

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

### **CDFCA Agreement Number 16-0615-SA**

#### **Title of Project: Biology and Spread of Grapevine Red Blotch-Associated Virus**

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**Reporting period: The results reported here are from work conducted from June 2015 to March 2018**

##### **Introduction**

Red blotch was described for the first time on Cabernet Sauvignon at the UC Oakville Research Field Station in 2008 (Calvi 2011; 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; 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 in New York (Ricketts et al., 2017). These estimates highlight the economic impact of red blotch disease in different grape-growing regions of the U.S.

Disease 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 (Sudarshana et al., 2015). Grapevine red blotch virus (GRBV), the causal agent of red blotch disease (Yepes et al., 2018), was documented in all major grape-growing US States (Krenz et al., 2014) and Canada (Poojari et al., 2017). GRBV was also isolated from numerous table grape accessions at the USDA germplasm repository in Davis, CA (Al Rwahnih et al., 2015) and hybrid accession at the USDA germplasm repository in Geneva, NY (Perry and Fuchs, unpublished). 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 2015), South Korea (Lim et al. 2016) and India (GenBank accession number KU522121).

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; Sudarshana et al., 2015). 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.

In spite of tremendous progress in recent years on the biology and ecology of GRBV, research on GRBV spread in vineyards is needed. This research is important not only to document the extent of vector-mediated transmission of GRBV but also to identify insect vectors. This is a prerequisite for the development of optimal disease management strategies. Also, limited information is available on the role of alternate hosts in disease epidemiology. Wild grapes have been identified as potential reservoirs of the virus in some locations in Napa Valley (Bahder et al., 2016b; Perry et al., 2016) but the extent of infection remains to be determined in riparian areas. Similarly, the occurrence of other alternate hosts, particularly among vineyard cover crops, needs to be evaluated. Finally, disseminating information to the industry is essential to extend research and share the latest knowledge on red blotch disease and GRBV, its causal agent.

## Objectives

The overarching goal of this project is to advance our understanding of red blotch disease and its causal agent, GRBV. Our specific objectives are to:

1. Monitor the spread of GRBV in selected vineyards in California and New York
  - Evaluate the presence of vector candidates in a selected vineyard in which spread of GRBV is documented in Napa Valley
  - Carry out controlled transmission experiments of GRBV with potential vector candidates
  - Determine the extent to which wild grapes and vineyard cover crops in Napa Valley harbor and serve as reservoirs of GRBV
2. Develop an innovative inoculation methodology of grapevines with GRBV in order to assess symptoms and the impact of clade I and II isolates of the virus
3. Disseminate research results to farm advisors and to the grape and wine industry

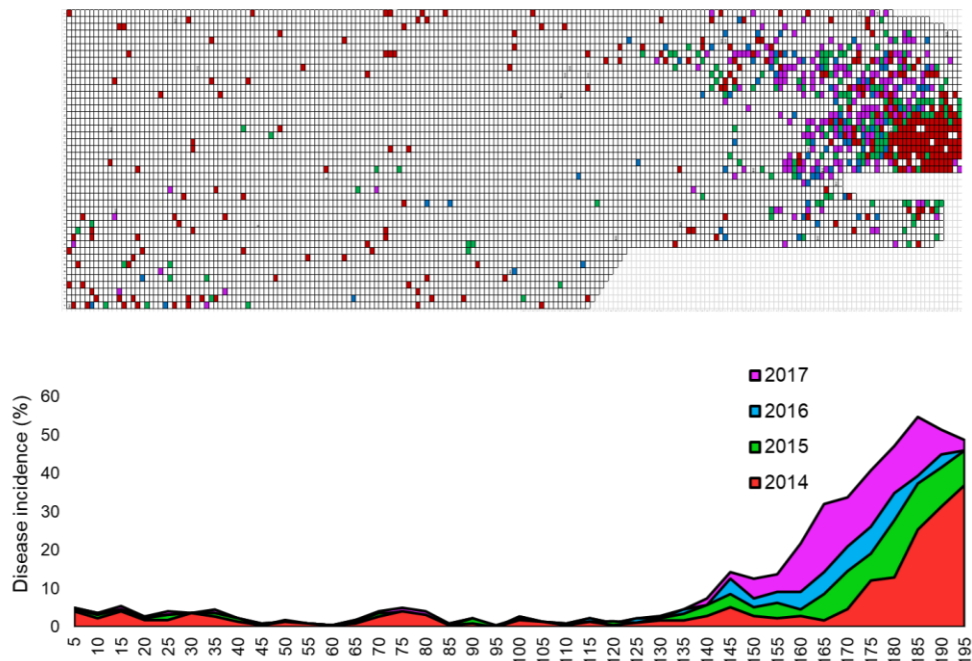
## Description of activities conducted to accomplish each objective, and summary of accomplishments and results for each objective

### Objective 1: Monitor the spread of GRBV in selected vineyards in California and New York

The goal of this objective is to quantitatively evaluate spread of GRBV in diseased vineyards, identify a vector of epidemiological importance for GRBV, and identified host reservoirs of GRBV in distinct vineyard ecosystems.

To quantitatively measure spread of GRBV, a 5-acre Cabernet franc vineyard and a 1-acre Merlot vineyard were selected for this study in California and New York, respectively. The two vineyards were chosen based on a relatively low disease incidence. The California and New York vineyards were planted in 2008. In 2013 and 2014, virus prevalence was determined in the two selected vineyards by visual monitoring of diseased vines and testing randomly selected vines for GRBV by multiplex PCR. This information was used as a baseline to determine the spatiotemporal incidence of GRBV.

