

Proposal for Nursery Research Grant Program 2025 Request

Title: Development of bioactive peptides as insecticidal agents for nursery pests

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Project Background: The discovery of new insecticides is typically accepted as a long high-risk process with a low probability of success. For decades, insect neuropeptides (NPs) and their G-protein coupled receptors (GPCRs) have been considered biological targets for new insect pest control methods, because they are involved in many key biological/physiological processes in insects. Thus, disruption of any of these functions will be critical for insect survival, leading to novel pest management strategies. Recently, Dr Choi developed a novel GPCR-based insecticide discovery method to identify bioactive peptides using short peptide library and insect cell expression system. The bioactive peptides strongly bind to the target GPCR, thus blocking the key physiological function. The first proof-of-concept for the technology is called Receptor interference (Receptor-i). The Receptor-i technology was patented (2017, U.S. Patent 9,771,393; 2018, U.S. Patent 10,017,538 B2), and the research results were published (2021, <https://www.mdpi.com/2218-273X/11/4/583>; 2023, <https://doi.org/10.3390/ijms241813978>), and is being commercialized to control the fire ants. The Receptor-i technology is generally applicable to all invertebrate pests.

Thrips: Thrips species are global insect pests with 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.

Lygus: *Lygus hesperus* (western tarnished plant bug) is a destructive polyphagous sucking pest found throughout western North America that severely damages a variety of horticultural and nursery crops. Economic losses due to Lygus pests in the U.S., including crop damage, control costs, and reduced market value, are huge and increasing every year.

Currently, these pests are 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.

Results from the previous projects:

1) developed a sustainable mass rearing method for WFT; 2) established a DNA marker for molecular identification for thrips species (2022, <https://doi.org/10.1653/024.105.0211>); 3) developed a novel nano-injection system for microinsects (< 3 mm) using WFT model that facilitate injection test with biological compounds into thrips without any immobilization tools (2022, <https://doi.org/10.1111/jen.13063>); 4) identified the thrips neurohormones for potential biological targets for thrips biological control (2023, <https://doi.org/10.1111/imb.12859>); 5) identified WFT specific pheromone biosynthetic mechanism for thrips pheromones (2023; <https://doi.org/10.1038/s41598-023-32833-9>); 6) identified neuropeptides and GPCRs for Lygus (2021, <https://www.mdpi.com/2075-4450/12/10/914>); 7) The bioactive peptides selected for four insect pests, including WFT and Lygus, were recently submitted for a USDA patent filed

Projective Objectives and Outcomes: Using Receptor-i technology, we identified potential bioactive peptides from WFT, *Lygus*, spotted-wing drosophila, and diamondback moth. We are also targeting other invasive and important agricultural pests, including box tree moth, spotted lanternfly, and slug. In this proposal, we will focus on WFT, and the following specific objectives need to be accomplished:

1. Injection of bioactive peptides into thrips and evaluation of the effect on WFT survival.
2. Feeding bioactive peptides on thrips and evaluation of the effect on WFT survival.

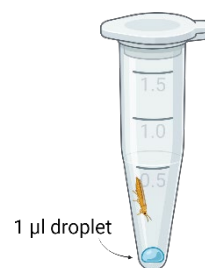
The objective is to screen bioactive peptides for WFT and identify insecticidal properties to develop novel management strategies. We, particularly, focus on WFT CAPA and modified peptides because these peptides are involved in a variety of biological functions: muscle contraction in the digestive track, and diuresis, and cuticle melanization in thrips life stages. Therefore, successful achievement of our long-term 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 also expected to address fundamental requirements for the application of biocontrol to other thrips pests.

Methods and Timeline

1. Injection of bioactive peptides into thrips and evaluation of the effect on WFT survival (0.5 yr):

Western flower thrips (WFT) will be obtained from the laboratory colony established in a sustainable mass rearing method above at the USDA ARS laboratory in Corvallis OR. All the bioactive peptides (>95% purity) in this study will be synthesized and injected into female thrips (1 – 3-day-old) using the novel injection method for WFT developed by PI Choi's group. Therefore, we do not expect any difficulties for this experiment. For evaluate survival rate, ten thrips per treatment will be injected, then transferred into bioassay containers (outer diam. 95 × H 40 mm) with a ventilation mesh screen, and kept in 25±1°C, 12 L:12D, at 60% RH. The thrips will be checked daily and mortality at 24 and 48 h. This will be repeated 3 times per treatment at least. Survival rates will be compared between treatments by log-rank analysis.

2. Feeding bioactive peptides on thrips and evaluation of the effect on WFT survival (0.5 yr): Thrips will be fed a 1% sucrose solution containing 10 nmol of bioactive peptide and mortality will be checked daily for two weeks (see **Figure**). The solution will normally last 5 days and will be replaced with fresh solution, but will be checked daily to decide whether to replace the droplet before or after 5 days. Water only without sucrose is used as a negative control. Thrips will be starved 1 h before feeding. Survival rates will be compared between treatments using log-rank analysis.



Budget Summary:

Salary & Benefit ¹	Travel ²	Materials & Supplies ³	Total
\$23,800	\$1,000	\$4,200	\$29,000

¹Salary & benefit (0.3FTE = \$17,800 + \$6,000 = \$23,800) for graduate student. ²Support the graduate student travel for commission and/or entomological meetings; ³Purchase synthetic bioactive peptides (\$2,500), nano-injector supplies including micro-needles (\$200), and thrips rearing supplies: soybeans, dish, fertilizer, and bedding (\$1,500). The USDA base fund and other grants will support this project.