SEROLOGICAL DETECTION OF *GRAPEVINE FANLEAF VIRUS* (GFLV) IN AMPELOGRAPHIC COLLECTION FROM USAMV IAŞI (ROMANIA)

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Abstract

Grapevine fanleaf virus (GFLV) is one of the most severe virus diseases in vineyards worldwide. It causes extensive leaf yellowing, stem and leaf deformation, reduced fruit quality, substantial crop loss and shortened longevity of vineyards. GFLV is transmitted specifically from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index*, and belongs to the genus *Nepovirus* in the family *Comoviridae*. During 2009 and 2011 a sanitary survey was conducted in the ampelographic collection of the University of Agricultural Sciences and Veterinary Medicine Iaşi (Romania) on 170 cultivars belonging to *Vitis* spp. Our objectives were to determine the presence and distribution of GFLV across the ampelographic collection, and to find out if the virus titre fluctuation is cultivar specific. Leaf samples were taken during spring season from vines exhibiting virus-like symptoms or general vine decline. Three mature leaves including the petiole from different sections of the vine, keeping between the first and fifth node from the base of the vine were collected in dry, cool weather. The results of DAS-ELISA test confirm that virus was present in 29 grapevine cultivars (17.1% of total) from the ampelographic collection. Infected vine cultivars with the highest OD (optical density) values were Gordan, Newburger, Cioinic, Galbenă de Odobeşti, Blauerzweigelt and Merlot.

Key words: *Vitis* spp., GFLV, ELISA, Romania

Grapevine fanleaf virus (GFLV) is one of the most destructive pathogens of grapevine worldwide. It causes extensive leaf yellowing, stem and leaf deformation, reduced fruit quality, substantial crop loss (up to 80%) and shortened longevity of vineyards (Andret-Link et al., 2004). Among 58 virus species that can infect grapevine, GFLV belongs to the plant virus genus *Nepovirus* of the family *Comoviridae* (Mayo and Robinson, 1996) with genome consisting of two single-stranded positive sense RNAs, separately encapsidated (Fritsch et al., 1993, Wellink et al., 2000). The soil-borne virus is transmitted exclusively by the ectoparasitic nematode *Xiphinema index* Thorne and Allen, which can survive in vineyard soils and retain GFLV for many years with or without host plants (Demangeat et al., 2005; Martelli, 2006).

Vineyards viruses control is currently based on preventive measures and cultural practices. Prophylactic measures intend to prevent introduction of diseased vine varieties in healthy vineyards, and cultural practices try to reduce the vectors population (Laimer, 2009). GFLV is controlled by soil disinfection with nematicides, but this procedure is only partially efficient and in many countries forbidden because of environmental toxicity (Demangeat et al., 2005; Raski and Goheen, 1988).

The aims of this study were to determine the presence and distribution of GFLV across the ampelographic collection of University of Agricultural Sciences and Veterinary Medicine (USAMV) Iaşi, and to find out if the virus titre fluctuation is cultivar specific.

MATERIAL AND METHODS

During 2009 and 2011 a sanitary survey was conducted in ampelographic collection belonging to USAMV Iaşi (N-E Romania) on 170 genotypes belonging to *Vitis* spp.

Leaf samples were taken each year during spring season from 34 vines exhibiting virus-like symptoms or general vine decline. Three mature leaves including the petiole from different sections of the vine, keeping between the first and fifth node from the base of the vine were collected in dry, cool weather. Totally, 368 samples of symptomatic leaves from surveyed varieties were collected, put into separate plastic bags, frozen with liquid nitrogen, transported to the laboratory, and stored at −80ºC until analysed.

Symptomatic grapevine samples were used for detection of GFLV by a double-antibody sandwich ELISA (DAS ELISA) using monoclonal and polyclonal antibodies. ELISA was performed with commercial

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kits (ADGEN Phytodiagnostics, UK), according to the manufacturer recommendation. Crude grapevine extracts were prepared by grinding 1 g leaves in 10 mL of ELISA extraction buffer. Leaf extracts were centrifuged at 2,000 g for 15 min and the supernatant was used as the antigen in DAS ELISA. 100 µL were loaded in each well on microtiter plates (Nunc Immunoplate I, Nunc, Denmark). Incubation steps lasted overnight at 4°C in closed dark boxes. Reactive were preincubated to the plate temperature.

Intermediate washings were done with PBS/Tween buffer. Values were recorded measuring absorbance at 405 nm with a microplate reader Sunrise (Tecan, Austria) powered by Magellan data analysis software.

DAS ELISA results were taken as mean absorbance value of three replicates per sample. Positive and negative controls were supplied with the kit. Each value was considered GFLV-positive when the average value was at least three times greater than the mean of healthy control.

