# Grapevine Leafroll: A Complex Viral Disease Affecting a High-Value Fruit Crop

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Grapevine (*Vitis* spp.) is one of the most widely grown fruit crops in the world. It is a deciduous woody perennial vine for which the cultivation of domesticated species began approximately 6,000 to 8,000 years ago in the Near East (119,152). Today, grapes are primarily produced from cultivars of *V. vinifera*, the Eurasian grapevine; *V. labrusca*, the Northeastern American grapevine; *Muscadinia rotundifolia* (syn. *Vitis rotundifolia*), the Southeastern United States grapevine; *V. amurensis*, the most important Asian species; and several interspecific hybrids. In most grapevine-growing areas of the world, the majority of cultivars are planted as grafted vines—consisting of a scion cultivar grafted onto a rootstock genotype—to improve survival and production with regard to increased vigor, to protect from soil-inhabiting pests such as phylloxera (*Daktulosphaira vitifoliae*) and nematodes, and to promote early ripening. In regions where phylloxera is absent, grapevines can be grown as own-rooted plants.

Grapes are used for the production of fresh fruits, wines, juices, raisins, and in several byproducts such as jellies, vinegars, and seed oils. In 2010, 7.19 million hectares were dedicated worldwide to the production of about 68 million metric tons (53). The four top grape producing countries in 2010 were the People's Republic of China (8.65 million metric tons), Italy (7.7 million metric tons), the United States (6.2 million metric tons), and Spain (6.1 million metric tons) (53). In the United States, the grape, wine, and juice industries are important and fast-growing segments of the economy with commercial operations present in nearly every state in the country. The total crop value was $3.47 billion in 2010, with California accounting for $3.0 billion (87%) followed by Washington ($214 million or approximately 6%) and New York ($68.4 million or approximately 2%) (124). Approximately 58% of the grape production in the United States is used for wines, 23% for raisins, 13.5% for fresh fruits, 5.2% for juice, and 0.3% for canned products (124). A recent impact study revealed that the grape and wine industry has become an economic catalyst contributing more than $162 billion annually to the American economy (117).

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Grapevines are broadly classified into red- and white-berried cultivars based on their fruit skin color, although yellow, pink, crimson, dark blue, and black-berried cultivars also exist. Red-berried cultivars have anthocyanin pigments in berry skin, whereas white-fruited cultivars lack this pigment since regulatory genes of the anthocyanin biosynthetic pathway are nonfunctional in these cultivars (162).

Grapevines can be subject to attacks by many different pests and pathogens, including graft-transmissible agents such as viruses, viroids, and phytoplasmas (109). Currently, more than 60 different viruses belonging to nearly 30 different genera have been documented in grapevines (108,126). On a worldwide basis, more viruses have been identified in grapevines than in any other woody perennial crop. All currently documented grapevine viruses are classified into four major groups based on the disease they cause or are associated with: viruses involved in the degeneration/decline disease complex, viruses associated with the leafroll disease complex, viruses associated with the rugose wood complex, and viruses associated with the fleck disease complex. Other grapevine viruses are apparently not associated with a disease and are suspected to have a minor, if any, impact (107,109,126). The majority of grapevine viruses have a RNA genome. Recently, two viruses with a DNA genome have been reported: a badnavirus associated with vein-clearing and vine decline syndrome (169), and a geminivirus associated with red blotch symptoms (8,92,133), with the latter emerging as an economically important constraint to grape production in the United States.

Among the virus and virus-like diseases, grapevine leafroll disease (GLD) is by far the most widespread and economically damaging viral disease of grapevines in many regions around the world (57,107,121,125). A recent economic study indicated that GLD, depending on the level of disease incidence, yield reduction, and impact on fruit quality, can cause an estimated loss of approximately $25,000 to $40,000 per hectare in the absence of any control measure (10). GLD was described in Europe as early as the mid-nineteenth century (107) and then in many other countries throughout the world, including in the United States (63,81,82). The graft-transmissibility of GLD was demonstrated in 1935 (144), and the presence of flexuous, filamentous virus particles was reported in a leafroll-affected grapevine in 1979 (123). A decade later, the ability of mealybugs to transmit one of the viruses associated with GLD was demonstrated (137,151). As improved diagnostic techniques became available, several viruses associated with GLD were characterized during the last three decades. These vi-

ruses are collectively referred to as Grapevine leafroll-associated viruses or GLRaVs (110,111).

The global expansion of the grape and wine industry has seen a parallel increase in the incidence and economic impact of GLD. Despite the fact that GLD was recognized as a potential threat to grape production for several decades, our knowledge of the nature of the disease is still quite limited due to a variety of challenges related to the complexity of this virus disease, the association of several genetically and serologically distinct GLRaVs, and contrasting symptoms in red- and white-berried cultivars. In view of the growing significance of GLD to wine grape production worldwide, this feature article provides an overview of the state of knowledge on the biology and epidemiology of the disease and describes management strategies currently deployed in vineyards.

## Symptoms

Only cultivars of European grapevines (viz. *V. vinifera*) and some Asian *Vitis* species (viz. *V. coignetiae*; 142) exhibit conspicuous symptoms of the disease. In contrast, *V. californica* and natural *V. californica* × *V. vinifera* hybrids (88), juice grapes (viz. *V. labruscana* 'Concord' and *V. labrusca* 'Niagara'), muscadine grapes (*M. rotundifolia*), and rootstocks (viz. *V. riparia*, *V. rupestris*, *V. berlandieri*, *V. champini,* and crosses thereof) are asymptomatic hosts. In *V. vinifera*, GLD produces contrasting symptoms on the foliage in red- and white-berried cultivars (Fig. 1; 64,106,121,165). In many red-berried cultivars, symptomatic leaves exhibit red or reddish-purple discolorations in inter-veinal areas, but a narrow strip of leaf tissue on either side of the main veins remains green, giving the appearance of "green veins" (Fig. 1A). The red and reddish-purple coloration of symptomatic leaves is due to the accumulation of specific classes of anthocyanin pigments (75). In contrast, white-berried cultivars show mild yellowing or chlorotic mottling of inter-veinal areas of leaves (Fig. 1C). These symptoms, however, are often subtle and may not be recognized in many white-berried cultivars. In both red- and white-berried cultivars, symptoms often appear first on mature leaves at the bottom portion of the canopy around *véraison* (a transitional phase representing the onset of berry ripening with change of berry skin color [35]) and progressively move upward to younger leaves as the season advances. In general, GLD symptoms become apparent in early to mid-summer, and symptomatic leaves of both types of cultivars usually show downward rolling of leaf margins toward the end of the season (Fig. 1B and D), giving the name leafroll disease (109,121).

It should be noted, however, that the extent of foliar symptoms and downward rolling of leaves varies considerably among cultivars. Some red-berried *V. vinifera* cultivars such as Pinot noir and Cabernet franc, and white-berried *V. vinifera* cultivars such as Chardonnay show pronounced downward rolling of leaves by harvest time. In other cultivars, downward rolling of leaf margins may not be apparent at all. As a result, visual diagnosis of GLD symptoms is difficult in the latter cultivars. In addition, expression of disease symptoms can be influenced by cultivar, scion-rootstock combination, and environmental factors prevailing in a given grapevine-growing region. In the case of red-berried *V. vinifera* cultivars, nutritional disorders (such as potassium deficiency), mechanical damage to the trunk inflicted during viticultural operations or wind abrasion between canes, and girdling of leaves by tendrils and shoots by buffalo leafhopper (*Ceresa bubalus*) oviposition and red blotch disease can produce discolorations that mimic GLD symptoms.

