The Impact of Grapevine Red Blotch Virus

Grapevine red blotch-associated virus found in grapevine nursery stock and established vineyards

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OVER THE PAST SEVERAL years vines have turned red in the fall for no obvious reason. This phenomenon has been observed in single vines dotted throughout vineyards in apparently random fashion, in small contiguous groups of vines and in whole vineyard blocks where vines have developed symptoms resembling those associated with grapevine leafroll disease in mid- to late-October through November. Detailed studies of affected vines have failed to provide satisfactory answers as to the cause of the foliar reddening. Affected red varieties develop symptoms that closely resemble those associated with grapevine leafroll virus (see **PHOTO 1, 2**) as do white varieties where foliar symptoms include leaf curling and chlorosis.

The foliage of virus-free red varieties should not turn red in the fall but pass through a series of yellows and browns typical of the senescence of many deciduous species. Typically, investigation of the fall reddening of red varietals would involve the following methodology:

a. Evaluation of graft unions: imperfect graft unions will cause vine stress.

b. Evaluation of physical soundness of vines: above or underground trunk/root damage can induce foliar reddening—especially in the fall. Field budding tape, gophers and voles can potentially girdle the vine.

c. Evaluation of root systems: J-rooted vines may turn red under stress.

d. Evaluation of viral status of vines: presence of leafroll viruses and other pathogens.

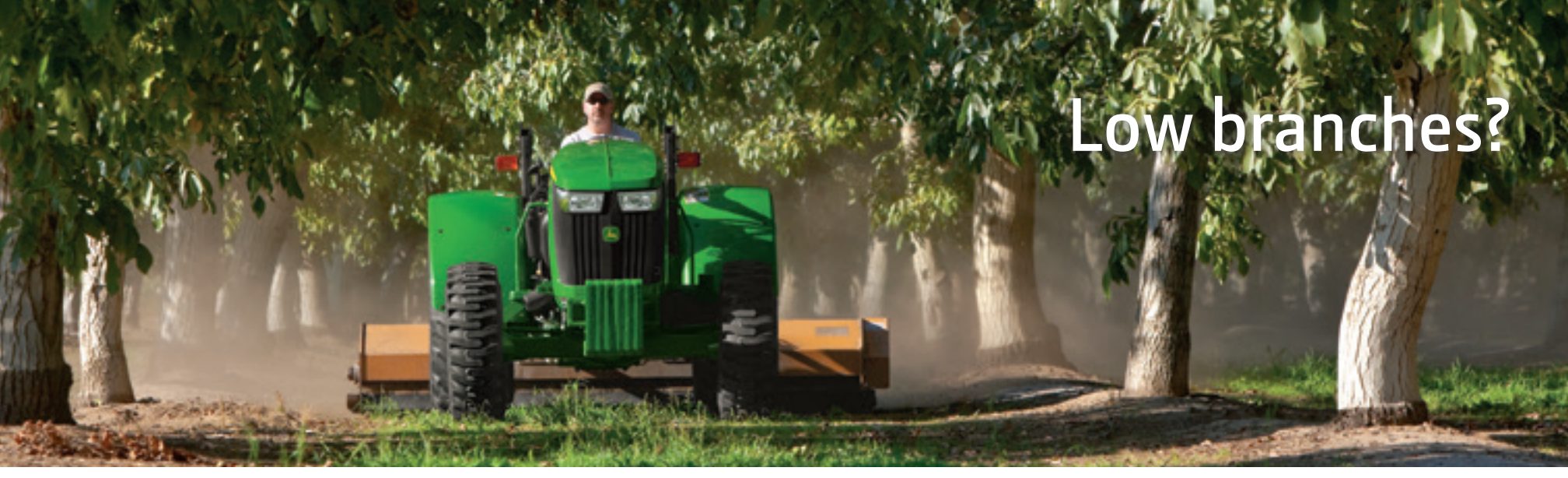
e. Evaluation of fungal pathogen status: examination of root and trunk tissues for evidence of pathogen activity. This is usually a last resort as all grapevines carry at least some load of pathogenic and saprophytic fungal species.

