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Short communication

Effect of entomopathogenic nematodes on *Plectrodera scalator* (Fabricius) (Coleoptera: Cerambycidae)

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Abstract

Entomopathogenic nematodes were screened for efficacy against the cottonwood borer, *Plectrodera scalator* (Fabricius). *Steinernema feltiae* SN and *S. carpocapsae* All killed 58 and 50% of larvae, respectively, in filter paper bioassays but less than 10% in diet cup bioassays. *S. glaseri* NJ, *S. riobrave* TX, and *H. indica* MG-13 killed less than 10% of larvae in both assays. *H. marelata* IN was ineffective in the diet cup bioassay and killed 12.9% of larvae in a filter paper bioassay. The nematode isolates we tested are not suitable for use as biological control agents against *P. scalator*.

Keywords: Biological control; Cottonwood borer; *Heterorhabditis*; Nematodes; *Plectrodera scalator*; Preconditioning; *Steinernema*

1. Introduction

Tree-boring cerambycid larvae damage host trees by tunneling through the wood cambium, eventually leading to the death of the tree (Forschler and Nordin, 1989; Smith et al., 2001). Effective methods to control cerambycid pests are constrained by the cryptic behavior of the larvae and, as a result, there is limited published research on methods for their control.

The cottonwood borer, *Plectrodera scalator* (Fabricius), is a native cerambycid beetle and an occasional pest in cottonwood nurseries in the southern United States (Forschler and Nordin, 1989); but it is a more serious pest of *Populus deltoides* (Marsh.) and *Salix* spp. in the eastern United States (Solomon, 1980). Entomopathogenic nematodes (EPN) in the genera *Heterorhabditis* and *Steinernema* have considerable potential as biological control agents of a number of cryptic insect pests (Kaya, 1985) and several strains showed activity against the closely related Asian longhorned beetle, *Anaplophora glabripennis* (Motchulsky) (Fallon et al., 2004). Our objective was to determine the susceptibility of *P. scalator* to EPNs.

2. Materials and methods

Isolates of *Steinernema feltiae* (Filipjev) SN from France, *S. glaseri* (Steiner) NJ from New Jersey; *S. riobrave* Cabanilla, Poinar, and Raulston TX from Texas; *S. carpocapsae* (Weiser) isolates Sal from Indiana and All from Georgia; *Heterorhabditis indica* Poinar, Karunakar, and David MG-13 from Hawaii; and *H. marelata* Liu and Berry IN from Indiana were cultured in *Galleria mellonella* (L.) at 24 °C. Infective juveniles (IJs) were harvested in White traps (Dutky et al., 1964), stored at 15 °C, and used within 3 weeks of emergence. *P. scalator* larvae were cultured using

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a modified artificial *Prionus* spp. diet (Payne et al., 1975) in 59-ml diet cups and reared to third and fourth stadia at the USDA Forest Service facility in East Lansing, Michigan. A randomized complete block design was used for the experiments and experimental animals were incubated at 24 °C. Each experiment was replicated three times. Insect mortality was monitored daily for 2 weeks; host insects were dissected 3 days after death and nematodes were counted. The percentage of larvae killed per treatment in each experiment was considered a replicate for analysis and was arcsine transformed prior to analysis using PROC GLM (SAS Institute, 1999). The number of nematodes per host larva was compared among treatments using PROC GLM.

Third- and fourth-instar *P. scalator* larvae were exposed to *S. feltiae*, *S. carpocapsae* All, *S. glaseri*, *S. riobrave*, *H. indica*, and a water control in filter paper bioassays and diet cup bioassays.

In the filter paper assay, four *P. scalator* larvae were placed in 60-mm Petri plates (Fisherbrand®, Hanover Park, IL), lined with two pieces of 55-mm Whatman® No. 1 filter paper. One-hundred IJs in 1 ml tap water were added to the filter paper and the plates were incubated for 24 h to allow invasion of the host by the nematodes. The host larvae were removed, washed and dried, and then transferred to clean 60-mm plates for further incubation.

In the diet cup assay; a 100-μl aliquot of 100 IJs was applied to the borehole cut into the nutritional media of cups containing larvae, one larva per cup, and the cups were incubated for 72 h. The nematode-exposed larvae were removed, washed, blotted dry, and transferred to 60-mm Petri plates for further incubation. Four larvae were used for each treatment.

To assess EPN dosages, *P. scalator* larvae were exposed to 10, 50, and 100 IJs per insect of the commercial nematode isolates *S. carpocapsae* Sal and *H. marelata* using a filter paper assay and a diet cup assay. Dosages in the diet cup assay were increased to 0, 100, 500, 1000, and 2000 IJs per insect as dosages below 100 IJs were ineffective in the filter paper assay. *S. feltiae* was included as an additional treatment in the diet cup assay. Seven insects were used per treatment.

3. Results and discussion

In the filter paper assay, *S. feltiae* and *S. carpocapsae* All produced the highest mortality in *P. scalator* third and fourth instar larvae ($F=22.00$ $df=4,10$ $P=0.0002$; Table 1). A mean of 1.3 *S. feltiae* adults were recovered from *P. scalator* larvae. A mean of less than one adult *S. carpocapsae* All, *S. glaseri*, *S. riobrave*, and *H. indica* was recovered per host larva. There was no mortality in control treatments.

