Susceptibility of the Strawberry Crown Moth (Lepidoptera: Sesiidae) to Entomopathogenic Nematodes

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ABSTRACT The objective of this study was to determine the susceptibility of the strawberry crown moth, *Synanthedon bibionipennis* (Boisduval) (Lepidoptera: Sesiidae) larvae to two species of entomopathogenic nematodes. The entomopathogenic nematodes *Steinernema carpocapsae* (Weiser) strain Agriotos and *Heterorhabditis bacteriophora* (Steiner) strain Oswego were evaluated in laboratory soil bioassays and the field. Both nematode species were highly infective in the laboratory bioassays. Last instars were extremely susceptible to nematode infection in the laboratory, even in the protected environment inside the strawberry (*Fragaria* × *ananassa* Duch.) crown. Infectivity in the laboratory was 96 and 94% for *S. carpocapsae* and *H. bacteriophora*, respectively. Field applications in late fall (October) were less effective with *S. carpocapsae* and *H. bacteriophora*, resulting in 51 and 33% infection, respectively. Larval mortality in the field from both nematode treatments was significantly greater than the control, but treatments were substantially less efficacious than in the laboratory. Soil temperature after nematode applications in the field (11°C mean daily temperature) was below minimum establishment temperatures for both nematode species for a majority of the post-application period. It is clear from laboratory data that strawberry crown moth larvae are extremely susceptible to nematode infection. Improved control in the field is likely if nematode applications are made in late summer to early fall when larvae are present in the soil and soil temperatures are more favorable for nematode infection.

KEY WORDS strawberry, microbial control, biological control, entomopathogenic nematodes

Strawberries (*Fragaria* × *ananassa* Duch.) are an important small fruit crop, particularly along the west coast of the United States. Strawberries were grown on 16,000 ha in California, Oregon, and Washington in 2006, with a value in excess of $1 billion (NASS 2007). Currently, the key insect pests associated with strawberries in the Pacific Northwest production region (Oregon and Washington) include strawberry crown moth, *Synanthedon bibionipennis* (Boisduval) (Lepidoptera: Sesiidae), a complex of root weevil species (Coleoptera: Curculionidae), and cyclamen mites *Stenotarsonemus pallidus* (Banks) (Acari: Tarsonemidae), among others.

The strawberry crown moth is a univoltine pest that occurs throughout the western United States and British Columbia, Canada, wherever strawberries are grown. Adults are a clearwing moth with a wing span of nearly 20 mm. The adult is brightly colored, with a black abdomen banded with yellow on the second, fifth, and sixth abdominal segments. Larvae, which are white with a dark brown head capsule, reach 20 mm in length when mature. Larvae of the strawberry crown moth feed primarily on strawberry crowns, resulting in stunting of plants and severe stand thinning under heavy infestations. Heavily infested fields can be completely destroyed in a single growing season. Last instars spend the winter in the crowns of strawberry plants. Overwintering larvae feed for a short time in early spring (April–May) before pupating. Adults emerge in late June and July, mate, and females lay eggs on leaves around the base of strawberry plants or directly on the crowns. Eggs hatch within 2 wk, and neonates initially feed on small roots before tunneling into the crowns (Berry 1978). Current strawberry crown moth management depends on the use of a pheromone (Nielsen et al. 1978) to help time insecticide applications. If the conventional management program is not successful, larvae are very difficult to control with conventional insecticides once they enter the crowns in late summer to early fall. In addition to strawberries, this moth also attacks raspberries, black raspberries, blackberries, and boysenberries throughout the Pacific Northwest (J. Todd, personal communication).

Entomopathogenic nematodes are attractive for use in biological control programs, because numerous species are commercially available and they have been

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used for control of a variety of insect pests (Georgis et al. 2006). Because of their sensitivity to UV light and desiccation, nematodes are most effective against pests in soil or other protected environments (Kaya and Gaugler 1993). Effective control relies on the successful matching of the most effective nematode with the target pest (Georgis and Gaugler 1991).

