Central Coast Vineyard News

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Recognizing Grapevine Leafroll Disease

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As neighboring growers work together to reduce the spread of grapevine leafroll virus, the visual recognition of virus infection in the field and confirmation of those visual symptoms by laboratory testing are valuable tools in the fight to prevent spread of this virus disease.

Grapevine Leafroll Disease

Leafroll is one of the more important virus diseases of grapevines. It occurs in every major grape growing area of the world. There are five grapevine leafroll associated viruses (GLRaVs) that are serologically distinct. These single stranded RNA viruses are placed in a family called Closteroviridae. The majority of these are grouped in the genus *Ampelovirus* (GLRaV-1, -3, and -4), most of the viruses in this genus have been demonstrated to be vectored by mealybugs and scale insects in vineyards. GLRav-2 is in the genus *Closterovirus*, and GLRaV-7 is in the genus *Velarivirus*, there is no known vector of these two genera.

These viruses can cause similar symptoms in infected grapevines. All the GLRaVs can be transmitted by vegetative propagation and grafting; GLRaVs in *Ampelovirus* can also be transmitted by the mealybugs and soft-scale insects in vineyards. GLRaV-3 is the predominant species found in most vineyards worldwide. Recent surveys in the north coast have shown 80% of symptomatic vines sampled were infected with GLRaV-3.

To further complicate matters there are variants that have been identified for given GLRaV species. For GLRaV-3 there are several distinct variants known to exist. What needs to be better understood is the significance of these GLRaV-3 variants and their interactions with other viruses when multiple infections exist in a vine.

