

## **California Department of Food and Agriculture - Interim Progress Report**

**CDFA Agreement Number 17-0517-000-SA**

**Title of Project: Ecology of Grapevine Red Blotch-Associated Virus**

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**Reporting period: The results reported here are from work conducted from July 2017 to January 2018**

### **Introduction**

Red blotch was described for the first time on Cabernet Sauvignon at the UC Oakville Research Field Station in 2008 (Calvi 2011; Cieniewicz et al., 2017a; Sudarshana et al., 2015). Diagnosis based on symptoms can be challenging because of several confounding factors, including striking similarities between foliar symptoms elicited by red blotch and leafroll diseases, as well as several other biotic and even abiotic factors. Because symptom variation makes visual diagnosis of diseased vines difficult, only DNA-based assays are reliable for accurate diagnosis (Cieniewicz et al., 2017a; Sudarshana et al., 2015)

Fruit ripening issues have been documented with diseased wine grapes. Reductions of 1-6°Brix have been consistently reported, as well as lower berry anthocyanin and skin tannins, particularly in red wine grapes such as Cabernet franc and Cabernet Sauvignon (Calvi 2011; Cieniewicz et al., 2017a; Sudarshana et al., 2015). Based on the effect of the virus on fruit quality and ripening, numerous vineyard managers are culling infected vines and replacing them with clean, virus-tested ones. The economic cost of GRBV is estimated to range from \$21,833 (for a 5% initial infection in year 3 and a 25% price penalty for infected grapes) to \$169,384 (for a 60% initial infection in year 3 and a 100% price penalty for the

proportion infected grapes) per acre in Napa Valley; from \$12,023 to \$93,067 per acre in Sonoma; and from \$5,468 to \$39,140 per acre on Long Island in New York (Ricketts et al., 2017). These estimates highlight the economic impact of red blotch disease in different grape-growing regions in the U.S.

GRBV was documented in all major grape-growing US States (Krenz et al., 2014). GRBV was also isolated from numerous table grape accessions at the USDA germplasm repository in Davis, CA (Al Rwahnih et al., 2015) and in Canada (Poojari et al., 2017; Xiao et al., 2015). The widespread occurrence of GRBV in North America suggests that propagation material has played a significant role in its dissemination. The virus was also described in Switzerland (Reynard et al., 2018), South Korea (Lim et al. 2016) and India (GenBank accession number KU522121).

Grapevine red blotch virus (GRBV) is a member of the genus *Grabovirus* in the family *Geminiviridae* (Varsani et al., 2017). It has a circular, single-stranded DNA genome that codes for six open reading frames (Al Rwahnih et al., 2013; Cieniewicz et al., 2017a; Krenz et al., 2012; Krenz et al., 2014; Sudarshana et al., 2015). We recently showed the causative role of GRBV in the etiology of red blotch disease using agroinoculation of tissue culture-grown grapevines with partial dimer or bitmer constructs of the GRBV genome (Yepes et al., 2018).

The Virginia creeper leafhopper (*Erythroneura ziczac* [Walsh]) (Poojari et al. 2013) and the three cornered alfalfa treehopper (*Spissistilus festinus* [Say]) (Bahder et al. 2016a) have been shown to transmit GRBV from infected to healthy vines under greenhouse conditions. The epidemiological significance of these findings is unknown, stressing the need to carry out studies in diseased vineyards for vector identification. Interestingly, the transmission ability of *E. ziczac* was refuted (Bahder et al. 2016b), highlighting the need for additional studies, particularly to determine the role of *S. festinus* in GRBV transmission in vineyards and assess whether any other insects can vector GRBV.

The overarching goal of this proposal is to advance our understanding of the ecology of red blotch disease and its causal agent, grapevine red blotch (GBRV), with a major emphasis on transmission attributes and the epidemiological role of vineyard cover crops.

