



RESEARCH FOCUS

Red Blotch Disease Ecology and Management

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Grapevine red blotch virus affects fruit ripening of a diseased (left) compared to a healthy (right) Cabernet franc vine on Long Island.

Photo by Alice Wise

Grapevine red blotch disease, caused by grapevine red blotch virus (GRBV), is a new threat to North American grape production, affecting fruit ripening and quality. Only recently identified (2011), little is known about the origin, spread, host range, and vectors of GRBV. We investigated these factors in vineyard studies in California and New York. In a Cabernet franc vineyard in California, GRBV disease incidence increased at a rate of up to 10-15% per year from 2014-2018. In contrast, we found no evidence of spread in a similar survey of a New York Merlot vineyard. Insect trapping in California vineyards confirmed the three-cornered alfalfa hopper as a GRBV vector. However, it is not a common grapevine pest and generally is infrequently found in vineyards. In broader surveys, we found GRBV in wild grapevines (*V. californica* and hybrids) throughout northern California, but not in three wild grapevine species in New York, and not in row-middle cover crops in diseased California vineyards.

Infected planting material appears to be the most important (and perhaps sole) source of GRBV in New York vineyards, and there is no evidence that it will spread to uninfected vines. Red blotch can be managed by reducing inoculum sources; i.e. by removing vineyards with disease incidence greater than 30%, roguing infected vines if disease incidence is less than 30%, and planting vines derived from virus-tested nursery stock.

KEY CONCEPTS

- Grapevine red blotch virus (GRBV) affects fruit ripening and quality and is an emerging threat to grape production in North America.
- GRBV is disseminated via grafting and vegetative propagation.
- Secondary spread of GRBV is occurring in California vineyards, but not currently in New York vineyards.
- The three-cornered alfalfa hopper, the only presently known vector of GRBV, is spatially associated with GRBV spread in California.
- GRBV is present in wild grapevines in California, but not in New York.
- Management efforts should be aimed at:
 - preventing the introduction of the virus into vineyards (by planting vines derived from virus-tested nursery stock).
 - reducing the virus inoculum through roguing and removal of infected vines.

Background. Red blotch disease has emerged in the last decade as one of the major viral diseases of grapevine in North America. Grapevine red blotch virus (GRBV), the causal agent of red blotch disease, is widespread in vineyards throughout the United States (1), including in the Finger Lakes and Long Island, and at other locations in eastern North America, including Virginia, New Jersey, Pennsylvania, Maryland, and Ontario (2).

GRBV affects the profitability of vineyards by substantially reducing fruit quality and ripening (3,4,5) (**Fig. 1**). Red blotch disease is difficult to visually identify in vineyards because symptoms are similar to those of leafroll disease and some nutrient disorders.



Figure 1. Reduction in fruit quality and ripening on (left) a red blotch diseased compared to (right) an asymptomatic Pinot noir vine.

Photos by Marc Fuchs

GRBV has likely been present in vineyards and nurseries for decades, but has been confused with other diseases with similar symptoms. Moreover, it often causes asymptomatic infections in rootstocks.

In red cultivars, red blotches form on the leaves early in the season, and they can coalesce across most of the leaf blade later in the season (**Fig. 2A-E**). In white cultivars, irregular chlorotic areas (yellow) of the leaves may form and become necrotic (brown) late in the season (**Fig. 2F**). Foliar symptoms usually appear first on older leaves at the base of the canopy in late spring/early summer, (late May to June), and progress up the shoot towards younger leaves later in the season (August to October).

