# Chapter 16
# Grapevine fleck and similar viruses

**S. Sabanadzovic, N. Aboughanem-Sabanadzovic, and G.P. Martelli**

**Abstract** Grapevine fleck virus (GFkV), grapevine red globe virus (GRGV), grapevine rupestris vein feathering virus (GRVFV), grapevine asteroid mosaic-associated virus (GAMaV), and grapevine Syrah virus 1 (GSyV-1) are a group of evolutionarily related viruses with similar morphological, physicochemical, and molecular properties. GFkV is the agent of fleck disease, and GAMaV and GRVFV are associated with asteroid mosaic and vein feathering diseases, respectively, while GRGV is not involved in any specific symptomatology. GSyV-1 is included in this chapter as it shares many traits with the four aforementioned viruses, but has not yet been associated with any particular syndrome. All these viruses are phloem limited, nonmechanically transmissible, and primarily spread through infected propagating material. GFkV is ubiquitous, while the other viruses have been reported only from certain geographical areas. Despite few unconfirmed reports of natural field transmission of GFkV, no vector has been identified for it, nor any of the other viruses. Viral genomes consist of a single molecule of a capped and polyadenylated, cytosine-rich, messenger-like RNA 6.5–7.5 kb in size. According to the current taxonomy, these viruses belong to recognized or putative species in the genera *Maculavirus* (GFkV and GRGV) and *Marafivirus* (GSyV-1, GRVFV, and GAMaV) in the family *Tymoviridae,* order *Tymovirales*.

**Keywords** Asteroid mosaic • Fleck • *Maculavirus* • *Marafivirus* • Phloem • *Tymovirales* • *Tymoviridae*

S. Sabanadzovic (✉)
Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762 USA
e-mail: ss501@msstate.edu

N. Aboughanem-Sabanadzovic
Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Mississippi State, MS 39762, USA

G.P. Martelli
Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy



B. Meng et al. (eds.), *Grapevine Viruses: Molecular Biology, Diagnostics and Management*, DOI 10.1007/978-3-319-57706-7_16

## Introduction and Historical Aspect

The grapevine fleck complex comprises several diseases, most of which can be detected by grafting onto the indicator *Vitis rupestris* St. George (Martelli 2014; Martelli et al. 2015). Asteroid mosaic (AM), the first recognized disease of this complex, is characterized by translucent/chlorotic starlike spots on the foliage of several cultivars of *Vitis vinifera* and clearing of primary and secondary veins on *V. rupestris.* It was described in California and successfully transmitted by grafting (Hewitt 1954). Fleck disease (FK) was reported, also in California, almost a decade later from symptomless *V. vinifera* vines which, upon grafting onto *V. rupestris*, induced symptoms distinct from those of AM (Hewitt et al. 1962, 1972).

No virus could be identified in vines affected by these two diseases for a couple of decades, prompting suggestions of prokaryote origin of fleck (Milkus 1974). Finally, in 1983, two contemporary but independent studies revealed the presence of an isometric nonmechanically transmissible virus in the phloem tissues of some leafroll-affected vines (Castellano et al. 1983; Verderevskaja et al. 1983). This virus, which was initially named grapevine phloem-limited isometric virus (GPLIV), was later purified, and the first virus-specific antiserum was produced (Castellano et al. 1985). Virus particles are isometric, ca. 30 nm in diameter, and have a rounded contour and prominent surface structure with clusters of CP subunits arranged as pentamers and hexamers. In 1990, the physicochemical properties of GPLIV were determined, and its association with leafroll disease was ruled out (Boulila et al. 1990). Shortly afterward, a study conducted in Switzerland reported the close association of an isometric virus with fleck symptoms shown on *V. rupestris* (Gugerli et al. 1991). At the same time, Boscia et al. (1991a,b) observed the consistent presence of GPLIV in fleck-affected *V. rupestris* in Italy, demonstrated the role of this virus in disease etiology, and renamed it grapevine fleck virus (GFkV) (Boscia et al. 1991b).

Soon afterward, an isometric virus with morphological traits resembling those of GFkV was recovered and partially purified from asteroid mosaic-affected *V. rupestris* from California that were kept in a virus collection at the University of Bari (Italy) (Boscia et al. 1994). Despite the morphological resemblance with GFkV, this virus proved to be serologically distinct and was named grapevine asteroid mosaic-associated virus (GAMaV) (Fig. 16.1). Further studies, based on the design and use of degenerate primers, allowed the amplification of two signature domains (i.e., methyltransferase and RNA-dependent RNA polymerase) of the viral replicase gene and their cloning and sequencing (Sabanadzovic et al. 2000). Molecular data confirmed that GAMaV is a distinct virus, evolutionary related to GFkV, which led to further molecular characterization by sequencing of the 3′ end of its genome (Abou Ghanem-Sabanadzovic et al. 2003a).

Virions resembling those of GFkV were found in Italy in a leafroll-diseased vine of cv. Red Globe. Since this vine was not infected by GFkV, a more in-depth investigation was conducted, which disclosed that the virus under study was distinct from both GFkV and GAMaV. Thus, it was given the name of grapevine red globe

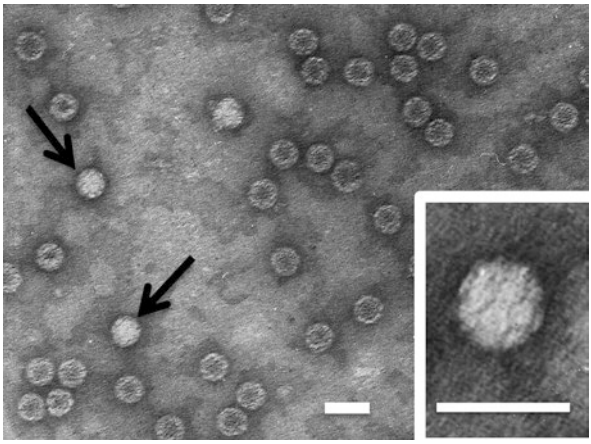

**Fig. 16.1** Electron micrograph of negatively stained partially purified preparation of grapevine asteroid mosaic-associated virus (GAMaV) containing two types of virus particles: empty particles (penetrated by the stain) and apparently intact particles with prominent surface structures (*arrows*). *Inset* shows a close-up of a virus particle. Magnification bars = 50 nm

virus (GRGV) (Sabanadzovic et al. 2000). GRGV is also biologically different from GFkV and GAMaV, as it does not elicit symptoms in *V. rupestris*.

Grafting on *V. rupestris* of budwood from a vine of cv. Sultanina of Greek origin with symptoms resembling those of asteroid mosaic (Kyriakopoulou 1991, Kyriakopoulou et al. 1993) resulted in the transient expression of mild chlorotic discoloration of the primary and secondary leaf veins (vein feathering), quite different from the reaction elicited in the same indicator by true asteroid mosaic sources (Boscia et al. 1994). Symptomatic *V. rupestis* vines contained another virus distantly related with those of members of the fleck complex (Elbeaino et al. 2001). This virus was later characterized and named grapevine rupestris vein feathering virus (GRVFV) (Abou Ghanem-Sabanadzovic et al. 2003a).

Grapevine Syrah virus 1 (GSyV-1) was discovered in 2009 as a result of a next-generation sequencing (NGS) run performed in the attempt to clarify the etiology of a decline syndrome affecting vines of cv. Syrah in California (Al Rwahnih et al. 2009). Contemporarily, during an investigation on the virome of native grapes in the Southeastern USA, an apparently new virus found in symptomless muscadine vines was described under the name grapevine virus Q (GVQ) (Sabanadzovic et al. 2009). Comparison of the GSyV-1 and GVQ genome organization and sequence disclosed that the two viruses are the same.

