Renewal Progress Report for CDFA Agreement Number 15-0428-SA

"Searching for Potential Vectors of Grapevine Red Blotch-Associated Virus"

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REPORTING PERIOD: The results reported here are from work conducted July 2015 to January 2016.

INTRODUCTION

In 2006 an increase in grapevine leafroll disease (GLD) and vines with "red leaf" symptoms was observed by growers in vineyards located within Napa Valley, CA. Symptoms were also observed at the Oakville Experimental Vineyard (OEV) by Jim Wolpert (UC Davis Viticulture Extension Specialist), Ed Weber (UCCE Viticulture Farm Advisor), and Mike Anderson (UC Davis Staff Research Associate). Tissue samples were collected from symptomatic vines and tested by commercial laboratories and UC Davis Foundation Plant Service. Test results were most often negative for known grapevine leafroll-associated viruses (GLRaVs), although not all GLRaV species can be detected with current PCR tools.

The increasing awareness of blocks containing vines with grapevine leafroll disease symptoms, primarily in Napa and Sonoma Counties, but testing negative for grapevine leafroll-associated viruses resulted in a renewed focus on virus species and strains causing GLD. New GLRaV-3 strains have been discovered (e.g., Sharma et al. 2011); however, this did not fully explain all of the observed symptomatic vines. In 2010, next generation sequencing analyses identified a new pathogen (Al Rwanhnih et al. 2013). Soon after a circular DNA virus, similar to members of the family Geminiviridae, was isolated (Krenz et al. 2012) and, concurrently, PCR primers were developed(Al Rwahnih et al. 2013) for this pathogen now known as Grapevine Red Blotch-associated Virus (GRBaV). GRBaV has since been isolated from vines throughout North America and in Switzerland (Krenz et al. 2014), highlighting either a rapid dissemination or, more likely, its long hidden presence (e.g., misidentified as GLD).

This proposal focuses on possible vectors of GRBaV. Multiple viruses in the Geminiviridae are insect transmissible (Ghanim et al. 2007, Chen and Gilbertson 2009, Cilia et al. 2012), and there has been some initial evidence that leafhoppers may transmit GRBaV (Poojari et al. 2013). However, there has been mixed evidence of GRBaV field spread in association with leafhoppers. Concern for the spread of GRBaV led to an off-cycle project in summer 2013, funded by the "Napa County Winegrape Pest and Disease Control District" to initiate appropriate scientific studies of possible insect vectors of GRBaV. The work was continued in 2014 with American Vineyard Foundation (AVF) and Napa County funds.

Table 1. Arthropods targeted for GRBaV tests

Common name	Scientific Name	Common Distribution
Western grape leafhopper	*Erythroneura elegantula*	North Coast (north of Tehachapi Mtns.)
Variegated leafhopper	*Erythroneura variabilis*	Central Valley (San Joaquin Co. to So. Cal.)
Virginia creeper leafhopper	*Erythroneura ziczac*	Northern CA
Potato leafhopper	*Empoasca* sp.	Sporadic vineyard populations
Vine mealybug	*Planococcus ficus*	California vineyards
Grape mealybug	*Pseudococcus maritimus*	North Coast and San Joaquin Valley
Obscure mealybug	*Pseudococcus viburni*	Central and North Coast
Blue-green sharpshooter	*Graphocephala atropunctata*	Northern CA
European fruit Lecanium scale	*Parthenolecanium corni*	North Coast
Grape phylloxera	*Daktulosphaira vitifoliae*	North Coast, Sacramento Delta, Foothills
Grape whitefly	*Trialeurodes vittatas*	California
Mites	*Tetranychus spp.*	California

Our goal is to test potential vectors to provide concrete evidence that organisms can or cannot move GRBaV among vines. Determining field epidemiology of GRBaV is critical in the development of a control program – whether the pathogen is moved via infected nursery material, mechanically or, as with the focus of this study, by a vector. There are ample California vineyard sites where the pathogen is present but does not appear to have moved from infected vines over a period of many years, but in a few vineyards vine to vine movement has been recorded. This difference – whether there is no vector movement and disease presence is exclusively from infected nursery material or there is a vector – completely changes the needed control programs.