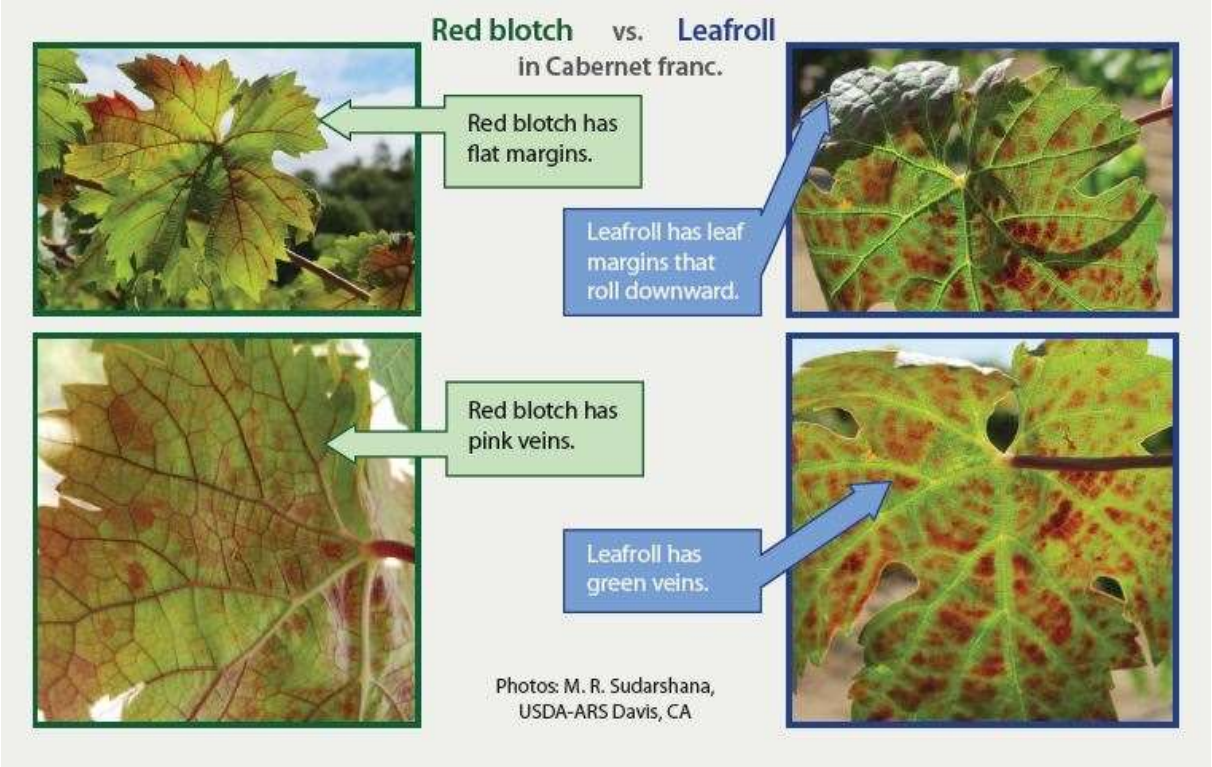
Recognizing Leafroll

Leaf symptoms become visually apparent by early summer and intensify into midsummer and fall. Physical stresses to the vine may increase symptom severity and there are similar symptoms caused by other abiotic and biotic injuries. On affected vines, the margins of the leaf blades roll downward, starting with the basal leaf on the cane. Areas between the major veins turn yellow or red, depending on whether the cultivar produces white or red fruit. In some cultivars, the area adjacent

to the major veins remains green until late fall. There can be a great deal of variation in symptoms expressed due to cultivar, rootstock, and the yearly differences in growing season weather.

Leafroll disease on Pinot noir (left) showing burgundy red between green main leaf veins accompanied by downward rolling of the leaf margins; on Chardonnay (right) leaves show a more generalized chlorosis and downward rolling of the leaf margins in late fall.

Other causes of red leaves can be mineral deficiencies (Mg top left or K top right) or pest feeding from mites (bottom left) or gopher damage to roots (bottom right).



Comparing red blotch to leafroll disease, leafroll symptoms are more uniform across the leaf blade, the veins remain green, and the there can be a downward rolling of the leaf margin. For more pictures of red blotch symptoms on different cultivars got to: GRBaV symptoms

The most important effect of leafroll disease is a reduction in the yield and quality of fruit from infected vines. Yield losses of 10 to 20% are fairly typical. Because leafroll viruses damage the phloem of infected vines, sugar accumulation is delayed, and color pigment production is reduced. Fruit from infected vines can be low in sugar, poorly colored, and late in ripening.

It is important to remember that the lack of symptoms in a grapevine does not guarantee freedom from infection by the viruses that are the causal agents of leafroll disease.

Lab Testing

Leafroll viruses may be diagnosed using ELISA and RT-PCR tests. Virus titer levels are variable not only within the year, but also within the vine. Collect petioles in late summer and fall, or shoots/canes for cambium scrapings in fall and winter. PCR and ELISA tests are not available for all GLRaVs. Check with the commercial lab you are submitting your samples to for their preferred sampling method and collection time prior to taking samples. **See the article below for an update on testing for GLRaV.**

Mealybug Vectors

The most common mealybug found in California vineyards is the grape mealybug (*Pseudococcus maritimus*). Obscure mealybug (*P. viburni*) is present in central coast vineyards but less common than the grape mealybug. The vine mealybug (*Planococcus ficus*) was introduced into California in 1994 and has now been found in most production area of the state. Less common is the long-tailed mealybug (*P. longispinus*) found primarily in the cooler areas of the central coast. The Gill's mealybug (*Ferrisia gilli*) is the fifth species found in California but is currently very limited in distribution with populations found in the Sierra foothills, in the northern coast (Lake County) and in the southern San Joaquin Valley.

All the above species are capable of being a vector for leafroll disease. Research has shown that mealybugs can become infective after one hour of feeding on a leafroll virus infected vine and can transmit the virus to a clean host after one hour of feeding. Although all female instars can transmit the virus once infected, the first instar is the most effective vector of the disease. The first instar or "crawler" moves to find a feeding spot and is considered to be the most common dispersal stage of a mealybug population. Wind, equipment, workers and infested nursery stock can also move mealybugs.

Management of Grapevine Leafroll Disease

1. Plant Material. The first management strategy should be to plant certified vines that have been grown and produced by a nursery participating in the California Grapevine Registration and Certification Program. Once virus infected a vine will remain infected, there is no cure. Commercial nurseries that produce certified grapevines and participate in the California Grapevine R&C Program obtain their clean stock from the Foundation Plant Services at the University of California, Davis. UC Davis has a foundation vineyard for grape cultivars and clones. Before being planted in the foundation vineyard, all vines are tested across biological indicators, and by ELISA and RT-PCR. The foundation vineyard is monitored by visual inspections in spring and fall, and a portion of it is retested every year by ELISA and RT-PCR for viruses known to spread naturally. This provides the highest level of confidence about the virus status of the selections.

