POTENTIAL FOR MANAGEMENT OF THE CLOVER CROWN BORER PEST IN RED CLOVER SEED PRODUCTION USING BIOLOGICAL CONTROL

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The clover crown borer (also known as the clover root borer), *Hylastinus obscurus*, is a major pest of red clover seed production in the Willamette Valley (Rockwood 1926, Rao et al. 2012). This bark beetle pest, native to Europe, was inadvertently introduced into the US over 100 years ago. Damage to red clover is caused by adult and larval feeding internally within roots. This disrupts nutrient and moisture transport within the plant, and as a result, infested plants turn brown, wilt and die (Rockwood 1926). The presence of five or more larvae per root can result in 43% reduction in above-ground foliage (Koehler et al. 1961). In addition, mining caused by the pest often becomes a site for infection for pathogens that also contribute to a decline in clover stands. Due to all of these factors, clover crown borer infestation leads to a reduction in plant population, forage yield, and seed yield in the subsequent year. Thus, even though red clover is a perennial plant, the crop can only be grown economically for two years (Rockwood 1926, Steiner and Alderman 2003).

Management tactics for the clover crown borer are critical in the Willamette Valley where red clover is raised for seed on approximately 20,000 acres, and economic losses can be extensive. However, the clover crown borer is a challenge to control due to its subterranean life cycle. In the past, it was managed with organochlorine insecticides such as aldrin, BHC, chlordane, dieldrin, heptachlor and lindane (Gyrisco and Marshall 1950, Gyrisco et al. 1954, Koehler et al. 1961). These insecticides were, however, subsequently banned due to negative impacts on the environment resulting from their persistence in the environment. No new insecticides were labeled for the pest (http://www.ipmcenters.org/pmsp/pdf/WestAlfalfa CloverSeed.pdf) due to the challenge of getting insecticide materials to reach clover crown borer larvae and adults feeding within the roots. In a field trial conducted in 2011, four insecticides labeled for red clover seed production were evaluated but none caused significant mortality compared with the controls (Rao et al. 2012).

For other subterranean pests, biological control with nematodes that are pathogenic to insects, which are known as entomopathogenic nematodes, has been effective (Shapiro-Ilan et al. 2012). Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are potent biocontrol agents that kill a wide variety of economically important insect pests, and are applied at a commercial scale in numerous cropping systems (Grewal et al. 2005). The two genera differ in the manner in which they seek hosts. *Steinernema* spp. are sedentary and use ambush tactics while *Heterohabditis* spp. actively seek out their hosts. Currently, there is no information on the impacts of these species on the clover crown borer. Hence the objective of this study was to determine the virulence of *Steinernema* and *Heterorhabditis* spp. nematodes when exposed to clover crown borer adults.

Material and Methods

Over 300 clover crown borer adults were obtained from two commercial red clover seed production fields in the Willamette Valley. Infested roots were placed in Berlese funnels at Oregon State University for collection of adults. The adults were kept cool in the refrigerator for 3-5 days until they were used in the experiments.

Two species each of commercial *Steinernema* and *Heterorhabditis* nematodes were evaluated in a laboratory bioassay. These included *H. marelata*, *H. bacteriophora*, *S. carpocapsae*, and *S. kraussei*. Nematodes were added to filter paper along with distilled water in petri dishes and exposed to ten clover crown borer beetles per dish. Each nematode species was evaluated at the following two concentrations, 25 infective juveniles per cm^2 and 75 infective juveniles per cm^2, with four replications for each treatment. Petri dishes containing clover crown borer adults but no nematodes served as the controls.

Clover crown borer beetles in each petri dish were monitored every other day for three weeks. The

numbers of dead adults in each petri dish were recorded to determine which nematode species and dose, if any, caused greater mortality compared with the controls.

Data Analysis
Data on mean mortality of clover crown borers per petri dish were analyzed using analysis of variance.

Results
Entomopathogenic nematodes belonging to both genera evaluated, *Steinernema* and *Heterorhabditis* were observed to cause mortality of adult clover crown borers in the laboratory bioassay. However, mean mortality of clover crown borer adults varied across the nematode species and dose tested (Figure 1; $P < 0.01$). Over 15% mean mortality was recorded with the higher dose (75 infective juveniles per cm^2) for all nematode species evaluated except *H. bacteriophora*. No mortality was observed in the controls, while mean mortality in dishes exposed to the lower dose (25 infective juveniles per cm^2) of all species was minimal, ranging from 0 to less than 5%.