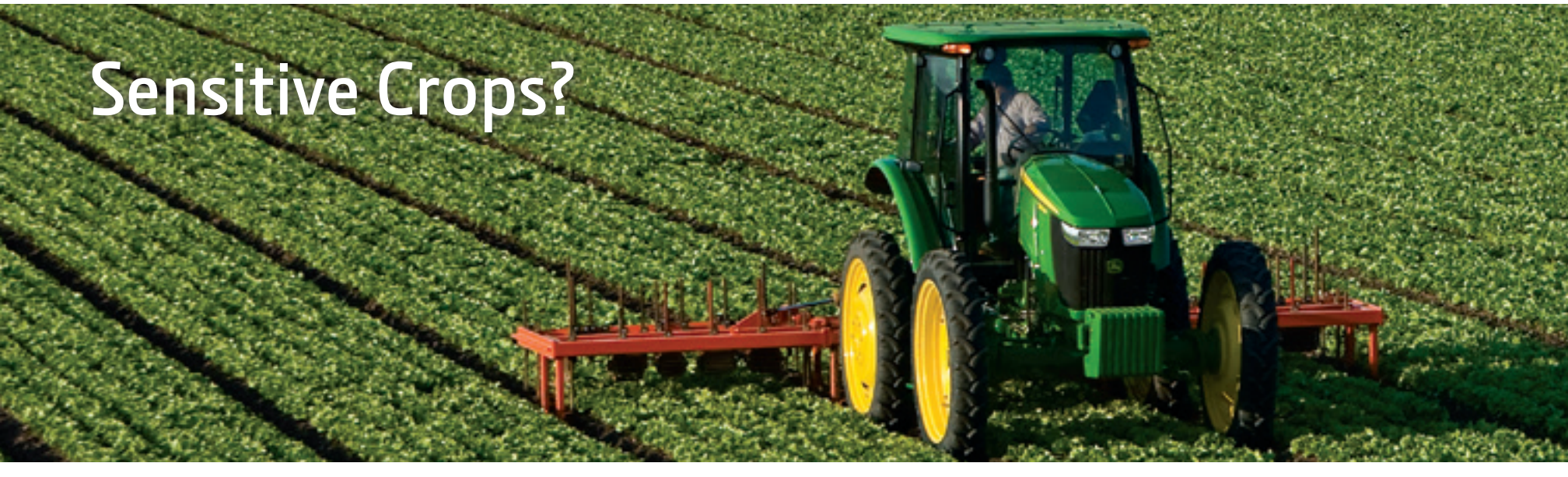
f. Evaluation of nematode status.

Given that field investigations frequently fail to determine any impact from the above factors, growers and scientists alike have been at a loss to determine the cause of this reddening foliage. The discovery of additional viruses in the past four years, specifically Rupestris Stem Pitting associated Virus (RSPaV) Syrah strain[3] and Grapevine Syrah Virus-1[1], has failed to resolve this issue with no evidence found to suggest that there is a causal relationship between the presence of these viruses and any type of vine decline or particular set of symptoms in either white or red varietals[7].

PHOTO 1. GLRaV-3 positive CS ENTAV 169 increase block vine

PHOTO 2. 2009 field grafted CS4/420A with GRBaV





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Identification of Grapevine Red Blotch-associated Virus in Grapevine Plants

For years, **Mike Anderson**, a **UC Davis** research associate, has been aware of growers that expressed frustration when vines showing leafroll disease returned negative RT-PCR results for known leafroll-associated viruses. In 2007, **Jason Benz** and Anderson began to categorize unexplained disease symptoms at the UC Davis Oakville Station in Napa Valley. One of the categories was termed Red Blotch. Leaves of vines in the Red Blotch category had irregular blotchy red leaves with red veins. Red Blotch vines were especially concerning because the fruit appeared to have lower sugar content than vines appearing healthy.

In 2008, Anderson was asked to cover viticultural responsibilities at the **Napa County Cooperative Extension** office and began receiving numerous calls from growers expressing concern about an unidentifiable "red leaf" disease. In spring 2009, Anderson asked **USDA-ARS** plant pathologists at Davis to visit vineyards displaying symptoms of the disease he was referring to as Red Blotch. Using Next Generation Sequencing technology to determine whether known or unknown virus species were involved in symptom development in Napa County Cabernet Franc and Cabernet Sauvignon vines, USDA and UC Davis scientists discovered what is now referred to as Grapevine Red Blotch-associated Virus (GRBaV)[12]. The DNA sequence of the virus was determined and primers developed that would allow rapid PCR screening of plant materials for GRBaV.

