Chapter 17
Grapevine Pinot gris virus

P. Saldarelli, V. Gualandri, U. Malossini, and M. Glasa

Abstract *Grapevine Pinot gris virus* (GPGV) is a new trichovirus identified in grapevine plants showing symptoms of chlorotic mottling and leaf deformations (GLMD: grapevine leaf mottling and deformation). The virus and/or the disease has been detected in different countries around the world although its association with symptoms and cultivar susceptibility are not deeply explored. GLMD is reproduced on vine indicators by grafting and GPGV is transmitted to healthy vines by the mite *Colomerus vitis*. However, the recent detection of GPGV on two herbaceous hosts makes the epidemiology of this virus more complex. Different studies suggest that GPGV genome variants exist, some of which are able to elicit GLMD on grapevine. As such GPGV represents an interesting candidate for the study of plant/virus interactions in grapevine. GPGV is a grapevine-emerging virus not listed in regulations for production of grapevine propagation materials, whose testing is recommended.

Keywords Virus • Grapevine • Chlorotic mottling • GLMD • Trichovirus

Introduction and Historical Aspects

The occurrence of a new virus-like disease in a continuously evolving and globalized agriculture is frequent, although it is sometimes difficult to identify the responsible agent(s). Broad detection tools based on High-Throughput Sequencing (HTS) techniques can help to quickly detect new and unknown pathogens (see Chap. 30 of this

P. Saldarelli (✉)
CNR Istituto per la Protezione Sostenibile delle Piante UOS Bari and Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi di Bari, via Amendola 165/A, Bari, Italy
e-mail: pasquale.saldarelli@ipsp.cnr.it

V. Gualandri • U. Malossini
Fondazione E. Mach, Via E. Mach 1, 38010, San Michele all'Adige, Italy

M. Glasa
Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, Dubravska cesta 9, 84505 Bratislava, Slovakia

B. Meng et al. (eds.), *Grapevine Viruses: Molecular Biology, Diagnostics and Management*, DOI 10.1007/978-3-319-57706-7_17

book). The discovery of *Grapevine Pinot gris virus* (GPGV), which is associated with symptoms of leaf mottling and deformation (Fig. 17.1) in *Vitis vinifera* (GLMD: Grapevine Leaf Mottling and Deformation) (Martelli 2014), largely benefitted from these technological advances.

GLMD is a new disease that was first observed in a commercial vineyard in Trentino (Northern Italy) in 2003. Symptoms were similar to those induced by mite infestations, although the involvement of these pests in disease expression was firmly excluded. In the originally studied vineyard, affected vines of cvs Pinot gris and Pinot blanc were planted in 1985 and 1970, respectively (Mauro Varner, unpublished information). Subsequently, the disease was observed in cvs Pinot blanc, Pinot noir, and Traminer. In this latter cultivar, a severe stunting and necrosis of the shoots were observed (Fig. 17.1). In the same year, GLMD symptoms were recorded in vineyards of cvs Pinot gris, Traminer, Friulano (Tocai), and Glera (Prosecco) in Friuli Venezia Giulia (Bianchi et al. 2015). The inability to associate any known virus or virus-like agent to GLMD symptoms led to investigate the involvement of boron deficiency, among other factors, but mineral deficiency was ruled out. The distribution of symptomatic vines in the whole Friuli Venezia Giulia region was scattered and limited, if any, temporal increase was observed. This was in contrast to a striking increase of symptomatic vines in the Gorizia province, which is close to the Slovenian border (Bianchi et al. 2015). In Slovenia symptoms of short internodes, leaf mottling, and deformation of cvs Pinot gris and Sauvignonasse were under investigation since 2001, and laboratory efforts were unsuccessful in associating the disease with known viruses using available serological and molecular detection tools (Mavric-Plesko et al. 2014).

The etiology of GLMD remained unsolved for about a decade even by using degenerate primers for the generic detection of closteroviruses, flexiviruses, and nepoviruses by RT-PCR. Because of continuous concerns expressed by grape growers, colleagues at the Fondazione Mach, San Michele all'Adige (FEM) sought the collaboration of several Italian laboratories. The timing of this initiative coincided with the advent of HTS in grapevine virology. Therefore, it was proposed to analyze tissues from symptomatic vines by HTS. A private winery, Cantine Cooperative di Mezzocorona, funded the research. Two libraries of small RNAs, extracted from a GLMD and a symptomless vine of cv Pinot gris, were sequenced by Illumina technology and analyzed by a bioinformatic pipeline based on the *de novo* assembly of sequenced reads into larger contigs. A coupled HTS and Sanger sequencing allowed the reassembly of the genome of a new virus, which resembled that of the trichovirus *Grapevine berry inner necrosis virus* (GINV) (Yoshikawa et al. 1997). Due to the lack of a yet established correlation with GLMD, this new virus was named *Grapevine Pinot gris virus,* based on the vine and the cultivar in which it was initially described (Giampetruzzi et al. 2012). Molecular relationships of GPGV isolate ZA505-1A with GINV were further confirmed by the existence of striking similarities among symptoms elicited by both viruses on grapevine leaves and shoots (Fig. 17.2a). However, GINV induces a necrosis of berries, which is not observed on GPGV-infected vines. Similarities between the two viruses raised a number of questions about the GPGV origin since GINV was only found (Yoshikawa

