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Title of Project: BIOLOGY AND ROLE OF TREEHOPPERS IN GRAPEVINE RED BLOTCH DISEASE

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Abstract: This one-year project was initiated July 1, 2017. It builds upon studies initiated earlier by the Zalom and Sudarshana labs at UC Davis, and the Daane lab at UC Berkeley. Results presented include monitoring the population dynamics of the three-cornered alfalfa hopper (3CAH), *Spissistilus festinus* Say in vineyards and surrounding landscapes over the 2017 season in vineyards and along transects from vineyards to natural areas, preliminary field transmission studies, greenhouse studies of the feeding and reproductive status of various weeds and cover crops found in vineyards as they relate to three cornered alfalfa hopper feeding and reproduction, and the status of grape as a reproductive host of 3CAH.

Lay Summary; The results of this project are intended to better define the role of the three-cornered alfalfa hopper in the epidemiology of GRBV, and to examine the role of grapevines, cover crops, and non-crop vegetation in and around vineyards in sustaining 3CAH populations. Studies to determine possible transmission by other treehoppers found in vineyards where GRBV is spreading were initiated. 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 where nearby GRBV source are known to occur.

Introduction: A grapevine disease with symptoms that resembled those of grape leafroll was found in Napa County vineyards in 2007 (Calvi 2011). The disease was named grapevine red blotch disease and further investigations revealed a new DNA virus initially named Grapevine red blotch-associated virus (GRBaV), tentatively grouped in the family Geminiviridae (Al Rwahnih et al., 2013; Sudarshana et al. 2015). The virus was also found independently in grapevines in New York, Oregon and Washington (Krenz, et al. 2012; Poojari et al., 2013; Seguin et al., 2014), and it is now known to be widely distributed in the US. California vineyards infected with the disease, especially those planted to red varieties, report substantial impact to grape quality, substantially reducing their value.

Red blotch disease epidemiology is not well known. Although some researchers initially believed that the virus did not spread to or within established vineyards, observations by growers, consultants, and other researchers strongly suggested spread was occurring in some vineyards in a pattern that was consistent with a motile insect vector. The virus has been isolated from wild grapevines, mainly open-pollinated *Vitis californica* (Bahder et al., 2016; Perry et al., 2016), even at a considerable distance from commercial vineyards. After surveying many of the hemipteran insect species found in commercial vineyards where there was evidence that red blotch disease was spreading, the three-cornered alfalfa hopper (3CAH), *Spissistilus festinus* Say, (Hemiptera: Membracidae) was found capable of transmitting GRBV under laboratory conditions by Bahder et al. (2016). Subsequently, other treehoppers of the genus *Tortistilus* were observed feeding on grapevines where red blotch disease was believed

to be spreading in California, southern Oregon and the Willamette Valley (Zalom and Sudarshana, unpublished; Walton, unpublished), but the status of these species as GRBV vectors has yet to be confirmed. Although some aspects of 3CAH biology is mentioned in the scientific literature, the majority of this information comes from legume cropping systems such as soybean, peanut and alfalfa where it is considered to be a pest (Wildermuth 1915, Beyer et al. 2017). The biology of 3CAH and more especially the other treehoppers found in vineyards is little known. A better understanding of their seasonal biology in and around vineyards and their role in virus transmission is essential for developing management guidelines to prevent spread of red blotch disease within and between vineyards. The research objectives addressed through this Pierce's Disease Program sponsored research began in 2016 with funding from the CDFA Specialty Crops Block Grant Program (to Sudarshana and Zalom), USDA-ARS National Program funds (to Sudarshana), and the American Vineyard Foundation (to Daane). Funding for this Pierce's Disease Program grant was finally received on October 10, 2017, due to complications in contracting between UC and CDFA, however we continued many elements of the proposed research prior to formally receiving funding in order to continue previously-ongoing research through the growing season. Therefore some of the results we report hereafter chronologically precede the initiation of this grant.

Objectives: The long-term objectives of this study address improved 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 red blotch disease can be developed. The primary goal is to document the abundance of treehoppers, focusing on the three-cornered alfalfa hopper and *Tortistilus* species, in California vineyards and the surrounding landscape, and to understand their role in the spread of GRBV between grapevines and regionally.

The specific objectives of this project are:

1. Monitor the population dynamics of 3CAH in vineyards and surrounding landscapes over the season.
2. Conduct GRBV transmission studies using treehoppers collected from vineyards with red blotch disease, and detect GRBV in the salivary glands of insects collected. Monitor field transmission by 3CAH.
3. Determine transmission efficiency of 3CAH to identify virus acquisition periods and persistence in the insect.
4. Evaluate the role of cover crops on the 3CAHs in vineyards.
5. Determine status of common weed and cover crops as feeding and reproductive hosts for 3CAH.

Results and Discussion:
Monitor the population dynamics of 3CAH in vineyards and surrounding landscapes over the season. This objective was addressed by both the Zalom and Sudarshana labs at UC Davis, and by the Daane Lab at UC Berkeley.

