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Short Communication

Susceptibility of the filbertworm (*Cydia latiferreana*, Lepidoptera: Tortricidae) and filbert weevil (*Curculio occidentalis*, Coleoptera: Curculionidae) to entomopathogenic nematodes

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Abstract

The objective of this study was to determine the susceptibility of the two primary direct insect pests of hazelnuts in Oregon to three species of entomopathogenic nematodes. The entomopathogenic nematodes (*Heterorhabditis marelatus* Pt. Reyes, *Steinernema carpocapsae* All and *Steinernema kraussei* L137) were used in laboratory soil bioassays to determine their virulence against filbertworm, *Cydia latiferreana* (Walsingham) (Lepidoptera: Tortricidae) and filbert weevil, *Curculio occidentalis* (Casey) (Coleoptera: Curculionidae). All three nematode species were infective in laboratory bioassays. Infectivity ranged from 73–100% and 23–85% for filbertworm and filbert weevil, respectively. Field results were similar to those found in the laboratory with filbertworm larvae being more susceptible to nematode infection.
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Hazelnut (*Corylus avellana* L.) is an important nut crop produced around the world including the United States (Olsen, 2002). Oregon's Willamette Valley accounts for about 99% of hazelnuts grown in the United States (3–5% of world production). Currently, the key insect pests associated with hazelnuts include filbertworm, *Cydia latiferreana* (Walsingham) (Lepidoptera: Tortricidae), filbert aphid, *Myzocallis coryli* (Goetze) (Homoptera: Aphidae), filbert leafroller, *Archips rosanus* L. (Lepidoptera: Tortricidae), obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) (AliNiazee, 1984, 1998; Viggiani, 1994) and filbert weevil, *Curculio occidentalis* (Casey) (*Curculio uniformis*) (Coleoptera: Curculionidae) (Dohanian, 1944; Lewis, 1992; Dunning et al., 2002).

Adult filbertworm moths emerge from early June to early October. During this period, eggs are laid close to hazelnut husks under favorable conditions (AliNiazee, 1983). Larvae bore into nuts and consume the flesh during development. Damaged nuts often drop early and fully developed larvae emerge from an enlarged entry hole. Filbertworm larvae over winter in organic material and soil to a depth of 5–8 cm (Dohanian, 1944). The biology of the filbert weevil differs from that of filbertworm in that weevils oviposit eggs into the nut after cutting small holes into the substrate (Lewis, 1992). After development inside the nuts, weevils exit from dropped nuts. Weevil larvae burrow 25 cm or deeper into the soil and hibernate up to three years, emerging as adults in the spring (Dohanian, 1944; Dunning et al., 2002). Both pests have been recorded as direct pests on hazelnuts and can cause between 20 and 40% damage if left untreated (Dohanian, 1944; AliNiazee, 1984). These pests are mostly controlled with synthetically produced insecticides and thus alternative control measures need to be investigated.

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

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biological control of a wide 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).

Prior to these studies, no data were available on the infectivity of nematodes against the filbertworm or filbert weevil. Laboratory testing and small scale field studies are important first steps to identify nematodes with potential for managing these pests. Therefore, the objective of this study was to determine the susceptibility of the two primary direct insect pests of hazelnuts in Oregon to three species of entomopathogenic nematodes. Additionally, a small-scale replicated field trial was performed to determine the efficacy of the two most effective nematodes species in the laboratory against both insects in the field.

Three species of entomopathogenic nematodes (*Heterorhabditis marelatus* (Liu and Berry) Pt. Reyes, *Steinernema carpocapsae* (Weiser) All and *Steinernema kraussei* (Steiner) L137) were used in laboratory soil bioassays to determine their virulence against filbertworm and filbert weevil. Nematodes were produced *in vivo* in last instar *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) at 22 °C (Woodring and Kaya, 1988). After collection, infective juveniles (IJs) were stored at 7 °C (*Steinernema* sp.) and 10 °C (*Heterorhabditis* sp.) for <4 weeks. Hazelnuts infested with filbertworm and filbert weevil were collected from the orchard floor of abandoned and organically managed hazelnut orchards in Benton county, Oregon. Nuts were returned to the laboratory and cracked or placed in large plastic containers with numerous holes (4–5 mm) in the bottom. Containers with infested nuts were then stacked inside empty containers without holes in which the emerged insects were collected.

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 < 2 weeks) in zip-lock bags until use. Fifty grams of oven dried soil was placed into plastic cups (5 cm h × 7.5 cm w). Sterile distilled water (6.5 ml) was added to each container and mixed with a sterile spatula until homogenous. Five final instars were placed into each container on top of the soil for 24 h and allowed to burrow. Nematodes (500 IJs/larva; 57 IJs/cm^2) were released onto the soil surface of each cup in 1 ml of water so that the final moisture content was standardized at field capacity (14% moisture). Containers were capped and placed into large zip-lock bags containing several pieces of moistened paper towel and incubated at 22 °C in complete darkness for 10 days. Each container was subsequently searched and the numbers of live and nematode-infected larvae determined. Dead larvae were dissected and nematode infections confirmed. All experiments contained an untreated control (water only) and were arranged in a randomized complete block design with four replications. This experiment was repeated (two complete trials), with each insect assayed separately against all nematodes species.