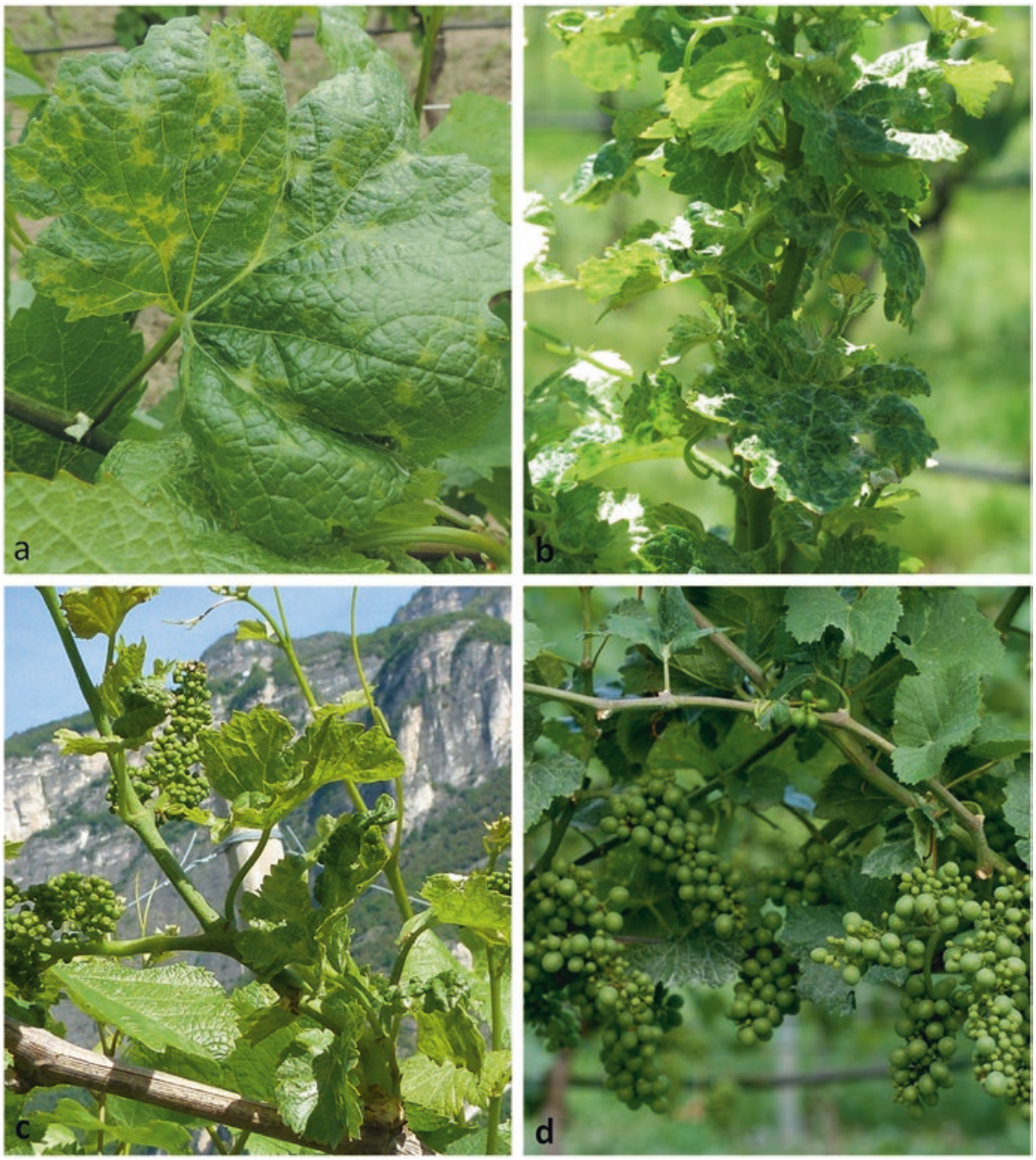

Fig. 17.1 Chlorotic mottling (**a**), stunting and bushy growth (**b**), deformations (**c**), and uneven fruit set (**d**) in leaves and shoot of cv Pinot gris (Courtesy of M. Varner)

et al. 1997) in Japan infecting diverse selections of *Vitis labrusca* (Takao, Kyoho and Pione). This first study showed that the virus was also found in symptomless vines. This finding did not conclusively associate GPGV with GLMD (Giampetruzzi et al. 2012).

The discovery of GPGV triggered a number of studies, which led to the report of the virus and/or the observed disease in several other regions in Italy and Europe (Table 17.1). Surprisingly, the first GPGV detection outside of Europe came from South Korea in the grapevine hybrid cv Tamnara (*V. vinifera x V. labrusca*), where, in addition to symptoms of leaf deformations and mottling, necrosis of the berries was also observed (Cho et al. 2013). Subsequently, the virus and/or the disease was

