Proposal for Oregon Department of Agriculture for Nursery Research (2021)

Title: Develop bioactive peptides as potential control agents for pest slugs

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Project Background and Justification: As generalist herbivores, many pest slugs, native and exotic, have detrimental impacts on almost all crops including nursery plants, and field and row crops in western Oregon and Washington. Slugs are a serious pest in container nurseries, and may be a contaminant when nursery products (ex: Christmas trees) are shipped to other places. The gray garden slug, *Deroceras reticulatum* (Gastropoda: Agriolimacidae), is the most destructive pest for a variety of greenhouse and nursery crops. The combined value in 2019 of two of the most important vulnerable commodities i.e. greenhouse and nursery ($955 million) and Christmas trees ($104 million) according to USDA and ODA is $1 billion. In recent years, slug damage has accounted for nearly $100 million in damage to the $500 million grass seed industry. Those economic impacts are increasing every year.

Currently the most common slug control methods rely on chemical pesticides that are mixed in pellet bait-based products for growers. For crop protection, the delivery and efficacy of this application is limited. There are also environmental risks associated with chemical residue in soil and water, their effect on non-target arthropods, human health, and development of resistance. Therefore, it is essential to develop appropriate management strategies, which focus on biologically-based environmentally friendly methods.

Neuropeptides and G-protein coupled receptors (GPCRs) are involved in almost all physiological processes including response to light, odorants, peptides, lipids, neurotransmitters, hormones , etc. The slug neuropeptides and their receptors provide great potential for developing new control approaches, including RNAi, receptor interference (Receptor-i), and bioactive peptides.

During the previous projects (OR seed Council and ODA), we identified a variety of neuropeptides and GPCRs for targets in the slug, and discovered potential bioactive peptides, which have significantly reduced slug survival in the laboratory tests (submitted a patent by Choi & Martin et al., 2020). In this proposal, therefore, we further explore those bioactive peptides through various tests: 1) *in vitro* analysis, and 2) *in vivo* assays to identify bioactive peptides.

Management of slugs under biological controls is one of top research priorities from nursery growers. Development of bioactive peptides for pest slugs proposed in this project will be a great potential to approach molecular and biological tools. The impact of developing a biologically-based slug control, which is a chemical pesticide alternative, would be significant for thousands of growers and stakeholders in the nursery industry. Our long-term goal – development of a biologically-based control for slugs will add a new environmentally-friendly slug management to the integrated pest management (IPM) tool box, contribute to the reduction of slug populations, and concomitantly reduce environmental impacts and losses in agriculture.

Objectives: Slug neuropeptides and GPCRs can potentially be used to develop a new class of

molluscicides. Recently, we found 16 small peptides translated from slug genes, and some peptides were actively bound to two slug receptors expressed in Sf9 cells (Ahn et al. 2017; Choi & Martin et al. 2020). When the bioactive peptides were injected into the gray garden slug, they triggered a noxious effect on the slug's behaviors such as fast initial movement, extrusion of tentacles, excess milky mucus production, and dehydration (Fig. 1). The peptide injection probably affected the receptors to cause such dramatic effects and did lead to slug death.

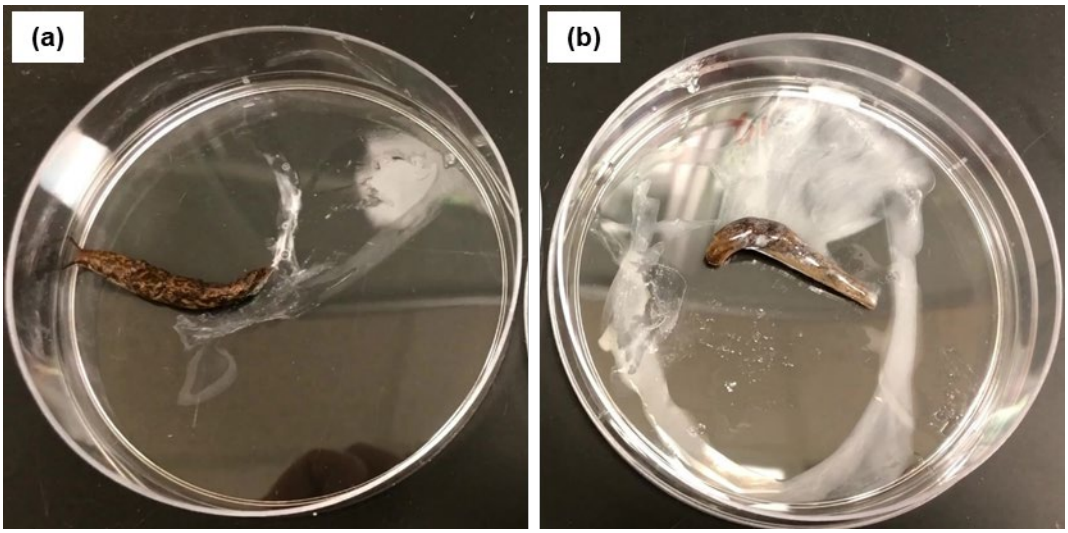

Figure 1. Effect of a small peptide on behavior of the gray garden slugs. (a), water control; (b), injected with the bioactive peptide.

Objective 1. Screening of bioactive peptides using *in vitro* system (0.5 yr)
Objective 2. Screening of bioactive peptides using *in vivo* assays (0.5 yr)

Methods and Timelines:
1. Screening of bioactive peptides using *in vitro* system (0.5 yr)
All peptides (purity > 95%) will be synthesized from Peptide 2.0. Experiments for all the peptides binding affinity to the GPCRs expressed in Sf9 insect cells will be modified as described in our previous publications. The *in vitro* screening will provide to select bioactive peptide candidates.

2. Screening of bioactive peptides using *in vivo* assays (0.5 yr)
2-1. Injection: To evaluate impact on the gray garden slug, various peptides identified from slugs or other invertebrates will be dissolved in 5 µl of purified water (10 nmol) and injected into 20 slugs per peptide. The slugs will be kept under dry or damp conditions for 24 h to evaluate mortality and weight loss every 3 h.

2-2. Feeding**:** Peptides will be dissolved in 10 µl water (20 nmol total), then applied onto a lettuce-leaf disk (2 cm diameter). Ten slugs will be fed each treatment. Slug mortality and weight loss will be recorded for 3 days. Mortality and weight loss will be analyzed as in injection tests above.

Budget summary:

Salary[1]	Travel[2]	Materials & Supplies[3]	Total
$17,300	$500	$3,500	$21,300

[1]Salary (0.3 FTE = $17,300) for research associate level. [2]Support the postdoc travel for commission and/or entomological meetings; [3]Molecular biology materials & supplies, and slug rearing materials ($3,500).