Statistic was performed with Microsoft Office Excel 2007. Analysis of variance was performed with the use of One-Way ANOVA test. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSIONS

The incidence of GFLV disease on ampelographic collection of USAMV Iaşi was visually monitored between 2009 and 2011. Out of 336 grapevine samples collected from 34 varieties with characteristic symptoms 279 were infected with *Grapevine fanleaf virus*. GFLV was present in 29 grapevine cultivars (17.1% of total) from the ampelographic collection. Foreign grapevine cultivars showed a higher sensitivity to GFLV in comparacy with the indigenous grapevine cultivars (Table 1).

Table 1

Occurrence of GFLV as determined by DAS ELISA on grapevine samples collected from ampelographic collection of USAMV Iaşi

Indigenous grapevine cultivars	No. total of samples	GFLV positives		Foreign grapevine cultivars	No. total of samples	GFLV positives	
		No.	%			No.	%
Ardeleanca	12	8	66.7	Aligoté	12	10	83.3
Armaş	12	8	66.7	André	12	10	83.3
Bicane	12	10	83.3	Andrevit	12	8	66.7
Cioinic	12	12	100.0	Bastarde de Magaraci	12	9	75.0
Creaţă de Banat	12	11	91.7	Blauerzweigelt	12	12	100.0
Fetească albă	12	7	58.3	Chasselas Doré	12	10	83.3
Fetească regală	12	9	75.0	Decabriski	12	8	66.7
Frîncuşa	12	8	66.7	Dimiat	12	7	58.3
Galbenă de Odobeşti	12	12	100.0	Gamay Beaujolais	12	8	66.7
Gordan	12	12	100.0	Merlot	12	11	91.7
Grasă de Cotnari	12	10	83.3	Newburger	12	12	100.0
Napoca	12	7	58.3	Pinot Noir	12	11	91.7
Răzăchie	12	7	58.3	R6/Chasselas Doré	12	12	100.0
Regina viilor	12	7	58.3	Riesling Aromat	12	11	91.7
				Traminer roz	12	12	100.0
TOTAL	168	128	76.2	TOTAL	168	151	83.9

The results show that not all cultivars with virus-like symptoms are caused by GFLV. Leaf yellowing, stem and leaf deformation could be caused by physical injury or some other disorder (fungicide, herbicide, insecticide). GFLV causes the grapevine fanleaf degeneration worldwide and severe losses up to 80%, poor fruit quality and reduced grapevine longevity (Andret-Link et al., 2004).

Infected foreign cultivars with the highest OD (optical density) values measured at 405 nm were Blauerzweigelt, Merlot and Newburger. Infection with high OD was also confirmed in some indigenous cultivars as Gordan, Cioinic and Galbenă de Odobeşti (Figure 1).

Differences in sensitivity to GFLV are known among cultivars of *V. vinifera*; some are resistant to infection and others recover one year after the appearance of symptoms. A fundamental importance in the development of the disease is played by environment and growing area, because the number of infected grapevines in vine plantations will increase dramatically in the presence of infected vineyards.

ANOVA did not reveal statistical differences among the mean values of extinction in GFLV-infected cultivars during 2009 and 2011 (data not show).

These results could be used for improving detection protocols to test grapevine propagative material in Romania and to eliminate the risk of

long distance spreading during international exchange of plant material.