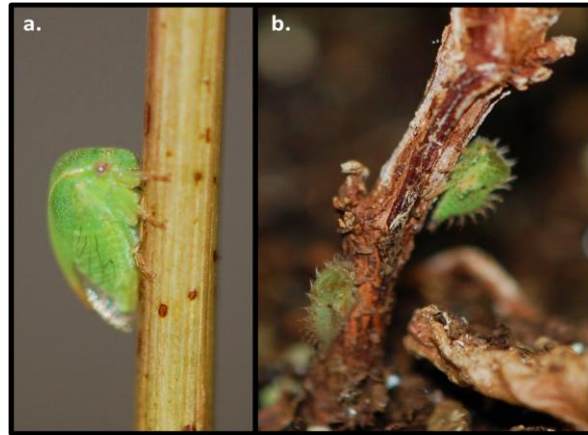
## Objectives

Our specific objectives are to:

1. Characterize the spread of grapevine red blotch virus (GRBV)
  - Describe the transmission mode of GRBV by *Spissistilus festinus*
  - Test sentinel vines established in a diseased vineyard where spread is documented for the presence of GRBV
  - Investigate the seasonal diversity and distribution of vector candidate populations in a diseased vineyard for which there is no evidence of spread
2. Determine if vineyard cover crops can host GRBV and/or *S. festinus*
  - Survey cover crops in Napa Valley vineyards for *S. festinus*
  - Survey cover crops in Napa Valley vineyards for GRBV
3. Determine the experimental host range of GRBV and *S. festinus*
  - Agroinoculate commonly used vineyard cover crop species with infectious GRBV clones and assess virus infection
  - Examine the reproductive potential of *S. festinus* on commonly used vineyard cover crop species
4. Disseminate research results to farm advisors and to the grape and wine industry

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

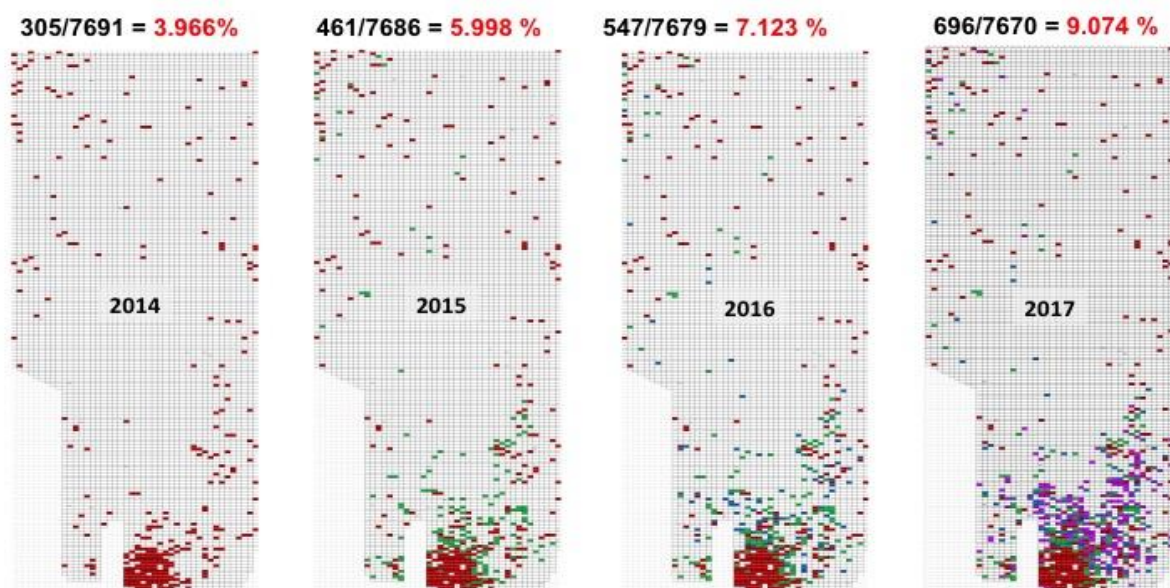
To address objective #1 - Characterize the spread of grapevine red blotch-associated virus (GRBV) -, specimens of *S. festinus* from alfalfa fields in Yolo and Kern counties were collected, transferred to Cornell University and established on alfalfa plants in a growth chamber with controlled temperature, humidity and photoperiod. To describe the transmission mode, *S. festinus* were allowed to feed on GRBV-infected grapevines for 72h to eight days (Figure 1). Then, *S. festinus* were moved to alfalfa plants for 2 weeks. Subsequently, *S. festinus* were collected and tested for GRBV by PCR. Results showed that most of the specimens (80%, 18 of 23) tested positive for GRBV in PCR after the 3-8 days acquisition period and some (40%, 6 of 15) tested positive for GRBV two to three weeks after acquisition, indicating that *S. festinus* can acquire the virus from infected vines in the greenhouse and retain it for extended time, even after feeding on healthy vine tissue.