In the study by the Zalom and Sudarshana labs and primarily conducted by PhD student Cindy Preto, ground cover located in and around a 53-row Cabernet Sauvignon block at the UC Davis Oakville Research Station and the perimeter of the reservoir pond at that site was sampled weekly by sweep net for two years starting March 2016 through March 2018. The vineyard block consists of 53 rows. All odd numbered rows were tilled late March of both years and were therefore not sampled. Each even numbered row was subdivided corresponding to the 6 proximal vines on each row border and the middle 18 vines, and ground cover within these areas were sampled separately for treehopper adults and nymphs. and the captured adults were sexed. The vineyard was removed removal due to increasing red blotch disease incidence following the last sampling date in March 2018. Similar late winter capture patterns were observed each year (2016-18). We now believe that this marks the initiation of activity of the overwintering generation into the vineyard. Bud break occurred in early April of both 2016 and 2017, about seven weeks after the first 3CAH adult was found in the vineyard. The first nymphs were collected on May 16 and May 23 of those years coinciding with an increase in adult 3CAH captures (Figure 1) and the grapevine phenological marker of bloom. Increase in captures of 4^{th} and 5^{th} instar nymphs increased in concert with adult captures, and we posit that this indicates the first in-field generation of 3CAH. Subsequent 3CAH generations overlap one another. Vineyard weeds, which constitute the ground cover sampled at Oakville, started to noticeably dry in early August of both years, corresponding with a drop in adult 3CAH. This also corresponded to an increase in the number of girdles on vines. (Figure 2). Girdles were only sampled in 2017, but we are counting girdles weekly again in 2018 in 3 commercial vineyards to see if the pattern remains similar and across locations.

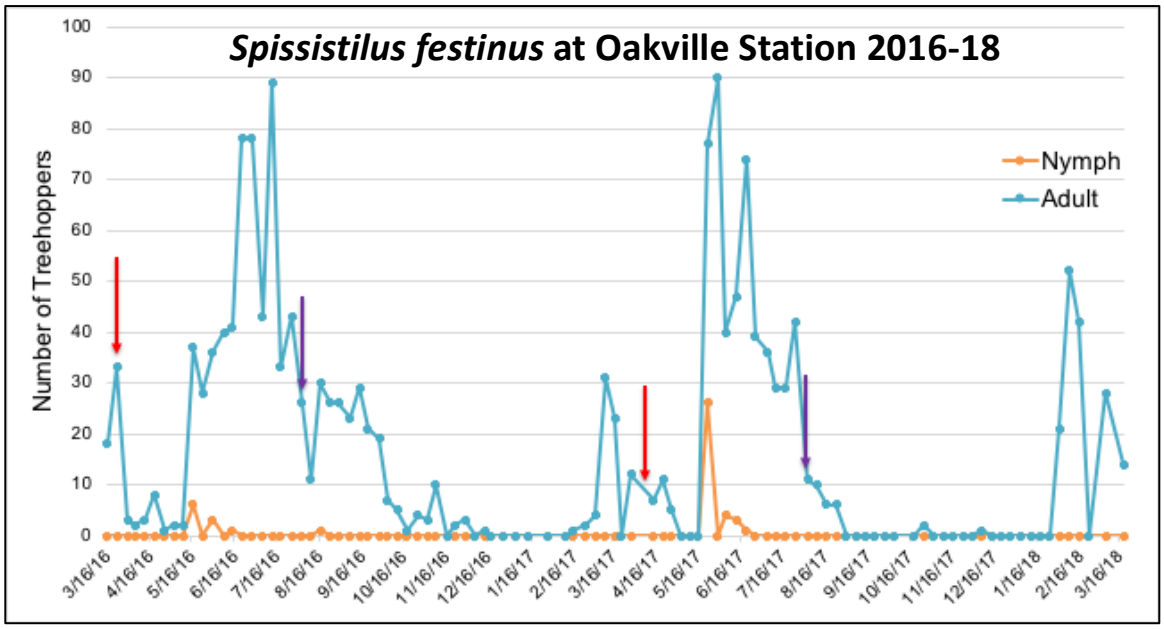

Figure 1. Weekly sweep net sampling of vineyard ground cover for three-cornered alfalfa hopper at Oakville, 2016-2018. Red arrows indicate bud break and purple arrows indicate time when ground cover was completely dried.

