Renewal Progress Report for CDFA Agreement Number 18-0398-000-SA

Title: Biology and Role of Treehoppers in Grapevine Red Blotch Disease

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Time period covered by the report: July 1, 2018 – February 22, 2019

Introduction:
A ssDNA virus, *Grapevine red blotch virus* (GRBV; family Geminiviridae), associated with Grapevine red blotch disease (Al Rwahnih et al. 2013; Sudarshana et al. 2015), is now recognized as the causal agent of red blotch disease (Cieniwicz et al. 2017). Because of its adverse effect on wine quality and resulting revenue loss, GRBV is becoming one of the most intensely studied grapevine viruses in California. A recent analysis on the economic impact indicated that the disease can cause economic losses of as much as $30,000 per acre in North Coast vineyards (Rickett et al. 2016).

Among the several insect species found in commercial vineyards with red blotch disease, the three-cornered alfalfa treehopper (3CAH), *Spissistilus festinus* Say, was found to be capable of transmitting GRBV under greenhouse conditions (Bahder et al., 2016). In studies conducted in California by Cornell University virologists, spatial patterns of red blotch distribution and *S. festinus* adults caught on yellow sticky traps that tested positive for GRBV by PCR indicated that this membracid is the most likely vector of significance to virus epidemiology (Cieniewicz et al. 2018). Our studies on GRBV transmission using 3CAH have not always produced consistent results. For example, our attempts to transmit GRBV using 3CAH under field conditions in an experimental vineyard at the UC Davis Plant Pathology Research Farm in Davis has yet to show success of transmission after an entire year. In the case of geminiviruses,

members of the genus Begomovirus are transmitted by a whitefly, *Bemisia tabaci*, (Hemiptera: Aleyrodidae). Recently, *B. tabaci* has been recognized as a cryptic species complex within which three biotypes have been recognized (Jiu et al. 2017). An added problem is differential transmission specificity by these cryptic species (Polston et al. 2014). We are curious whether our inconsistency in getting transmission by 3CAH might be due to the fact that different sources of field collected insects have been used in different studies conducted in different years. Use of a laboratory colony of insects raised from a single gravid female would be preferred when cryptic species or biotypes might be present. Our recent study of the suitability of grapevines and other plants as reproductive hosts of 3CAH now enables us to obtain successful oviposition and rearing of insects in culture, so one of the goals of our project is to develop methods to allow us to establish treehopper colonies from different infected vineyards for transmission studies.

In California's North Coast and in southern Oregon, the Zalom and Sudarshana labs as well as Dr. Walton's lab at Oregon State University have found colonization of grapevines by other treehoppers of the genus *Tortistilus* in vineyards where virus spread is occurring (Dalton et al. 2018). However, the status of *Tortistilus* as vectors of GRBV has not been established and preliminary attempts to transmit GRBV by field-collected *Tortistilus* have not been successful to date. In spring 2017, we made an extensive collection of *Tortistilus* adults from a Napa County vineyard and found morphs of brown and green color both with and without horns from the same host plants on the same day. These insects have previously been identified as *T. albidosparsus*, *T. pacificus* and *T. wickhami* based on the presence or absence of a suprahumeral horn characteristic and to some extent their coloration. That three closely related species would seemingly occupy the same feeding niche at the same time and location seemed odd to us, so we sent them to an expert on the family Membracidae, Dr. Dennis Kopp at the Smithsonian Natural History Museum in Washington DC, who now believes that these represent the same species based on morphological characteristics. As part of this project we are collaborating with Dr. Kopp to unravel the identification of *Tostistilu*s treehoppers and learn more about their seasonal biology which is little known to date, as well as their possible role in GRBV transmission.

We first began intensive and systematic sampling for 3CAH in early 2016 as soon as its potential status as a vector was determined. Continuous weekly monitoring from March 2016 to present has allowed us to characterize seasonal population dynamics of that species. We began observations of *Tortistilus* species treehoppers shortly thereafter in May 2016 when their presence was called to our attention in two different vineyards where GRBV spread was suspected. Biological studies of *Tortistilus* have not been seriously attempted in California, and we propose to do this in the coming season to determine seasonal phenology in vineyards and the surrounding landscape which is essential to develop an understanding of how, when, and where their management could be most effective, especially as efforts to confirm their status as vectors of GRBV continue in our labs and at Oregon State University.

Treehopper feeding symptoms are easy to recognize on grapevines and appear as girdled young shoots and petioles. Even though 3CAH had been considered a minor pest of grapevines because of girdling damage, it had not previously been determined if grape is a reproductive host for 3CAH. Cindy Preto recently completed dissertation research in our lab that identified feeding and reproductive hosts of 3CAH that included cover crops and common weeds (Preto et al. 2018a). A related study that was part of her dissertation also included a field study that confirmed grape as a reproductive host for 3CAH and documented successful oviposition and nymphal emergence (Preto et al. 2018b). Our current project will utilize her foundational studies on feeding and reproductive hosts of 3CAH as a basis for developing management approaches for this insect that would reduce risk of GRBV in vineyards most at risk of transmission by this species, and we also hope to develop useful information on the role of woody host plants on *Tortistilus* treehoppers as well.

In summary, this proposal is intended to unravel the role of treehoppers (Hemiptera: Membracidae) found

in California vineyards in the spread of GRBV by building on our recently completed studies, and developing sustainable management guidelines.

