

The Ceres Trust 2011 Graduate Student Organic Research Grant: Interim Report

1. Project Title: The impact of entomopathogenic nematode applications on mortality of codling moth larvae on the orchard floor

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4. Accomplishments and Projected Activities:

Abstract

Entomopathogenic nematodes are tiny parasites of insects that can be applied in a water mixture and can kill their hosts within only a few hours after infection. The codling moth (*Cydia Pomonella* [L.]) is a serious pest of apples worldwide and is of critical concern in organic apple production. Codling moth larvae pupate and overwinter in silk cocoons in soil surface litter and under the bark on tree trunks or branches. Entomopathogenic nematodes have potential for management targeting codling moth larvae since they actively search out hosts in protected locations. We have been evaluating the entomopathogenic nematodes species, *Steinernema feltiae* (Filipjev), at three Michigan organic apple orchards to test its effectiveness for codling moth management. Our previous research indicated that nematode applications were more effective in orchards planted with smooth-barked tree varieties, presumably because the codling moth larvae in these orchards are forced to overwinter on the ground where they may be more susceptible to infection by entomopathogenic nematodes. This study was designed to test the hypothesis that codling moth larvae in cocoons on the ground are more susceptible to nematode sprays than those in other locations in the orchard. We found that nematodes applied with a backpack sprayer were able to successfully infect codling moth larvae in a variety of locations in the orchard. However, larvae that were in cocoons under wooden shelters on the soil surface did not exhibit increased infection in nematode treated plots compared to those in untreated plots. This result implies that entomopathogenic nematodes may need to be applied at higher rates to kill codling moth larvae in cocoons on the orchard floor in smooth barked orchards with dense undergrowth.

Study Sites

This study was carried out in three organic apple orchards, in Michigan's southern lower peninsula. These included AlMar orchards located near Flushing, MI, The Country Mill located near Charlotte, MI and the Michigan State University (MSU) Clarksville horticultural research center.

Objective 1: Determine the survival of codling moth larvae in sheltered locations on apple tree trunks in EPN treated and untreated plots.

Methods

Nematode rearing

Nematodes were reared in the lab by introducing infective juvenile stage nematodes (*S. feltiae*) in a water mixture into filter paper lined petri dishes containing wax worms (*Galleria mellonella*). The petri dishes were then incubated in complete darkness for 7-10 days at room temperature. Subsequently, petri dishes containing nematode infected wax worms were floated in larger dishes containing water to make a trap for the emerging infective juvenile nematodes. These traps were held at room temperature and checked daily for emerging nematodes. Infective juveniles collected in the traps were transferred in water to a 5 gallon jug equipped with an aquarium bubbler to provide aeration. Infective juvenile nematodes were held in this container for 3 weeks at 55°F before being applied to orchard plots.

Nematode applications were made on the evening of October 28, 2011 between the hours of 5 and 10 p.m. Applications were made with a gasoline powered backpack sprayer at a rate of 600 million infective juveniles/acre/20 gallons of water. All field sites received rainfall the day of application, either immediately before, during, or after application.

Sentinel prey

Sentinel fifth-instar codling moth larvae were allowed to build cocoons in wooden “shelters” in the lab. The shelters were made from aged (~1 year) apple prunings that were cut into 2-inch sections, drilled, and split lengthwise. A piece of kraft paper was then affixed to the flat side of the shelter with hot melt glue to leave a space that would be attractive to fifth-instar codling moth larvae seeking a cocoon location (Figure 1). Individual larvae were then placed in 500 mL plastic containers containing shelters and left overnight to build cocoons in the shelters. In the field, half of the shelters with codling moth larvae in them were placed with the paper covered surface against the tree trunk. The remaining half of the shelters were oriented to leave the paper exposed. This orientation of the shelter left the larvae in a more exposed (unsheltered) situation. Shelters were placed in experimental plots at our three study sites and half of those plots received nematode applications. The remaining plots were left unsprayed to serve as control plots. Three days after application codling moth larva survival in the experimental shelters was assessed. Upon return to the laboratory, the larvae were removed from their cocoons and placed in a petri dish with a piece of moistened filter paper. These dishes were sealed with paraffin film and the mortality of the larvae was assessed daily to determine nematode infection status.