The sequence of the virus and its discovery in grapevines showing Red Blotch disease was first presented at the **17th Congress of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine** (ICVG) in October 2012 by two groups: one comprising of the USDA and UCD scientists[12] and the other by Drs. **Keith Perry** and **Mark Fuchs** of **Cornell University**[4]. The genome of the virus suggests that it may be related to geminiviruses. Unlike many of the known grapevine viruses, the geminivirus is a DNA virus potentially vectored by leafhoppers and whiteflies. We emphasize "potentially" because the RBaV vectors are not yet identified. The virus has a relatively small genome of 3,206 nucleotides in size, which is about 15 percent of the RNA genome of Grapevine leafroll-associated virus-3.

GRBaV is the second DNA virus found in grapevines; the first one was called the grapevine vein-clearing virus, and was found in the Midwest regions of United States[11]. Recently, a virus isolate, called Grapevine geminivirus, was reported by a Canadian research group[5]. Thus far, the gene sequences of three isolates reported from New York, California and Canada are believed to be almost identical. Using the sequence information in public domain, commercial diagnostic laboratories have developed a test for detecting the presence of GRBaV in grapevine plant materials. Immediately after ICVG, Agri-Analysis issued newsletters to inform its customers of this important discovery and its potential impact[9,10]. Growers responded overwhelmingly to this news. Working closely with leading researchers in Davis and Cornell, Agri-Analysis was the first laboratory to have applied these new knowledge and discoveries to develop tests for detecting the presence of GRBaV in grapevine plant materials, including rootstocks and bud woods.

Work undertaken by the USDA and UCD scientists suggested that there was a strong correlation between the presence of GRBaV, foliar symptoms (red leafing), reduced Brix and change of flavor in ripe fruit: in the vast majority of tested vines, plants with foliar symptoms were infected by the virus; plants without foliar symptoms were not infected by GRBaV.

PHOTO 3. Reddened veins on underside of CS4/420A leaf with GRBaV

PHOTO 4. CS4/420A leaf with GRBaV

Symptoms Associated with GRBaV

REDDENING OF FOLIAGE AND REDUCED BRIX AT HARVEST TIME

1. In the following discussion, it is important to note that where foliar symptoms of Red Blotch disease have been correlated with the presence of GRBaV, sampled vines have tested negative for all other economically important viruses.

2. As noted earlier, foliar reddening appears very similar to symptoms associated with leafroll disease, especially as symptoms become more pronounced in November. In early- to mid-October 2012, vines exhibiting symptoms associated with GRBaV were lightly colored pink-red in a blotchy fashion and a distinguishing feature was that unlike leafroll disease (where the veins remain green), the underside of the veins turned a light pink/red color (PHOTO 3, 4).

3. However, as the season progressed, affected vines began to look more like they were infected with GLRaV.

4. Preliminary observations made by the USDA-ARS and UCD researchers have indicated that symptomatic grapevines that tested positive for GRBaV recorded 3 to 5 Brix units lower than the asymptomatic grapevines in which the virus was not detected.

5. Data collected by a prominent grower in northern Napa Valley is presented in TABLE 1 (PHOTO 5). This grower planted 420A rootstock in spring 2009 and field grafted to Cabernet Sauvignon FPS 04 in spring 2010.

TABLE 1. Symptoms, virus presence and Brix readings in 5 vines with and 5 vines without symptoms of Red Blotch

Scion/Rootstock (5 adjacent symptomatic and none-symptomatic vines)	GRBaV	Red foliar symptoms	Average of 5 vines			Additional virus/pathogen test results (all 5 vines)							
			Brix	TA	pH	GLRaV-1	GLRaV-2	GLRaV-3	SyV-1	GVB	GFLV	Xf	RSP-Syrah
CS04/420A	**POS (5/5)**	**YES (5/5)**	21.90	7.80	3.34	neg	neg	neg	neg	neg	neg	neg	POS
CS04/420A	neg (5/5)	No (5/5)	27.80	4.91	3.68	neg	neg	neg	neg	neg	neg	neg	POS