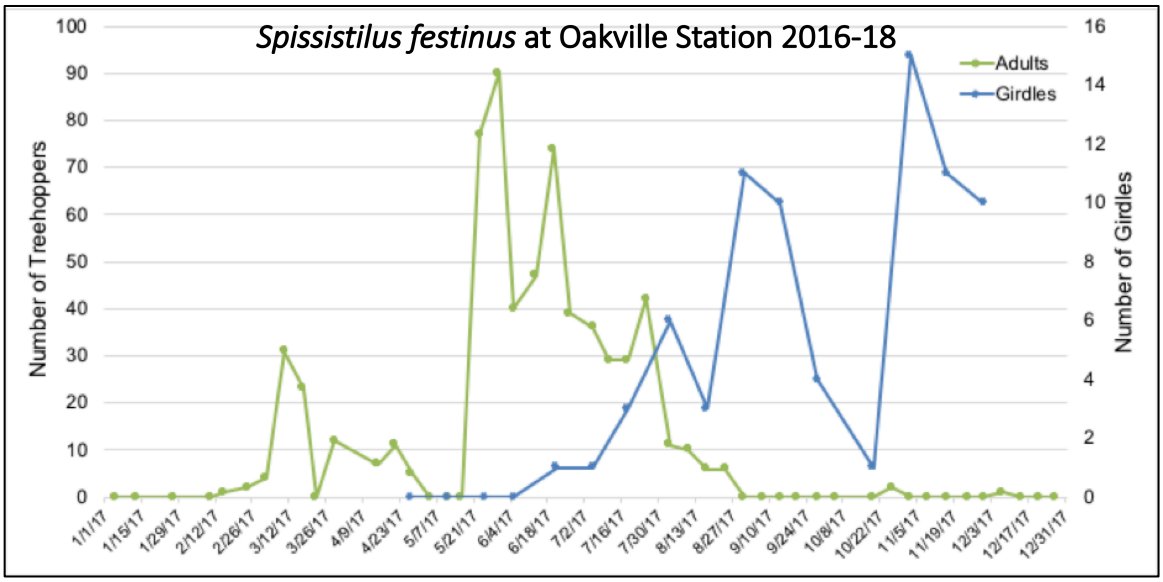

Figure 2. Weekly sweep net sampling of vineyard ground cover for three-cornered alfalfa hopper and weekly number of girdles on 30 study vines at Oakville in 2017.

Salivary glands were extracted from the 3CAH collected at the Oakville vineyard to test for presence of GRBV biweekly beginning March 3, 2017, just prior to bud break, and throughout the season. A total of 96 usable samples were collected. Salivary glands from 3CAH reared from eggs were dissected on each collection date, and these served as negative controls. The salivary glands were removed, placed in 180uL ATL and 20uL proteinase K incubated 4 h at 56^{o}C, and stored in a -80^{o}C freezer until they were analyzed by qPCR for GRBV detection in February 2018. None of the salivary glands tested positive for GRBV.

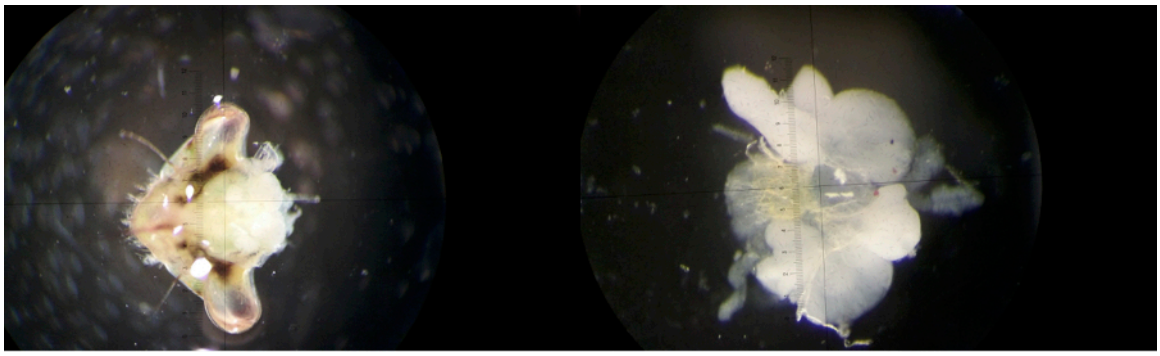

Figure 3. Three-cornered alfalfa hopper salivary gland dissections showing salivary glands within head capsule (left) and removed from head capsule (right).

In a related study conducted by Dr. Houston Wilson of the Daane lab, 3CAH populations and crop damage were sampled along transects that extend out from natural habitats into vineyards. At each sample point along the transect, *S. festinus* densities were measured on both ground covers and in the crop canopy along with petiole girdling. were evaluated at approximately 2 week intervals beginning in March 2017 using a combination of yellow sticky-traps, sweep-nets and beat-sheet sampling. Field sites consisted of five vineyard blocks >2 acres in size adjacent to riparian and/or oak woodland habitat located in Napa and Sonoma counties. All vineyard blocks were red varietals that were at least 5 years old and located on level ground with similar trellis and irrigation systems. All plots were maintained insecticide free throughout the course of the study. At each site, insects were sampled along five parallel transects (positioned 20 m apart) that extended out from the riparian or oak woodland habitat (i.e. "natural habitat") into the vineyard. Each transect was 160 m long – going 10 m into the natural habitat and 150 m into the vineyard. Along each transect, samples were 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. Two yellow sticky-traps (16 x 10 cm, Seabright Laboratories, Emeryville, CA) were placed at each transect point in the vine canopy and on the drip irrigation line at ~0.3 m above the soil surface. In the natural habitat, two sticky-traps were hung from a pole at each transect point at a height above the ground surface equivalent to those in the vineyard. On each sampling date, proportion of ground cover to bare soil was recorded along with species composition and ground cover status. At each transect point, a set of 30 sweep net samples were used to sample the ground cover. A modified beat-sheet was 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 consisted of a 1 m^2 nylon funnel that fed into a detachable 1 gallon plastic bag. For each sample, the funnel was held beneath the canopy while vigorously shaking the plant (or vine) for 30 sec. in order to dislodge insects into the funnel and plastic collection bag.