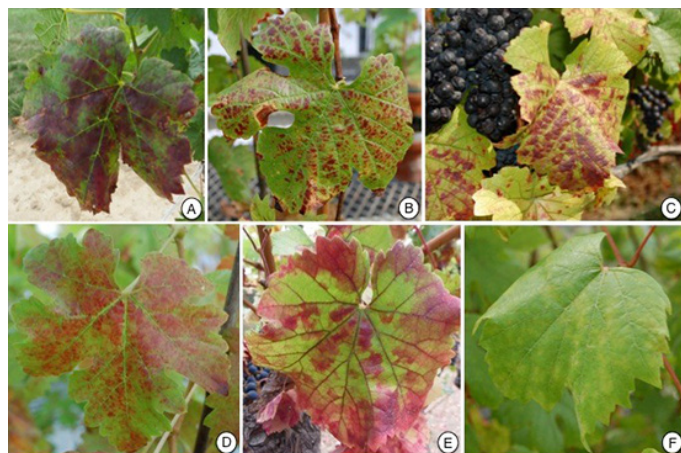


Figure 2. Close up of foliar symptoms of red blotch on Cabernet franc (A), Chambourcin (B), Pinot noir (C), Syrah (D), Cabernet Sauvignon (E) and Chardonnay (F).

Photos by Marc Fuchs

Currently, the only available detection techniques are DNA-based tests such as the polymerase chain reaction (PCR). Red blotch can cause losses up to \$170,000 per acre over the lifespan of a vineyard, depending on the initial disease incidence, cultivar, region, and price penalty for low quality fruit (6). Optimal management of red blotch requires a comprehensive understanding of the disease epidemiology and ecology.

Spread of red blotch in a California vineyard. We monitored a Cabernet franc vineyard in Rutherford, California from 2014 to 2018 to track the incidence and spread of red blotch disease.

The 5-acre study vineyard was planted in 2008—before the discovery of GRBV. By 2012 the vineyard manager noticed a gradient of vines exhibiting foliar reddening symptoms at the edge of the vineyard next to the Napa River (**Fig. 3**). In 2013, PCR confirmed the presence of GRBV in some of the symptomatic vines in this area. Every October from 2014 to 2018, we monitored symptoms throughout the whole vineyard.



Figure 3. Red blotch secondary spread in a gradient down the row of a Cabernet franc vineyard in Rutherford, California (background). GRBV is transmitted in vineyards by the three-cornered alfalfa hopper (lower left inset)

Photos by Libby Cieniewicz

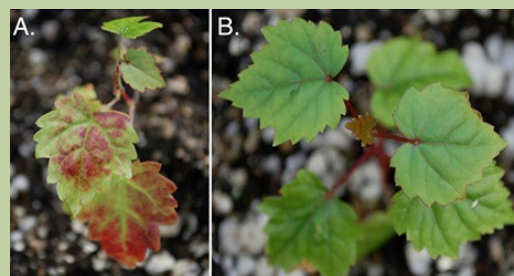
In 2014, the disease incidence (percentage of vines infected) over the whole vineyard was 4.0% (305/7,691 vines diseased). In 2015 the incidence increased to 6% (461/7,691 vines diseased). By 2016 it was 7.1% (547/7,691 vines diseased), in 2017 it was 9.1% (696/7,691 vines diseased), and by 2018 it was 13.8% (1,058/7,691 vines diseased). While the increase of disease incidence in the whole vineyard increased by only 10% over five years, the spread occurred much more rapidly (40% over five years) in the area where the disease was initially aggregated (**Fig. 4**) (7).

Establishing a Causal Relationship Between GRBV and Red Blotch Disease Using Koch's Postulates

Koch's postulates provide a framework by which researchers can establish the causal relationship between a pathogen and a disease. First presented by bacteriologist Robert Koch in 1883, the idea behind the postulates is to first isolate the pathogen in the laboratory, then re-infect a host to see if the laboratory strain causes the same symptoms, and finally re-isolate the pathogen to be sure it is the same as previously isolated.

We followed these steps to demonstrate that GRBV is the causal agent of red blotch disease:

1. GRBV was independently recognized at Cornell University and UC-Davis in 2011.
2. The virus is found in the majority of vines manifesting red blotch disease symptoms.
3. We engineered an infectious GRBV clone in the laboratory.
4. Healthy vines inoculated with the infectious GRBV clone in the laboratory became infected and exhibited typical red blotch disease symptoms.
5. The virus isolated from inoculated vines that became diseased is identical to the infectious GRBV clone.