Our proposed work will screen all common vineyard arthropods, as well as the "long shots" that are potential GRBaV vectors, thereby providing the proper target for control. Table 1 provides a partial list of the common vineyard insect species that should be screened as potential vectors of GRBaV, based on their incidence and distribution in California vineyards.

Once tested organisms are either identified as vectors or our work shows that they are either not vectors or that they are so inefficient that spray programs are not needed, this information will be disseminated to farmers, PCAs and extension personnel, thereby having a practical, direct and immediate impact on control decisions to "spray or not to spray".

OBJECTIVES

To screen potential vectors for their ability to acquire and transmit Grapevine Red Blotch-associated Virus (GRBaV) and, if a vector is discovered, to determine vector efficiency. Objectives for this research program are as follows:

1. Screen common vineyard insects and mites as potential vectors for GRBaV.
2. Screen uncommon organisms that feed on vines as potential vectors for GRBaV.
3. Follow disease progression in established vineyard plots to collect preliminary data on field epidemiology.

Objective 1. Screen common vineyard insects and mites as potential vectors of GRBaV.

<u>2013-2014 – Initial Transmission Trials with Potted Vines</u>

In 2013 and 2014, we prioritized the screening of leafhoppers (*E. elegantula* and *E. ziczac*), grape whitefly (*Trialeurodes vittatas*), mealybugs (*Planococcus ficus* and *Pseudococcus maritimus*), and blue-green sharpshooter (*Graphocephala atropunctata*) because of the published work by Poojari et al. (2013), their prevalence in California vineyards, and/or their phloem feeding (this category of viruses [often Geminiviridae] are phloem-limited, although the biology and ecology of GRBaV is not fully understood).

In both years, canes were collected from Cabernet Sauvignon (clone 6) and Cabernet Franc (clone 04) vines in vineyard blocks where vines are known to have tested positive for GRBaV, and negative for all known GLRaVs and other known grapevine viruses. PCR test results for these vines were made and canes negative for all viruses except GRBaV and RSP (UC Berkeley and FPS test results) were transferred to UC Berkeley Oxford Tract Greenhouse and established in pots on a mist bench. Vines were maintained in the greenhouse, strictly treated to be insect and mite-free, and isolated from other vines that may have harbored viral pathogens. As indicators for these studies, we used Cabernet Sauvignon vines propagated from material provided by FPS and maintained under similar conditions.

Initial tests were conducted using the most mobile stages of key species, including adults of the *Erythroneura* (leafhopper) species and the grape whitefly, and crawlers of the vine mealybug crawlers and grape phylloxera. We employed standard transmission protocols to evaluate the potential of these insects to transmit GRBaV, as has recently been done for GLRaVs (Tsai et al. 2008, Tsai et al. 2011) and Pierce's Disease (Almeida and Purcell 2003a, b). We used a standard Acquisition Access Period (AAP) and Inoculation Access Period (IAP) of 120 hours (5 d) each for all tested insect species except the more delicate grape whitefly, which was allowed to feed on plants for an AAP and IAP of 48 hours (2 d) each. In the "controlled trials", known infected source plants or uninfected control plants in pots (1 liter size) were inoculated with 30-50 insects for the AAP, and surviving insects were then transferred to uninfected plants for the IAP. Field-collected leafhopper adults and blue-green sharpshooter adults were taken from an insectary

colony and released on plants that were placed singly in 61 x 61 x 61 cm BugDorm cages. Grape whitefly adults reared from pupae were collected in Napa County vineyards and then released into nylon bags enclosing 5 leaves on potted grape plants. Mealybug crawlers were moved onto individual grape leaves (3 leaves per plant) using a brush, and grape leaves were then enclosed with white paper bags. Following the IAP, all vines were treated with a contact insecticide to kill any remaining insect species. All insects were collected and tested for GRBaV within 48 hours after the AAP period. Every four months thereafter, three petioles were collected from each host plant and assayed for GRBaV infection. A total of 20 test vines were inoculated for each of the above insect species in the 2014 trials.