**Figure 1.** *S. festinus* adult female (left) and 5<sup>th</sup> instar nymphs feeding on potted grapevines in a greenhouse.

These gut cleansing experiments were repeated twice with similar results: the majority of *S. festinus* specimens (60%, 12 of 20 and 55%, 6 of 11) tested positive for GRBV following a two weeks on alfalfa after feeding 5 days on GRBV-infected grapevines. This documented the ability of *S. festinus* to acquire and retain GRBV for several days following a gut cleansing experiment on alfalfa, a non-host of GRBV (Cieniewicz et al., unpublished). Retaining the virus for a couple of weeks is indicative of a circular transmission.

Disease incidence was monitored from 2014 to 2017 in a 5-acre diseased Cabernet franc vineyard in Napa County. This vineyard was established in 2008 and first disease symptoms were observed in 2012. The presence of GRBV was confirmed in a subset of symptomatic vines in 2013. Disease increased by 1-2% annually throughout the vineyard in 2014-2017, although it was more pronounced (with a 5-20% annual increase) in the section of the vineyard close to a riparian area (Figure 2). Approximately 150 sentinel vines, i.e. healthy Cabernet franc vines for which the mother stocks from which scion budwood and rootstock (3309C) canes were collected tested negative for GRBV, were planted in the area of the Cabernet franc vineyard with strong aggregation of diseased vines in spring 2015. Sentinel vines were established to gain direct evidence of insect-mediated GRBV spread if they become infected. Sentinel vines replaced existing Cabernet franc vines that were weak or had died, regardless of their GRBV infectious status. Each sentinel vine was tested for the presence of GRBV by PCR in fall 2017. The 2017 test results confirmed those obtained in 2016 with no sentinel vines becoming infected. It remains unknown why none of the sentinel vines accumulated detectable levels of GRBV from 2015-2017 in spite of being proximal to infected vines.



**Figure 2.** Cumulative spatial distribution of symptomatic vines in a red blotch diseased 5-acre Cabernet franc vineyard in 2014 (red cells), 2015 (green cells), 2016 (blue cells) and 2017 (purple cells) in Napa county, California.