A Cabernet Sauvignon plant inoculated with grapevine red blotch virus, manifesting typical disease symptoms (left) compared to a mock inoculated Cabernet Sauvignon plant (right).

Photos by Marc Fuchs

We used computer models to better understand how the disease is spreading and the likely origin of the earliest infections in the Cabernet franc vineyard. The models showed that a vine is more likely to become infected if it is in proximity to an infected vine, and that disease is primarily due to localized, within-vineyard sources rather than inoculum sources outside of the vineyard.

This result was confirmed when we extracted DNA from symptomatic vines and characterized GRBV isolates in the study vineyard to determine how similar they were to each other. All of the vines in the area where the disease is aggregated had nearly identical GRBV isolates, further supporting the claim that spread occurred from within-vineyard sources of inoculum. In the Cabernet franc vineyard, the inoculum likely originated from the rootstock of the planting stock (7, 8).

Survey of potential insect vectors in a California vineyard. To determine which insects might be associated with the spread of GRBV, we conducted surveys in the Cabernet franc vineyard in 2015 and 2016. We hung insect sticky cards in the canopy in a grid pattern within the Cabernet franc area of extensive GRBV spread (Fig. 6). We refreshed the traps weekly from April through November. We identified the insects trapped on the sticky cards, first based on morphological characteristics, and later by DNA-based identification methods. We then tested the insects for GRBV to determine which species were capable of ingesting the virus by feeding on the vines. Although the ability to ingest GRBV from infected vines does not mean these insects are vectors, it does narrow down the pool of potential insect vector candidates and provide insights into timing of feeding and potential transmission.

In 2015, over 700 insect specimens from 40 different species/taxa were tested for GRBV. Of the 40 taxa evaluated, only four species consistently tested positive for GRBV. In 2016, we tested fewer specimens (n=271) but results were consistent with the 2015 survey. The four insects we identified are *Spissistilus festinus* (three-cornered alfalfa hopper), currently the only confirmed vector of GRBV (9), two leafhoppers (*Colladonus reductus* and *Obsornellus borealis*) and a planthopper (*Melanoliarus* spp.) (8).

These insects are all phloem-feeders, and were all found in low relative abundance (~5-40 individuals per year) compared to other hemipteran insects such as the grape leafhopper, variegated leafhopper, aphids, and potato leafhoppers, which were frequently detected on sticky cards (~500 to 1,500 individuals per year).

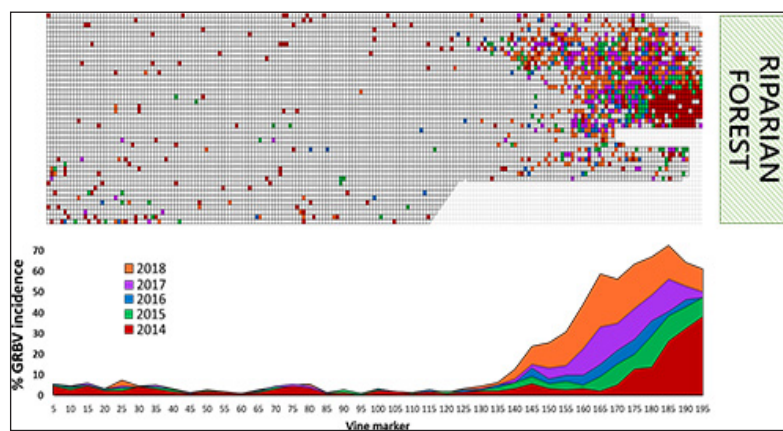


Figure 4. GRBV spread in a 5-acre Cabernet franc vineyard in California over 5 years. The top graph shows the entire study vineyard with each cell representing a single vine that is asymptomatic (blank) or symptomatic (colored). The bottom graph shows the distribution of diseased vines in 5-vine panels across rows.