Objectives:

1. Determine timing and distribution of treehopper girdling in relation to red blotch incidence in vineyards.
2. Conduct field and greenhouse GRBV transmission studies using three cornered alfalfa hopper (3CAH) and *Tortistilus* spp. collected from vineyards with red blotch disease, and detect GRBV presence in the salivary glands of insects collected.
3. Confirm taxonomic identification and monitor *Tortistilus* spp populations in California vineyards and determine their seasonal biology in vineyards and surrounding landscapes.

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

Objective 1: *Determine timing of treehopper girdling in relation to red blotch incidence in vineyards.*

We initiated weekly monitoring of 3CAH activity in March 2016 in a block of Cabernet Sauvignon at the UC Davis Oakville Experimental Station where red blotch disease spread has been observed since 2011. Data obtained included sweep net sampling of resident vegetation in the vineyard to establish treehopper phenology and girdling incidence. The block was removed due to the amount of Grapevine red blotch disease incidence in March 2018, and the results (including the incidence of girdling in the block) was accepted for publication in the *Journal of Economic Entomology* (Preto et al. 2019) in fall 2018. Beginning in 2017, we began sampling a commercial Cabernet Sauvignon vineyard west of CA-29 near Oakville (Oak-1) every two weeks. Also in 2017, we recorded treehopper girdles every two weeks in a Cabernet Sauvignon research vineyard that we established using tested GRBV-free vines at the UC Davis Armstrong Tract in Solano County. The block has a 3 meter wide strip of alfalfa planted adjacent to the southern edge of the planting that has become naturally infested with 3CAH and serves as a source for natural migration to the vineyard. In 2018, we continued to sample girdles in both the Armstrong and Oak-1 vineyards in order to obtain a second year of data. We also began to sample girdles in a third commercial Cabernet Sauvignon vineyard located along Oakville Cross Rd. just east of Oakville (Oak-2), a replanted block close to a riparian area where new GRBV infections had been noticed.

Figure 1. Girdles on petioles from feeding by *Spissistilus festinus* (left) and *Tortistilus* spp. (right)

Girdle counts from all three vineyards commenced approximately three weeks after bud break and continued every two weeks until the sample date prior to leaf drop in fall. Girdles were counted and removed (in order to ensure that the girdles were not counted again in subsequent field visits) every two weeks from six rows containing five vines each located within the same vineyard. Girdles (Figure 1) were documented as being located on the apical shoot or leaf petiole and counted only if necrosis extended around the entire petiole or shoot.

Figure 2 presents the results of our girdle sampling in the Oak-1 vineyard. Similar to our results from 2017, girdles were first observed in June with peaks of new girdles occurring in July and late September, coinciding with emergence of adult 3CAH. More girdles were found on petioles than apical shoots.

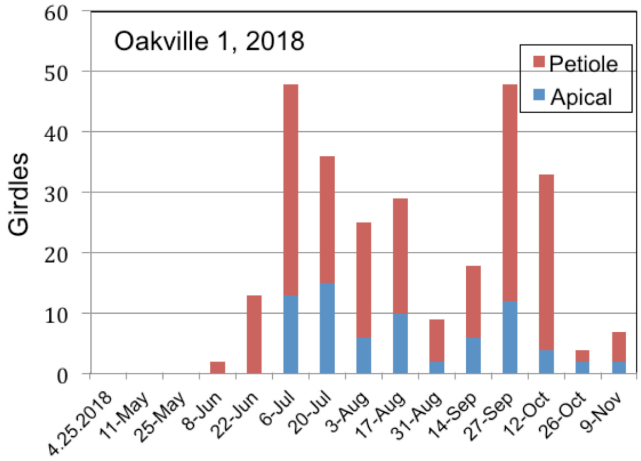

Figure 2. Total new petiole and apical girdles found on 30 vines at the Oak 1 site in 2018. Sampling was terminated following the November 9 sampling date as this entire block was removed because of high red blotch disease incidence.

Figure 3 presents the results of our girdle sampling in the UC Davis Armstrong Tract vineyard. The seasonal occurrence of new girdles was similar to what was observed in 2017, with the first girdles observed in late July and a single peak of new girdles occurring in late September through October. The number of petiole girdles was similar to the number of girdles found on the apical shoots.

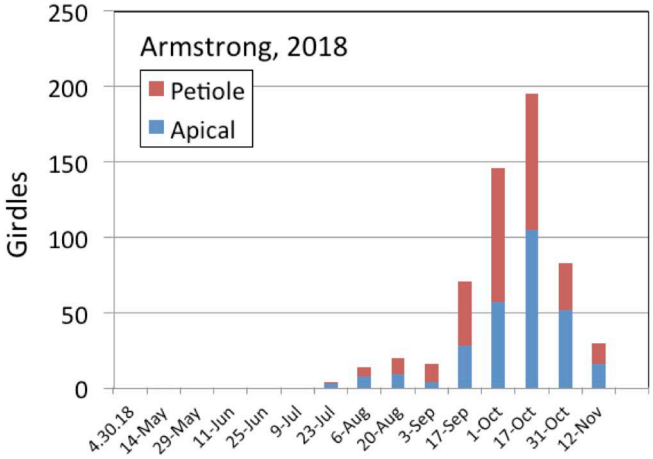

Figure 3. Total new petiole and apical girdles found on 30 vines at the UC Davis Armstrong site in 2018. Sampling was terminated following a severe wind storm that occurred on October 30 that removed most of the remaining leaves from the vines.