Results from the 2013/2014 trials have not indicated that any of these insects (i.e. leafhoppers [*E. elegantula* and *E. ziczac*], grape whitefly [*Trialeurodes vittatas*], mealybugs [*Planococcus ficus* and *Pseudococcus maritimus*], and blue-green sharpshooter [*Graphocephala atropunctata*]) are capable of transmitting GRBaV to uninfected grape vines. Inoculated vines from these trials are being held for a two year period, during which petioles are tested for GRBaV every four months and vines are visually evaluated for symptoms every fall. All insects that fed on infected plant material in these trials have tested negative as well. That said, we have recently begun to redesign our insect testing procedures in order to improve the sensitivity and accuracy of these laboratory tests. Insects from the 2013/2014 trials will be re-tested once the new protocol is developed and verified.

2015 – Improved "Bouquet" Transmission Trials

In 2015, protocols for these transmission experiments were modified due to concerns about (a) potentially low virus titer levels in the potted vines grown from cuttings of GRBaV-positive vines at vineyard field sites and (b) small number of insects per trial. Our concern is that candidate vector ability to transmit GRBaV is confounded by low titer levels in the GRBaV-positive vines used in previous trials and/or inadequate insect sample size.

The new approach involves using "bouquets" of mature grape leaves collected from GRBaV-positive vines at vineyard field sites that were not sprayed with insecticides. Each bouquet consists of ten mature grape leaves held in a 16 oz. plastic container that contains moist perlite. Ten leaves were collected from each of ten GRBaV-positive vines (nodes 1-5) in an established vineyard in Napa County (100 leaves total). Each bouquet consisted of one leaf from each of the ten vines, totaling ten leaves per bouquet and ten total bouquets (i.e. one bouquet per replicate). Furthermore, each trial now contains at least 100 insects/replicate and 10 replicates per treatment. There were no prior insecticide applications in this block.

Since July 2015, we have completed trials using the bouquets with Virginia Creeper leafhopper adults (*Erythroneura ziczac*), vine mealybug crawlers (*Planococcus ficus*), and foliar form grape phylloxera crawlers (*Daktulosphaira vitifoliae*). Due to concerns about bouquet degradation, these experiments used an AAP of 48 hours (2 day) and an IAP of 72 hours (3 d). Clip-cages (7 cm diameter x 2 cm height) were used to confine 10 insects/leaf to each bouquet (100 insects/bouquet). Bouquets with insects were placed in a 61 x 61 x 61 cm BugDorm cage and there were a total of 10 replicates per treatment. After the 48 hour AAP, clean potted vines were introduced into the cages. The clip cages were then removed, thus allowing the insects to move onto the clean vine. Bouquets were also removed at this time, after ensuring that they were free

of the candidate vectors. Petioles from the bouquets were then collected for GRBaV testing as well as a sub-sample of the candidate vectors (10-50 insects per replicate). After the 72 hour IAP, another subsample of the candidate vectors was collected for testing (10-50 insects per replicate) and the potted vines were then treated with a contact insecticide to kill any remaining insects. Three petioles were sampled from each vine (nodes 1-5) for immediate testing. Vines are now being maintained for a two year period and petioles tested for GRBaV every four months.

Bouquet experiments with grape phylloxera were initially unsuccessful due to their rejection of the bouquet material. Following the 48 hour AAP it was observed that none of the phylloxera crawlers had settled on the leaves and instead were mostly desiccated inside the cages. As such, we reverted to the previous experimental approach utilizing potted vines that were confirmed to be GRBaV positive. This time, two year old GRBaV-positive vines were used in these trials to possibly provide vines having elevated virus titer levels. Negative control source vines were one year old. Vines were placed in 61 x 61 x 61 cm BugDorm cages and inoculated by pinning ten leaf discs containing a large number of galls (>15) on each vine. The galls on these discs had been cut open with a razor in order to encourage movement of the crawlers onto the vine. After 25 days all of the potted vines exhibited >50 galls (i.e. 25 day AAP). At this point, clean vines were introduced into the cages and sub-samples of grape phylloxera adults, eggs and crawlers were collected for testing. Acquisition and inoculation vines remained together in the cages until the inoculation vines had >50 galls/vine, which resulted in a 38 day IAP. At this point vines were treated with both a contact and systemic insecticide. As before, vines will be held for a two year period and tested every four months.

So far, our 2015 “bouquet” trials have shown no transmission of GRBaV by either the Virginia Creeper leafhopper or vine mealybug. Similarly, the trial with foliar form grape phylloxera on two year old GRBaV-positive vines did not show any transmission.