Both the fruiting scion and the rootstock need to come from certified mother plants. A very common spread of leafroll is the use of infected bud wood from commercial vineyards. The lack of symptoms in the source vineyard cannot be relied upon as a guarantee that there is no virus; many of the major grapevine viruses show no symptoms during some or all of the season. Particularly if wood is collected during the dormant season, it is unlikely that the source vines will show distinct symptoms of virus infection. Selected grapevines should also be pre-tested for virus by a competent diagnostic laboratory if this type of material is going to be used. Even with vine testing, sourcing bud wood from established vineyards carries a risk of introducing virus into a new planting.

2. Learn to recognize leafroll symptoms. Leafroll symptoms become visually apparent by early summer and intensify into midsummer and fall as noted above. Symptoms can vary by leafroll

species, multiple virus infections, and by cultivar and rootstock combination. Symptoms are more apparent in cultivars producing red or black fruit than in white fruiting cultivars. **Remember that the lack of symptoms in a grapevine does not guarantee freedom from infection by the viruses that are the causal agents of leafroll disease.**

3. Recognize and be aware of potential leafroll vectors. As discussed above mealybugs and scale insects are known vectors of some species of GLRaVs. Monitor and be aware of which insect vectors may be in your vineyards. More information on these insects is available in Grape Pest Management UCANR publication 3343 or in the online UC IPM guideline for grapes, http://www.ipm.ucdavis.edu. Know which species of mealybugs are present in your vineyards, their population dynamics are different and will influence the timing of any needed control practices. European fruit lecanium scale (*Parthenolecanium corni*) is a common insect found in California vineyards, it and other scale insects have been shown to transmit some GLRaV species.

4. Be aware of potential spread from leafroll infected blocks. Leafroll infected blocks can be a source for vector and disease spread into adjacent clean plantings. Consider if plant removal is a viable option to reduce further spread for both the source and clean blocks. Vector control is a management decision to consider. Recent research suggests the rate of disease spread of GLRaV-3 is greater when higher mealybug population levels are present. Treatment of virus source blocks should minimize the infective vectors leaving the block; the treatment of clean blocks should be targeted to kill infective vectors quickly upon entering the block and to reduce secondary spread to adjacent vines.

5. Area-wide management. When both mealybug populations and the virus causing leafroll disease are present in an area, cooperation between neighboring vineyard owners will be necessary to improve on reducing the spread of disease from infected source blocks to non-infected vineyards.

*The following article is reprinted from a **Foundation Plant Services News** release, March 22, 2018.*

Two new assays address the challenge of reliably detecting the genetically diverse variants of Grapevine leafroll-associated virus 3

Grapevine leafroll-associated virus 3 (GLRaV-3) is the main etiological agent of grapevine leafroll disease, one of the most important virus diseases of grapevine which is distributed worldwide. The long-distance spread of GLRaV-3, caused by the movement of infected vines, can be controlled effectively if GLRaV-3 is accurately identified and virus-tested clean stock is made available to growers. In turn, accurate GLRaV-3 identification and the production of a large amount of tested planting stock require a high throughput testing method that is sensitive and specific for GLRaV-3. As the source of all California Registered or Certified grapevines, Foundation Plant Services (FPS),

has met the need for reliably detecting GLRaV-3 in large sample numbers by focusing on the development of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) assays.

RT-qPCR assays detect viruses by amplifying a small section of the virus genome to levels that can be easily detected. Amplification is achieved by identifying regions of the genome that are unique to any given virus yet are conserved among all the genetic variants of that particular virus. While identifying conserved regions across genetic variants can be difficult with any RNA virus, due to its relatively high mutation rate, GLRaV-3 has an exceptional number of highly diverse variants. Recent studies based on genome-wide phylogenetic analysis demonstrated that GLRaV-3 variants can be divided into eight distinct groups, six of which have been identified in California. This level of genetic diversity makes it almost impossible to identify a conserved region common to all isolates for design of a single qPCR-based assay. Up to now, FPS has dealt with this problem by designing variant-specific assays, six to date. However, it isn't feasible to test large numbers of vines using six different assays. In addition, the California Department of Food and Agriculture (CDFA), which works closely with FPS on the grapevine certification process, recently adopted RT-qPCR assays. CDFA tests thousands of grapevine from nursery increase blocks every year, highlighting the need for a reliable single GLRaV-3 assay.