At the Oak-2 site, an established 9-year-old replanted vineyard adjacent to where an older vineyard with high incidence of GRBV infection had been removed in fall 2017, we received approval to plant younger vines in the vine row between the established vines both to evaluate GRBV spread as well as to compare preferential feeding of treehoppers, if any, on younger as compared with older vines. In May 2018, fifteen recipient Cabernet Sauvignon vines (qPCR tested GRBV negative) were planted directly between the established field vines, with 5 interplanted vines in each of three vine rows. The recipient vines were approximately four years old and maintained in 1gallon pots in a greenhouse until planting. The newly planted recipient vines were watered by hand twice a week. The fifteen interplanted vines and fifteen adjacent established vines were examined every two weeks for treehopper girdles. Figure 4 presents the total number of new girdles found on each sampling date on the older vines and the younger vines at the Oak-2 site. The first girdle was found on June 22 (on a young vine), with peak new girdles occurring in late September. In general, more girdles were found on the older established vines than on the younger interplanted vines, but this could simply be due to their relative size difference. The occurrence of girdles on each of the young vines is now known and will provide some background on when treehopper feeding occurred should GRBV be detected in one of these previously tested GRBV negative vines.

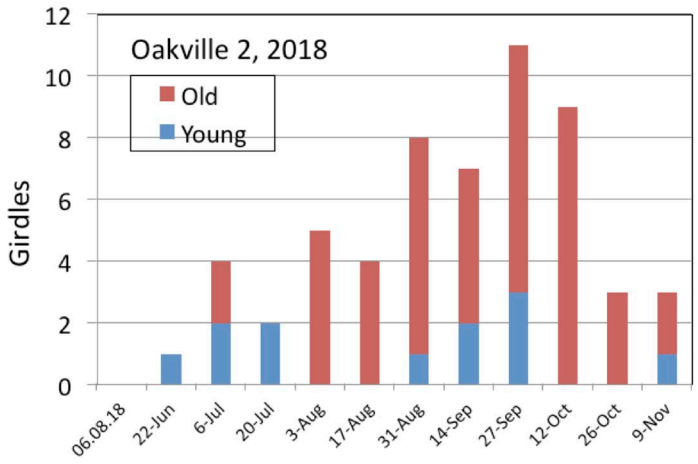

Figure 4. Total number of new petiole and apical shoot girdles found on established (old) and interplanted (young) vines at the Oak-2 vineyard.

Objective 2: *Conduct field and greenhouse GRBV transmission studies using three cornered alfalfa hopper (3CAH) and Tortistilus spp. treehoppers collected from vineyards with red blotch disease, and detect GRBV presence in the salivary glands of insects collected.*

In December 2017, we collected qPCR tested GRBV-infected cuttings from a Zinfandel vineyard in Amador County that we had studied for the previous three years and had documented GRBV spread. The new source material has been rooted and is now available for our transmission studies. Sequencing of amplicons obtained by qPCR assays on these GRBV isolates indicate that they belong to clades I and II. We will refer to this source material as we discuss certain field and laboratory transmission studies during the course of our project. In addition, we currently have ~500 potted Cabernet Sauvignon grapevines maintained in our greenhouse for use in our field and laboratory transmission studies.

Field spread and transmission studies. We have previously established a Cabernet Sauvignon vineyard using qPCR-tested GRBV negative vines at the UC Davis Armstrong Tract in Solano County for use in field transmission experiments. The vineyard consists of 30 vine rows with 55 vines per row and is

Figure 5. Experimental Cabernet Sauvignon transmission and spread block planted at Armstrong Tract, Solano County.

oriented east to west. As mentioned earlier in Objective 1, we have also established an alfalfa strip along the southern edge of the vineyard that has become naturally infested with 3CAH (Figure 5). On September 8, 2018, we planted rooted Zinfandel vines from the Amador County vineyard infected with clade I and II GRBV isolates between our established Cabernet Sauvignon GRBV-free vines in the third vine row north of the alfalfa strip in order to provide a virus source within the established vineyard to monitor possible infection and spread. Five plants of each GRBV clade were interplanted in this manner, and are being watered from the pre-existing drip-lines. qPCR tests to determine if GRBV spread from newly planted GRBV infected vines to neighboring GRBV-free vine will begin in March 2019, and will continue throughout this project.