Testing Plant Material for GRBaV

For all plant material, a standard DNA extraction protocol was used in order to extract DNA from grapevine petioles potentially infected with red blotch disease (Sharma et al. 2011). Three petioles were randomly selected from nodes 1-5, and 0.1 g of tissue was macerated in 1.8 ml Grape ELISA grinding buffer in Mo-Bio 2.0 ml tough tube containing a Boca chrome steel ball bearing (Sharma et al. 2011). Using a Precellys 24 Tissue Homogenizer at 6,500 Hz for two 10-second cycles with a 30-second intermission between cycles, the samples were centrifuged for 10 minutes at 13,200 rpm at 20°C. 1 ml of the supernatant was pipetted into 1.5 ml Eppendorf tubes and stored at -20°C. After briefly vortexing, the DNA extracts were denatured prior to performing qPCR; 8uL of extract was denatured in 99 uL of GES Denaturing Buffer plus 1 ul 1% beta-mercaptoethanol, by incubating at 95°C for 10 minutes and 4°C for 5 minutes (Sharma et al. 2011).

The qPCR was performed using Promega GoTaq qPCR Master Mix (Al Rwahnih et al. 2013). Two ul of each denatured sample were added to 12.5 ul Promega GoTaq master mix, 2.5 ul of 10 uM primers GVGF1 and GVGR1 (Al Rwahnih et al. 2013) , or with 10 uM primers RB-F and RB-R (developed by the lab for this study), 0.25 ul CXR reference dye, and 8ul water (Al Rwahnih et al. 2013). An Applied Biosystems qPCR machine with 7500 Fast System SDS Software was used for qPCR and to analyze the results. Thermocycling conditions include one

cycle of 95°C for 2 minutes; forty cycles of 95°C for 15 seconds, 58°C for 1 minute; and one cycle of 72°C for 10 minutes, followed by a final dissociation cycle. The PCR product was analyzed by the 7500 Fast System SDS Software, accounting for the Ct values, melting temperatures, and component curves.

Testing Insects for GRBaV

All insects used in these studies were frozen (-80 ºC) and later tested for GRBaV. The Qiagen DNeasy Blood and Tissue Kit was used for extractions and the bench protocol was followed to prepare the insect samples for the QIAcube; 25mg of insect were used for each extraction. The New England Biolabs Phusion High Fidelity kit was used for PCR. For each sample, 10 µL 5x Phusion buffer, 1µL of 10 mM dNTP, 2.5 µL of 10uM forward primer, 2.5 µL of 10 uM reverse primer, 100 ng of DNA, and 0.5 µL of Phusion DNA polymerase were used and diluted to 50 ul total reaction volume with water. After the samples were prepared, they were briefly centrifuged before being placed in a thermal cycler (DNA Engine Peltier, Biorad) with a heated lid. The thermal cycler conditions was as follows: 1) Initial Denaturation at 98^{o}C for 30 seconds, 2) Cycle of denaturing step at 98^{o}C for 10 seconds, annealing step at 62^{o}C for 30 seconds, and extension step at 72^{o}C for 30 seconds, repeated 30 times, and 3) Final Extension at 72^{o}C for 10 minutes. To visualize PCR product, a 2% agarose gel was used in 1x TAE buffer. A Qiagen GelPilot100bp Plus ladder was used. The gel was stained with ethidium bromide, and visualized on a GelDoc XR using the Quantity One program under UV light.

Conclusion – 2013-2015 – No Transmission Observed to Date

Since 2013, we have evaluated a total of 7 vector candidates, which includes grape leafhopper (*Erythroneura elegantula*), Virginia Creeper leafhopper (*E. ziczac*), grape whitefly (*Trialeurodes vittatas*), mealybugs (*Planococcus ficus* and *Pseudococcus maritimus*), blue-green sharpshooter (*Graphocephala atropunctata*) and foliar form grape phylloxera (*Daktulosphaira vitifoliae).* In 2015 we modified experimental protocols that were designed to overcome perceived limitations in previous transmission experiments from 2013-2014. This led to the re-evaluation of 2 candidates, Virginia Creeper leafhopper and vine mealybug, as well as evaluation of a new candidate, foliar form grape phylloxera. To date, none of the candidate vectors have tested positive for GRBaV and no transmission has been observed, although testing of insect and plant material from these experiments is on-going; in 2016 we plan to continue testing other candidate vectors listed in Table 1 as well as novel vectors identified from field collections in Objective 2 (see below).

