Laboratory Assay of Entomopathogenic Nematodes Against Clearwing Moth (Lepidoptera: Sesiidae) Larvae[1]

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Abstract The susceptibility of clearwing moth *Paranthrene diaphana* (Dalla Torre & Strand) last-instar larvae to the entomopathogenic nematodes *Steinernema feltiae* (Filipjev), *Steinernema carpocapsae* (Weiser), and *Heterorhabditis bacteriophora* (Poinar) was determined in laboratory assays. Larval mortality was assessed at 0, 6, 12, 24, 48, and 72 h after exposure of the larvae to six concentrations (0, 50, 100, 200, 400, and 800) of infective juveniles (IJs) per bioassay arena. The median lethal concentration (LC_{50}) for each nematode species was 35.97 IJ/ml for *S. feltiae*, 44.0 IJ/ml for *S. carpocapsae*, and 104.5 IJ/ml for *H. bacteriophora*. The median lethal time (LT_{50}) for each nematode species at the concentration of 100 IJ/ml was 43.94 h for *S. feltiae*, 60.88 h for *S. carpocapsae*, and 82.3 h for *H. bacteriophora*. Based on these and other results, we conclude that research should be expanded on the prospects for using entomopathogenic nematodes, especially *S. feltiae*, in managing *P. diaphana*.

Key Words biological control, entomopathogenic nematode, *Heterorhabditis*, *Steinernema*, *Paranthrene diaphana*

A clearwing moth *Paranthrene diaphana* (Dalla Torre & Strand) (Lepidoptera: Sesiidae) is an important global pest of ornamental trees. Adult moths typically lay eggs on the trunk and limbs of trees, in cracks in the bark, in tree crotches, or adjacent to injured areas on the tree (Dreistadt and Perry 2004). Emerging larvae bore into the inner bark and cambium where they feed and develop within excavated tunnels. Damage from larval feeding reduces tree vigor and, in high infestations, can lead to loss of tree limbs or render the entire tree unsalvageable (Špatenka et al. 1999). Tactics employed in its management include pheromones, physical methods, chemical insecticides, and biological control agents (Dreistadt and Perry 2004), but organochlorine, organophosphate, and synthetic pyrethroid insecticides are most frequently used. And, hence, there is an effort to find efficacious alternatives to avoid development of resistance to insecticides while addressing human and environmental health concerns with chemical insecticide use (Maniania et al. 2008).

Biological control agents, including the entomopathogenic nematodes (EPNs), should be evaluated and considered as alternatives (Chandler et al. 2005). Of the

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EPNs, members of the families Steinernematidae and Heterorhabditidae are reportedly the most effective and useful biological agents against insects (Adams and Nguyen 2002, Rechcigl and Rechcigl 2000) and are effectively used to manage a variety of economically important insect pests (Grewal et al. 2005, Klein 1990, Shapiro-Ilan et al. 2009).

Previous studies indicate that applications of EPNs to trees and other aboveground features can yield high levels of control of a variety of sesiid pests, including several *Synanthedon* spp. (Cottrell and Shapiro-Ilan 2006, Kaya and Brown 1986, McKern et al. 2007, Parvizi 2003, Shapiro-Ilan et al. 2009, Williams et al. 2002). However, virulence or efficacy against sesiids can vary among different plant hosts and EPN species or strain (Bedding and Miller 1981, Cossentine et al. 1990, Deseö and Miller 1985, Kaya and Brown 1986, Nachtigall and Dickler 1992, Saunders and All 1982, Smith-Fiola et al. 1996, Williams et al. 2002).

Our overall goal in this study was to determine the potential for use of EPNs for *P. diaphana* suppression. The susceptibility of *P. diaphana* to EPNs has not been reported previously. A critical component for success in any biocontrol program with entomopathogenic nematodes is matching the most suitable nematode with the target host, and relative virulence among different nematodes to the target pest is one of the important factors to consider in determining suitability (Georgis and Gaugler 1991, Shapiro-Ilan et al. 2009). Thus, herein, we report the susceptibility of last-instar *P. diaphana* larvae to *Steinernema feltiae* (Filipjev), *Steinernema carpocapsae* (Weiser), and *Heterorhabditis bacteriophora* (Poinar) as determined in laboratory bioassays.

Materials and Methods

Insects. Last-instar *P. diaphana* larvae were collected from the field in early February 2016 from Babylon willow trees, *Salix babylonica* (L.), of various ages and sizes growing in Tehran city, Iran. Each larva was removed from its feeding gallery, placed individually in a 9-cm petri dish containing a moistened paper towel, and transported to the laboratory where larvae were held for not more than 5 d at 10°C until a sufficient number could be collected on successive days for the bioassays. Larvae were all last instars of uniform size due to the occurrence of a single generation of *P. diaphana* in central Iran.

