**Interim progress report for CDFA Agreement # 11-0146-SA**

**Project Title: Development and use of recombinant *Homalodisca coagulata* virus-1 for controlling GWSS**

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**Time period covered by the report:** 7/1/13-3/24/14

**Introduction**: Pierce’s disease is a common and devastating disease of grapevines caused by the bacterium *Xylella fastidiosa*. The bacterium is often transmitted to plants by the glassy winged sharpshooter (GWSS), *Homalodisca vitripennis* (Hemiptera: Cicadellidae) which has become a high profile pest insect targeted for control efforts. Novel strategies are under investigation for modifying or enhancing the insecticidal activity of a naturally occurring dicistrovirus (*Homalodisca coagulata* virus-1 or HoCV-1) infective to GWSS. We report our activities and objectives below.

**List of objectives and description of activities conducted to accomplish each objective:**

The specific objectives of our effort are:

1. Development and evaluation of recombinant HoCV-1 for inducing systemic RNAi in GWSS.
2. Development of cell culture and baculovirus-based systems for recombinant virus production.

**Description of activities, accomplishments and results for each objective:**

In our previous report we described the research aimed at accomplishing **objective 1**: the development of a plasmid containing three constructs of HoCV1 and the development of hairpin RNAi cassettes against Green Fluorescent Protein (control), *H. vitripennis* actin, and *H. vitripennis* chitin deacetylase in the pGEM-13Zf+ vehicle. We also showed the production of polyclonal antibodies to HoCV1 capsid polyprotein for future identification of capsid proteins in our work. In the current reporting period we have been working on optimizing transfection assays in whole insects and the GWSS cell line Z15.

In our current research, we have been working to achieve **objective 2,** the development of cell culture based systems for recombinant virus production. We received cell pellets and cell culture supernatant from Iowa State to use in insect experiments. We attempted to inoculate healthy GWSS with the GWSS-Z15 transfected cells both by injection and oral acquisition. RNA from five infectious clones of HoCV1 and two controls were used to transfect GWSS-Z15 cells: HoCV-3’Rz, HoCV-3’Rz old, Rz-HoCV-3’Rz, Rz-HoCV-3’Rz old, mutant Rz-HoCV-3’Rz, elongation factor RNA, and transfection buffer. An additional negative control for the GWSS infection experiments was untreated GWSS-Z15 cell suspension. For the injections, 1µL of needle homogenized cell suspension in injection buffer (10 mM Tris-HCl, pH 7.0,1 mM EDTA) was injected into adult GWSS between tergites 3 and 4 of the ventral aspect using a 33 gauge needle. Three insects were injected per HoCV-3’Rz construct. After injection the insects were put in cages with a basil plant for one week, and then RNA collected as a treatment group. For feeding assays we used basil cuttings approximately 5cm in length and submerged the cut end in a suspension of cell pellet and supernatant of approximately 1.5mL volume. Three insects per treatment were given an acquisition period of three days on the basil cuttings, then moved to basil plants for four days, after which RNA was extracted from each group. One-tube RT-PCR was used to detect infection with primer pairs specific to inter-genomic region 1 and coat protein of HoCV-1. The positive control was GWSS RNA from a naturally infected insect. All treatment groups tested negative for HoCV-3’Rz.

We are currently working to optimize cell line transfection to generate infectious virus, however have not had positive results. We have Z15 cell cultures and the plasmid constructs of HoCV1 and are utilizing different transfection reagents and conditions. So far we have primarily been working with the initial protocol and plasmids developed at Iowa State University, in which single stranded RNA is generated from plasmid template for transfection experiments. We used *in vitro* transcription kits that generated both capped and uncapped messenger RNA (Figure 1). This was followed by cell transfection and analysis at 3 days post transfection. Using two-step RT-PCR we were unable to find both positive and negative sense HoCV1 RNA from these experiments, indicating that virus replication was not occurring. Currently, we do not believe that the plasmid constructs generate infectious RNA.

Figure 1. RNA gel of capped (left) and uncapped (right) HoCV1 from in vitro transcription that was used for transfection of Z15 cells.

In the final months of the grant period we are going to try a different approach to developing an infectious clone. We will examine utilizing a Baculovirus promoter, *ie1*, to use plasmid template for the transfection experiments instead of single stranded RNA. DNA is a more robust template to work with, and we already have a vector system that works with SF9 cells using this promoter. Previous research has shown this system to work in *Bombyx moorei* and *Drosophila melanogaster*, so we will design experiments to see if it is effective for the Z15 cell line (1). Initially, we will use the promoter with the *lac-z* gene to determine if it can drive production of messenger RNA and protein in Z15 cells. If we get positive results from that we will combine the HoCV1 sequence from the HoCV-3’Rz and Rz-HoCV-3’Rz constructs with the vector containing *ie1* to conduct transfection experiments in Z15 cells. We should have enough remaining time to examine this line of inquiry before the final project report in June.

**Publications produced and pending:**

Nandety, R. S., Nouri, S., Kuo, Y.-W., Pitman, T. L. and Falk, B. W. 2014. Novel strategies for target design and gene silencing in the insect vectors of plant pathogens. Abstract W596. Plant and Animal Genome XXII. San Diego, CA.

**Layperson summary:** Our efforts here are to take advantage of a naturally-occurring virus, *Homalodisca coagulata virus – 1* (HoCV-1), which occurs in the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, and to develop it as a tool to help manage GWSS. HoCV-1 is non-pathogenic in our studies. We attempted to make recombinant DNA-based forms of HoCV-1 that could be delivered back to GWSS. If successful we hoped to insert specific cDNA sequences into the recombinant HoCV-1 such that they would debilitate, or confer negative phenotypes on GWSS. These could be reducing the ability of GWSS to reproduce on plants, or even death. So far we have been unable to achieve consistent infectivity with the recombinant HoCV-1. We are using whole GWSS insects and cultured GWSS cells and will continue efforts until the end of the grant period.

**Status of funds:** This year was a no-cost extension. All funds are now spent.

**Summary of intellectual property:** We will work with UC for managing any intellectual property or technologies that may arise from this effort. We submitted an overview of our work for evaluation by the UC Davis Technology Transfer team, they declined to pursue it at this time.

**Reference**: Masumoto, Mika, et al. "A Baculovirus Immediate-Early Gene, ie1, Promoter Drives Efficient Expression of a Transgene in Both Drosophila melanogaster and Bombyx mori." *PloS one* 7.11 (2012): e49323.