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THE DETECTION OF VIRUSES AND PHYTOPLASMAS IN DWARFED SHOOTS OF GRAPEVINE VARIETIES AURELIUS AND NEUBURGER

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Abstract

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The survey of occurrence of six chosen virus pathogens and phytoplasmas complex was done on plants of grapevine (*Vitis vinifera* L.) which showed symptoms of short-shoot syndrom. The results of serological and molecular tests did not confirm either virus or phytoplasma infection as the main source of short-shoot syndrom. The presence of 6 viruses in samples taken from 45 affected plants of grapevine on 4 habitats. The highest occurrence of viruses was found out on habitat Moravská Nová Ves, where all taken samples were infected by *Grapevine leafroll-associated virus* GLRaV-1 (100 %). In 66 % of the samples taken from that habitat were detected mixed infection of *Grapevine virus A* and GLRaV-1. These 2 pathogens but were not detected in samples from affected plants from the other habitats or very sporadically – just in 2 plants. Another 4 virus pathogens were detected either sporadically (*Grapevine fleck virus*, *Grapevine leafroll-assoc. virus* GLRaV-3, *Arabis mosaic virus*) or not at all (*Grapevine fanleaf virus*). From 270 tests made to 6 viruses were only 20 positive, e. g. 7.4 %. It means that from 45 plants were 15 infected at least by 1 virus (33 %). The phytoplasmas complex was tested in 28 plants. The result was positive only in 1 plant, by another test the Potato stolbur phytoplasma was confirmed.

ELISA, nested-PCR, symptoms, shoot, *Vitis*

The research for presence of viruses in plants which were affected by growth disfunctions and harvest decrease of grapevine started in laboratory for diagnostic of plant viruses and phytoplasmas in institut Mendeleum in 2003. The growers pointed out the shortened growth of internodias which caused zig-zag growth of shoots (Fig. 1), low crop, and shortened clusters and stalks (Fig. 2). These symptoms were noticed mainly by varieties Neuburger and Aurelius and later by Sauvignon blanc and Saint Laurent. During the years with low precipitation and high temperatures during spring and summer was shortened growth of shoots very significant, bunch of grapes minimised and their development limited.

Bovey *et al.* (1980) describe short internodia and harvest decrease as a symptom of virus infection by *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV). The detection of these viruses by serological method DAS-ELISA in 2003–2009 did not confirm definitely that these viruses caused that growth defect, in oposite – in some plants with strong symptoms these viruses were not detected at all. That's why was the spectrum of tested viruses broaden for another 4 virus pathogens and phytoplasmas complex. Canadian Food Inspection Agency mentions that by phytoplasma Grapevine yellows diseases can appear tight clustering of young shoots, zig-zag growth and early rolling of leaves. Also can be noticed bad development of flowers and flower stalk (2006). The occurrence of stolbur phytoplasma in grapevine was officially confirmed in Czech Republic by diagnostics laboratory of State Phytosanitary Administration in 2006 at varieties

1: *Significant shortening of internodia on grapevine shoot*

2: *Dwarfed claster*

Lemberger (Blaufränkisch), Zweigeltrebe and Saint Laurent in districts Břeclav, Znojmo and Brno (Červená *et al*., 2007).

The spread of rust mite (*Calepitrimerus vitis* Nal.) and bud mite (*Colomerus vitis* Pag.) in the vineyards is considered as the source of short shoot syndrome in Australia (Bernard *et al*., 2005) and also in Oregon and Washington (Walton *et al*., 2007).

MATERIAL AND METHODS

The samples of dormant shoots were taken from vineyards in 4 habitats in South Moravia from plants, which showed above mentioned symptoms of growth abnormalities. They were tested by serological method DAS- ELISA (Clark & Adams, 1977) first to presence of GFLV and ArMV viruses and then to Grapevine leafroll complex GLRaV-1 and GLRaV-3, *Grapevine fleck virus* and *Grapevine*

I: *Overview of habitats and tested varieties*

Habitat	Variety	Year of testing
Čejkovice	Aurelius	2009, 2010
Dolní Dunajovice	Aurelius, Neuburger	2010
Dolní Kounice	Neuburger	2005, 2010
Moravská Nová Ves	Neuburger	2010

virus A. The diagnostics of Bioreba company (Switzerland) were used. The methodology was followed according to the producer's instructions. The list of habitats and varieties is in the Tab. I.