Each month, vines along each vineyard transect point were 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 was visually inspected for leaf girdling. Total leaf nodes and leaf girdles per cane were recorded for each vine. Findings to date (March 2017 – May 2018) indicate that 3CAH activity showed a strong temporal trend, with densities generally increased between June and August along with some activity observed in March and again in October and November (Figures 4A-D). While there was no clear gradient of 3CAH activity across the transect points, densities on the yellow sticky traps and in sweep net 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 (Figures 4C and 4D). Changes in 3CAH densities between the ground covers and vine canopy were not always clearly reflected in the data. Comparing the different sampling techniques for 3CAH from the vine canopy and natural habitat, the highest 3CAH densities were recorded on yellow sticky traps, followed by sweep-nets and beat sheets. Changes in 3CAH 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 (Figure 5A), some 3CAH could still be found on the little bit of ground cover that remained later in the season (Figure 5B). Surprisingly, these late season 3CAH adults were most frequently encountered on ground covers in the vineyard interior (Figure 4B). Finally, petiole girdling became apparent in August, with a higher proportion of girdles located at the vineyard interior (Figure 6). This increase in girdling in August follows increased 3CAH densities observed in the vine canopy between June and August.

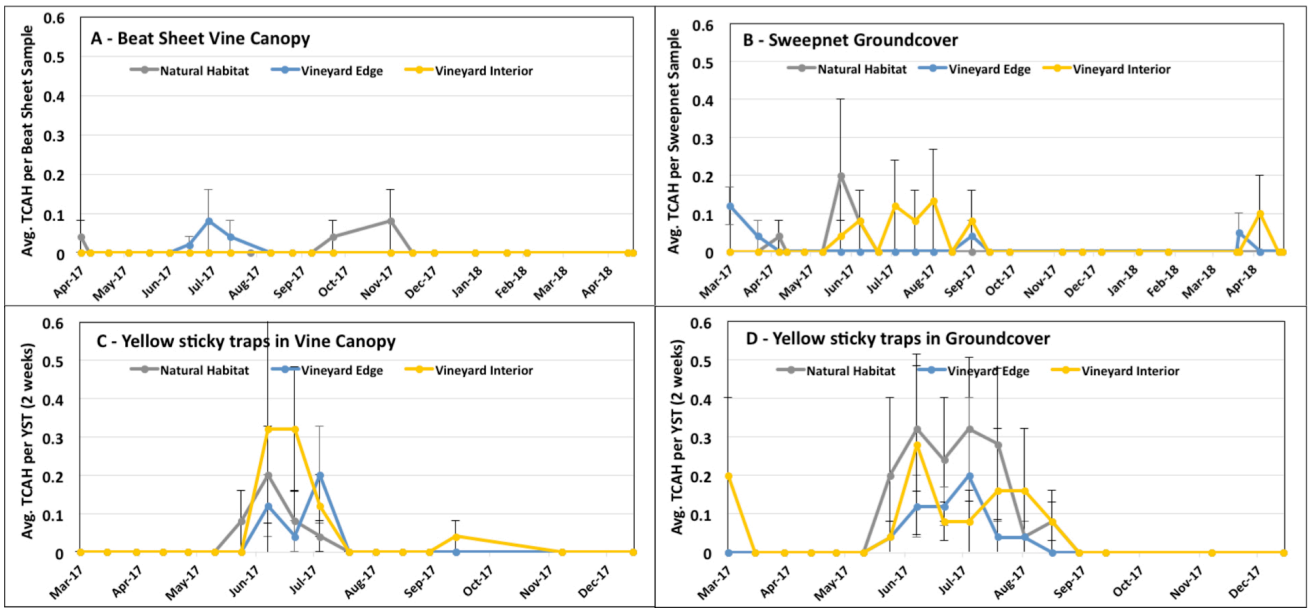

Figure 4. Three cornered alfalfa hopper densities sampled along the transect using (A) beat sheet in the vine canopy or perennial vegetation canopy; (B) sweep-net on ground covers; (C) yellow sticky traps in the vine canopy or at vine canopy height; and (D) yellow sticky traps at ground cover height (~0.3 m).

