AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2016 – 2018

TITLE: Screening of RNAi targets to develop a novel RNAi-based control method for spotted wing drosophila

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SUMMARY: Spotted wing drosophila (SWD), *Drosophila suzukii*, is a serious invasive pest from Asia that is now in the United States, Canada, and Europe. The severe damage caused by this destructive pest affects ripening all small fruits, and the infestation area is rapidly spreading through North America as well as Europe. Growers are facing economic losses by increased spending on management costs, the loss of production and market values, and rejection of exports if unacceptable levels of insecticide residues and damage are found. Major control of SWD relies on the spray of chemical insecticides which have negative impacts on agricultural ecosystems affecting non-target insects and human health. In addition, there is an inevitable risk that SWD populations in the field will develop insecticide resistance from the continuous use of chemical controls. Therefore, the heavy reliance on chemical insecticides should be replaced or at least complemented with biologically-based environmentally friendly alternatives.

RNA interference (RNAi) technology is a new direction of the insect pest management, a biologically-based and target specific strategy. During the past decade the availability of insect genomics and computational biology has further enabled the implementation of RNAi technology to target economically important insect pests. One of the key advantages of RNAi technology is its high degree of species-specificity for the target pests; this is a unique point compared other conventional insecticides. Recently it has shown striking results in various insect groups, suggesting that it will be a promising tool for the next generation of pest management. To date, a variety of RNAi targets are being screened and evaluated for specific impacts applicable to pest management of agricultural crops or insect vector-borne diseases. However, virtually nothing is known for RNAi targets for SWD although a lot of RNAi tests has been done to identify biological functions in *D. melanogaster*.

To successfully develop RNAi applications, a critical initial step is screening for appropriate RNAi target genes because degrees of gene silencing impacts vary from RNAi target genes and insects. The challenge with gene selection is to select suitable insect-specific target genes that provide fast-acting mortality or suppression and long-term population suppression without affecting other non-target organisms. Therefore, it is important to screen multiple and key RNAi candidates to improve the chance for identifying an effective RNAi target. This is the initial step towards our long-term-goal that is to develop a novel RNAi-based control for SWD. Successful achievement of our *goal* will add a new environmentally-friendly SWD control method to the IPM tool box and contribute to the reduction of SWD populations and concomitant reduction in environmental impacts and losses in agriculture.

Our 2016-18 **OBJECTIVES** are as follows:

Objective 1. RNA sequencing SWD midgut, and analyze the RNA expression profile.
Objective 2. Select and identify RNAi targets – ten (10) genes from the midgut.
Objective 3. Design and synthesis dsRNAs for RNAi materials.
Objective 4. Bioassay to measure RNAi impacts on SWD.

PROCEDURES:

RNA sequencing and analyze RNA expression profile on SWD midgut (2016): A SWD colony was maintained with an artificial diet in the laboratory. Adult midguts were dissected, and collected in an RNase/DNase free tube on dry ice and stored at -80 °C to isolate total RNA. Total RNAs were isolated

from SWD whole body, midgut tissue and whole body. The isolated total RNA samples were analyzed to determine RNA concentration and immediately used for cDNA synthesis. Purified total RNA samples (whole body and midgut) was prepared for the RNA sequencing and differentially expressed gene (DEG) analysis.

Select and identify RNAi targets (2016): With RNA sequencing data and DEG analysis from the midgut and the whole body specific genes for RNAi targets were selected and identified from the midgut. In this initial project, we decided ten candidate genes for SWD RNAi targets. The selection of targets was based on the previous studies for insect RNAi targets and biological functions. These target genes were classified into essential housekeeping genes that are required for the maintenance of basic cellular functions, neuropeptide hormones and G-protein-coupled receptors.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:
We completed Obj. 1 &2 in 2016. RNA-sequences for SWD midgut tissue, female ovary and whole body were competed and analyzed up and down regulated transcripts, mRNA copies. The methodology we outlined in the proposal has been successful; we have been able to dissect and collect target tissues, then total RNAs have been done for RNA sequencing 2016.

We found 873 genes are up-regulating in the midgut compare to the whole body whereas almost two times of genes (1,921 genes) are up-regulating in the ovary compare to the whole body. We completed to classify these all genes for further consideration and characterization for RNAi targets. This is very important data because selection of RNAi targets can be narrowed down. Based on our RNAi experience, knowledge and previous RNAi reports, we selected 11 potential candidates (Table 1) including neuropeptide hormones, receptors and housekeeping genes for SWD RNAi target(s).

Table 1. SWD RNAi candidates from three different groups and GFP, and nucleotide lengths of dsRNAs.

RNAi candidates	**DNA template for RNAi synthesis**	**Gene group**
SWD ID1	296 nucleotides	Neurohormone
SWD ID2	195 nucleotides	Neurohormone
SWD ID3	399 nucleotides	Hormone receptor
SWD ID4	244 nucleotides	Housekeeping
SWD ID5	253 nucleotides	Housekeeping
SWD ID6	255 nucleotides	Housekeeping
SWD ID7	253 nucleotides	Housekeeping
SWD ID8	250 nucleotides	Housekeeping
SWD ID9	251 nucleotides	Housekeeping
SWD ID10	254 nucleotides	Housekeeping
SWD ID11	254 nucleotides	Housekeeping
Green fluorescence protein (GFP)	350 nucleotides	To be used for control gene – unrelated in SWD

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: PI has received additional funding from Northwest Center Small Fruit Research, Oregon Blueberry commission, and Oregon Rasp-Blackberry commission.

FUTURE FUNDING POSSIBILITIES: We have just been notified another funding from WA Tree Fruit Research Commission & Oregon Sweet Cherry Commission Fruit, and Washington Blueberry commission for screening of more RNAi targets from SWD.