The presence of phytoplasmas was tested by molecular method nested-PCR (polymerase chain reaction) from the sample, which contained mix of tissues of leaf, petiole with veins of 1st line and phloem from summer shoots. Isolation of DNA was made by modified protocol (Ahrens & Seemüller, 1992). In the first PCR reaction were used primers P_1/P_7 in the second PCR reaction were used primers $R_{16}F2n/R_{16}R2$ (Gundersen & Lee, 1996). The samples were later separated by electrophoresis.

Positive samples from this test to phytoplasmas complex were then tested on presence of Potato stolbur phytoplasma. In this test were used following primer combinations: first PCR reaction – $STOL_{11}F2/STOL_{11}R1$ (Daire *et al*., 1997) and second PCR reaction – $STOL_{11}F3/STOL_{11}R2$ (Clair *et al*., 2003).

The samples were loaded into agarose gel (1.2%) coloured by GelRed (Biotium) and separated by electrophoresis. Tests were made in three repetition (A, B, C). As a positive sample was considered that one with two positive results from three repetition.

RESULTS AND DISCUSSION

For the presence of 6 virus pathogens were tested 45 plants in 2010, from that 10 plants of variety Aurelius were tested in 2009 and 10 plants of the variety Neuburger were tested in 2005. The highest occurrence of virus pathogens was showed by Neuburger in the habitat Moravská Nová Ves, where leafroll virus GLRaV-1 was detected in 6 samples (100%) and also GVA virus in 4 samples (66 %). It is possible to assume, that in that vineyard was planted infected propagation material. The mixed infection GVA and GLRaV-1 was found out in one plant Neuburger variety from Dolní Dunajovice and one plant of Neuburger from Dolní Kounice. Next most frequent occurrence was found out by GFkV at 3 plants of Neuburger in Dolní Kounice. Leafroll GLRaV-3 was found out at 2 plants, ArMV at one and GFLV at none plant. Altogether were 15 plants from 45 tested infected at least by one virus (33.3 %), from 270 made tests for viruses presence were 20 positive, e. g. 7.4% (see Tab. II).

The phytoplasmas were detected only in one plant from 28 tested. In this plant was Potato stolbur phytoplasma confirmed by other tests (Fig. 3). The plant was variety Aurelius from Dolní Dunajovice. It is clear, that phytoplasma is not the reason of abnormal dwarf growth of shoots. There were no typical visual symptoms of phytoplasma infection visible on these tested plants like sectorial yellowing of leaf or irregular ripening of shoots and gummy shoots (Červená *et al*., 2007).

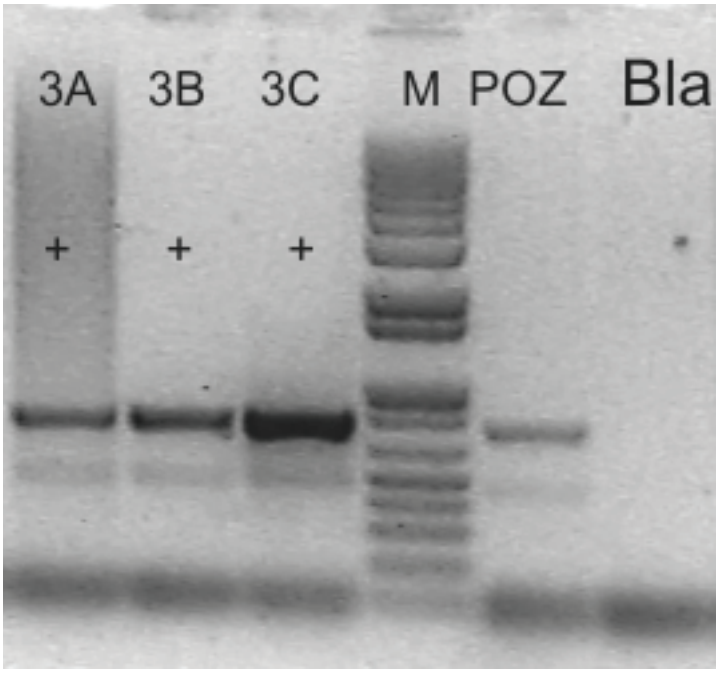
The affected plants were slightly damaged by *Colomerus vitis* Pag. causing felting of leaves in habitats Dolní Dunajovice and Dolní Kounice in September. The reason of zig-zag growth of the branches could be in buds damage by mites during winter. The shoot from main bud sproots, but looses apical dominance, grows zig-zag and often dies. The secondary branches from adventive buds become dominant later on and have little or none grapes (Walton *et al*., 2007).

