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RESEARCH ARTICLE

Virus surveys of commercial vineyards show value of planting certified vines

In the North Coast wine-growing region, mixed infections were predominant in older vineyards, while recently planted certified vines did not have mixed infections.

by Kari L. Arnold, Neil McRoberts, Monica L. Cooper, Rhonda Smith and Deborah A. Golino

Abstract

Viruses are of great concern in vineyards. They cost the California wine grape industry as much as $91,661 per acre over the life of a vineyard, according to a 2015 economic study of the North Coast wine-growing region. As a first step toward managing viruses, growers are encouraged to plant certified material regulated by the California Grapevine Registration and Certification program. There are risks in sourcing plant material from stocks that are not subject to the same level of regulation. We surveyed vineyards of varying ages for eight common viruses to demonstrate the value of selecting certified material for new plantings.

Grapevines have the highest number of viruses and virus-like diseases ever discovered in a single crop (Martelli 2014), and they may cost California wine grape growers as much as $91,661 per acre over the life of a vineyard (Ricketts et al. 2015) by affecting yield, color, sugar and other qualitative parameters (Goheen and Cook 1959). Some viruses are spread by insects and nematodes (Golino et al. 2002; Raski and Hewitt 1960), but human activities such as the propagation of infected material also distribute viruses into vineyards. Viruses are graft transmissible, meaning they can move from scion to rootstock, or from rootstock to scion during topworking (Alley and Golino 2000; Olmo 1951).

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Author Kari Arnold examines a virus-positive vine at a UC Davis research project. Viruses cost the California wine grape industry as much as $91,661 per acre over the life of a vineyard. Planting certified material regulated by the California Grapevine Registration and Certification program is a first step toward managing viruses.

Marita Madeloni, UC Regents

CGR&C program

The first step in virus management is sourcing virus-screened material. Under the auspices of the California Grapevine Registration and Certification (CGR&C) program (www.cdfa.ca.gov/plant/pe/nsc/nursery/grapevine.html), virus-screened material is provided through a systematic supply chain regulated by the California Department of Food and Agriculture (CDFA): CGR&C-certified vines are derived from registered nursery increase blocks where samples are collected and screened for viruses on a rotational basis and maintained in accordance with CDFA guidelines; the vines in registered nursery increase blocks are derived from foundation material at the Foundation Plant Services (FPS) department at UC Davis, where every vine is screened on a rotational basis and maintained in accordance with CDFA guidelines (Golino et al. 2017). Since its establishment in the 1950s, the CGR&C program has grown to provide an economic benefit of up to $50 million per year for the North Coast region alone (Fuller et al. 2015).

Virus screened, however, does not mean virus free; unknown viruses may not be detected and some viruses may not have a negative impact on the crop (Al Rwahnih, Rowhani et al. 2015; Al Rwahnih et al. 2013; Al Rwahnih et al. 2016).

Screening activities occur at different points in the supply chain. The initial screening of a grapevine selection is a series of biological assays designed to detect disease symptoms related to certain detrimental virus diseases (Rowhani et al. 2005). These assays require the grafting of the selection onto healthy indicator vines. If certain viruses are present in the selection, the indicator vines will indicate virus infection by developing disease symptoms (Rowhani et al. 2005). For example, red, rolling leaves in the Cabernet Franc indicator assay is indicative of grapevine leafroll disease.

Material that passes the initial screening is tested by DNA analysis to ensure it is true to type, then advanced to foundation status and planted into the foundation vineyard at FPS. That vineyard is subject to spring and fall inspections, and every vine is tested on a rotational basis using PCR (polymerase chain reaction) assays and ELISA (enzyme linked immunosorbent assay) because viruses can enter the vineyard by way of insects and other vectors.

Registered increase block material at commercial nurseries originates from foundation material, and therefore it does not need to be subjected to biological assays. However, virus-specific assays such as PCR and ELISA are used to repeatedly sample the blocks, and visual inspections are made to monitor the virus status of the plant material. PCR and ELISA are reliable assays, but their specificity is a drawback: They give positive results only for known viruses and virus strains, not for unknown viruses. When grapevine red blotch virus (GRBV) and grapevine Pinot gris virus (GPGV) were recently discovered, they had to be sequenced before researchers could construct new diagnostic assays to detect them (Al Rwahnih et al. 2013; Al Rwahnih et al. 2016).