Objective 2. Screen uncommon organisms that feed on vines as potential vectors for GRBaV.

Vineyard Insect Survey

We used the same methodologies described for Objective 1 to screen lessor known vineyard organisms or unlikely vectors. Insects were collected 1x/month from 5 established vineyards where movement of GRBaV has been observed or reported (assumed to have happened). Samples were collected from grape vines, ground covers and non-crop vegetation in the surrounding landscape using a combination of sweep-nets (on ground covers, 5 samples per site, 30 sweeps per sample) and a D-Vac type suction sampling machine (on grape vines and non-crop vegetation), which consisted of a 25cc gas blower/vacuum (Craftsman) fitted with a 5

gallon (18.9 liter) bucket on the vacuum tube to create a 1 ft^2 (0.093 m^2) sampling cone. Each D-Vac sample consisted of five thrusts with the D-Vac running at full speed (5 samples of grape vine per site, 5-10 samples of non-crop vegetation). All samples were held in a cooler and brought to the laboratory for immediate processing. Specimens were incapacitated using CO_2 gas, sorted and identified to species or genus, and then stored in 95% EtOH and stored at -80^o C until testing. So far we have collected leafhoppers in the genera *Ceratagallia* sp., *Acinopterus* sp., *Alconeura* sp., *Colladonus* sp., *Empoasca* spp., *Macrosteles* sp., *Osbornellus* sp., *Scaphytopius* spp., as well as the species *Deltocephalus fuscinervosus*, *Dikrella californica*, and *Euscelidius schenki*. Other organisms include members of the families Acanaloniidae, Cixidae, Membracidae, Miridae, Lygaeidae, Psyllidae, and Tingidae.

Many novel insects have been collected from vineyard sites where movement of GRBaV is suspected, but to date none have tested positive for GRBaV, although many specimens are still in the process of being tested, and as mentioned above, we are still in the process of refining our laboratory techniques to improve sensitivity of detection for insect material.

Non-crop Plant Survey

As a complement to the insect collection/testing, plant material was also collected from non-crop vegetation and tested for GRBaV in order to identify plant species that serve as reservoirs of GRBaV outside of the vineyard. Plant material was sampled from maple (*Acer* sp.), California buckeye *(Aesculus californica*), alder (*Alnus rhombifolia*), madrone (*Arbutus menziesii*), coyotebrush (*Baccharis pilularis*), toyon (*Heteromeles arbutifolia*), California walnut (*Juglans californica*), olive (*Olea europaea*), plum (*Prunus* sp.), coast oak (*Quercus agrifolia*), blackberry (*Rubus* spp.), willow (*Salix* sp.), elderberry (*Sambucus* sp.), California bay (*Umbellularia californica*), periwinkle (*Vinca major*), and wild grape (*Vitis californica*).

Plant material from the non-crop vegetation at these same sites has primarily tested negative for GRBaV, with the exception of wild grape which has tested positive fairly consistently across multiple sites. It should be noted that "wild grape" at these sites may actually be a hybrid form *Vitis californica* x *vinifera* due to its proximity to commercial vineyards.

Objective 3. Follow disease progression in established vineyard plots to collect preliminary data on field epidemiology.

Large Block Mapping (1 site, 2009-present)

We have been studying grapevine leafroll disease (GLD) movement at one particular site in Napa Valley, beginning in 2009. The block is a 20 ha newly planted (in 2008) block of Cabernet Sauvignon. Each year in September, incidence of GLD and more general "red leaf" symptoms were mapped at this site and location recorded with GPS. As early as 2009, many of the vines displayed "red leaf" symptoms but tested negative for grapevine leafroll-associated virus (GLRaV). In our subsequent surveys these symptoms appeared to spread through the vineyard, although a majority of these "red leaf" symptom vines continued to test negative for GLRaV over this period. We began testing vines for both GLRaV and grapevine red blotch-associated virus (GRBaV) in 2014 and found that 136 vines tested positive for red blotch, 9 tested positive for leafroll and 11 tested positive for both red blotch and leafroll. Plant material from the 2015 survey is still in the process of being tested, but we recorded about 250 "red leaf" symptomatic

vines, all of which had tested negative for GLRaV in 2014. With the development of new and more complete primers for both leafroll and red blotch, we are now in the process of re-testing plant material from the 2009-2013 survey to verify whether or not GRBaV is present in the "red leaf" symptom vines that previously tested negative for GLRaV.

