#### **Research Final Report**

# OREGON DEPARTMENT OF AGRICULTURE OAN NURSERY RESEARCH COMMITTEE

Title: Snail and slug-killing nematodes in Oregon nurseries

#### **Principle Investigators:**

Rory Mc Donnell, Assistant Professor, Dept of Crop and Soil Science, Oregon State University, Corvallis OR 97333; E-mail: <u>rory.mcdonnell@oregonstate.edu</u>; Tel: 541-737-6146

Dee Denver, Professor, Dept of Integrative Biology, Oregon State University, Corvallis OR 97333; E-mail: <u>denvedee@cgrb.oregonstate.edu;</u> Tel: 541-737-3698

#### **Start and End Dates**

January 1<sup>st</sup> 2018 to December 31<sup>st</sup> 2019. Research project funded for 2018, granted an extension through 2019.

#### Background

Snails and slugs are among the most damaging pests of horticultural production in Oregon. In fact, various states in the US have quarantine restrictions concerning plant materials imported from the Pacific Northwest. Despite the significant economic losses caused by these organisms, control measures are focused heavily on the use of chemical molluscicides. However, growers report considerable variation in efficacy of the active ingredients (metaldehyde, iron phosphate, sodium ferric EDTA, and methiocarb). Metaldehyde is the most widely used, but in baited form it is attractive to dogs and can be fatal if ingested. Methiocarb is a restricted use material with the DANGERPOISON signal word. It is neurotoxic to humans, and highly toxic to birds, aquatic taxa particularly fish, and bees. Methiocarb has also been recently banned in Europe because of its damaging non-target effects (Gov. UK, 2018). Iron phosphate and sodium ferric EDTA are stomach poisons and there is evidence to suggest that they can be harmful to earthworms (Edwards et al., 2009). There is hence an urgent need to identify and develop alternative control practices for growers in the region. One such strategy is biological control, which is the use of natural enemies to control a pest.

Slugs and snails have a diverse range of natural enemies (Barker, 2004) but their biological control potential has been largely overlooked in the US. In Europe, a nematode called *Phasmarhabditis hermaphrodita* is currently being used as a commercially available biological control option called Nemaslug®. The latter is lethal to a wide range of pest slugs and snails. Furthermore, in Europe Nemaslug® has been successfully used to reduce gastropod damage in a diverse range of horticultural crops. For example slug damage to orchids is consistently reduced by >90% when this product is used (Rae et al., 2007). However, Nemaslug® is not currently available in the US because, until recently, the nematode was never found here.

During 2017, we discovered this nematode for the first time in Oregon (Mc Donnell et al., 2018) and this ground-breaking discovery opens the door to serious consideration of *P. hermaphrodita* as a potential biocontrol agent for the management of significant snail and slug pests in Oregon nurseries. However, many important questions, central to the eventual development of nematode-based gastropod management products and practices in the Pacific Northwest, remain to be answered. A key question is how widespread are *P. hermaphrodita* and other species of *Phasmarhabditis* in Oregon? This provided the incentive for the current study.

## **Project Objective**

The primary objective of this study is to determine the incidences and species richness of malacopathogenic nematodes associated with key snail and slug pests in Oregon nurseries with a focus on *Phasmarhabditis*.

### **Final Results**

### A. Surveys for snails and slugs in Oregon nurseries

We collected slugs and snails from 20 different nurseries throughout the state. The nurseries were located in Medford, Grants Pass, Roseburg, Eugene, Corvallis (two locations), Portland, Brookings, Florence, Seaside, Astoria, Tillamook, Hermiston, Island City, Baker City, Klamath Falls, Bend, Redmond, Madras and Hood River. At each nursery, specimens were collected by hand in areas offering shelter, and abundant moisture e.g. under potted plants. Signs of slugs and snails including eggs, mucus trails, and ribbon-like feces also helped direct surveys to areas of active gastropods. Specimens were collected into plastic containers and stored in a cooler and then returned to the laboratory at Oregon State University.

### B. Nematode isolation and identification

Collected gastropods were maintained in colonies in the laboratory and as they died they were examined under light microscopy. M9 buffer was also pipetted onto dead slug and snail specimens and the solution was then transferred to a Petri dish to look for the presence of nematodes. Nematodes were pipet-transferred to NGM agar plates and allowed to grow at 20°C. Nematode cultures were subcultured to new plates every two weeks to ensure lab populations did not die out due to overgrowth.