GRBV was discovered in 2013 (Al Rwahnih et al. 2013), but the testing of a dried leaf sample collected from Sonoma County and stored in the UC Davis herbarium for 70 years dates the presence of GRBV in California as far back as 1940 (Al Rwahnih, Rowhani et al. 2015), when that leaf sample was first collected by Dr. Harold Olmo, a UC Davis professor of viticulture. GRBV shares common symptoms with grapevine leafroll disease, which is caused by multiple grapevine leafroll associated viruses (GLRaV-1, -2, -3, -4 and -7). These virus diseases were likely detected as a single disease by the biological assay due to similar symptomology. Vine selections showing leafroll-like symptoms, whether they were infected with GRBV or GLRaVs, would not have advanced into the foundation vineyard at FPS. Yet, if GRBV were somehow introduced to a registered increase block, the virus could go undetected because a more virus-specific screening assay like ELISA or PCR for GLRaV-3 would not detect it.

Grower concern, as well as recent work involving economic impacts (Ricketts et al. 2017), led to the addition of GRBV to the regulations of the CGR&C

Grapevine viruses

GRBV: grapevine red blotch virus
GPGV: grapevine Pinot Gris virus
GLRaV-1: grapevine leafroll associated virus-1
GLRaV-2: grapevine leafroll associated virus-2
GLRaV-3: grapevine leafroll associated virus-3
GVA: grapevine virus A
GVB: grapevine virus B
GFkV: grapevine fleck virus
GFLV: grapevine fanleaf virus

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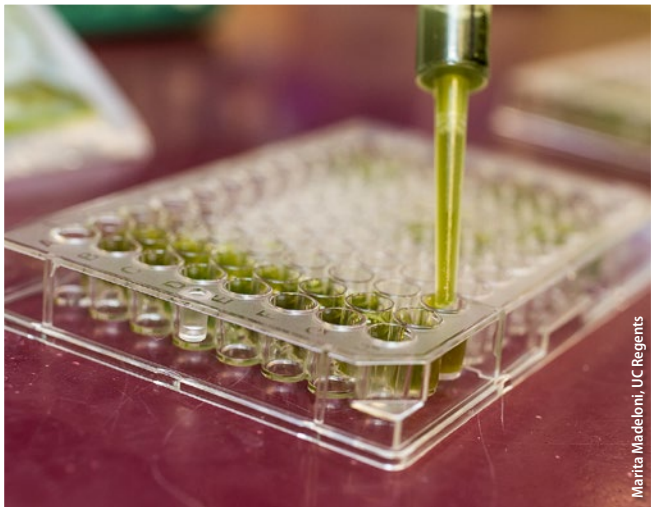
Right, the initial screening of a grapevine selection requires the grafting of the selection onto healthy indicator vines. If certain viruses are present in the selection, the indicator vines will develop disease symptoms. Material that passes initial screenings is tested by virus-specific assays such as PCR and ELISA (*below*, ELISA plate with sample).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program and the addition of PCR detection techniques because GRBV is not detectable by ELISA methods. Using PCR-based assays increases efficiency by screening a sample for multiple viruses at once and enhances reliability by screening registered increase blocks for viruses not previously targeted. The updated sampling protocol also provides analytics to measure success and facilitate future improvements (Arnold et al. 2017; McRoberts et al. 2003). With the support of industry stakeholders, FPS has also invested in genetic sequencing technology to improve unknown virus detection in plant material (Al Rwahnih, Daubert et al. 2015).

There are great risks in selecting material for new vineyard plantings from sources that are not subject to the same level of regulation as the CGR&C program. Multiple viruses spread naturally in the vineyard by way of insects and nematodes, and the longer a vine remains exposed to nearby infected vines and their virus vectors, the more likely that vine is to be infected (Arnold et al. 2017). For example, mealybugs and some scale insects spread GLRaV-3, and dagger nematodes (*Xiphinema index*) spread grapevine fanleaf virus (GFLV). To demonstrate the risks of not using certified planting material, we surveyed commercial wine grape

Zinfandel grafted to St. George rootstock appears healthy, although it is infected with GLRaV-2 and grapevine virus A and B.

Chardonnay infected with both GLRaV-3 and GRBV.