Row/Vine	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	Total
R1			G		G	G											G					4
R2		G	G										G						G		G	5
R3		G	G		G	G					G										G	6
R4		G		G	G		G	*		*	G		G				G	G			G	9
R5										G								G				2
R6	G	G							G		G	G			G			G		G	G	9
R7						G		G		G		G							G		G	6
R8							G	G	G	G				G			G			G		7
R9	G			G				G	G				G					G		G	G	8
R10	G											G		G						D		3
R11	G	G		G	G		G	G			G				G						G	9
R12	G	G	G	G											G	G	G	G	G		G	10
R13	G	G	G	*			G					G			G					G		7
R14	G	G			G	G			G		G		G							G	G	9
R15	G							G	G				G	G							G	6
R16	G	G	G	G	G		G	G	G	G		G						G				11
R17										G					G						G	3
R18	G		G	G			G	G				G		G	G	G					G	10
R19											G	G	G	G		G					G	6
R20											G											1
R21	G						G	G	G	G						G	G	G	G		G	10
R22					G					G	G					G	G		G		G	7
R23	G				G	G			G		G			G	G	G	G				G	10
R24	G	G	G	G	G			G	G		G						G	G	G	G	G	13
R25	G			G		G	G		G	G		G				G	G				G	10
R26	G						G		G			G			G			G	G	G	G	9
R27					G								G							G		3
R28					G				G						G							3
R29	G				G	G					G				G		G				G	6
R30			G		G		G								G							4
R31			G				G	G	G	G	G						G	G	G			9
R32			G				G		G		G					*		G		G	G	7
R33	G		G	G	G				G		G	*	G			G			G			9
R34	G				G		G	G						G	G						G	7
R35	G		G	G							G				G							5
R36					G		G			G	G			G					G			6
R37				G	G		G	G	G					G			G	G	G	G	G	11
R38		G	G	G		G	G	G					G		G			G	G			10
R39						G		G	G			G			G	G	G			G		8
R40	G	G	G		G	G			G		*	G									G	8
R41	G	G				G		G		G	G		G		G		G	G	G			11
R42				G	*			G												G	G	4
R43				G				G	G	G	G			G	G					G	G	10
R44	G			G			G		G		G		G	G		G		G			G	10
R45	G			G		G	G	G	G		G	G			G				G			10
R46					G	G									G						G	4
R47	G			G	G							G					G	G				6
R48			G	G	G		G				G						G					6
R49																						0
R50			G															G		G		3
R51					G			*						*						G	G	3
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	353

Figure 6. Map of treehopper girdles on a new Cabernet Sauvignon block (A block) at the UC Davis Oakville Experimental Station. (G=vine with girdles, *=rootstock only, and dark rectangle=missing vine)

In another study, we observed that a third of the vines in a block of newly planted Cabernet Sauvignon vines (A block) at the UC Davis Oakville Experimental Station (1,066 vines of CS clone 7 on C3309) had girdling damage in fall 2017, and we mapped girdle incidence in the block (Figure 6). This block is adjacent to blocks that had a history of grapevine red blotch disease occurrence since the disease was first recognized as different than leafroll virus, and is therefore at high risk of becoming infected with GRBV by vector-mediated transmission. None of the vines had grapevine red blotch disease symptoms at that time. We will test all 1066 grapevines for GRBV by qPCR again this year and we intend to continue monitoring girdles and testing these vines for GRBV during the course of this project.

In June 2018, we were directed to a Sauvignon Blanc vineyard in the Gordon Valley area of Napa County that had a large resident population of *Tortistilus albidosparsus* treehoppers (Figure 7). Later in 2018, we qPCR tested all of the vines in the most heavily infested of the vineyard blocks for GRBV infection, and have mapped which vines have tested positive for the virus in preparation for a transmission study we intend to do at this site in spring 2019. The grower will allow us to deploy potted 50 of our tested GRBV-free Cabernet Sauvignon vines in cages between the existing vines within vine rows. We will collect *T. albidosparsus* from known infected vines and cage them on infected vines for a minimum of 48 hours, and then transfer them directly to the caged recipient virus-free vines where they will remain for an additional 72 hours. The recipient vines will be returned to our UC Davis greenhouse and qPCR-tested periodically for GRBV presence. The study will be repeated if it is possible to continue to collect *T. albidosparsus* adults.

Figure 7. Morphs of *Tortistilus albidosparsus* (left) and infestation of *Tortistilus albidosparsus* feeding on Sauvignon Blanc shoots in the Gordon Valley vineyard on June 18, 2018 (right).

3CAH and Tortistilus collections and colony establishment. We have planned to establish 3CAH laboratory colonies originating from individuals collected from GRBV-infected vineyards where spread consistent with a motile vector is believed to be occurring that we could use for better-controlled transmissions studies. In the past, the source of 3CAH used for transmission studies by us and other groups that have studied GRBV transmission were typically not well-documented so it seems possible that biotypes or even cryptic species could confound resulting outcomes. To date, it has been quite difficult for us to maintain a reproductive lab colony of 3CAH, although we were able to achieve modest success in rearing, particularly on common vetch, in our previous work on feeding and reproductive hosts (Preto et al. 2018a). To our knowledge, it has not been possible for any researchers to maintain a

reproductive laboratory colony of *Tortistilus*. We believe that both a woody host and an annual host are required for consistent successful completion of a life cycle, especially by *Tortistilus* treehoppers, and we began testing this hypothesis in late summer 2018 by introducing both treehopper species into cages containing potted oak seedlings together with a legume in a greenhouse at UC Davis. We have observed successful oviposition of both 3CAH and *Tortistilus* (Figure 8) on the oak seedlings, and have recently had 3CAH nymphs emerge. We have not yet seen *Tortistilus* nymphs emerge, but this is not surprising because many treehopper species are limited to a single generation each year. We are continuing to monitor these cages to determine sustainability of 3CAH populations and *Tortistilus* nymphal emergence.

Figure 8. *Tortistilus albidosparsus* oviposition scar on oak seedling in our laboratory study.

3CAH greenhouse transmission and salivary gland studies. In July 2018, we collected 3CAHs to be used for GRBV greenhouse transmission work directly off of grapevines in two GRBV-infected Napa County vineyards. Other 3CAHs for use as controls were collected at the same time in July from an organic alfalfa field near UC Davis. All treehoppers were collected by sweep net, aspirated into collection vials, separated into males and females, and then placed as individual mating pairs into nylon insect cages housing a mature alfalfa plant. This study included seven "GRBV-infected grapevine" Napa County collected mating pairs and seven "GRBV healthy" alfalfa-sourced mating pairs. Unfortunately, our attempt to establish isofemale line colonies using single female and male combinations was not successful.