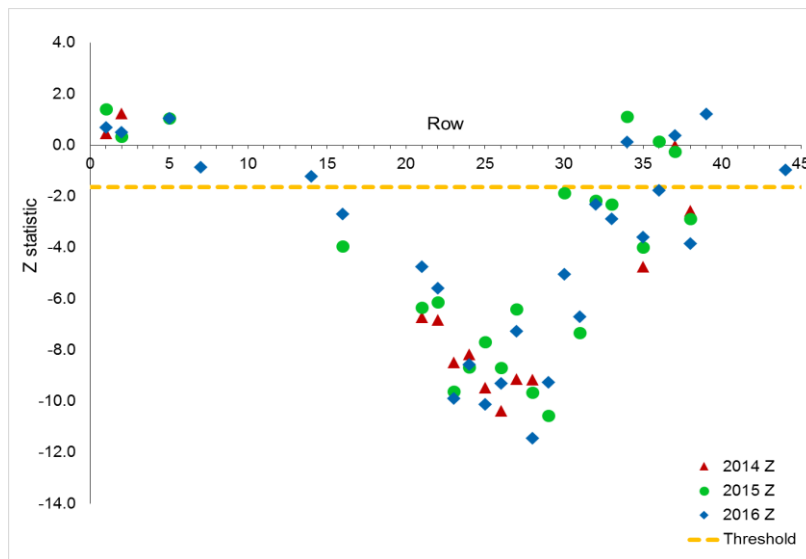
Disease incidence was 4% (305/7,691 vines) in 2014, 6% (461/7,686 vines) in 2015, 7% (547/7,679 vines) in 2016, and 9% (696/7,670 vines) throughout the entire 5-acre Cabernet franc vineyard in California. This result revealed an overall 1-2% increase in disease incidence in 2014-2017, respectively. The magnitude of the annual increase in number of symptomatic vines was most substantial in the section of the vineyard adjacent to the riparian habitat (**Fig. 1**). Plotting disease incidence along the long axis of the vineyard for each year highlighted two major points: i) the absolute magnitude of increase in red blotch incidence was greater between 2014 and 2015 (2.1%) than between 2015 and 2016 (1.1%), and ii) the annual increase in incidence was primarily localized to the section of the vineyard nearest the riparian area (**Fig. 1**). In this section, disease incidence increased by 16% from 2014 to 2015, 4.8% from 2015 to 2016, and 12% from 2016 to 2017 (Cieniewicz et al., 2017b).



**Figure 1.** Spatial pattern of diseased vines over a four-year period (2014-2017) in a 5-acre Cabernet franc vineyard affected by red blotch in California. (a) Colored cells indicate diseased vines in 2014 (red), 2015 (green), 2016 (blue) and 2017 (purple). (b) Annual disease incidence plotted over the long axis in 5-vine panel increments.

Similar work in a Merlot vineyard in New York provided no evidence of GRBV spread from 2014 to 2017 (data not shown). These findings suggested that a vector does not exist in the New York vineyard ecosystem or it eventually exists but at a very low population density or it exists but does not visit the vineyard. Alternatively, the plant protection program used by the vineyard manager in New York is effective at reducing the vector population.

In the Cabernet franc vineyard in California, ordinary runs analysis indicated a significant aggregation ( $Z_u \leq -1.64$ ) of diseased vines in at least two of the three years in rows 16, 21-33, 35, and 38, as well as randomly distributed diseased vines in the remaining rows. Aggregation was observed in 23%, 36%, and 39% of the rows in 2014, 2015, and 2016, respectively. The level of aggregation of diseased vines, as indicated by the magnitude of the Z-statistic, was higher in rows 21 through 33 than in the other rows analyzed (**Fig. 2**). Based on the results of ordinary runs analysis, the spatial pattern of diseased vines in rows 21 through 33 was selected for analysis using SADIE.



**Figure 2.** Z-statistics derived from testing for spatial aggregation of grapevine red blotch virus diseased vines within rows using ordinary runs analysis of a 2-hectare Cabernet franc vineyard in California. Ordinary runs analysis was only implemented if disease incidence within an individual row was greater than 5%. Spatial aggregation of diseased vines was concluded if the  $Z_u$  was less than or equal to -1.64 (yellow horizontal dotted line).

Spatiotemporal analysis between consecutive years within the association function of SADIE revealed a strong overall association among all three years ( $X = 0.874-0.945$ ). In addition, significant spatial associations ( $P < 0.001$ ) were detected between the local clustering indices between successive seasons, suggesting the degree of spatial aggregation of diseased vines was associated with the spatial position of diseased vines in the previous year. This result also indicated that GRBV can spread over time within and between rows in a vineyard area where diseased vines are aggregated. Analysis of epidemic spread fitting a stochastic spatiotemporal model using the Monte Carlo Markov Chain method identified strong evidence for localized (within vineyard) spread. Altogether, a spatial pattern consisting of a combination of strongly aggregated and randomly isolated symptomatic vines within 8-years post-planting suggested unique epidemic attributes compared to those of other grapevine viruses vectored by mealybugs and soft scales or by dagger nematodes for which typical within-row spread and small-scale autocorrelation are well documented. These findings were consistent with the existence of a new type of vector for a grapevine virus (Cieniewicz et al., 2017b).

