

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Identification of antennal odorant receptors for biological targets to control spotted-wing *Drosophila*

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EXECUTIVE SUMMARY: Spotted-wing drosophila (SWD) is a destructive invasive pest from Asia that attacks almost all small fruits. Annual losses have been estimated at \$800 million for the small fruits industry in US, and increase every year. While numerous biological, cultural, and mechanical strategies are being developed for SWD control, current management relies primarily on chemical pesticides despite health and environmental risks.

In this project, we analyzed SWD antennal transcriptomes to identify sex-biased genes by comparison of differential gene expressions between male antennae and female antennae. Among 13,583 total genes of the fly genome, 11,787 genes were expressed in either male or female antennae. There are only 132 genes (9 in male antennae, 7 in female antennae and 116 in both, FPKM>1) were expressed in antennae exclusively, and 2,570 genes (9 in male antennae, 0 in female antennae and 2,561 in both) were enriched in antennae containing 185 and 113 sex-biased genes in male and female antennae, respectively.

Interestingly, many immune-related genes were highly expressed in the male antennae, whereas several chemosensory genes were at high rank in the female antennae. We identified twenty-seven sex-biased chemosensory genes including odorant and gustatory receptors, odorant-binding proteins, chemosensory proteins, ionotropic receptors and cytochrome P450s, and validated the gene expressions using the real-time PCR. The highly expressed sex-biased genes in antennae are likely involved in the fly specific mating, host-finding behaviors, or sex-specific functions. The molecular results demonstrated here will facilitate to find the unique chemoreception of SWD, as well as on the development of new management strategies for this pest.

OBJECTIVES: The long-term goal of this research objectives is the development of biologically-based insecticide and non-transgenic strategy to control SWD. We have recently initiated the next generation sequencing from a variety of SWD tissues including midgut to screen RNAi targets. We have also identified and characterized four G protein-coupled receptors (GPCRs) that would be expected similar to olfactory receptors.

In this project, therefore we particularly focused on the adult antenna because it is the most important olfactory organ to detect volatiles from host plants. To achieve this goal the following specific objectives need to be accomplished in this project:

1. Collection of the antennae from SWD female and male adults and RNA isolation.
2. RNA sequencing of SWD antennae and gene expression analysis.
3. Identification of SWD specific odorant receptors.

PROCEDURES:

1. Collection of SWD adult antenna and RNA isolation (Obj. 1): SWD adults were obtained from the laboratory colony that had been maintained for several years in USDA ARS lab in Corvallis OR. Approximately 2,000 antennae from each sex were carefully dissected out from live adults (1-5 days old) using a fine forceps and collected in separate tubes on dry ice. The detached antennae and the whole body (as a reference) will be stored at -80 °C until RNA extraction. Total RNA was extracted from the three frozen samples (female antenna, male antenna and female whole body). The quantity of the RNA was assessed and the quality was analyzed on the basis of RNA Integrity Number (RIN) obtained through an Agilent 2100 Bioanalyzer.

2. RNA sequencing SWD antennae, and analyze the RNA expression profile (Obj. 2): The cDNA libraries were prepared using TruSeq Stranded Total RNA Library Prep Kit (Illumina) and sent for the Illumina sequencing by Macrogen Inc. The raw sequence reads generated by Illumina sequencing were checked by FastQC for quality control and trimmed by Trimmomatic tool. Overlapping high-quality reads were de novo assembled to create longer contiguous fragments (contigs) using the Trinity (version r2014-07-17). Transcript abundance was estimated using RSEM (ver. 1.2.15) to generate FPKM (fragments per kilobase per million mapped reads). The functional annotation of the transcripts were performed by sequence similarity searches against NCBI non-redundant protein sequences (nr) database. The BLAST hits were grouped into major gene families based on their putative functions in fly biology. The organism information obtained by the BLAST hit was collected to confirm the sequence similarity of the transcripts to closely related species. All the *D. melanogaster* chemosensory protein sequences from the latest version of FlyBase FB2017_04 were used as queries to perform local tblastn searches using BioEdit against SWD antenna transcriptome database produced by de novo assembly in this study. The candidate transcripts were assembled with corresponding genome scaffolds downloaded from the SpottedWingFlyBase to verify exon-intron boundaries.

3. Identify SWD specific odorant receptors (Obj. 3): Gene expression levels were estimated by FPKM for each sample of male antenna, female antenna, and female whole body. Clean data were mapped back onto the assembled transcriptome, and read count for each gene was obtained from the mapping results. FPKM considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels. Thus, the RPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. Sometimes gene models can be split across subcomponents in the Trinity assembly. Our target genes including olfactory receptors for full length transcripts were amplified and identified from a routine PCR and molecular biology methods mentioned above.

SIGNIFICANT ACCOMPLISHMENTS:

- Annotated 11,787 genes total from *Drosophila suzukii* antennae
- Identified 132 genes in antennae exclusively
- Identified 185 and 113 sex-biased genes in male and female antennae, respectively
- Validated gene expressions of 27 sex-biased chemosensory genes by qRT-PCR
- The molecular data facilitate to identify the unique chemoreception in SWD

BENEFITS & IMPACT:

The impact of this project provides significant results to develop a new control strategy which is a chemical pesticide alternative. From the project we have selection potential biological targets for further evaluation to apply for future funding on our long-term goal of controlling SWD with novel biologically-based insecticides. The outcomes of this project were significant to provide a molecular foundation for developing a biological approach for the fly control as an alternative to the current chemical pesticides approach. In addition, the research results have been published in the peer-reviewed journal entitled on 'Sex-biased gene expression in antennae of *Drosophila suzukii*' by Ahn, S-J., H-W, Oh, J. Corcoran, J-A. Kim, K-C. Park, C-G. Park, M-Y. Choi, in Archives Insect Biochemistry and Physiology (<https://doi.org/10.1002/arch.21660>).

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: Additional funding has been made by multiple small fruits commissions including Oregon blueberry, and raspberry & blackberry commissions, and Washington blueberry and red-raspberry commissions.

FUTURE FUNDING POSSIBILITIES: Results obtained from this project will continue to apply for the SWD research for Northwest Center Small Fruits Research and USDA grants, and currently collaborate with interdisciplinary scientists from OSU.