The advent of new technologies and the increased interest in grapevine research are continuing to add knowledge on GFkV and related viruses, especially about their distribution (Table 16.1) and genetic diversity. Hence, the ubiquitous nature of GFkV (Martelli 2014) was further confirmed by recent reports of this virus from India (Kumar et al. 2013), former Yugoslavian Republic of Macedonia (Kostadinovska et al. 2014), the UK (Immanuel et al. 2015), and Canada (Poojari

**Table 16.1** Current geographic distribution of grapevine fleck virus-like viruses

| Virus | Country (state) |
|---|---|
| GFkV | Worldwide |
| GRVFV | Greece, Italy, USA (California, New York), South Africa[GB], Brazil[GB], Spain[GB] |
| GAMaV | USA (California, New York), Canada |
| GRGV | Greece, Albania, Italy, USA (New York), France, China, Brazil[GB] |
| GSyV-1 | USA (California, Washington, Mississippi, North Carolina, New York), Italy, France, Slovakia, Czech Republic, Chile, South Africa, Brazil, Canada, Hungary, Australia[a] |

GB – data available in GenBank
[a]– reported as GFkV 'variant 416' by Shi et al. (2003)

**Table 16.2** Percent amino acid identity among RdRp (above diagonal) and CP (below diagonal) encoded by grapevine fleck virus (GFkV) and related viruses: grapevine red globe virus (GRGV), grapevine rupestris vein feathering virus (GRVFV), grapevine asteroid mosaic-associated virus (GAMaV), and grapevine Syrah virus 1 (GSyV-1)

| | GFkV | GRGV | GRVFV | GAMaV | GSyV-1 |
|---|---|---|---|---|---|
| GFkV | | 62.2 | 62.2 | 64.4 | 65.5 |
| GRGV | 38.6 | | 61.2 | 65.3 | 61.2 |
| GRVFV | 31.2 | 30.6 | | 73.0 | 68.4 |
| GAMaV | 30.6 | 31.8 | 39.7 | | 68.9 |
| GSyV-1 | 32.0 | 31.6 | 57.7 | 36.2 | |

et al. 2016). Published reports on the presence of GSyV-1 in South America (Engel et al. 2010), Central Europe (Glasa et al. 2015; Czotter et al. 2016), and South Africa (Oosthuizen et al. 2016) and unpublished data from Brazil (GenBank Acc No KX130754) suggest a distribution pattern similar to that of GFkV. The occurrence of GRGV is documented from Greece, Italy (Pantaleo et al. 2010), France (Beuve et al. 2015), China (Fan et al. 2016), Spain (Cretazzo et al. 2017), and Brazil (GenBank Acc No KR107538), while GRVFV appears to have a more restricted distribution. GAMaV, until recently known to infect vines only in the USA (California, New York), has been reported from Canada (Xiao and Meng 2016) as well as from Uruguay (Jo et al. 2015) (Table 16.2).

## Taxonomy and Nomenclature

The current classification (Dreher et al. 2012) assigns GFkV to the homonymous type species of the genus *Maculavirus*, while GSyV-1 belongs to a recognized definitive species in the genus *Marafivirus* (both in the family *Tymoviridae,* order *Tymovirales*).

Other GFkV-like viruses have not been officially classified yet, mainly due to the lack of complete genome sequences. Nevertheless, the tentative taxonomic position of these viruses could be inferred with a relatively high confidence based upon the

available information on particle morphology, physicochemical properties, partial genome sequences, pairwise comparisons, and phylogenetic analyses (Martelli et al. 2002a, b). Grouping of GAMaV and GRVFV with recognized species in the genus *Marafivirus* in phylogenetic trees constructed with taxonomically relevant genes (i.e., RdRp and CP) strongly suggests that they represent distinct species worth classification in the said genus. This taxonomic allocation has been supported by recent data on GAMaV and GRVFV complete genome sequences (Vargas-Asencio et al. 2017; N. Aboughanem-Sabanadzovic, unpublished data). Therefore, their formal recognition as members of the genus *Marafivirus* is expected to happen soon. Likewise, similarities in the organization of the 3′ end of genomic RNA and the phylogenetic affiliation with GFkV in both RdRp and CP genes support the notion that GRGV belongs in the genus *Maculavirus* (family *Tymoviridae*) as a member in its own right. Nevertheless, at present, GAMaV, GRVFV, and GRGV are still awaiting their official taxonomic recognition.

## Genome Structure, Genome Expression, and Replication

Complete genome sequences are currently available for GFkV (Sabanadzovic et al. 2001), GSyV-1 (Al Rwahnih et al. 2009; Sabanadzovic et al. 2009; Glasa et al. 2015), GAMaV (Vargas-Asencio et al. 2017), and GRVFV (N. Aboughanem-Sabanadzovic, unpublished information). Partial data on GRGV genome comprises nucleotide sequences of the genes involved in replication (MTR and RdRp), as well as the viral coat protein (CP) cistron.

Albeit there are differences in the organization of the different GFkV-like virus genomes, all of them share several common features, as they are (i) made up of a single molecule of positive-sense single-stranded RNA, (ii) polyadenylated at the 3′ end, (iii) presumably capped at the 5′ terminus, (iv) rich in cytidine (exceeding 40% of the nucleotide content), and (v) expressed via a combination of posttranslational processing of a large precursor polyprotein into several mature proteins involved in viral replication and synthesis of 3′ coterminal subgenomic RNA molecules as templates for CP translation (Fig. 16.2).

## Grapevine fleck virus *(GFkV)*

The GFkV genome is the largest of all (Fig. 16.3a). It consists of 7.5-kb-long, polyadenylated, and extremely cytosine-rich (50% of the total content) RNA molecule, comprising four open reading frames (ORFs) and untranslated regions (UTRs) of 291 and 35 nt at the 5′ and 3′ ends, respectively. ORF1 codes for a putative polyprotein with estimated molecular mass of 215 kDa that contains signature motifs of several domains involved in the replication cycle of viruses belonging in the family *Tymoviridae* (order *Tymovirales*), namely, methyltransferase (MTR), papain-like