The shoot base of plants of variety Aurelius in Čejkovice showed not only zig-zag growth, but also

II: *The results of virus and phytoplasmas detection in the plants of grapevine from 45 tested plants*

Pathogen/variety		Habitats				Number of infected plants
		Čejkovice	D. Dunaj.	D. Kounice	M. N.Ves	
ArMV	AUR	0	0	0	0	1
	NEU	0	0	1	0	
GFkV	AUR	0	0	0	0	3
	NEU	0	0	3	0	
GFLV	AUR	0	0	0	0	0
	NEU	0	0	0	0	
GLRaV-1	AUR	0	0	0	0	8
	NEU	0	1	1	6	
GLRaV-3	AUR	1	0	0	0	2
	NEU	0	1	0	0	
GVA	AUR	0	0	0	0	6
	NEU	0	1	1	4	
Phytoplasmas complex	AUR	0	1	0	0	1
	NEU	0	0	0	0	
Potato stolbur phytoplasma	AUR	0	1	0	0	1
	NEU	0	0	0	0	

Consideration: ArMV *Arabis mosaic virus*; GFkV *Grapevine fleck virus;* GFLV *Grapevine fanleaf virus*; GLRaV-1, 3 *Grapevine leafroll assoc. virus* 1 and 3; GVA *Grapevine virus A;* AUR Aurelius variety; NEU Neuburger



3: *Picture of the agarose gel with samples positive to presence of Potato stolbur phytoplasma*

blushing and bark-cracking, which can be caused not only by mites, but also by fungi disease which damage the xylem, or by nutrition lack.

CONCLUSION

Viral pathogens *Arabis mosaic virus*, *Grapevine fleck virus, Grapevine fanleaf virus*, *Grapevine leafroll assoc. viruses* 1 and 3; *Grapevine virus A* and Potato stolbur phytoplasma were detected minimally in the tested plants *Vitis vinifera* L. damaged by dwarf shoots and clusters. This was not general occasion so-called short shoot syndrom.

SUMMARY

The target of this work was verification if the reason of grapevine shoot shortening is infection by virus pathogens and phytoplasmas. By serological method DAS-ELISA was checked the presence of 6 viruses in samples taken from 45 affected plants of grapevine on 4 habitats. The wood was tested during dormancy period, the leaves during vegetation. The presence of phytoplasmas was checked by method nested-PCR in the leaf samples. The highest occurrence of viruses was found out on habitat Moravská Nová Ves, where all taken samples were infected by *Grapevine leafroll assoc. virus* GLRaV-1 (100 %). In 66 % of the samples taken from that habitat were detected mixed infection of *Grapevine virus A* and GLRaV-1. These 2 pathogens but were not detected in samples from affected plants from the other habitats or very sporadically – just in 2 plants. It is possible to assume, that in that vineyard was planted infected propagation material.

Another 4 virus pathogens were detected either sporadically (*Grapevine fleck virus*, *Grapevine leafroll assoc. virus* GLRaV-3, *Arabis mosaic virus*) or not at all (*Grapevine fanleaf virus*). From 270 tests made to 6 viruses were only 20 positive, e. g. 7.4 %. It means that from 45 plants were 15 infected at least by 1 virus (33 %).

The phytoplasmas complex was tested in 28 plants. The result was positive only in 1 plant, by another test the Potato stolbur phytoplasma was confirmed. The phytoplasmas do not cause that abnormal grapevine shoot shortening.

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