Close to 100 sentinel vines, i.e. healthy vines for which the mother stocks from which scion budwood and rootstock canes were collected from tested negative for GRBV were planted in the Cabernet franc

vineyard in California in spring 2015. None of these vines flagged for GRBV in 2016. These vines will be further monitored in 2017 and tested for GRBV to gain direct evidence of insect-mediated GRBV spread if they become infected. Sentinel vines replaced existing vines that were weak, regardless of their GRBV infectious status.

To evaluate the presence of vector candidates in a selected vineyard in which spread of GRBaV is documented in Napa Valley, insect yellow sticky traps were placed at the edge of the selected vineyard in California where clustering of diseased vines is occurring proximal to the riparian area (**Fig. 1a**). Traps were positioned on the middle trellis wire throughout a sampling area that spanned 12 rows, and six 4-vine panels per row.

**Table 1.** Grapevine red blotch virus detection in insects trapped on sticky cards in 2015 and 2016 in a *Vitis vinifera* ‘Cabernet franc’ vineyard in which secondary disease spread is documented.

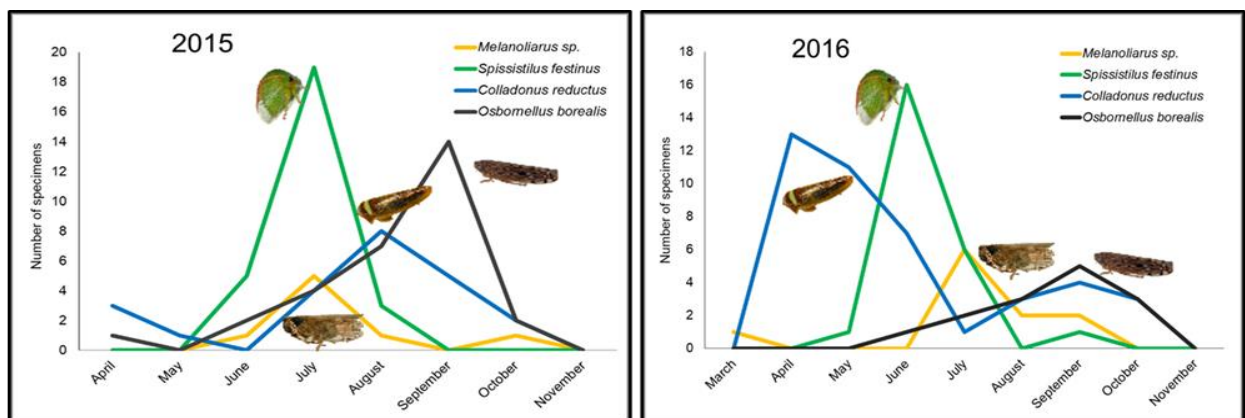
			GRBV detection in				Cumulative		
			2015		2016		GRBV detection		
Hemiptera	Membracidae	<i>Spissistilus festinus</i>	12/25	<b>48%</b>	13/25	<b>52%</b>	25/50	<b>50%</b>	
	Cixiidae	<i>Melanoliarius</i> sp.	4/8	<b>50%</b>	10/12	<b>83%</b>	14/20	<b>70%</b>	
	Cicadellidae	<i>Osbornellus borealis</i>	13/31	<b>42%</b>	4/11	<b>36%</b>	17/42	<b>40%</b>	
		<i>Colladonus reductus</i>	14/23	<b>61%</b>	12/41	<b>29%</b>	26/64	<b>41%</b>	
		<i>Scaphytopius magdalensis</i>	3/45	7%	2/17	12%	5/62	8%	
		<i>Empoasca</i> sp.	1/28	4%	1/16	6%	2/44	5%	
		<i>Graphocephala atropunctata</i>	1/23	4%	0/14	0%	1/37	3%	
		<i>Erythroneura variabilis</i>	0/22	0%	0/22	0%	0/44	0%	
		<i>Euscelis</i> sp.	0/33	0%	0/11	0%	0/44	0%	
		<i>Erythroneura elegantula</i>	0/41	0%	0/24	0%	0/65	0%	
		<i>Japananus hyalinus</i>	0/4	0%	-	-	0/4	0%	
		<i>Deltocephalus</i> sp.	0/15	0%	-	-	0/15	0%	
		<i>Sophonia orientalis</i>	0/5	0%	-	-	0/5	0%	
		<i>Draeculacephala minerva</i>	0/4	0%	-	-	0/4	0%	
		<i>Xestocephalus</i> sp.	0/5	0%	-	-	0/5	0%	
		<i>Erythroneura ziczac</i>	- <sup>a</sup>	-	0/10	0%	0/10	0%	
		<i>Erythroneura tricincta</i>	-	-	0/2	0%	0/2	0%	
		<i>Typhlocyba</i> sp.	0/5	0%	0/6	0%	0/11	0%	
		Unidentified species #1	0/23	0%	0/6	0%	0/29	0%	
		Unidentified species #2	0/16	0%	-	-	0/16	0%	
		Unidentified species #3	0/4	0%	-	-	0/4	0%	
		Unidentified species #4	0/1	0%	0/1	0%	0/1	0%	
		Unidentified species #5	0/7	0%	-	-	0/7	0%	
		Unidentified species #6	0/4	0%	-	-	0/4	0%	
		Unidentified species #7	0/3	0%	-	-	0/3	0%	
		Unidentified species #8	0/2	0%	-	-	0/2	0%	
		Unidentified species #9	0/2	0%	-	-	0/2	0%	
		Unidentified species #10	0/12	0%	-	-	0/12	0%	
		Unidentified species #11	0/1	0%	-	-	0/1	0%	
		Delphacidae	Unidentified species	0/10	0%	0/9	0%	0/19	0%
		Cercopidae	Unidentified species	0/2	0%	-	-	0/2	0%
		Aleyrodidae	Unidentified species	0/52	0%	0/17	0%	0/69	0%
		Aphididae	Unidentified species	1/46	2%	1/12	8%	2/58	3%
	Phylloxeridae	Unidentified species	0/22	0%	0/10	0%	0/32	0%	
	Psyllidae	Unidentified species	0/25	0%	0/5	0%	0/30	0%	
	Miridae	<i>Lygus</i> sp.	1/16	6%	-	-	1/16	6%	
	Lygaeidae	<i>Nysius raphanus</i>	0/8	0%	-	-	0/8	0%	
Thysanoptera		Unidentified species	0/36	0%	-	-	0/36	0%	
Psocoptera		Unidentified species	0/12	0%	-	-	0/12	0%	
Coleoptera		Unidentified species	0/24	0%	-	-	0/24	0%	
Diptera		Unidentified species	0/24	0%	-	-	0/24	0%	
Hymenoptera		Unidentified species	0/24	0%	-	-	0/24	0%	