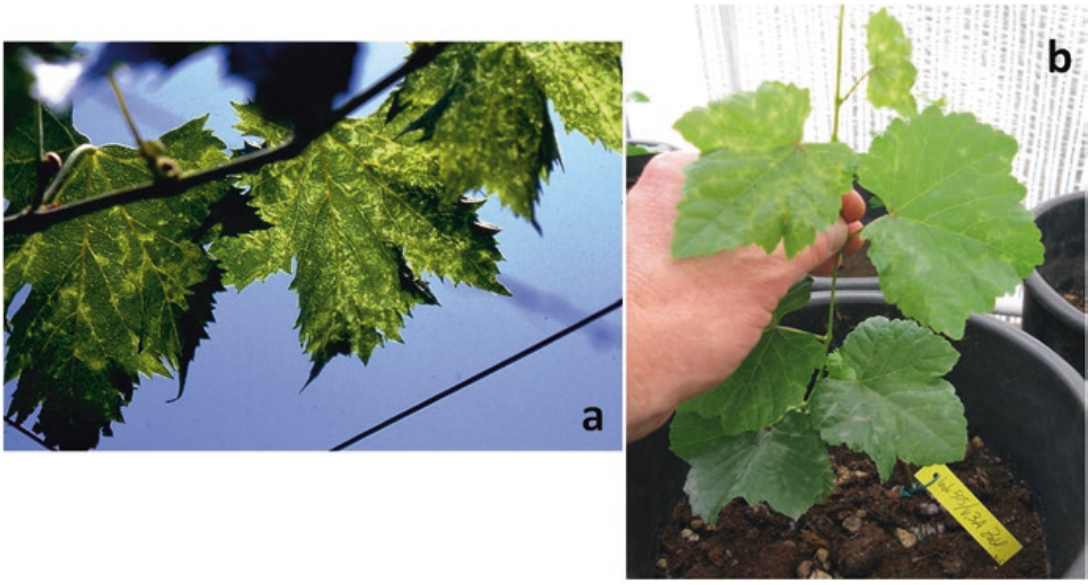

Fig. 17.2 Chlorotic mottling (**a**) on cv Pione in Japan (courtesy of Dr. Terai). (**b**) Symptoms of chlorotic mottling of a *Pinot gris* vine grafted on a GPGV-infected vine of the same cv

described in several white- and red-berry cultivars in different European, South and North American, and Asian countries, although the association between the presence of the virus and GLMD symptoms was not always established due to multiple infections of the analyzed vines (Table 17.1).The presence of GPGV was documented in archival grapevine samples from different European countries that were stored before 2005, suggesting an eastern European origin of the virus and subsequent introduction to viticultural areas of Veneto (northeast Italy) (Bertazzon et al. 2016).

Taxonomy and Nomenclature

Grapevine Pinot gris virus is an established member of the genus *Trichovirus* (family *Betaflexiviridae*, order *Tymovirales*) (Giampetruzzi et al. 2012), whose type species is *Apple chlorotic leaf spot virus* (ACLSV) (Martelli et al. 2007). Besides GINV, GPGV is the second species of the genus infecting grapevine. All members of the genus [*Apricot pseudo-chlorotic leaf spot virus* (APCLSV), *Cherry mottle leaf virus* (CMLV), *Peach mosaic virus* (PcMV), except *Phlomis mottle virus* (PhMV)] infect woody plants. To date, the complete or near-complete genome sequence of seven GPGV isolates (SK30-1, SK13, SK01, and SK30 from the Slovak Republic; Tannat, from Uruguay; ZA505-1A from Italy; and Merlot from France) has been described.

Table 17.1 GPGV distribution in different countries

Country (region)	Cultivar	References
Italy (Trentino Alto Adige)	Pinot blanc, Pinot noir, Traminer	Giampetruzzi et al. (2012)
Italy (Friuli Venezia Giulia)	Pinot gris, Traminer, Friulano (Tocai), Glera	Bianchi et al. (2015)
Italy (Emilia Romagna)	Chardonnay	http://archives.eppo.int/EPPOReporting/2014/Rsf-1401.pdf
Italy (Veneto)	Glera	Raiola et al. (2013) and Bertazzon et al. (2016)
Italy (Lombardia)	Chardonnay, Pinot noir	Casati et al. (2014)
Italy (Apulia)	Supernova, Black Magic	Morelli et al. (2014)
South Korea	Tamnara (*V. vinifera x V. labrusca*)	Cho et al. (2013)
Slovenia	Pinot gris, Sauvignonasse	Mavric-Plesko et al. (2014)
Slovakia	Veltliner, Dornfelder, Muller Thurgau, Welschriesling, André, Alibernet	Glasa et al. (2014)
Czech Republic (South Moravia)	Laurot (interspecific hybrid)	Glasa et al. (2014)
Czech Republic (South Moravia)	Kodrjanka, Pamjati Negrula, Muller Thurgau, Chardonnay	Eichmeier et al. (2016)
France	Merlot, Carignan	Beuve et al. (2015)
Greece	Unknown	V. Maliogka, personal information
Switzerland	Chasselas	Reynard (2015)
China	Red Globe, Merlot, Muscat Hamburg, Cabernet Franc, Moldova, Beta (rootstock)	Fan et al. (2015)
United States	Touriga Nacional	Al Rwahnih et al. (2016)
United States	Cabernet Franc, Cabernet Sauvignon and Chardonnay	Angelini et al. (2016)
Canada	Cabernet Franc, Riesling	Xiao et al. (2016)
Turkey	Pinot noir, Chardonnay, Muscat of Hamburg and two local cultivars	Gazel et al. (2016)
Georgia	Local cultivars	Casati et al. (2016)
Uruguay	Tannat	Jo et al. (2015)
Canada	Pinot gris	Poojari et al. (2016)

