Proposal for Oregon Department of Agriculture for Nursery Research (2023)

Title: Identification of bioactive peptides as potential targets for thrips control in nursery crops

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Project Background and Justification: Pest thrips have hundreds of host plants, including many ornamental and nursery crops. Thrips not only cause direct damage from feeding and oviposition on leaves, flowers and fruits, they also transmitted economically important plant viruses, such as the tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV). Western flower thrips (WFT), *Frankliniella occidentalis*, is one of the most economically important pests due to its serious damage to nursery crops worldwide. Numerous studies of WFT have been documented on the species complex, and biological aspects including feeding and oviposition behaviors, control options, and resistance of chemical insecticides. Due to their extremely small size and broad host range, detecting and preventing the spread of WFT is extremely difficult. Currently, this pest is primarily controlled with conventional chemical insecticides, which could result in a detriment to human and environmental health as well as development of pesticide resistance. It is essential to develop appropriate management strategies, which focus on environmentally friendly alternatives.

Recently, biologically derived tools, such as RNA interference (RNAi), have been suggested as potential thrips management tactics. The WFT genome (published 2021) revealed insight onto a variety of molecular and evolutionary processes including neuropeptides and their receptors. Insect neuropeptides and G-protein coupled receptors (GPCRs) are involved in almost all physiological processes including response to light, odorants, peptides, lipids, neurotransmitters, hormones, etc. Therefore, the thrips neuropeptides and their receptors provide great potential for developing new control approaches, including RNAi, receptor interference (Receptor-i), and bioactive peptides.

In the previous projects, we developed a sustainable mass rearing method for WFT, including optimized rearing conditions and techniques to minimize mite contamination and mold infestation in the lab and greenhouse (2022, Paper 1). We also established a DNA marker for molecular identification for thrips species. The mass rearing technique facilitates identifying biological targets using various '-omics' tools such as genomics, proteomics, and metabolomics to develop alternative options for pest thrips. In addition, we developed a novel nano-injection system (Fig. 1) and protocol for microinsects (< 3 mm) using the thrips model (2022, Paper 2). We found a suitable injection site with minimal injury to the thrips without physiological stress on the insects. Our method will facilitate injection bioassay with biological compounds into micro-live insects such as thrips without any immobilization tools.

In this proposal, we will explore bioactive peptides to find potential biological targets to develop novel management strategies. We, particularly, focus on identification of *capa* and *pyrokinin* genes and peptides from the genes because these peptides are involved in a variety of biological functions: hindgut muscle contraction in the digestive track, feeding, pheromone biosynthesis, cuticle melanization, and diapause for different life stages. Therefore, successful achievement of our goal will add a new environmentally friendly thrips control, and would be significant for thousands of growers and stakeholders in the nursery and horticulture industry. Thus, outcomes of this research are expected to address fundamental requirements for the application of biological tools for controlling other thrips.

Projective Objectives: The long-term goal of this research objectives is the development of a new biologically-based strategy to control pest thrips. We have recently initiated to identify the neuropeptide genes and corresponding receptors, that notably, the peptide profiles of thrips look very different from the other insect groups. In this proposal, the following specific objectives need to be accomplished:

1. Molecular cloning and sequencing for *capa* and *pyrokinin* genes.

2. Gene expressions during different life stages of thrips to find biological function(s) in thrips.

3. Determination of mature peptides and synthesize the peptides to be tested.

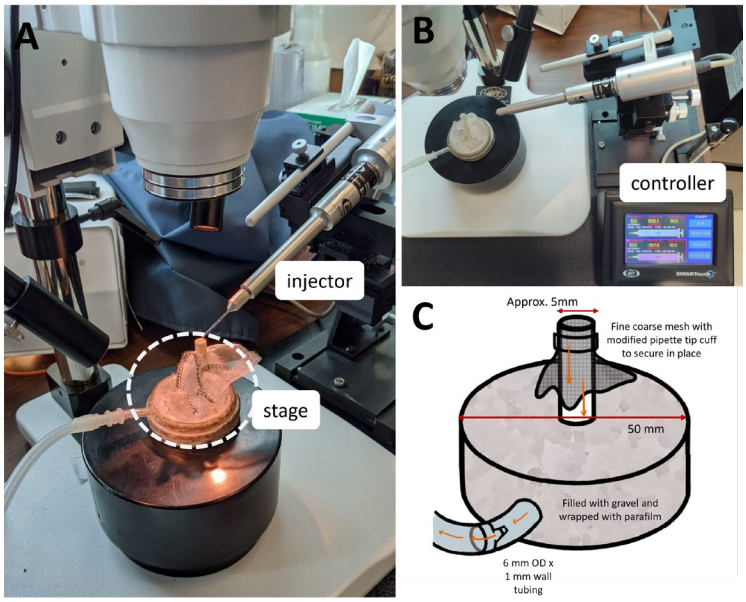

Figure 1. Picture of the nano-injection system: Stereomicroscope with standard injector used with a nanoliter with injector head, and micro-manipulator connected with a controller, and customized screened vacuum stage (circled) see detain in paper 2.

Overview of Methods and Timelines:

1. **Molecular cloning and sequencing for *capa* and *pyrokinin* genes (0.5 yr):** Western flower thrips (WFT) will be obtained from the laboratory colony that had been maintained for several years in USDA ARS lab in Corvallis OR. Total RNA will be extracted from WFT samples. BLAST searches for WFT orthologs of *capa* and *pyrokinin* genes will be performed against the USDA databases and NCBI GenBank. Sequences of the *capa* and *pyrokinin* genes will be amplified using the cDNA prepared from the total RNA above.
2. **Gene expressions during different life stages of thrips (0.3 yr):** Developmental stages of WFT will be collected separately from: eggs, 1st nymphs, 2nd nymphs, female prepupae, male prepupae, female pupae, male pupae, female adults, and male adults. Gene expression levels of these genes might provide critical clue to find their biological functions.
3. **Determination of mature peptides and synthesize the peptides (0.2yr):** Based prepropeptides encoded by the mRNAs of the two genes, amino sequences of mature peptides will be predicted. All the peptides (>95% purity) in this study will be synthesized and tested *in vitro* and *in vivo* assays in the future study.

Budget summary:

Salary & Benefit[1]	Travel[2]	Materials & Supplies[3]	Total
$19,000	$800	$5,200	$25,000

[1]Salary & benefit (0.3 FTE for 12m = $13,860 + $5,140 = $19,000) for postdoc or graduate research assistant. [2]Support the graduate student travel for commission and/or entomological meetings; [3]Molecular biology materials & supplies ($5,000), and thrips rearing materials – dish and soybean ($200).