From the above discussion, it should be emphasized that GLD symptoms are highly variable between cultivars and even within the same cultivar. In addition, some strains of both GLRaV-2 and -7 can cause asymptomatic infections in certain wine grape cultivars (9,16,86,134). Thus, testing suspect samples for GLRaVs

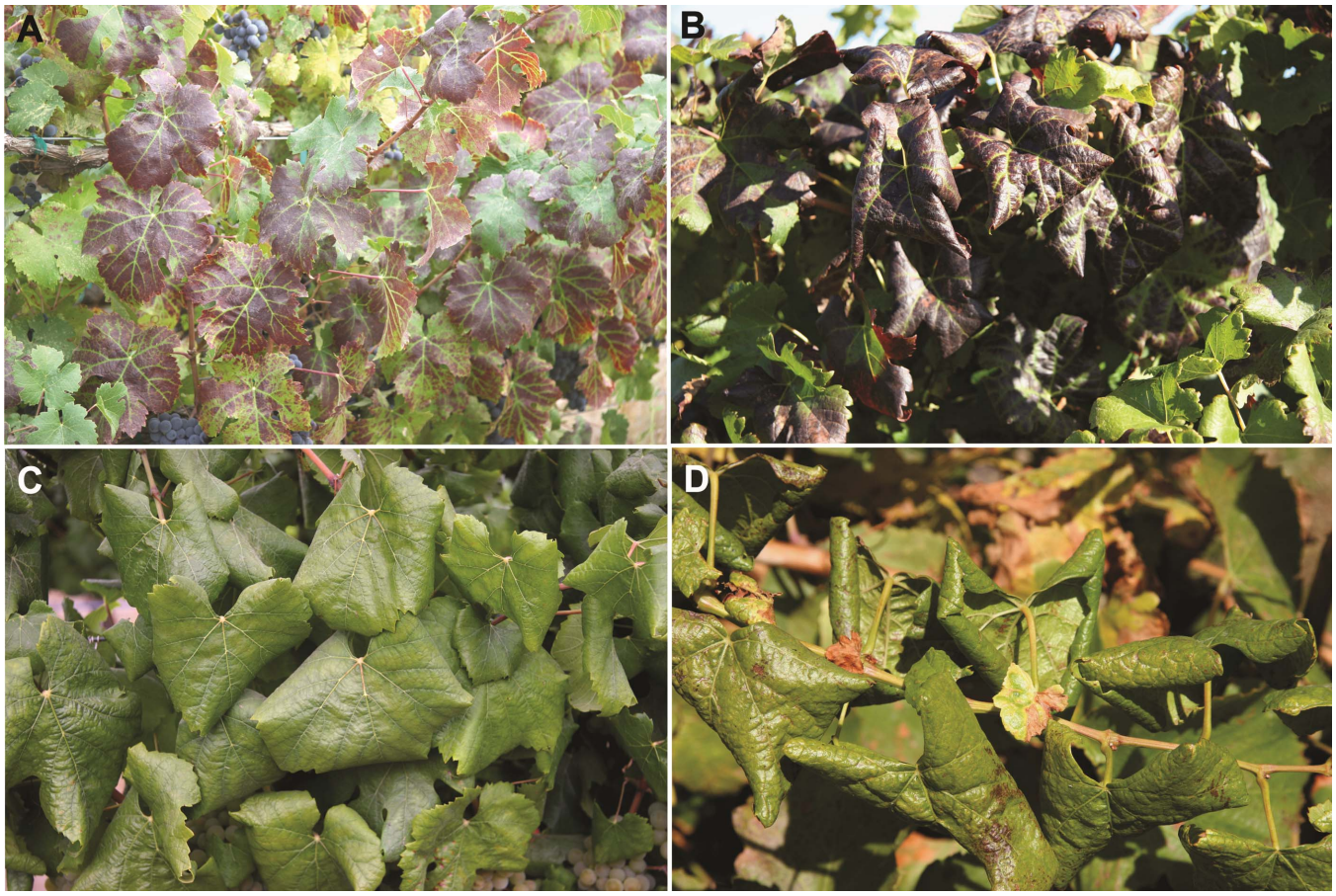


**Fig. 1.** Foliar symptoms of grapevine leafroll disease in **(A and B)** red- and **(C and D)** white-berried wine grape cultivars. Downward rolling of leaf margins (B and D) is commonly visible in both types of cultivars toward the later part of the season. A and B = Merlot, C and D = Chardonnay.

using sensitive diagnostic methods described below is a vital step in determining the viral nature of symptoms and for making appropriate disease management recommendations to growers.

## Viruses Associated with GLD

GLRaVs are morphologically similar but serologically and genetically distinct viruses. They are numbered serially as GLRaV-1, -2, -3, -4, and so on (110), and belong to the plant virus family *Closteroviridae* (87,110,111). GLRaV virions are flexuous rods, with lengths ranging between 1,400 and 2,200 nm and a diameter of about 12 nm. They encapsidate a linear, positive-sense, single-stranded RNA genome with a possible cap structure at the 5′ terminus and no poly(A) track at the 3′ terminus. Similar to other closteroviruses (87), the genomic RNA of GLRaVs may constitute about 5% of the total mass of virions. Like other members of the family *Closteroviridae*, GLRaVs may have bipolar virions with most of the genome encapsidated by the coat protein (CP) and the 5′ extremity of virions having a segmented "tail" structure made up of several other virus-encoded proteins (130,143). It has been hypothesized that the segmented tail of a closterovirus functions as a movement device guiding directional transport of viral genomes between neighboring cells (46). Likewise, the segmented tails of GLRaVs may have similar functions. Based on the current understanding of the functional roles of proteins encoded by *Beet yellows virus* (45) and *Citrus trizteza virus* (44), proteins encoded by GLRaVs could be multifunctional, involved in various aspects of the virus life cycle. GLRaVs are limited to phloem-associated cells, replicate in companion and phloem parenchyma cells, and can affect the cytology of differentiating sieve tubes, parenchyma, and companion cells (27,54), a characteristic of plant infection by members of the family *Closteroviridae* (170).

In general, GLRaVs are unevenly distributed in grapevines and exist in low concentrations (157). They are usually present in an infected grapevine as mixed infections of several GLRaVs or GLRaVs with other viruses. Mixed infections may have resulted from the continual vegetative propagation of grapevines over many centuries and grafting between different scion and rootstock materials (58,120,136). Such mixed infection of GLRaVs may lead to either synergistic or antagonistic interactions, commonly observed between plant viruses co-infecting the same host (150). Among the currently known GLRaVs, GLRaV-3 appears to be the most widespread, as single or mixed infections with other GLRaVs, grapevine viruses and viroids, in areas where GLD is present (28,105).

## Taxonomy, Genome Organization, and Diversity of GLRaVs

GLRaVs belong to distinct genera in the family *Closteroviridae* (Fig. 2; 110,111). GLRaV-1, -3, and -4 are assigned to the genus *Ampelovirus* (derived from ampelos, meaning grapevine in Greek) with GLRaV-3 as the type species, whereas GLRaV-2 belongs to the genus *Closterovirus* (from kloster, meaning thread in Greek) typified by *Beet yellows virus*. GLRaV-7 is assigned to the newly proposed genus *Velarivirus* (from velari, meaning cryptic in Latin) (9). Recent taxonomic revisions have designated GLRaV-5, -6, -9, GLRaV-Pr, GLRaV-De, and GLRaV-Car as genetically divergent strains of GLRaV-4 due to similarity in their overall genome size and genetic organization. Consequently, these former virus species are now grouped under the umbrella term "GLRaV-4-like" viruses (110).