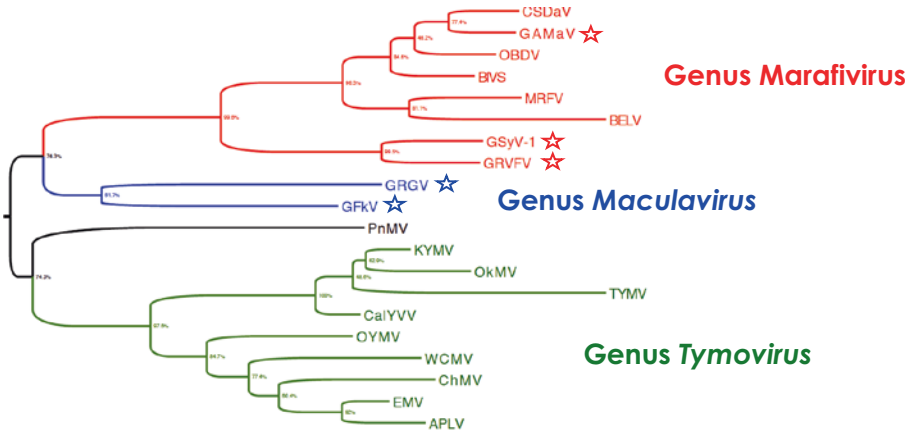


**Fig. 16.2** Maximum likelihood-based phylogenetic tree showing the relationships of grapevine fleck virus and related viruses (indicated by *stars*) with approved and putative members in the family *Tymoviridae*. The tree is based on the amino acid sequences of viral RdRps and was generated with MEGA 6.06. (Tamura et al. 2013) under the best-fit substitution model (LG + G) for amino acid dataset. Bootstrap percentage values out of 1000 replicates are shown on the nodes. The three genera of the family *Tymoviridae* are color coded. Names, abbreviations, and GenBank accession numbers of viruses used for generating the tree are Andean potato latent virus (APLV, AF035402), Calopogonium yellow vein virus (CalYVV, AAC58458), chayote mosaic virus (ChMV, AF195000), citrus sudden death-associated virus (CSDaV, NC_006950), eggplant mosaic virus (EMV, J04374), grapevine asteroid mosaic-associated virus (GAMaV, AJ249358), grapevine fleck virus (GFkV, NC_003347), grapevine red globe virus (GRGV, AJ249360), grapevine rupestris vein feathering virus (GRVFV, AY128949), Kennedya yellow mosaic virus (KYMV, NC_001746), maize rayado fino virus (MRFV, AF265566), oat blue dwarf virus (OBDV, U87832), okra mosaic virus (OkMV, AF035202), ononis yellow mosaic virus (OYMV, J04375), poinsettia mosaic virus (PnMV, NC_002164), turnip yellow mosaic virus (TYMV, NC_004063), and wild cucumber mosaic virus (WCMV, AF035633)

protease (PRO), helicase (HEL), and RNA-dependent RNA polymerase (RdRp). Because of the similarity in domain content with similar proteins encoded by tymoviruses, it is assumed that mature proteins are produced via autocatalytic cleaving of the 215 kDa precursor polyprotein. An in-frame ORF2 is separated from ORF1 by a double stop codons and potentially codes for a 24.5 kDa protein identified as the viral CP. ORF3 and ORF4 are 3′ coterminal and potentially code for proteins of 31 and 16 kDa, respectively, both rich in proline and serine. The role of these two putative proteins in the life cycle of GFkV is still unknown. Expression of ORFs coding for the putative CP, p31, and p16 is likely ensured by the synthesis of at least two subgenomic RNAs produced in infected tissue and occasionally encapsidated in virus particles (Sabanadzovic et al. 2001). However, expression of these two putative ORFs is yet to be experimentally confirmed.

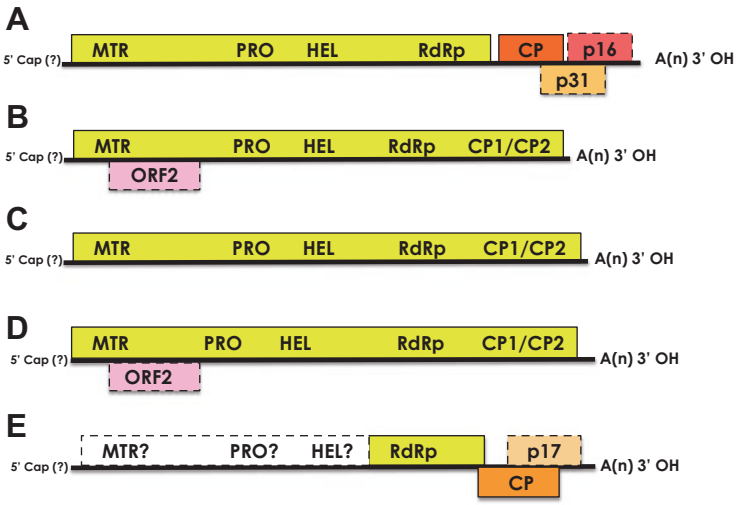


**Fig. 16.3** Diagrammatic representation of complete genomes of grapevine fleck virus (**a**), grapevine Syrahvirus 1 (**b**), grapevine rupestris vein feathering virus (**c**), grapevine asteroid mosaic-associated virus, (**d**) and the partially sequenced genome of grapevine red globe virus (**e**). *Boxes* represent large ORFs and corresponding putative products; *lines* represent untranslated genomic regions (UTRs) at the genome extremities. Known genome sequences are depicted with *full lines*, while *dotted lines* represent parts of genomes yet to be sequenced, or ORFs yet to be confirmed functional/expressed in plants. Schemes are not to scale. Abbreviations: *MTR* = methyltransferase, *PRO* = endopeptidase/protease, *HEL* = helicase, *RdRp* = RNA-dependent RNA polymerase, *CP* = coat protein. Not to scale

## Grapevine Syrahvirus1 *(GSyV-1)*

Genomes of GSyV-1 isolates from cv. Syrah affected by a decline syndrome (Al Rwahnih et al. 2009) and from symptomless muscadines (Sabanadzovic et al. 2009) are colinear; consist of 6481 nucleotides, excluding the polyA tail at the 3′ end; and contain two ORFs (Fig. 16.3b). ORF1 represents the majority (95%) of the genome and potentially codes for a polyprotein with an estimated molecular mass of 230 kDa. The three fourths of this polypeptide, located at the N-terminus, is characterized by the presence of conserved motifs of MTR, P-PRO, HEL, and RdRp, while the carboxy coterminal part encodes two CPs with an estimated size of 23 kDa and 21 kDa, respectively. An additional, small ORF, potentially coding for a polypeptide with a molecular mass of 26–27 kDa, is present near the 5′ end. It is not clear whether this ORF is expressed *in planta* or not and what its role is. However, its expression product is rich in proline and serine and shares 43% identical residues with p43, a protein putatively expressed by an ORF present in the genome of maize rayado fino virus (MRFV), a typical *Marafivirus* (Hammond and Ramirez 2001).

The unique feature of the GSyV-1 genome is the structural permutation of the characteristic RdRp motifs A-B-C that form the active site (Sabanadzovic et al.

2009). Unlike other plant viruses, the 21 amino acid long motif C of the viral RdRp is relocated upstream of the motif A to form an unusual C-A-B sequence. Such permutation does not seem to occur in other positive-sense alphavirus-like RNA viruses. Its biological implications are yet to be understood.

Full genome sequences of additional three GSyV-1 isolates from Central Europe have recently been published (Glasa et al. 2015), two of which contain an extra nucleotide compared with the isolates from California and Mississippi.

## Grapevine rupestris vein feathering virus *(GRVFV)*

Published partial nucleotide sequences comprising the viral MTR, RdRp, and CPs (Abou Ghanem-Sabanadzovic et al. 2003a) substantiated by unpublished data on its complete genome (S. Sabanadzovic and N. Aboughanem-Sabanadzovic, unpublished information) indicate the close relationships of GRVFV with extant members of the genus *Marafivirus* (family *Tymoviridae*). The 6.7 kb GRVFV genome (Fig. 16.3c) is monocistronic and closely resembles that of oat blue dwarf virus (OBDV) in organization. The large ORF codes for a putative polyprotein of approx 234 kDa containing the conserved motifs of proteins involved in virus replication and, possibly, two CPs with estimated molecular masses of 23 kDa and 21 kDa. The marafibox precedes two AUG codons, the putative initiation sites for the translation of CPs.