5 vines with/without symptoms all testing negative (neg) or positive (POS) for GRBaV

Sample Date: 10/19/2012

Rootstock planted in 2009 and field grafted in 2010

Symptoms first observed in November 2010

GLRaV: Grapevine leafroll associated viruses

SyV-1: Grapevine Syrah virus-1

GVB: Grapevine Vitivirus B (causative agent of Corky Bark Disease)

GFLV: Grapevine fanleaf virus

Xf: Xylella fastidiosa (causative bacteria associated with Pierce's Disease)

RSP-Syrah: Rupestris Stem Pitting-Syrah virus

See photo

PHOTO 5. Vineyard block subject of data presented in Table 1

The Impact of Grapevine Red Blotch Virus

Symptoms were first observed in a few vines in fall 2011 but by November 2012, 80 percent of vines in this block of 12,000 exhibited Red Blotch leaf symptoms. Data presented in TABLE 1 indicate:

a. Five adjacent symptomatic GRBaV-infected vines possessed an average Brix of 21.90.

b. Five adjacent non-symptomatic GRBaV-negative vines possessed an average Brix of 27.80.

c. Titratable acidity was significantly increased in the GRBaV-positive vines while pH was moderately reduced.

d. All vines tested negative for several leafroll strains, Syrah Virus-1, Grapevine Vitivirus B, Fanleaf virus and *Xylella fastidiosa* (the bacterium associated with Pierce's disease).

e. In all, this Northern California grower tested 25 vines with symptoms in two different blocks; all were positive for GRBaV. Ten vines without symptoms all tested negative. He observed a perfect correlation between symptoms, presence of virus and delayed fruit ripening in both blocks.

The Importance of Koch's Postulates

Over 100 years ago, **Robert Koch** introduced his ideas about how to prove a causal relationship between a microorganism and a disease (SIDEBAR 1). Koch's postulates have played an important role in microbiology, yet they have major limitations. For example, viral diseases were not yet discovered when Koch formulated his postulates. In the case of grapevine viruses, most of them cannot be propagated outside of the grapevine phloem environment and are not mechanically transmissible. Even for the widely studied grapevine leafroll viruses, Koch's postulates have not yet been met as of today.

SIDEBAR 1 Koch's Postulates

The key requirements of Koch's postulates include:

1) The microorganism must be isolated from a diseased host and grown in pure culture.

2) The cultured microorganism should cause disease when introduced into a healthy host.

3) The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

More recently, modern nucleic acid-based microbial detection methods such as PCR and DNA sequencing have revealed previously uncharacterized, fastidious or uncultivated, microbial pathogens that resist the application of Koch's original postulates, but they also provide new approaches for proving disease causation. In particular, the increasing reliance on sequence-based methods for microbial identification requires a reassessment of the original postulates and the rationale that guided Koch and later revisionists. **Fredricks** and **Relman**[2] of **Stanford University** suggested a revised set of Koch's postulates for the 21st century that encompasses seven criteria. We believe the following experimental evidence provides support that GRBaV is the causal agent for the Red Blotch disease under the modern version of Koch's postulates.

MODERN KOCH'S POSTULATES #3: CORRELATION OF SEQUENCE COPY NUMBER WITH SEVERITY OF DISEASE

Samples from Cabernet Sauvignon FPS 07/VR039-16 vines showing varied severity of foliar symptoms were analyzed by conventional PCR and the intensity of the "PCR band" was recorded, what represents the relative amount of viral genetic materials in plant tissue. The samples were taken from vines with green leaves (no PCR band), speckled red leaves (weak to medium band) and completely red leaves (intense PCR band). Mysore Sudarshana[8] tested these samples by quantitative PCR in order to determine the viral DNA copy numbers in each one. Results below suggest that the virus sequence copy number strongly correlates with the severity of the disease symptoms observed in the vines (see SIDEBAR 2).

SIDEBAR 2. Relationship between GRBaV DNA copy number and severity of symptoms in CS7/VR039-16 vines (Samples collected on same date October 2012)

Sample #1 **Green foliage**
No gel band was seen by conventional PCR. No copy number identified by quantitative real-time PCR analysis (qRT-PCR).