Red variety infected with GLRaV-2 andGLRaV-3 (*left*), and grapevine viruses A and B (leaf closeup, *right*).

vineyards of varying age and origin and screened for viruses regulated by the CGR&C program.

Survey of 24 vineyards

We selected vineyards for the survey based on the history of grapevine material in the North Coast region of California and defined them by planting year range: 1880–1980, 1981–1995, 1996–2010 and 2011–2014 (table 1). We surveyed 24 vineyards in the Carneros, Oakville, Rutherford, Calistoga and St. Helena viticulture areas in the Napa Valley and the Healdsburg region of Sonoma County to account for multiple regions.

We collected five to 15 vine samples, including both white- and red-fruited varieties, from 27 to 29 randomly selected blocks in each planting year range. Sample size was adjusted to account for variability in block size, which ranged from 2 to 20 acres. Vines expressing symptoms and also vines not expressing symptoms were sampled. A total of 980 samples were collected from 112 blocks. Survey collection began in August 2014 and continued for 3 months. We sampled in a W pattern in the field to account for spatial patchiness in the incidence of the viruses (Hughes and Madden 1992, 1993; Madden et al. 2007).

We tested each sample individually using molecular assays (quantitative real-time polymerase chain reaction, or qRT-PCR) to acquire accurate diagnostic information. We tested for GLRaV-1, -2 and -3, GRBV, grapevine virus A and B (GVA, GVB), grapevine fleck virus (GFkV) and GFLV. Samples consisted of either four petioles or four canes (equivalent when using qRT-PCR for viruses in this survey) from each individually tested vine. Primers used were courtesy of FPS (Klaassen et al. 2011; Weber et al. 2002).

The survey data were subjected to three analyses to compare samples originating from older vineyards to those originating from vineyards planted between 2011 and 2014 with CGR&C program material. First, we analyzed the percentage of positive vines for each virus tested in each planting year range. Second, we performed a distributional analysis (Madden et al. 2007). Third, we analyzed the presence of mixed infections in the surveyed vines because many viruses regulated by the CGR&C program may exist as mixed infections in a single vine. Some viruses are synergistic (interacting with one another and producing dramatic increases in symptoms), potentially leading to greater economic impact (Syller 2012).

Value of certified stock confirmed

At the block level, the percentage of positive vines for each virus tested ranged from 0% to 100%; each block contained only a subset of the eight viruses. On average, more viruses were present in old material than in recently planted certified material (fig. 1).

The distributional analysis (fig. 2) shows the frequency of different levels of infection. For example, in the 1880–1980 planting year range, seven blocks had 80% infection by GLRaV-2. In that year range, there was a relatively even number of blocks infected at the different percentage levels for the eight viruses. This is considered a uniform or even distribution.

Infection in the later planting year ranges was not evenly distributed at different levels; most blocks had low incidence, and in the planting year range 2011–2014 the incidence was mostly zero. The incidences in certified material planted in year range 2011–2014 of GFLV, GLRaV-3, GVA and GRBV (fig. 2) were likely related to spread by insects and nematodes (Bahder et al. 2016; Golino et al. 2002; Raski and Hewitt 1960; Tsai et al. 2008) and the recent discovery of GRBV (Al Rwahnih et al. 2013).

Of the vines tested from vineyards planted between 1880 and 1980, 85% contained mixed infections. None of the vines in the 2011–2014 planting year range

TABLE 1. Planting years and history of surveyed vineyard blocks

Planting year range	Surveyed vineyard blocks
1880–1980	Many blocks replanted on AXR-1 and St. George in response to an epidemic of grape phylloxera (*Daktulosphaira vitifoliae*), an aphidlike root pest (Wolpert et al. 1994).
1981–1995	Many blocks replanted due to the failure of AXR-1 to phylloxera biotype B (Wolpert et al. 1994).
1996–2010	AXR-1 failure–related replacement vines planted on rootstocks of American species parentage after viruses in infected scion mother material expressed symptoms previously masked by AXR-1 and St. George.
2011–2014	2008–2009 recession resulted in many blocks in fallow, which have since been planted/replanted with certified material.

The benefit of using certified material sourced from virus-tested, monitored vines is seen in the absence of mixed infections.