Before these studies, no data were available on the infectivity of nematodes against the strawberry crown moth. However, other *Synanthedon* spp. have been shown to be highly susceptible to entomopathogenic nematodes in the laboratory and field (Bedding and Miller 1981, Miller and Bedding 1982, Deseö and Miller 1985, Cossentine et al. 1990, Shapiro-Ilan and Cottrell 2006, McKern et al. 2007). Laboratory testing and small-scale field studies are important first steps to identify nematodes with potential for managing pests of unknown susceptibility. This is particularly important for a pest such as the strawberry crown moth that is difficult to manage once established in the strawberry crown. Entomopathogenic nematodes are ideally suited to attack larvae overwintering in such an environment. Nematodes move within *Synanthedon tipuliformis* (Clerk) (Lepidoptera: Sesiidae) tunnels in black currants, *Ribes nigrum* L. (Miller and Bedding 1982). The cryptic overwintering site inside pistachios, *Pistacia vera* L., proved to be an ideal environment to target navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), with entomopathogenic nematodes (Siegel et al. 2006). Although filbertworm, *Cydia latiferreana* (Walsingham) (Lepidoptera: Tortricidae) and the filbert weevil *Curculio occidentalis* (Casey) (Coleoptera: Curculionidae) generally do not overwinter inside the nut, they do emerge from the nut once it falls on the ground and overwinter in hibernacula in the soil under infested trees (Dohanian 1944). These overwintering larvae were also ideally situated for targeting by entomopathogenic nematodes (Bruck and Walton 2007). Therefore, the objective of this study was to determine the susceptibility of strawberry crown moth larvae to two species of entomopathogenic nematodes. Additionally, a replicated field trial was performed to determine the efficacy of these nematodes against this insect in the field.

Materials and Methods

Two species of entomopathogenic nematodes, *Steinernema carpocapsae* (Weiser) Agriotos strain and *Heterorhabditis bacteriophora* Poinar Oswego strain, were used in laboratory soil bioassays to determine their virulence against strawberry crown moth larvae. Nematodes were produced in vivo in last instar *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) (Kaya and Stock 1997). After collection, infective juveniles (IJs) were stored at 4°C for <1 wk before use. Strawberry crowns infested with last instar strawberry crown moth were collected from an infested strawberry field (28 September 2007) in Benton County Oregon. Crowns were returned to the laboratory, and the larvae were removed. Larvae were individually placed into plastic cups (5 cm in height by 7.5 cm in width) with an uninfested strawberry crown along with ≈20 g of field soil and held at 21°C in complete darkness for 2 wk.

Laboratory bioassays were performed using field soil (Canderly Sandy Loam; 66.3:22.5:11.2, sand–silt–clay) autoclaved (1.1 kg/cm^2; 121°C) for 2 h, left overnight, and autoclaved for an additional hour. The soil was then placed in a drying oven at 70°C for 24 h and stored (4°C) in zip lock bags until use. Fifty grams of oven-dried soil was placed into plastic cups (5 cm in height by 7.5 cm in width). Sterile distilled water (7 ml) was added to each container and mixed with a sterile spatula until homogenous. Strawberry crowns infested with an individual last instar were placed into each container, and half were covered with soil to simulate conditions in the field. Nematodes (500 infective juveniles (IJs) per cup; 12 IJs per cm^2) were released onto the surface of each cup in 1 ml of water so that the final moisture content was standardized at field capacity (15% moisture). Containers were capped and placed into large zip-lock bags containing several pieces of moistened paper towel, and then containers were incubated at 22°C in complete darkness for 8 d. Subsequently, each container was thoroughly searched, and the numbers of live and nematode-infected larvae (confirmed by dissection) were determined. Each experiment contained an untreated control (water only) and was arranged in a randomized complete block design with four replications and five larvae per replicate. This experiment was repeated (two complete trials) using both nematodes species. Because of a shortage of larvae, the control treatment in the second run of the experiment only contained three replications.

An experiment was also performed to determine the efficacy of *S. carpocapsae* and *H. bacteriophora* against strawberry crown moth larval infestations in the field. Six replicates, consisting of 1.5 m of row (≈10–15 plants) arranged in a randomized complete block design, were marked out in an infested field of 'Totem' strawberries located in Benton County, OR. On 11 October 2007, each length of row was treated with nematodes (100 IJs per cm^2) with a volume of water equivalent to 1,900 liters water/ha by using a backpack CO^2 sprayer fitted with a fan nozzle. The experiment also contained an untreated control (water only). Soil temperatures were recorded at a depth of 5 cm (HOBO U12, Onset Computer Corporation, Cape Cod, MA). Twenty-one days after nematode application, seven soil cores (10.8 cm in diameter; 5–7 cm in depth) were randomly dug from each length of row, and the cores were returned to the laboratory. Each sample was thoroughly searched, and the numbers of live and nematode-infected larvae (confirmed by dissection) were determined.