The list reports the cultivars infected and the corresponding references

Morphology, Genome Structure, Genome Expression, and Replication

GPGV particles have not been observed yet in infected grapevine tissue (P. Saldarelli unpublished), but its classification in the genus *Trichovirus* suggests a filamentous morphology. GPGV genome is a (+)-sense single-stranded RNA molecule of 7259 nucleotides in length excluding the 3′ polyA terminus. The 5′ untranslated region is 104 nucleotides long in isolates SK13, SK01, and SK30, as determined by using 5′-RACE RT-PCR. The 3′ untranslated region is 82 nucleotides long in isolates ZA505-1A and SK30. The 5'UTR and 3'UTR of Slovak GPGV isolates share ca. 78 and 85% identity, respectively, with that of GINV (Giampetruzzi et al. 2012; Glasa et al. 2014).

Nucleotide sequence identity of GPGV with other trichoviruses for which complete genome sequences are available reaches 69.0% (GINV, NC_015220), 49.0% (ACLSV, X99752), 48.7% (APCLSV, AY713379), 47.7% (CMLV, NC_002500), and 48.6% (PcMV, NC_011552).

The GPGV genome is organized in three open reading frames (ORF) encoding, in the 5′→ 3′ direction (Fig. 17.3), a putative viral replicase (ORF1, RdRp ca. 214 kDa), movement (ORF2, MP ca. 42 kDa), and coat (ORF3, CP ca. 22 kDa) proteins (Giampetruzzi et al. 2012). The replicase contains basic methyltransferase, helicase, and RdRp domains of the replication proteins of (+) ssRNA viruses. It also contains an AlkB-like domain for protecting the viral RNA from methylation. An additional putative small ORF has been identified within ORF1 at nucleotide positions 3538–3840 in an overlapping reading frame. The deduced 11.5 kDa protein has no conserved domains or homology to known proteins in the databases and is present in the genome of the three Slovak and Italian GPGV isolates, but not in GINV (Glasa et al. 2014). Side-by-side comparisons of the GPGV RdRp, MP, and CP proteins with counterparts from other species in the family *Betaflexiviridae* classify the virus in the genus *Trichovirus* with the closest homology with GINV (Giampetruzzi et al. 2012).

Studies of genome expression of ACLSV (German et al. 1992) suggest that the GPGV strategy of RNA translation and replication likely relies on polyprotein processing and production of subgenomic RNAs.

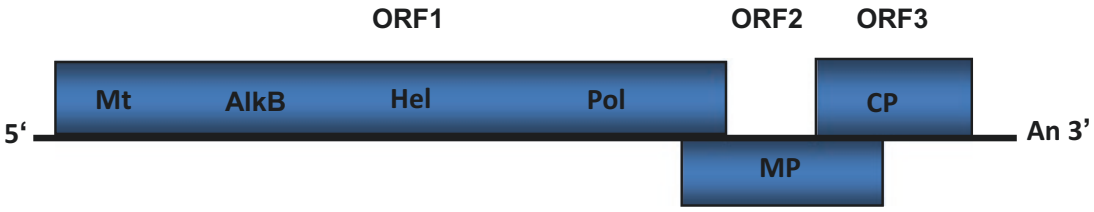

Fig. 17.3 Organization of *Grapevine Pinot gris virus* genome RNA. Open reading frames encoding the replicase (ORF1), movement protein (ORF2, MP), and coat proteins (ORF3, CP) are showed. Replicase domains corresponding to methyltransferase (Mt), AlkB, helicase (Hel), and polymerase (Pol) domains are indicated

Genetic Diversity of GPGV

The alignment of the genome sequence of Italian (ZA505-1A) and Slovak (SK30) isolates revealed a high percentage of homology (95.5–95.8% identity), but unusual patterns of local divergences were noticed. Nucleotide differences were mainly localized in the 5′ part of the genomic RNA containing the 5′UTR and the very beginning of ORF1 up to nucleotide position 233 as well as in three short regions of ORF1 (Glasa et al. 2014). These differences in the 5′ region were also found on a larger group of isolates (Bertazzon et al. 2016). A strong recombination signal between the 5′UTR and ORF1 sequences of the Slovak isolates SK01, SK13, and SK30 of GPGV and GINV was found using the RDP4 program with breakpoints predicted at nt positions 92 and 208. This potential recombination event would explain the high divergence in the amino acid portion 4–43 between the Slovak and Italian ZA505-1A isolates. It cannot however be discounted that this detection represents the local divergence of the sequence of isolate ZA505-1A rather than from a true recombination event (Glasa et al. 2014). According to Glasa et al. (2014), local nucleotide divergences in the three regions of ORF1 may have originated from sequencing errors or real polymorphisms in genome regions not subjected to evolution constraints.

With the two additional recent genomes identified in cvs Merlot (Beuve et al. 2015) and Tannat (Jo et al. 2015), the current GPGV phylogeny confirms the clustering of all GPGV isolates distinctly from other trichovirus species and from GINV. It also confirms the limited divergence of the ZA505-1A isolate and the grouping of the two French (Mer and Tannat) and the four Slovak isolates (Fig. 17.4).

