Interim Progress Report for CDFA Agreement Number 11-0146-SA**.**

Title of project: Development and use of recombinant *Homalodisca coagulata Virus-1* for controlling *Homalodisca vitripennis*, the glassy-winged sharpshooter.

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Introduction

The glassy-winged sharpshooter (GWSS, *Homalodisca vitripennis*) transmits the bacterium, *Xylella fastidiosa*, which causes Pierce's disease of grapevines. We are attempting to use natural, GWSS-infecting viruses as part of a strategy to control GWSS population. We are attempting to engineer viruses to deliver toxic peptides and/or deliver GWSS RNAs that will activate the RNA interference (RNAi)-based immune system. We hope that one or both approaches will result in GWSS mortality thereby preventing the spread of *Xylella fastidiosa*. We are using recombinant HoCV-1, a naturally occurring virus that specifically infects GWSS, and FHV, an insect virus that has a wide-host range and also easy to manipulate in the lab. If successful, our studies may lead to new and effective methods to help control the GWSS population.

List of objectives

1. Development of *Homalodisca coagulata virus-1* (HoCV-1) infectious cloned cDNAs;
2. Expression of GFP or other stable sequences in GWSS-Z15 cells or whole *H. coagulata* insects by using HoCV-1.

Description of activities conducted to accomplish each objective, and summary of accomplishments and results for each objective

Development of *Homalodisca coagulata virus-1* (HoCV-1) infectious cloned cDNAs

We have already cloned full length HoCV-1 cDNAs (Fig. 1A). *In vitro* transcription was performed using these constructs to generate infectious HoCV-1 transcripts and delivered to GWSS Z-15 cells (Fig. 1B and C). After transfection with HoCV-1 transcripts with extended or un-extended 5'-ends, Z-15 cells showed severe cytopathic effects (CPE; Fig. 1B). Both the genomic-sense strand (positive-strand) and its complementary strand (negative-strand) of HoCV-1 RNAs were amplified by RT-PCR analysis following the transfection indicating that the virus was replicating (Fig. 1C). pT7-Rz-HoCV1-3'Rz generated

