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Ecological influence of the entomopathogenic nematode, *Steinernema carpocapsae*, on pistachio orchard soil arthropods

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ABSTRACT

The entomopathogenic nematode, *Steinernema carpocapsae*, can reduce pesticide reliance in pistachios by controlling overwintering larvae of the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). But, beyond this, their influence in pistachio soil food webs is unclear. Given soil food webs' complexity, *S. carpocapsae* likely interact with more species than just their intended target, infecting alternate hosts or providing food for native predators. This study quantifies the nematodes' effects on soil arthropod and surface arthropod diversity in two large orchards in Madera County, California. We found significantly more isotomid collembolans, predatory anystid mites and gnaphosid spiders under trees where nematodes were applied indicating either direct predation or indirect trophic effects. Significantly fewer *Forficula auricularia* (Dermaptera: Forficulidae) and *Blapstinus discolor* (Coleoptera: Tenebrionidae) were found under treated trees, suggesting a possible non-target infection. Nematode persistence was limited but positively correlated with pitfall catches of the tenebrionid beetles, *Nyctoporis cristata* and *B. discolor*.

Introduction

Entomopathogenic nematodes (EPNs) live in soil and lethally parasitize insects, infecting the larvae of many pest species. As an alternative to chemical insecticides, EPNs are used against pests of several fruit and nut crops (Lacey and Shapiro-Ilan 2008). The EPN, *Steinernema carpocapsae*, can control populations of navel orangeworm (*Amyelois transitella*) in pistachios, a crop with high and increasing value in California (Boriss 2005; Starrs and Goin 2010). Navel orangeworm moths oviposit during the summer and the larvae spend winter feeding on nuts remaining on trees or the ground after harvest, infesting new nuts the next year (Bentley et al. 2008). Throughout most of the pistachio growing season, navel orangeworm is controlled with insect growth regulators and pyrethroid insecticides (Bentley et al. 2008; Zalom et al. 1984), but these methods do not affect overwintering larvae. In the field, *S. carpocapsae* can locate and kill >72% of navel orangeworm larvae when applied at a rate of 10^5 infective juveniles (IJs) $\times$ m^{-2} by manual spraying (Siegel et al. 2004). However, applying EPNs through the irrigation system is more economically feasible and less labor intensive. While pistachio acreage continues to increase (Boriss 2005), the pesticides used to control navel orangeworm are increasingly regulated so that as pistachio producers reduce their pesticide use, EPN applications will likely become a more widely used tactic.

Applied *S. carpocapsae* may interact with native arthropods in several ways. First, they may serve as food for predators. Adding *S. carpocapsae* at the standard rate (2500 m^{-2}) in these ecosystems increases total nematode densities to 35 times above normal (Hodson, unpublished data), representing a substantial pulse of resources for nematode feeding arthropods. Many mesostigmatid mites will eat nematodes (Walter and Ikonen 1989) and astigmatid mites (*Sancassania* sp.) can also consume IJs emerging from insect cadavers, thereby decreasing their ability to persist and reducing the number of infected hosts (Ekmen et al. 2010). Forschler and Gardner (1991) found increases in predatory mites (family Rodarariidae) 1–4 weeks after field-application of EPNs and poor persistence of EPNs has been positively correlated with numbers of total mites and collembolans (Epsky et al. 1988; Gilmore and Potter 1993) indicating that these organisms may be an important mortality factor for naturally occurring and applied EPNs.

Alternatively, EPNs may parasitize non-target insects in the orchard. Bathon (1996) reported that the EPNs, *Steinernema feltiae* and *Heterorhabditis megidis*, generally had little impact on non-target arthropods, but some studies finding no effects only identified insects to the family or order level (Georgis et al. 1991; Campbell et al. 1995), perhaps hiding effects on individual species. In a large scale multi-year study in citrus, microarthropods and enchytraeid worm densities were reduced in *Steinernema riobrave*

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treated plots (Duncan et al. 2007). Other studies measuring changes in individual species abundance found that, while most groups remained unchanged, the densities of four non-target species of chrysomelid and carabid beetles were reduced in *S. feltiae* treated plots, and one species of Curculionidae (*Barypeithes* spp.) was reduced in *H. megidis* treated plots (Buck and Bathon 1993; Koch and Bathon 1993). These results indicate the importance of examining as much of the ecological community as possible when assessing non-target effects.

One concern of applying EPNs is that they will disrupt native communities and ecosystem services provided by soil biodiversity. For example, Millar and Barbercheck (2001) found that when the exotic *Steinernema riobrave* was applied, detection of the endemic EPN *Heterorhabditis bacteriophora* decreased, with possible effects on long term pest suppression. While soil ecosystems are often thought to be resilient (Wardle et al. 1995; De Ruiter et al. 1995), agricultural intensification reduces the abundance of soil biota (Postma-Blaauw et al. 2010) and such losses in biodiversity may leave ecosystems vulnerable to disturbance (Bengtsson et al. 2000). As a potential biological disturbance in pistachios, *S. carpocapsae* could negatively affect species diversity and community composition either through direct trophic interactions, or indirectly through its mutualistic bacteria. These bacteria, in the genus *Xenorhadbus* for Steinernematids, secrete toxins which kill the insect host and also antimicrobial and fungicidal metabolites that protect it from degrading (Isaacson and Webster 2002). The effects of *S. carpocapsae* could also vary within an orchard. For example, if areas of the orchard where diversity is high are more resistant to biological disturbance, *S. carpocapsae* might have stronger effects in low diversity microsites than in higher diversity microsites.