On September 9, 2018, we again collected 3CAHs from alfalfa fields to test GRBV transmission in a greenhouse study that used our new qPCR tested GRBV-infected source material from Amador County described earlier. For these transmission assays, 3CAHs were placed into three separate insect cages housing a single leafed-out source vine planted in a 1gallon pot. One cage had a healthy grapevine, qPCR tested negative for GRBV; one cage had a grapevine with GRBV clade I, and one cage had a grapevine with GRBV clade II. The 3CAHs were starved for three hours prior to being transferred to the cages to facilitate feeding. Acquisition access period (AAP) was 48 hours. Insects removed from each cage were individually placed in clip-cages on qPCR confirmed GRBV healthy recipient CS vines and allowed an inoculation access period (IAP) of 48 hours. This study included 10 recipient vines as replicates for both of the clades as well as 10 recipient vine replicates for the healthy control. A total of 30 vines are included in this study. Testing of these vines for GRBV by qPCR will begin in March 2019.

GRBV detection in salivary glands. In August 2018, hundreds of 3CAHs were collected by sweep-net from an alfalfa field near UC Davis. We also collected hundreds of Virginia creeper leafhoppers (VCLH), *Erythroneura ziczac,* from an untreated UC Davis campus CS vineyard to serve as a negative control.

After starving both groups of insects for three hours, half of the collected insects of each species were placed into mesh cages containing a GRBV-infected source vine (392-II), and the other half of the insects of each species were placed into a mesh cage containing a GRBV-free healthy (Ghv-35) source vine. All insects were allowed an AAP of 48 hours. Beet leafhopper, *Circulifer tenellus*, adults from a laboratory colony (courtesy: Dr. R.L. Gilbertson, at UC Davis, functioned as an internal positive control. These leafhoppers were fed on sugar beet plants infected with Beet curly top virus (BCTV), a ssDNA geminivirus known to be circulative in its vector.

In order to overcome the possibility of environmental contamination while also providing a level of external cuticular sterilization for insects with documented self fecal "grooming" behavior, all insects were vortexed in a 20% bleach solution prior to dissection. After a 48 hr AAP, individual 3CAH and VCLH were aspirated off source vines, placed singly into 1.5 ml centrifuge tubes containing 1 ml of 20% bleach solution and vortexed on high for five seconds. Individual insects were then placed into another 1.5 ml centrifuge tube containing 1 ml sterile Millipore water and vortexed again for five seconds. Insects were then removed from the centrifuge tubes using a #3 Bioquip insect pin and placed ventral side up onto a sterile Petri dish situated directly under a Leica 12.5 stereo-microscope.

Two insect pins were used to extract salivary glands from each insect. The first pin was used to press against the insect, stabilizing it so that the second pin could easily locate the area between the insect's first and second coxae. Once this area was located, the second pin was pressed through the entire insect, effectively severing the head/first coxae region from the rest of the insect's body. After the insect heads were removed, the heads were placed onto another sterile Petri dish and a single drop of Millipore water was pipetted onto the insect's head. Immersing insect's head entirely in fluid facilitates salivary gland extraction as the water droplet exerts a suspension force that forces salivary glands to "float" outside the insect head cavity (Figure 9).

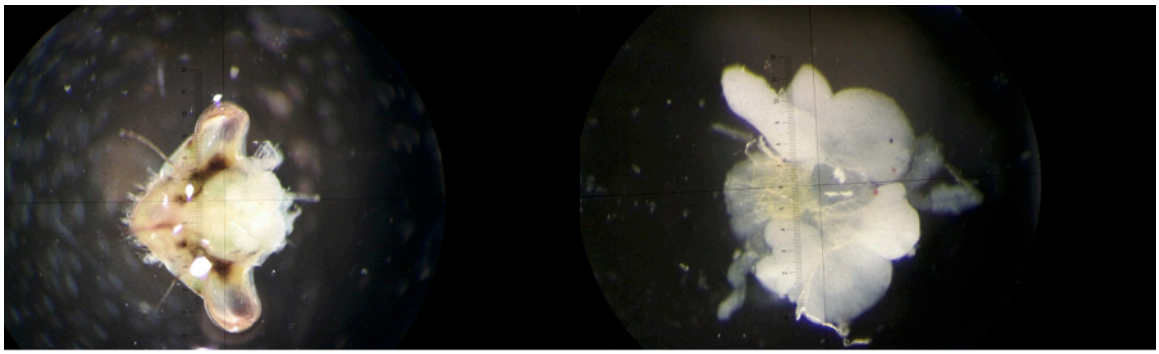

Figure 9. Three-cornered alfalfa hopper salivary gland dissections showing salivary glands within head capsule (left) and removed from head capsule (right).

After" teasing out" the insect's salivary glands with insect pins, sterile 10uL pipette tips attached to a 0.1uL-20uL pipette were used to sever the salivary glands from the insect's head. After the salivary glands were removed, they were individually placed in another 1.5 mL centrifuge tube containing 180uL ATL buffer (Qiagen Inc) and 20uL proteinase K. All vials were then incubated for 30 minutes at 65 °C. In all, 60 vials of insect salivary glands were tested, a test that included ten 3CAHs and ten VCLHs feeding on GRBV-infected (392-II) source vines, ten 3CAHs and ten VCLHs feeding on our GRBV-free Ghv-35 source vine, and ten BCTV positive and ten BCTV negative beet leafhoppers. In this assay, none of the salivary gland sample from 3CAHs tested positive for GRBV in qPCR tests. However, salivary gland extracts from 9 of 10 beet leafhoppers fed on BCTV-infected sugar beets, tested positive for BCTV.