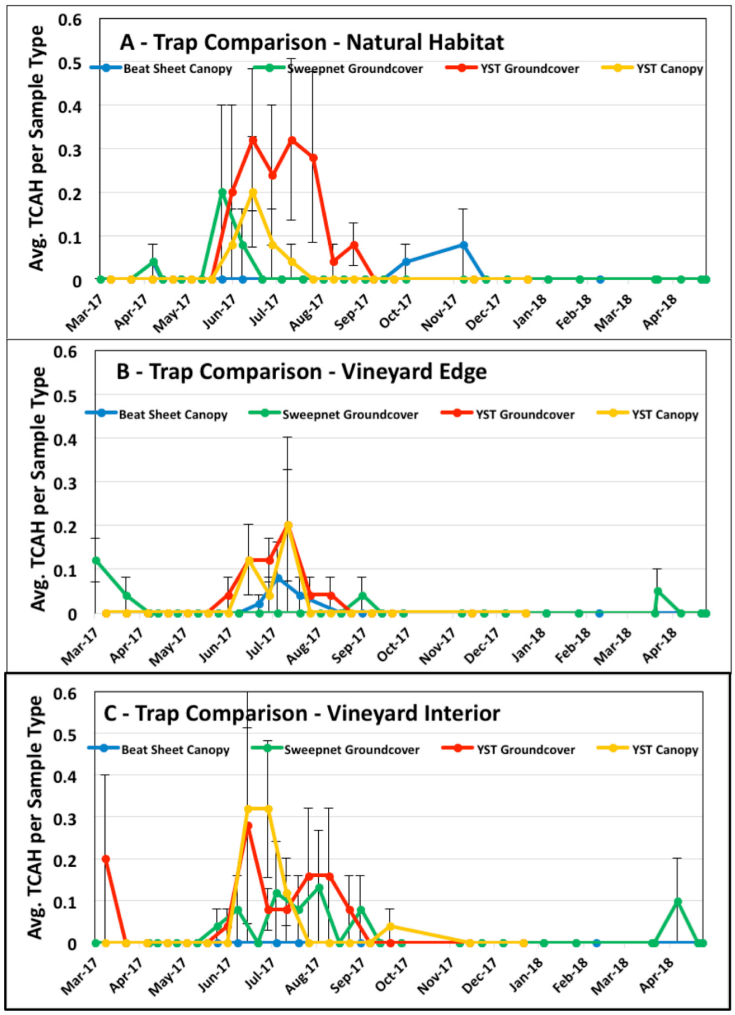

Figure 5. Three cornered alfalfa hopper densities varied according to sampling technique across the (A) natural habitat; (B) vineyard edge; and (C) vineyard interior. Generally the yellow sticky traps picked up more TCAH than sweep-nets or beat sheets.

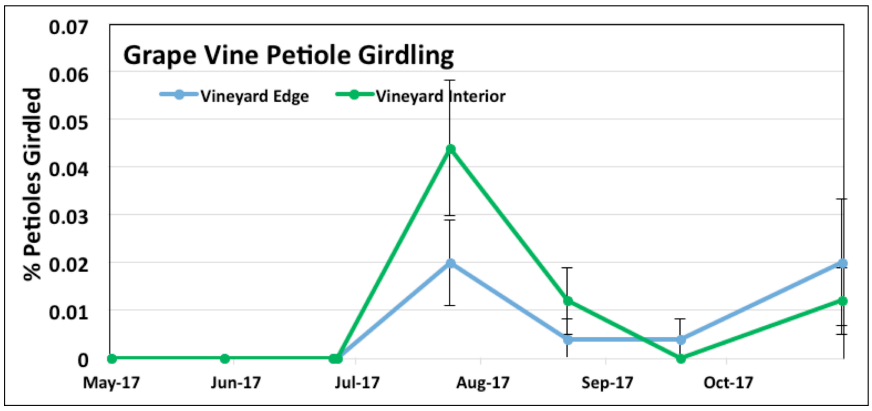

Figure 6. Petiole girdling became apparent in late July and early August 2017, with a higher proportion of girdles located at the vineyard interior.

Changes in 3CAH densities along these transects may provide evidence of seasonal movement of the insect between natural habitats and vineyards, while differences in 3CAH abundance on ground covers and in the crop canopy, along with petiole girdling, may indicate the timing of vine colonization and feeding.

Conduct GRBaV transmission studies using treehoppers collected from vineyards with red blotch disease, and detect GRBaV in the salivary glands of insects collected. Monitor field transmission by 3CAH.
Michael Bollinger of the Zalom lab at UC Davis has been collecting *Tortistilus* treehoppers in Napa and Sonoma county vineyards where GRBV has been occurring since May 2016 when we became aware of a large population of adults present and actively feeding on grapevines, but despite considerable effort we have been unable to establish a reproducing colony in the laboratory. We attempted GRBV greenhouse transmission studies with field-collected 'horned' and 'unhorned' *Tortistilus* during 2016, and we continue to test the grape plants for transmission using qPCR. A larger study was initiated on May 24, 2017, when a very large population of wild *Tortistilus* was found feeding on vines in a Pope Valley vineyard. *Tortistilus* collected on that date and for several weeks thereafter were separated into 'horned' and 'unhorned' morphs, and individuals of each were placed onto qPCR GRBV confirmed positive Ghv-24-392 (Clade II) and onto Ghv-32-377 (Clade I) Cabernet Sauvignon source vines. qPCR confirmed test healthy Ghv-37 Cabernet Sauvignon source vines served as a negative control. Transmission was attempted both by placing individuals of both morphs that had fed on GRBV infected source vines into clip cages on the uninfected vines or in large cages containing 8 uninfected vines and 20 male and 20 female *Tortistilus* of each morph. Subsequent qPCR analysis of these plants has failed to detect presence of GRBV. We also attempted to conduct a transmission assay in a more natural environment using field captured *Tortistilus* collected on May 30, 2017 were placed inside cages wrapped around separate qPCR confirmed positive and negative Cabernet Sauvignon field vines located at the Pope Valley vineyard and similarly on qPCR GRBV confirmed wild grapevine located in the vicinity for an AAP of 6 days then transferred to qPCR confirmed healthy Cabernet Sauvignon recipient vines and allowed an IAP of 6 days. Testing of these plants will begin in July 2018.