Positive: RSP and RSP-SY

qRT-PCR copy #: 0

Sample #2 **Blotchy red foliage**
Weak-to-intermediate gel band was observed with conventional PCR.

Positive: GRBaV

qRT-PCR copy #: 200+/-

Sample #3 **Complete red foliage**
Strong gel band was observed on conventional PCR

Positive: GRBaV

qRT-PCR copy #: 80,000+/-

MODERN KOCH'S POSTULATES #1 AND #6: PATHOGEN NUCLEIC ACID SHOULD BE PRESENT IN MOST CASES OF THE DISEASE

Since October 2012, Agri-Analysis has analyzed well over 4,000 samples for GRBaV. The presence of target GRBaV nucleotide sequences has been consistently detected in diseased tissues by PCR and DNA sequencing. GRBaV has been found in samples from Napa, Sonoma, Paso Robles, San Luis Obispo, Monterey, Mendocino and Santa Barbara as well as in Virginia and Maryland. Infected grape varieties include not only reds, such as Merlot, Zinfandel, Mouvedre, Petite Sirah, Cabernet Franc, Cabernet Sauvignon, Malbec, Pinot Noir and Petit Verdot, but also white varieties, including Chardonnay, Sauvignon Blanc and Riesling. Rootstocks are also contaminated (TABLE 3). The virus appears to be widely spread in California and other wine growing regions in the U.S.

TABLE 2.
Incidence of GRBaV and GLRaV in 2013 season nursery production lots propagated in 2012

Rootstock	Scion	Product	Virus detected	
			GRBaV	GLRaV-3
420A A		RTG	POS	nt
Riparia Gloire A		RTG	neg	nt
1616C A		RTG	neg	nt
3309C A blk1		RTG	neg	nt
3309C A blk2		RTG	neg	nt
3309C F		RTG	neg	neg
1616C A	MB09 E	DBG	POS	nt
1616C F	PV400 F	DBG	neg	POS
1616C F	CH17 F	DBG	neg	neg
3309C F	PN91 F	DBG	neg	neg
420A A	ZIN XX	DBG	POS	nt
420A C	ZIN XX	DBG	neg	nt
420A C	DURIF D	DBG	neg	nt
420A C	MB09 E	DBG	POS	nt
420A C	CS33 A	DBG	POS	nt
420A F	ME18 F	DBG	neg	neg
420A F	PN90 F	DBG	neg	POS
420A F	PN943 F	DBG	neg	POS
Riparia Gloire A	PN667 A	DBG	neg	nt
Riparia Gloire F	SEM02	DBG	neg	POS
VR 039-16 A	SB01 A	DBG	POS	nt
VR 039-16 A	CS07 B	DBG	POS	nt
VR 039-16 C	CF214	DBG	POS	neg

Product: RTG: dormant rootings. DBG: dormant bench grafts
POS: positive for virus. neg: negative for virus A-F: Individual nurseries
All materials CDFA certified except XX private selections
XX: private selections nt: not tested

The GRBaV sequence is very reproducible in different laboratories. To confirm observations, Agri-Analysis sent eight samples (seven GRBaV positive and one negative) from a prominent Napa vineyard to the laboratory of Drs. Keith Perry and Marc Fuchs at Cornell University where they were able to reproduce detection results exactly, using primers from Cornell (see **SIDEBAR 3**).

SIDEBAR 3.
Reproducibility of GRBaV results using primers from different sources

Samples from a Napa Vineyard	PCR using Cornell Primers	PCR using UCD Primers	PCR Agri-Anal. Primers	DNA Sequencing Results
Chardonnay	POS	POS	POS	All amplified sequences were found to be identical to the published sequence of GRBaV isolate JRT 456 found in New York.
Pinot noir	POS	POS	POS	
Cabernet Sauvignon	POS	POS	POS	
Malbec	POS	POS	POS	
Petit Verdot	POS	POS	POS	
Cabernet franc	POS	POS	POS	
White Riesling	POS	POS	POS	
Merlot	NEG	NEG	NEG	

GRBaV in Nursery Production Lots and CDFA-certified Increase Blocks

With the availability of an efficient and accurate diagnostic procedure for the detection of GRBaV, and with the late fall 2012 observation of symptoms in production lots and increase blocks that might previously have been considered to be nutritional or leafroll associated, several 2013 dormant product nursery production lots and several increase block sources for green 2013 and dormant 2014 pending orders were tested for GRBaV in October/November 2012. New GLRaV-3 infections were detected in increase blocks at some nurseries in fall 2012 and so production lots from these nurseries were tested for GLRaV-3 and GRBaV.