At present, the full-length genome sequence of almost all known GLRaVs is available in public databases. GLRaVs are genetically diverse with open reading frames (ORFs) encoded by each virus varying in size and number (Fig. 3). Current sequence data indicate that GLRaV-4 strain 'Car' is the smallest of the GLRaVs with 13,626 nucleotides (nt) encoding six ORFs (1), and GLRaV-3 is the largest and the most complex with 18,671 nt encoding 12 ORFs (55,85,105). Although the genome organization of GLRaVs has similarities to the gene modules characteristic of closterovirids, some differences are notable and appear to be unique to viruses associated with GLD. Similar to members of the genus *Closterovirus* (46), all GLRaVs have a signature replication gene block (RGB) that covers a large portion of the genome toward the 5′ terminus. The RGB is made up of replication-associated proteins encoded by ORFs 1a and 1b. The polymerase module encoded by ORF1a is made up of domains for one or two papain-like leader proteases, methyl transferase- and helicase-like domains with large inter-domain region. Unlike other GLRaVs, the ORF1a of GLRaV-3 and GLRaV-4 and its strains contains an AlkB domain within the large inter-domain region (2,105,110,154). In all GLRaVs, ORF1b expresses the RNA-dependent RNA polymerase-like domain via a +1 frameshift translation. The other ORFs are located downstream of RGB toward the 3′ terminus of the genome. A signature quintuple gene module, consisting of a 6-kDa protein (p6 or its homologue), a heat-shock protein 70 homolog (HSP70h), a ~60-kDa protein (p55 or its homologue), the CP and a minor CP (CPm), is present in GLRaV-1, -2, and -3, but not in GLRaV-4 and its strains -5, -6, -9, -Pr, -De, and -Car, and GLRaV-7. As expected, the arrangement of CP and CPm in GLRaV-2 is similar to that in members of the genus *Closterovirus*. In contrast, GLRaV-1, -3, and -7 genomes have CP and CPm ORFs in the reverse order relative to GLRaV-2. Further, GLRaV-1 shows an additional peculiarity in that its genome has two divergent copies of CPm. In contrast, CPm is absent in GLRaV-4 and its strains -5, -6, -9, -Pr, -De, and -Car. Thus, it is clear that all characterized GLRaVs show remarkable differences in number and arrangement of ORFs that appear to be characteristic of each virus species. Based on the genome size, gene organization, and genetic variability, GLRaVs in the genus *Ampelovirus* have been divided into two subgroups, with GLRaV-1 and -3 in subgroup I, and GLRaV-4 and it strains -5, -6, -9, -Pr, -De, and -Car in subgroup II (1,2,103, 110,154).

Studies on genetic diversity of GLRaV-1, -2, and -3 have indicated the presence of genetically diverse but closely related variants in several grapevine-growing regions (3,20,30,70,84,146,160). In the case of GLRaV-3, the presence of seven possible variant groups has been reported in different cultivars and vineyards worldwide based on phylogenetic analysis of full-length CP gene sequences (105). Further, a pairwise comparison of the full-length genome of several GLRaV-3 isolates indicated an uneven distribution of sequence variation along the virus genome (105). An analysis of natural populations of GLRaV-1 and -2 from California, Washington, and New York, based on the partial HSP70h and CP gene sequences, revealed the existence of genetic variants that segregate into phylogenetically distinct groups (three in the case of GLRaV-1 and six in the case of GLRaV-2) independent of their geographic origin (3,84). Since grapevines are clonally propagated and no resistance is known in *Vitis* spp. (95,126), variants of GLRaVs could be perpetuated without being subjected to stringent purifying selection or bottleneck events. The intrinsically error-prone nature of the viral RNA-dependent RNA polymerase (149) further contributes to global genetic variability of GLRaVs leading to accumulation of genetically diverse but closely related variants of each virus, often termed "quasi-species" (47). Consequently, an individual grapevine may harbor a myriad of variants whose evolutionary dynamics can be influenced by host- and vector-imposed bottleneck events. More specifically, the genetic diversity and fitness of genetic variants among ampeloviruses (GLRaV-1, -3, and -4 and its strains) can be influenced to a greater extent by constraints imposed by horizontal vector-mediated transmissions in comparison to GLRaV-2 and GLRaV-7, which are not yet known to be transmitted by vectors.

## Impact of GLD

GLD can cause reduced plant vigor and longevity, and significant losses in both fruit yield and quality. Crop losses between 14 and 40% due to GLD infection have been reported (62,167,168). The magnitude of yield losses appears to be dependent on cultivar-rootstock combinations, age of vines when infection occurs, causal virus(es), single or mixed virus infection, and environmental condi-

tions (12,26,74,89,90,167). GLD is also reported to affect photosynthesis in some red-berried cultivars under field conditions (15,36) as well as modulation of host genes involved in a wide spectrum of biological functions (51,52).

Reduced cluster size, loose clusters, and small berries are commonly observed in GLD-affected vines (Fig. 4). One of the major impacts of GLD in red-fruited *V. vinifera* cultivars is asynchronous fruit ripening and poor color development of berries (4,69, 140,161), altered fruit maturity indices (soluble solids or °Brix, titratable acidity or TA, and pH), and modifications of individual and total anthocyanins, total phenolics, as well as total tannins (4,69,96,97,104,161,167).

## Diagnosis

Visual observation of GLD symptoms in affected vines is largely unreliable for consistent diagnosis of the disease under vineyard conditions. This is largely due to the highly variable nature of GLD symptoms (Fig. 1). Foliar symptoms of GLD are apparent only during late summer and fall, but not in spring. Some abiotic and biotic stresses described earlier can mimic foliar GLD symptoms, especially in red-fruited *V. vinifera* cultivars. The lack of obvious symptoms in white-fruited cultivars, latent infection in American *Vitis* species, interspecific hybrids, and rootstocks further complicates symptom-based diagnosis of GLD in vineyards. With improved understanding of intricacies associated with field-based