An arcsine transformation of the percentage of larval infection in the laboratory bioassay was performed to stabilize variance (Snedecor and Cochran 1989). A test of homogeneity of variance was performed to detect variation between the two laboratory bioassays with each insect–nematode combination (Little and

Table 1. Mean percentage (± SD) of strawberry crown moth larvae infected with each species of entomopathogenic nematode from laboratory bioassays and field trials

	Treatment		Control
	S. carpocapsae	*H. bacteriophora*	
Laboratory	96 (5.9)b	94 (5.25)b	0 (0)a
Field	51 (19)b	33 (18)b	0 (0)a

Means with different letters in the same row are significantly different ($P \leq 0.05$) (SAS Institute 1999).

Hills 1978). Variability was not significant between bioassays, and data were combined for analysis. Laboratory data were analyzed using the General Linear Models procedure, with Tukey's multiple range test used to separate means (SAS Institute 1999). Chi-square analysis was used to compare nematode efficacy in field applications (SAS Institute 1999). The reference probability used throughout was $P \leq 0.05$.

Results and Discussion

Both nematode species were highly infective in laboratory bioassays ($F = 66.87$; df = 2, 20; $P < 0.0001$) (Table 1). All larvae in laboratory assays were inside a strawberry crown at the beginning of the assay and all were found inside the crown at the conclusion of the assay. This behavior seemed to be typical, because larvae were rarely (<10%) located outside of the crown when collected from the field (28 September 2007). Their presence in the crown in the laboratory assays did not afford them any protection from nematode infection.

Conditions in the laboratory bioassays were optimal for nematode infection, and they may not be an accurate reflection of what occurs in the field. Therefore, we determined the efficacy of *S. carpocapsae* and *H. bacteriophora* against strawberry crown moth larvae in the field. Although the field experiment performed was limited in scale, the results indicated that even under far less than ideal environmental conditions, both nematodes were infective in the field (Table 1). There was a significant difference in the number of dead larvae (all due to nematode infection) between the control and applications of *S. carpocapsae* ($\chi^2 = 10.72$; df = 1; $P = 0.001$) and *H. bacteriophora* ($\chi^2 = 23.00$; df = 1; $P = 0.0001$). There was no significant difference in larval infection between *S. carpocapsae* and *H. bacteriophora* applications ($\chi^2 = 2.82$; df = 1; $P = 0.09$) (Table 1). Soil temperature postnematode applications (Fig. 1) in the field steadily declined, and they were below the minimum establishment temperatures for both nematode species (12 and 15°C for *S. carpocapsae* and *H. bacteriophora*, respectively) for a majority of the postapplication period (Grewal et al. 1994). Soil temperature, 2 wk before nematode application (i.e., immediately after larval collection), was more moderate, with warmer low temperatures and less variation between the high and low temperature. Had we been able to apply nematodes earlier in the fall, they are likely to have been more efficacious. However, we did not have the quantity of each species of nematode available when larvae were initially collected in September to perform field trials, and we were forced to wait until a fresh batch of nematodes could be produced in vivo.