After nematode cultures were established for ~two weeks in the lab, we performed DNAbased species identification of nematodes in culture. This was achieved by PCR-amplifying and sequencing a region of the 18S rDNA gene. DNA sequences were compared to sequences present in the GenBank database to find species matches.

### C. Nematodes discovered

During our surveys, we discovered the gastropod-killing nematode, *Phasmarhabditis californica* for the first time in Oregon. This nematode species was first discovered in California in 2013 as a species new to science (Tandingan De Ley et al. 2016) and has subsequently been shown to be a lethal parasite of slugs and snails (Tandingan De Ley et al. 2017). Our discovery of this nematode in slugs collected in nurseries in Brookings, Eugene, Corvallis and Portland (Table 1)

suggests that it is more widespread in Oregon than *P. hermaphrodita*, which has only been found on the main OSU campus in Corvallis (Mc Donnell et al. 2018).

Location	City	County
Chet's Garden Center	Brookings	Curry
Fox Hollow Creek Nursery	Eugene	Lane
Shonnards Nursery, Florist and Landscape	Corvallis	Benton
Home Grown Gardens	Corvallis	Benton
Marbott's Greenhouse and Nursery	Portland	Multnomah

**Table 1.** Collections of *Phasmarhabditis californica* from garden centers and nurseries duringthis project. No additional species of *Phasmarhabditis* were recorded during surveys.

### **Benefit to Nursery Industry**

There is an urgent need for the development of novel tools for controlling snails and slugs in plant nurseries in Oregon. One such option is biological control using nematodes in the genus *Phasmarhabditis*. As mentioned above *P. californica* was discovered in Oregon for the first time during this project and it appears to be more widespread (four counties) than *P. hermaphrodita* (one county). Given the more widespread distribution of *P. californica*, it may be a better candidate for biological control than *P. hermaphrodita*. However, before an informed decision can be made on the efficacy and safety of these nematodes as biological control agents in Oregon, there are many knowledge gaps that need to be filled e.g. host range testing of different *Phasmarhabditis* species on both pest and native gastropod species.

#### References

Barker, G.M. (2004) Natural Enemies of Terrestrial Molluscs. CABI Publishing, Oxfordshire, United Kingdom

Denver, D.R., Morris, K., Thomas, W.K. (2003) Phylogenetics in *Caenorhabditis elegans*: an analysis of divergence and outcrossing. *Molecular Biology and Evolution* **20**: 393-400.

Edwards, C.A., Arancon, N.Q., Vasco-Bennett, M., Little, B. and Askar, A. (2009) The relative toxicity of metaldehyde and iron phosphate based molluscicides to earthworms. *Crop Protection* **28**: 289-294

Mc Donnell, R.J., Lutz, M., Howe, D., and Denver, D. (2018) First Report of the Gastropod-Killing Nematode, *Phasmarhabditis hermaphrodita*, in Oregon, U.S.A. *Journal of Nematology* **50**: 77-78.

Rae, R., Verdun, C., Grewal, P.S., Robertson, J.F. and Wilson, M.J. (2007) Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita*--progress and prospects. *Pest Management Science* **63**: 1153-1164

Tandingan De Ley, I., Mc Donnell, R.J., Paine, T. and De Ley, P. (2017) *Phasmarhabditis*: The Slug and Snail Parasitic Nematodes in North America. In: *Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes* (Eds. M. M.M. Abd-Elgawad, T. Hassan Askary, and J. Coupland). Pages 560-578. CABI Publishing.

Tandingan De Ley, I., Holovachov, O., Mc Donnell, R.J., Bert, W., Paine, T. and De Ley, P. (2016) Description of *Phasmarhabditis californica* new species and first report of P. *papillosa* (Nematoda: Rhabditidae) from invasive slugs in the United States. *Nematology* **18**: 175-193.

Tandingan De Ley, I., Mc Donnell, R.J., Lopez, S., Paine, T., De Ley, P. (2014) First record of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae), a potential biocontrol agent isolated for the first time from invasive slugs in North America. *Nematology* **16**: 1129 – 1138.