piggott et al. 2000). Piggott et al. (2000) reported a reduced desiccation rate for *S. feltiae* on the surface of leaves treated with a formulation containing a polymer humectant similar to Stockosorb. A survival time of 16 h was observed for *S. feltiae* in the field when using the polymer formulation against leafminers (*Liriomyza* spp.) on tomatoes. One of the caveats of using Stockosorb is the slippery hazard created on concrete that is splashed with drop tank water containing this polymer. Also use of the circulation pump in the drop tank with Silwet can create substantial foaming. Use of another wetting agent that is acceptable to organic producers also warrants further attention.

Additional research on formulation components that enhance IJ penetration of cryptic habitats and retard desiccation is warranted. Also, the effect of various chemicals that come into contact with fruit bins in and before the packing line (e.g., chlorine and antiscalding agents) on IJ viability and infectivity should be investigated before widespread implementation of EPNs for control of codling moth in fruit bins.

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