

RENEWAL REPORT FOR CDFA AGREEMENT NUMBER 18-0338-SA

“SEASONAL ECOLOGY AND TRANSMISSION EFFICIENCY OF THREE-CORNERED ALFALFA HOPPER (*SPISSISTILUS FESTINUS*) AND OTHER NOVEL INSECT VECTORS OF GRAPEVINE RED BLOTCH ASSOCIATED VIRUS (GRBAV)”

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REPORTING PERIOD: The results reported here are from work conducted November 2018 – February 2019

INTRODUCTION

Grapevine red blotch virus (GRBV) is a circular, single-stranded DNA virus (Geminiviridae: Grablovirus) and is associated with red blotch disease in wine grapes (*Vitis vinifera* L.) (Krenz et al. 2012, Varsani et al. 2017). Symptoms of red blotch include reddening of leaf veins and the appearance of blotchy red areas on the leaf surface and/or at the leaf margin. Red blotch disease negatively impacts crop vigor, yield and quality. Diseased vines typically exhibit reduced photosynthesis and stomatal conductance, delayed fruit maturation, decreased accumulation of sugars and anthocyanins, as well as lower pruning and berry weights (Al Rwahnih et al. 2013, Sudarshana et al. 2015, Blanco-Ulate et al. 2017).

While this disease was first reported in 2008 in a Napa County vineyard, subsequent surveys found GRBV to be widespread throughout North America (Krenz et al. 2014) and testing of archival plant material revealed the virus has been present in California since at least 1940 (Al Rwahnih et al. 2015). The wide geographic distribution of GRBV implicates that this virus was likely distributed via infected nursery material, although many have also reported in-field spread of red blotch disease. While increased incidence of red blotch disease over time within vineyards and/or clustering of symptomatic vines gave reason to believe in the existence of one or more vectors, it could be argued that such trends were the result of environmental factors leading to latent expression of symptoms in some GRBV-positive vines. Yet the argument for an insect vector was strengthened by surveys that revealed the presence of GRBV in wild *Vitis* spp. naturally established outside of vineyards (Bahder et al. 2016a, Perry et al. 2016) and shortly thereafter it was shown that a treehopper, the threecornered alfalfa hopper (TCAH) (Membracidae: *Spissistilus festinus* Say), could successfully transmit GRBV between grapevines (Bahder et al. 2016b).

All characterizations of GRBV to date have placed it within the Geminiviridae (Krenz et al. 2012, Al Rwahnih et al. 2013, Sudarshana et al. 2015, Varsani et al. 2017). The only known vectors of viruses in this family are hemipterans, in particular leafhoppers, treehoppers and whiteflies (Bridson and Stanley 2015, Bahder et al. 2016b). Key vineyard hemipterans that are known to regularly feed on grape vines include *Erythroneura* leafhoppers (Cicadellidae: *E. elegantula*, *E. variabilis*, and *E. ziczac*), mealybugs (Pseudococcidae: *Planococcus ficus*, *Pseudococcus maritimus*, *Ps. viburni* and *Ferrisia gilli*), blue-green sharpshooter (Cicadellidae: *Graphocephala atropunctata*), and to a lesser extent phylloxera (Phylloxeridae: *Daktulosphaira vitifoliae*), grape white fly (Aleyrodidae: *Trialeurodes vittatas*) and lecanium scale (Coccidae: *Parthenolecanium corni*). While many of these candidate vectors are frequently encountered and/or in high abundance in vineyards, so far experiments have shown that only TCAH can successfully transmit GRBV between grape vines (Daane et al. 2017).

While the ecology and management of TCAH has been well defined for multiple leguminous crops like alfalfa, soybeans and peanuts (Meisch and Randolph 1965, Mueller and Dumas 1975, Moore and Mueller 1976, Mitchell and Newsom 1984, Wilson and Quisenberry 1987, Johnson and Mueller 1989, Wistrom et al. 2010, Beyer et al. 2017), very little is known about this insect in vineyards. Facing a lack of information, growers concerned about the spread of GRBV in their vineyards may be inclined to preemptively apply chemical controls for TCAH. As such, new information on TCAH population dynamics, transmission efficiency and economic thresholds in vineyards will be critical to the development of sustainable IPM programs.

