Vitis **59**, 29–34 (2020) DOI: 10.5073/vitis.2020.59.29-34

# Fast transmission of grapevine 'Pinot gris' virus (GPGV) in vineyard

N. Bertazzon, V. Forte and E. Angelini

CREA Research Centre for Viticulture and Enology, Conegliano (TV), Italy

## Summary

**Grapevine 'Pinot gris' virus (GPGV) is a newly emergent virus associated with the appearance of grapevine leaf mottling and deformation disease (GLMD). The worldwide spreading of this virus, and sometimes of the associated disease, that has occurred in the last years, requests further epidemiological studies to verify the progress of natural infection in the field. In the present paper, GPGV infection and spatiotemporal spreading of GLMD, investigated in two vineyards with high disease occurrence, confirmed an elevated presence of the virus in vineyards of Northeastern Italy, and revealed an increasing of symptomatic plants over the time. At the same time, the progress of natural infection was monitored after the placement of new grafted plants near the symptomatic grapevines in the infected vineyards. After three years, 76 % of the plantlets that were initially GPGV-free became GPGV-infected, giving an evidence of the fast transmission of GPGV in the field. Only 14 % of the plantlets, all collocated inside a patch with diseased plants, showed typical GLMD-symptoms. Interestingly, some plantlets, which were already GPGV-infected with the "asymptomatic" GPGV variant before planting in the field, did not become infected with the "symptomatic" viral wild variant after three years and never showed GLMD symptoms.**

K e y w o r d s : GPGV detection; GPGV variant; grapevine; 'Pinot gris' disease; spatiotemporal spreading.

## Introduction

Grapevine leaf mottling and deformation (GLMD) is a grapevine pathology identified for the first time in 2003 in 'Pinot gris' (Giampetruzzi *et al.* 2012). Symptoms include chlorotic mottling, mosaic and deformation of leaves, shortened internodes, stunting, and reduced yields of production. The trichovirus named grapevine 'Pinot gris' virus (GPGV) has been associated with the GLMD disease, and the manifestation of the symptoms has been correlated with the presence of different viral variants and/or with high viral titre (Giampetruzzi *et al.* 2012, Saldarelli *et al.* 2015, Bertazzon *et al.* 2017).

After its first discovery, the virus has been later detected in the main European viticultural regions (Martelli 2014, Pleško *et al.* 2014, Beuve *et al.* 2015, Bertazzon *et al.* 2016, Eichmeier *et al.* 2016 and 2017, Reynard *et al.* 2016, Ruiz-García and Olmos 2017, Abou Kubaa *et al.* 2018, Silva *et al.* 2018). The virus has also been reported in Korea, Pakistan, China, Uruguay, Canada, United States, Brazil and Australia, proving to have a high worldwide distribution (Cho *et al.* 2013, Jo *et al.* 2015, Al Rwahnih *et al.* 2016, Angelini *et al.* 2016, Fan *et al.* 2016, Poojari *et al.* 2016, Xiao *et al.* 2016, Fajardo *et al.* 2017, Wu *et al.* 2017). The finding of GPGV in different countries and in many grapevine varieties has been sometimes associated with observation of symptoms of leaf mottling and deformation (Cho *et al.* 2013, Pleško *et al.* 2014, Fan *et al.* 2016, Poojari *et al.* 2016, Reynard *et al.* 2016, Moran *et al.* 2018, Spilmont *et al.* 2018). In some cases, due to the frequent presence of multiple viral infections, the association of specific symptoms with GPGV infection has not been possible (Glasa *et al.* 2014, Beuve *et al.* 2015, Fajardo *et al.* 2017).

Surveys performed on a number of grapevine samples in different countries revealed variable incidence of GPGV infection. Low viral prevalence (less than 3 %) was reported in Australia, Pakistan and Spain (Rasool *et al.* 2017, Ruiz-Garcia *et al.* 2017, Wu *et al.* 2017). Higher prevalence (between 10 and 20 %) of GPGV infection were detected during surveys carried out in Canada and Brazil (Xiao *et al.* 2016, Fajardo *et al.* 2017). Elevated presence of GPGV (more than 20 %) was described in investigations performed in China, Slovakia and Poland (Glasa *et al.* 2014, Fan *et al.* 2016, Eichmeier *et al.* 2017). However, the highest prevalence of GPGV infections (more than 78 %) were reported in vineyards of Northeastern Italy, with larger values on samples collected from plants displaying GLMD symptoms (Bianchi *et al.* 2015, Saldarelli *et al.* 2015, Bertazzon *et al.* 2017).