Discussion
This is the first study that has evaluated the potential for biological control of the clover crown borer with entomopathogenic nematodes. The laboratory bioassay documented that adult clover corn borers can be killed when exposed to *H. marelata*, *H. bacteriophora*, *S. carpocapsae*, and *S. kraussei* in petri dishes. However, the dose is critical; for all species tested except *H. bacteriophora*, the higher dose (75 infective juveniles per cm^2) caused considerably greater mortality compared with the controls.

Based on the promising results of this study, further research is needed to determine the impact of *Steinernema* and *Heteorhabditis* spp. in commercial red clover seed fields. Nematodes have been shown to effectively locate and infect another Willamette valley pest, the strawberry crown borer, *Synanthedon bibionipennis*, larvae of which are located in the strawberry crown (Bruck et al. 2008). However, as the clover root borer develops within red clover roots, there may be limited impact when the nematodes are applied to the soil but this needs to be evaluated. A more effective strategy could be autoinoculation, a tactic in which pest insects are used to vector the biological control pathogen to conspecifics, other members of the same species, after they have acquired the pathogen (Vega et al. 1995). For this tactic, the beetles need to be lured initially to traps containing the entomopathogenic nematode. Typically, food baits are used as the lure, but host plant volatiles or pheromones may be effective for the clover root borer since bark beetles are known to respond effectively to olfactory cues from hosts and conspecifics.

For other pest species, much higher doses than the ones included in the current study have been used. High doses were not evaluated in the current study due to the low cash value of red clover seed crops and high cost of entomopathogenic nematodes. However, with the autoinoculation approach, entomopathogens will need to be employed in relatively small quantities and thus costs will be greatly reduced. Given the lack of alternative management tactics, and the positive results documented in this study, this approach warrants further investigation.

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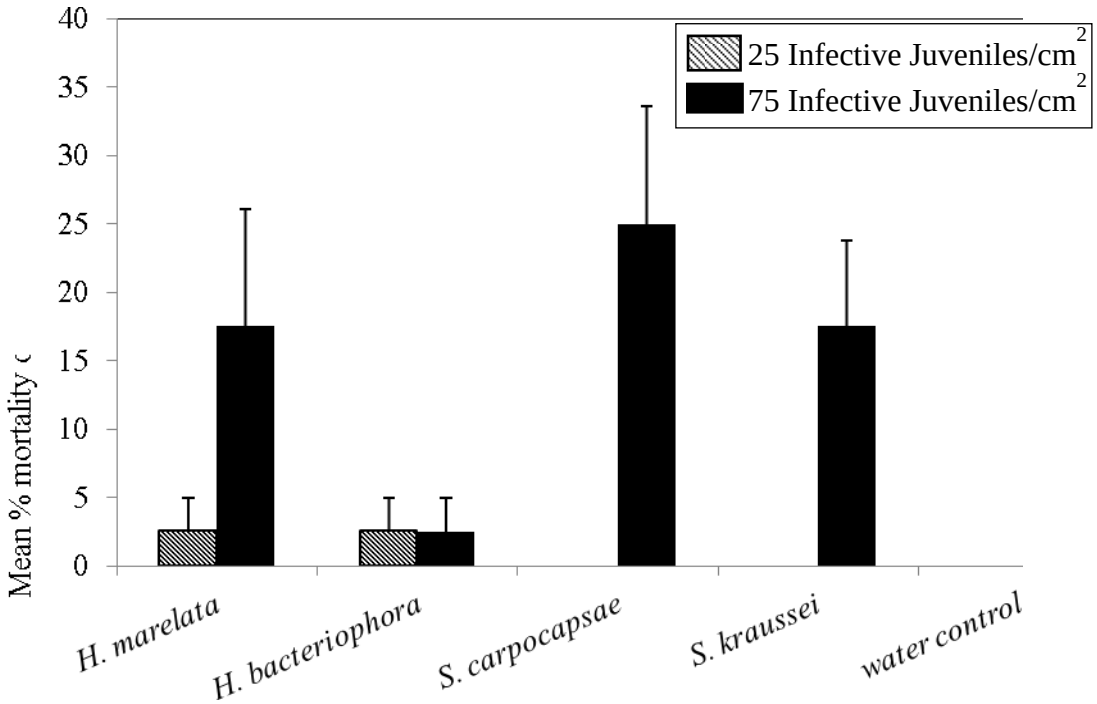

Figure. 1. Impact of entomopathogenic nematodes on adult clover crown borers in a laboratory bioassay ($P < 0.01$).