In addition to TCAH, broad testing of numerous non-economic insects in vineyards has revealed a number of potentially novel candidate vectors, including *Melanoliarius* sp. (Cixiidae), *Osbornellus borealis* (Cicadellidae) and *Colladonus reductus* (Cicadellidae) (Cieniewicz et al. 2017, Fuchs et al. 2017). Like TCAH, these organisms are typically found in low abundance in vineyards but are none-the-less present in and around these systems (Wilson et al. 2016, Daane et al. 2017).

While we know that TCAH can reproduce on certain leguminous annual ground covers found in vineyards (Zalom et al. 2017), the role of perennial non-crop plants found outside of or adjacent to vineyards is less clear.

Recent work has demonstrated that TCAH densities in vineyards do not appear to be influenced by proximity to natural habitats such as oak woodland and riparian areas (Zalom et al. 2017). While many of the perennial plants found in such habitats can likely serve as suitable overwintering sites, or even reproduction sites (less likely), the TCAH do not appear to have an obligate relationship with any particular perennial species. That said, they do appear to make some use of these plants and more information on this will contribute to a better understanding of their seasonal ecology and movement between vineyards and natural habitats.

OBJECTIVES

- (1) Identify TCAH overwintering and reproduction sites
- (2) Determine timing of vineyard colonization by TCAH, including movement into the vine canopy and cane girdling
- (3) Evaluate novel insect vector candidates
- (4) Quantify TCAH transmission efficiency

DESCRIPTION OF ACTIVITIES AND RESULTS

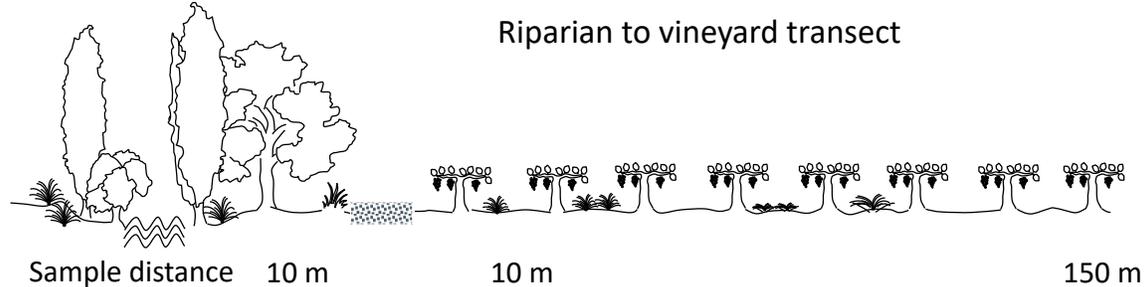
(1) TCAH overwintering sites and reproduction on non-crop perennial plants

TCAH populations have been sampled in natural vegetation surrounding vineyards with known TCAH populations. During the winter, it is assumed that TCAH is in the adult stage but breeding hosts other than legumes have been difficult to identify in the field. To date we have recovered TCAH adults on

toyon (*Heteromeles arbutifolia*), wild grape (*Vitis* spp.) and various ground covers, primarily legumes during the summer, but TCAH has only been recovered from ground covers during the winter.

(2) Timing of TCAH colonization, movement into the vine canopy and cane girdling

We have sampled vineyards in Napa and Sonoma County vineyards to evaluate the activity of TCAH populations along transects that extend out from large patches of natural habitat into vineyards. Field sites consist of vineyard blocks >2 acres adjacent to riparian and/or oak woodland habitat. There are 5 total study sites. All vineyard blocks are red varietals that are at least 5 years old and located on level ground with similar trellis and irrigation systems. All plots are maintained insecticide free throughout the course of the study.