## Grapevine asteroid mosaic-associated virus *(GAMaV)*

For more than a decade, the knowledge about GAMaV genome was limited to partial sequences of isolate USA9 encompassing a fragment of the viral MTR and the 3′ region comprising the RdRp domain and two CPs (Abou Ghanem-Sabanadzovic et al. 2003a). Nevertheless, genome of GAMaV isolate GV30 has been completely sequenced very recently (Vargas-Asencio et al. 2017). The complete genome of GAMaV is 6719 nt long excluding a poly(A) tail and shares 94% identical nucleotides with isolate USA9. Computer analysis identified the presence of a unique large ORF encoding a possible polyprotein of 2158 aa with recognized conserved domains of MTR, PRO, HEL, RdRp, and CPs and a genome organization similar to that of the marafiviruses (Dreher et al. 2012). The large ORF is preceded and followed by 129 and 116 nt long 5′ and 3′ untranslated regions, respectively (Vargas-Asencio et al. 2017). The putative subgenomic RNA promoter (“marafibox”) has been identified in the GAMaV genome upstream of the possible initiation sites for translation of two putative CPs with estimated molecular mass of 24 kDa and 21 kDa, respectively (Abou Ghanem-Sabanadzovic et al. 2003a; Vargas-Asencio et al. 2017). An additional 5′ proximal ORF, with a potential of coding for a putative product of 38.5 kDa but lacks a canonical AUG start codon, has been identified in GAMaV genome.

## Grapevine red globe virus *(GRGV)*

The available nt sequence of GRGV comprises a stretch of approximately 2 kb at the 3′ end of the genome (Fig. 16.3e). This genomic fragment is characterized by a high cytosine content (exceeding 41%); comprises three putative ORFs, which are followed by a 139-long 3′ NCR; and terminates with a polyA tract (Abou Ghanem-Sabanadzovic et al. 2003a). The first ORF is partially sequenced and codes for the C-proximal part of the viral replicase, containing the RdRp domain. The second ORF partially overlaps ORF1 and encodes a 25 kDa peptide identified as CP. The 3′-proximal possible ORF3 codes for a putative 17 kDa protein characterized by an unusually high proline content (31%). Whether ORF3 is expressed *in planta* and what could be the possible role of protein p17 in the life cycle of GRGV is still unknown (Abou Ghanem-Sabanadzovic et al. 2003a).

## Genetic Diversity and Population Structure

More than 80 partial nt sequences, along with the complete genome of isolate MT48 from Italy (Sabanadzovic et al. 2001), are currently available for GFkV in GenBank (database accessed in late November 2015). The majority of deposited sequences are representative of viral RdRp and CP genes. However, many of them are too short and not sufficiently informative.

The most significant study on GFkV population targeting the viral CP gene was carried out in Slovakia (Glasa et al. 2011). Sequencing this gene from 36 GFkV isolates collected from local grapevines and its comparison with sequences from databases revealed nt identities ranging from 88 to 100%, with a maximum divergence of 12% between a few Slovakian isolates and the type strain MT48. Most of the mutations were silent, and a maximum divergence of 5% was found in the amino acid (aa) content. The same study showed that the analyzed virus isolates clustered in two distinct molecular groups and suggested that the CP gene is under a negative selection pressure (Glasa et al. 2011). The presence of two distinct molecular groups with a significant range of intergroup variability (up to 10%) was also reported for a dozen of GFkV isolates from Idaho (Kanuya et al. 2012). The CP of several GFkV isolates from Washington State in the USA showed 94–95% nt sequence identity with the type isolate (Mekuria and Naidu 2010).

Eight complete (or nearly complete) nt sequences are available for GSyV-1, including those of two isolates from California (Acc. no. FJ436028; Al Rwahnih et al. 2009) and Mississippi (Acc. No. FJ977041; Sabanadzovic et al. 2009) and of six additional isolates: two from Slovakia, one from the Czech Republic (Acc. nos. KP221255-257; Glasa et al. 2015), plus two sequences of Brazilian isolates (Acc. nos. KT037017 and KR153306) and one from Canada (Acc. no. JX513896). Direct comparison shows that significant nt differences exist among these isolates. In particular, the Brazilian isolate “MH” was the most divergent and uniformly distinct

from all the others (nt identity 82.5–83.8%). Interestingly, the three GSyV-1 isolates from Central Europe were more diverse (max nt difference 7.1%) than the three representatives from North America (only 1.2% intragroup divergence) (Glasa et al. 2015).

As to GRVFV, based upon available data from four isolates (two complete or near-complete genome sequences from Greece and California and two partial CP sequences from Brazil), this virus appears to be rather divergent, as pairwise comparisons between CP sequences indicated an inter-isolate variability of up to 14%.

Comparison of CP sequences between two of GAMaV sequences (one from the original source and one from Italy) showed a diversity of 5% at the nt level (4% in the aa content) in a very small genome segment. Additionally, GRGV has been recently reported from France upon Illumina sequencing of a Cabernet franc plant showing fanleaf-like symptoms (Beuve et al. 2015). Two contigs matched the viral replicase and shared 85% nt identity with the "type" isolate (Abou Ghanem-Sabanadzovic et al. 2003a), while partial CP sequences of the two isolates shared 92% common nucleotides.

## Detection and Diagnosis

Diverse methods, spanning from traditional to modern ones, have been developed and are currently available for detection and identification of GFkV and related viruses.

### *Biological Methods*

Infections by GFkV and some related viruses (i.e., GAMaV and GRVFV) can readily be discriminated from those of any other grapevine disease (and from each other) by the specific responses of *V. rupestris* in which fleck usually induces localized translucent spots of the young leaves due to clearing of the veins of lower (third and fourth) order (Fig. 16.4a). Severe forms of fleck cause stunting, poor rooting, and reduced graft take of nursery productions (Triolo and Materazzi 1987; Credi and Babini 1996), whereas asteroid mosaic elicits creamy-yellow bands along the major veins of the leaves, which are twisted and asymmetric (Fig. 16.4b). A transient chlorotic discoloration (feathering) of the primary and secondary veins is the reaction to GRVFV (Fig. 16.4c). GRGV does not seem to induce any particular symptomatology, and the possible effects of GSyV-1 are yet to be studied.

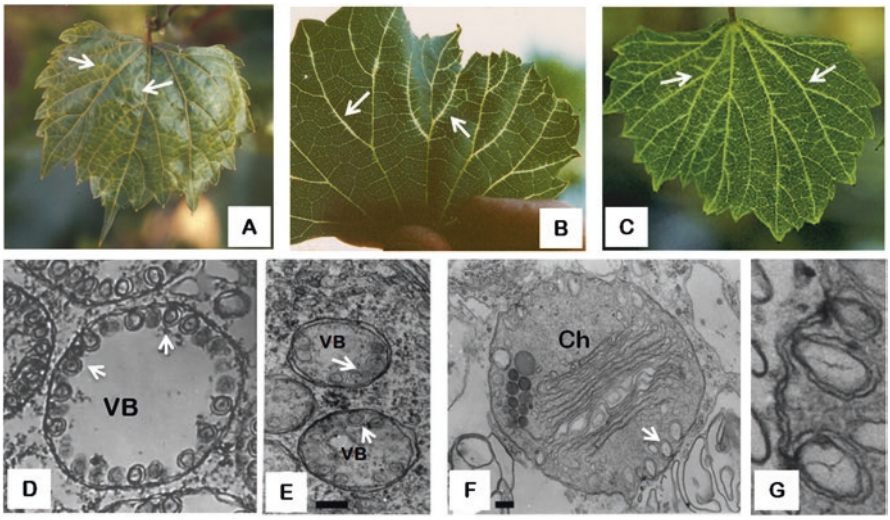


**Fig. 16.4** Effects of infections by grapevine fleck virus and related viruses in grapevines. Symptoms of different diseases of the grapevine fleck complex induced in the specific indicator *Vitis rupestris* "St. George": fleck, clearing of the tertiary and quaternary veins (**a**); asteroid mosaic, strong clearing of primary and secondary veins (**b**); and vein feathering, transient vein discoloration (**c**). Cytopathic structures associated with grapevine infections by GFkV and similar viruses. Vesiculated bodies (VB), originating from mitochondria, present in differentiating sieve tubes and companion cells of GFkV- (**d**) and GAMaV-infected (**e**) grapevines. VBs are characterized by the presence of peripheral double-membrane vesicles (arrows) that line the mitochondrial bounding membrane. Rounded and altered chloroplasts (Ch) with numerous double-membrane vesicles (**f**) containing fine fibrillar material in sieve tubes and companion cells of GRGV-infected plants. A close-up of the chloroplast vesicles is in panel (**g**) (Images c and d–g reproduced from Martelli et al. 2015 and Sabanadzovic et al. 2000, respectively)

