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Short communication

Monitoring of stone fruit viruses in Serbia

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Abstract. The results of the survey on the presence of seven viruses (cherry necrotic rusty mottle virus, cherry leaf roll virus, cherry rasp leaf virus, tomato black ring virus, tomato ringspot virus, prunus necrotic ringspot virus and apple mosaic virus) infecting stone fruits are presented in this paper. A total number of 107 samples from mother blocks and nurseries in 30 locations were collected and analyzed. Samples were analyzed with ELISA and RT-PCR methods. Cherry necrotic rusty mottle virus was detected in six samples (one sweet and one sour cherry, two plums and two apricots). No other viruses were detected in analyzed samples.

Key words: *Prunus* sp., viruses, ELISA, RT-PCR

Introduction

More than 65% of fruit orchards in Serbia are planted with different stone fruit species (*Prunus* spp.), with plum (*P. domestica* L.) as a dominant culture (Keserović et al., 2014). Stone fruits are cultivated in all parts of the country. Wide presence of stone fruits is a result of favorable agro and climatic conditions for their cultivation, proximity to processing facilities, demand for fresh, frozen and dry fruit. Serbia is a significant producer and exporter of stone fruits. According to statistics division of the Food and Agriculture Organization (FAO) of the United Nations, Serbia is ranked first in Europe on area under plums, fourth for sour cherry, fifth for peach and nectarine, seventh for apricot and fifteenth for sweet cherry (FAOSTAT, 2021).

Numerous viruses infect stone fruits. Economically the most important and detrimental is plum pox virus (PPV) that is endemically present in Serbia for more than 85 years (Josifović, 1937). In sensitive cultivars, as 'Požegača', PPV causes great yield losses and lowers fruit quality. PPV is one of the 10 most important plant viruses around the globe (Scholthof et al., 2011). Other viruses are much less presented and distributed. Prunus necrotic ringspot virus (PNRSV) was detected in low prevalence in sweet and sour cherry orchards in Serbia (Paunović et al., 1994). The information on other viruses, as cherry necrotic rusty mottle virus (CNRMV), cherry leaf roll virus (CLRV), cherry rasp leaf virus (CRLV), tomato black ring virus (TBRV), tomato ringspot virus (ToRSV) and apple mosaic virus (ApMV), is limited.

The aim of our work was to investigate the presence of seven viruses infecting stone fruits in mother blocks and nurseries.

Material and Methods

Plant material. During 2021, the number of leaf samples (107) from stone fruits were collected from mother blocks and nurseries (Tab. 1). Samples were collected from 30 locations. A number of 35 sour cherry, 32 sweet cherry, 21 plum, nine apricot, eight peach, one mahaleb cherry and one hybrid (*P. avium* × *P. cerasus*) were analyzed. Each sample consisted of 15–25 leaves collected from one tree. One portion of the samples was stored at +4°C and another at -20°C.

Serological tests. All samples were tested on the presence of cherry leaf roll virus, tomato black ring virus, tomato ringspot virus, prunus necrotic ringspot virus and apple mosaic virus by double antibody sandwich – enzyme-linked immunosorbent assay (DAS-ELISA) with the reagents of BIOREBA AG (Switzerland). Fresh leaf samples were prepared at 1:20 ratio in the PBS-Tween + 2% PVP extraction buffer. Color development was measured at 405 nm on ELISA reader (MULTISKAN MCC/340) after 30–120 min. Samples were considered as positive when optical density (OD) values were at least two times higher than the OD values of the negative control.

Molecular tests. Total nucleic acids (TNA) were extracted from frozen leaves with a modified CTAB method as described by Li et al. (2008). After extraction TNAs were stored at -20°C until use. The samples were analyzed for the presence of cherry necrotic rusty mottle virus and cherry rasp leaf virus by reverse transcription-polymerase chain reaction (RT-PCR). The first-strand cDNAs were generated by reverse transcription reactions using Maxima Reverse Transcriptase (ThermoFisher Scientific, USA) following the manufacturer's protocol. The obtained cDNAs were used as templates in PCR reactions with specific CNRMV primers (NEG1U/NEG1L) that amplify a 255 bp fragment (Rott & Jelkmann, 2001). The PCR conditions were: 94°C for 2 min for initial denaturation; followed by 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min; and final extension at 72°C for 5 min. For the detection of CRLV, a primer pair FAVR1-7F/FAVR1-8R was used for PCR amplification of 447 bp fragment of RNA-dependent RNA polymerase gene of the CRLV genome (James & Upton, 2005). The program for PCR was as follows: 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 50°C for 30 s and 72°C for 1 min, followed by a final extension step at 72°C for 7 min. PCR reactions were performed in TPersonal thermocycler (Biometra GmbH, Germany). PCR products were analyzed by 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light by Gel Doc EZ System (Biorad laboratories, USA).