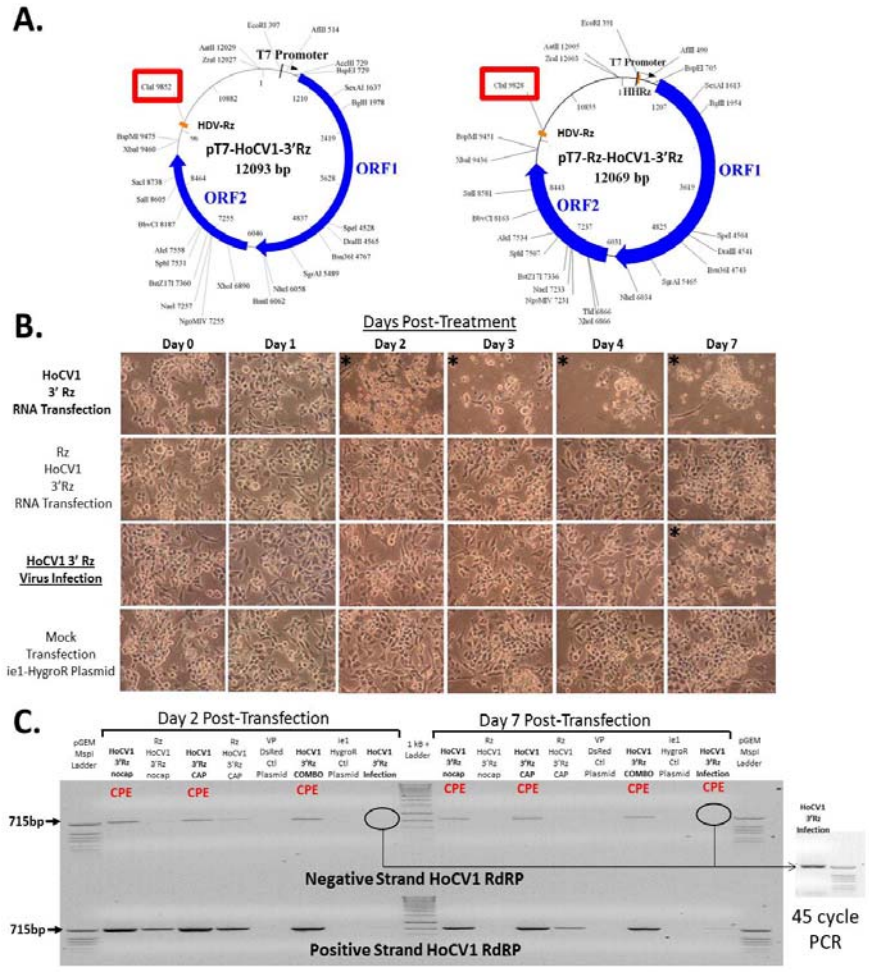

Figure 1. A. Vector diagram of pT7-HoCV1-3'Rz and pT7-Rz-HoCV1-3'Rz. The T7 promoter is indicated by the black bar and arrow. Hammerhead (HHRz) and Hepatitis Delta Virus (HDFV-Rz) ribozymes are indicated as orange boxes. pT7-HoCV1-3'Rz lacks the HHRz. HoCV-1 open reading frames (ORF) 1 and 2 are indicated as blue arrows. The *ClaI* restriction site (red box) is used to linearize plasmid for *in vitro* transcription. **B.** Cytopathic effects (black asterisks) were induced in GWSS Z-15 cells after transfection using HoCV-1 RNA transcripts and with HoCV-1 virus generated from the pT7-HoCV1-3'Rz plasmid. **C.** Negative and positive RNA strands for the HoCV-1 RNA-dependent RNA-polymerase (RdRP) were detected by 30 cycle RT-PCR for HoCV-1 RNA transfected from both plasmids, although only transfections with pT7-HoCV1-3'Rz RNA caused strong cytopathic effects (indicated by red CPE) in GWSS-Z15 cells. Weak signals for positive (30 cycles) and negative (45 cycles-indicated) strand RdRP RNA were also detected in virus overlayed from the HoCV-3'Rz transfection onto new GWSS-Z15 cells. Similar results were also obtained with RT-PCR against the HoCV-1 intergenic region and capsid sequences (not shown)

transcripts were less efficient possibly due to enhanced RNA degradation following ribozyme cleavage at the 5' end.

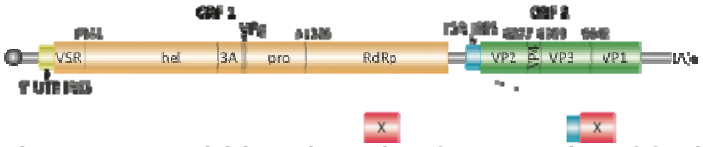

Figure 2. Potential insertions sites for expression of foreign proteins and RNAs from the RhPV and HoCV-1 genomes. Genome organization shows ORF 1 (orange) and ORF 2 (green) which encode proteins separated by cleavage sites indicated at estimated positions by vertical lines. Precise cleavage sites, estimated by alignment are labeled by the first amino acid downstream of the cleavage (e.g. P241 = proline at amino acid 241 in ORF 1). A1226 and S642 cleavages are from Nakashima and Nakamura (2008). We predict Q219 using alignment in Nayak et al. (2010), and G277, G359 using VP structures in Tate et al. (1999). Sequence encoding the protein of interest (e.g. toxin or fluorescent protein) indicated by red box (protein X) may be inserted at as a separate ORF, preceded by the IGR IRES (blue) at noncoding regions (gray bars) at sites indicated by dashed lines. Host RNA sequences can also be inserted at these sites for the RNAi strategy (objective 3). Alternatively, protein X coding region can be inserted within ORFs, at cleavage sites indicated by solid lines. Predicted functions of polyprotein cleavage products are viral suppressor of RNAi (VSR), helicase (hel), picornavirus-like protease 3A (3A), genome-linked protein (VPg), 3C-like protease (pro), RNA-dependent RNA polymerase (RdRp), virion proteins (VP1-4). Non-coding features include the VPg protein (sphere), 5' untranslated region IRES (5' UTR IRES), intergenic region IRES (IGR IRES), and poly(A) tail [(A)n)].