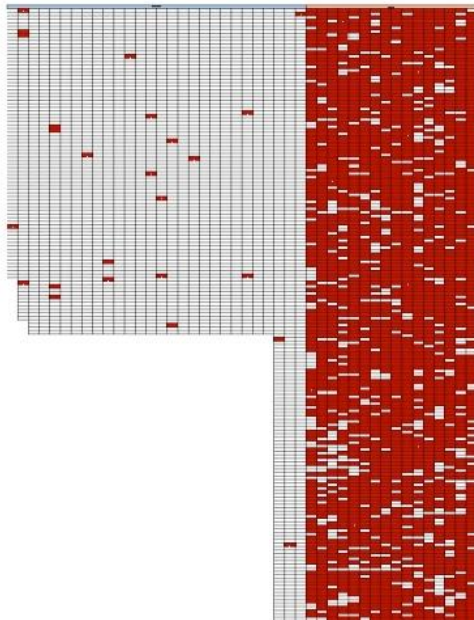
Our 2014-2017 epidemiological studies focused on a 5-acre Cabernet franc vineyard in Napa county (Perry et al., 2016; Cieniewicz et al., 2017; Cieniewicz et al., 2018) (Figure 2, middle right). Adjacent to this Cabernet franc vineyard (60 ft away) is a 4-acre Cabernet Sauvignon vineyard (Figure 2, lower left). This vineyard was planted in 2008 with Cabernet Sauvignon clones 4 and 169. Almost every vine of clone 4 exhibited typical red blotch symptoms soon after planting whilst vines of clone 169 remained asymptomatic (Figure 2). Vines of the two Cabernet Sauvignon clones were purchased from two different nurseries. GRBV isolates belonging to clade 1 and clade 2 are present in clone 4 vines (Cieniewicz and Fuchs, unpublished).

From 2008 to 2016, GRBV was not observed in any of the clone 169 vines in spite of the (i) proximity (60 ft away) of this vineyard to the Cabernet franc vineyard where spread is occurring (Figure 2), and (ii) presence of GRBV in most of the very proximal clone 4 vines.



**Figure 2.** Proximity of a Cabernet franc vineyard for which spread of GRBV was reported in 2015-2017) and a Cabernet Sauvignon vineyard for which an extremely limited rate of spread was documented in 2017. Red blotch diseased vines are depicted in red.

In fall 2017, 0.9% (25 of 2,796) of clone 169 vines exhibited suspicious red blotch symptoms whilst 96% (2,657 of 2,768) of clone 4 vines were symptomatic (Figure 3). Testing symptomatic clone 4 vines for



**Figure 3.** Distribution of diseased vines in a Cabernet Sauvignon vineyard with clone 4 (right) and clone 169 (left). Red blotch diseased vines are depicted in red.

pathogens indicated that 16 of them were infected with GRBV, seven with Pierce disease and two with grapevine leafroll-associated virus 1. This was the first evidence of the presence of GRBV in clone 169 vines (0.6%, 16 of 2,796) nine years post-planting. This low incidence of GRBV is likely resulting from an extremely low spread of GRBV in the section of the Cabernet Sauvignon vineyard established with clone 169 vines. Interestingly, GRBV-infected clone 169 vines were scattered throughout the clone 169 section of the Cabernet Sauvignon vineyard rather than distributed in proximity to the infected clone 4 vines. This suggested that secondary spread likely occurred from external inoculum sources.

To better understand why spread is extremely low in the Cabernet Sauvignon vineyard compared to the Cabernet franc vineyard, we hypothesized that a reduced population of vector candidates is visiting the Cabernet Sauvignon, thus reducing opportunities for transmission. To address this hypothesis, yellow sticky cards were placed from March thru November 2017 on diseased and asymptomatic vines of clones 169 and 4, across six rows of each clone, in the area of the vineyard facing the Cabernet franc vineyard (Figure 3, white grid). Sticky

cards were rotated on a weekly basis. Each card was analyzed for the presence of insects to establish a census population and identify them at the species level. Then, a subset of each insect family, genus or species that was caught was removed from the traps and tested for the presence of GRBV by PCR. Emphasis was placed on the four species that were identified as vector (*S. festinus*) or as vector candidates in the Cabernet franc vineyard (Cieniewicz et al. 2018).