<sup>a</sup>(-) No specimen tested.

Sticky cards were removed weekly, placed in plastic bags, shipped overnight from the vineyard to the laboratory in Geneva, NY for evaluation, and replaced with new sticky cards. The survey was conducted from April to November 2015, and March to November 2016 to span the entire growing season (Cieniewicz et al., 2018b). Insects caught on sticky card traps were identified to genus and species where possible based on morphological characteristics. Specimens were identified and counted while still impacted on sticky cards. The number and identity of specimens was recorded for each sticky card to evaluate the abundance and diversity of flying insects.

Of approximately 134,000 insects caught on yellow sticky traps in both years, 960 (700 and 260 in 2015 and 2016, respectively) were tested for GRBV by multiplex PCR (Krenz et al. 2014). Specimens were individually removed from sticky cards using Goo Gone liquid degreaser to dissolve the adhesive and loosen the specimens. Individual specimens were stored at -20°C until testing by multiplex PCR for GRBV detection (Krenz et al. 2014) and/or species identification by sequencing of the mitochondrial DNA barcode region. GRBV was detected in at least 40% of *S. festinus* (Membracidae), *C. reductus* (Cicadellidae), *O. borealis* (Cicadellidae) and a *Melanoliarius* species (Cixiidae) by multiplex PCR (**Table 1**). This result revealed that specimens of these four hemipteran species visited the study vineyard and ingested GRBV over two consecutive years. GRBV was not found by multiplex PCR in the majority of other insects tested over two consecutive years, or it was found in only a few specimens (3 to 8%) of a limited number of insects (**Table 1**) (Cieniewicz et al., 2018b).

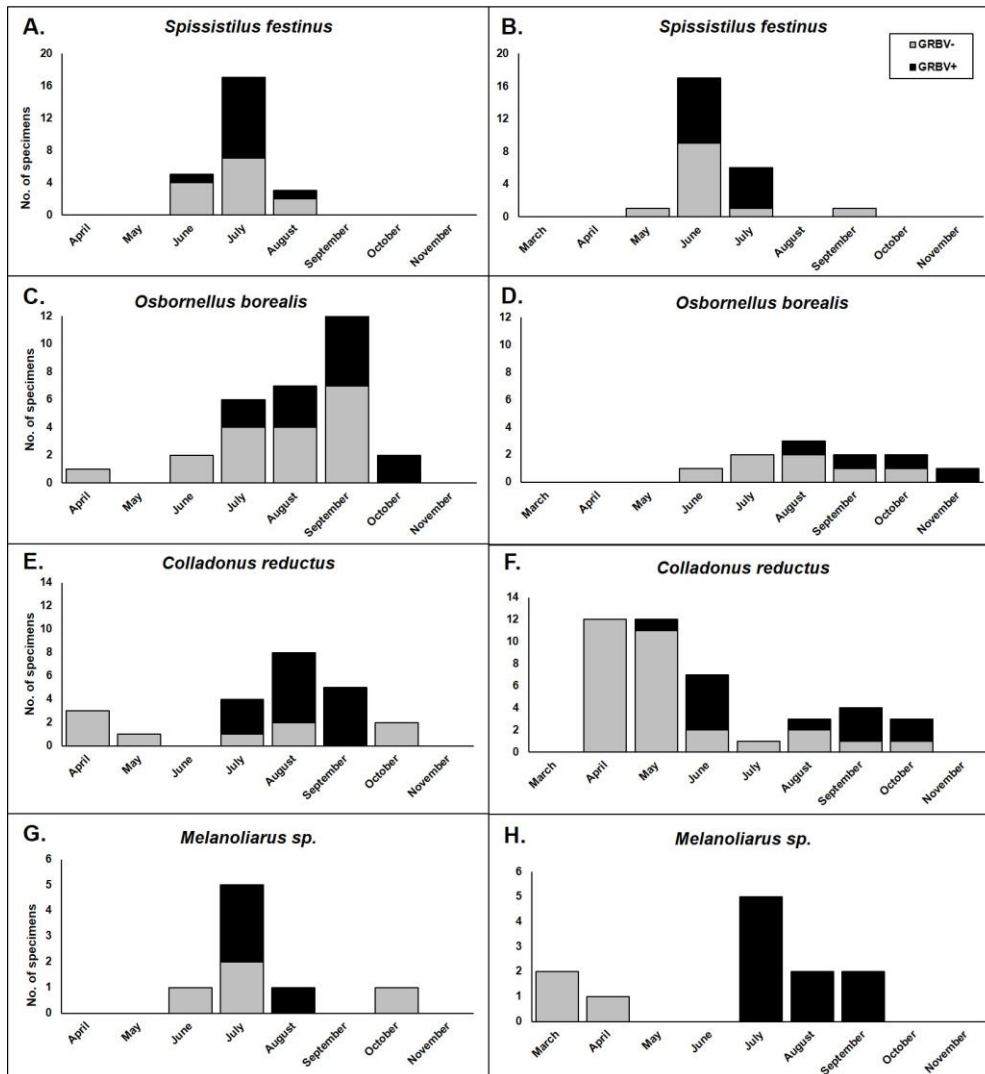
The four insect vector candidates (*S. festinus*, *C. reductus*, *O. borealis* and a *Melanoliarius* spp.) collectively comprised only 0.14% (87 of 62,128 in 2015 and 99 of 72,242 in 2016) of specimens on sticky cards in both years, and 0.4% (87 of 18,525) and 0.6% (99 of 16,060) of Hemiptera on sticky cards in 2015 and 2016, respectively (Cieniewicz et al., 2018b). The relative abundance of the four vector candidates captured on sticky cards was low with only 87 and 99 specimens in 2015 and 2016, respectively. Populations of the four vector candidates peaked between June and September during both years. Populations of *S. festinus* peaked during early July 2015 (**Fig. 3A**) and late June 2016 (**Fig. 3B**), with populations quickly tapering after July. Populations of *C. reductus* peaked in August 2015 (**Fig. 3A**) and in April and September 2016 (**Fig. 3B**). Populations of *Melanoliarius* sp. peaked in June and July, while *Osbornellus borealis* was captured infrequently in June and July, and increased in August and September of both years (**Fig. 3**) (Cieniewicz et al., 2018b).



