I. Project Title: RNA-interference and control of the glassy-winged sharpshooter (*Homalodisca vitripennis*) and other leafhopper vectors of *Xylella fastidiosa*

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III. List of objectives and description of activities conducted to accomplish each objective:
The specific objectives of our effort are:

1. To evaluate existing transgenic plants for their ability to generate RNAs capable of inducing RNAi effects vs. *Homalodisca vitripennis*.

2. To identify GWSS interfering RNAs for practical application.

   2.a. To utilize transgenic *Arabidopsis thaliana* plants as efficient alternatives for identifying, delivering, and evaluating efficacious interfering RNAs.

   2.b. To enhance production of interfering RNAs *in planta*.

   2.c. To evaluate alternative strategies to deliver and screen high number of RNAi inducers to *Homalodisca vitripennis.*

IV. Summary of major research accomplishments and results for each objective:

We have made very good progress during this past year and are in excellent position to make more and complete most of our objectives during the upcoming year. We have published one refereed journal article (Rosa et al., 2010), one symposium report (Falk et al., 2010), and have another refereed article submitted (Rosa et al., submitted; see section V). In addition we gave several presentations of our work at various national and international meetings during this past year (see section VI). There is a good amount of interest now in RNA interference applications for controlling insect pests and vectors, and our work here is very timely. We now have transgenic grape, potato and *Arabidopsis thaliana* plants expressing various RNA-interference (RNAi) constructs. We used grape initially to generate transgenic control plants (expressing the gene for *β*-glucoronidase), but are using potatoes and *A. thaliana* as herbaceous models because of the speed in generating and testing these plants. We are directly evaluating the glassy-winged sharpshooter (GWSS) on these plants and evaluating for survival, life history and for molecular markers of RNAi.

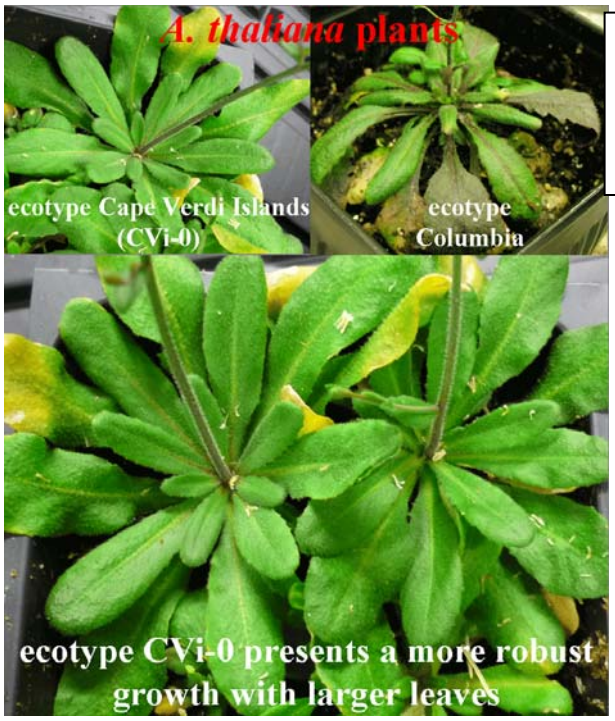

Figure 1. Top photos show comparison of Arabidopsis thaliana ecotypes Cape Verdi Islands (left) and Columbia (right). A close-up of Cape Verde Islands is shown at the bottom.

We are using the *A. thaliana* ecotype Cape Verdi Islands (Cvi), as it has larger leaves and presents a more robust growth, and will be more appropriate in supporting insects of large size such as *H. vitripennis* (Fig 1). In order to generate dsRNAs that can target GWSS, GWSS target sequences (Table 1) were cloned into a gateway-compatible binary vector pCB2004B (Figure 2). The target sequences are cloned in head to tail direction in the gateway vector with a non-homologous sequence between them. Upon transcription in transgenic plants, these constructs will yield double-stranded, hairpin RNAs of the desired sequence. The expression vectors carrying the insect target sequences of interest were first cloned into *E.coli* and *Agrobacterium tumefaciens* and they have been sequence verified. *A. tumefaciens* cultures carrying the sequences of interest were used to transform *A. thalianas* Cvi plant ecotypes through floral dip process. Arabidopsis T_0 plants were screened for resistance against the selectable marker *BAR* gene, and we are in the process of confirming the T_1 transgenics. The homozygous transgenic *A. thaliana* plants will be used for the screening against GWSS.