Results

Survival analysis was performed with the Kaplan-Meier method using the statistical software package *survival* in R (v.2.9.2). Mortality of codling moth larvae was significantly higher in nematode treated plots compared with control plots regardless of whether larvae were in shelters in unsheltered or sheltered orientations on the tree trunk ($P < 0.05$) (Figure 2).

Objective 2: Determine the survival of codling moth larvae in sheltered locations on the orchard floor in EPN treated and untreated plots.

Methods

The methods for this objective were identical to the methods for Objective 1 except that the placement of shelters was changed. Shelters containing sentinel larvae for this objective were placed on the orchard floor in direct contact with soil surface. These shelters were also placed either with the paper surface exposed (unsheltered) or in direct contact with the substrate.

Results

Survival analysis was performed with the Kaplan-Meier method using the statistical software package *survival* in R (v.2.9.2). Mortality of codling moth larvae was significantly higher in nematode treated plots compared with control plots in the unsheltered orientation on the orchard floor ($P < 0.05$). Mortality of codling moth larvae in the sheltered orientation in treated plots was not significantly different from mortality of larvae in the same orientation in untreated plots ($P > 0.05$) (Figure 3).

Objective 3: Develop extension materials and online resources to educate growers about EPNs for pest management.

The results from this study were presented in a poster at the Great Lakes Fruit Vegetable and Farm Market Expo in Grand Rapids, MI (December 2011) and at the Midwest Organic and Sustainable Education Service conference in LaCrosse, WI (February 2012). This poster is available for download as a .pdf file on our website (www.opm.msu.edu). We are presently working with the Michigan State University College of Agriculture and Natural Resources communications staff to prepare an extension bulletin that will contain the results of this research.

Figures

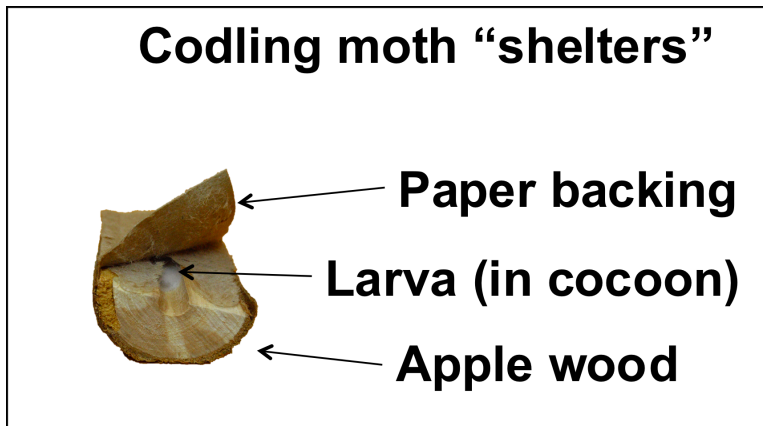


Figure 1. Diagram showing the composition of a codling moth shelter used to evaluate sentinel codling moth larval mortality in different locations in apple orchards for this study.