**Figure 3.** Seasonal population dynamics of candidate insect vectors of GRBV based on specimens captured on sticky cards in a diseased Cabernet franc vineyard in (A) 2015 and (B) 2016.

GRBV was not detected in vector candidates until June (**Fig. 4**), with the exception of one *C. reductus* specimen that tested positive for GRBV in early May 2016 (**Fig. 4F**). The incidence of viruliferous *S. festinus* was highest in July in 2015 (**Fig. 4A**) and June in 2016 (**Fig. 4B**). Viruliferous *O. borealis* were

detected from July to November in 2015 (Fig. 4C) and 2016 (Fig. 4D), while viruliferous *Melanoliarus* sp. were captured on sticky cards only from July to September (Fig. 4G & H) (Cieniewicz et al., 2018b).



**Figure 5.** Seasonal dynamics of ingestion of GRBV by candidate insect vectors in a diseased Cabernet franc vineyard in Napa County, California in (A, C, E, and G) 2015 and (B, D, F, and H) 2016.

Spatial pattern analyses indicated aggregated patterns of GRBV-infected vines and populations of *S. festinus* and *O. borealis*. No significant aggregation was found for *C. reductus* and the *Melanoliarus* sp. (data not shown). Moreover, there was a significant spatial association between the distribution of infected vines and viruliferous *S. festinus*. No significant spatial associations were identified between populations of alternative insect vector candidates and GRBV-infected vines (data not shown). The spatial distribution of vector candidates on sticky cards also indicated a gradient of higher *S. festinus* (N=50) and *O. borealis* (N=42) at the edge of the vineyard next to a riparian area and decreasing *S. festinus* and *O. borealis* populations distant from the edge. Additionally, both *S. festinus* and *O. borealis* populations of section 1 near the edge of the vineyard (0 to 10 m from the edge of the vineyard) had a higher proportion of viruliferous insects than the inner-vineyard section 2 (10 to 20 m within the vineyard) and section 3 (20 to 30 m within the vineyard), in which the proportions of viruliferous specimens were lower. The spatial distribution of *C. reductus* (N = 64) and *Melanoliarus* (N = 20) was not dependent upon proximity to the edge of the vineyard (Cieniewicz et al., 2018b).



To carry out controlled transmission experiments of GRBV with potential vector candidates, 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. Alfalfa is a host of *S. festinus* but not of GRBV (Cieniewicz et al., unpublished). Conditions to rear *S. festinus* colonies were optimized so that a full development cycle, including oviposition, and the production of nymphs (**Fig. 5**) and adults, could be completed within two months.



**Figure 5.** Nymphs of *S. festinus* on alfalfa plants in a growth chamber.

The transmission mode of GRBV by *S. festinus* is hypothesized to be nonpropagative, circulative. To address this issue, *S. festinus* were allowed to feed on GRBV-infected grapevines for 48-72 h. Then, groups of 2-4 individuals were transferred to alfalfa and allowed to feed for two weeks. These assays were duplicated. Subsets of *S. festinus* were tested for the presence of GRBV after the acquisition and alfalfa feeding steps. After the acquisition period, 6 out of 8 *S. festinus* in experiment 1, and 3 of 5 *S. festinus* in experiment 2 were positive for GRBaV in multiplex PCR, confirming that *S. festinus* can ingest GRBaV. After feeding on alfalfa, most specimens tested (12 of 20 in experiment 1 and 6 of 11 in experiment 2) were positive for GRBaV, revealing that *S. festinus* is capable of keeping the virus even after a gut-clearing episode on a nonhost plant of GRBV. These findings suggested a persistent transmission of GRBaV. To further our understanding of the transmission mode of GRBaV, additional work is under way to localize the virus in organ tissue of *S. festinus*.