The widespread occurrence of GPGV in vineyards of many countries seem to be relatively recent. Indeed, Bertazzon *et al.* (2016) suggested the spreading of GPGV from some Eastern European countries to the other parts of Europe to happen after 2010. In Italy, a comparative analysis of samples collected in different years in the Veneto region (Northeast Italy) revealed the absence of GPGV in the grapevines tested before 2010, and a wide presence of the virus in samples collected in subsequent years (Bertazzon *et al.* 2016). Gentili *et al.* (2017) confirmed the recent introduction of GPGV in Sardinia and Lazio (Southern and Central Italy), as the virus was not detected in grapevines older than 10 years. A recent GPGV spreading was hypothe-

Correspondence to: Dr. N. Bertazzon, CREA Research Centre for Viticulture and Enology, 31015 Conegliano (TV), Italy. E-mail: nadia.bertazzon@crea.gov.it

© The author(s).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Share-Alike License (http://creative-commons.org/licenses/by-sa/4.0/).

sized also outside of Europe. Indeed, a survey performed on Brazilian grapevines revealed an elevated GPGV prevalence (78.3 %) in recently imported grapevine cuttings, and lower viral presence in the oldest imported plants (3.8 %) and in germplasm collections (11 %) (Fajardo *et al.* 2017).

The spatiotemporal distribution of GPGV likely suggests a role of trading of infected grapevine propagation material (Fajardo *et al.* 2017), and locally a vine-to-vine spread operated by vectors. Some studies reported the apparent lack of natural transmission of the virus, as assessed by the analysis of vines surrounding infected plants (Al Rwahnih *et al.* 2016, Wu *et al.* 2017). In other works, an increase of infected vines in a 3-year period suggested the active spreading of the virus in vineyards (Martelli 2014, Bertazzon *et al.* 2018). Natural transmission of the disease in vineyards could be caused by the eriophyid grape bud mite *Colomerus vitis*, a monophagous vector of the virus in controlled conditions (Malagnini *et al.* 2016). Moreover, another polyphagous vector could be involved in the epidemiology of GPGV, given the presence of the virus in vineyard's neighboring woody and herbaceous hosts (Gualandri *et al.* 2017, Demian *et al.* 2018). Further studies are needed to understand the involvement of putative vectors in the epidemiology of GPGV and the associated disease.

In the present study, GPGV infection and spatiotemporal spreading of GLMD were investigated in two vineyards with high virus and disease occurrence. At the same time, the progress of the natural infection was monitored after the placement of new grafted plants near the symptomatic grapevines in the infected vineyards. The appearance of GLMD symptoms and GPGV infection were checked over three years.

## Material and Methods

Plant material and visual survey: The study was carried out in the Veneto region, in two vineyards, named CIS and COL, with high occurrence of symptomatic plants and a significant clustering of grapevines with symptoms, which had been verified by spatial analysis with PATCHY program according to Bertazzon *et al.* (2017). Both vineyards, cultivated with 'Glera', were older than 30 years and subject to frequent replacement of individual vines. In 2014, from each vineyard, leaf samples were collected from 15 grapevines and frozen at -80 °C for further analysis of GPGV. Ten samples were collected inside the patch with the disease: five from symptomatic and five from asymptomatic plants. Further five samples were collected from asymptomatic plants located far from the patch with the diseased plants.

In the same year, 66 grafted plants of 'Glera' had been sprouted during early spring in a greenhouse and tested for presence of GPGV and other grapevine viruses. In late spring, the plantlets were planted into the previously described vineyards: 20 plantlets were placed in the CIS vineyard inside the patch with symptomatic plants, so that every new plantlet was close to at least one diseased plant along the row, and 46 plantlets were placed in the COL vineyard, 18 of them in the area where symptomatic plants were clustered, and 28 far from the patch with the diseased plants. Visual surveys of GLMD symptoms were performed in "old" and newly planted vines for three years: at the end of the vegetative season in 2014, and in the middle of the vegetative season in 2015 and 2016. Plants with symptoms were identified and marked, and every year a bidimensional map was produced, using the software QGIS version 3.4.4. (www.qgis.org). At the same time, from each plantlet, leaf samples were collected and frozen at -80 °C for molecular analysis.