Results indicated the presence of *S. festinus*, *C. reductus* and *O. borealis* but no *Melanoliarus* sp. on the traps from the Cabernet Sauvignon vineyard (Table 1). Interestingly, the population of *S. festinus* showed a 8-fold reduction in the Cabernet Sauvignon vineyard (3 specimens) compared to the Cabernet franc vineyard (25 specimens in 2015 and 25 specimens in 2016) (Cieniewicz et al. 2018). The population of *C. reductus* in the Cabernet Sauvignon vineyard was higher in 2017 (63 specimens) compared to the Cabernet franc vineyard in 2015 (41 specimens) and 2016 (23 specimens), while the population of *O. borealis* was lower in 2017 (6 specimens) compared to 2016 (11 specimens) and 2015 (31 specimens) (Cieniewicz et al., 2018). Additionally, among the three specimens of *S. festinus* caught on traps in 2017 only one tested positive for GRBV (Table 1); and this viruliferous specimen was caught on a trap placed in the clone 4 section of the Cabernet Sauvignon vineyard. In contrast, similar to the 2015-2016 data, 30% (19 of 63) of *C. reductus* and 67% (4 of 6) of *O. borealis* tested positive for GRBV in PCR (Table 1). These preliminary results suggested an association between the dynamics of GRBV spread and the composition of the insect community visiting the Cabernet Sauvignon vineyard, including the population density of *S. festinus*.

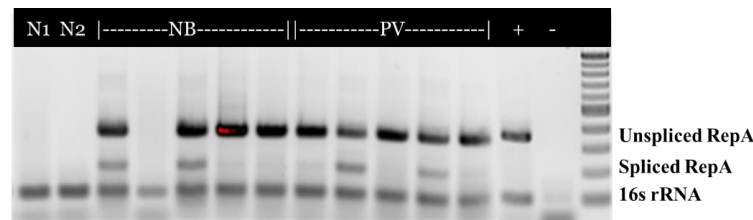
**Table 1:** Grapevine red blotch virus (GRBV) detection in insects trapped on sticky cards from March to November in 2017 in a Cabernet Sauvignon vineyard in which secondary disease spread is extremely low.

Family/Order	Species	Common name	# tested	# positive	% positive
Membracidae	<i>Spissistilus festinus</i>	Three-cornered alfalfa treehopper	3	1	33
Cicadellidae	<i>Colladonus reductus</i>		63	19	30
	<i>Osbornellus borealis</i>		6	4	67
	<i>Scaphytopius</i> sp.	Sharp-nosed leafhopper	50	10	20
	<i>Euscelis</i> sp.		7	1	14
	<i>Empoasca</i> sp.	Potato leafhopper	18	0	0
	<i>Erythroneura variabilis</i>	Variegated leafhopper	25	0	0
	<i>Erythroneura elegantula</i>	Western grape leafhopper	15	0	0
	Deltocephalinae		8	0	0
	<i>Xestocephalus</i> spp.		1	0	0
	<i>Japananus hyalinus</i>	Japanese maple leafhopper	2	0	0
	<i>Erythroneura ziczac</i>	Virginia creeper leafhopper	2	0	0
Delphacidae		Delphacid planthopper	2	0	0
Psyllidae		Psyllids	6	0	0
Thysanoptera		Thrips	13	0	0
Aphididae		Aphids	28	1	4
Miridae		Plant bugs	12	0	0
Lygaeidae		Seed bugs	2	0	0
Hymenoptera		Wasps, bees, ants	6	0	0
Diptera		True flies	10	0	0
Coleoptera		Beetles	6	0	0
Psocoptera		Barklice	8	0	0
Aleyrodidae		Whiteflies	8	0	0
Phylloxeridae		Phylloxera (foliar-form)	8	0	0

To address objective # 2 -Determine if vineyard cover crops can host GRBV and/or *S. festinus* -, vineyard cover crop species were collected between rows of diseased Cabernet franc, Cabernet Sauvignon, Merlot and Sauvignon blanc vineyards in Napa County and in unmanaged areas adjacent to these vineyards in spring 2017. These samples were tested for GRBV by PCR. Emphasis was placed on legume cover crop species such as vetch, clover, bean, pea and Medicago. Our 2017 surveys showed that none of 223 legume samples collected in diseased vineyards tested positive for GRBV. These results confirmed the 2015 and 2016 findings. Similarly, no *S. festinus* was identified by sweep netting middle rows of GRBV-infected vineyards in March 2017.