*S. festinus* specimens reared on alfalfa were collected and deposited on GRBV-infected vines in insect-proof cages in the greenhouse. *S. festinus* were allowed to feed for 2-6 days. After the virus acquisition access period, individual *S. festinus* were moved to healthy vines (2-3 specimens per vine) for a 4-6 day transmission access period. Individual insects were then collected and tested for GRBV for multiplex PCR and vines were monitored for symptom expression and presence of GRBV. Preliminary results showed that *S. festinus* transmits GRBV to healthy vines in the greenhouse, confirming previous findings (Badher et al., 2016a).

To determine the extent to which wild grapes and vineyard cover crops in Napa Valley harbor and serve as reservoirs of GRBV, wild grapes were surveyed in California and New York, and eight California



**Figure 6.** Cover crops in a GRBV-infected vineyard surveyed for GRBV and *S. festinus*.

vineyards were selected for surveys of cover crops. The eight California vineyards are infected with GRBV or proximal to vineyards infected with GRBV. In addition, they carry legumes in their cover crop stands sown in November 2016 (**Fig. 6**). Legumes, i.e. bell beans, peas, vetch, clover, alfalfa, medicagos, etc. are known hosts of *S. festinus*, and most of these species, i.e. bell beans, peas, vetch, clover, etc., are used as cover crops in vineyards. Three additional vineyards were selected because they are not infected with GRBV and carry legumes species in their cover crops stands. Alfalfa samples from unmanaged areas proximal to GRBV-infected vineyards were also sampled. A total of over

300 legume samples from vineyard middle-row cover crops have been collected in early March 2017 for



GRBV testing by multiplex PCR. Results showed that all the samples were negative for GRBV in PCR. These results suggested that legume cover crops are not infected with GRBV in the diseased vineyards selected for this study, and thus are unlikely to serve as reservoirs of GRBV. These preliminary data will need to be confirmed during a second growing season.

Based on earlier findings on the occurrence of GRBV in free-living grapes (Bahder et al., 2016a; Perry et al. 2016), the distribution and diversity of grapevine red blotch virus (GRBV) was determined in free-living *Vitis* species in northern California and New York from 2013 to 2017. GRBV was detected by PCR in 21.5% (43/203) of samples from California but in none of the 163 samples from New York (Table 2).

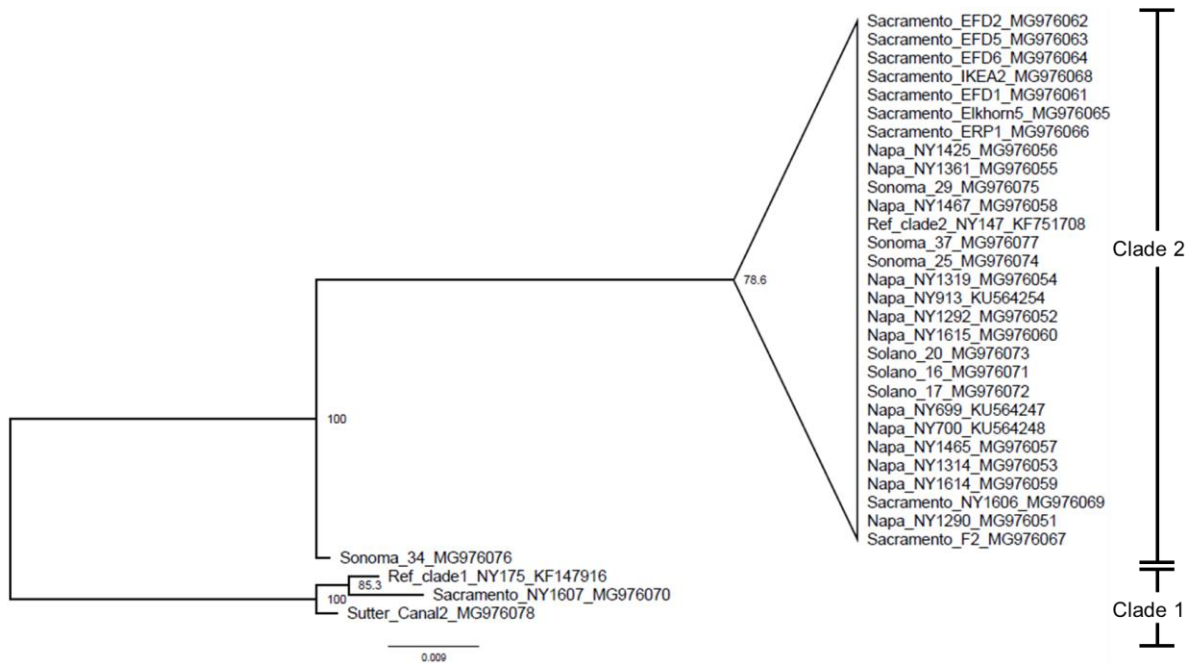
**Table 2.** Detection of grapevine red blotch virus in free-living grapes in California and New York.