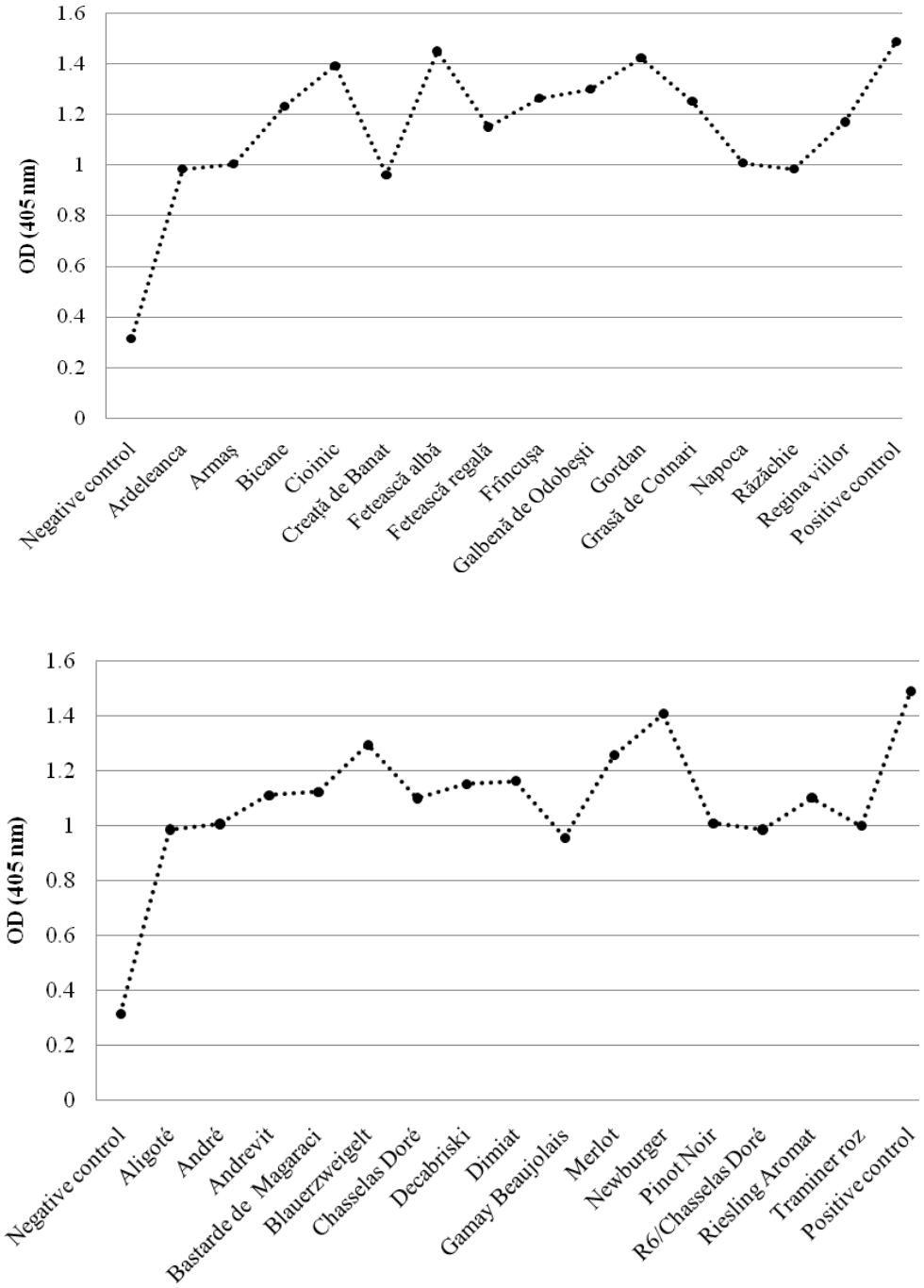

Figure 1: **Average values of extinction with DAS ELISA for indigenous cultivars and foreigner grapevine.**

CONCLUSIONS

Grapevine cultivars from ampelographic collection of USAMV Iaşi (N-E Romania) have been examined by visual symptom assessment for typical GFLV symptoms between 2009 and 2011 and serological tests (DAS ELISA) revealed that incidence of GFLV disease ranged 17.1%.

Infected grapevine plants from the 29 varieties should be removed and replaced after reducing of vector populations. Also, the usage of clean planting material and surveying the neighbouring viticulture areas are measures that should be implemented to maintain the disease under control.

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REFERENCES

Andret-Link, P., Laporte, C., Valat, L., Ritzenthaler, C., Demangeat, G., Vigne, E., Laval, V., Pfeiffer, P., Stussi-Garaud, C., Fuchs, M. 2004 - *Grapevine fanleaf virus: still a major threat to the grapevine industry,* Journal of Plant Pathology 86, p. 183-195.

Demangeat G., Voisin R., Minot J.C., Bosselut N., Fuchs M., Esmenjaud D. 2005 - *Survival of Xiphinema index in vineyard soil and retention of Grapevine fanleaf virus over extended time in the absence of host plants*, Phytopathology 95, p. 1151–1156.

Fritsch, C, Mayo, M., Hemmer, O. 1993 - *Properties of the satellite RNA of nepoviruses,* Biochimie. 75, p. 561-567.

Laimer, M., Lemaire, O., Herrbach, E,. Goldschmidt, V., Minafra, A., Bianco, P., Wetzel, T. 2009 - *Resistance to viruses, phytoplasmas and their vectors in Europe: a review*, Journal of Plant Pathology 91, p. 7-23.

Martelli, G.P. 2006 - *Grapevine virology highlights 2004–2005.* Extended abstracts 15th Meeting of ICVG, Stellenbosch, South Africa, p. 13–18.

Mayo, M.A., Robinson, D.J., 1996 - *Nepoviruses: molecular biology and replication.* In The Plant Viruses, vol. 5: Polyhedral Virions and Bipartite RNA Genomes. Edited by B. D. Harrison & A. F. Murant. New York: Plenum Press.

Raski D.J., Goheen A.C., 1988 - *Comparison of 1, 3-dichloropropene and methyl bromide for control of Xiphinema index and grapevine fanleaf degeneration complex.* Am J Enol Vitic. 39, p. 334–336.

Wellink, J., Le Gall, O., Sanfacon, H., Ikegami, M., Jones, A.T., 2000 - In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (Eds) *Virus Taxonomy. Seventh report of the International Committee on Taxonomy of Viruses.* Academic Press, San Diego, Ca, p. 691-701.