Results and Discussion

During inspection, leaves on the plants in the surveyed mother blocks and nurseries did not show any symptoms indicating virus presence.

The results of the ELISA tests on the presence on PNRSV, ApMV, CLRV, ToRSV and TBRV revealed that none of 107 analyzed samples was positive (Tab. 1). The OD values of all samples for each tested virus were at the level of negative control. A positive result was obtained only in the positive control for each tested virus, with OD values at least two times higher than the value for negative control.

PNRSV is widely distributed virus infecting stone fruits that is commonly found in stone fruits in Serbia. It is widely distributed among sweet and sour cherries in the country with low incidence in the orchards (Paunović et., 1994; Mandić et al., 2007; Paunović, unpublished results). Mother blocks of stone fruits in Serbia are regularly inspected and tested on the PNRSV presence (1/3 of the trees each year). Infected trees are immediately removed after the confirmation of virus presence.

Apple mosaic virus is common virus infecting apples and hazelnuts. ApMV can infect most cultivated *Prunus* species, as plum, peach, apricot, almond, cherry, and sour cherry (Paunović et al., 2011). Infections of different *Prunus* spp. are confirmed worldwide. ApMV was confirmed in apple and hazelnut in Serbia, but not on other sweet and sour cherries (Paunović, unpublished results).

Cherry leaf roll virus is present on all continents, except Africa. In sensitive cultivars CLRV induces chlorosis, yellow vein netting, chlorotic ring spots and mottling, leaf roll, dieback of branches or entire trees (Bandte & Büttner, 2001). Symptoms vary in host species, virus strain, and plant predisposition due to environmental stresses and interaction with biotic factors. CLRV is present in Serbia, but with no details (EPPO, 2021a). In previous surveys CLRV was not confirmed in sweet and sour cherry orchards in Serbia (Paunović, unpublished results).

Tab. 1. The results of ELISA and RT-PCR analysis of leaf samples of stone fruits
Tab. 1. Rezultati ELISA i RT-PCR analize uzoraka lišća koštičavih vrsta voćaka