Analyses of GPGV: Collected frozen leaf samples, maintained at -80 °C (100 mg), were homogenized in liquid nitrogen, and total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) with a protocol described by MacKenzie *et al.* (1997). One µg of RNA was then reversely transcribed at 42 °C for 50 min using Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen) and DNA random primers (Roche Diagnostic).

GPGV detection was carried out by means of SYBR Green real-time RT-PCR assays with primer pair CPF3/R3, targeting the coat protein region of the virus (Bertazzon *et al.* 2017). The assays were carried out on a Bio-Rad thermal cycler (model CFX96) in 96-well plates using the 2X Platinum SYBR Green qPCR Supermix-UDG (Invitrogen). PCR reactions were performed at least in duplicate, in a total volume of 10 µL, including 0.3 µM of each primer and 1 µL of cDNA. The thermal protocol consisted of a decontamination step of 3 min at 50 °C to allow for optimal UDG (Uracil DNA Glycosylase) enzymatic activity, followed by 3 min at 95 °C in order to activate the Platinum Taq polymerase, to deactivate the UDG and to denature the DNA samples. Subsequently, 50 cycles of a two-step protocol, consisting of 5 sec of denaturation at 95 °C and 30 sec of annealing/extension at 60 °C, were performed.

Samples that tested positive for GPGV with real-time RT-PCR assays were subsequently amplified by means of conventional RT-PCR with primer pair DetF-DetR, using *Taq* DNA Polymerase (Sigma-Aldrich, USA) and cycling condition reported by Saldarelli *et al.* (2015). Characterization of GPGV variants was performed by means of RFLP analysis, which allowed the discrimination of isolates belonging to clade A or to clade B/C, mainly associated to absence and presence of symptoms, respectively, according to Bertazzon *et al.* (2017). In detail, aliquots of the obtained DNA products were digested with *BamH*I (MBI Fermentas) according to the manufacturer's instructions. Restriction products were then separated by electrophoresis on 1 % agarose gel stained with GelRed (Biotium Inc.) and visualized with GelDoc XR UV transilluminator (Biorad).

Statistical tests were performed with the chi-square ($\chi^2$) test in the IBM Statistical Package for Social Science (SPSS) program.

## Results and Discussion

Evolution of GPGV infection and GLMD symptoms in the "old" grapevines: The presence of GPGV in the two vineyards was at first assessed in 30 samples collected in 2014. The virus occurred

in 100 % of the samples collected from symptomatic plants (10) and in 100 % of those collected from asymptomatic plants inside and far from the patch with diseased grapevines (20) (Table). GPGV isolates belonging to clade B/C, generally associated with symptoms, were detected in all the samples collected from symptomatic grapevines and in eight samples collected from plants without any symptoms of GLMD. Another 12 samples coming from asymptomatic plants contained isolates of clade A, generally associated to absence of symptoms. No significant difference on the prevalence of GPGV variants infecting grapevines inside or far from the patch with the disease was detected. These results confirmed that elevated rates of GPGV infection are harboured in vineyards with a lot of symptomatic plants, as reported in Bertazzon *et al.* (2017). Moreover, GPGV detection on both symptomatic and asymptomatic plants, and the presence of "symptomatic" viral variants on asymptomatic plants, confirmed the ambiguous correlation between the occurrence of the disease and the virus (Bianchi *et al.* 2015, Saldarelli *et al.* 2015, Bertazzon *et al.* 2017, Spilmont *et al.* 2018)

Table

Numbers of GPGV positive grapevines and characterization of viral isolates ("asymptomatic" A, or "symptomatic" B/C) on samples collected from "old" plants on CIS and COL vineyards in 2014. For each vineyard, 10 samples were collected inside the patch with the disease: five from symptomatic and five from asymptomatic plants. Another five samples were collected from asymptomatic plants located far from the patch with the diseased plants

| Vineyard | CIS | | COL | |
|---|---|---|---|---|
| Viral variant | A | B/C | A | B/C |
| No. of sympt. in the patch | 0 | 5 | 0 | 5 |
| No. of asympt. in the patch | 2 | 3 | 3 | 2 |
| No. of asympt. far from the patch | 4 | 1 | 3 | 2 |
| Total | 6 | 9 | 6 | 9 |

The monitoring of GLMD-symptoms revealed an increase of symptomatic plants in the three years, with a different extent between the two vineyards (Fig. 1, suppl. Fig. 1). The highest increase was observed in the CIS vineyard (76 %): the number of symptomatic plants (out of 480 surveyed) raised from 48 in 2014 to 65 in 2015 and 77 in 2016, with the new symptomatic plants aggregated in close proximity to the formerly diseased grapevines. Conversely, an increase of only 19 % of symptomatic plants was detected in the COL vineyard, in which the number of symptomatic plants (out of 900 monitored) ranged from 66 in 2014 to 68 in 2015 and 79 in 2016.