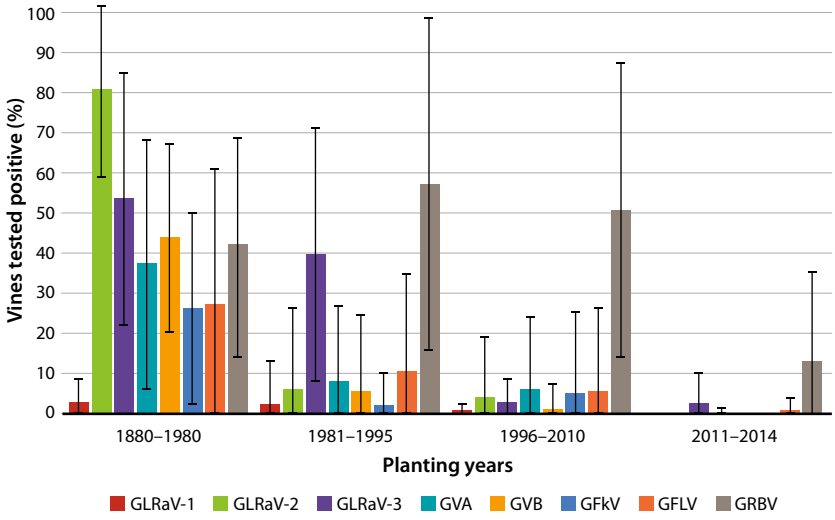

FIG. 1. Average percentage of positive vines for each virus tested in each planting year range. Standard error bars are in black.