Small Block Mapping (5 sites, 2015-present)

Additionally, in September 2015, we began to map and test for GRBaV (using the protocols described previously) at the same 5 established vineyards mentioned in Objective 2. At each site, an area consisting of 6 rows by 20 vines per row (120 vines/site total) was visually evaluated for GRBaV and petiole samples collected from each vine for diagnostic testing. The idea is to return to these same blocks in September 2016 and 2017 to repeat this detailed mapping in order to evaluate if the virus appears to be spreading from vine to vine. In October 2015 we learned that 1 of these established vineyard sites was going to be removed due to intolerable levels of GRBaV incidence. In November 2015, we located an alternate site to replace the lost site and conducted the same detailed mapping protocol. Results from this new mapping effort will not be available until follow-up mapping in fall 2016.

Red Blotch Titers Survey

Concerns about the possibility of low GRBaV titer levels in potted vines used in the transmission trials (see Objective 1) led us to initiate a broader survey to quantify GRBaV titer levels throughout grapevines over the course of the year. Starting in April 2015, plant material is collected each month from various parts (roots, trunk, canes etc.) of at least 10 GRBaV positive vines at each of 3 vineyard sites in Napa Valley. The goal is understand whether or not the virus localizes in certain regions of the grapevine during the year. If this is the case, it could improve the focus of our search for novel vectors (i.e. vectors that preferentially feed on parts of the vine with high GRBaV titer levels).

PUBLICATIONS PRODUCED AND PENDING, AND PRESENTATIONS MADE THAT RELATE TO THE FUNDED PROJECT.

Publications:

No peer-reviewed publications to report.

K. M. Daane, V. Hochman Adler, T. M. Lutz, H. Wilson, J. Hutchins, M. L. Cooper, B. Hogg, K. Blaisdell, G. Dervishian, S. Van Zyl, K. Kurtural, J. Chen, H. Oh, N. Fonseca-Espinoza, R. Oneto, D. Golino, and R. Almeida. 2016. Mealybug research – from pesticide movement in the vine to their role as vectors of plant viruses. In R. L. Smith (compiler), Proceedings, Sonoma County Winter Grape Day, Sonoma, CA.

Hochman Adler, V., Lutz, T. M., Hutchins, J. Cooper, M. L., and **Daane, K. M**. 2016. Identification and control of vine mealybug, pp. 6-11. In: *Proceedings, San Joaquin Valley Grape Seminar, January, 2015*. University of California Cooperative Extension and Allied Grape Growers. Easton, CA.

Presentations:

K.M. Daane: The importance of insects in the transmission of 'red-leaf' virus pathogens. *San Joaquin Valley "Grape Day"*. Parlier, CA. Aug. 2015.

K.M. Daane: Current research on vine mealybugs (and mealybugs as vineyard pests in general). *UC Davis Viticulture and Enology "On the Road in Santa Barbara)*. Santa Maria, CA. Nov. 2015.

K.M. Daane: Mealybug research – from pesticide movement in the vine to their role as vectors of plant viruses. *Sonoma County Grape Day*. Santa Rosa. CA. Feb. 2016.

RESEARCH RELEVANCE STATEMENT

Findings from this research will help improve our understanding of GRBaV transmission and field epidemiology in order to develop better recommendations and control programs for commercial growers. Greenhouse trials to evaluate GRBaV transmission by both suspected and novel insects aims to clarify which, if any, insects can transmitthis virus. Similarly, screening insects from field sites with suspected spread of GRBaV allows us to identify additional novel vectors for subsequent evaluation in greenhouse trials. Testing plant material from non-crop species in the natural habitats surrounding vineyards provides new information on potential reservoirs of GRBaV outside of the vineyard. Closer evaluation of the insects associated with non-crop reservoirs of GRBaV will further reinforce efforts to identify novel vectors. Detailed mapping of GRBaV at multiple sites where spread of this virus has been suspected will allow us to confirm if this is actually the case as well as evaluate spatial trends of infected vines relative to pertinent landscape features, such as riparian habitats or adjacent vineyard blocks with high levels of GRBaV infection. Finally, quantifying GRBaV titer levels throughout the vine will aid in the search for novel vectors that may feed on areas of the vine where the virus is concentrated.