The genetic diversity of GPGV was studied to investigate its association with GLMD symptoms. The nucleotide sequences of MP/CP and RdRpol genome regions from 45 and 20 GPGV isolates, respectively, from Trentino (Italy) were analyzed (Saldarelli et al. 2015). This study showed a clear clustering of GPGV isolates originating from symptomless as compared to those from symptomatic vines. Intriguingly, the presence of six extra amino acid residues in the MP of isolates from symptomless grapevines was observed due to a C/T polymorphism in the stop codon. This polymorphism was also found in a survey of GPGV in Switzerland (Reynard 2015). However, the lack of experimental demonstration of the involvement of this nucleotide polymorphism in symptom expression does not allow this feature to be used as a marker to distinguish symptomless from symptomatic isolates.

GPGV surveys in two viticultural regions in the north of Italy also documented the virus presence in symptomless vines. In Trentino, GPGV was found in 79% and 21% of symptomatic and symptomless vines, respectively (Saldarelli et al. 2015). In Friuli Venezia Giulia, a 95% GPGV incidence in symptomatic vines was observed, but the virus was also present in 61.5–87.1% symptomless vines (Bianchi et al. 2015).

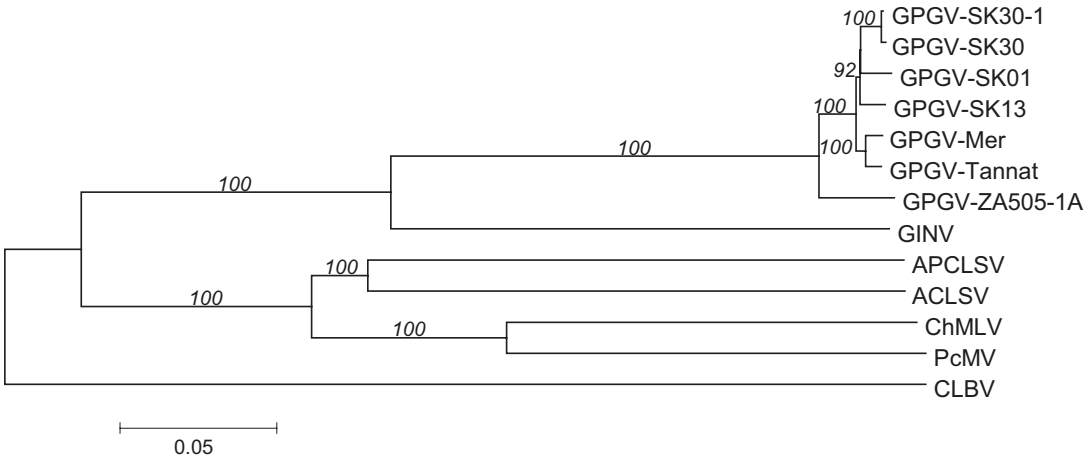

Fig. 17.4 GPGV phylogeny performed by maximum likelihood analysis using the full-length nucleotide sequence of virus species in the genus *Trichovirus*. Genome accession numbers of the different species are *Grapevine Pinot gris virus* (GPGV), isolate SK30-1 (KF686810.1), GPGV-SK30 (KF134123.1), GPGV-SK01 (KF134124.1), GPGV-Mer (KM491305.1), and GPGV-ZA505-1A (NC _15782.1); *Grapevine berry inner necrosis virus* (GINV) (NC_015220.1); *Apricot pseudo-chlorotic leaf spot virus* (APCLSV) (AY713379.1); *Apple chlorotic leaf spot virus* (ACLSV) (X99752.1); *Cherry mottle leaf virus* (ChMLV) (NC_002500.1); *Peach mosaic virus* (PcMV), (NC 011552.1); and *Citrus leaf blotch virus* (CLBV) (NC_003877.1). Bootstrap values at relevant nodes and scale of nucleotide substitutions *per* site are indicated

Detection and Diagnosis

The presence of GPGV in symptomless grapevines and the lack of information about the diverse susceptibility of grapevine cvs to the disease make visual diagnosis of GPGV unreliable.

Commercial antibodies are not yet available but a polyclonal serum raised against an *Escherichia coli*-expressed GPGV CP was obtained in rabbit (Gualandri et al. 2015). This serum was unsuitable for ELISA but recognized a putative GPGV CP in samples from infected vines when used on denatured proteins in western blot analysis (Saldarelli unpublished).

Several primer pairs designed in the MP/CP (588 bp, Saldarelli et al. 2015), MP (302 bp, Glasa et al. 2014; 770 bp, Beuve et al. 2015), and CP (412 bp, Glasa et al. 2014; 430 bp, Bertazzon et al. 2016) genomic regions have been developed for efficient detection of GPGV using end point RT-PCR. A real-time RT-qPCR assay was developed with primers and probes targeting the RdRp and CP genes (Bianchi et al. 2015). This assay also developed in a multiplex format targeting both the RdRpol and CP regions, proved sensitive for detecting GPGV in infected grapevine tissues and was used for an extensive virus survey in the Friuli Venezia Giulia region in Italy.