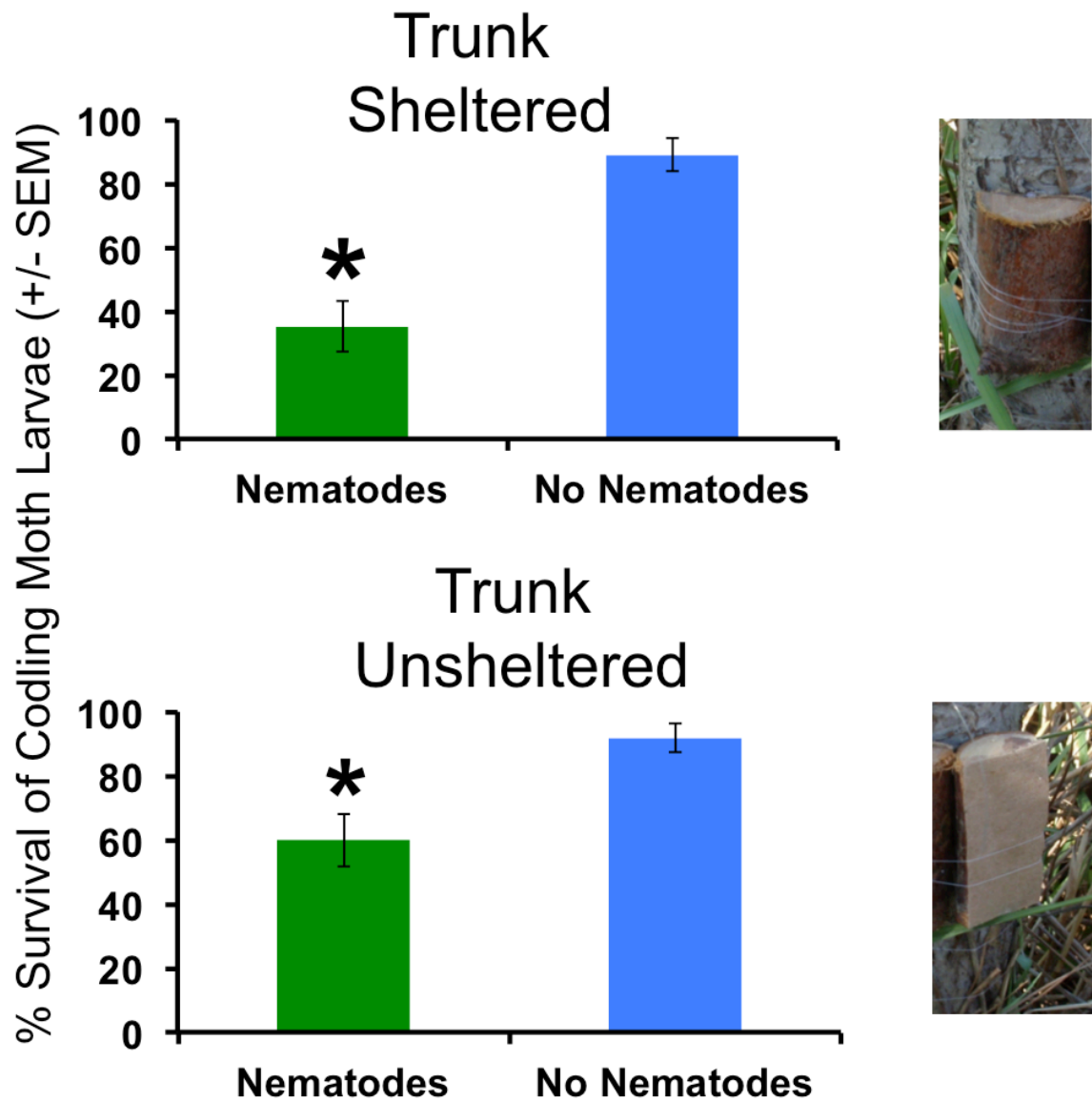


Figure 2. Percent survival of codling moth larvae in wooden shelters placed on apple tree trunks that received nematode applications (Nematodes) or didn't (No Nematodes).

*Larvae in nematode treated plots had a significantly ($P < 0.05$) lower percent survival than those in the same orientation in untreated plots.