Evolution of GPGV infection and GLMD symptoms in the "new" plantlets: The detection of GPGV was initially performed on samples collected after plantlet sprouting in greenhouse. At this stage, the detection of GPGV is already possible on one year-infected grafted plants. Indeed, during our previous studies the presence of GPGV could be clearly identified in the first year after grafting on grafted plants obtained from

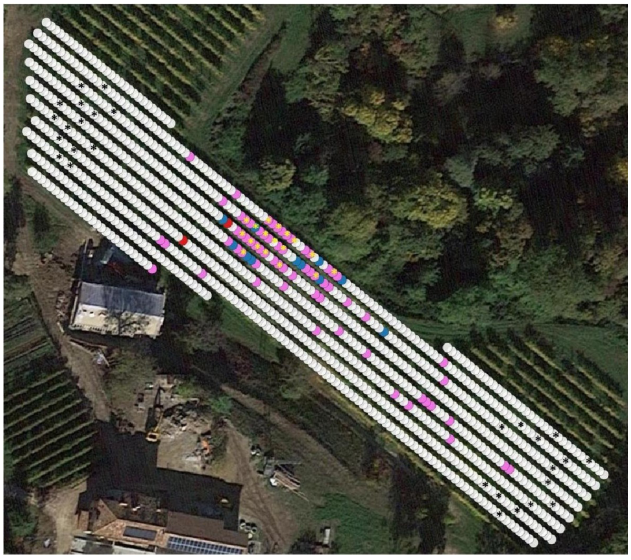

Fig. 1: Cumulative spatial distribution of symptomatic vines in COL vineyard in 2014 (pink cells), 2015 (red cells) and 2016 (blue cells). Asterisks indicate the collocation of "new" plantlets: in the area where symptomatic plants were clustered (yellow asterisks), and far from the patch with the diseased plants (black asterisks).

GPGV-infected scion or rootstocks (data not shown). Twelve out of 66 grafted plants were found to be already GPGV-infected with the "asymptomatic" viral variant (clade A), and they were equally divided, during plantation, between CIS and COL vineyards. Analysis performed on the remaining 54 healthy plants during the three years of trial revealed that the overall percentage of GPGV infections increased from 18 % after one season, to 31 % in the second year, and reached 76 % in the third year.

In detail, in the CIS vineyard the greatest increase of viral infection occurred after only one vegetative season, with 43 % of healthy plants that became infected (Fig. 2). Afterwards, 67 % and 82 % of plantlets were found to be GPGV-infected in the second and third year, respectively. The characterization of the GPGV isolates revealed the presence of the "symptomatic" viral variant (clade B/C) in all the grapevines that became infected.

A high occurrence of GPGV was also observed in the COL vineyard, with 75 % of plants that became infected during the three years (Fig. 3). Only four out of 40 plants were GPGV-infected at the end of the vegetative season in 2014. The highest increase of infected plants was recorded in the third year. Indeed, the percentage of plants that tested positive for GPGV rose from 22 % in 2015 to 75 % in 2016. A high infection rate was detected for plants located inside the patch with the disease, where 19 %, 37 % and 87 % of the vines tested positive for GPGV in the course of the three years. GPGV variants of both A and B/C clades were detected in the newly infected plants. In grapevines planted far from the area with the aggregation of symptomatic plants, the percentage of GPGV-infected plants increased from 4 % to 12 % and to 67 % in the first, second and third year, respectively (Fig. 3b). In this case, viral variants belonging to clade A were predominant in the newly infected grapevines (nearly 77 %). The differences in GPGV infection rates measured between plantlets placed inside or far from the patch with the disease were not statistically significant (suppl Fig. 2). Over-