All *Tortistilus* removed from the grapevines post-inoculation were placed inside 1.5 ml tubes filled with 95% ethanol for salivary gland removal and GRBV testing. Salivary glands from *Tortistilus* collected from the test positive Cabernet Sauvignon in the field have not yet been tested for presence of the virus, In addition, 15 salivary glands removed and from *Tortistilus* collected from test positive wild grapevine have been tested with only one of the 15 testing positive.

In Winter 2018, we collected cuttings from Zinfandel grapevines in an Amador county vineyard that tested positive for GRBV, and have rooted and potted the cuttings for transmission studies that will begin in summer 2018. We have been working in the Amador county vineyard for the past 3 years and have documented GRBV spread. It is particularly interesting since no known grape viruses other than GRBV have been found in the vineyard. One concern that we have is that perhaps the GRBV in the source plants that we had been using are no longer capable of being transmitted via a vector, and we hope that the new source plants will allow us to test that hypothesis.

The Sudarshana lab planted a Cabernet Sauvignon vineyard planted on Freedom rootstock using nursery plants that were determined to be free of GRBV by qPCR at the UC Davis Plant Pathology Field Station in 2015 for the

purpose of documenting transmission and spread (Figure 7, left photo). At three intervals during summer and fall 2016, ten adult 3CAH that were allowed to feed on clade 1 or clade 2 GRBaV infected vines for at least 3 days were caged on each of 5 grapevines. A 3 meter wide alfalfa strip was planted on the edge of the vineyard nearest the 'infected' vines in Summer 2016, and 3CAH were found in the alfalfa planting by mid-summer. Testing of the recipient vines for GRBV presence during 2017 though June 2018 has not documented GRBV presence in any of the vines, but testing will continue through 2018. A survey of the vineyard for 3CAH girdles conducted during 2017 indicated the presence of girdles throughout the block beginning in August and increasing into Fall (Figure 8). If transmission was successful from the caged inoculation attempts, we anticipate that this site will provide a controlled model for studying details of GRBV spread by both clades.

Figure 7. Cabernet Sauvignon grapevines on Freedom planted in 2015 at the UC Davis Plant Pathology Field Station. Above left: Caged grapevines for 3CAH release. Above right: Grapevines showing treehopper feeding damage with girdled shoots that turned red.

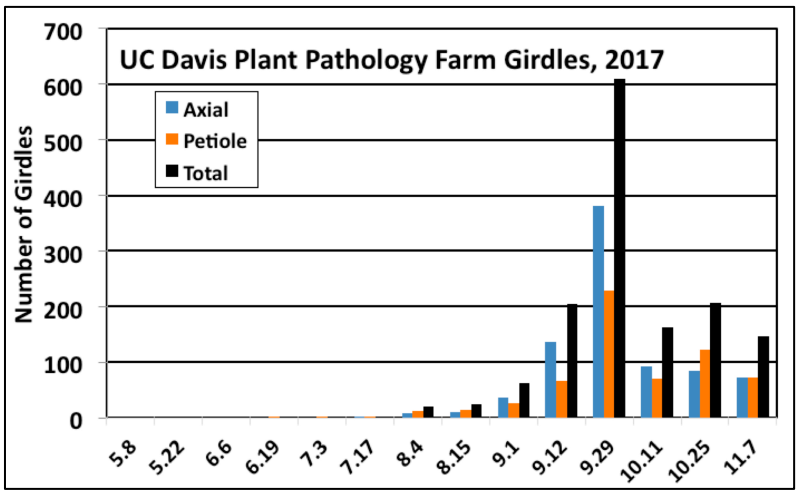

Figure 8. Weekly 3CAH girdle counts in a Cabernet Sauvignon at the UC Davis Plant Pathology Field Station in 2017.

Determine transmission efficiency of 3CAH to identify virus acquisition periods and persistence in the insect. Studies related to this objective, proposed to be conducted by the Daane lab, have been initiated, but no results are available as yet in part because the length of time required to initiate a study and then obtain results is beyond the timeframe of this project.

Evaluate the role of cover crops on the 3CAHs in vineyards. In 2016-17, common cover crops were planted in replicated plots at 3 vineyard locations, and sampled by sweep net for presence of treehoppers. Unfortunately, most of the cover crop species we planted at the two commercial sites in Napa and Yolo counties only produced a very sparse stand that was largely indistinguishable from resident vegetation. At the third site at UC Davis we were able to successfully establish cover crops (Figure 9), but we did not capture any 3CAH adults or nymphs in our weekly sweep sampling of the ground cover from January through April 2017. Given this experience, for 2017-18 we decided to concentrate the study at UC Davis where we had the option of establishing the cover crops with irrigation and better maintaining them, and intended to cage adult 3CAH on twelve individual plants in each

plot three times during the winter to assess overwintering success and reproduction. On October 24, 2017, we planted five cover crops, bell beans, magnus peas, blando brome, California red oats, and mustard, in a randomized block design with 4 replicates in a Syrah vineyard at UC Davis. A resident vegetation plot within each replicate served as a control. However, the source of the 3CAH for the study, a colony that we had established the previous summer, crashed, so we did not have a source of insects for the study.