Objective 3: *Confirm taxonomic identification for and monitor Tortistilus spp. populations in California vineyards and surrounding landscapes.*

Identification. In spring 2016, our labs found colonization of grapevines by treehoppers we identified to belong to the genus *Tortistilus* in vineyards where GRBV spread was occurring. *Tortistilus* treehoppers had not been associated with grapevines prior to that time, although there was mention of the "buffalo leafhopper' belonging to a different treehopper genus, as feeding on California grapevines in Smith (2013). Later that year, Dr. Walton's lab at the Oregon State University also found *Tortistilus* treehoppers in Oregon vineyards where Grapevine red blotch disease was spreading (Dalton et al. 2018). Both 3CAH and *Tortistilus* spp. belong to the Ceresini tribe of the family Membracidae. However, preliminary attempts to transmit GRBV by field-collected *Tortistilus* have not been successful to date. In spring 2017, we made an extensive collection of *Tortistilus* adults from a Napa County vineyard and found morphs of brown and green color both with and without horns from the same host plants on the same day. These insects had been tentatively identified as *Tortistilus albidosparsus*, *T. pacificus* and *T. wickhami* based on the presence or absence of a suprahumeral horn characteristic and to some extent their coloration. These three closely-related species would seemingly occupy the same feeding niche at the same time and location seemed very odd to us, so we sent them to a specialist on the family Membracidae (i.e. Kopp and Yonke 1979) Dr. Dennis Kopp at the Smithsonian Natural History Museum in Washington DC. The four morphs; brown horned, green horned, brown unhorned and green unhorned, were all identified as being the same species based on microscopic observations of genitalia and the characteristic spots on the front of their head (which was used in the original description of *T. albidosparsus* as a species in 1860). He also noted significant variation in the development of the horns among individuals (Figure 10). In 2018, we performed shotgun DNA sequencing on some of the voucher specimens of these morphs, all collected on the same hosts on the same date and from the same Napa County Vineyard (Table 1), and found them to possess the identical Cytochrome oxidase 1 gene (CO1), a mitochondrial gene used in insect taxonomy and identification. The genomes described were deposited at NCBI under bioproject (BIOPROJ00090900) as the first genomic resource for the genus *Tortistilus*. These results indicate that the morphs indeed all belong to *T. albidosparsus*. Based on their morphology, we are preparing a paper as junior authors with Dr. Kopp that will also synonymize *T. pacificus* as a junior synonym of *T. albidosparsus.* We are currently trying to obtain specimens that have been previously identified as *T. pacificus* from insect collections for molecular analysis to support the morphological observations.

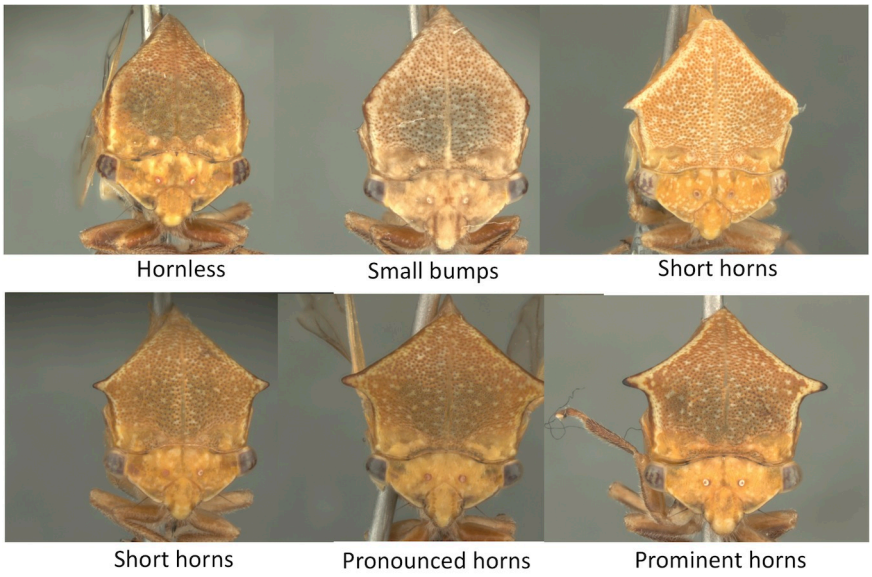

Figure 10. Clinal variation of suprahumeral horns in females from a single collection of *Tortistilus albidosparsus* from a Napa County vineyard.

Table 1. Sample information and sequence reads obtained by Illumina sequencing of DNA from four morphotypes of insects now described as *Tortistilus albidosparsus*, and collected in a grape vineyard in Napa County, CA.