## *Serological Methods*

Polyclonal antisera and monoclonal antibodies to GFkV have been raised (Boscia et al. 1991a, 1995; Schieber et al. 1997), and commercial ELISA kits are now available for routine diagnosis of this virus. There are no serological tools for the detection of the other GFkV-related viruses.

## *Molecular Methods*

Molecular detection of GFkV was initially based on the use of a nonradioactive cRNA probe for virus identification in dot spot, Northern blot, and tissue blot assays (Sabanadzovic et al. 1996). The same approach was used for GAMaV and GRGV (Elbeaino et al. 2001).

The first RT-PCR detection system was developed in the mid-1990s (Sabanadzovic et al. 1996). It was followed by the design of a number of virus-specific or "universal" primers allowing RT-PCR-based detection of GFkV and allied viruses, either in single- or multiple-target formats. Sets of degenerate primers targeting the MTR and RdRp genes were designed and successfully used on purified viral dsRNAs or total nucleic extracts for the nondiscriminative recognition of GFkV, GAMaV, and GRGV, the three viruses known at that time (Sabanadzovic et al. 2000). Another set of degenerate primers, targeting the viral helicase gene, was developed soon afterward and used for the simultaneous detection of these viruses, while, in the same study, DIG-RNA probes were successfully employed for the selective identification of the different viruses in dot spot hybridization tests (Elbeaino et al. 2001).

Degenerate primers targeting the viral RdRp, developed in 2000 and modified in 2009, proved most useful for the routine and nondiscriminative detection of these viruses, as well as other marafi- and maculaviruses in plants other than grapevines, such as blackberry and ranunculus (Sabanadzovic et al. 2009; S. Sabanadzovic, unpublished information). Because of the specific targeted region that encompasses the permuted motif in GSyV-1, these primers discriminate GSyV-1 from related viruses because of the different size of the amplified products due to an insertion of 63 nt (344 bp in GFkV, GRVFV, GRGV, and GAMaV vs 407 bp in GSyV-1).

Several sets of GFkV-specific primers targeting different parts of the viral genome were described and used in two-step or single-tube assays for the recognition of this virus in single (Sabanadzovic et al. 1996; Osman and Rowhani 2006; Mekuria and Naidu 2010; Glasa et al. 2011) or mix infections with other viruses (Gambino and Gribaudo 2006). A quantitative real-time PCR (qRT-PCR) assay based on TaqMan chemistry was developed for GFkV in single (Osman et al. 2008; Bertolini et al. 2010) or multiple reactions (López-Fabuel et al. 2013). In addition, Low Density Arrays have been designed for GFkV along with other 12 grapevine viruses (Osman et al. 2008). The TaqMan-based real-time RT-PCR approach was successfully developed and applied for quantitation of several viruses in field-grown samples of cv. Nebbiolo, including GFkV (Pacifico et al. 2011).

GAMaV-, GRVFV-, and GRGV-specific RT-PCR assays have also been finalized (Sabanadzovic et al. 2000; Abou Ghanem-Sabanadzovic et al. 2003b), whereas another set of GRGV-specific primers has more recently been employed for its detection in France (Beuve et al. 2015).

Although several sets of primers had been designed for the RT-PCR detection of GSyV-1 immediately following the virus discovery and characterization (Al Rwahnih et al. 2009, Sabanadzovic et al. 2009; Mekuria and Naidu 2010), the availability of sequences of multiple virus isolates has led to conceiving improved primers targeting the viral CP gene, which are based on the highly conserved nucleotide sequences shared by diverse GSyV-1 isolates (Glasa et al. 2015). These primers confirmed the presence of an inter-isolate molecular diversity and allowed the detection of 29 divergent GSyV-1 isolates in a survey carried out in Central Europe (Glasa et al. 2015), compared to only 15 identified by the primers designed on the original sequence of the Californian virus isolate (Al Rwahnih et al. 2009).

Finally, multiple virus-specific probes were designed for GFkV-like viruses in the framework of an "universal" crop-specific microarray platform, aimed at detecting any of the 38 most important or emerging grapevine viruses, which successfully identified the targeted viruses in single or multiple infections, including GFkV and allied viruses (Thompson et al. 2014).

## Host Range and Transmission

GFkV and related viruses are known to infect only *Vitis* spp., except for GSyV-1, which has a natural host range comprising also wild blackberries (*Rubus* sp.), muscadine (*Vitis rotundifolia*), and summer grape (*Vitis aestivalis*) (Sabanadzovic et al. 2009).

Reports from Japan (Yamakawa 1989), South Africa (Engelbrecht and Kasdorf 1990), and Italy (Fortusini et al. 1996) of natural field spread of GFkV have not been experimentally confirmed, nor the putative vector has been identified. Mechanisms of natural spread of GRGV, GRVFV, and GAMaV, if any, are also unknown. Although GSyV-1 was detected in adults of the variegated leafhopper (*Erythroneura variabilis*) collected from infected grapevines in California (Al Rwahnih et al. 2009), no transmission trials have been reported.

Thus, the primary way of long-distance dissemination of GFkV-like viruses is through propagative material (budwood, rootstocks, nursery productions) and, at a site, by grafting. None of these viruses is mechanically transmissible to herbaceous indicators, and GFkV is not seed transmissible (Hévin et al. 1973). Dodder-mediated grape-to-grape transmission of the fleck agent to 50% of acceptor *V. rupestris* plants was obtained in Australia (Woodham and Krake 1983). In some cases, typical fleck symptoms appeared 3 weeks after dodder bridge establishment. Anyway, dodder-mediated transmission from the same grape donors to ten different herbaceous hosts failed.

## Cytopathology, Tissue Tropism, and Virus-Host Interactions

The cytopathology of grapevine cells infected by GFkV, GAMaV, and GRVFV has been described (Castellano et al. 1983; Castellano and Martelli 1984; Sabanadzovic et al. 2000). Ultrastructural modifications are restricted to phloem tissues (differentiating sieve elements, companion cells, phloem parenchyma cells) in which virus particles usually occur, either in a scattered form, in disorderly aggregates, or in crystalline arrays.