A small-scale field experiment was also performed to determine the efficacy of *S. carpocapsae* and *H. marelatus* against both insects. Plastic PVC pipes (15 cm in length 10 cm in diameter) were driven half way into the soil in a hazelnut orchard located in Benton county, OR. On 23 October, 2006 ten final instar filbertworm and filbert weevil were added to each pipe section. The pipe ends were sealed with aluminum foil to prevent insect escape after which the pipes were covered with leaf debris to simulate field conditions. Nematodes were added in 10 ml of water (200 IJs/cm^2 soil surface inside each pipe) on 24 October, 2006. An additional 10 ml of water was added to each section of pipe and the foil and leaf debris replaced. The experiment contained an untreated control (water only) and was arranged in a randomized complete block design with five replications. Soil temperatures were recorded at a depth of 5 cm on the in- and outside of pipe sections (HOBO U12, Onset Computer Corporation, Cape Cod MA). Twenty four days after nematode application, pipe sections were dug and placed in a large zip-lock bag and returned to the laboratory. Each sample was thoroughly searched and the numbers of live and nematode-infected larvae determined. Dead larvae were dissected to confirm nematode infection.

An arcsine transformation of the percentage larval infection was performed to stabilize variance (Snedecor and Cochran, 1989). A test of homogeneity of variance was performed to detect variation between the two bioassays performed with each insect-nematode combination (Little and Hills, 1978). Variability was not significant between bioassays and data were combined for analysis. A weighted analysis of the arcsine transformation of the percentage larval infection was performed to compare treatments in the field experiment. Data were analyzed using the General Linear Models procedure (GLM) with Tukey's multiple range test used to separate means (SAS Institute, 1999). The reference probability used throughout was $P \leqslant 0.05$.

All nematode species were infective against both insects in laboratory bioassays (Table 1). Filbertworm appeared to be more susceptible to nematode infection than filbert weevil. Filbertworm larvae produced hibernacula during the laboratory bioassays and larvae were often (>50%) found infected inside their hibernacula. All filbertworm larvae were infected with *S. carpocapsae* and *H. marelatus* and 73% infected with *S. kraussei* (Table 1). *Steinernema kraussei*, while infective, caused significantly less larval infection to filbertworm and filbert weevil than *S. carpocapsae* and *H. marelatus* (Table 1). The cause of the overall reduced efficacy of *S. kraussei* is unclear. *Steinernema carpocapsae* and *H. marelatus* have been assayed against a wide range of insect pests (Kaya and Gaugler, 1993) while applied studies of the efficacy of *S. kraussei* are limited to Coleop-

Table 1
Mean percentage (±SD) of filbertworm and filbert weevil larvae infected with each species of entomopathogenic nematode from laboratory bioassays in field soil

	Treatment			
	S. carpocapsae	*H. marelatus*	*S. kraussei*	Control[a]
Filbertworm	100(0)c	100(0)c	73(21)b	0(0)a[b]
Filbert weevil	85(14.1)b	70(15.1)b	23(27)a	0(0)a[b]

[a] No larval mortality (nematode infection or natural mortality) occurred in the control treatment.
[b] Means within the same row with different letters are significantly different ($P \leqslant 0.05$) (SAS Institute, 1999).

tera (Willmott et al., 2002). There are no other published reports on the infectivity of entomopathogenic nematodes against either of these insects to compare with our results. However, application of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) in the fall to infested soil under trees resulted in 99.5% filbert weevil mortality the following spring (Paparatti and Speranza, 2005). Entomopathogenic nematodes have a limited history of use against direct pests of nut crops. Studies have been performed on navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) in pistachios and almonds (Agudelo-Silva et al., 1987; Lindegren et al., 1987; Agudelo-Silva et al., 1995; Siegel et al., 2004; Siegel et al., 2006) as well as pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae) in pecans (Shapiro-Ilan, 2001a; Shapiro-Ilan, 2001b). Studies in almonds are limited to applications to nuts left on trees and not fallen nuts (Agudelo-Silva et al., 1995). The cryptic overwintering site inside pistachios proved to be an ideal environment to target navel orangeworm with entomopathogenic nematodes (Siegel et al., 2004; Siegel et al., 2006). While filbertworm and filbert weevil do not generally overwinter inside the nut, they do emerge from the nut once it falls on the ground and overwinter in the soil under infested trees (Dohanian, 1944; Lewis, 1992). These overwintering larvae are ideally situated for targeting by entomopathogenic nematodes. Pecan weevil larvae also overwinter in the soil and are low to moderately susceptible to nematode infection in the laboratory (Shapiro-Ilan, 2001a). Pecan weevil adults spend 9 months in the soil (Harris, 1985) and are more susceptible to nematode infection than larvae (Shapiro-Ilan, 2001b). The biology of filbertworm and filbert weevil is not conducive to targeting adults with nematodes, as they spend very little time in the soil.