Sample ID	Code	Color	Horned	Illumina reads (million)	Coverage Gbp)
DS17-01	BH-	Brown	No	46.0	13.8
DS17-02	BH-	Brown	No	44.8	13.4
DS17-03	GH-	Green	No	47.3	14.2
DS17-04	GH-	Green	No	45.8	13.7
DS17-05	BH+	Brown	Yes	43.9	13.2
DS17-06	BH+	Brown	Yes	45.9	13.8
DS17-07	GH+	Green	Yes	44.5	13.4
DS17-08	GH+	Green	Yes	45.3	13.6

In a related study, on April 25, 2018, we collected third and fourth instar *T. albidosparsus* nymphs from common vetch growing in a riparian area 30 m away from a confirmed GRBV infested vineyard in Pope Valley, Napa County. The nymphs were then brought to UC Davis where they were transferred into individual clip cages containing potted vetch plants placed within field cages (Figure 11), and allowed to reach maturity. As the treehoppers emerged as adults, they were placed into separate cages according to their color and suprahumeral horn morphotypes. These morphotypes were subsequently placed in various combinations according to their morphotype in cages on oak plants to test the heritability of the color and suprahumeral horn characteristics. We are anticipating the nymphs resulting from these combinations will emerge in spring 2019.

Figure 11. Field-collected *Tortistilus albidosparsus* nymphs individually caged on common vetch plants in larger cages at UC Davis awaiting adult emergence, 2018.

Field studies. According to our 2016-18 observations, adult *T. albidosparsus* are more common in vineyards during a brief period in spring of the year, May and June. We have found nymphs on groundcover in and around vineyards. In spring 2019, we will sample groundcover in and around two vineyards where we have consistently collected adults on grapevines beginning in March to determine

when nymphs are present and to record the associated plant hosts. We will also cage adults that we collect on woody hosts on which we have collected them in previous years including oak, vetch, grape, toyon, burdock, and privet to determine their suitability as ovipositional hosts. On June 26, 2018, we swept *T. albidosparsus* adults from grapevines and transferred them to cages on oak trees adjacent to one of the vineyards (Figure 12), and we have observed successful oviposition. Some of the cages contained males of the horned morphotype and females of the unhorned morphotype, while some contained both sexes of only the unhorned morphotype. The eggs have yet to hatch at the time of writing this report, but we anticipate hatching in March or April 2019 at which time.

Figure 12. *Tortistilus albidosparsus* caged on oak trees adjacent to our Gordon Valley vineyard site.

Publications and Presentations:

Publications –

Dalton, D.T., R.J. Hilton, C. Kaiser, K.M. Daane, M.R Sudarshana, J. Vo, F.G. Zalom, J.Z. Buser, and V.M. Walton. 2018. Spatial associations of vines infected with grapevine red blotch virus in Oregon vineyards. Plant Dis. https://doi.org/10.1094/PDIS-08-18-1306-RE

Preto, C.R., M.R Sudarshana, and F.G. Zalom. 2018. Feeding and reproductive hosts of *Spissistilus festinus* (Hemiptera: Membracidae) found in Californian vineyards. J. Econ. Entomol. 111(6): 2531-2535. https://doi.org/10.1093/jee/toy236

Preto, C.R., M.R Sudarshana, M.L. Bollinger and F.G. Zalom. 2018. *Vitis vinifera* as a reproductive host of *Spissistilus festinus* (Say) (Hemiptera: Membracidae). J. Insect Sci. 18(6): 20, https://doi.org/10.1093/jisesa/iey129s

Preto, C.R., B.W. Bahder, E.N. Bick, M.R Sudarshana, and F.G. Zalom. 2019. Seasonal dynamics of *Spissistilus festinus* (Say) (Hemiptera: Membracidae) in a Californian vineyard. J. Econ. Entomol. https://doi.org/10.1093/jee/toz022 (accepted)

Preto, C.R., M.R Sudarshana, and F.G. Zalom. 2019. A Grapevine red blotch virus vector: the three-cornered alfalfa hopper (3CAH), *Spissistilus festinus*, in Californian vineyards. American Vineyard Magazine. (accepted)

Sudarshana, M.R., M.L. Bollinger, J. Vo, K.A. Stevens, B.W. Bahder, D.D. Kopp and F.G. Zalom. 2019. Four morphotypes of a treehopper in a California vineyard belong to a single species, *Tortistilus albidosparsus* Stål (Hemiptera: Membracidae). J. Insect Sci. (submitted)

Presentations –

8-07-18 The three-cornered alfalfa hopper and their association with vineyards. Paso Robles Vineyard

Technical Group. Paso Robles, CA.
8-07-2018 Grapevine red blotch disease: Recognition and Management. Invited talk hosted by the Paso Robles Wine Country Alliance, Paso Robles, CA.
9-17-2018 Grapevine red blotch disease: Biology and impact on wine grape production in California. Invited seminar hosted by the Department of Plant Pathology, Washington State University, Pullman, WA.
9-21-18 Biology and behavior of *Spissistilus festinus* in Californian vineyards. University of California Berkeley Essig Presentation. Berkeley, CA.
10-17-18 Red blotch and the three-cornered alfalfa hopper. Leafroll virus tailgate talk - microscope and sweep net demonstration. Lodi-Woodbridge Winegrape Commission. Lodi, CA.
11-09-2018 Virus diseases of Grapevines. An overview presented to UC Davis/University of Montpellier symposium hosted by the Department of Viticulture and Enology, University of California, Davis, CA.
12-05-18 Biology and behavior of *Spissistilus festinus* in California vineyards. University of California Davis Entomology & Nematology department seminar. Davis, CA.
12-18-18 Biology and role of treehoppers in grapevine red blotch disease, CDFA Pierce's Disease Conference, San Diego, CA
2-27-19 Seasonal Dynamics of the three-cornered alfalfa hopper, a vector of GRBV, in Californian vineyards, Current Wine and Winegrape Research Conference, UC Davis Extension, Davis, CA (pending)