Host Range and Epidemiology

GPGV infects grapevine but was recently detected in the two herbaceous hosts *Silene latifolia subsp. Alba* (Mill) and *Chenopodium album* L (Gualandri et al. 2016). The virus was detected by RT-PCR in plants of both species collected in the field, which showed symptoms of chlorotic mottling of the leaves. GPGV infection was confirmed by the amplification and cloning of the whole viral RNA genome. Repeated attempts to transmit the virus to the herbaceous hosts *Chenopodium quinoa*, *Nicotiana benthamiana*, and *N. occidentalis* and to dodder (*Cuscuta europaea*) were unsuccessful (Saldarelli, unpublished and Beber 2012). The virus is transmitted by grafting; this makes the infected plant material the main source of dissemination (Giampetruzzi et al. 2012; Saldarelli et al. 2015). A recent study reports that GPGV is present in the body of the eriophyid mite *Colomerus vitis* and is transmitted to healthy vines through mite infestation (Malagnini et al. 2016), although symptoms were not observed in the recipient vines throughout the time of the experiment. This finding is in line with the involvement of a vector that transmits the virus in vineyards, as suggested by observations of an aggregated pattern of GPGV symptomatic vines in the vineyard (Malossini et al. 2015; Bertazzon et al. 2015b). Therefore, a likely epidemiological model of GPGV infections relies on the introduction of the virus in the vineyard through planting material and a subsequent spread from vine-to-vine by a slow-moving vector such as mites. This model is in agreement with the transmission of GINV by *C. vitis*, which was demonstrated in confined conditions and in the field (Kunugi et al. 2000). However, the recent GPGV detection in wild plants in the vineyard reveals the existence of an alternative route of transmission. It is now compelling to understand how it occurs and whether these new GPGV hosts are a dead end for the virus or have a role in the epidemiology.

Cytopathology, Tissue Tropism, and Virus-Host Interactions

The virus has yet to be observed in grapevine tissues and information on cytopathology, tissue tropism and virus-host interactions is lacking.

Pathological Properties, Associated Diseases, and Their Impact

GPGV is easily transmitted to *Vitis* spp. by grafting, but the susceptibility to GLMD of grapevine cultivars is variable and unexplored to date. As an example, cv Teroldego seems to be tolerant to GLMD in Trentino (Malossini, unpublished observations). GLMD symptoms were reproduced by bud- and green grafting (Fig. 17.2b) in cvs Pinot gris and Traminer (Saldarelli et al. 2015).

The distinct genetic diversity between GPGV isolates from symptomatic and symptomless vines supports the existence of viral variants responsible for eliciting disease symptoms (Saldarelli et al. 2015). In support of this hypothesis, a statistically significant higher virus titre was found in symptomatic compared to symptomless vines (Bertazzon et al. 2015a, b; Bianchi et al. 2015). This observation was ascertained by measuring GPGV concentration in cv Glera, which decreased in both symptomatic and symptomless vines with the progress of the vegetative season.

GLMD symptoms appear in early spring and are followed by a period of poor vegetation of the infected shoots. Shoot necrosis is observed in cvs Traminer and Pinot gris but not in cv Glera (Bertazzon et al. 2015a, b; Giampetruzzi et al. 2012). Vines with GLMD have fewer canes and a lower number of clusters as well as reduced cluster weight (Malossini et al. 2012). Moreover, a reduced fruit set and an uneven ripening is observed in cv Glera (Bertazzon et al. 2015a). During the summer, the new vegetation completely recovers from symptoms, making the observation of foliar symptoms unreliable for diagnosis.

The impact of the disease on enological parameters related to wine production is mainly related to the lower weight of clusters (Malossini et al. 2012) which is more evident in cv Pinot gris than in Glera (Bertazzon et al. 2015a).

Strategies for Control and Management

Management of GPGV relies on a preventive approach that needs integrated actions based on a thorough knowledge of GPGV epidemiology, availability of GPGV-free plant propagation material, and vector control. Preliminary studies indicate that elimination of GPGV from infected vines is possible through in vitro culture and meristem-tip excision with or without thermotherapy (Gualandri et al. 2015). Since *C. vitis* mites likely play a role in virus transmission, the management of this pest in vineyards is recommended.

Conclusions and Future Research Directions

GPGV is an emerging virus associated with GLMD, a recently recognized disease of grapevine. Its widespread occurrence and its likely recent introduction in several premium viticultural areas in Europe call for further research efforts to evaluate its impact.

To date, studies of GLMD and the discovery of GPGV were scattered and funded by regional government and private organizations in response to growers' requests. Desirable research trends should be directed to the study of the epidemiology and etiology of the disease, its impact, and management. The mode of transmission of GPGV by *C. vitis* would be an interesting area of research, bearing in mind that PcMV is transmitted by *Eriophyes insidiosus* in a semipersistent manner (Gispert

et al. 1998). The finding that two herbaceous hosts are a reservoir for the virus suggests a more complex epidemiology, which should be explored and eventually considered in the design of management strategies.

The existence of GPGV viral variants should lead to research on virus/host interactions, particularly on virus determinants of symptoms expression. In response to stakeholder's needs, efforts should be devoted to the sanitation of infected vines and knowledge of GLMD impact in different grapevine cvs.

The production of certified grapevine propagation materials does not currently include GPGV in the list of viruses and diseases of consideration. Based on the impact of GLMD and globalized nursery activities, testing for GPGV is recommended.

Acknowledgments The research presented in this chapter was supported by:

"Progetto di ricerca per una nuova malattia della vite in Trentino" funded by FEM-IASMA, Centre for Technology Transfer, via E. Mach,1 San Michele all'Adige (TN), Italy.