In the diet cup assay, *S. glaseri* killed 16% of *P. scalator* larvae. *S. feltiae*, *S. carpocapsae* All, *S. riobrave*, and *H. indica* killed fewer than 10% of the larvae. Of the larvae killed, only one was infected with a single adult of *S. feltiae*, the remaining dead insects did not produce adult or juvenile nematodes. The host diet was compared to that of *Anoplophora glabripennis*, a host against which *S. carpocapsae* Sal and *S. feltiae* produced 71–100% mortality in similar bioassays (*S. feltiae* was the same nematode strain) under the same conditions (Fallon et al., 2004). Only choline chloride and ascorbic acid were added to the *P. scalator* diet, both of which have been reported to favor development in other nematode species (Narian, 1992; Rajan et al., 2003; Strauch et al., 2000), although Osmun (1993) reported mortality of a plant nematode *Meloidogyne javanica* (Tylenchidae) when exposed to aqueous solutions of ascorbic acid.

Table 1
Percent mortality of third- and fourth-instar *Plectrodera scalator* larvae by entomopathogenic nematodes applied at a dosage of 100 IJs per host larva in a filter paper assay

Nematode isolate	% Mortality
Heterorhabditis indica MG-13	0 ± 0 A[a]
Steinernema feltiae SN	58.3 ± 8.3 B
Steinernema carpocapsae All	50.0 ± 8.3 B
Steinernema glaseri NJ	0 ± 0 A
Steinernema riobrave TX	8.3 ± 8.3 A

[a] Values within a column followed by the same letter are not different among the treatments according to least mean squares analysis ($P \leqslant 0.05$).

In the filter paper assay to evaluate dosages, exposure of *P. scalator* to *S. carpocapsae* Sal produced an LD_{50} of 83 IJs (95% CI 44–3928 $P=0.0335$). *H. marelata* did not produce mortality at the dosages tested. There was no mortality in control treatments. The LT_{50} of *S. carpocapsae* Sal was 15 days at 100 IJs per insect (95% CI 8–527 days $P=0.0138$) and 20 days at 50 IJs per insect ($P>0.05$). *S. carpocapsae* Sal was significantly more virulent ($F=9.50$ $df=1,16$ $P=0.013$) and penetrated and developed more successfully ($F=8.11$ $df=1,16$ $P=0.019$) in *P. scalator* than *H. marelata*; the mean larval mortality produced by *S. carpocapsae* Sal was $38.9 \pm 9.4\%$ compared to $12.9 \pm 5.2\%$ for *H. marelata* over all dosages. Although *S. carpocapsae* Sal was more infective than *H. marelata*, a mean of less than one adult *S. carpocapsae* nematode per *P. scalator* larva was recovered.

In the diet cup assay to evaluate dosages, the length of time necessary for *S. feltiae* and *S. carpocapsae* Sal to kill *P. scalator* was highly variable (Table 2). At 2000 IJs per host larva, the LT_{50} of *S. feltiae* was lower than that of *S. carpocapsae*; but at dosages below 1000 IJs per larva there was no difference in the LT_{50} of the two isolates. There was no mortality in control treatments. Fewer *S. feltiae* IJs than *S. carpocapsae* Sal IJs were required to kill *P. scalator* larvae. The LD_{50} of *S. feltiae* was 805 IJs (95% CI 460–1316 $P=0.0012$) compared to 2683 IJs for *S. carpocapsae* Sal ($P>0.05$). The number of *S. feltiae* that penetrated *P. scalator* larvae increased as the nematode dosage increased ($F=17.12$, $df=1,11$ $P=0.0020$), but there was no such correlation for *S. carpocapsae* Sal ($P>0.05$). *H. marelata* was excluded from the analysis because no larvae died at dosages up to 2000 IJs per insect.

Table 2
LT_{50} of *Steinernema feltiae* SN and *S. carpocapsae* Sal applied against third- and fourth-instar *Plectrodera scalator* larvae at concentrations of 2000, 1000, 500, 100, and 0 nematodes per insect in a diet cup bioassay

Isolate	Concentration	LT_{50}[a]	95% Interval	*P*
Steinernema	2000	9	7–15	0.0006
feltiae SN	1000	18	11–131	0.0066
	500	74	26–1.6×10^{18}	0.0400
	100	nm^2	nm^2	>0.05
	0	nm^2	nm^2	>0.05
Steinernema	2000	39	17–1.8×10^{10}	0.0363
carpocapsae	1000	47	19–3.0×10^{14}	0.0393
Sal	500	nm^2	nm^2	>0.05
	100	nm^2	nm^2	>0.05
	0	nm^2	nm^2	>0.05

nm, no mortality.
[a] Lethal time in days to kill 50% of the insect hosts.

Plectrodera scalator was a poor host for the entomopathogenic nematodes we tested. In the filter paper bioassays, *S. feltiae* and *S. carpocapsae* All were the most virulent of the five nematode isolates screened, but no isolate caused 100% mortality. Results from the diet cup bioassays at rates of 100 IJs per insect were inconsistent, and no isolate killed greater than 20% of larvae at this dosage. *P. scalator* larvae were highly resistant to nematode penetration and died slowly once infected. High LT_{50} values for *S. feltiae* and *S. carpocapsae* Sal against *P. scalator* suggests that there is a high level of resistance but not immunity to the entomopathogenic-nematode complex. *S. feltiae* and *S. carpocapsae* were able to complete a life cycle in a *P. scalator* host, but the total development time from infection to emergence was 3–4 months. As a comparison, development time of *S. carpocapsae* Sal in the related cerambycid *A. glabripennis* is approximately 3–4 weeks (Solter, unpublished data). The entomopathogenic nematodes we tested have limited potential for the control of *P. scalator*.

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