To address objective # 3 - Determine the experimental host range of GRBV and *S. festinus* -, legume species (clover, vetch, bean, Medicago and peas) were agroinoculated with GRBV infectious clones in the greenhouse, as previously described (Yepes et al., 2018). Agroinoculation was employed because it is more efficient than the use of insect vectors. Plant samples were assayed for GRBV 1-2-weeks post-agroinoculation by PCR in inoculated tissue and by RT-PCR in apical leaves (Yepes et al., 2018). The

RT-PCR assay was designed to detect the accumulation of spliced transcripts. It is critical to determine virus replication in agroinoculated plants and distinguish virus infection from the GRBV genetic information in *Agrobacterium tumefaciens* carrying infectious clones. Our results showed that bean (*Phaseolus vulgaris*) sustains the replication of GRBV with 25% (2 of 8) of the plants becoming infected following agroinoculation, as shown by RT-PCR (Figure 4). Similarly, *Nicotiana benthamiana* is a host of GRBV (Figure 4). Other legumes did not get consistently infected with GRBV; none of the clover, vetch and pea plants tested so far acted as alternate host for GRBV. Replicated experiments are needed to validate these preliminary results. Agroinoculated bean plants infected by GRBV were used in transmission assays with *S. festinus*. Data showed transmission of GRBV from infected to healthy bean. This is indicating that bean and eventually *N. benthamiana* are excellent model hosts for virus-vector and virus-host interaction studies.



**Figure 4.** Detection of grapevine red blotch virus (GRBV) by RT-PCR in apical leaves of *Phaseolus vulgaris* (PV) and *Nicotiana benthamiana* (NB) two weeks post-agroinoculation with infectious clones of GRBV. Lanes N1 and N2 correspond to *P. vulgaris* and *N. benthamiana* agroinoculated with empty vectors.

Examining the reproductive potential of *S. festinus* on legume cover crops species indicated that bean is a host of *S. festinus*. This work needs to be confirmed in a duplicated experiment. Studying other legume cover crops species commonly used in vineyard middle rows, such as clover, vetch, Medicago and field peas, is under way.

To address objective # 4 - Disseminate information to farm advisors and to the grape and wine industry -, research results were be communicated to farm advisors, extension educators, crop consultants, researchers, vineyard managers and regulators at winter school meetings in California, New York and Oregon. The targeted venues in 2017 were (i) the Cornell Recent Advances in Viticulture and Enology (CRAVE) conference on November 14, in Ithaca, NY (60 participants), (ii) the Sustainable Ag Expo on Nov. 13-15 2017 in San Luis Obispo, CA (1,500 participants), (iii) the Summer Grape Conference and Field Day on July 25 in Dunkirk, NY (75 participants), (iv) the Eastern Winery Exposition on March 23, in Syracuse, NY (40 participants), (v) the 3<sup>rd</sup> Annual Southern Oregon Grape Symposium on March 14 in Central Point, OR (106 participants), and (vi) Unified Symposium on January 25 in Sacramento, CA (250 participants).

## **Publications produced and pending, and presentations made that relate to the funded project**

### Publications:

1. Yepes, L.M. Cieniewicz, E., Krenz, B., Mclane, H., Thompson, J.R., Perry, K.L. and Fuchs, M. 2018. Causative role of grapevine red blotch virus in red blotch disease. *Phytopathology*, in press.
2. Perry, K.L., Mclane, H., Thompson, J.R. and Fuchs, M. 2018. A novel grablovirus from non-cultivated grapevine (*Vitis* Sp.) in North America. *Archives Of Virology*, 163:259-262.
3. Cieniewicz, E., Pethybridge S.J., Loeb, G.M., Perry, K.L., And Fuchs, M. 2018. Insights into the ecology of grapevine red blotch virus in a diseased vineyard. *Phytopathology*, 108:94-102.