At each site insects are sampled along five parallel transects (positioned 20 m apart) that extend out from the riparian or oak woodland habitat (i.e. “natural habitat”) into the vineyard. Each transect is 160 m long – going 10 m into the natural habitat and 150 m into the vineyard. Along each transect samples are taken at the interior of the natural habitat (10 m into the habitat) as well as at the edge and interior of the vineyard (10 and 150 m into the vineyard, respectively). The edge of the vineyard and natural habitat are typically separated by a roadway or path that is about 5 m wide. Densities of TCAH, *Erythroneura* leafhoppers and other hemipterans are being monitored along the transects approximately every 2 weeks using a combination of yellow sticky-traps, sweep-nets and beat-sheet sampling. Two yellow sticky-traps (16 x 10 cm, Seabright Laboratories, Emeryville, CA) are placed at each transect point. In the vineyard, one trap is placed in the vine canopy (approximately 4 feet above the ground surface) and another trap is hung from irrigation lines (approximately 1 foot the above ground surface). In the natural habitat, two sticky-traps are hung from a pole at each transect point at a height equal to those in the vineyard (i.e. one trap 4 feet and the other 1 foot above the ground surface). Traps are replaced approximately every 2 weeks between March 2017 and March 2019. Sweep-nets are used to sample ground covers. At each transect point, a set of 30 unidirectional sweeps are collected from the groundcovers using a 30.5 cm diameter sweep-net (BioQuip Products, Rancho Dominguez, CA). Proportion of ground cover to bare soil is recorded along with species composition and ground cover status (i.e. proportion of cover that was still green/healthy). A modified beat-sheet is used at each transect point to sample the canopy of grape vines (in the vineyard) and non-crop species (in the natural habitat). The beat-sheet consists of a 1 m² nylon funnel that feeds into a detachable 1- gallon plastic bag. For each sample, the funnel is held beneath the canopy while vigorously shaking the plant (or vine) for 30 seconds to dislodge insects into the funnel and plastic collection bag. Each month, vines along each vineyard transect point are evaluated for signs of TCAH feeding damage (i.e. girdling of leaf petioles). At each vineyard transect point, 1 cane from each of 10 randomly selected vines is visually inspected for leaf girdling. Total leaf nodes and leaf girdles per cane were recorded for each vine.

The study is near the two-year end (March 2019) and we report here on preliminary findings, with a full analyses at the end of the collection to be prepared for publication. TCAH activity showed a strong temporal trend, with densities generally increased between June – August along with some activity in March and October/November. Comparing the different sampling techniques, the highest TCAH densities were recorded on yellow sticky traps (YST), followed by sweep-nets and then beat-sheets.

While there was no clear gradient of TCAH activity across the transect points, densities on the YSTs and in the sweep samples were slightly elevated in natural habitats in early June just prior to increases observed in the vine canopy at both the vineyard edge and interior in the following round of sampling. Changes in TCAH densities between the ground covers and vine canopy were not always clearly reflected in the data. While densities in the vine canopy did increase as the proportion of healthy/green ground covers diminished, some TCAH could still be found on the little bit of ground cover that remained later in the season. Surprisingly these late season TCAH were most frequently encountered on ground covers in the vineyard interior. Finally, petiole girdling became apparent in August, with a higher proportion of girdles located at the vineyard interior. This increase in girdling in August follows increased TCAH densities observed in the vine canopy between June – August.

(3) Evaluation of novel insect vector candidates

Candidate vectors are those insects collected in a previous survey that tested positive for GRBaV, which includes *Melanoliarus* sp. (Cixiidae), *Osbornellus borealis* (Cicadellidae), *Colladonus* spp. (Cicadellidae) and *Scaphytopius* spp. (Cicadellidae). While these species can be found in vineyards, they are generally very low in abundance. As such, robust colonies of each species will need to be established in order to conduct adequate transmission experiments, and this will be the focus of our efforts in 2019-2020, primarily working with the *Scaphytopius* sp. that was both commonly found and often positive for the GRBaV virus. We will also conclude are trials with TCAH.

Organism (Family: Species)	Tested Positive / Total	% Positive (Frequency infected)	General population density
Cicadellidae: <i>Erythroneura elegantula</i>	1 / 161	<1%	High
Cicadellidae: <i>Aceratagallia</i> spp.	1 / 59	2%	High
Cicadellidae: <i>Empoasca</i> sp.	1 / 51	2%	High
Membracidae: <i>Spissistilus festinus</i>	5 / 39	13%	Med.
Cicadellidae: <i>Scaphytopius</i> sp.	14 / 24	58%	Med.
Cicadellidae: <i>Acinopterus angulatus</i>	1 / 31	3%	Med.
Cixiidae: <i>Melanoliarus</i> sp.	1 / 23	4%	Med.
Cicadellidae: <i>Caladonus coquilletti</i>	5 / 8	63%	Low
Cicadellidae: <i>Colladonus montanus reductus</i>	1 / 2	50%	Low

(4) TCAH transmission efficiency

Previous transmission experiments (2015-2017) were conducted under greenhouse conditions using potted grape vines. Candidate vectors evaluated included western grape leafhopper (*Erythroneura elegantula*), Virginia creeper leafhopper (*Erythroneura ziczac*), grape whitefly (*Trialeurodes vittatas*), vine mealybug (*Planococcus ficus*), blue-green sharpshooter (*Graphocephala atropunctata*) and foliar form grape phylloxera (*Daktulosphaira vitifoliae*). To date, none of these candidates have been able to move GRBV between potted vines.