DORMANT VINE PRODUCTION LOTS DESTINED FOR PLANTING IN SPRING 2013

Dormant vines destined for planting in spring 2013 were grafted in spring 2012, callused for approximately four weeks in warm, humid conditions (to encourage vascular connection at the graft union and root initiation) and then planted in nursery row conditions at approximately 17,000 vines per acre (see **TABLE 2**). 2013 dormant product production lots were inspected at the time of grafting (to ensure correct rootstock and scion materials were used) and then again in late July and early October 2012. It is rare to observe disease or stress symptoms in July (unless graft unions are significantly imperfect), but not unusual to see symptoms of disease in known diseased stock in early October (for example, Cabernet Sauvignon ENTAV 337 is known to be contaminated with GLRaV-2 and when this is grown in the production field symptoms of leafroll disease can be observed in early fall).

Analysis of comparative data from production lots under cultivation in October and November 2012 revealed the following:

1. Northern California produced vines were far more likely to be contaminated with GRBaV than southern California vines (**PHOTO 6**).

a. Fifty percent (seven of 14) of northern California production lots were contaminated with GRBaV.

b. Only one of nine southern California production lots was contaminated with GRBaV.

PHOTO 6.
Certified Malbec 09/420A production vines GRBaV positive (Table 2)

TABLE 3.
Incidence of GRBaV and GLRaV in CDFA certified nursery Increase Blocks

Clone/Rootstock from nurseries A-G	Production vines/IB	Virus detected	
		GRBaV	GLRaV-3
420A A	Production	POS	nt
420A C	IB	neg	neg
1616C A	Production	neg	nt
1616C C	IB	neg	neg
3309C A	IB	neg	nt
3309C F	Production	neg	neg
5C A	IB	POS	nt
Riparia Gloire A	Production	neg	nt
VR 039-16 A	IB	POS	nt
VR 039-16 C	IB	neg	neg
CS ENTAV 15 F	IB	POS	neg
CS ENTAV 15 G	IB	POS	nt
CS ENTAV 169 F	IB	neg	POS
CS ENTAV 169 G	IB	POS	neg
CS ENTAV 338 G	IB	POS	neg
CS ENTAV 412 G	IB	POS	neg
CS31 F	IB	neg	POS
CS33 A	IB	POS	nt
MB06 A	IB	neg	nt
ME ENTAV 181 G	IB	POS	neg
PV02 A	IB	POS	nt

IB: increase block Production: vines tested in production
POS: positive for virus. neg: negative for virus
nt: not tested A-G: Individual nurseries All materials CDFA certified

2. Red foliage was minimally apparent in production lots when examined in late September 2012. It was clear, however, that symptoms became progressively more severe when blocks were re-examined in mid-October and early November 2012.

3. Rootstock and grafted lots were both contaminated with GRBaV. Comparison of data from grafted and non-grafted vines of identical and different origins indicated that contamination was introduced from the rootstock in some cases and scion in others.

4. Forty-four percent (four of nine) of sampled production lots tested positive for GLRaV-3. These vines were derived from rootstock and scion increase blocks that had previously tested negative for GLRaV-3 (and all other economically important viruses) on several occasions and had exhibited no symptoms of virus contamination when blocks were examined in the fall (PHOTO 7).

PHOTO 7.
Certified PN90/420A production vines GLRaV-3 positive (Table 2)

CONTAMINATION OF INCREASE BLOCKS WITH GRBaV AND GLRaV-3

Best practice calls for regular virus testing of increase blocks and late season examination for symptoms associated with stress and disease. This process is usually undertaken in October and November. The following observations were noted when increase blocks were examined in fall 2012. (see TABLE 3).

1. Both rootstock and scion increase blocks from northern California nurseries were far more likely to be contaminated with GRBaV than those from southern California nurseries.