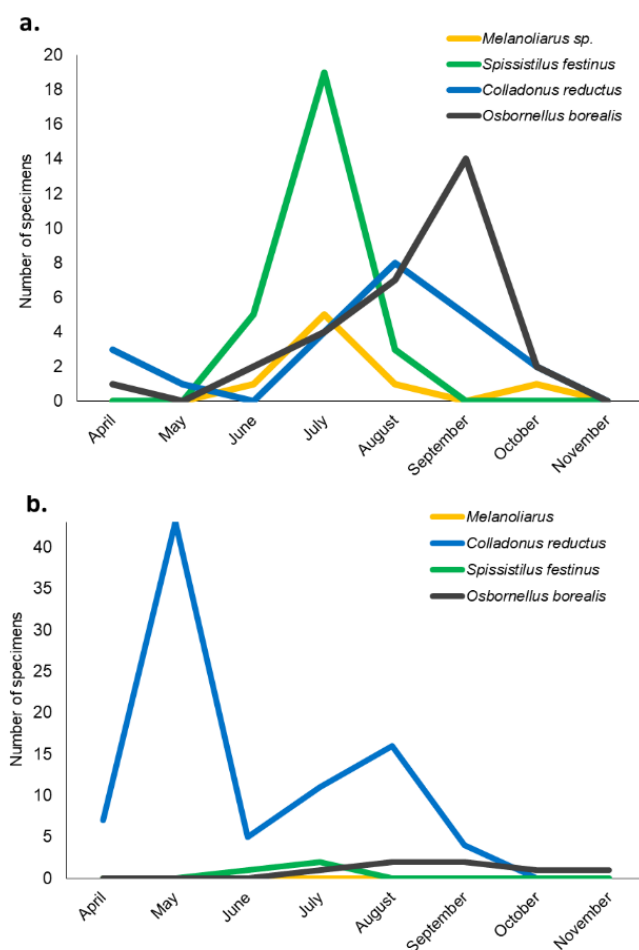


Figure 5: Populations of vector candidates in (a) a Cabernet franc vineyard where GRBV was readily spreading and (b) an adjacent Cabernet Sauvignon vineyard where limited spread of GRBV was occurring.



Figure 6. Landscape view of GRBV spread in Cabernet franc and Cabernet Sauvignon vineyards in Rutherford, California. Colored overlay indicates GRBV-infected vines. White grids indicate area of surveys for insects in 2015-16 in the Cabernet franc vineyard and 2017-18 in Cabernet Sauvignon vineyard

Photo credit: Google earth satellite imagery and Libby Cieniewicz

The populations of the insects peaked at different times in the season, but the population dynamics were consistent between 2015 and 2016 with predominant populations of the TCAH in July (Fig. 5a). Populations of the TCAH in this survey were higher in the sections closest to the edge, and tapered off further into the vineyard (8).

Limited spread of GRBV in an adjacent California vineyard. While GRBV spread from vine to vine in the Cabernet franc vineyard, that doesn't mean this is the case in every vineyard with GRBV-infected vines. In fact, immediately to the west of the Cabernet franc block is a 5-acre Cabernet Sauvignon block, also planted in 2008 (Fig. 6). Vines were derived from two different clones sourced from two different nurseries. The southern portion of the vineyard is heavily infected with GRBV, while the northern portion is mostly GRBV-negative and asymptomatic (Fig. 6).

Despite the large potential source of GRBV inoculum in roughly 40% of the vineyard, we haven't seen much spread of GRBV from the southern portion to the northern portion in five years. This led us to ask:

Why is there a differential spread of GRBV between the Cabernet franc and Cabernet Sauvignon vineyards? Why is GRBV readily spreading in the Cabernet franc vineyard but not much in the Cabernet Sauvignon vineyard in spite of the availability of a very low inoculum source (1%) in the former and a very high inoculum source (40%) in the latter following planting?

Since GRBV shows equally striking symptoms on both Cabernet franc and Cabernet Sauvignon, and the TCAH can transmit GRBV from infected to healthy Cabernet Sauvignon (9), we hypothesized that there is a difference in population or behavior of the TCAH vector (or other potential vectors) in these two vineyards that resulted in the observed differential GRBV spread. To address these questions, we conducted a sticky card survey in 2017 and 2018 in the Cabernet Sauvignon vineyard.