Relevance Statement:
The primary aim of this proposed study is to gain a better understanding of the ecology and epidemiology of Grapevine red blotch virus (GRBV) in California vineyards so that appropriate measures for preventing infection and spread of the virus can be developed. This will be accomplished by documenting the role of treehoppers (Hemiptera: Membracidae) in the spread of GRBV in California vineyards, and how their use of the surrounding landscape influences the spread of the virus

Layperson Summary of Project Accomplishments: The results of this project are expected to better define the possible role of the three-cornered alfalfa hopper (3CAH) and other vineyard treehoppers in the epidemiology of GRBV, including management of virus spread, by determining feeding on grapevines seasonally and their phenology in relation to cover crops and non-crop vegetation in and around vineyards. Possible transmission by other treehoppers found in vineyards where GRBV is spreading will also be confirmed. This essential information will contribute to the management of red blotch disease by cultural methods such as reducing plant hosts favorable to sustaining vector populations or precise treatment timings based on treehopper biology in vineyards and when transmission is most likely to occur.

Status of Funds: We have expended $44,934 as of the end of December with most expenses to date for a graduate student (salary plus fees and benefits) and a Postdoctoral Researcher (salary plus benefits). We anticipate expending most of the remaining first year funding by the end of June, primarily for these same individuals in addition to charges for travel and supplies (including UC Davis land charges) that will greatly increase as we get into the field season.

Summary and status of intellectual property associated with the project: There have been no intellectual property considerations with this project.

Literature Cited:
Al Rwahnih, M., Dave, A., Anderson, M., Rowhani, A., Uyemoto, J.K., Sudarshana, M.R. 2013. Association of a DNA virus with grapevines affected by red blotch disease in California. Phytopathology 10:1069-1076.
Bahder, B.W., Zalom, F.G., Jayanth, M., Sudarshana, M.R. 2016. Phylogeny of Geminivirus coat protein

sequences and digital PCR aid in identifying *Spissistilus festinus* as a vector of Grapevine red blotch-associated virus. Phytopathology. 106(10):1223-1230.
Cieniewicz E., Perry K., Fuchs M. 2017. Grapevine Red Blotch: Molecular Biology of the Virus and Management of the Disease. pp. 303-314, In: Meng B., Martelli G., Golino D., Fuchs M. (eds) Grapevine Viruses: Molecular Biology, Diagnostics and Management. Springer Verlag, Berlin.
Cieniewicz, E.J. Pethybridge S.J., Loeb, G., Perry, K., Fuchs, M. 2018. Insights into the ecology of Grapevine red blotch virus in a diseased vineyard. Phytopathology 108:94-102.
Dalton, D,T., Hilton, R.J., Kaiser, C., Daane, K.M., Sudarshana, M.R., Vo, J., Zalom, F., Jessica Z. Buser, J.Z., Walton, V.M. 2018. Distinct patterns of distribution and spread of Grapevine red blotch virus in comparison with Grapevine leafroll-associated virus-3 distribution in Oregon vineyards. Plant Dis. (online preprint) https://doi.org/10.1094/PDIS-08-18-1306-RE
Jiu, M., Hu, J., Wang, L.J., Dong, J.F., Song, Y.Q., Sun, H.Z. 2017. Cryptic species identification and composition of Bemisia tabaci (Hemiptera: Aleyrodidae) complex in Henan Province, China. J. Insect Sci.:78:1-7.
Kopp, D.D., Yonke, T.R. 1979. A taxonomic review of the tribe Ceresini (Homoptera: Membracidae). Entomol. Soc.Amer. Misc. Publ. 11(2):1-97.
Polston, J.E., De Barro, P., Boykin, L.M. 2014. Transmission specificities of plant viruses with the newly identified species of the Bemisia tabaci species complex. Pest Manag. Sci., 70:1547-1552.
Preto, C.R., Sudarshana, M.R, Zalom, F.G. 2018a. Feeding and reproductive hosts of *Spissistilus festinus* (Hemiptera: Membracidae) found in Californian vineyards. J. Econ. Entomol. 111(6): 2531-2535.
Preto, C.R., Sudarshana, M.R, Bollinger, M.L., Zalom, F.G. 2018b. *Vitis vinifera* as a reproductive host of *Spissistilus festinus* (Say) (Hemiptera: Membracidae). J. Insect Sci. 18(6): 20, https://doi.org/10.1093/jisesa/iey129s
Preto, C.R., Bahder, B.W., Bick, E.N., Sudarshana, M.R, Zalom, F.G. 2019. Seasonal dynamics of *Spissistilus festinus* (Say) (Hemiptera: Membracidae) in a Californian vineyard. J. Econ. Entomol. https://doi.org/10.1093/jee/toz022 (accepted)
Ricketts, K.D., Gomez, M.I., Fuchs, M.F., Martinson, T.E., Smith, R.J., Cooper, M.L., Moyer, M., Wise, A. 2016. Mitigating the economic impact of grapevine red blotch: Optimizing disease management strategies in U.S. vineyards. Amer. Jour. Enol. Vitic. 68:127–135.
Smith, R. 2013. Three-cornered alfalfa hopper, pp. 286-287. *In* L. J. Bettiga (ed.), Grape Pest Management, 3rd ed. University of California, Division of Agriculture and Natural Resources Publication 3343.
Sudarshana, M.R., Perry, K.L., Fuchs, M.F. 2015. Grapevine red blotch-associated virus, an emerging threat to the grapevine industry. Phytopathology. 105:1026-32.