While previous studies have identified *S. carpocapsae* as tool against navel orangeworm in pistachios (Siegel et al. 2004, 2006), its broader ecological effects in these systems remain unknown. This study examines the response of two pistachio orchard ecosystems to the addition of high densities of *S. carpocapsae* and quantifies the relationship between arthropod population densities and nematode persistence. By understanding how *S. carpocapsae* interacts with the ecosystems into which it is applied, we can better predict where it will be able to most successfully survive, persist, and reduce pest populations. Understanding the ecological effects of *S. carpocapsae* in pistachio orchards could also have broader implications on its effects in other high value perennial crops.

Predicting that large numbers of *S. carpocapsae* would affect the orchard soil ecosystem, we posed the following hypotheses:

1. If *S. carpocapsae* IJs are eaten by predatory arthropod species, these arthropods' abundance/activity should increase in treated areas and be negatively correlated with nematode persistence.
2. If *S. carpocapsae* IJs parasitize some arthropod species, host species abundance/activity should decrease in treated areas and be positively correlated with nematode persistence.
3. If adding *S. carpocapsae* IJs represents an ecological disturbance, species diversity, richness, and evenness values may be negatively affected in treated areas.
4. If orchard microsites with low diversity are more susceptible to biological disturbance, *S. carpocapsae* application should have stronger effects in those areas.

Methods

Nematode application

We conducted two field experiments at the commercially managed S & J ranch in Madera County, California. The field experiments were done in different years in separate pistachio orchards (2008: 36°53′58.68″ N, 119°48′08.89″ W; 2009: 36°54′26.91″ N, 119°48′31.34″ W). Soils in the experimental areas (classified primarily as Ramona Sandy Loam) possessed the following characteristics: 63.3% sand, 27.14% silt, 9.57% clay, 0.73% organic matter, and 6.93 pH (from an average of 7 samples). In each experiment, we used *S. carpocapsae* formulated product containing 250 million IJs (supplied by Becker Underwood, Ames, IA), which had been shipped chilled in a plastic tub. The product was stored for no longer than 1 month at 8 °C until used in the experiments. The day of application, nematodes were mixed with water in buckets and viability was confirmed by their movement under a dissecting microscope. Before application, plots were irrigated for several hours. Nematodes were applied through the irrigation system at a rate of $12.5 \times 10^4 \times m^{-1}$ and their viability re-confirmed following passage through the microsprinklers. Microsprinklers deposited nematodes onto a berm, a raised area extending 91–121 cm from the pistachio tree trunks, which is free of vegetation but often contains moss, decomposing nuts and leaves. Since the microsprinklers' range did not extend into the areas between tree rows, the local rate of application on the berm was approximately 25 IJs cm^{-2}.

Experimental design

The 2008 orchard measured 31.8 ha while the 2009 orchard measured 19 ha. Rows of trees were separated by drive lanes approximately 6 m wide containing weeds that were mowed each season. Within each orchard, we randomly chose seven pairs of rows and sampled five trees in each row. We randomly designated one tree in each row pair as a control, plugging its microsprinkler while nematodes were applied. We removed the plugs after ~3 h, which gave the pulse of nematodes time to clear from the irrigation system. All trees were then irrigated for an extra ~4 h to offset the extra water that treatment trees received. Soil sampling dates for each year were March 10, March 19, April 2, April 16 and May 21, which corresponded to 2 days before *S. carpocapsae* application and 1, 3, 5, and 10 weeks after. For each date we sampled soil under 70 trees within the orchard (35 treatments, 35 controls) using different methods (outlined below) for EPN isolations and measurement of arthropod communities. All soil samples were placed in a chilled cooler for transport back to the laboratory and stored at 10 °C until processing.

Soil properties

For all sample time-points, we measured gravimetric soil water content on ~40 g soil as the percent of oven dry soil after 24 h at 105 °C (Black 1965). Using samples from week 10, we measured the electrical conductivity (EC) of soil under each tree using a Hi 255 combination pH/mV electrode and EC/TDS/NaCl Meter (Hanna Instruments Woonsocket, RI). To test if sand content was related to EPN persistence, we also measured particle size on 8 representative trees from each year where EPNs persisted for varying times (0–70 days). The total sand content of each sample was split into five particle size categories (very coarse sand ≥ 1.000 mm; 1.000 mm > coarse sand ≥ 0.500 mm; 0.500 mm > medium sand ≥ 0.250 mm; 0.250 mm > fine sand ≥ 0.106 mm; 0.106 mm < very fine sand ≥ 0.053 mm) using a sieve-shaker apparatus (Ro-Tap®, model RX-29, W.S. TYLER, OH).

S. carpocapsae re-isolation and identification

To determine if *S. carpocapsae* persisted in the orchards, soil samples were taken from the 70 trees immediately after application and at the aforementioned time-points. We also took soil samples 2 days before application to survey for native EPNs. For EPN isolation, we used a spade to sample an area 15 × 15 cm × 3 cm