Although many of the same insects were present in both vineyards, the relative abundance of many of the species/taxa differed. For example, we found 25 TCAH in the Cabernet franc vineyard throughout the growing season both in 2015 and 2016 (Fig. 5a), but we only found three and two TCAH in the Cabernet Sauvignon vineyard in 2017 and 2018, respectively (Fig. 5b).

Similarly, there were fewer *Osbornellus borealis* and *Melanoliarius* spp. in the Cabernet Sauvignon vineyard. However, there was a greater abundance of *Colladonus reductus* in the Cabernet Sauvignon vineyard. The difference in insect community dynamics, particularly of the TCAH, could explain the differential spread of GRBV in the two study vineyards. The difference in populations of the TCAH could be due to the proximity of the Cabernet franc vineyard to the Napa River and wooded natural area, compared to the Cabernet Sauvignon vineyard, which is about 800 ft. from the riparian habitat.

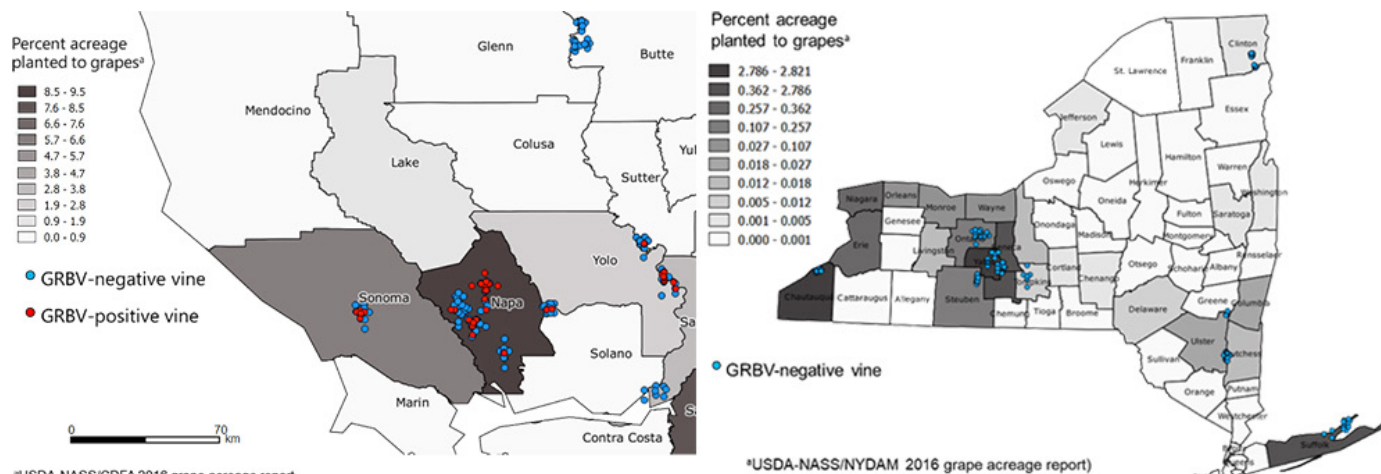


Figure 7: Map of wild vines in northern California (left) and New York (right) surveyed for the presence of GRBV. Counties are colored according to the percentage of acreage planted to grapevines.

Survey of red blotch in a New York vineyard. To complement the study of GRBV spread in California, we surveyed a Merlot vineyard on Long Island during the 2014 to 2018 growing seasons for spread of GRBV over time. We annually tested a subset of vines from this vineyard for GRBV. Any vines that were GRBV-negative were resampled each year. GRBV-positive vines were confirmed by re-testing in at least one more year (at least two years of GRBV-positive test result).

Over the five years of sampling in this vineyard, negative vines consistently tested negative. In other words, no vines that tested negative one year tested positive in a subsequent year. This indicates that, although GRBV is prevalent in this vineyard with 60% disease incidence, the evidence does not support secondary spread. Our conclusion is that the observed incidence of GRBV resulted from infected planting material.