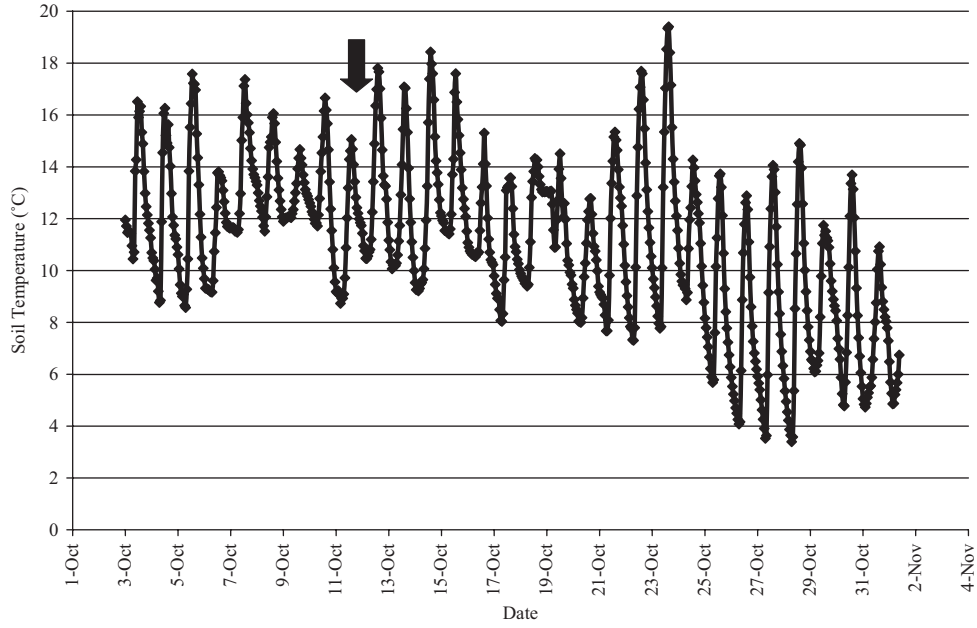

Fig. 1. Soil temperatures (°C) in field experiments performed in 2007 to determine the efficacy of entomopathogenic nematodes against strawberry crown moth larvae. Downward arrow indicates when nematodes were applied.

S. carpocapsae and *H. bacteriophora* have been assayed against and are infective toward a wide range of insect pests (Kaya and Gaugler 1993). However, there are no other published reports on the infectivity of entomopathogenic nematodes against strawberry crown moth larvae with which to compare our results. Entomopathogenic nematodes are highly infective against a number of other Lepidoptera (Williams et al. 2002, Cottrell and Shapiro-Ilan 2006, Shapiro-Ilan and Cottrell 2006, McKern et al. 2007). Shapiro-Ilan and Cottrell (2006) found that steinernematids were more virulent toward the lesser peach tree borer, *Synanthedon pictipes* (Grote & Robinson) (Lepidoptera: Sesiidae), than the heterorhabditids evaluated in the laboratory. Applications of *S. carpocapsae* and *H. bacteriophora* in the field reduced the number of raspberry crown borer, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), another pest of small fruit also commonly located in the crown, 53 and 33%, respectively (McKern et al. 2007). The results of McKern et al. (2007) mirror our field data; however, the nematode rate used in their trial was significantly lower ($\approx$5.4 IJs per cm^2). Applications targeting *P. marginata* in Arkansas were made in April when soil temperatures were reported to range between 8 and 16°C, but the duration of time at those temperatures was not reported. In addition, *P. marginata* trials were not evaluated for a full 2 mo after nematode application in which time soil temperatures would be increasing in addition to ample time for nematode recycling to occur. Our trials were performed in late fall when soil temperatures were becoming less conducive for nematode infection and recycling over the course of the trial. Entomopathogenic nematodes have been evaluated previously in strawberries primarily for control of root weevil larvae (Coleoptera: Curculionidae) (Berry et al. 1997, Wilson et al. 1999, Booth et al. 2002, Willmott et al. 2002). Nematode efficacy for root weevil control in strawberries can be in excess of 75%. The efficacy of entomopathogenic nematodes for weevil control in the Pacific Northwest is also severely limited by the cool soil temperatures that are prevalent throughout the region when the susceptible stages of most soil pests of strawberries are targeted. In addition to cool soil temperatures, large strawberry crowns also have been implicated for their ability to protect root weevil larvae against nematode infection (Booth et al. 2002). Our laboratory data in particular indicate that the strawberry crown may not provide an adequate refuge for strawberry crown moth larvae to avoid nematode infection. It may prove efficacious to target overwintering root weevil and strawberry crown moth larvae simultaneously infesting strawberry planting, particularly if applications are made in early fall (September) when soil temperatures are more conducive to nematode infection.

These initial studies indicate that entomopathogenic nematodes have good potential for managing strawberry crown moth. It is clear from the laboratory data, that strawberry crown moth larvae are extremely susceptible to nematode infection, even in the protected environment inside the crown. However, control in the field may be improved if nematode applications are made before larvae enter the crowns. Earlier nematode application will not only target potentially exposed larvae but also increased soil temperatures at that time, late summer to early fall, would likely lead to increased levels of nematode infection. Future work will focus on larger scale field studies with nematodes applied at various times to determine the efficacy, feasibility, and economics of managing strawberry crown moth larvae with entomopathogenic nematodes on a commercial scale. Based on these results and other published reports of nematode efficacy against Sesiidae (Shapiro-Ilan and Cottrell 2006, McKern et al. 2007), applications of steinernematids are likely to be most efficacious in the field.

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