AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Development of Blueberry shock virus as a Vector for Delivery of RNAi to Control Spotted Wing Drosophila and Blueberry Scorch Virus in Blueberries

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COOPERATORS: Dr. Man-Yeon Choi, new Research Entomologist in our group that works with RNAi in insect pests. Dr. Jana Lee, Research Entomologist in our group that works with SWD

SUMMARY: RNAs 1, 2 and 3 of Blueberry shock virus (BlShV) were obtained using standard cDNA cloning methods using RNA extracted from purified virus. The 3' and 5' ends were obtained using 5' race from double-stranded RNA templates of BlShV extracted from infected tissues. For ilarviruses (BlShV is in this genus) the coat protein is involved in the replication complex, which means that the coat protein gene present as the second gene in bicistronic RNA-3 RNA needs cloned as a monocistronic RNA so that it will be translated using host translational machinery before the viral replicase is produced. The 5' end of RNA-4 was determined using a modified 5' race. The RNAs were cloned into a vector with a T7 promoter to produce each of the viral RNAs and these RNAs were then combined and used to inoculate *Nicotiana benthamiana,* a host of BlShV. After numerous attempts we have not been able to get infectivity. We resequenced the virus and did not find any errors in the sequence. The next steps will be to clone each RNA into an Agrobacterium vector and attempt to get infectivity with agro-infection. One of the difficulties is that we need all four RNAs getting into the same cell to get an infection in the plant. If this fails, will be using Blueberry scorch virus or Strawberry mild yellow edge virus, they each have a single RNA and should be much easier to develop infectious clones. Either one can be used for a proof of concept, the problem is that we do not have an herbaceous host system to work with for these two viruses. We have developed an infectious clone of Strawberry mild yellow edge virus that is cloned into Agrobacterium and with this system we can inoculate strawberry directly.

Design and Synthesis dsRNA (= RNAi material) - Using routine molecular biology skills and software, specific primers set with 5'-T7 promoter appended (TAATACGACT CACTATAGGG) will be designed to amplify partial lengths between 200- 400 nucleotides of the each target gene found in the SWD genome data. Amplified actual nucleotides of the genes, then cloned into an appropriate vector for sequencing. Once confirmed the sequence DNA fragments were served as the templates for dsRNA synthesis using a dsRNA synthesis kit. The negative dsRNA control (dsGFP) was also constructed by the same method described above for SWD.

We identified DNA sequences for 11 candidate genes including PBAN and green fluorescence protein (GFP), designed and synthesized 12 dsRNAs (Fig. 1). We found some genes identified in this study were very different from DNA sequences published on the SWD genome data, indicating a wrong annotated or uncompleted the SWD genome that should need to be confirmed actual sequences for each target genes. The length of dsRNA for SWD dsRNA1 was designed a

short because the size corresponds roughly to full sequence (~200 base pairs). Double-stranded RNA2 is a receptor for a neuropeptide hormone functioning to egg development in the female SWD.

Eight housekeeping genes as constitutive genes are expressed in all cell types at a level that does not fluctuate with the cell cycle. Functional examples of housekeeping genes for RNAi targets are related in the muscle physiology, detoxification, ATP metabolism, protein sorting and transporting, and cell membrane structure in cells. These genes have been selected for RNAi candidates to develop RNAi-based control for spotted wing drosophila.

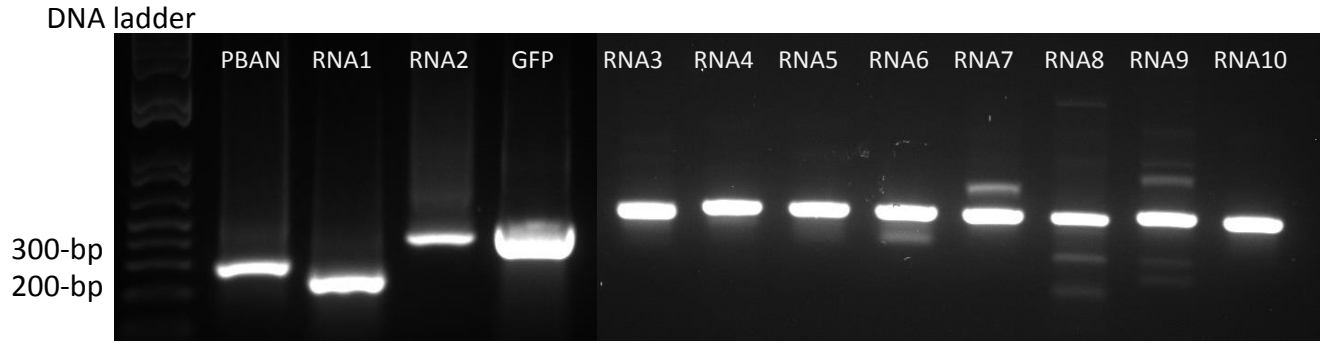

Figure 1. Electrophoresis pictures of synthesized dsRNAs of SWD dsRNAs including PBAN and GFP. Amounts of dsRNAs on the gel are not equal. Sequences of each RNAi targets are not shown in this report.

Recently, established a microinjection system with a nano-litter volume (50 nL = 0.02 uL) using a Nanoliter 2010™ injector fitted with custom-pulled borosilicate needles, and a homemade vacuumed tube to hold fly alive (> 90%). This technique allows us enabling to fast screening for more RNAi targets from the fly.

OBJECTIVES:

1. Develop infectious clone of BlShV using strategy shown to work for viruses in the same family
2. Insert sequences of GFP in BlShV clone to demonstrate functionality of VIGS vector to silence a gene in herbaceous host, ensure the vector works as planned
3. Insert sequences of Blueberry scorch virus in vector and demonstrate silencing after infection of blueberry with the Shock VIGS Scorch vector. Infection will be via agroinfiltration, mechanical inoculation of young TC plants, or by grafting from herbaceous host to blueberry
4. Clone PBAN gene from Drosophila suzukii and develop constructs for inducing RNAi to this pest in the BlShV VIGS vector to develop a BlShV SWD construct. Inoculate the BlShV SWD construct to young blueberries.

PROCEDURES: Standard molecular biology procedures was used for most of this work. The microinjection of D. suzukii was developed in-house.

SIGNIFICANT ACCOMPLISHMENTS: Full sequence of the 4 RNAs of BlShV obtained
Development of mechanism to reliably inject Drosophila with a 95% survival rate
Identification of 11 target genes for Drosophila specific RNAi application
Production of dsRNAs for the induction of RNAi for these 11 target genes

BENEFITS & IMPACT: This work is continuing with other funding sources on both the RNAi and virus fronts. Identifying target genes and being able to test them in insect injection studies is essential first step in developing and deploying this technology for SWD control. The development of infectious virus provides a tool to deliver target sequences for RNAi insect control when insects feed on fruit.

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FUTURE FUNDING POSSIBILITIES: Continued commission funding, AFRI, SCRI