Figure 9. A grass (left) and legume (right) cover crop plot from our winter 2016-17 study.

Determine status of common weed and cover crops as feeding and reproductive hosts for 3CAH. Feeding and reproductive common vineyard weed and cover crop hosts of 3CAH were determined in the greenhouse in a series of no-choice experiments. This study represents part of the dissertation research of Cindy Preto in the Zalom lab at UC Davis. Three female and three male 3CAH were caged onto individual pots of weeds or cover crops (Figure 10). The cages were opened weekly for 4 weeks to determine adult survival (defined as percent survival on caged plants for 2 weeks), girdling, oviposition, and nymph emergence. Purple vetch was used as a positive standard in each run of the no choice experiment because of our previous laboratory and field observations of successful feeding and oviposition. The weeds and cover crops evaluated as feeding and reproductive hosts of 3CAH are presented in Tables 1 and 2. This study identified plant species in the families Asteraceae, Convolvulaceae, Fabaceae, and Poaceae that are capable of serving as feeding and reproductive hosts. Plants in the family Fabaceae were previously reported as their preferred hosts in the Southern US (Wildermuth 1915, Mueller and Dumas 1987). Spanish clover, dandelion, birdsfoot trefoil, common groundsel, field bindweed, magnus peas, bell beans, blando brome, purple vetch, black medick, subterranean clover, crimson clover, and woollypod vetch were all found to be reproductive hosts in our study. Our results also indicate that buckhorn plantain, Kentucky bluegrass, wild carrot, mustard, oats, and Bermuda grass are poor feeding hosts, not reproductive hosts and likely would not be of significance for maintaining *S. festinus* populations in vineyards where more suitable hosts are present.

Figure 10. Weeds and cover crops caged with three cornered alfalfa hoppers in a greenhouse study at UC Davis.

Table 1: Weed species tested as feeding and reproductive hosts for *Spissistilus festinus*.

Scientific Name	Common Name	Family	Girdles	Nymphs	% Survival[a]
Acmispon americanus	Spanish clover	Fabaceae	Yes	Yes	92
Taraxacum officinale	Dandelion	Asteraceae	Yes	Yes	71
Lotus corniculatus	Birdsfoot trefoil	Fabaceae	No	Yes	58
Poa pratensis	Kentucky bluegrass	Poaceae	Yes	No	25
Senecio vulgaris	Common groundsel	Asteraceae	Yes	Yes	21
Plantago lanceolata	Buckhorn plantain	Plantaginaceae	No	No	8
Daucus carota	Wild carrot	Apiaceae	Yes	No	4
Convolvulus arvensis	Field bindweed	Convolvulaceae	Yes	Yes	4
Kickxia elatine	Sharppoint fluvellin	Plantaginaceae	No	No	0
Cynodon dactylon	Bermuda grass	Poaceae	No	No	0

[a] Survival of adults for first 2 weeks on plants

Table 2: Cover crop species tested as feeding and reproductive hosts for *Spissistilus festinus*.

Scientific Name	Common Name	Family	Girdles	Nymphs	% Survival[a]
Pisum sativum	Magnus Peas	Fabaceae	Yes	Yes	92
Vicia faba	Bell beans	Fabaceae	No	Yes	83
Bromus hordeaceus	Blando brome	Poaceae	Yes	Yes	33
Vicia benghalensis	Purple vetch	Fabaceae	Yes	Yes	30
Medicago lupulina	Black medick	Fabaceae	Yes	Yes	25
Trifolium subterraneum	Subterranean clover	Fabaceae	Yes	Yes	17
Trifolium incarnatum	Crimson clover	Fabaceae	Yes	Yes	13
Vicia villosa ssp. varia	Woollypod vetch	Fabaceae	Yes	Yes	13
Brassica sp.	Mustard	Brassicaceae	No	No	0
Avena sativa	California red oats	Poaceae	No	No	0

[a] Survival of adults for first 2 weeks on plants

In an effort to evaluate preference of 3CAH to confirmed reproductive cover crop and weed reproductive hosts when presented a choice, three groups of 5 plants (four known reproductive hosts from the completed no-choice experiment were randomly arranged in a large dome-shaped cage in the greenhouse and replicated three times (Figure 11). Purple vetch was included in each evaluation as a standard. Ten male and ten female 3CAH were released into each cage and allowed to freely feed and oviposit. All adults were removed from the cages after one week. Nymphs were counted and collected from individual plants on weeks two and three. Destructive sampling of all plants and collection of nymphs were conducted at week four. The plant species that exhibited the greatest nymph emergence in each of the three groups tested were all in the family Fabaceae (Figure 12). Interestingly, while the five plant species tested in cover crop group 1 were all in the family Fabaceae there were differences among them, suggesting that even within the Fabaceae reproductive preference exists with the two vetch species tested being preferred over the two clover species (Figure 12A). Cover crop group 2 consisted of four plants in the Fabaceae family and one in the Poaceae family. Two of those Fabaceae had significantly greater nymph emergence than did the plant species in the family Poaceae (Figure 12B). Group 3 which consisted of vineyard weeds included three species in the family Fabaceae, one in the family Asteraceae and one in the family Convolvulaceae. The three Fabaceae plant species yield significantly more nymphs than did the plants in the other two families (Figure 12C). These results further support a hypothesis that plants of the family Fabaceae are preferred hosts of *S. festinus.*

Figure 11. Four reproductive hosts plus purple vetch as a standard caged with three cornered alfalfa hoppers in a greenhouse preference study at UC Davis.