State	County	N. positives/N. tested	%
California	Napa	24/87	28
	Sonoma	5/23	22
	Solano	3/20	15
	Sacramento	9/31	29
	Sutter	1/19	5
	Butte	0/15	0
	Glenn	0/12	0
New York	Suffolk	0/21	0
	Ulster	0/31	0
	Clinton	0/20	0
	Ontario	0/20	0
	Chautauqua	0/20	0
	Tompkins	0/6	0
	Yates	0/18	0
	Steuben	0/14	0
	Seneca	0/25	0

None of the infected samples exhibited disease symptoms. Genetic fingerprinting of a subset of GRBV-infected samples from California identified them as hybrids of *V. californica* x *V. vinifera* cv. Syrah or Durif or hybrids of *V. californica* x rootstocks *V. rupestris* St. George and Ruggeri 140 (*V. berlandieri* x *V. rupestris*). The incidence of GRBV in free-living vines was significantly higher in samples from California counties with high grape production compared to low grape production ( $\chi^2 = 83.09$ ;  $P < 0.001$ ), and in samples near to (< 5km) compared to far from (> 5km) vineyards ( $\chi^2 = 57.58$ ;  $P < 0.001$ ). These results suggested a directional spread of GRBV inoculum predominantly from vineyards to free-living *Vitis* species. When considering individual counties, GRBV incidence was significantly higher in Napa (29%, 24 of 83), Sonoma (22%, 5 of 23) and Sacramento (32%, 10 of 31) compared to Solano (15%, 3 of 20) and Sutter (5%, 1 of 19) counties ( $\chi^2 = 22.79$ ;  $P < 0.001$ ). GRBV was not detected in any of the free-living vines collected in Glenn and Butte counties, two counties with extremely low commercial grape production (less than 0.09% of the total acreage) and not bordering counties with major viticulture acreage. GRBV-infected samples in Solano County were less than 10 km away from vineyards in Napa County, a neighboring county with the highest acreage planted to grapes. The single GRBV-infected free-living *Vitis* spp. sample from Sutter County is an exception because it was far away from any production vineyard but close to nursery vineyards.

Two distinct phylogenetic clades were identified for GRBV from free-living *Vitis* species (Fig. 7). A similar diversity pattern was described for GRBV isolates both from commercial vineyards (Krenz et al. 2014) and insect vector candidates in an infected vineyard (Cieniewicz et al. 2018). The majority of

GRBV isolates, including all those from Napa, Sonoma and Solano counties and most from Sacramento County, belong to clade 2 in which the nucleotide sequence identity ranges from 96.9 to 100% identity.



**Figure 7.** Maximum likelihood phylogeny of grapevine red blotch virus (GRBV) diversity fragment nucleotide sequences of isolates from free-living *Vitis* spp. in California. Recombinant regions corresponding to the contribution of the minor parent were removed from isolates Sonoma\_34 and Sacramento\_F2 for phylogenetic analyses. Numbers at branches indicate bootstrap support (1000 bootstrap replicates, random seed=3). Branches with less than 70% bootstrap support were collapsed.

One isolate from Sacramento County and the only isolate from Sutter County grouped in clade 1, with 98.3% nucleotide sequence identity. The nucleotide sequence identity between clades 1 and 2 ranges between 88.3 to 90.9%. Additionally, intra-specific recombination events was confirmed in GRBV isolates. The prevalence of GRBV in California free-living vines highlights the need for vigilance regarding potential virus inoculum sources in order to protect new vineyard plantings and foundation stock vineyards in California.

**Objective 2:** Develop an innovative inoculation methodology of grapevines with GRBV in order to assess symptoms and the impact of clade I and II isolates of the virus

The goal of this objective is to develop a biolistic inoculation method of grapevine with GRBV to facilitate the evaluation of vines singly infected with GRBaV, while avoiding manipulations using recombinant DNA. Two approaches to infect grapevines with GRBV were tested: 1) direct mechanical inoculation of grape seedlings with GRBV DNA amplified from infected vines using rolling circle amplification (RCA), and 2) biolistic bombardment of grape seedlings with GRBV DNA from RCA. Among plants mechanically inoculated with GRBV DNA, none were infected at 6 months post inoculation. Biolistic bombardment conditions for seedlings are being established using a reporter gene construct. Grapevine seedlings were agroinoculated with bitmer constructs and will be tested for infection at 6 months post-inoculation. To facilitate our ability to detect the replication of inoculated GRBV, we refined a quantitative PCR assay. Petioles were consistently found to contain higher amounts of GRBV compared to their corresponding leaves. Leaves proximal to the main stem were found to

contain higher amounts of GRBV compared to leaves located in the apical part of the cane. Based on these findings, it is recommended that total nucleic acid extracted from multiple petioles of fully developed leaves be used for reliable GRBV diagnostics.

### **Objective 3: Disseminate research results to farm advisors and to the grape and wine industry**

The goal of this objective is to raise awareness of the impact of red blotch and to inform stakeholders of research progress.

