Proposal for Oregon Department of Agriculture for Nursery Research (2022)

Title: Identification of antennal chemosensory genes for thrips management in nursery crops

Principle Investigator: Man-Yeon Choi - Research Entomologist; USDA ARS Horticultural Crops Research Unit, Corvallis, OR 97330
Phone 541-738-4026; email man-yeon.choi@usda.gov

Cooperators: Dr. Hyo Sang Jang (hyosang.jang@oregonstate.edu), OSU Horticulture, Corvallis, OR; Dr. Seunghwan Yun (ysh1986@gg.go.kr), Gyeonggido Agricultural Research & Extension Services (GARES), Suwon, South Korea.

Project Background and Justification: Pest thrips have hundreds of host plants, including many ornamental and nursery crops. One of the most economically important pests is the Western flower thrips (WFT), *Frankliniella occidentalis*, owing to its serious damage on horticultural crops worldwide. Not only direct damage from feeding on flowers and fruits, they also transmit tomato spotted wilt virus (TSWV) that is economically the most important. Control methods for thrips mainly relied on chemical insecticides. This has led to the development of WFT resistance, toxicity towards beneficial insects, contamination of the environment, and human health risks. Therefore, it is essential to develop appropriate management strategies, which focus on environmentally friendly alternatives.

Thrips rely primarily on their sense of smell to find the plants that they cause economic damage. In the case of thrips, the thrips detect volatile odorants that are emitted by plants including flowers and find their host plants. Thrips have specialized tiny antennae (Fig. 1) that contain chemosensory receptors that are responsible for detecting volatile compounds. When the odorant molecules that are emitted by these plants are detected by the receptors in the thrips antennae, its brain tells it to "follow that smell!" and the thrips literally follow the trail of the volatile compounds back to the source. To find mating partners, thrips are also expected to using volatile compounds such as sex and/or aggregation pheromones. To date, nothing is known about thrips chemosensory receptors in the antennae.

Recently, we dissected thousands of spotted-wing drosophila (SWD) antennae, and identified antennae-enriched genes including chemosensory genes in the male and female using a transcriptome analysis (Ahn and Choi et al., 2019). In this publication, we evaluated their expression levels between two sexes, whether they are chemosensory or non-chemosensory genes, and found the fly host preference has possibly evolved from a unique olfactory adaptation. Therefore, we selected genes encoding chemosensory genes such as odorant receptors and chemosensory proteins, and validated the gene expressions. The molecular results significantly facilitate finding the unique chemoreception of the fly, as well as on the development of new management strategies for this pest.

The goal of this research is to identify the antennal chemosensory genes that are used by thrips to detect the volatile compounds. This is the initial step towards our *long-term-goal* that is to screen novel biological targets for pheromone application in thrips chemical communication. Identification of specific receptor antagonists will develop novel biologically-based insecticide, which is a chemical pesticide alternative. 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 biologically-based and non-transgenic strategy to control thrips. We have recently initiated the next generation sequencing from a variety of insect tissues including SWD and slug antennae to identify

pheromone/olfactory receptors. We have also identified and characterized many G protein-coupled receptors (GPCRs) that would be expected similar to olfactory receptors. In this project, therefore we particularly focus on the adult antennae because it is the most important olfactory organ to detect volatiles from host plants and finding mating partners. To achieve this goal the following specific objectives need to be accomplished in this project:

1. Dissection and collection of the antennae from thrips male and female adults.
2. Isolation of total RNA, and RNA sequencing of thrips antenna genes.

Overview of Methods and Timelines:

1. Dissection and collection of the antennae from thrips male and female adults (0.5 yr): Thrips adults will be obtained from the laboratory colony that had been maintained for several years in USDA ARS lab in Corvallis OR. Based on our experience to extract RNA from insect antennae, we expect approximately >6,000 antennae total from male and female adults (1-5 days old) are required because thrips antenna (Fig. 1) is much smaller than fly antennae. Thrips antennae will be carefully collected in separate tubes on dry ice using a fine forceps. The detached antennae and the whole body (as a reference) will be stored at -80 °C until RNA extraction. Total RNA will be extracted from the three frozen samples (male antenna, female antenna, and female whole body) using an RNA Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The quantity of the RNA will be assessed using a NanoDrop Spectrophotometer ND-2000 (Thermo Fisher Scientific).

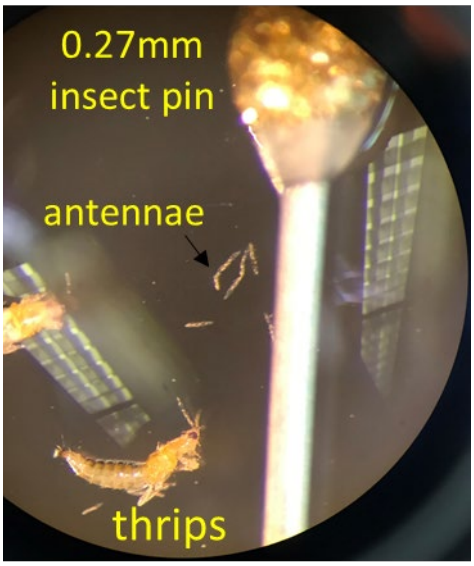

Figure 1. Photos of thrips antennae. Tiny antennae will be dissected and collected in dry ice under microscope.

2. Isolation of total RNA, and RNA sequencing of thrips antenna genes (0.5 yr): The cDNA libraries will be prepared using TruSeq Stranded Total RNA Library Prep Kit (Illumina) and sent for the Illumina sequencing by Psomagen Inc (Rockville, MD). The functional annotation of the transcripts will be performed by sequence similarity searches against National Center for Biotechnology Information (NCBI) non-redundant protein sequences (nr) database. The BLAST hits will be grouped into major gene families based on their putative functions in thrips biology. The organism information obtained by the BLAST hit will be collected to confirm the sequence similarity of the transcripts to closely related species.

Budget summary:

Salary & Benefit[1]	Travel[2]	Materials & Supplies[3]	Total
$17,000	$500	$6,500	$24,000

[1]Salary & benefit (0.3 FTE = $15,600 + $1,400 = $17,000) for research associate or graduate research assistant. [2]Support the postdoc or student travel for commission and/or entomological meetings; [3]RNA sequencing and analysis for thrips antenna samples ($800 x 6 rep = $4,800), Molecular biology materials & supplies ($1,500), and thrips rearing materials – dish and soybean ($200).