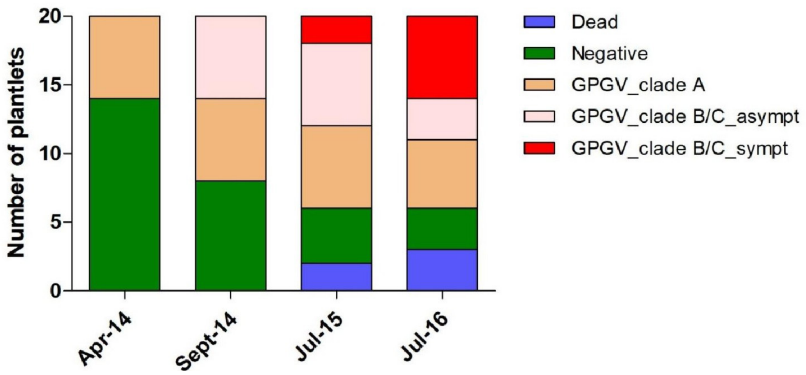


Fig. 2: Results of molecular analyses performed on young plantlets collocated in the CIS vineyard. The numbers of different GPGV isolates ("asymptomatic" – A, or "symptomatic" - B/C) is reported. The number of plantlets showing GLMD symptoms is also indicated.

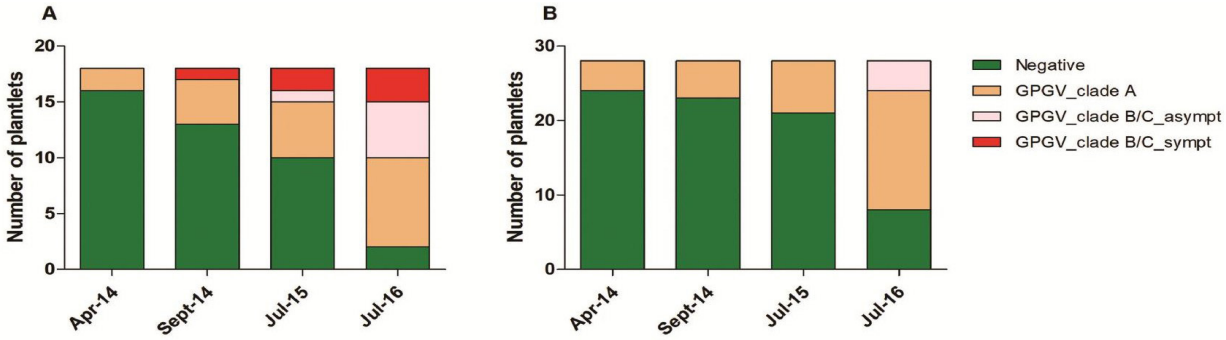


Fig. 3: Results of molecular analyses performed on young plantlets planted in the COL vineyard inside the patch with the disease (**a**) or far from this area (**b**). The number of plants infected with different GPGV isolates ("asymptomatic" – A, or "symptomatic" - B/C) is reported. The number of plantlets showing GLMD symptoms is also indicated.

all, one of 12 grafted plants, already infected with GPGV before plantation, died after two years. On the remaining plantlets, the same initially detected "asymptomatic" viral isolate was recorded after three years from planting, whereas the "symptomatic" variant was not detected.

In this work several plantlets, that were initially GPGV-free, became GPGV-infected soon after the planting into the vineyards with high prevalence of GLMD syndrome, giving an evidence of the fast transmission of GPGV in the field. The high rate of new GPGV infection observed in the present study reveals that the spreading of the virus could be very fast in the field, and could explain the high prevalence of GPGV infection reported by many authors in vineyards of Northeastern Italy (Bianchi *et al.* 2015, Saldarelli *et al.* 2015, Bertazzon *et al.* 2017). Moreover, the rapid insurgence of several new infections suggests the involvement of a very efficient transmission. The patchy distribution of "old" GLMD-symptomatic grapevines in CIS and COL vineyards fits with the involvement of a vector, such as *C. vitis*, in the field transmission of the virus (Bertazzon *et al.* 2017). Although a detailed monitoring of *C. vitis* was not performed, leaf erinea were observed every year in both vineyards on old and newly planted grapevines. The presence of "symptomatic" and "asymptomatic" variants in the newly infected plantlets, observed in the COL vineyard, agrees with the ability of *C. vitis* to transmit GPGV from both infected symptomatic and asymptomatic grapevines, as recently reported by Malagnini *et al.* (2018). Endemic plants growing on the edges surrounding the vineyards, abundant near the patch with symptomatic plants in both CIS and COL vineyards (Fig. 1, suppl. Fig. 1), could have a role in the dissemination of the virus (Gualandri *et al.* 2017, Demian *et al.* 2018) and should be further analysed for the presence of GPGV.