This conclusion is strengthened by the results of an insect survey conducted in 2017 and 2018 concurrent with the California studies. Although several phloem-feeding treehoppers and leafhoppers were found in the Long Island Merlot vineyard, none of them tested positive for GRBV. The TCAH was not found in the Merlot vineyard on Long Island, nor has it been found in any vineyards in New York to our knowledge. Evidence of GRBV spread from vine to vine by insect vectors is absent in New York.

GRBV in wild grapevines surveyed in California and New York. GRBV was detected in wild *Vitis* spp. near commercial vineyards in Napa, California, including in the riparian area proximal to the Cabernet franc vineyard (10). This led us to ask more questions: Is GRBV widespread in wild grapevines? Is it an important source of inoculum for GRBV introduction into vineyards? To address these questions, we expanded the collection of wild grapevines throughout northern California and also in New York. We tested them for GRBV, then analyzed the genetic diversity of the virus populations.

In New York, we collected samples from 161 wild *V. riparia*, *V. aestivalis* and *V. labrusca* vines from western NY, Finger Lakes region, Champlain Valley, Hudson Valley, and Long Island. All of these samples tested negative for GRBV.

In California, we found GRBV in 21% (43 of 203) of wild *V. californica* vines and *V. californica* hybrids sampled. The virus was more prevalent in counties with more grape production than in counties with less or no grape production (Fig. 7). The genetic diversity among GRBV isolates from wild vines was nearly identical to the diversity of GRBV isolates found in commercial vineyards.

The fact that virus strains found in wild grapevines matched those in adjacent commercial plantings suggests that the direction of spread of GRBV inoculum is from commercial vineyards to adjacent wild vines, rather than the opposite (11). Nonetheless, our surveys covered only four recent years (2014-2017), so we cannot rule out that GRBV may have originated from wild vines in California in the more distant past.



Figure 8. Riparian areas adjacent to vineyards can harbor wild vines infected with GRBV and/or insect vectors

Photo credit: Google earth satellite imagery

Cover crops and TCAH. TCAH is not considered a pest of grapes and does not complete its reproductive cycle on grape, but rather prefers legumes. Therefore, we wanted to determine if legume cover crops, which are commonly sown in row middles in vineyards, can serve as a habitat for the TCAH or reservoir for GRBV. To do this, we sampled cover crops in the spring (before they were tilled) in Napa, California vineyards. All cover crop samples (clovers, field peas, fava beans, grasses) tested negative for GRBV and no TCAH were found in these springtime surveys in 2016, 2017, and 2018.

Legumes in row-middles are probably not contributing to GRBV inoculum or within-vineyard spread of the disease in the study vineyards in Napa County, where the ground is typically left bare from March to November. However, this may not hold true for other vineyards in Napa or other viticulture regions where under-canopy and between-row management practices differ. It is important that the role of cover crops and weeds in red blotch epidemiology be explored in other regions where GRBV is prevalent.

Management recommendations. In New York, we have no evidence that insect vectors are spreading grapevine red blotch disease from vine to vine. All evidence to date points toward introduction of GRBV solely through infected planting stock. This means that GRBV is not likely to spread to adjacent healthy vines. Nonetheless, growers should remain vigilant for GRBV and scout for virus disease symptoms frequently. GRBV-infected vines will likely not attain optimum maturity, so it makes sense to rogue and replace them as soon as it is feasible.

In western production areas, current evidence suggests that the TCAH is infrequent in vineyards and doesn't seem to reproduce on or to prefer grapevine as a host, so insecticide application or management strategies aimed at reducing TCAH populations are not recommended. Particularly in California where secondary spread of GRBV has been documented, vigilance and prompt removal of inoculum sources, i.e., production vines and wild vines (Fig. 8), adjacent to new vineyard and nursery plantings is critical. Overall roguing is recommended if disease incidence is less than 30% and entire vineyard removal is advised if disease incidence is higher than 30% (6).

As is the case for other virus diseases, prevention is the key to management of red blotch disease. Planting vines derived from virus-tested nursery stock is critical.

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