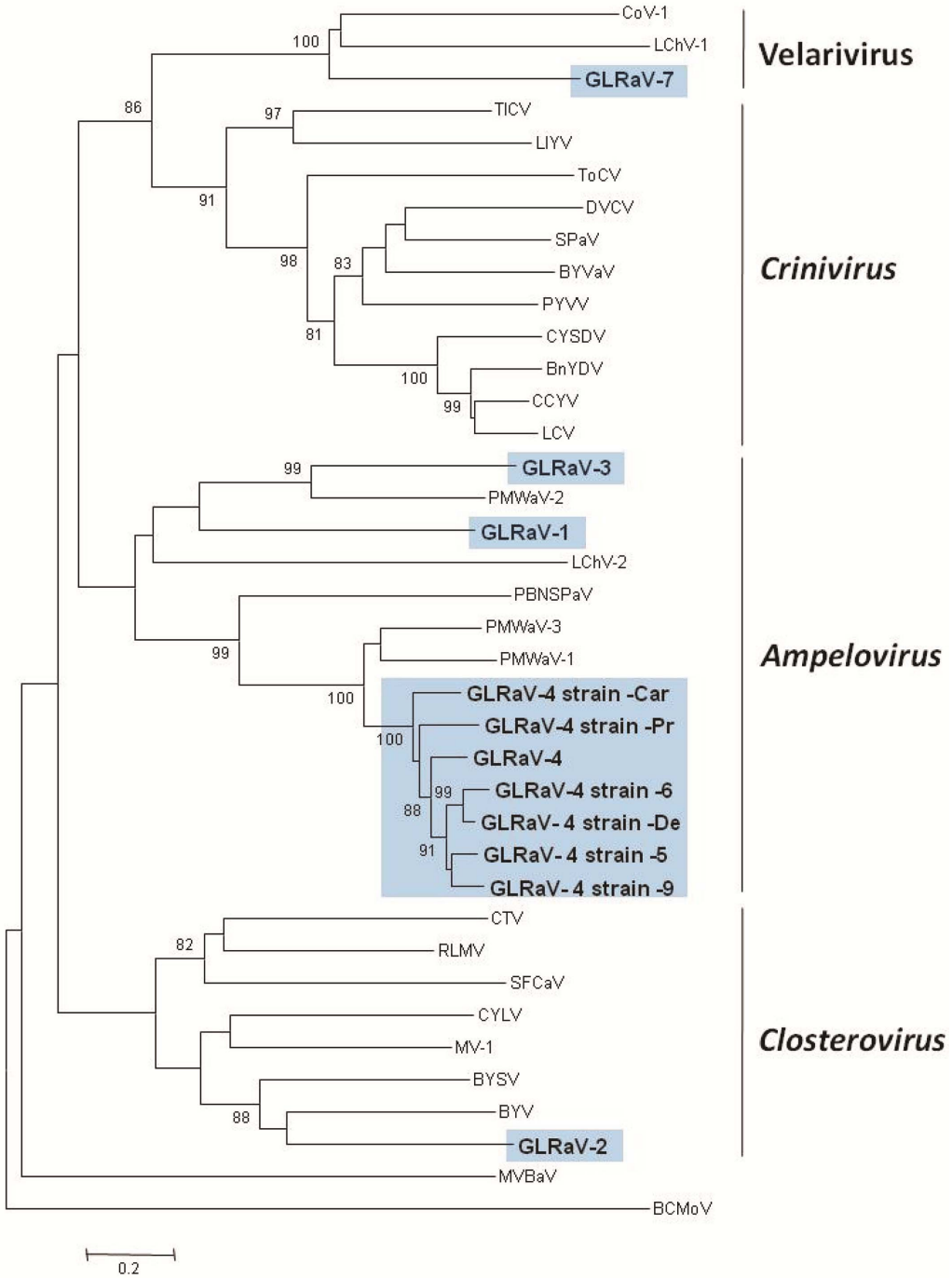


**Fig. 2.** Phylogenetic analysis of the coat protein (CP) gene sequence of Grapevine leafroll-associated viruses (GLRaVs) in the family *Closteroviridae*. The majority of GLRaVs belong to the genus *Ampelovirus*. Bootstrap values less than 70% are not shown. The CP gene sequence of *Bean calico mosaic virus* (BCMoV, NC 003504) was used as an outgroup. Accession numbers of GLRaVs used in this figure are: GLRaV-1 = AF195822; GLRaV-2 = AF039204; GLRaV-3 = NC004667; GLRaV-4 = FJ467503; GLRaV-4 strain -5 = FR822696; GLRaV-4 strain -6 = FJ467504; GLRaV-4 strain -9 = AY297819; GLRaV-4 strain -Car = FJ907331; GLRaV-4 strain -De = AM494935; GLRaV-4 strain -PR = NC_011702; GLRaV-7 = JN383343. Figure modified from Al Rwahnih et al. (9) with permission of the publishers and the corresponding author (A. Rowhani).

diagnosis and the nature of GLRaVs, methodologies for the specific and accurate detection of individual GLRaVs have evolved through the years to include highly sophisticated and sensitive detection techniques that can target individual GLRaVs and their molecular variants. Diagnostic methods include biological indexing using woody indicator hosts, serological assays (enzyme-linked immunosorbent assay [ELISA]), and molecular methods (reverse transcription-polymerase chain reaction [RT-PCR] and PCR) and recent approaches such as micro- and macro-arrays as well as next-generation sequencing (NGS), as described below.

**Biological indexing.** Biological indexing has been used as an important and fundamental assay for the diagnosis of GLD. It is also a standard method for establishing graft transmissibility of GLRaVs and other graft-transmissible pathogens associated with a disease, and further characterization of hitherto unknown/uncharacterized agents. Biological indexing for GLD is routinely used in clean plant programs.

In biological indexing, budwood from a candidate vine suspected of GLD infection is grafted onto an indicator plant by chip-, bench-, or micro-grafting, and the grafted plant is observed for symptoms over 2 to 3 years in a field setting (141). *V. vinifera* 'Cabernet franc' is a common diagnostic indicator for GLD. Other indicators used for GLD indexing are cultivars Pinot noir, Mission, Cabernet Sauvignon, and Barbera. Selection of a specific cultivar as an indicator host depends upon personal preferences of people performing indexing and on climatic conditions under which indicator plants are grown. On Cabernet franc, symptoms are interveinal reddening of the leaf blade, beginning in late summer and intensifying thereafter, with prominently green primary veins and downward rolling of leaf margins. Because GLRaVs and their strains may produce similar symptoms on Cabernet franc, it is impossible to identify a specific virus present in a candidate vine through biological indexing. Furthermore, the asymptomatic nature of some strains of both GLRaV-2 (5,16,134) and GLRaV-7 (9,86) limits the reliability of biological indexing. In addition, biological indexing is influenced by various factors including the efficient spread of virus(es) from the budwood piece to the recipient indicator host and climatic conditions under which field indexing is performed (34). Thus, biological indexing is not always reliable. It is also labor-intensive, requires large field or greenhouse space to grow grafted vines, and takes 2 to 3 years for vines to grow and express disease symptoms in a field setting.

**Serological assays.** The most common serological method used for rapid detection of GLRaVs is ELISA. This technique is based on the recognition of virus antigens with immunoglobulins or monoclonal antibodies produced against purified virions or the virus CP expressed in *Escherichia coli* cells. Serological reagents are commercially available and routinely used in ELISA for the detection of GLRaV-1, -2, -3, -4, and -7 in grapevine tissue (18,56,138). ELISA is sensitive, reliable, and adapted to high throughput applications for testing large numbers of samples. However, ELISA-based diagnosis of GLRaVs has some limitations and can be influenced by various factors (166). These factors include sensitivity (when a virus is present at extremely low concentrations), specificity (presence of variants of GLRaVs that may not be recognized by available antibodies), and quality of antibodies. Nevertheless, ELISA remains a reliable diagnostic tool in large-scale surveys for GLD and for research purposes.

**Molecular assays.** Over the past two decades, diagnosis of GLRaVs using molecular assays has rapidly advanced such that a broad range of techniques is available for a more reliable detection. RT-PCR-based technologies have increased sensitivity compared to ELISA (33,100,139). RT-PCR is estimated to be 100 to 1,000 times more sensitive than ELISA (139). In recent years, real-time

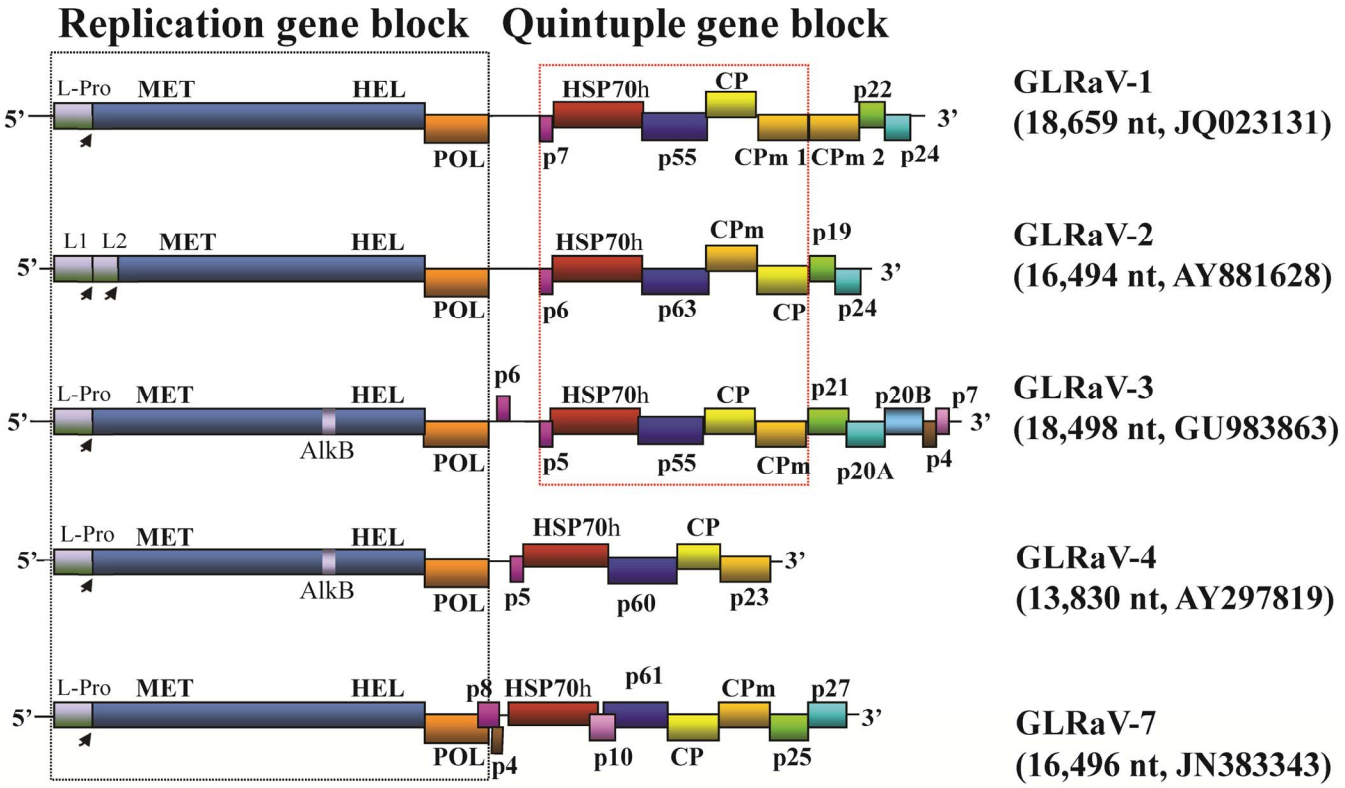


**Fig. 3.** Genome maps of select Grapevine leafroll-associated viruses (GLRaVs). Names of viruses, their genome size, and GenBank accession numbers are shown on the right side. The open reading frames (ORFs) are shown as boxes with associated protein designations. L-Pro, papain-like leader protease; AlkB, AlkB domain; MET, HEL, and POL, methyltransferase, RNA helicase, and RNA dependent RNA polymerase domains of the replicase, respectively; HSP70h, a HSP70-homologue; CP, the major capsid protein; CPm, the minor capsid protein. The other ORFs are designated with approximate molecular weight and a common "p" designator (46,110). Note that the 3′-most ORF of GLRaV-4 and its strains is not a CPm, it is denoted as p23. Unlike other GLRaVs, GLRaV-2 encodes two papain-like leader proteases, designated in the map as L1 and L2. Black box shows replication gene block encoded by all GLRaVs, and red box indicates quintuple gene block proteins encoded by GLRaV-1, -2, and -3. Figures are not drawn to scale.