Despite the high number of new GPGV infections, only few plantlets showed GLMD symptoms during the three years of trial (suppl. Fig. 3). Overall, 14 % of the grapevines newly planted in the two vineyards showed typical GLMD-symptoms (Figs 2 and 3). All of them were infected with GPGV variants belonging to clade B/C and were located near symptomatic plants in the vineyard. A large number of plantlets became symptomatic in the CIS vineyard, characterized by the highest increase of diseased grapevines registered over three years. In the COL vineyard, only one plantlet manifested symptoms in the first year of plantation, and further two plants became symptomatic the year later. In parallel, in the CIS vineyard symptoms of GLMD appeared on two plants in the second year of plantation, and on another four plants in the third year. Recently, Tarquini *et al.* (2019) hypothesized an involvement of the plant-mediated RNA silencing in the recovery of GPGV infected plants, as a possible explanation of the frequent absence of GLMD symptoms in GPGV infected grapevines in the field. However, they reproduced GPGV infection using viral clones under controlled conditions, without the influence of environmental factors, such as the interaction with other pathogens, agronomical factors, vectors and abiotic stresses. Interestingly, in the present study, during the three years of trial, the appearance of GLMD symptoms, both in "old" and "new" previously asymptomatic plants, occurred

exclusively inside/near the patch with the disease in both vineyards, thus suggesting an involvement of environmental factors in the appearance of recordable symptoms. It can be supposed that external factors in the vineyard may inhibit the activation of the RNA silencing machinery, and maintain the viral titre at high level, allowing the manifestation of the disease (Bertazzon *et al.* 2017).

Interestingly, plantlets that were already GPGV-infected with the "asymptomatic" viral variant before planting in the field, never showed GLMD symptoms. Characterization of GPGV isolates, performed three years later, revealed that none of these plants became infected with the "symptomatic" variant. Despite the restricted number of plants, it could be speculated that infection with the "asymptomatic" isolate of GPGV could protect plants against subsequent infection with the "symptomatic" isolate. This phenomenon, known as cross-protection, has been reported between closely related strains of several viruses, and it seems to depend on triggering RNA silencing (Gal-On *et al.* 2006). In grapevine, cross-protection was observed for Nepoviruses: indeed, mild strains of Grapevine Fan leaf Virus (GFLV) or Arabis Mosaic Virus (ArMV) were able to protect plants against severe GFLV strains (Huss *et al.* 1989, Komar *et al.* 2008). In some cases, cross-protection has been exploited to protect plants from infection with severe viral strains and consequent production losses (Ziebell *et al.* 2010). Further studies are in progress to investigate the mechanisms that take place during mixed infections of different GPGV variants, in order to describe a possible involvement of "asymptomatic" isolates in preventing the appearance of GLMD symptoms.

## Acknowledgements

The authors gratefully acknowledge M. Gallo and Agridinamica SRL for realization of maps with spatial distribution of symptomatic vines. The authors also thank G. Sbardellotto de Bona and G. F. Palmitessa for their contribution in graphic elaboration of data.

## References

Abou Kubaa, R.; Lanotte, P.; Saldarelli, P.; 2019: First report of grapevine 'Pinot gris' virus in grapevine in Moldavia. J. Plant Pathol. **101**, 441.

Al Rwahnih, M.; Golino, D.; Rowhani, A.; 2016: First Report of Grapevine 'Pinot gris' virus Infecting Grapevine in the United States. Plant Dis. **100**, 1030.

Angelini, E.; Bertazzon, N.; Montgomery, J.; Wang, X.; Zinkl, A. J.; Stamp, A.; Wei, A.; 2016: Occurrence of grapevine 'Pinot gris' virus in commercial vineyards in the United States. Plant Dis. **100**, 1254.

Bertazzon, N.; Filippin, L.; Forte, V.; Angeliuni, E.; 2016: Grapevine 'Pinot gris' virus seems to have recently been introduced to vineyards in Veneto, Italy. Arch. Virol. **161**, 711-714.

Bertazzon, N.; Forte, V.; Filippin, L.; Causin, R.; Maixner, M.; Angelini, E.; 2017: Association between genetic variability and titre of Grapevine 'Pinot gris' virus with disease symptoms. Plant Pathol. **66**, 949-959.

Bertazzon, N.; Forte, V.; Di Gaspero, M.; Angelini, E.; 2018: Temporal spread of Grapevine leaf mottling and deformation in the field, 20-21. Proc. 19th Congress of ICGV, Santiago, Chile.