LAYPERSON SUMMARY OF PROJECT ACCOMPLISHMENTS

Grapevine Red Blotch-associated Virus (GRBaV) is a newly identified vineyard pathogen causing vine damage similar to other Grape Leafroll Diseases (GLD). There has been some initial laboratory evidence that leafhoppers are a potential vector of GRBaV; however, there have been mixed reports of possible vector-borne movement in vineyards. Our goal is to identify and test potential vectors to provide concrete evidence that organisms can or cannot move GRBaV among vines. This work must be completed to develop a control program for "Red Blotch" and develop accurate information on the epidemiology of this newly reported pathogen. To date, we have tested leafhoppers (*E. elegantula* and *E. ziczac*), grape whitefly (*Trialeurodes vittatas*), mealybugs (*Planococcus ficus* and *Pseudococcus maritimus*), blue-green sharpshooter (*Graphocephala atropunctata*) and foliar form grape phylloxera (*Daktulosphaira vitifoliae*). So far none of these insects have moved the pathogen from an infected plant or plant material to a clean plant in laboratory studies. Our field studies have surveyed insects and potential non-crop reservoirs in vineyards with suspect movement of red blotch. None of the herbivores in this survey have tested positive for the virus responsible for red blotch, although many samples are still being processed in the laboratory. We have also conducted detailed mapping of red blotch in vineyards where movement of the virus is suspected in order to evaluate spatial trends related to

virus spread. Similarly, we are also mapping GRBaV titers levels within the vine itself to help with the identification of novel vectors which may preferentially feed on regions of the vine where the virus is localized.

STATUS OF FUNDS

Funds are being spent appropriately and are on schedule – as of January 2016, approximately $30,000 has been spent on employee wages and supplies. UC Berkeley is often slow to invoice the granting agency.

SUMMARY AND STATUS OF INTELLECTUAL PROPERTY ASSOCIATED WITH THE PROJECT

There is no intellectual property associated with this project.

LITERATURE CITED

Almeida, R. P. P., and A. H. Purcell. 2003a. *Homalodisca coagulata* (Hemiptera, Cicadellidae) transmission of *Xylella fastidiosa* to almond. Plant Disease 87: 1255-1259.

Almeida, R. P. P., and A. H. Purcell. 2003b. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera : Cicadellidae). Journal of Economic Entomology 96: 264-271.

Al Rwahnih, M. A., A. Dave, M. M. Anderson, A. Rowhani, J. K. Uyemoto, and M. R. Sudarshana. 2013. Association of a DNA Virus with Grapevines Affected by Red Blotch Disease in California. Phytopathology 103:1069–1076.

Krenz, B., J. R. Thompson, M. Fuchs, and K. L. Perry. 2012. Complete genome sequence of a new circular DNA virus from grapevine. Journal of virology 86:7715–7715.

Krenz, B., J. R. Thompson, H. McLane, M. Fuchs, and K. L. Perry. 2014. Grapevine red blotch-associated virus is widespread in the United States. Phytopathology.

Poojari, S., O. J. Alabi, V. Y. Fofanov, and R. A. Naidu. 2013. A Leafhopper-Transmissible DNA Virus with Novel Evolutionary Lineage in the Family Geminiviridae Implicated in Grapevine Redleaf Disease by Next-Generation Sequencing. PLoS ONE 8:e64194.

Sharma, A. M., J. B. Wang, S. Duffy, S. M. Zhang, M. K. Wong, A. Rashed, M. L. Cooper, K. M. Daane, and R. P. P. Almeida. 2011. Occurrence of Grapevine Leafroll-Associated Virus Complex in Napa Valley. Plos One 6.

Tsai, C. W., J. Chau, L. Fernandez, D. Bosco, K. M. Daane, and R. P. P. Almeida. 2008. Transmission of grapevine leafroll-associated virus 3 by the vine mealybug (*Planococcus ficus*). Phytopathology 98: 1093-1098.

Tsai, C. W., A. Rowhani, D. A. Golino, K. M. Daane, and R. P. P. Almeida. 2010. Mealybug transmission of grapevine leafroll viruses: an analysis of virus-vector specificity. Phytopathology 100:830–834.