GFkV-infected cells show secondary vacuolation and proliferation of membranes appearing as vesicles scattered in the cytoplasm or aggregated in paramural bodies. Whereas nuclei are apparently unaltered, chloroplasts and mitochondria are modified. Mitochondrial alterations are much more prominent and common, often affecting the totality of such organelles present in a cell. The ultrastructural changes suffered by mitochondria consist of a series of modifications that initiate with the

development of peripheral double-membraned vesicles, originated by the invagination of the organelle's bounding membrane. The increase in the number of the vesicles is accompanied by the progressive loss of the cristae and thinning of the stroma (Castellano and Martelli 1984). The outcome of these transformations is a cytopathic structure with an apparently empty electron-lucent center, peripherally lined with vesicles, to which the name of vesiculated body (VB) was given (Castellano et al. 1983) (Fig. 16.4d). VB vesicles are flask shaped, their neck opening to the surrounding cytoplasm, and contain a network of fine fibrils which, by analogy with the comparable material present in the vesiculated organelles typical of tombusvirus (Rubino et al. 2014) and tymovirus (Lesemann 1977) infections, are thought to be viral RNA.

Cells from foliar and root tissues of *V. rupestris* affected by asteroid mosaic possess an apparently normal and well-preserved organization and structure. However, some of the root cells contained round, double-membrane structures with peripheral vesicles of *ca* 80 nm in diameter, resembling the VB associated with GFkV infections (Fig. 16.4e). Unlike GFkV-infected cells, which usually contain plenty of virions, these were not seen in the examined samples of GAMaV-infected *V. rupestris* (Sabanadzovic et al. 2000).

Differentiating sieve tubes and companion cells of GRVFV-infected grapevine tissues contained chloroplasts with a rounded shape, a heavily modified internal structure and flask-shaped double-membraned vesicles lining their periphery (Fig. 16.4f, g) (Sabanadzovic et al. 2000). These vesicles had the fibrillar content suggestive of viral dsRNA. Thus, contrary to GFkV and GAMaV infections, where VB originate from altered mitochondria, GRVFV appears to target chloroplasts.

## Pathological Properties, Associated Diseases, and Their Impact

Infections by GFkV and related viruses are latent or semi-latent in most *V. vinifera* cultivars and American rootstocks (Martelli 2014; Martelli et al. 2015). The exceptions are the Californian asteroid mosaic and asteroid-like disease from Greece, both of which are characterized by chlorotic star-shaped spots on the leaves of some cultivars (Hewitt 1954; Kyriakopuolou 1991). As mentioned, putative agents of these two diseases are GAMaV and GRVFV (Sabanadzovic et al. 2000, Elbeaino et al. 2001, Abou Ghanem-Sabanadzovic et al. 2003a). GRGV was isolated from a vine of cv. Red Globe and did not induce any specific symptom upon grafting on *V. rupestris* (Abou Ghanem-Sabanadzovic et al. 2003a and unpublished information). GSyV-1, originally discovered in a decline-affected vine, does not seem to have a bearing in this disorder (Al Rwahnih et al. 2009) and was also found in muscadines with no obvious symptoms (Sabanadzovic et al. 2009).

There are few additional diseases described in the literature that suggest the involvement of GFkV-like and still undescribed virus(es). One of them is "rupestris necrosis," reported from Japan (Matsumoto and Ohki 1998), which owes its name to

the reaction of *V. rupestris* grafted with buds from a symptomless vine of cv. Abujiaoxi of Chinese origin, consisting of localized necrosis of shoots, leaf petioles, and secondary veins. Symptomatic indicators contained isometric virus particles morphologically resembling those of GFkV and mitochondria-derived VB were observed in thin-sectioned cells. Finally, studies carried out in Brazil suggest the presence of at least three GFkV-like viruses (referred to as "three distinct strains") based upon the differential reaction of *V. rupestris* and other indicators (Kuniyuki and Costa 1995).

## Strategies for Control and Management

Because of the latency of symptoms, sanitary selection of *V. vinifera* cultivars and most American rootstock hybrids is ineffective. However, GFkV-free mother stocks are readily obtained as this virus is efficiently eliminated by an array of different techniques, i.e., heat therapy (Ottenwaelter et al. 1973), fragmented shoot apex culture (Barlass et al. 1982), meristem-tip culture (Boscia et al. 1991a), and micrografting of shoot apices (Spilmont et al. 2012). The same sanitation procedures may also operate successfully with the other viruses of the complex, but no experimental data are available.

## Conclusions and Future Research Directions

Pathogenicity and epidemiology are two little explored aspects of the behavior of viruses of the fleck complex. As a whole, these viruses are retained as being little or no pathogenic. For example, the grapevine certification scheme enforced in the European Union considers only GFkV for exclusion, limitedly to the American rootstocks (Maliogka et al. 2015). Nevertheless, the heavy damage that some of the viruses inflict to major organelles, such as mitochondria (GFkV in particular) and chloroplasts, could have a bearing on plant health that may be worth investigating.

Likewise, experimental confirmation should be sought of the field observations from Japan (Yamakawa 1989), South Africa (Engelbrecht and Kasdorf 1990), and Italy (Fortusini et al. 1996), reporting natural spread of GFkV in vines showing leafroll symptoms. It seems plausible to hypothesize that GFkV, which occupies the same ecological niche (sieve tubes) of ampeloviruses and vitiviruses, may be moved by the same vectors that spread these pathogens (Martelli 2014).

**Acknowledgments** The authors would like to thank all scientists that contributed to the current knowledge on these viruses, providing an inspiration and a valuable source of information for the preparation of this chapter. Thanks are extended to various funding agencies that allowed studies on these viruses at the University of Bari and Mississippi State University in particular to Mississippi Agricultural and Forestry Experiment Station (MAFES) Strategic Research Initiative and NIFA Hatch Projects for financial support to SS and NA.

## References

Abou Ghanem-Sabanadzovic, N., S. Sabanadzovic, and G.P. Martelli. 2003a. Sequencing of the 3′ end of three grapevine fleck virus-like viruses. *Virus Genes* 27: 11–16.

Abou Ghanem-Sabanadzovic, N., S. Sabanadzovic, A. Rowhani, and G.P. Martelli. 2003b. Multiplex RT-PCR detection of grapevine fleck virus-like viruses in grapevine with coamplification of control plant mRNA. Extended abstracts 14th meeting of ICVG, Locorotondo, Italy: 195.

Al Rwahnih, M., S. Daubert, D.A. Golino, and A. Rowhani. 2009. Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that included a novel virus. *Virology* 387: 395–401.

Barlass, M., K.G.M. Skene, R.C. Woodham, and L.R. Krake. 1982. Regeneration of virus-free grapevines using in vitro apical culture. *Annals of Applied Biology* 101: 291–295.

Bertolini, E., J. Garcia, A. Yuste, and A. Olmos. 2010. High prevalence of viruses in table grape from Spain detected by real-time RT-PCR. *European Journal of Plant Pathology* 128: 283–287.

Beuve, M., T. Candresse, M. Tannières, and O. Lemaire. 2015. First report of grapevine redglobe virus in grapevine in France. *Plant Disease* 99: 422.

Boscia, D., G.P. Martelli, V. Savino, and M.A. Castellano. 1991a. Identification of the agent of grapevine fleck disease. *Vitis* 30: 97–105.

Boscia, D., V. Savino, G.P. Martelli, and M.A. Castellano. 1991b. Association of a phloem-limited non mechanically transmissible isometric virus with grapevine fleck disease. Proceedings 10th meeting of ICVG, Volos, Greece: 173–174.

Boscia, D., S. Sabanadzovic, V. Savino, P.E. Kyriakopoulou, and G.P. Martelli. 1994. A non mechanically transmissible virus associated with asteroid mosaic of the grapevine. *Vitis* 33: 101–102.

Boscia, D., V. Elicio, V. Savino, and G.P. Martelli. 1995. Production of monoclonal antibodies to grapevine fleck virus. *Plant Pathology* 44: 160–163.