PCR, using detection chemistries such as TaqMan, is increasingly being used for the detection and quantification of GLRaVs in plant tissue and insect vectors compared to conventional RT-PCR assays (127). Low-density PCR arrays (LDA) using the real-time TaqMan PCR primers/probes complexes in 384-well plates were developed for the simultaneous detection and quantification of different GLRaVs (128). Overall, the TaqMan-based real time RT-PCR and LDA detection methods are rapid and quantitative, and can provide the required robustness in processing a large number of samples for the detection of GLRaVs (129). RT-PCR in combination with high-resolution melting curve analysis has been used for monitoring the incidence of GLRaVs and their variants in vineyards (19,135). Recently, a RT loop-mediated isothermal amplification assay was developed for the rapid detection of GLRaV-3 (163). Microarray- (50) and macroarray- (153) based detection methods have been developed for the multiplex detection of GLRaVs. Although these methods are useful due to their relative simplicity and robustness, limitations include time, expertise, and costs in running the assay. One of the more exciting diagnostic developments in recent years is the application of NGS for mining sequences of viruses and virus strains in grapevines in an unbiased manner (7,8,32,61,133,169). Although NGS is relatively expensive for use as a routine tool in virus diagnostics, the information generated from this emerging technology can be effectively used in molecular diagnostics for reliable and sensitive identification of viruses.

To summarize, ELISA and RT-PCR are routinely used in a complementary manner for the detection of GLRaVs. Irrespective of the method used, although molecular assays may be more sensitive than serological assays, it should be remembered that reliable detection by either of the methods depends on sampling strategy and proper controls (118,157). Sampling appropriate tissue at the optimal time is one of the most critical factors influencing reliable detection of GLRaVs due to their localization in phloem-associated tissue, low concentration, and uneven distribution in infected vines. In general, petiole samples collected during late summer and fall, and cambial scrapings obtained from dormant woody canes in winter, are used for GLRaVs testing by ELISA or RT-PCR (166).

## Dissemination of GLRaVs

**Propagation material.** Grapevines are vegetatively propagated to maintain clonal integrity and trueness-to-type, and planted as either own-rooted or grafted vines. Because of this propagation practice, GLRaVs can spread from one location to another when cuttings derived from infected vines are used for propagation (106,107). This means that GLRaVs can be disseminated along with scion and/or rootstock materials used for propagation, grafting, or planting new vineyards. Dissemination of GLRaVs via these practices can happen with both red- and white-berried *V. vinifera* cultivars, native American *Vitis* species, as well as interspecific hybrids and rootstocks. Therefore, the use of infected cuttings or budwood for propagation, bench grafting, chip budding, and top working provide many avenues for the introduction of GLRaVs into vineyards. Because GLRaVs are not mechanically transmissible between grapevines, their spread in vineyards via pruning shears, trimmers, thinners, harvesters, or saws does not occur.

**Insect vectors.** Plant-to-plant dissemination of GLRaVs in the genus *Ampelovirus* occurs via mealybug (Pseudococcidae) and scale insect (Coccidae) vectors. Several species of mealybugs belonging to the genera *Heliococcus, Phenacoccus, Planococcus,* and *Pseudococcus* and scale insects belonging to the genera *Pulvinaria*, *Neopulvinaria,* and *Parthenolecanium* have been identified as vectors of GLRaV-1, -3, and -4 and its strains (Table 1). There are no known insect vectors for GLRaV-2 and -7. Despite the fact that several members of the genus *Closterovirus* are transmitted by different species of aphids (87,111), there is no evidence so far for transmission of GLRaV-2 by aphids under experimental or natural conditions. Aphids are not common pests of grapevine, although the grapevine aphid (*Aphis illinoisensis*) has been reported in several grapevine-growing areas (80,159). However, current evidence indicates that *A. illinoisensis* is unlikely to serve as a vector for GLRaV-2. Nevertheless, GLRaV-2 can be mechanically transmitted with some difficulty from grapevine tissue to *Nicotiana benthamiana* (71). Recently, GLRaV-7 was demonstrated to be transmitted experimentally by the parasitic dodder *Cuscuta reflexa* to *Tetragonia expansa* and *Cuscuta europea* to *Nicotiana occidentalis* (113).

Most of the information on transmission of ampeloviruses is obtained with mealybugs rather than scale insects. Hence, the following discussion pertains to our current knowledge of ampelovirus transmission by mealybug vector species. Mealybugs show gender-specific distinction in their ability to transmit ampeloviruses. Male mealybugs are winged and capable of flight, but have only vestigial mouthparts not suitable for feeding and acquiring virus (37). Female mealybugs have functional mouthparts allowing acquisition of virus while ingesting plant sap from phloem, and subsequent transmission. However, females are wingless and are largely seden-

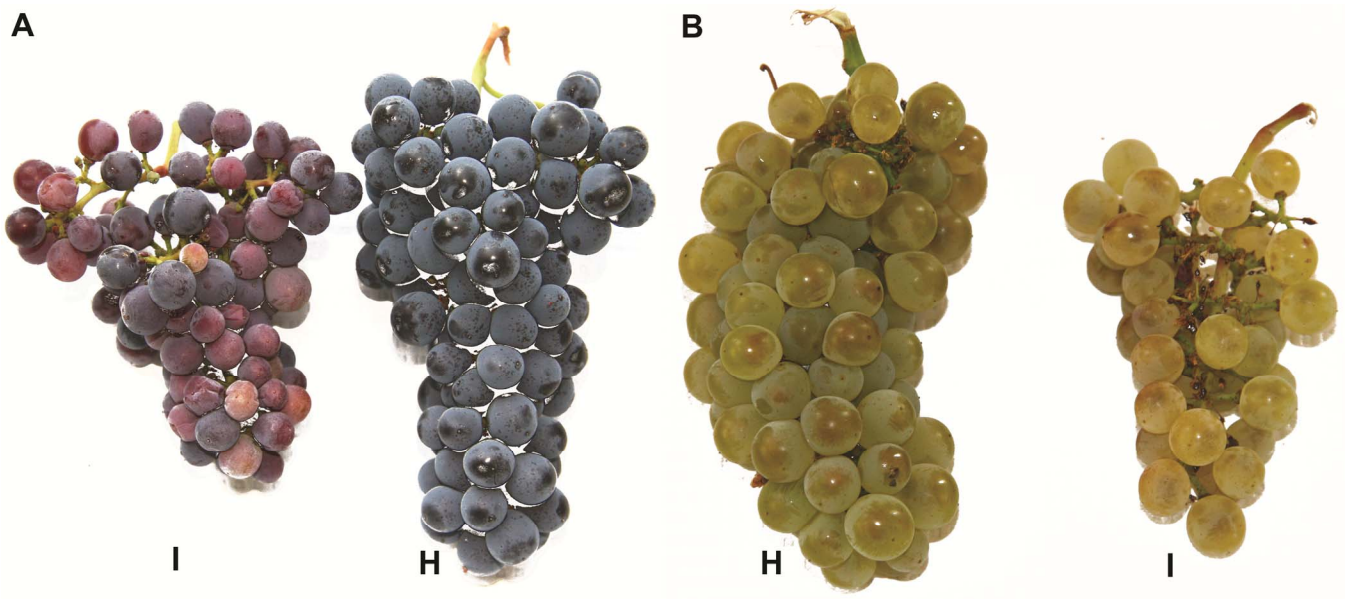


**Fig. 4.** Clusters produced by grapevine leafroll disease-affected vines are smaller and less compact, with berries showing uneven size and ripening. **A,** Red-berried *Vitis vinifera* cultivar (Cabernet franc), and **B,** white-berried cultivar (Chardonnay). H = Cluster from healthy vine, I = Cluster from infected vine.

