AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
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TITLE: Development of RNAi-based biopesticide to control slugs on agricultural crops

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SUMMARY: Slugs are a worldwide problem on agriculture. In Oregon, a variety of slugs identified including native and invasive species, attacking a broad spectrum of crops and agricultural industries. The gray garden slug (GGS), *Deroceras reticulatum*, is the most destructive and omnivorous pest on a variety of crops in the greenhouses and fields. The infestations and problems of the slug are getting worse and affecting a wide range of seed growers, field crops, row crops, Christmas trees, and horticultural nurseries. The estimated economic impact from crop yield loss, drop in market value, re-planting and higher management cost is over fifty million dollars in the Oregon alone, and increasing every year. Currently the most commonly 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 when the pressure of slug population is light, but does allow the population to recover over time. There are also environmental risks including chemical residue, non-target invertebrate, and human health. Therefore, we are faced to develop appropriate management strategies which are focused to develop biologically-based environmentally friendly alternatives.

RNA interference (RNAi) for pest management is a new direction of the pest control, a biologically-based and target specific strategy. The mechanism of RNAi action is a specific knockdown of target gene expression, degradation of a target messenger RNA (mRNA), by double-stranded RNA (dsRNA) that blocks target protein synthesis, leading to failure of normal physiological functions in the organism. In the past ten years, the application of RNAi techniques has progressed rapidly, and shows great potential for novel insect pest control alternatives because it poses little or no negative impact on the environment. We will employ a novel RNAi technology to apply for slug control. To develop an RNAi-based biopesticide the major key step is to identify suitable RNAi target(s) from specific pest. Therefore, the screening of effective RNAi target(s) with multiple candidates is a critical initial step because RNAi impacts vary depending on target genes and pests.

OBJECTIVES: Our 2016-18 objectives are as follows:
The long-term goal of this research objectives is the development of RNAi-based biopesticide as an environmentally-friendly control that is non-toxic and non-transgenic slug control method. In the present proposal, therefore we focus on the screening and identification of suitable RNAi target(s) from the gray garden slug. Based on our RNAi experience, knowledge and previous RNAi reports, we will select at least ten (> 10) potential candidates for initial screening from the slug.

Objective 1. RNA sequencing to investigate RNA expression profile in the slug.
Objective 2. Identify target genes (~10 genes) from the slug and design dsRNA of each genes.
Objective 3. Evaluate RNAi impacts (i.e. mortality or developmental delay) on the slug.

PROCEDURES:
1. RNA sequencing (1st yr.): To search suitable RNAi targets from the slug the first basic approach is to study the next generation sequencing (called RNA sequencing or transcriptome analysis) technology, to identify target gene expression profile from the slug. This approach and result provided key biological

data to determine RNAi targets and identify specific target gene sequences. To establish the slug colony GGSs were collected from horticultural gardens in Corvallis area, OR, and reared with cabbages in a rearing room at 15±1ºC under a dim light. Total RNA was isolated from either fresh or frozen samples collected.

2. Identify RNAi target genes from the slug (1st yr.): Based on our experience and the previous reports that most of those genes have been targeted for RNAi-based approach to control insect pests. As constitutive or essential genes, housekeeping genes are expressed in all cell types at a level that does not fluctuate with the cell cycle. We selected RNAi target (~10 housekeeping genes). Specific primers and/or degenerate (mixed) primer set designed with 5'-T7 promoter appended was designed to amplify partial lengths between 200- 400 nucleotides of the each target gene found in the slug sequence data.

3. Design and construct dsRNAs for RNAi material: Once confirmed the gene sequence the DNA fragments were served as the template to synthesis dsRNA (2nd yr.).

4. Bioassay and evaluation of RNAi impacts on slugs: Each RNAi targets will be injected into juvenile or adult stages of the slugs. After injection, phenotypic changes will be monitored (2nd yr.).

SIGNIFICANT ACCOMPLISHMENTS TO DATE:
We completed Obj. 1 &2 in 2016. We selected more than 10 candidate RNAi targets and identified actual DNA sequences (Table 1). Housekeeping genes as constitutive genes are expressed in all cell types at a level that does not fluctuate with the cell cycle. Functional examples of housekeeping genes for RNAi targets are related in the muscle physiology, detoxification, ATP metabolism, protein sorting and transporting, and cell membrane structure in cells. These genes have been selected for RNAi candidates to develop RNAi-based control for insect pests.

Table 1. RNAi target sequences identified from the slug

No.	Gene	Contig	Length (bp)
1	Target 1	c7637_g1_i1	655
2	Target 2	c66177_g1_i1	3928
3	Target 3	c69242_g1_i1	4584
4	Target 4	c65057_g1_i1	1752
5	Target 5	c66214_g1_i1	3517
6	Target 6	c69575_g1_i1	863
7	Target 7	c50123_g1_i1	1260
8	Target 8	c68383_g1_i1	3062
9	Target 9	c113158_g1_i1	1636
10	Target 10	c67742_g3_i1	1662
11	Target 11	c61113_g1_i1	1329
12	Target 12	c53555_g1_i1	666
13	Target 13	c27224_g1_i1	346
14	Target 14	c42322_g1_i1	238
15	Target 15	c55007_g1_i2	571

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: PI has received additional funding from Oregon Nursery Association in Oregon Department of Agriculture, and Oregon Seed Council (OSC).

FUTURE FUNDING POSSIBILITIES: We continue to submit a research proposal for OSC and ODA, and currently collaborate with OSU scientists.