2. GRBaV was found in a wide range of scion selections (PHOTO 8, 9, PHOTO 10).

3. GRBaV was only detected in one southern increase block: Cabernet Sauvignon ENTAV 15 (PHOTO 11).

4. GLRaV-3 was found in two increase blocks (Cabernet Sauvignon ENTAV 169 and Cabernet Sauvignon FPS 31) in vines that showed symptoms of leafroll (PHOTO 1). On previous occasions, these blocks had both tested negative for GLRaV-3 and showed no symptoms of leafroll.

PHOTO 8.

CS33 increase block vine negative for GRBaV adjacent to vine in photo 9 (CS33 increase block vine positive for GRBaV)

PHOTO 9.

CS33 increase block vine positive for GRBaV adjacent to vine in photo 8 (CS33 increase block vine negative for GRBaV)

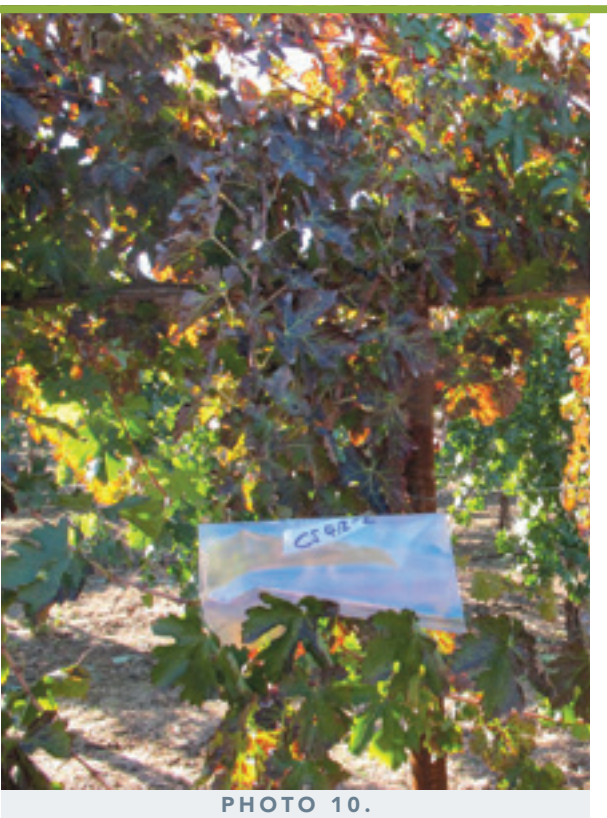

PHOTO 10.

CS412 increase block vine positive for GRBaV (Table 3)

Possible Routes of Infection of Vines by GRBaV

Clearly, specific rootstock and scion CDFA-certified increase blocks are infected with GRBaV. Observations from evaluation of nursery increase blocks, production lots and established vineyards suggest that the following routes might be involved in the spread of the virus:

1. The proposed biology of the thought-to-be geminivirus-related GRBaV suggests that the virus might be vectored by leafhoppers and aphids.
2. Observed vine-to-vine, in-row spread of the disease in increase blocks would support infection by pruning. However, observations indicate that vines are also contaminated in an apparently random manner—with single vine contaminations occurring away from existing infected vines.
3. Development of disease symptoms in previously asymptomatic blocks suggests that the virus may be transmitted on farming equipment or by other human activity.
4. The spread of symptoms in blocks seems to be quite rapid. Blocks that were previously absent all symptoms of disease in 2010 possessed a few symptomatic vines in 2011, but this number more than tripled by 2012.

Industry Reaction to the Identification of GRBaV

Although it is not scientifically proven that GRBaV is the causative agent of Red Blotch disease of grapevines, the discovery of this new virus with seemingly very strong correlation between its presence, foliar reddening and reduced sugars is both enlightening and frustrating. If GRBaV is proven to be the cause of Red Blotch disease, this will provide closure to the concerns of many growers who have experienced this phenomenon but had no real leads as to its cause. In the short term, this will prove very challenging to nurseries that are undoubtedly in the process of determining how to handle

tens of thousands of vines infected with this virus. For the growers, however, discovery of GRBaV provides the opportunity to evaluate difficult blocks and to step back and determine whether they should plant material infected with the virus.