METAGERM (in the frame of "Conoscenze Integrate per la Sostenibilità e l'Innovazione del made in Italy Agroalimentare," Ministry of Economy, Italy). The NGS activity was hosted by the Illumina platform at Rete di laboratori pubblici di ricerca SELGE (www.selge.uniba.it).

M.G. received the support from the Scientific Grant Agency of the Ministry of Education and Slovak Academy of Sciences (grant VEGA 2/0036/16).

References

Al Rwahnih, M., D. Golino, and A. Rowhani. 2016. First report of *Grapevine Pinot gris virus* infecting grapevine in the United States. *Plant Disease* 100: 1030. http://dx.doi.org/10.1094/PDIS-10-15-1235-PDN.

Angelini, E., N. Bertazzon, J. Montgomery, A. Zinkl, X. Wang, J.A. Stamp, and A. Wei. 2016. Occurrence of *Grapevine Pinot gris virus* in commercial vineyards in the U.S. *Plant Disease* 100: 1254. http://dx.doi.org/10.1094/PDIS-01-16-0055-PDN.

Beber R. 2012. Studi su una nuova virosi della vite in Trentino. Dissertation, University of Bologna

Bertazzon, N., V. Forte, L. Filippin, and E. Angelini. 2015a. Studies on *Grapevine Pinot gris virus* (GPGV) presence and its association with an emergent disease of grapevine. In *Proceedings of the 18th Congress of ICVG*, Ankara, Turkey, 7–11 September 2015, 78–79

Bertazzon, N, M. Maixner, L. Filippin, I. Bazzo, V. Forte, and E. Angelini. 2015b. Survey on a new emergent grapevine disease and *Grapevine Pinot gris virus* (GPGV) in Veneto, Northeast Italy. In *Proceedings of the 18th Congress of ICVG*, Ankara, Turkey, 7–11 September 2015, 201–202.

Bertazzon, N., L. Filippin, V. Forte, and E. Angelini. 2016. *Grapevine Pinot gris virus* seems to have recently been introduced to vineyards in Veneto, Italy. *Archives of Virology*. doi:10.1007/s00705-015-2718-2.

Beuve, M., T. Candresse, M. Tannieres, and O. Lemaire. 2015. First report of *Grapevine Pinot gris virus* (GPGV) in grapevine in France. *Plant Disease* 99: 293.

Bianchi, G.L., F. De Amicis, L. De Sabbata, N. Di Bernardo, G. Governatori, F. Nonino, G. Prete, T. Marrazzo, S. Versolatto, and C. Frausin. 2015. Occurrence of *Grapevine Pinot gris virus* in Friuli Venezia Giulia (Italy): field monitoring and virus quantification by real-time RT-PCR. *Bulletin OEPP/EPPO Bulletin* 45 (1): 22–32.

Casati, P., G. Durante, F. Quaglino, E. Zacchi, and P.A. Bianco. 2014. Preliminary data on the presence of *Grapevine Pinot gris virus* in Lombardy. *Journal of Plant Pathology* 96 (suppl): S4.46–S4.47.

Casati, P., D. Maghradze, F. Quaglino, A. Ravasio, O. Failla, and P.A. Bianco. 2016. First report of *Grapevine Pinot gris virus* in Georgia. *Journal of Plant Pathology*. doi:10.4454/JPP.V98I1.003.

Cho, I.S., S.M. Jung, J.D. Cho, G.S. Choi, and H.S. Lim. 2013. First report of *Grapevine Pinot gris virus* infecting grapevine in Korea. *New Disease Report* 27: 10.

Eichmeier, A., E. Penazova, R. Pavelkova, Z. Mynarzova, and P. Saldarelli. 2016. Detection of *Grapevine Pinot gris virus* in certified grapevine stocks in Moravia, Czech Republic. *Journal of Plant Pathology* 98: 155–157.

Fan, X.D., Y.F. Dong, Z.P. Zhang, F. Ren, G.H. Hu, Z.N. Li, and J. Zhou. 2015. First report of *Grapevine Pinot gris virus* in grapevines in China. *Plant Disease* 100: 540. http://dx.doi.org/10.1094/PDIS-08-15-0913-PDN.

Gazel, M., M. Çağlayan, E. Elçi, and L. Ozturk. 2016. First report of *Grapevine Pinot gris virus* in grapevine in Turkey. *Plant Disease* 100: 657. http://dx.doi.rg/10.1094/PDIS-05-15-0596-PDN.

German, S., T. Candresse, O. Le Gall, M. Lanneau, and J. Dunez. 1992. Analysis of the dsRNAs of apple chlorotic leaf spot virus. *Journal of General Virology* 73: 767–773.

Giampetruzzi, A., V. Roumi, R. Roberto, U. Malossini, N. Yoshikawa, P. La Notte, F. Terlizzi, R. Credi, and P. Saldarelli. 2012. A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in cv Pinot gris. *Virus Research* 163: 262–268.

Gispert, C., G.N. Oldfield, T.M. Perring, and R. Creamer. 1998. Biology of the transmission of *Peach Mosaic Virus* by *Eriophyes insidiosus* (Acari: Eriophyidae). *Plant Disease* 82: 1371–1374.

Glasa, M., L. Predajna, P. Kominek, A. Nagyova, T. Candresse, and A. Olmos. 2014. Molecular characterization of divergent *Grapevine Pinot gris virus* isolates and their detection in Slovak and Czech grapevines. *Archives of Virology* 159: 2103–2107.