Beuve, M.; Candresse, T. M.; Tannières, A.; Lemaire, O.; 2015: First Report of Grapevine 'Pinot gris' virus (GPGV) in grapevine in France. Plant Dis. **99**, 293.

Bianchi, G. L.; De Amicis, F.; De Sabbata, L.; Di Bernardo, N.; Governatori, G.; Nonino, F.; Prete, G., Marrazzo, T., Versolatto, S.; Frausin, C.; 2015: Occurrence of grapevine 'Pinot gris' virus in Friuli Venezia Giulia (Italy): field monitoring and virus quantification by real-time RT-PCR. EPPO Bull. **45**, 22-32.

Cho, I. S.; Jung, S. M.; Cho, J. D.; Choi, G. S.; Lim, H. S.; 2013: First report of grapevine 'Pinot gris' virus infecting grapevine in Korea. New Dis. Rep. **27**, 10.

Demian, E.; Czotter, N.; Varallyya, E.; 2018: Detection of Grapevine 'Pinot gris' virus in different non-*Vitis* hosts in Hungary, 24-25. Proc. 19th Congress of ICGV, Santiago, Chile.

Eichmeier, A.; Komínková, M.; Komínek, P.; Baránek, M.; 2016: Comprehensive virus detection using next generation sequencing in grapevine vascular tissues of plants obtained from the wine regions of Bohemia and Moravia (Czech Republic). PLoS ONE **11**, 1-15.

Eichmeier, A.; Pieczonka, K.; Peňázová, E.; Pečenka, J.; Gajewski, Z.; 2017: Occurrence of Grapevine 'Pinot gris' virus in Poland and description of asymptomatic exhibitions in grapevines. J. Plant Dis. Protect. **124**, 407-411.

Fajardo, T. V. M.; Eiras, M.; Nickel, O.; 2017: First report of Grapevine 'Pinot gris' virus infecting grapevine in Brazil. Australas. Plant Dis. Notes **12**, 2015-2018.

Fan, X. D.; Dong, Y. F.; Zhang, Z. P.; Ren, F.; Hu, G. J.; Li, Z. N.; Zhou, J.; 2016: First report of grapevine 'Pinot gris' virus in grapevines in China. Plant Dis. **100**, 540.

Gal-On, A.; Shiboleth, Y. M.; 2006: Cross-protection. In: G. Loebenstein, J. P. Carr (Eds): Natural resistance mechanisms of plants to viruses, 261-288. Springer, Dordrecht, Netherlands.

Gentili, A.; Prota, V.; Moro, G.; Schianchi, N.; Di Lucca, E.; Luigi, M.; Faggioli, F.; 2017: Identification of grapevine 'Pinot gris' virus in Sardinia and Lazio (South and Central Italy). J. Plant Pathol. **99**, 527-530.

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

Glasa, M.; Predajňa, L.; Komínek, P.; Nagyová, A.; Candresse, T.; Olmos, A.; 2014: Molecular characterization of divergent grapevine 'Pinot gris' virus isolates and their detection in Slovak and Czech grapevines. Arch. Virol. **159**, 2103-2107.

Gualandri, V.; Asquini, E.; Bianchedi, P.; Covelli, L.; Brilli, M.; Malossini, U.; Bragagna, P.; Saldarelli, P.; 2017: Identification of herbaceous hosts of the Grapevine 'Pinot gris' virus (GPGV). Eur. J. Plant Pathol. **147**, 21-25.

Huss, B.; Walter, B.; Fuchs, M.; 1989: Cross-protection between Arabis Mosaic Virus and Grapevine Fan leaf Virus isolates in *Chenopodium quinoa*. Ann. Appl. Biol. **114**, 45-60.

Jo, Y.; Choi, H.; Kyong Cho, J.; Yoon J. Y.; Choi, S. K.; Kyong Cho, W.; 2015: *In silico* approach to reveal viral populations in grapevine cultivar Tannat using transcriptome data. Sci. Rep. **5**, 1-11.

Komar, V.; Vigne, E.; Demangeat, G.; Olivier Lemaire, A.; Fuchs, M.; 2008: Cross-protection as control strategy against grapevine fanleaf virus in naturally infected vineyards. Plant Dis. **92**, 1689-1694.

MacKenzie, D. J.; McLean, M. A.; Mukerji, S.; Green, M.; 1997: Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. Plant Dis. **81**, 222-226.