While Bahder et al. (2016b) demonstrated that TCAH can transmit GRBV between potted grape vines in a greenhouse, it remains unclear how well TCAH can move this virus under field conditions. As

such, we are currently evaluating TCAH transmission using field vines for virus acquisition. That is, TCAH are caged on known positive vines in commercial vineyards for a 48-hour period and then moved to clean potted vines in the greenhouse.

In 2018, we attempted to show transmission using a new experimental design that considered virus titer in the vine. In a commercial vineyard with known positive GRBV vines, we used organically grown cages to cage TCAH adults on virus-free and virus-infested vine branches for a 48-h acquisition period. We then cut the entire branch (cage and all) and transferred the tested TCAH to the UC Berkeley Laboratory. There, we transferred the tested TCAH to clean vines for a 48-h transmission period. This study considered changes in virus titer in the vine during the season because we repeated this work four times during the season (we suspect that virus titer increases during the season). This also considers the possibility that earlier studies with potted vines supplied by FPS had GRBV but the titer in these potted vines was not comparable to field conditions where some vines have had the virus for years if not decades.

To date, we have successfully shown TCAH on virus-infested vines have been positive for the GRBV (e.g., acquisition). However, we have not yet observed transmission to clean vines at UC Berkeley, although we note that symptoms are often slow to appear. These vines will be held for two years, being observed regularly and PCR-tested periodically for the GRBV.

PUBLICATIONS AND PRESENTATIONS DURING REVIEW PERIOD

During the review period, the CDFA/PD Proceedings report was the only publication produced. Presentations were:

Wilson, Daane et al. “Searching for Potential Vectors of Grapevine Red Blotch Virus” *CDFA Pierce’s Disease Glassy-winged Sharpshooter Symposium*. San Diego, CA. Dec 2018.

Daane, Wilson et al. “Grapevine Virus and Vector Management” *San Joaquin Valley Grape Symposium*. Easton, CA. Jan 2019.

Daane, Wilson et al. “Grapevine Virus and Vector Management – Is Red Blotch Being Spread by Insect Vectors” *Vineyard IPM – Central Coast Grape Seminar*. San Luis Obispo, CA. Feb 2019.

RELEVANCE STATEMENT

Over the past five years we have drastically improved our understanding of GRBV epidemiology, host plants and insect vectors. We have effectively defined a narrow list of non-crop reservoirs for this virus and whittled down the range of candidate insect vectors. While it has been demonstrated that TCAH can transmit GRBV between vines, many questions remain about transmission efficiency, especially under field conditions and, more generally, TCAH seasonal ecology in vineyards. Additional candidate vectors remain to be tested as well, including *Colladonus* spp. and *Scaphytopius* spp. As we enter this second phase of research, our goal is to better characterize TCAH activity in vineyards and adjacent natural habitats, quantify transmission efficiency and test any remaining candidate vectors.

LAYPERSON SUMMARY

Grapevine red blotch virus (GRBV) is associated with red blotch disease in wine grapes (*Vitis vinifera* L.) and negatively impacts crop vigor, yield and quality. Surveys have revealed that the virus only infects grapes (*Vitis* spp.). While multiple insects have tested positive for GRBV, only the three-cornered alfalfa hopper (TCAH) (Membracidae: *Spissistilus festinus*) has been shown to actually transmit the virus between grapevines. We are now in the process of developing a better understanding of the seasonal ecology and transmission efficiency of TCAH in vineyards. Additionally, we plan to test the ability of any remaining candidate insect vectors to transmit GRBV. Our goal is to use this information to develop

actionable management strategies for commercial grape growers to help reduce the incidence and spread of GRBaV in vineyards.

STATUS OF FUNDS

Funds are currently provided by only the CDFA PD/GWSS program. We are working with a graduate student that has been awarded a USDA grant that will leverage our current funding, and we will work with a larger national group on a USDA SCRI grant. At this time, funds are being spent appropriately, albeit a little behind schedule as the initial use was delayed while other grants were being spent to seed this work. We expect all funds to be spent by the granting end period.

SUMMARY STATUS OF INTELLECTUAL PROPERTY

We are claiming no intellectual property associated with this project and are reporting our findings as they are developed.

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