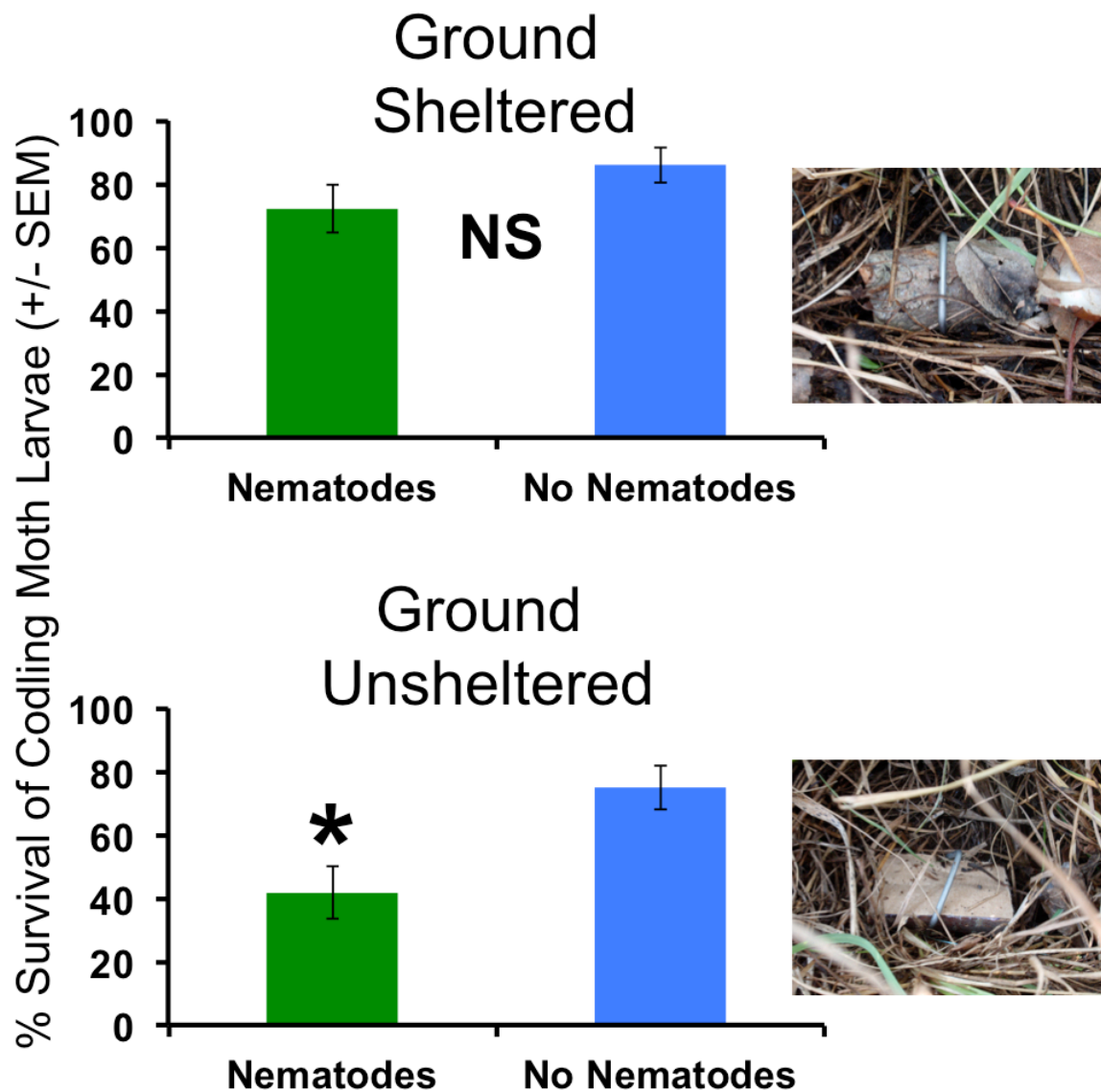


Figure 3. Percent survival of codling moth larvae in wooden shelters placed on the orchard floor that received nematode applications (Nematodes) or didn't (No Nematodes).

*Larvae in nematode treated plots had a significantly ($P < 0.05$) lower percent survival than those in the same orientation in untreated plots.

^{NS} No significant difference ($P > 0.05$) was found between the percent survival for larvae in shelters on the ground in nematode treated vs. untreated plots.