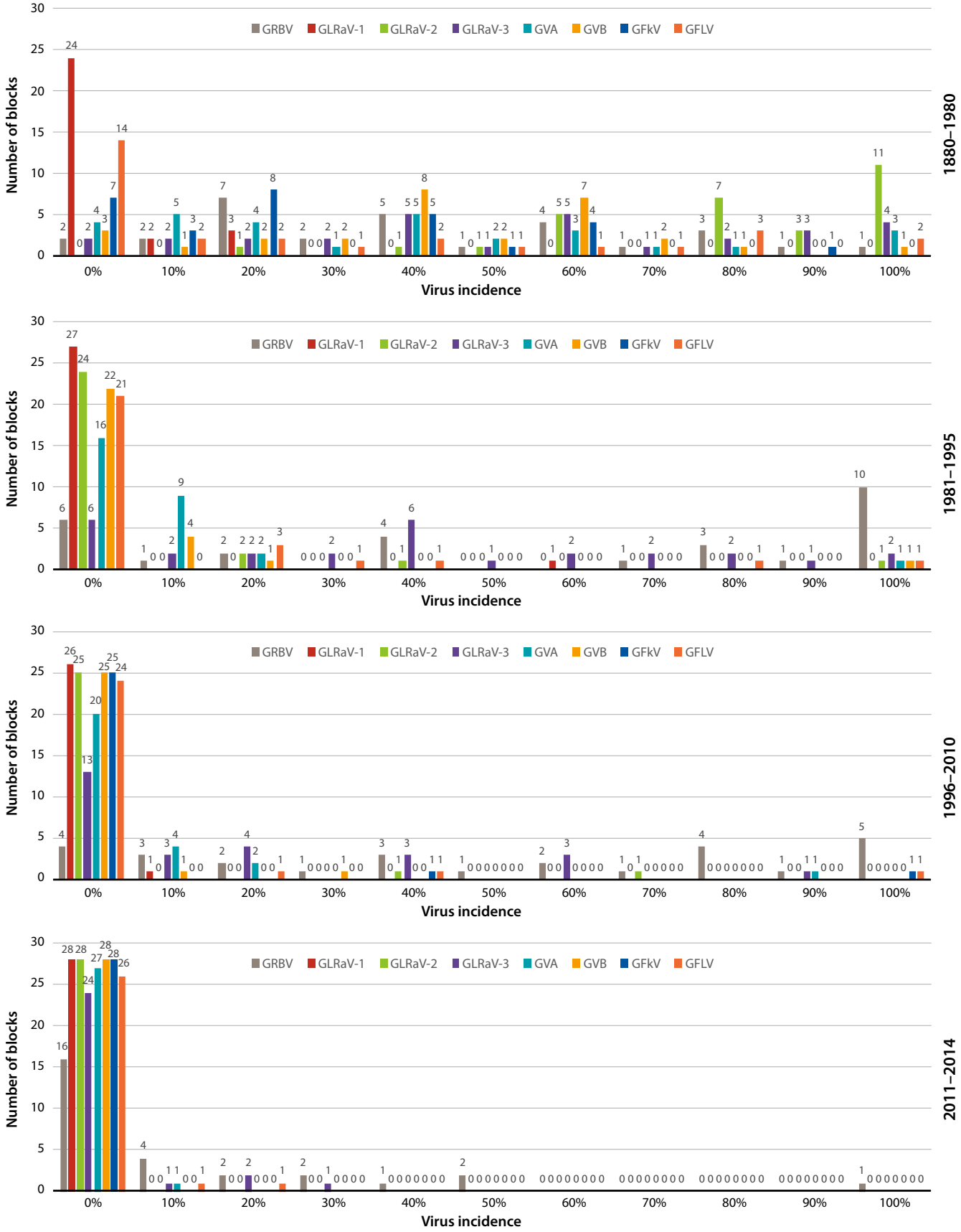

FIG. 2. Distributional analysis showed an even distribution of viruses in material planted between 1880 and 1980, while certified material planted between 2011 and 2014 showed predominantly zero infection. The few viruses present in certified material were viruses with known vectors and GRBV, which was unregulated until 2013.

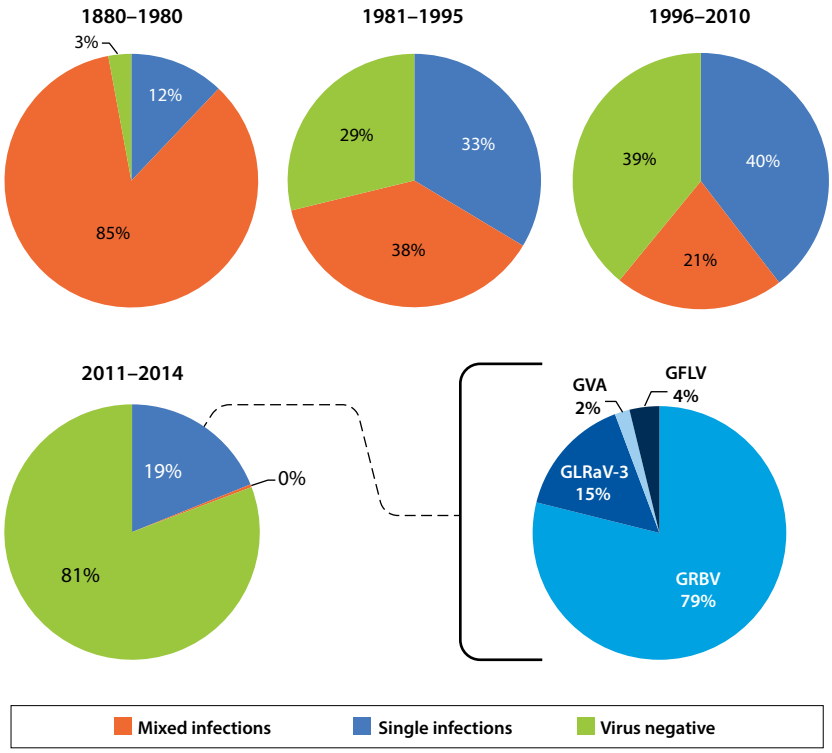

FIG. 3. Mixed infections were predominant in older vineyards. Recently planted certified vines did not have mixed infections. Only 19% of recently planted certified material had single infections and of those 79% were GRBV, a recently discovered virus, 15% were GLRaV-3, an insect transmitted virus and 4% were GFLV, a nematode-transmitted virus.

contained mixed infections (fig. 3). More than 80% of the certified vines tested negative for all eight viruses, and the remaining approximately 20% of vines contained single virus infections. Of those single infections in new material, nearly 80% tested positive for GRBV, which was not regulated by the CGR&C program until 2013, and 15% tested positive for GLRaV-3, which is spread by mealybugs and scale insects.

The results of this survey suggest that there are risks associated with the use of nonregulated vineyard material for establishment of new vineyards. Without regulated sampling, screening and monitoring provided by the CGR&C program as well as the research involved in discovering viruses, future planting material would decline as viral infections spread. CA

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