tary, limiting movement and spread of virus to short distances covered by crawling between immediately adjacent vines within a row and between neighboring rows (72). As a consequence of this limited mobility, the spread of GLD within a vineyard is slow unless the insect is dispersed by other means, such as human activities, or being blown by the wind or transported by foraging birds. Despite the limited mobility of mealybugs, they can be dispersed relatively long distances on wind-blown infested leaves, carried on vineyard workers' clothing, or spread by harvesting equipment carrying leaf or bark material infested with mealybugs. The first-instar nymphs or crawlers are well adapted to dispersal, whereas the later life stages and adult females are relatively immobile and make little contribution to distant spread of GLD. Although all life stages of mealybug vectors are capable of transmission, first-instar nymphs appear to be more efficient in transmission and thus play a more significant role compared to other developmental stages in the epidemiology of GLD (131,156).

Studies on mealybug transmission suggest that transmission is semi-persistent (158). GLRaV-3 can be transmitted by different mealybug species and, conversely, a single mealybug species (e.g., *Planococcus ficus*) is capable of transmitting different ampeloviruses. This would suggest a lack of virus-vector specificity of transmission between different species of mealybug vectors and ampeloviruses (98,158). Detection of GLRaV-3 in salivary glands of *Planococcus citri* suggests a circulative mechanism of transmission (31), in disagreement with the previous conclusions (87,110). Based on studies with GLRaV-3 transmission by *Pl. ficus*, acquisition and inoculation of the virus can occur within a 1-hour plant access period with a transmission efficiency of ca. 10% per individual per day under greenhouse conditions (156). However, higher transmission rates were obtained with first- and possibly second-instar nymphs than with adults of *Pl. ficus* and *Pseudococcus longispinus* (48,131). A single viruliferous mealybug nymph is capable of infecting a healthy vine with GLRaV-3 (48), although variation in transmission efficiency of ampeloviruses among different mealybug species is possible (158). Source tissue, virus titer, spatial distribution of virus, and seasonal variations can also influence transmission of GLRaV-3 (155,157).

A few studies have shown differences in transmission efficiency of ampeloviruses, with GLRaV-3 transmitted at higher rate than GLRaV-4 strain -5 by *Pl. ficus* and *Ps. longispinus* (65,102). Also, *Pl. ficus* transmitted GLRaV-4 and its strain -9, but *Ps. longispinus* transmitted strain -9 but not GLRaV-4 (158). Thus, in-depth research is needed to elucidate virus-vector interactions and factors influencing the relative transmission efficiency of ampeloviruses by different mealybug vector species for a better understanding of the transmission biology of ampeloviruses.

**Vineyard spread of ampeloviruses.** An understanding of ampelovirus spread in vineyards is essential for developing effective control measures. Primary spread or introduction of GLRaVs into a newly planted vineyard can occur either via planting virus-infected vines or be initiated by alighting viruliferous vectors. Either of these modes generally results in a random spatial distribution of infected grapevines in a vineyard. As mentioned above, it should be noted that planting infected vines results in primary spread of all GLRaVs, and viruliferous vectors can contribute to the primary spread of only ampeloviruses. Consequently, grapevines infected with GLRaV-1, -3, and -4 and its strains can serve as an initial source for secondary spread within a vineyard by mealybug or scale insect vectors. In contrast, the secondary spread of GLRaV-2 and -7 is unlikely to occur due to the absence of insect vectors known to spread these viruses.

Several studies on the secondary spread of GLRaV-3 involving transmission by different mealybug vector species were conducted (23–25,28,68,77,78). In general, the spatio-temporal spread dynamics over multiple years indicates two common patterns of secondary spread (28,148). One is aggregation or clustering of infected vines, suggestive of virus spread between adjacent vines within a row and across adjacent rows as a consequence of vine-to-vine movement of mealybugs around the primary foci of infection. These random patches of infection expand with time, often involving many seasons, and ultimately coalesce to cover the entire vineyard. The second common pattern of secondary spread is "edge effects" contributed mainly by incoming viruliferous vectors from sources of infection either far from or near the newly planted vineyard. Such an infection pattern results in disease gradients with a high percentage of symptomatic vines in rows alongside nearby sources of infection and a gradually decreasing incidence of the disease with increasing distance from the source of infection. Such a pattern is an indication that ampeloviruses initially spread into a newly planted vineyard from heavily infected neighboring vineyards and then secondarily inside the newly infected vineyard. These patterns of virus spread appear to be similar across grapevine-growing regions, irrespective of the vector species present in a given region.

Within-field spread of GLRaV-3 can occur even at low vector population densities, and few insects feeding on infected vines have the potential to spread the virus over time. GLRaV-3 spread by *Pl. citri* or *Pseudococcus maritimus* at low vector population density was reported with a 35 to 97.5% and a 23 to 66% increase of infected vines over 8 and 5 years, respectively (24,68). Natural spread of GLRaV-3 was reported in New Zealand (21), Australia (77,78), South Africa (48), Spain (22), Italy (17), and the United States (68,158). Other GLRaVs that spread in vineyards include

**Table 1.** Mealybug (Pseudococcidae) and scale insect (Coccidae) species currently reported as vectors for grapevine-infecting ampeloviruses

| Virus | Mealybugs | Scale insects | References |
|---|---|---|---|
| GLRaV-1 | Bohemian mealybug (*Heliococcus bohemicus*)<br>Apple mealybug (*Phenacoccus aceris*)<br>Obscure mealybug (*Pseudococcus viburni* [formerly *Ps. affinis*])<br>Citrophilous mealybug (*Pseudococcus calceolariae*)<br>Grape mealybug (*Pseudococcus maritimus*)<br>Comstock mealybug (*Pseudococcus comstocki)* | *Pulvinaria vitis*<br>*Parthenolecanium corni*<br>*Neopulvinaria innumerabilis* | 14, 17, 22, 48, 49, 65, 66, 67, 73, 83, 93, 98, 102,108,112,122,145, 147,156,158,171 |
| GLRaV-3 | Bohemian mealybug (*Heliococcus bohemicus*)<br>Vine mealybug (*Planococcus ficus*)<br>Citrus mealybug (*Planococcus citri*)<br>Longtailed mealybug (*Pseudococcus longispinus*)<br>Citrophilous mealybug (*Pseudococcus calceolariae*)<br>Grape mealybug (*Pseudococcus maritimus*)<br>Obscure mealybug (*Pseudococcus viburni*)<br>Comstock mealybug (*Pseudococcus comstocki*)<br>Apple mealybug (*Phenacoccus aceris*) | *Pulvinaria vitis*<br>*Neopulvinaria innumerabilis*<br>*Parthenolecanium corni*<br>*Coccus hesperidium*<br>*Coccus longulus, Saissetia* sp.<br>*Parasaissetia nigra*<br>*Ceroplastes rusci* | |
| GLRaV-4 and its strains -5, -6, and -9 | Vine mealybug (*Planococcus ficus*) | *Ceroplastes rusci* | |

GLRaV-1 by *Heliococcus bohemicus* in Italy (17) and *Phenacoccus aceris* in France (98), and GLRaV-4 strain -9 in Australia (79).