Boulila, M., D. Boscia, B. Di Terlizzi, M.A. Castellano, A. Minafra, V. Savino, and G.P. Martelli. 1990. Some properties of a phloem-limited non mechanically transmissible grapevine virus. *Journal of Phytopathology* 129: 151–158.

Castellano, M.A., G.P. Martelli, and V. Savino. 1983. Virus-like particles and ultrastructural modifications in the phloem of leafroll-affected grapevines. *Vitis* 22: 23–39.

Castellano, M.A., and G.P. Martelli. 1984. Ultrastructure and nature of vesiculated bodies associated with isometric virus-like particles in diseased grapevines. *Journal of Ultrastructure Research* 89: 56–64.

Castellano, M.A., G.P. Martelli, V. Savino, and D. Boscia. 1985. Progress in the study of the phloem-limited isometric virus-like particles associated with leafroll-diseased grapevines. *Phytopathologia Mediterranea* 24: 165–169.

Credi, R., and A.R. Babini. 1996. Effect of virus and virus-like infections on the growth of grapevine rootstocks. *Advances in Horticultural Science* 10: 95–98.

Cretazzo E., C.V. Padilla, and L. Velasco, 2017. First report of grapevine red globe virus in grapevine in Spain. Plant Disease (in press) Doi:org/10.1094/PDIS-06-16-0932-PDN

Czotter, N., E. Szabo, J. Molnar, L. Kocisz, T. Deák, G. Bisztray, G. Tusnády, J. Burgyán, and Várallyay E. 2016. First description of grapevine Syrah virus-1 from grapevine in Hungary. Journal of Plant Pathology 97 (in press).

Dreher, T.W., M.C. Edwards, A.J. Gibbs, A.-L. Haenni, R.W. Hammond, I. Jupin, R. Koenig, S. Sabanadzovic, and G.P. Martelli. 2012. Family *Tymoviridae*. In *Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses*, ed. A.M.Q. King, M.J. Adams, E.B. Carstens, and E.J. Lefkowitz, 944–952. Amsterdam: Elsevier-Academic Press.

Elbeaino, T., S. Sabanadzovic, M. Digiaro, N. Abou Ghanem-Sabanadzovic, A. Rowhani, P.E. Kyriakopoulou, and G.P. Martelli. 2001. Molecular detection of grapevine fleck virus-like viruses. *Vitis* 40: 65–68.

Engel, E.A., P.A. Rivera, and P.D.T. Valenzuela. 2010. First report of grapevine Syrah virus 1 in Chilean grapevines. *Plant Disease* 94: 633.

Engelbrecht, D.J., and G.G.F. Kasdorf. 1990. Field spread of corky bark, fleck, leafroll and Shiraz decline diseases and associated viruses in South African grapevines. *Phytophylactica* 22: 347–354.

Fan, X.D., Y.F. Dong, Z.P. Zhang, F. Ren, G.J. Hu, Z.N. Li, and J. Zhou. 2016. First report of grapevine red globe virus (GRGV) in China. *Plant Disease* 100: 2340.

Fortusini, A., G. Scattini, S. Cinquanta, and S. Prati. 1996. Diffusione naturale del virus 1 (GLRaV-1) e del virus 3 (GLRaV-3) dell'accartocciamento fogliare e del virus della maculatura infettiva o "fleck" (GFkV) della vite. *Informatore Fitopatologico* 46: 39–43.

Gambino, G., and I. Gribaudo. 2006. Simultaneous detection of nine grapevine viruses by multiplex reverse transcription-polymerase chain reaction with coamplification of a plant RNA as internal control. *Phytopathology* 96: 1223–1229.

Glasa, M., L. Predajňa, and P. Kominek. 2011. Grapevine fleck virus isolates split into two distinct molecular groups. *Journal of Phytopathology* 159: 805–807.

Glasa, M., L. Predajna, K. Soltys, S. Sabanadzovic, and A. Olmos. 2015. Detection and molecular characterization of grapevine Syrah virus 1 isolates from Central Europe. *Virus Genes* 51: 112–121.

Gugerli, P., B. Rosciglione, J.-J. Brugger, S. Bonnard, M.-E. Ramel, and F. Tremea. 1991. Further characterization of grapevine leafroll disease. Proceedings 10th meeting of ICVG, Volos, Greece: 59–60.

Hammond, R.W., and P. Ramirez. 2001. Molecular characterization of the genome of maize rayado fino virus, the type member of the genus *Marafivirus*. *Virology* 282: 338–347.

Hévin, M., M.M. Ottenwaelter, J.P. Doazan, and M. Rives. 1973. Investigating the transmission of marbrure and fanleaf through the seed in the grapevine. *Rivista di Patologia Vegetale (Ser. IV)* 9: 253–258.

Hewitt, W.B. 1954. Some virus and virus-like diseases of grapevines. *Bulletin California Deparment of Agriculture* 43: 47–64.

Hewitt, W.B., A.C. Goheen, D.J. Raski, and G.V. Gooding Jr. 1962. Studies on virus diseases of the grapevine in California. *Vitis* 3: 57–83.

Hewitt, W.B., A.C. Goheen, L. Cory, and C. Luhn. 1972. Grapevine fleck disease, latent in many varieties, is transmitted by graft inoculation. Annalen der Physik, NHS 43–47.

Immanuel, T.M., C. Delmiglio, L.I. Ward, J.O. Denton, and G.R.G. Glover. 2015. First reports of grapevine virus A, grapevine fleck virus and grapevine leafroll-associated virus 1 in the UK. *Plant Disease* 99: 898.

Jo, Y., H. Ghoi, J.K. Cho, J.-Y. Yoon, S.-K. Choi, and W.K. Cho. 2015. *In silico* approach to reveal viral populations in grapevine cultivar Tannat using transcriptome data. *Scientific Reports* 5: 15841.

Kanuya, E., L.A. Clayton, R.A. Naidu, and A.V. Karasev. 2012. First report of grapevine fleck virus in Idaho grapevines. *Plant Disease* 96: 1705.

Kostadinovska, E., S. Mitrov, P.A. Bianco, P. Casati, and D. Bulgari. 2014. First report of grapevine virus A and grapevine fleck virus in the former Yugoslav Republic of Macedonia. *Plant Disease* 98: 1747.

Kumar, S., L. Singh, L. Ferretti, M. Barba, A.A. Zaidi, and V. Hallan. 2013. Evidence of grapevine leafroll-associated virus 1–3, grapevine fleck virus and grapevine virus B occurring in Himachal Pradesh, India. *Indian J. Virology* 24: 66–69.

Kuniyuki, H., and A.S. Costa. 1995. Occorrencia de mais um isolado do virus do mosaico da nervuras da videira que no causa sintomas no porta-enxerto Kober 5BB. *Fitopatologia Brasileira* 20: 618–622.

Kyriakopoulou, P.E. 1991. Symptoms of grapevine asteroid mosaic in Greece. Proc. 10th meeting ICVG, Volos, Greece: 143–146.

Kyriakopoulou, P.E., S. Tzortzakaki, and M. Tsagris. 1993. Grapevine asteroid mosaic in Greece: positive indexing results and viroids associated. Extended abstracts 11th meeting ICVG, Montreux, Switzerland: 41.

Lesemann, D.E. 1977. Virus group-specific and virus-specific cytological alterations induced by members of the tymovirus group. *Phytopathologische Zeitschrift* 90: 315–336.

López-Fabuel, I., T. Wetzel, E. Bertolini, A. Bassler, E. Vidal, B.L. Torres, A. Yuste, and A. Olmos. 2013. Real-time multiplex RT-PCR for the simultaneous detection of five main grapevine viruses. *Journal of Virological Methods* 188: 21–24.