We have used three of these constructs (see bottom of Table 1) also for transformation of potato plants. Transformation/regeneration was performed via recharge at the UC Davis Ralph M. Parsons plant transformation facility (http://ucdptf.ucdavis.edu/) and only a few plants have

been so far received. We are in the process of screening these for insert composition. In contrast to the approach with *A. thaliana*, we will directly vegetatively propagate the T_0 plants and use them for RNAi experiments with GWSS. Potatoes are an excellent host plant for GWSS so we expect them to be very useful for our efforts here.

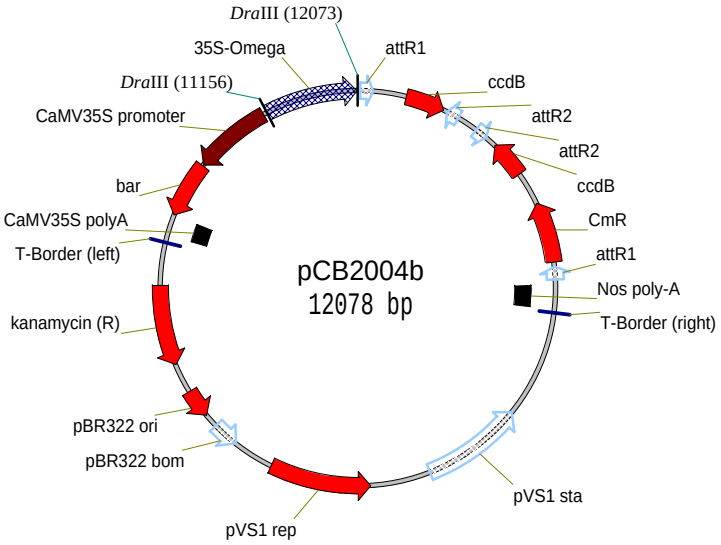

Figure 2. Diagram depicting binary gateway-compatible plasmid used for *Arabidopsis thaliana* and potato transformation.

Table 1. Glassy-winged sharpshooter sequences used for generating transgenic *A. thaliana* and potato plants.

Construct Name*	**Protein Encoded**	**Length of PCR Product (bp)**	***E. coli* DH5-α Sequence Verified**	***A. tumefaciens* EHA105 PCR Verified**
GWSS 965	Zinc Metalloproteinase	443	Yes	Yes
GWSS 989	Glucosyltransferase	576	Yes	Yes
GWSS 1591	Sugar Transporter	668	Yes	Yes
GWSS 1377	Serine Proteaseserpin	645	Yes	Yes
GWSS 364	Trypsin	605	Yes	Yes
GWSS 975	Transaldolase	800	Yes	Yes
GWSS 366	Sugar Transporter	888	Yes	Yes
GWSS 500	Serpin	418	Yes	Yes
GWSS 745	Trypsin	756	Yes	Yes
GWSS 512	Transketolase	1435	Yes	Yes
GWSS Actin	Actin	1100	Yes	
GWSS Cuticle	Cuticle Regeneration Protein		Yes	
GWSS DN198443	Chitin Deacetilase	484	Yes	

*All of the constructs have so far been used to transform *A. thaliana*, but only the bottom three, shown in bold, so far have been used for potatoes.

In addition to the transgenic plant approaches, based on recent reports in the literature (Killiny and Almeida, 2009, PNAS 106:22416), and personal communications from other scientists, we have evaluated *in vitro* feeding approaches for GWSS. If successful this will allow for much more rapid screening of candidate sequences for their abilities to induce RNAi effects via oral acquisition. We have a number of candidate sequences which we are testing for RNAi (Table 2). These are used for in vitro transcription and known quantities of dsRNAs are used for *in vitro* acquisition.