Many factors can influence the rate of spread and the distances over which ampelovirus spread occurs over time. Differences in the species composition of mealybug and scale insect vectors in a given grapevine-growing region and their fecundity and transmission efficiency have important epidemiological consequences. It has been reported that *Parthenolecanium corni*, *Ps. maritimus*, *Pseudococcus viburni,* and *Pl. ficus* have one, two, three, and four to six generations annually, respectively (6,37,60,76), and such species-specific variability in number of generations can play a significant role in the spread of ampeloviruses. Thus, a comprehensive monitoring and sampling program (60) involving diagnostic methods (42) and synthetic sex pheromones (11,114) for the collection and identification of mealybug species in vineyards can lead to a better understanding of ampelovirus epidemics. Vector feeding behavior, vine-to-vine movement, presence of ampeloviruses as single or mixed infections in source grapevines, age of grapevine at which virus acquisition or inoculation occurs, and cultivar preferences can contribute additively to the field spread of ampeloviruses. Although all mealybug vector species are known to feed and thrive on the trunk, canes, leaves, and berries (37), some like *Pseudococcus calceolariae* and *Ps. viburni* in New Zealand (13) and *Pl. ficus* in South Africa (37,132) survive on grapevine roots in the soil and transmit GLRaV-3 from remnant roots surviving in uprooted vineyards to new plantings. The rapidity with which ampeloviruses can spread is also influenced by regional environmental and landscape variables as well as vineyard management practices that can influence the survival and dispersal of mealybug vectors (37).

## Strategies for Management of GLD

Since grapevine is the only natural host known for GLRaVs, no sources of resistance to GLRaVs are known in *Vitis* spp., and species-specific differences occur in vector transmission (95,126,158), options for disease management involve a combination of preventive, cultural, sanitary, and vector control strategies. In general, preventive measures such as establishing new vineyards with planting materials derived from virus-tested stocks, cultural practices like roguing, and control of mealybug and scale insect vectors of ampeloviruses are practiced for the management of GLRaVs. The current state of knowledge of GLD management has recently been reviewed (6), and strategies commonly deployed in vineyards are briefly discussed here.

The first line of defense against GLD is a careful selection of the planting material (both rootstock and scion) when establishing a new vineyard. Planting material, irrespective of whether it is grafted or own rooted, should originate from virus-tested, clean foundation stocks. Due to a lack of curative measures to eliminate viruses from an infected grapevine in a vineyard, macroshoot tip or meristem culture is widely adopted in generating grapevines free from GLRaVs (141). In the United States, USDA-APHIS has recently established a network of clean plant centers to facilitate the production of such pathogen-tested plant materials for nurseries and growers (http://nationalcleanplantnetwork.org/). Such a coordinated approach enables the maintenance of uniform standards in producing and supplying virus-tested planting stocks benefiting nurseries and grape growers across the country. A recent study by Fuller et al. (59) highlighted the significant economic benefits from using certified virus-free planting materials for new plantings in grower vineyards. Outside the United States, a comparable approach had been adopted by the European Union, which in 2005 issued a revision of Directive 68/193/CEE for the sanitary certificate of grapevine nursery productions.

In addition to using planting material derived from virus-tested stocks, postplanting management strategies should be implemented for reducing the spread of ampeloviruses in vineyards. Roguing or removal of infected vines is one such strategy recommended to effectively slow disease spread while ensuring profitable grape production (10,148). Although symptom-based roguing can be implemented in red-berried cultivars, caution should be exercised while practicing this strategy for GLD management in white-berried cultivars, since they express no visual symptoms. Grapevines suspected of having GLD should be sampled and tested for GLRaVs followed by roguing of virus-positive plants. Roguing is successful and effective when implemented during early stages of vineyard life, even when only a limited number of vines are infected. In the case of GLRaV-2 and -7, roguing will lead to elimination of virus-infected grapevines since these two viruses are not known to be transmitted by insect vectors. In the case of ampeloviruses (GLRaV-1, -3, and -4 and its strains), roguing ensures removal of infected vines leading to substantial reduction of infection sources for secondary spread by mealybug and scale insect vectors. In the presence of suitable vector(s), ampeloviruses can spread quickly in the absence of roguing and reach near 100% infection rates in 5 to 10 years after planting (24,25,77). Roguing should be practiced annually so that infected vines can be removed soon after the appearance of visual symptoms. Since newly infected vines can take a long time (1 to 3 years) to express visible symptoms, removing asymptomatic infected vines, using sensitive diagnostic assays, will further reduce the availability of inoculum for secondary spread. As a precaution, removal of at least one adjacent vine on either side of GLD-affected vines in a given row is recommended to reduce possible risk of residual inoculum present in the vineyard. A recent study in Israel showed that roguing infected vines can significantly reduce the rate of GLD spread from 30 to 8.6% over a 7-year period (148). Roguing was also shown to be economically viable if disease prevalence is less than 25% in Cabernet franc vineyards in New York (10). If disease prevalence is higher than 25%, a full vineyard removal is recommended. Such measures not only help maintain the profitability of vineyards but also minimize collateral damage due to virus spread from heavily infected blocks to neighboring healthy plantings by resident vector populations. For roguing to be effective, vineyards should be inspected regularly between *véraison* and harvest, which is the optimal time period for the manifestation of disease symptoms, particularly in red-berried *V. vinifera* cultivars. In the case of white-berried *V. vinifera, V. labrusca, V. amurensis,* and interspecific hybrid cultivars as well as rootstocks, distinguishing between healthy and infected vines is difficult, necessitating diagnostic assays for identifying infected vines.

After a vineyard removal, new vines can be planted following conventional vineyard establishment practices unless vectors such as the vine (*Pl. ficus)* and obscure (*Ps. viburni*) mealybugs are present in the vineyard and its vicinity. If the vine and obscure mealybugs are of concern, a fallow period of at least 1 year is recommended to break the disease cycle. Since vine and obscure mealybugs can thrive on vine roots located more than 0.5 m deep in the soil (13,40,132), fallow allows roots to decay and helps in reducing the population of viruliferous vectors prior to planting new vines. Fallowing may not be necessary in vineyards where the grape mealybug (*Ps. maritimus*) is the only vector present, because this mealybug species does not thrive on residual roots in the soil (37). To aid in the reduction of viruliferous vine and obscure mealybugs, systemic herbicides can be applied prior to vine removal with the goal of reducing the persistence of live tissue in the soil that can act as reservoirs of vector populations (132).

In regions infested with viruliferous mealybugs and soft scales, the careful selection of planting material, roguing, and vineyard removal may not be sufficient to achieve acceptable disease control. This is because mealybug nymphs and adult females can crawl along the vine canopy and transmit ampeloviruses over short distances. Mealybug vectors also can be dispersed by wind currents and other means such as vineyard equipment and workers moving from an infected to a healthy vineyard. Therefore, the management of mealybug and scale insect vectors may be important in the fight against GLD. Control of mealybug populations can be achieved with natural enemies (43), parasitoids (39,41), and mating disruption (164) based on pheromone traps (114–116). However,

although attractive to reduce vector populations, biological control of mealybugs is not sufficient to restrict virus spread (76). More aggressive means based on roguing in combination with sanitary measures and timely applications of insecticides (38,132) are needed to restrict virus dissemination and reduce vector populations, respectively. Several chemicals are registered for mealybugs on grapevines. Among them, the systemic insecticide Movento (spirotetramat) can lower vector populations (>70% reduction) but may not be completely effective at reducing ampelovirus spread since mealybugs do not die immediately after exposure (feeding) and may have time to acquire and transmit viruses. Mealybug control with contact chemicals is another option, but this approach is limited because certain stages of mealybugs often live in locations protected from nonsystemic insecticides such as under bark of trunks, spurs and canes, and on roots (for the vine and obscure mealybugs). A combination of a systemic insecticide for reducing overall populations of mealybugs and a quick-acting contact insecticide targeting more exposed crawlers, which have the highest virus transmission efficiency (156), may have a better chance of vector control. Nevertheless, more research is needed to optimize the chemical control of mealybugs and scale insects as part of GLD management.

Sanitary measures should also be considered, especially in extreme situations, where GLD is widespread and mealybug and scale insect vector populations are abundant (132). A practical approach would be for field crews to visit first healthy vineyards and subsequently infected vineyards. Vineyard workers should be encouraged to change clothes before moving between infected and healthy vineyards. Furthermore, equipment dedicated to healthy vineyards or equipment sanitation prior to moving from infected to healthy vineyards should be practiced. All these hygiene measures have synergistic value in reducing the likelihood of dissemination of mealybugs and scale insects (132).

