AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2017 – 2019

TITLE: Malacopathogenic nematodes associated with the key slug pest, *Deroceras reticulatum*, in Oregon agriculture

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EXECUTIVE SUMMARY:

OBJECTIVES: The primary objective of this study is to determine the incidences and species richness of malacopathogenic nematodes associated with the invasive, gray field slug, *Deroceras reticulatum*, which is the most damaging slug species in Oregon agriculture. The supporting objectives are
1) survey for and collect specimens of the gray field slug from a variety of vulnerable crops throughout the state
2) identify nematodes associated with this key slug pest using a molecular-genetic strategy
3) generate preliminary data on the geographic range of these nematodes

PROCEDURES:
1. Surveys for the gray field slug

Gray field slug specimens have been collected from a number of vulnerable crops throughout the state (Table 1). A minimum of 30 specimens were either collected by hand at each site during times when slugs were active i.e. during wet weather or using slug refuge traps (Figure 1). Specimens collected at each site were placed in labelled Ziploc bags and returned to the laboratory.

Table 1. Sites and crops where *Deroceras reticulatum* has been collected to screen for malacopathogenic nematodes.

Site number	Crop	Location	Sampling
1.	Annual ryegrass (No till)	North of Harrisburg (Peoria Rd)	Trap
2.	Annual ryegrass (No till)	North of Harrisburg (Jenson Lane)	Trap
3.	Annual ryegrass (No till)	Shedd (Boston Mill Dr)	Trap
4.	Annual ryegrass (Tilled)	Shedd (Boston Mill Dr)	Trap
5.	Annual ryegrass (Tilled)	Tangent (Hampton Lane)	Trap
6.	Annual ryegrass (tilled)	Tangent (Midway Dr)	Trap
7.	Christmas trees (Douglas fir)	Oregon City (Carus Rd)	Trap

8.	Christmas trees (Douglas fir)	Oregon City (Leland Rd)	Trap
9.	Christmas trees (Douglas fir)	Stayton (Lower Wagner)	Trap
10.	Christmas trees (Douglas fir)	Lyons (Upper Wagner)	Trap
11.	Christmas trees (Douglas fir)	Corvallis (Beaver Creek Rd)	Trap
12.	Christmas trees (Douglas fir)	Cheshire (Hwy 36)	Trap
13.	Ornamentals (potted plants)	Shonnards Nursery, Corvallis (Hwy 20)	Hand
14.	Ornamentals (potted plants)	Schmidt's Garden Center, Corvallis (NW 29th St)	Hand
15.	White clover	Halsey (Powerline Rd)	Hand

Figure 1. A slug refuge trap used to collect specimens for nematode screening.

2. Nematode isolation

In the original proposal we stated that we would use two different approaches for screening field-collected slugs for nematodes i.e. examining their frass and as the slugs die their cadavers under a microscope. However initial preliminary studies with slug frass resulted in a number of false positives and consequently we decided to focus on the cadaver method. This latter approach was used successfully by Rory Mc Donnell and colleagues to collect malacopathogenic nematodes including *Phasmarhabdits hermaphrodita* from slugs in California (Tandingan De Ley et al. 2012; 2016).

All field collected slugs were kept in plastic containers (12" x 8" x 4") lined with moist paper towels and the slugs were fed organic carrot. A maximum of 30 slugs were kept per container and to avoid cross contamination only slugs from the same field site were kept together. The food and towels were replaced twice weekly. Each box was checked for slug cadavers three times weekly and when found the cadavers were examined carefully under a dissecting light microscope for nematodes. The latter are typically visible swimming through the decomposing slug flesh and often number in the thousands. Surprisingly we have not collected any nematode specimens to date.

3. Nematode identification

When we discover nematodes, they will be first photo-documented using a light-microscope camera assembly available in the Denver lab, and then undergo a DNA extraction followed by polymerase chain amplification (PCR) procedure targeting the 18S ribosomal RNA (rRNA) gene, which is a standard for nematode molecular species identification. PCR products will be directly sequenced at the OSU Center for Genome Research and Biocomputing; DNA sequences will then be compared to the GenBank database for species identification. 18S rRNA sequences that are at least 98.5% identical to database sequences are considered the same species; those with lower percent identity are considered candidate new nematode species.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

Although we have collected >1,500 *D. reticulatum* in total from fifteen study sites comprising four different crops throughout the state, we have not recovered any malacopathogenic nematodes from these specimens to date. This is surprising because we know that *P. hermaphrodita* and other malacopathogenic nematodes are present in Oregon i.e. we have found *P. hermaphrodita* in specimens of *D. reticulatum* collected on the main OSU campus in Corvallis. Over the coming 12 months we will continue to collect slugs from additional crops throughout the state.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

Mc Donnell and Denver have submitted a grant proposal to US Department of Agriculture for Farm Bill funding, and with colleagues from University of California Riverside, we have submitted a separate proposal for US Department of Agriculture Multi-state Specialty Crop Block Grant funding. These proposals are currently being reviewed by the funding agencies.

FUTURE FUNDING POSSIBILITIES:

We are in the process of writing a grant proposal with Man-Yeon Choi (USDA-ARS- Horticultural Crops Research) and Ruth Martin (USDA-ARS-Forage Seed and Cereal Research) for submission to the Oregon Department of Agriculture Specialty Crop Block Grant Program. The focus of the proposal will be to assess novel approaches for controlling slug pests including biological control using nematodes.

When we find additional populations of *Phasmarhabditis* or other gastropod-killing nematodes we envision submitting a new proposal for ARF funding to assess the infectivity of Oregon strains of these nematodes to pest and non-pest (native) gastropod species.