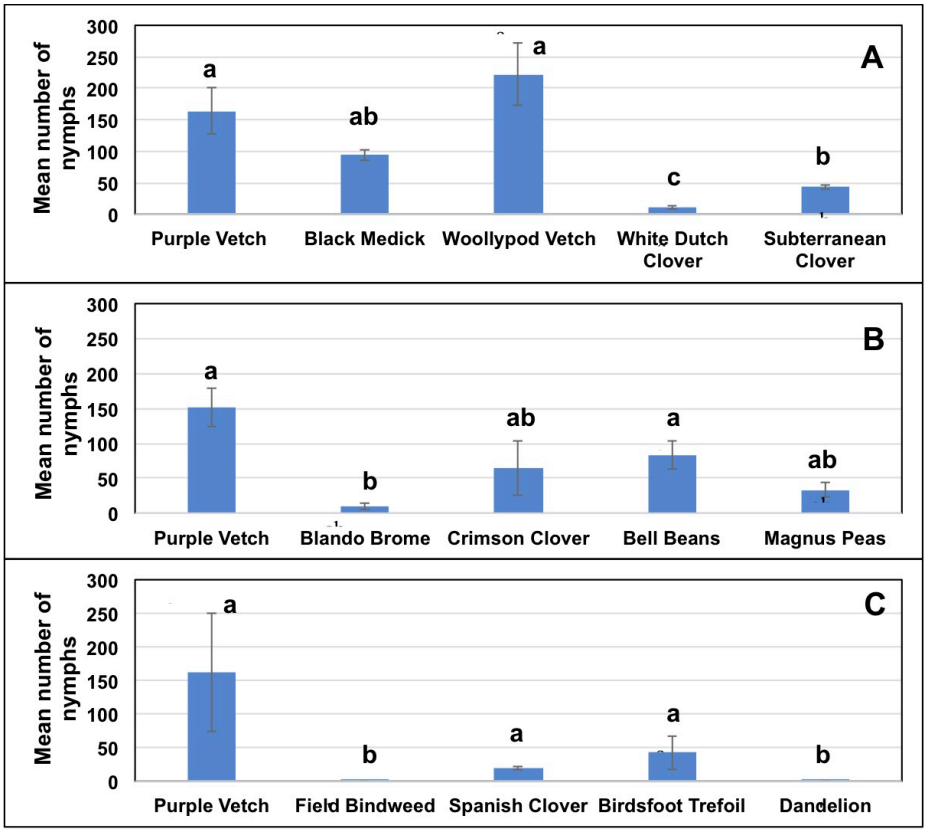

Figure 12. Results of post-hoc pairwise comparisons of mean number (±SEM) of *Spissistilus festinus* nymphs emerging from A) cover crop - group 1 (purple vetch, black medick, woollypod vetch, white Dutch clover, subterranean clover), B) cover crop - group 2 (purple vetch, blando brome, crimson clover, bell beans, magnus peas) and C) weeds (purple vetch, field bindweed, Spanish clover, birdsfoot trefoil, dandelion). Means followed by the same letter are not significantly different (Tukey HSD test, $P \leq 0.05$).

Conclusions:

The studies of the seasonal population dynamics of 3CAH in vineyards and surrounding landscapes presented in this report represents the first extensive study of timing of vineyard colonization, movement between ground covers and the crop canopy, and seasonal occurrence of girdling. Sampling data are presented both from an intensive sampling of a vineyard and from transects that extend out from vineyards into natural habitats. The transect sampling also allowed for comparison of different sampling methods that could prove useful in establishing guidelines for *S. festinus* monitoring by consultants and growers in the future. We have also initiated

studies to identify the role, if any, of *Tortistilus* treehoppers that occur in vineyards where GRBV spread is confirmed and 3CAH are not found or occur at very low densities. Studies using *Tortistilus albidosparsus* were initiated in 2017, but have not yet indicated successful GRBV transmission. However, we do not know the timeframe necessary to first detect the presence of new infections in the field. Our results on the association of *S. festinus* in relation to cover crops and resident vegetation expands the confirmed list of feeding and reproductive hosts, and represents the first study evaluating common plant species used as cover crop or present as weeds in California vineyards. An associated preference study based on results of this no choice test confirmed that plants of the family Fabaceae are preferred hosts of *S. festinus.* Knowledge of plant species present in vineyards that serve as alternative hosts for *S. festinus* is an additional contribution in understanding the relationship of GRBV and its presumed vector *S. festinus*. Our studies will provide a needed and sound foundation for developing management strategies for *S. festinus* to mitigate GRBV spread.

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