The use of cultivars and rootstocks with resistance to GLRaVs and their vectors would be an ideal alternative for combating GLD. Unfortunately, no sources of resistance are known in *Vitis* species (95,126). Research efforts are under way to develop host resistance to viruses (99) and their vectors through biotechnological approaches. Producing and using virus-resistant (91) and/or vector-resistant grapevine rootstocks and cultivars will greatly reduce production costs and allow growers to use more environmentally friendly production techniques by relying less on chemical, cultural, and sanitary measures for reducing virus sources and controlling their vectors. While awaiting the development of leafroll-resistant cultivars, growers should continue adopting the most appropriate strategies for their vineyards to mitigate the impact of GLD.

Overall, the most efficient management strategy for containing the spread of viruses associated with GLD should be based on careful selection of certified planting material followed by post-planting management by replacing diseased vines with healthy vines in young plantings, replanting unproductive blocks, implementing sanitary practices, and judicious and targeted application of insecticides for vector control. A concerted and cooperative effort between growers in a given grapevine-growing area is likely to be more effective for successful implementation of a mix of cultural, chemical, and sanitary measures for a synergistic effect in containing the spread of GLD between vineyards. Education and outreach efforts to disseminate science-based information through a variety of communication pathways are necessary to increase awareness of the negative impacts of GLD and appreciate the benefits of using certified planting stock among the grape community and to ensure active adoption of best management practices to advance sustainability and profitability of the grape industries. This approach has been successfully implemented in South Africa (132) and can be adopted for growers' advantage in other grapevine-growing regions.

## Summary

Among the numerous virus and virus-like diseases of grapevine, GLD is one of the most economically important diseases affecting the sustainability of the grape and wine industry in the United States and in other grapevine-growing countries. GLD is a complex viral disease and produces distinct symptoms in red- and white-berried *V. vinifera* cultivars. Despite the fact that GLD was recognized in Europe in the mid-nineteenth century and in the United States in the mid-twentieth century, our knowledge of different aspects of the disease and GLRaVs is still quite limited (29,85,94,101,105). The data on genome organization of GLRaVs indicate that these viruses make up one of the most diverse and unusually complex group of viruses infecting a single agriculturally important crop species, representing a unique virus pathosystem. Future studies should focus on the molecular biology of GLRaVs (viz. development and use of infectious genomic cDNA clones in reverse genetics approaches) for studying gene functions and host-virus-vector interactions, the relative efficiency with which ampeloviruses are transmitted by different vector species, as well as the role of genetically divergent GLRaVs in the biology and epidemiology of GLD. The lack of resistance to GLRaVs in *Vitis* species and challenges in developing disease resistance by conventional breeding necessitates innovative strategies of disease control. A holistic, multidisciplinary team approach employing the contemporary tools of molecular biology, genomics, and cell biology is likely to help elucidate different aspects of this complex disease. In addition, research is needed to fill gaps in the knowledge of ecology and epidemiology of GLD and socio-economic analysis of disease impacts for implementing integrated crop management approaches. A combination of using certified virus-tested planting material, roguing and sanitation on a consistent basis, and adopting environmentally benign vector control measures should be encouraged to implement knowledge-based, sustainable management strategies against GLD. Strong partnerships between research and extension faculty and the grape and wine industry stakeholders should be an integral part of these endeavors to translate research knowledge for practical applications, and to deploy improved disease control strategies.

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Rayapati Naidu (aka Naidu Rayapati) is associate professor of plant pathology at Washington State University's Irrigated Agriculture Research and Extension Center, Prosser. He is conducting fundamental and applied research on virus and virus-like diseases of grapevines. In addition, he is pursuing multidisciplinary, trans-institutional collaborative research on insect-transmitted virus diseases affecting agricultural and horticultural crops in the United States. His current international R&D activities are focused on implementing crop improvement strategies against virus diseases in subsistence agriculture in developing countries. Naidu has established a robust extension and outreach program translating research-based knowledge into practical applications for managing virus diseases in Washington vineyards and developing countries. He teaches the course "Diseases of Fruit Crops" alternate years to provide undergraduate and graduate students a comprehensive understanding of diseases of grapevines, fruit trees, and small fruit crops. Naidu has served the American Phytopathological Society (APS) as Chair of the Virology Committee, Chair of the Plant Pathogen and Disease Detection Committee, and Associate Editor of Phytopathology, and currently serves as Section Chair – Biology of Pathogens, Annual Meeting Board of the APS. He received the International Service Award from the APS in 2007.

Dr. Rowhani is a plant virologist in the Department of Plant Pathology at the University of California, Davis. He obtained his Ph.D. in plant pathology from the University of British Columbia, Canada in 1980. His research interests are in the etiology, molecular characterization, and detection of viruses of horticultural crops with an emphasis on grapevine viruses. Dr. Rowhani also serves as a plant pathologist at Foundation Plant Services. He is responsible for the evaluation of the disease status of newly introduced and quarantined grapevine accessions, the inclusion of those plants in the grapevine certification program, and the assessment of the health status of foundation certified vines in the field.

Dr. Fuchs is an associate professor in the Department of Plant Pathology and Plant Microbe Biology at the Cornell University New York State Agricultural Experiment Station, and has been conducting research and extension on plant virus diseases in Geneva, NY since 2004. He received his B.S., M.S., and Ph.D. in life sciences and molecular biology from the University Louis Pasteur in Strasbourg, France. His main areas of research include diagnosis, epidemiology, resistance, and disease control. He has responsibilities in virus disease management in fruit and vegetable crops. He is leading a multidisciplinary team effort to determine the impact of leafroll disease and devise strategies to prevent its extension in vineyards. He is currently serving as the vice-chair of the National Clean Plant Network for grapes, director of the Northeast Plant Diagnostic Network, senior editor for Phytopathology and Journal of Plant Pathology, and associate editor for Transgenic Research.

Dr. Golino has been the director of Foundation Plant Services (FPS) since 1994. FPS is a unit of the College of Agriculture and Environmental Sciences at University of California, Davis. FPS is dedicated to the distribution of disease-tested, true-to-identity plant materials produced by UC researchers or improved by technology developed by UC researchers. At this time, FPS is responsible for programs for grapes, strawberries, fruit trees, nut trees, sweet potatoes, and roses. The grape importation, quarantine, and clean stock programs are the largest in the United States. These programs have played a key national and international role in distributing new crop varieties and healthy planting stocks. In addition to her position as FPS Director, she is a cooperative extension specialist in the Department of Plant Pathology, UC Davis, participating in teaching, extension, and service. Her research program is directed at controlling the virus and virus-like diseases of grapevines with a focus on improved methods of pathogen detection and streamlining virus elimination techniques for clean stock and quarantine programs. She is an author of more than 100 scientific publications in plant pathology.

Dr. Martelli is professor emeritus at the University of Bari, Italy, where he graduated in 1956 in agricultural sciences, later receiving the national doctorate (Libera Docenza) in plant pathology. In 1957, he joined the University of Bari, working on fungal and bacterial diseases until he moved for a postdoctoral stay at the University of California, Davis, where he was exposed to virology and nematology under the guidance of W. B. Hewitt and D. Raski, respectively. Since then, he continued virological studies aimed at the detection, characterization, epidemiology, and cytopathology of viruses responsible for diseases of grapevine, stone fruits, citrus, vegetables, and ornamentals. His contributions to grapevine virology won him the chairmanship of the International Council for the Study of Virus and Virus Diseases of the Grapevine (ICVG), which he still heads. He has contributed to the description of new virus species, genera, families, and one order, headed the Plant Virus Subcommittee of the International Committee on Taxonomy of Viruses (ICTV), served on the executive committee of ICTV, and currently is a life member. He founded and headed till retirement the Research Center on Virus Diseases of Mediterranean Crops of the National Research Council, and chaired the Department of Plant Pathology of the University of Bari. He served as president of the Italian Phytopathological Association (AFI) and of the Italian Society for Plant Pathology (SIPaV), and was a member of the executive committee of the Italian Society for Virology. He is the editor-in-chief of the Journal of Plant Pathology, the international journal of the SIPaV, and a member of several Italian Academies, among which is the Accademia Nazionale dei Lincei. He has been an APS member since 1963 with the status of Fellow since 1997.

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