Malagnini, V.; De Lillo, E.; Saldarelli, P.; Beber, R.; Duso, C.; Raiola, A.; Zanotelli, L.; Valenzano, D.; Giampetruzzi, A.; Morelli, M.; Ratti, C.; Causin, R.; Gualandri, V.; 2016: Transmission of grapevine 'Pinot gris' virus by *Colomerus vitis* (Acari: Eriophyidae) to grapevine. Arch. Virol. **161**, 2595-2599.

Malagnini, V.; Duso, C.; Valenzano, D.; Pozzebon, A.; Simonetti, L.; Bianchedi, P. L.; Saldarelli, P.; Kubaa, R. A.; De Lillo, E.; Gualandri, V.; 2018: Role of *Colomerus vitis* (Pagenstecher) in the epidemiology of grapevine leaf mottling and deformation in North-eastern Italy, 22-23. Proc. 19th Congress of ICGV, Santiago, Chile.

Martelli, G. P.; 2014: Directory of virus and virus-like diseases of the grapevine and their agents. J. Plant Pathol. **96**, 110.

Morán, F.; Olmos, A.; Lotos, L.; Predajňa, L.; Katis, N.; Glasa, M.; Maliogka, V.; Ruiz-García, A. B.; 2018: A novel specific duplex real-time RT-PCR method for absolute quantitation of grapevine 'Pinot gris' virus in plant material and single mites. PLoS ONE. **13**, 1-14.

Pleško, I. M.; Marn, M. V.; Seljak, G.; Žežlina, I.; 2014: First report of grapevine 'Pinot gris' virus infecting grapevine in Slovenia. Plant Dis. **98**, 1014.

Poojari, S.; Lowery, T.; Rott, M.; Schmidt, A. M.; Úrbez-Torres, J. R.; 2016: First Report of Grapevine 'Pinot gris' virus in British Columbia, Canada. Plant Dis. **100**, 1513.

Rasool, S.; Naz, S.; 2017: First report of grapevine 'Pinot gris' virus infecting grapevine in Pakistan. Plant Dis. **101**, 1958.

Reynard, J. S.; Schumacher, S.; Menzel, W.; Fuchs, J.; Bohnert, P.; Glasa, M.; Wetzel, T.; Fuchs, R.; 2016: First Report of grapevine 'Pinot gris' virus in German vineyards. Plant Dis. **100**, 2545.

Ruiz-García, A. B.; Olmos, A.; 2017: First Report of grapevine 'Pinot gris' virus in Grapevine in Spain. Plant Dis. **101**, 1070.

Saldarelli, P.; Giampetruzzi, A.; Morelli, M.; Malossini, U.; Pirolo, C.; Bianchedi, P.; Gualandri, V.; 2015: Genetic variability of grapevine 'Pinot gris' virus and its association with grapevine leaf mottling and deformation. Phytopathology **105**, 555–563.

Silva, G.; Lecourt, J.; Clover, G. R. G.; Seal, S. E.; 2018: First record of grapevine 'Pinot gris' virus infecting *Vitis vinifera* in the United Kingdom. New Dis. Rep. **38**, 7.

Spilmont, A. S.; Sevin, A. F. ; Guinard, J.; Beuve, M.; Alliaume, A.; Marais, A.; Faure, C.; Candresse, T. L. O.; 2018: Occurrence of grapevine 'Pinot gris' virus (GPGV) and grapevine leaf mottling and deformation (GLMD) syndrome in France: genetic diversity and field monitoring in diverse viticulture areas, 52-53. Proc. 19th Congress of ICGV, Santiago, Chile.

Tarquini, G.; Zaina, G.; Ermacora, P.; De Amicis, F.; Franco-Orozco, B.; Loi, N.; Martini, M.; Bianchi G. L.; Pagliari, L.; Firrao, G.; De Paoli, E.; Musetti, R.; 2019: Agroinoculation of grapevine 'Pinot gris' virus in tobacco and grapevine provides insights on viral pathogenesis. PLoS ONE. **14**, e0214010.

Wu, Q.; Habili, N.; 2017: The recent importation of Grapevine 'Pinot gris' virus into Australia. Virus Genes. **53**, 935-938.

Xiao, H.; Shabanian, M.; McFadden-Smith, W.; Meng, B.; 2016: First report of grapevine 'Pinot gris' virus in commercial grapes in Canada. Plant Dis. **100**, 1030.

Ziebell, H.; Carr, J. P.; 2010: Cross-protection: a century of mystery. Adv. Virus Res. **76**, 211-264.

*Received April 26, 2019*
*Accepted October 11, 2019*