4. Li, R., Fuchs, M., Perry, K.L., Mekuria, T., and Zhang, S. 2017. Development of a fast Amplifyrp Acceler8 diagnostic assay for grapevine red blotch-associated virus. *Journal of Plant Pathology*, 99:657-662.
5. Cieniewicz, E., Pethybridge S., Gorny, A., Madden, L., Perry, K.L., Mclane, H. and Fuchs, M. 2017. Spatiotemporal spread of grapevine red blotch-associated virus in a California vineyard. *Virus Research*, 230:59-62.
6. Varsani, A., Roumagnac, P., Fuchs, M., Navas-Castillo, J., Moriones, E., Idris, I., Briddon, R.W. Rivera-Bustamante, R., Murilo Zerbini, F. and Martin, D.P. 2017. *Capulavirus* and *Grablovirus*: Two new genera in the family *Geminiviridae*. *Archives of Virology*, 162:1819-1831.
7. Ricketts, K.D., Gómez, M.I., Fuchs, M.F., Martinson, T.E., Smith, R.J., Cooper, M.L., Moyer, M. and Wise A. 2017. Mitigating the economic impact of grapevine red blotch: Optimizing disease management strategies in U.S. vineyards. *American Journal of Enology and Viticulture*, 68:127-135.
8. Cieniewicz, E.J., Perry, K.L. and Fuchs, M. 2017. Grapevine red blotch virus: Molecular biology of the virus and management of the disease. In: *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. Meng, B., Martelli, G.P., Golino, D.A. and Fuchs, M.F (eds). Springer Verlag, pp. 303-314.

#### Presentations:

1. Clarke, A., Cieniewicz, E. and Fuchs, M. 2017. Searching for alternative hosts of grapevine red blotch virus. Summer Scholars Poster Presentation, July 28, Geneva, NY.
2. Onwumelu, A., Cieniewicz, E. and Fuchs, M. 2017. Investigating potential insect vectors of grapevine red blotch virus in a Napa County, CA vineyard. Summer Scholars Poster Presentation, July 28, Geneva, NY.
3. Cieniewicz, E. and Fuchs, M. 2017. Ecology of grapevine red blotch disease. APS Annual Meeting, August 8, San Antonio, TX.
4. Poplaski, V., Cieniewicz, E. and Fuchs, M. 2017. Genetic diversity of *Spissistilus festinus*. 2<sup>nd</sup> Graduate Student Symposium, Cornell University, New York State Agricultural Experiment Station, June 23, Geneva, NY.
5. Cieniewicz, E. and Fuchs, M. 2017. Grapevine red blotch virus in free-living *Vitis* sp. Cornell Recent Advances in Viticulture and Enology (CRAVE) conference, November 14, Ithaca, NY (participants = 60).
6. Fuchs, M. 2017. Update on the ecology of red blotch virus. Sustainable Ag Expo on Nov. 14, San Luis Obispo, CA (participants = 500).
7. Fuchs, M. 2017. Viruses: Biology, ecology and management. Sustainable Ag Expo on Nov. 13, San Luis Obispo, CA (participants = 550).
8. Fuchs, M. 2017. Leafroll and red blotch viruses. Open house, Sept. 8, Niagara-on-the-Lake, Ontario, Canada (participants = 51).
9. Fuchs, M. 2017. Leafroll and red blotch viruses. Open house, Sept. 7, Harrow, Ontario, Canada (participants = 30).
10. Fuchs, M. 2017. Updates on leafroll and red blotch diseases. Eastern Winery Exposition, March 23, Syracuse, NY (participants = 40).
11. Fuchs, M. 2017. Updates on red blotch disease. 3<sup>rd</sup> Annual Southern Oregon Grape Symposium, Southern Oregon Research and Extension Center, March 14, Central Point, OR (participants = 106).
12. Fuchs, M. 2017. Management of red blotch disease. 2017 Ontario Fruit and Vegetable Convention, Scotiabank Convention Centre, Niagara Falls, Canada, February 22-23 (participants = 140).
13. Fuchs, M. 2017. Looking forward: How grapevine clean plant strategies can be improved? Unified Symposium January 25, Sacramento, CA (participants = 250).