The overwhelming reaction of growers informed about this emerging disease is that they would prefer to hold off on planting or select different, perhaps less exotic, but clean materials. As noted earlier, GRBaV-free rootstock and scion materials can be found. Growers in the know are scrambling to test rootstock, scion blocks and grafted vines and are looking for alternatives as necessary. In many cases growers do not have the luxury of deferring planting and so scarce planting materials are under even greater demand during this current planting cycle boom.

Given the evidence available to date, it seems likely that GRBaV has affected vines for many years. Symptom development is obvious in red varieties but more difficult to spot in white-fruited clones. Symptoms observed in Riesling, Chardonnay, Semillon and Sauvignon Blanc closely resemble those associated with leafroll disease. Noting that at least one important nursery source of VR 039-16 was infected with GRBaV may be relevant to the decline of some recently planted Chardonnay vineyards grafted to this rootstock. Vines failed to establish properly, looking sickly with curled and chlorotic foliage.

Conclusions

Although the discovery of this putative disease may be a relief for many, it is also a nightmare for growers and especially nurseries who are faced with potential rejection of vines contaminated with a virus that is not recognized by the CDFA nursery certification program.

It is imperative that funding be generated to support the research work of USDA, ARS and UC Davis scientists. Characterization of the virus and potential variants, establishment of Koch's Postulates and study of the biology and transmission of the virus are essential if we are to understand how to control and ultimately overcome this seemingly significant challenge.

The upcoming availability of new Protocol 2010 rootstock and scion materials might provide the best opportunity to source clean vines in the near future (*http://ngr.ucdavis.edu/russellranch.cfm?setdisclaimer=yes*). These plants are the cleanest grapevine stock to be released from FPS and UC Davis, and there are great expectations for the quality of these materials designed to replace often 10- to 20-year-old increase blocks known to contain a wide range of economically important viruses, crown gall and fungal pathogens[6].

"The UC Davis Russell Ranch Foundation Vineyard contains grapevine selections from the Classic Foundation Vineyard that were qualified for planting under the new 'Protocol 2010' standard, as well as new varieties and clones processed and tested through FPS. These plants came out of meristem tissue culture and were extensively tested free from all known

PHOTO 11. CS ENTAV 15 increase block vine positive for GRBaV

harmful viruses," said **Dr. Deborah Golino**, director of FPS. Golino noted, however, "since GRBaV was not discovered and reported until October of 2012, Protocol 2010 was unable to include GRBaV for testing. Efforts are under way to test and screen materials from Russell ranch for GRBaV. More testing results are expected from FPS in the next the few months." Anecdotally, Agri-Analysis recently tested three Protocol 2010 derived samples from Russell Ranch submitted by a nursery. They were negative for GRBaV.

Proper handling of these new materials or indeed GRBaV negative stock must be carefully considered. It is understood that nurseries should place new Protocol 2010 increase blocks in isolated locations to prevent infection of the blocks from external influences. It is likely that nurseries will place a premium on such materials and that the cost of vines will increase. However, if Protocol 2010 cuttings are generated in isolated vineyards, shouldn't they be handled, propagated and finished in facilities and growing fields dedicated specifically to these plant materials? Similarly, if a grower goes to the trouble to select GRBaV-free materials, it would be far preferable for these materials to be handled, propagated and finished in different facilities and growing fields than the GRBaV-infected stock.

Ultimately, it is timely to consider the very nature of grapevine propagation and improvement in California and beyond our borders. Existing propagation methods are quite backward in comparison with the techniques used in other crop species. For example, all potato "seed" used in North America is annually derived from tissue cultured, virus-tested stock. Virus pathogens are so important in this industry that there are no accepted alternatives to sourcing potato seed from virus-tested tissue culture clones every year.

Perhaps it is time to reconsider the pioneering experiment of **Agritope, Inc.**, an Oregon-based biotechnology company challenged with producing the cleanest, most physically perfect grafted grapevine stock in the early 1990s. All materials were produced from tissue culture and grown and propagated in clean greenhouse environments. Increase block vines were also maintained in clean greenhouses and finished product only left the facilities when ready for delivery. Growers complained that they couldn't find the graft unions. WBM

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