Gualandri, V., P. Bianchedi, M. Morelli, A. Giampetruzzi, P. Valenzano, G. Bottalico, and A. Campanale, P. Saldarelli. 2015. Production of *Grapevine Pinot gris virus*-free germplasm: techniques and tools. In *Proceedings of the 18th Congress of ICVG*, Ankara, Turkey, 7–11 September 2015, 246–247.

Gualandri, V., E. Asquini, P. Bianchedi, L. Covelli, M. Brilli, U. Malossini, P. Bragagna, P. Saldarelli, and A. Si-Ammour. 2016. Identification of herbaceous hosts of the *Grapevine Pinot gris virus* (GPGV). *European Journal of Plant Pathology*. doi:10.1007/s10658-016-0989-4.

Jo, Y., H. Choi, 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. doi:10.1038/srep15841.

Kunugi, Y., S. Asari, Y. Terai, and A. Shinkai. 2000. Studies on the *Grapevine berry inner necrosis virus* disease. 2. Transmission of grapevine berry inner necrosis virus by the grape erineum mite Colomerus vitis in Yamanashi. Bull. *Yamanashi Fruit Tree Experimental Station* 10: 57–63.

Malagnini, V., E. de Lillo, P. Saldarelli, R. Beber, C. Duso, A. Raiola, L. Zanotelli, D. Valenzano, A. Giampetruzzi, M. Morelli, C. Ratti, R. Causin, and V. Gualandri. 2016. Transmission of *Grapevine Pinot Gris virus* by *Colomerus vitis* (Acari: Eriophydae) to grapevine. *Archives of Virology*. doi:10.1007/s00705-016-2935-3.

Malossini, U., R. Moscon, M. Ferrazza, P. Bianchedi, M. Varner, and R. Credi. 2012. Caratteristiche vegeto-produttive di viti Pinot grigio e Traminer aromatico affette da una nuova virosi segnalata in Trentino. IV Convegno Nazionale di Viticoltura CONAVI.TO Asti, 10-11-12 luglio 2012 pp 37. http://www.unito.it/unitoWAR/ShowBinary/FSRepo/D046/Allegati/CONAVI_2012/CONAVI_2012_Libro_dei_riassunti.pdf

Malossini, U., P. Bianchedi, R. Beber, R. Credi, P. Saldarelli, and V. Gualandri. 2015. Spread of GPGV-associated disease in two vineyards in Trentino (Italy). In *Proceedings of the 18th Congress of ICVG*, Ankara, Turkey, 7–11 September 2015, 212–213.

Martelli, G.P. 2014. Directory of virus and virus-like diseases of the grapevine and their agents. *Journal of Plant Pathology* 96 (1, Supplement): 110–111.

Martelli, G.P., M.J. Adams, J.F. Kreuze, and V.V. Dolja. 2007. Family *Flexiviridae*: A case study in virion and genome plasticity. *Annual Review of Phytopathology* 45: 73–100.

Mavric-Plesko, I., M.V. Marn, G. Seljak, and I. Zezlina. 2014. First report of *Grapevine Pinot gris virus* infecting grapevine in Slovenia. *Plant Disease* 98: 1014.

Morelli, M., Catarino A. de Moraes, L. Susca, P. Saldarelli, V. Gualandri, and G.P. Martelli. 2014. First report of *Grapevine Pinot gris virus* from table grapes in Southern Italy. *Journal of Plant Pathology* 96: 439.

Poojari, S., T. Lowery, M. Rott, A.-M. Schmidt, and J.R. Úrbez-Torres. 2016. First report of *Grapevine Pinot gris virus* in British Columbia, Canada. *Plant Disease* 100: 1513. http://dx.doi.org/10.1094/PDIS-02-16-0178-PDN.

Raiola, A., C. Scopel, D. Ferrigo, F. Taglietti, C. Duso, and R. Causin. 2013. First report of *Grapevine Pinot gris virus* infecting cv. Glera in the Conegliano-Valdobbiadene area. Abstracts of the IXX Congress of the Italian Phytopathological Society, Padua 23–25 September 2013. *Journal of Plant Pathology* 95 (4, Supplement): S4.58.

Reynard, J.S. 2015. Survey of emerging viruses in Switzerland. In *Proceedings of the 18th Congress of ICVG*, Ankara, Turkey, 7–11 September 2015, 223–224

Saldarelli, P., A. Giampetruzzi, M. Morelli, U. Malossini, C. Pirolo, P. Bianchedi, and V. Gualandri. 2015. Genetic Variability of *Grapevine Pinot gris virus* and Its Association with Grapevine Leaf Mottling and Deformation. *Phytopathology* 105(4): 555–563

Xiao, H., M. Shabanian, W. McFadden-Smith, and B. Meng. 2016. First report of *Grapevine Pinot gris virus* in commercial grapevines in Canada. *Plant Disease* 100: 1030. http://dx.doi.org/10.1094/PDIS-12-15-1405-PDN.

Yoshikawa, N., H. Iida, S. Goto, H. Magome, T. Takahashi, and Y. Terai. 1997. *Grapevine berry inner necrosis*, a new trichovirus: comparative studies with several known trichoviruses. *Archives of Virology* 142: 1351–1363.