Conditions in the laboratory bioassays were optimal for nematode infection and may not be an accurate reflection of what occurs in the field. Therefore, we determined the efficacy of *S. carpocapsae* and *H. marelatus* against filbertworm and filbert weevil in the field. While the field experiment performed was small in scale, the results indicated that both nematodes were infective in the field. Soil temperatures inside of the pipe sections were similar to the surrounding field soil (Fig. 1). Soil temperatures were well below optimum for both nematode species the first 10–12 days after application (Grewal et al., 1994; Berry et al., 1997). Soil temperatures moderated later in the experiment and nematode infection occurred in both insects. There was a significantly higher percentage (mean ± SD) of filbertworm larvae infected in the *S. carpocapsae* treatment (90.5 ± 19.5) than the *H. marelatus*

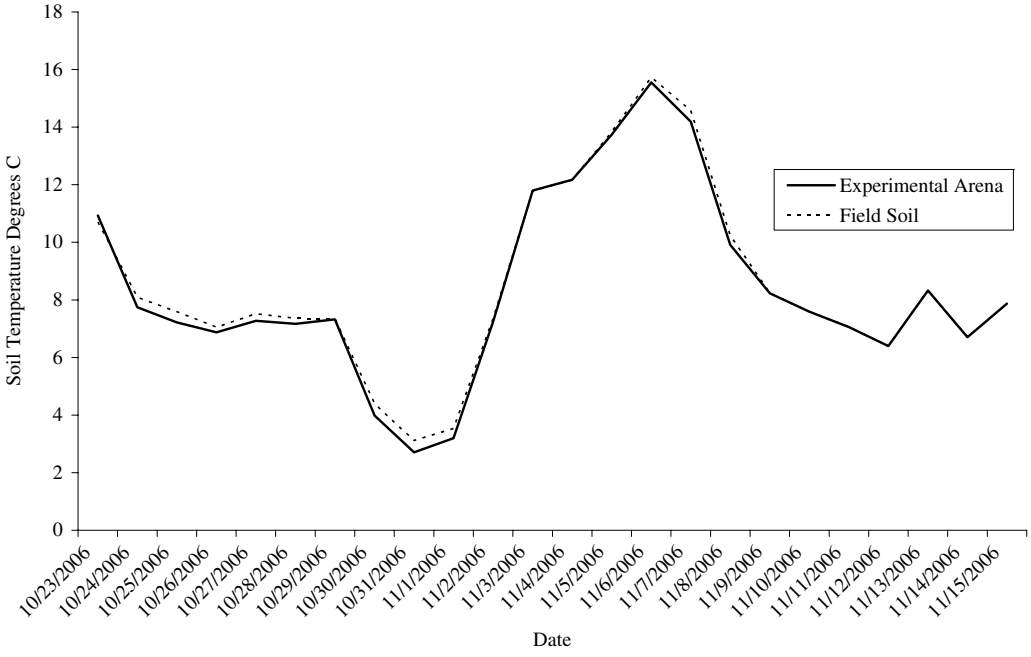

Fig. 1. Soil temperatures (°C) inside experimental arenas and in surrounding field soils from field experiments performed to determine the efficacy of entomopathogenic nematodes against filbertworm and filbert weevil larvae.

treatment (20.0 ± 33.5) or the untreated control (0.0 ± 0.0) ($F = 41.18$; d.f. $= 2, 11$; $P < 0.0001$). There was no significant difference in filbertworm infection between the *H. marelatus* and the untreated control treatment. There were no significant differences in the percent filbert weevil larval infection between treatments ($F = 3.21$; d.f. $= 2, 11$; $P = 0.08$). Weevil larvae were infected in both the *S. carpocapsae* (26.8 ± 51.9) and *H. marelatus* (37.5 ± 49.1) treatments but not the control (0.0 ± 0.0). Filbertworm and filbert weevil larvae were tested simultaneously in the field and the difference in infection levels may have been due to nematode preference towards filbertworm. Field results were similar to those found in the laboratory with filbertworm larvae being more susceptible to nematode infection. Filbertworm larvae build hibernacula while overwintering in the soil, but the hibernacula did not appear to be an effective defense to nematode infection. Filbertworm larvae were often found infected inside their hibernacula. However, it is possible that infection took place prior to the hibernacula being constructed. Filbert weevil larvae do not construct hibernacula, but do construct pupation chambers in the soil. These chambers of tightly packed soil may inhibit nematodes from gaining access to the weevil larvae.

These initial studies indicate that entomopathogenic nematodes have potential for managing these direct pests of hazelnuts in the field. Future work will focus on larger-scale field studies to determine the efficacy and feasibility of managing filbertworm and filbert weevil larvae on a commercial scale.

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