In 2017, Dr. Maher Al Rwahnih, Diagnostic and Research Laboratory Director, at FPS obtained funds from the Pierce's Disease and Glassy-winged sharpshooter Research Board to conduct the research project, "Survey and analysis of grapevine leafroll-associated virus-3 genetic variants and application towards improved RT-qPCR assay design". As part of this study, FPS reconstructed the near complete genomes of four new GLRaV-3 variants using high-throughput sequencing (HTS) and incorporated new genetic data into a more complete characterization of genetic variation across GLRaV-3 variants. A small but highly conserved region was identified that was then used to construct a single RT-qPCR assay for detecting all GLRaV-3 variants characterized to date.

The work behind designing and validating the new assay, called FPST, is described below:

- The FPST assay was designed using publicly available GLRaV-3 sequences as well as our own divergent GLRaV-3 variants that were sequenced at FPS. Once a large number of sequences from different genetic variants had been obtained, multiple alignments were used to identify regions with low sequence diversity that were suitable for assay design. In this case, FPST targeted the 3' terminal region of the virus genome, a highly conserved region that was now supported by the large amount of new sequence data.
- The FPST assay has been empirically tested and validated using single isolate positive controls, representing all the GLRaV-3 groups. When compared with previous GLRaV-3 assays, FPST was the one RT-qPCR assay that detected ALL variants obtained to date (Table 1).
- To further test the new assay, 1,872 samples showing grapevine leafroll disease symptoms were collected from grapevine populations with a historically high incidence of GLRaV-3. These populations included the USDA National Clonal Germplasm Repository in Winters, CA, the Davis Virus Collection at UC-Davis, the FPS pipeline of foreign and domestic introductions, selected vineyards in the main grape-growing areas of California, and samples provided by international collaborators.
- Of the 1,872 samples, 1,148 (61%) samples tested positive for GLRaV-3 using the FPST RT-qPCR assay. These samples corresponded to domestic selections or international plants

originating from Israel, Croatia, Portugal, Italy, Hungary, Canada, Japan, Turkmenistan, Spain, South Korea, France or Greece. The large number of samples analyzed resulted in a very rich geographic representation of GLRaV-3 variants.

- Further verification of the FPST assay was obtained by testing the above population with a new GLRaV-3 Enzyme-linked immunosorbent assay (ELISA) kit that was developed using funds from the Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board, managed by CDFA. This new ELISA kit has detected all known GLRaV-3 variants characterized to date. The side-by-side comparison indicated that all samples testing positive by the FPST RT-qPCR assay also tested positive by the new ELISA kit. The 100% match between the results suggests that both assays have similar efficiencies.

Moving forward, we will continue to test more samples and challenge both the ELISA kit and the FPST RT-qPCR assay against possible new divergent variants of GLRaV-3. Additionally, we will characterize more GLRaV-3 variants using HTS. The availability of more complete GLRaV-3 genome sequences will aid in further characterizing the genetic diversity covered by the assay which will be updated upon finding any new divergent variants. We cannot predict if the FPST assay will detect all the GLRaV-3 variants found in the future, however, the improved assay detects all known variants to date. Finally, FPS is committed to sharing these new detection tools with stakeholders, thus benefiting growers, researchers, and diagnostic labs involved in the grapevine industry in the US and around the world.

Table 1. Test results of divergent GLRaV-3 variants from FPST and individual GLRaV-3 RT-qPCR assays. The results demonstrate the challenge of designing a RT-qPCR assay to a genetically diverse genome. Until FPST was designed, variant-specific assays led to false negative test results.

GLRaV-3 Isolate	Original Source	Location in CA	GLRaV 3 RT-qPCR Assays						
			Original assay	"e"	"f"	"NZ2"	"GH24"	"NdA"	FPST
Common	Various countries	Widespread in vineyards throughout CA	POS	NEG	NEG	NEG	NEG	NEG	POS
"e"	Napa Valley	Primarily Napa Valley	NEG	POS	NEG	NEG	NEG	NEG	POS
"f"	Napa Valley	Five vineyards in regions throughout CA	NEG	NEG	POS	NEG	NEG	NEG	POS
"NZ2"	New Zealand	Santa Barbara	NEG	NEG	NEG	POS	NEG	NEG	POS
"GH24"	South Africa	FPS virus collection	NEG	NEG	NEG	NEG	POS	NEG	POS
"NdA"	Italy	FPS virus collection	NEG	NEG	NEG	NEG	NEG	POS	POS

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