Research results were communicated to stakeholders at the following venues:

- Fuchs, M. 2018. Leafroll and red blotch: What should I be aware of and what can I do? Show me grape and wine conference, March 7, Columbia, Missouri, (participants = 52).
- 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)
- Fuchs, M. 2017. Update on the ecology of red blotch virus. Sustainable Ag Expo on Nov. 14, San Luis Obispo, CA (participants = 500).
- Fuchs, M. 2017. Leafroll and red blotch viruses. Open house, Sept. 8, Niagara-on-the-Lake, Ontario, Canada (participants = 51).
- Fuchs, M. 2017. Updates on leafroll and red blotch diseases. Eastern Winery Exposition, March 23, Syracuse, NY (participants = 40).
- 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).
- 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).
- Fuchs, M. 2017. Looking forward: How grapevine clean plant strategies can be improved? Unified Symposium January 25, Sacramento, CA (participants = 250).
- Cieniewicz, E.J. and Fuchs, M. 2016. Spatiotemporal spread of Grapevine red blotch-associated virus, Cornell Recent Advances in Viticulture and Enology (CRAVE) conference, November 2, Ithaca, NY (participants = 60).
- Fuchs, M. 2016. Research updates on leafroll and red blotch diseases. Grape Growers of Ontario, August 16, Brock University, St Catharines, Ontario, Canada (participants = 25).
- Fuchs, M. 2016. Updates on leafroll and red blotch diseases, March 4, Riverhead, NY (participants = 15).
- Fuchs, M. 2016. Etiology of red blotch. Grapevine red blotch disease: What you need to know. Webinar organized by Regional IPM Centers, February 26, (participants = 310).
- Fuchs, M. 2015. Red blotch, Plant Diseases: Vineyard RX, Napa Continuing Education Class Series 3, Napa Farm Bureau, UC Cooperative Extension and Napa County Agriculture Commissioner, Yountville, CA, November 10 (participants = 250).
- Cieniewicz, E.J. and Fuchs, M. 2015. Epidemiology of red blotch, Cornell Recent Advances in Viticulture and Enology (CRAVE) conference, Ithaca, NY, November 4, (participants = 60).

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

#### Publications:

- Cieniewicz, E., Thompson, J.R., McLane, H., Perry, K.L., Dangl, G.S., Corbett, Q., Martinson, T., Wise, A., Wallis, A., O'Connell, J., Dunst, R., Cox, K. and Fuchs, M. 2018a. Prevalence and diversity of grabloviruses in free-living *Vitis* spp. Plant Disease, submitted.
- 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, <https://apsjournals.apsnet.org/doi/pdf/10.1094/phyto-12-17-0419-R>.
- Cieniewicz, E., Pethybridge S., Loeb, G., Perry, K.L., and Fuchs, M. 2018b. Diversity and spatial distribution of vector candidates of grapevine red blotch virus in a diseased vineyard. Phytopathology, 108:94-102.
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- 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.
- 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.

#### Presentations:

- Fuchs, M. 2018. Leafroll and red blotch: What should I be aware of and what can I do? Show me grape and wine conference, March 7, Columbia, Missouri, (participants = 52).
- 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)
- Fuchs, M. 2017. Update on the ecology of red blotch virus. Sustainable Ag Expo on Nov. 14, San Luis Obispo, CA (participants = 500).
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- 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).
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- Fuchs, M. 2015. Red blotch, Plant Diseases: Vineyard RX, Napa Continuing Education Class Series 3, Napa Farm Bureau, UC Cooperative Extension and Napa County Agriculture Commissioner, Yountville, CA, November 10 (participants = 250).
- Cieniewicz, E.J. and Fuchs, M. 2015. Epidemiology of red blotch, Cornell Recent Advances in Viticulture and Enology (CRAVE) conference, Ithaca, NY, November 4, (participants = 60).

#### **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. They also 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. Our research also documented the potential role of free-living *Vitis* spp. in California as reservoirs of GRBV, suggesting cautionary approaches when establishing new vineyards, particularly foundation vineyard and increase nursery vineyards.

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

Limited information is available on biology and epidemiology of grapevine red blotch disease for grapevine red blotch virus (GRBV) is the causal agent. Analysis of the spatial incidence of GRBV over a three-year period (2014–2016) in a California vineyard was consistent with the occurrence of virus spread. The increase of disease incidence was 1-2% annually. By contrast, no evidence of spread was obtained in

a New York vineyard. To determine the diversity and distribution of potential vector candidates in California, sticky cards were placed from March to November in the vineyard area where disease incidence increased by nearly 20% between 2014 and 2016. GRBV was consistently detected in four species caught on traps in 2015 and 2016: *Spissistilus festinus* (Membracidae), *Colladonus reductus* (Cicadellidae), *Osbornellus borealis* (Cicadellidae) and a *Melanoliarius* species (Cixiidae). Populations of these four candidate vectors peaked from June to September with viruliferous *S. festinus* culminating from late June to early July in both years. These findings revealed the epidemiological significance of *S. festinus* as a vector of GRBV and the need for testing the transmission capability of *C. reductus*, *O. borealis*, and the *Melanoliarius* species. A search of alternate hosts of GRBV in vineyard ecosystems revealed a high virus incidence in free-living grapes in diseased California but not in New York. Surveys of legume cover crops, i.e. bell beans, peas, vetch, clover, etc., in California vineyards in spring 2017 did not document a single GRBV-infected plant. Collectively, our insights into the spread of GRBV and population dynamics of *S. festinus* and three other candidate vectors informed epidemiological features of red blotch disease and helped devise disease management strategies based on vineyard management.

### Status of funds

Funds were spent for salaries of key personnel (postdoctoral associate, graduate student and technicians) involved in the research, supplies and 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

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