Bioassays. Commercial preparations of *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* were initially obtained from Koppert BV (The Netherlands, Berkel en Rodenrijs). These were cultured in last-instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), as per methods of Kaya and Stock (1997). Each nematode species was passed through *G. mellonella* less than seven times before use in bioassays. After harvesting, infective juveniles (IJs) were stored in 250-ml flasks at 13°C and at a maximum concentration of 5000 IJs per ml for $<$1 week before use. IJ stock cultures were serially diluted to achieve concentrations of 50, 100, 200, 400, and 800 IJs per ml of distilled water. The control solution was distilled water alone. Microscopic examinations found that $\geq$95% of IJs were viable in each of the nematode preparations in all bioassays.

Assay arenas were 60-mm inverted petri dishes with filter paper lining the lid as described by Kaya and Stock (1997). The design was a factorial in a randomized

complete block with three replications of five larvae per treatment. Individual concentrations (0, 50, 100, 200, 400, 800 IJs per ml) for each nematode species were deposited onto the filter paper of appropriate arenas in a 1-ml droplet of the suspension. Five last-instar *P. diaphana* larvae were placed in each arena. Control arenas received 1 ml of distilled water. Arenas were maintained in an environmental chamber at 25 ± 1°C, 65 ± 5% relative humidity, and in constant darkness. Arenas were examined and larval mortality recorded at 6, 12, 24, 48, and 72 h.

Data analysis. Mortality data were normalized by square root transformation. The effects of the main factors of species, concentration, and exposure time were subjected to analysis of variance (ANOVA) by using SPSS (Statistical Package for the Social Sciences, Version 15.0, Chicago, IL), with a level of significance at $P < 0.05$. Probit analysis of the mortality–concentration and the mortality–time responses were performed to estimate lethal concentrations and lethal times. Mean comparisons among significantly different treatments were performed using Tukey's honestly significant difference test.

Results and Discussion

Mortality of last-instar *P. diaphana* increased with increasing IJ concentration and with increasing exposure time for *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* ($F = 442.32$; $df = 5, 50$; $P < 0.001$). Interactions among all three main factor effects (species, concentration, exposure time) also were significant ($F = 1.22$; $df = 2, 50$; $P < 0.001$). Thus, the mortality response of *P. diaphana* last instars was similar for each of the nematode species for the concentrations tested (Figs. 1, 2).

Median lethal concentrations (LC_{50}s) for the three nematode species against *P. diaphana* last instars were 35.97 IJs per ml for *S. feltiae*, 44.0 IJs per ml for *S. carpocapsae*, and 104.05 IJs per ml for *H. bacteriophora* (Table 1). These estimates did not differ significantly based on the overlap of the 95**%** confidence intervals (Table 1). However, cumulative larval mortality was significantly higher following exposure to *S. feltiae* at 200, 400, and 800 IJs per ml than with either *S. carpocapsae* or *H. bacteriophora* (Fig. 1). Likewise, mortality was significantly higher with *S. feltiae* than with *S. carpocapsae* or *H. bacteriophora* at each of the observation times of 12, 24, 48, and 72 hours after exposure (Fig. 2).

Median lethal times (LT_{50}s) for each of the nematode species (100 IJs per ml) in *P. diaphana* last instars were estimated as 43.94 h for *S. feltiae*, 60.88 h for *S. carpocapsae*, and 82.3 h for *H. bacteriophora* (Table 2). These values did not differ significantly based on the overlap of the 95**%** confidence intervals (Table 2).

Based on these results, we conclude that *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* killed last-instar *P. diaphana* in laboratory arenas by using the IJ concentrations reported herein. We found no previous reports of assays of these EPNs against *P. diaphana*; thus, this represents the first report of the use of these EPN species against this pest. These results further corroborate the relative activity of EPN species against the lesser peach tree borer, *Synanthedon pictipes* (Clerck) (Lepidoptera: Sesiidae), by Shapiro-Ilan and Cottrell (2006) who found that *S. feltiae* and *S. carpocapsae* performed better than *H. bacteriophora*. Others also have found that steinernematid nematodes performed better than heterorhabditid