Three different in vitro oral acquisition approaches were attempted to deliver dsRNAs to GWSS. These included using a modified membrane feeding approach based on Killiny and Almeida, a modified tygon tubing delivery system, and using basil infusion (basil stems directly

inserted into dsRNA solutions). We have used the basil infusion in the past and although good for oral acquisition, it requires large amounts of dsRNAs. Each of these approaches has shown some promise, and we are so far having more consistent success in delivering specific sequences via the parafilm membrane feeding (Fig. 3). The latter approach is very economical in terms of dsRNAs required and if we can make it consistent, this will allow us to rapidly screen target sequences without having to develop transgenic plants, thereby saving time and effort towards our ultimate goal.

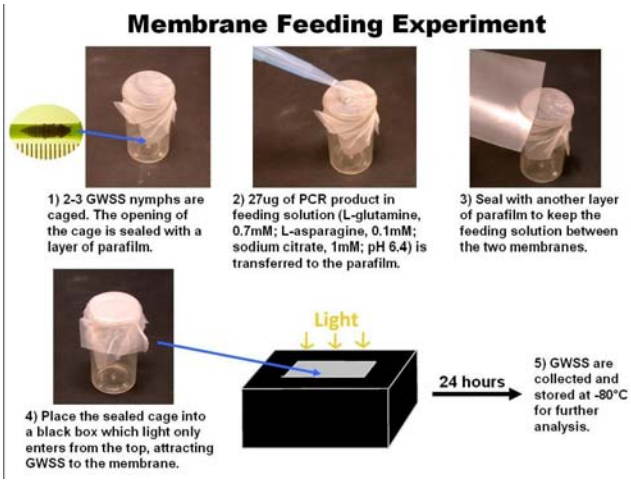

Figure 3. *In vitro* acquisition/membrane feeding approach for delivering dsRNAs to GWSS. 3 – 4th instar nymphs are introduced into vials and dsRNAs plus PCR product (to quantify uptake) in sterile diet solution are sandwiched between parafilm pieces. GWSS are allowed to feed overnight and then analyzed for dsRNA uptake and RNAi

Table 2: List of GWSS target sequences cloned into pGEMT-easy plasmids and used for *in vitro* transcription to generate dsRNAs.

S.No:	List of GWSS target genes	Cloned into plasmid	dsRNA prepped
1	Actin	pGEMT-easy	Yes
2	Ferritin	pGEMT-easy	Yes
3	Ubiquitin	pGEMT-easy	Yes
4	Lian2	pGEMT-easy	Yes
5	SAR1	pGEMT-easy	Yes
6	Fructose1,6 aldolase	pGEMT-easy	Yes
7	RAB1	pGEMT-easy	Yes
8	Tropomyosin	pGEMT-easy	Yes
9	Delta 9 desaturase	pGEMT-easy	Yes
10	Mitochondrial porin	pGEMT-easy	Yes
11	GFP	pJL24	XX

We also evaluated whether we could increase the amount of dsRNAs and/or siRNAs in plants by supplying a target plus the RNAi inducer sequence (Objective 2b). We tested our proposed strategy, by using transient expression to supply the RNAi inducer and a corresponding

GWSS target sequence simultaneously to *N. benthamiana* plants and then we evaluated these plants for accumulation of specific siRNAs. We failed to see any increase in siRNA accumulation from these treatments relative to when only the RNAi inducer (dsRNAs generated via *in planta* transcription) was used. We are no longer following this approach as it seems to not be necessary for our long term goals.

V. Publications or reports resulting from the project:

Rosa, C., Kamita, S. G., Dequine, H., Wuriyanghan, H., Lindbo, J. A., and Falk, B. W. 2010. RNAi effects on actin mRNAs in *Homalodisca vitripennis* cells. J. RNAi Gene Silencing 6:361 – 366.

Rosa, C., Kamita, S. G., Tsui, H., Falk, B. W. RNA-interference induced in the glassy-winged sharpshooter, *Homalodisca vitripennis*. To be submitted by March 31.