### **Research relevance statement, indicating how this research will contribute towards finding solutions to red blotch disease in California**

We provided new insights into the spread of GRBV and the population dynamics of *S. festinus* and three other candidate vectors. These insights informed epidemiological features of red blotch disease. We also started to better understand the dynamics of GRBV spread in vineyards with regard to the population density of *S. festinus*. Additionally, evidence of a circulative transmission of GRBV by *S. festinus* was obtained. Surveys of cover crops in middle rows of diseased vineyards, particularly of legumes, provided no evidence of GRBV infection or the presence of *S. festinus*, suggesting a limited role in the epidemiology of GRBV. This work provided a solid foundation for the development of disease management strategies, which, based on our knowledge, are currently focusing on vineyard management, i.e. roguing or vineyard removal, depending on the level of disease incidence, and removal of free-living vines proximal to vineyards.

### **Layperson summary of project accomplishments**

Grapevine red blotch virus (GRBV) is the causal agent of red blotch disease (Yepes et al., 2018). GRBV is present in diseased grapevines throughout US vineyards (Cieniewicz et al., 2017a; Sudarshana et al., 2015). Analysis of the spatiotemporal incidence of GRBV in a selected Cabernet franc vineyard in California in 2014-2017 was consistent with the occurrence of virus spread (Cieniewicz et al., 2017b). From March to November in 2015-2016, insect sticky traps placed in the section of the Cabernet franc vineyard with extensive clustering of diseased vines showed a diversity of insect species that visited the vineyard. Among the insects caught on traps in the Cabernet franc vineyard, four species consistently tested positive for GRBV by PCR and were considered vector candidates. These were members of the Membracidae (*Spissistilus festinus*), Cicadellidae (*Colladonus reductus* and *Osbornellus borealis*) and Cixiidae (*Melanoliarus* spp.) (Cieniewicz et al., 2018). Colonies of *S. festinus* - the three cornered alfalfa treehopper - were established on alfalfa in a growth chamber and transmission experiments revealed vectoring of GRBV by *S. festinus* from infected to healthy plants in the greenhouse. This finding revealed the epidemiological significance of *S. festinus* in vineyards. Interestingly, substantially less *S. festinus* visited a Cabernet Sauvignon vineyard for which extremely low spread of GRBV is documented in 2014-2017 compared to the adjacent Cabernet franc vineyard where spread readily occurs, suggesting an association between the dynamics of spread and vector population density. Additionally, bean (*Phaseolus vulgaris*) is an alternate experimental host of GRBV and *S. festinus*, as shown by agroinoculation experiments with infectious clones, but neither the virus nor *S. festinus* were found in legumes species, including bean, in diseased vineyard middle rows in spring 2017. Research results were communicated to growers, farm advisors, extension educators, crop consultants, researchers, vineyard managers and regulators at winter school meetings in California, New York and Oregon.

### **Status of funds**

Funds were spent for salaries of key personnel (postdoctoral associate, graduate student and technicians) involved in the research, materials and supplies, greenhouse rent, travel from labs to and from vineyards for sample collection and monitoring of virus spread, and travel to grower's meetings to present research progress.

### **Summary and status of intellectual property associated with the project**

No intellectual property is associated with the project.

### **Literature Cited**

Al Rwahnih, M., Dave, A., Anderson, M., Rowhani, A., Uyemoto, J. K. and Sudarshana, M.R. 2013. Association of a DNA virus with grapevines affected by red blotch disease in California. *Phytopathology* 103:1069-1076.

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