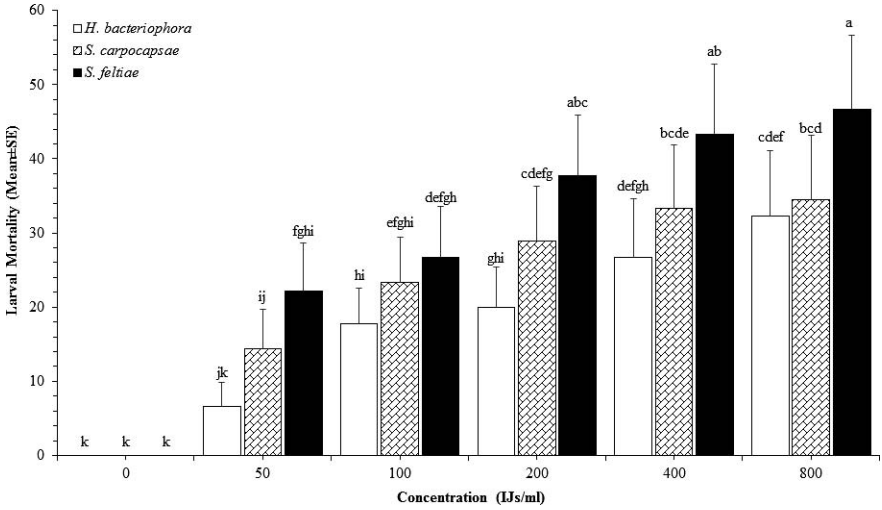

Fig. 1. Mean (± SE) mortality of last-instar larvae of the clearwing moth, *P. diaphana*, in response to concentrations of the entomopathogenic nematodes *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora*. Bars above each mean represent SE; means accompanied by the same lowercase letter are not significantly different ($P = 0.05$) according to Tukey's honestly significant difference test.

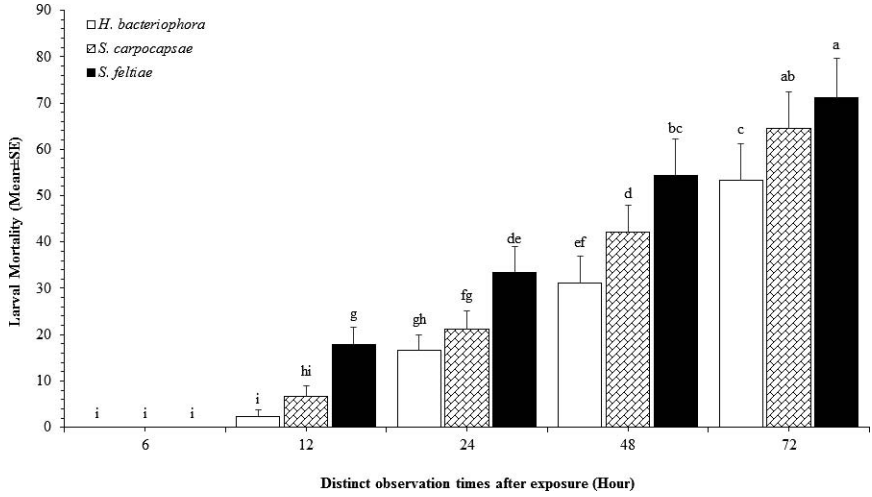

Fig. 2. Mean (± SE) mortality of last-instar larvae of the clearwing moth, *P.diaphana*, at different times after initial exposure. Bars above each mean represent SE; means accompanied by the same lowercase letter are not significantly different ($P = 0.05$) according to Tukey's honestly significant difference test.

Table 1. Concentration–mortality response of last-instar *P. diaphana* to *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora*.

Nematode species	LC_{50}		LC_{90}		χ^2	*df*	Slope ± SE
	IJ*	CI**	IJ*	CI**			
S. feltiae	35.97	3.50–65.17	182.13	110.67–713.87	1.49	3	1.82 ± 0.62
S. carpocapsae	44.00	4.07–86.30	411.44	216.49–3525.85	0.40	3	1.32 ± 0.44
H. bacteriophora	104.05	47.53–165.37	687.10	368.09–3585.82	1.73	3	1.56 ± 0.41

*Infective juveniles per ml.
**95% confidence limits.

Table 2. Time–mortality responses of last-instar *P. diaphana* to *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* expressed as lethal time (LT).

Nematode species	IJ*	LT_{50}		LT_{90}		χ^2	*df*	Slope ± SE
		Time (h)	CI**	Time (h)	CI**			
S. feltiae	100	43.94	29.86–76.37	194.87	101.57–953.56	6.929	3	1.981 ± 0.186
S. carpocapsae	100	60.88	51.80–74.12	233.41	169.09–370.24	1.904	3	2.196 ± 0.216
H. bacteriophora	100	82.30	68.01–106.01	322.61	218.85–581.15	2.839	3	2.160 ± 0.241

*Infective juveniles (IJ) per ml.
**95% confidence limits (CI).

nematodes against other sesiid larvae (Capinera et al. 1986, Deseö and Miller 1985, Nachtigall and Dickler 1992). Furthermore, *S. feltiae* has proven effective in field efficacy testing against the dusky clearwing borer, *P. tabaniformis* (Rottemburg) (Cavalcaselle and Deseö 1984) and the raspberry crown borer, *Pennisetia marginata* (Harris) (Capinera et al. 1986), and also members of the family Sesiidae. Bruck et al. (2008) also reported that *S. carpocapsae* and *H. bacteriophora* caused mortality levels of 96**%** and 94**%**, respectively, of the strawberry crown moth, *Synanthedon bibionipennis* (Boisduval) (Bruck et al. 2008).

Our data coupled with these previous research results indicate that EPNs might be developed for management of *P. diaphana*. Future research will focus on field efficacy, field application, and more.

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