Maliogka, V.I., G.P. Martelli, M. Fuchs, and N.I. Katis. 2015. Control of viruses infecting grapevine. *Advances in Virus Research* 91: 175–227.

Martelli, G.P. 2014. Directory of virus and virus-like diseases of the grapevine and their agents. *Journal of Plant Pathology* 96 (1 Supplement): 136.

Martelli, G.P., A. Rowhani, and S. Sabanadzovic. 2015. The fleck complex. In *Compendium of grape diseases*, ed. W.F. Wilcox, W.D. Gubler, and J.K. Uyemoto, 2nd ed., 115–117. St. Paul: APS Press.

Martelli, G.P., S. Sabanadzovic, N. Abou Ghanem-Sabanadzovic, and P. Saldarelli. 2002a. *Maculavirus*, a new genus of plant viruses. *Archives of Virology* 147: 1847–1853.

Martelli, G.P., S. Sabanadzovic, N. Abou Ghanem-Sabanadzovic, M.C. Edwards, and T. Dreher. 2002b. The family *Tymoviridae*. *Archives of Virology* 147: 1837–1846.

Matsumoto, T., and S.T. Ohki. 1998. A possible new necrotic diseases of grapevine associated with small isometric particles and novel membrane-bound large particles. *Annals of the Phytopathological Society of Japan* 64: 560–564.

Mekuria, T.A., and R.A. Naidu. 2010. First report of grapevine virus sequences highly similar to Grapevine Syrah virus 1 from Washington vineyards. *Plant Disease* 94: 787.

Milkus, B. 1974. Mycoplasma- or chlamydia-like bodies in grape, affected by marbour. *Acta Physiologica Academiae Scientiarum Hungaricae* 9: 385–388.

Osman, F., and A. Rowhani. 2006. Application of a spotting sample preparation technique for the detection of pathogens in woody plants by RT-PCR and real-time PCR (TaqMan). *Journal of Virological Methods* 133: 130–136.

Osman, F., C. Leutenegger, D. Golino, and A. Rowhani. 2008. Comparison of low-density arrays, RT-PCR and real-time TaqMan RT-PCR in detection of grapevine viruses. *Journal of Virological Methods* 149: 292–299.

Oosthuizen, K., B. Coetzee, H. Maree, and J. Burger. 2016. First report of grapevine Syrah virus-1 in South African vineyards. *Plant Disease* 100: 1252.

Ottenwaelter, M.M., M. Hévin, P. Leclair, J.P. Doazan, and M. Rives. 1973. Heat therapy eliminates the ability to transmit the causal agent of "marbrure" in several *V. vinifera* clones and in *V. rupestris* "du Lot" (St. George). Rivista Di Patologia Vegetale. (Ser. IV) 9:281–285.

Pacifico, D., P. Caciagli, S. Palmano, F. Mannini, and C. Marzacchi. 2011. Quantitation of grapevine leafroll-associated virus 1, grapevine virus A, grapevine fanleaf virus and grapevine fleck virus in field-collected *Vitis vinifera* L. 'Nebbiolo' by real-time reverse transcription PCR. *Journal of Virological Methods* 172: 1–7.

Pantaleo, V., P. Saldarelli, L. Miozzi, A. Giampetruzzi, A. Gisel, S. Moxon, T. Dalmay, G. Bisztray, and J. Burgyan. 2010. Deep sequencing analysis of viral short RNAs from an infected Pinot Noir grapevine. *Virology* 408: 49–56.

Poojari, S., T. Lowery, M. Rott, A.-M. Schmidt, N. DeLury, J. Boulé, and J.R. Úrbez_Torres. 2016. First report and prevalence of grapevine fleck virus in grapevines (*Vitis vinifera* L.) in Canada. *Plant Disease* 100: 1028.

Rubino, L., M. Russo, and G.P. Martelli. 2014. Tombusvirus-induced multivesicualar bodies: Origin and role in virus-host interactions. In *Plant-virus interaction. Molecular Approaches and Viral Evolution*, ed. R.K. Gaur, T. Hon, and P. Sharma, 163–175. Amsterdam: Elsevier-Academic Press.

Sabanadzovic, S., P. Saldarelli, and V. Savino. 1996. Molecular detection of grapevine fleck virus. *Vitis* 35: 137–140.

Sabanadzovic, S., N. Abou Ghanem, M.A. Castellano, M. Digiaro, and G.P. Martelli. 2000. Grapevine fleck virus-like viruses in *Vitis*. *Archives of Virology* 145: 553–565.

Sabanadzovic, S., N. Abou Ghanem-Sabanadzovic, P. Saldarelli, and G.P. Martelli. 2001. Complete nucleotide sequence and genomic organization of grapevine fleck virus. *Journal of General Virology* 82: 2009–2015.

Sabanadzovic, S., N. Abou Ghanem-Sabanadzovic, and A.E. Gorbalenya. 2009. Grapevine virus Q: the first plant virus with a permuted active site of RNA-dependent RNA polymerase. *Virology* 394: 1–7.

Schieber, O., A. Seddas, C. Belin, and B. Walter. 1997. Monoclonal antibodies for detection, serological characterization and immunopurification of grapevine fleck virus. *European Journal of Plant Pathology* 103: 767–774.

Shi, B.J., N. Habili, and R.H. Symons. 2003. Nucleotide sequence variation in a small region of grapevine fleck virus replicase provide evidence for two sequence variants of the virus. *Annals of Applied Biology* 142: 349–355.

Spilmont, A.S., A. Ruiz, and S. Grenan. 2012. Efficiency of micrografting of shoot apices as a sensitive sanitation method against seven grapevine viruses (ArMV, GFLV, GLRaV-1, -2, -3, GFkV, GVA). Proc. 17th Cong. of ICVG, Davis, USA, 270–271.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.

Thompson, J.R., M. Fuchs, H. McLane, F. Celebi-Toprak, K.F. Fischer, J.L. Potter, and K.L. Perry. 2014. Profiling viral infections in grapevine using a randomly primed reverse transcription-polymerase chain reaction/microarray multiplex platform. *Phytopathology* 104: 211–219.

Triolo, E., and A. Materazzi. 1987. La maculatura infettiva della vite: Influenza di isolati diversi sull'attitudine alla propagazione vegetativa di *Vitis rupestris* St. George. *Rech. Agronom. Suisse* 26: 320–324.

Vargas-Asencio, J., K. Wojciechowska, M. Baskerville, A.L. Gomez, K.L. Perry, and J.R. Thompson. 2017. The complete nucleotide sequence and genomic characterization of grapevine asteroid mosaic associated virus. *Virus Research* 227: 82–87.

Verderevskaja, T.D., V.G. Marinesku, and E.S. Semtschik. 1983. Etiologie und diagnose der marmorierung der weinrebe. *Archiv Für Phytopathologie Und Pflanzenschutz* 19: 221–226.

Woodham, R.C., and L.R. Krake. 1983. Investigations on transmission of grapevine leafroll, yellow speckle and fleck diseases by dodder. *Phytopathologische Zeitschrift* 106: 193–198.

Xiao, H., and B. Meng. 2016. First report of grapevine asteroid mosaic-associated virus and grapevine rupestris vein feathering virus in grapevines in Canada. *Plant Disease* 100: 2175.

Yamakawa, Y. 1989. Virus reinfection of virus-free Cabernet sauvignon and Cabernet franc vines. *Journal of the Japanese Society for Horticultural Science* 58: 297–302.