Expression of GFP or other stable sequences in GWSS-Z15 cells or whole *H. coagulata* insects by using HoCV-1

We are in the process of engineering infectious clones to express YFP and mCherry reporters as part of the transcribed viral sequence (refer to Fig. 2) and anticipate that this will be completed within the next month or two. We next will convert HoCV-1 into a highly specific, lethal virus by engineering it to express a peptide that is toxic only when expressed intracellularly within target sharpshooters. This includes primarily GWSS, but as HoCV-1 has a host range that is reported to include other sharpshooters, (Hunnicutt, 2008), our success here has broader applicability for *X. fastidiosa* vector control. The toxic peptides to be employed are lethal only to insects, and only when expressed intracellularly. Thus, only sharpshooters infected by the modified virus will be negatively affected by the peptides.

In addition to utilizing HoCV-1, we have been exploring the possibility of using another virus, *Flock house virus* (FHV), to augment our study of using viruses to express toxic peptides and induce RNAi in GWSS to control its population. FHV belongs to the family *Nodaviridae*, and is a non-enveloped, positive-sense RNA virus that has a bipartite genome. This virus been shown to multiply in insects from four different orders (Hemiptera, Coleoptera, Lepidoptera, Diptera). We have infectious, recombinant constructs producing FHV genomic RNAs 1 and 2 (pMT FHV RNA1 and pMT FHV RNA2; gift from Dr. Shou-wei Ding, UC Riverside). The plasmid backbone (pMT) of the constructs contains a copper-inducible *Drosophila* metallothionein promoter that drives an efficient transcription of FHV genomic RNA. We have already demonstrated that FHV can infect both adult GWSS and Z-15 cells (Fig. 3).

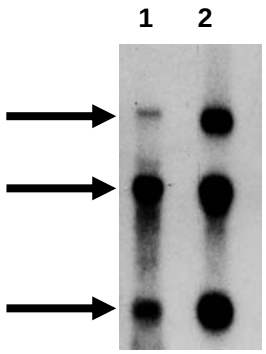

Figure 3. Lanes contain 1; FHV-inoculated S2 cells, 2; FHV-inoculated GWSS Z-15 cells. Arrows at left indicate FHV RNAs 1, 2 and 3, from top to bottom, respectively. RNA 3 is a subgenomic RNA from replicating RNA 1.

In adult mosquitoes, FHV has been engineered to systemically express GFP (Dasgupta et al., 2003). A defective interfering (DI) RNA of FHV originating from the genomic RNA 2 retains the RNA replication sites and packaging signal. We have subcloned the DI RNA into pMT plasmid vector, and it is currently being tested for infectivity in Z-15 cells and GWSS. Once we verify that the DI RNA is infectious, we will subclone GFP into the DI RNA in the pMT construct in a way that it will not disrupt the replication sites and the packaging signal, and then we will use it to produce FHV virions containing DI RNA that express GFP (DIeGFP). This will be accomplished by transfecting *Drosophila* Schneider 2 (S2) cells with the pMT vectors expressing FHV RNA1, RNA2, and DIeGFP. Once we verify the GFP expression in S2 cells, we will purify the virions from the infected S2 cells to inoculate Z-15 cells and inject GWSS to check for the GFP expression. Once the expression is verified, the same region on the DI RNA that was used for inserting the eGFP sequence will be used for subcloning various DNA sequences. Since this FHV system is easier to work with than the HoCV-1 system, it will be used to perform initial experiments screening for suitable peptides for expression and RNAs for inducing RNAi in GWSS.