Falk, B. W. and Rosa, C. RNA-Interference and control of the glassy-winged sharpshooter and other leafhopper vectors of *Xylella fastidiosa*. 2010. Pp 39 – 43, Symposium Proceedings, Pierce's Disease Research Symposium. Dec. 15 – 17, 2010. Manchester Grand Hyatt, San Diego, CA.

VI. Presentations on research:

Falk, B W. RNAi approaches for helping to control insect vectors of plant pathogens. Invited Lecture. XVIII Conference of the IOCV, Campinas/SP/Brazil, November 7 – 12, 2010.

Rosa, C., Kamita, S. G., Dequine, H., Ethier, K., and Falk, B. W. RNA interference (RNAi) in *Homalodisca vitripennis*. Abstract 1147. 58th Annual Meeting of the Entomological Society of America. Dec 12 – 15, 2010. Town and Country Convention Center, San Diego, CA.

Falk, B. W. RNA interference (RNAi) efforts against insect vectors of plant pathogens. Abstract 0779. 58th Annual Meeting of the Entomological Society of America. Dec 12 – 15, 2010. Town and Country Convention Center, San Diego, CA

Falk, B. W. RNAi strategies for insect vectors of plant pathogens. Keynote lecture 1. 2nd International Research conference on Huanglongbing. Jan 10 – 14, 2011. Caribe Royale Hotel and Convention Center, Orlando, Florida.

Falk, B. Keynote address. RNA interference (RNAi) strategies for insect vectors of plant pathogens. 3rd Biennial ISU aphid research symposium. Seed Science Center, Iowa State University, Feb 15, 2011.

VII. Research relevance statement:

RNAi is a natural biological activity for controlling gene expression and anti-viral defense in a majority of eukaryotic organisms, including insects. The application of RNAi directed toward the control of different types of insect plant pests is becoming more feasible and promising. In our efforts, we were able to induce RNAi in *H. vitripennis* cells lines and whole insects, and are evaluating using transgenic plants as a means to initiate RNAi to help control the glassy winged sharpshooter and other leafhopper vectors of *Xylella fastidiosa*. RNAi is already used in commercial agriculture for plant virus control, and the many new publications demonstrating experimental successes with various plant-feeding insects suggest that RNAi could have a role in helping to manage Pierce's Disease of grapevines.

VIII. Lay summary of current year's results:

This work presents fundamental efforts towards understanding the feasibility of applying RNA interference (RNAi), to help combat Pierce's Disease of grapevines. Pierce's Disease is a significant threat to grape production in California and other parts of the U.S., and the causal agent, *Xylella fastidiosa*, a xylem-limited bacterium, also causes several other extremely important plant diseases worldwide. Our effort here does not directly target *Xylella fastidiosa*, but instead targets one of its most significant insect vectors, the Glassy-winged sharpshooter, *Homalodisca vitripennis*, and other sharpshooter vectors of *X. fastidiosa*. We focused our efforts this year on generating transgenic plants to evaluate their potential for inducing RNAi effects in *H. vitripennis*, and for developing and identifying optimal RNAi inducer delivery systems. We are using herbaceous plants as opposed to grapes for our transgenic approaches. These are easier and faster to transform and regenerate than are grapes, and the glassy-winged sharpshooter feeds readily on these plants. We have several transgenic plants and these will be evaluated during the upcoming year. We also have made good progress toward developing an efficient, rapid non-plant-based delivery system. If successful this will allow us to more easily evaluate candidate sequences for inducing desired RNAi effects in GWSS.

IX. Status of funds:

We were awarded two years funding to support one postdoctoral scientist (originally Co-PI Rosa who has accepted a position at Pennsylvania State University, now Raj Nandety is the lead postdoc on this project), a graduate student/part time technician, an undergraduate intern, plus funds for standard benefits. We also requested funds for routine supplies, recharge facility (Biosafety 3P Contained Research Facility) recharge costs and limited travel. We were awarded two years of funding including; $121,037 and $126,773 for years one and two, respectively. We are on track, spending wise, to use the year one funds as proposed in our original proposal budget, and anticipate that the funds requested for year two are appropriate for our project.

X. Summary and status of intellectual property produced during the research project:

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.