Publications produced and pending, and presentations made that relate to the funded project

Rosa C, Kamita, S. G., and Falk, B. W. 2012. RNA-interference is induced in the glassy-winged sharpshooter Homalodisca vitripennis by actin dsRNA. *Pest management science.* Dec 13, 2011 (online print available; DOI 10.1002/ps.3253).

Falk, B. W., Bonning, B., Miller, W. A., Stenger, D., Choi, S. H., and Kamita, S. G. Development and use of recombinant Homalodisca coagulate virus-1 for controlling the glassy-winged sharpshooter. 2011. pp 32-36, Symposium Proceedings, Pierce's Disease Research Symposium. Dec 13-15, 2011. Sheraton Grand Sacramento Hotel, Sacramento, CA.

Falk, B.W., Choi, S. H., Pitman, T. L., Nandety, R. S., Kamita, S. G., Bonning, B., Miller, W. A., Kroemer, J., Stenger, D., and Spear, A. Hemipteran-infecting viruses as tools for vector management. December 13-15, 2011. Pierce's Disease symposium, Sacramento, CA.

Nandety, R. S., Pitman, T. L., Lin, M., Kiss, S., Song, K., and Falk, B.W. Next Generation sequencing and RNAi approaches for the control of Glassy winged Sharpshooters. December 13-15, 2011. Pierce's Disease symposium, Sacramento, CA.

Choi, S. H., Kamita, S. G., Pitman, T. L., and Falk, B. W. Development and use of recombinant *Homalodisca coagulata virus-1* for controlling *Homalodisca vitripennis*, the glassy-winged sharpshooter: Testing for FHV infection in Glassy-winged sharpshooter. December 13-15, 2011. Pierce's Disease symposium, Sacramento, CA.

Research relevance statement

We have made excellent progress during the past year. We have published one peer-reviewed article (Rosa et al., 2011) and one symposium report (Falk et al., 2011), in addition to giving several presentations of our work at various meetings during the past year (see above). In

addition to verifying the infectivity of the lab-generated HoCV-1, we have in our lab another virus system using FHV for gene delivery and RNAi induction in GWSS. This FHV system will complement our HoCV-1 system to expedite our work in controlling GWSS population to prevent spread of *Xylella fastidiosa* and expand our knowledge in virus-mediated pest management.

Layperson summary of project accomplishments

During the past funded year, we successfully engineered infectious clone of HoCV-1 and verified its biological activity in GWSS Z-15 cell-line. We also developed another virus-system using FHV to be used in conjunction with the HoCV-1 system to expedite our efforts to control GWSS population in California to prevent the spread of *Xylella fastidiosa*. We are currently modifying our virus constructs for delivering RNAs that can express toxic peptides or induce RNAi in GWSS insects and Z-15 cell-line. The knowledge gained from this study will be used further to develop a virus system to control the population of *Xylella fastidiosa* residing inside GWSS.

Status of funds

We were awarded one years funding ($112,427) to support research efforts at UC Davis, Iowa State University and the USDA Parlier Research Center. This included personnel at all three locations and funds for supplies, travel, etc., plus limited travel. We are on track, spending wise, to use the funds as proposed in our original proposal budget, and anticipate that the funds awarded to us will be spent by the end of this project year.

Summary and status of intellectual property produced during this research project

We will work with UC for managing any intellectual property or technologies that may arise from this effort.

Literature cited

Dasgupta, R., Cheng, L.L., Bartholomay, L.C., Christensen, B.M., 2003. Flock house virus replicates and expresses green fluorescent protein in mosquitoes. The Journal of general virology 84, 1789-1797.

Hunnicutt, L.E., Mozoruk, J., Hunter, W. B., Crosslin, J. M., Cave, R. D., and Powell, C. A., 2008. Prevalence and natural host range of Homalodisca coagulata virus-1 (HoCV-1). Arch Virol 153, 61-67.

Rosa, C., Kamita, S.G., Falk, B.W., 2011. RNA interference is induced in the glassy winged sharpshooter Homalodisca vitripennis by actin dsRNA. Pest management science.