Number *Broj*	Species *Vrsta*	Cultivar *Sorta*	Locality *Lokalitet*	ELISA test results/*Rezultati ELISA testa*					RT-PCR test results *Rezultati RT-PCR testa*	
				TBRV	ToRSV	PNRSV	CLRV	ApMV	CNRMV	CRLV
1.	*P. domestica*	'Stanley'	Sevojno	–	–*	–	–	–	–	–
2.	*P. cerasus*	'Oblačinska'	Dubnica	–	–	–	–	–	–	–
3.	*P. cerasus*	'Oblačinska'	Dubnica	–	–	–	–	–	–	–
4.	*P. cerasus*	'Oblačinska'	Dubnica	–	–	–	–	–	–	–
5.	*P. cerasus*	'Oblačinska'	Dubnica	–	–	–	–	–	–	–
6.	*P. cerasus*	'Oblačinska'	Dubnica	–	–	–	–	–	–	–
7.	*P. cerasus*	'Sofija'	Čačak	–	–	–	–	–	–	–
8.	*P. cerasus*	'Šumadinka'	Čačak	–	–	–	–	–	–	–
9.	*P. cerasus*	'Čačanski Rubin'	Čačak	–	–	–	–	–	–	–
10.	*P. cerasus*	'Sofija'	Čačak	–	–	–	–	–	–	–
11.	*P. cerasus*	'Šumadinka'	Čačak	–	–	–	–	–	–	–
12.	*P. avium*	'Burlat'	Čačak	–	–	–	–	–	–	–
13.	*P. avium*	'Regina'	Čačak	–	–	–	–	–	–	–
14.	*P. cerasus*	'Keleris 9'	Čačak	–	–	–	–	–	–	–
15.	*P. avium*	'Burlat'	Čačak	–	–	–	–	–	–	–
16.	*P. cerasus*	'Šumadinka'	Čačak	–	–	–	–	–	–	–
17.	*P. avium*	Wild cherry	Negotin	–	–	–	–	–	–	–
18.	*P. persica*	'Redhaven'	Negotin	–	–	–	–	–	–	–
19.	*P. avium*	'Burlat'	Negotin	–	–	–	–	–	–	–
20.	*P. avium*	'Giorgia'	Miokus	–	–	–	–	–	–	–
21.	*P. avium*	'Regina'	Miokus	–	–	–	–	–	–	–
22.	*P. avium*	'Karina'	Miokus	–	–	–	–	–	+**	–
23.	*P. mahaleb*	'Magriva'	Bresnica	–	–	–	–	–	–	–
24.	*P. domestica*	'Stanley'	Mojković	–	–	–	–	–	–	–
25.	*P. domestica*	'Stanley'	Presečina	–	–	–	–	–	–	–
26.	*P. avium*	'Early Lyons'	Presečina	–	–	–	–	–	–	–
27.	*P. cerasus*	'Oblačinska'	Presečina	–	–	–	–	–	–	–
28.	*P. cerasus*	'Oblačinska'	Vučje	–	–	–	–	–	–	–
29.	*P. cerasus*	'Oblačinska'	Presečina	–	–	–	–	–	–	–
30.	*P. cerasus*	'Oblačinska'	Vučje	–	–	–	–	–	+	–
31.	*P. cerasus*	'Oblačinska'	Presečina	–	–	–	–	–	–	–
32.	*P. cerasus*	'Oblačinska'	Presečina	–	–	–	–	–	–	–
33.	*P. domestica*	'Stanley'	Vučje	–	–	–	–	–	–	–
34.	*P. avium*	'Germersdorfska'	Vučje	–	–	–	–	–	–	–
35.	*P. domestica*	'Grosa di Felicia'	Sukovo	–	–	–	–	–	–	–
36.	*P. domestica*	'Stanley'	Klenje	–	–	–	–	–	–	–
37.	*P. cerasus*	'Oblačinska'	Vranište	–	–	–	–	–	–	–
38.	*P. avium*	'Burlat'	Gradište	–	–	–	–	–	–	–
39.	*P. avium*	'Burlat'	Pirot	–	–	–	–	–	–	–
40.	*P. domestica*	'Čačanska Rana'	Svilajnac	–	–	–	–	–	–	–
41.	*P. domestica*	'Stanley'	Svilajnac	–	–	–	–	–	–	–
42.	*P. domestica*	'Čačanska Rodna'	Svilajnac	–	–	–	–	–	–	–
43.	*P. domestica*	'Čačanska Lepotica'	Svilajnac	–	–	–	–	–	–	–
44.	*P. cerasus*	N/A	Svilajnac	–	–	–	–	–	–	–
45.	*P. avium*	N/A	Svilajnac	–	–	–	–	–	–	–
46.	*P. armeniaca*	N/A	Svilajnac	–	–	–	–	–	–	–
47.	*P. armeniaca*	'Novosadska Rana'	Dubnica	–	–	–	–	–	–	–
48.	*P. persica*	'Redhaven'	Vranje	–	–	–	–	–	–	–
49.	*P. domestica*	'Čačanska Lepotica'	Vranje	–	–	–	–	–	–	–
50.	*P. domestica*	'Čačanska Lepotica'	Vranje	–	–	–	–	–	–	–
51.	*P. avium*	'Sunburst'	Pančevo	–	–	–	–	–	–	–
52.	*P. avium*	'Rani Burlat'	Pančevo	–	–	–	–	–	–	–
53.	*P. avium*	'Lapins'	Pančevo	–	–	–	–	–	–	–

54.	*P. cerasus*	'Keleris 16'	Pančevo	–	–	–	–	–	–	–
55.	*P. cerasus*	'Oblačinska'	Pančevo	–	–	–	–	–	–	–
56.	*P. persica* var. *nucipersica*	'Krimson Gold'	Smederevo	–	–	–	–	–	–	–
57.	*P. persica*	'Redhaven'	Smederevo	–	–	–	–	–	–	–
58.	*P. avium*	'Regina'	Smederevo	–	–	–	–	–	–	–
59.	*P. armeniaca*	'Roxana'	Smederevo	–	–	–	–	–	–	–
60.	*P. domestica*	'Čačanska Rodna'	Smederevo	–	–	–	–	–	–	–
61.	*P. persica*	'Cresthaven'	Konjuh	–	–	–	–	–	–	–
62.	*P. avium*	'Sam'	Konjuh	–	–	–	–	–	–	–
63.	P. domestica	'Avalon'	Konjuh	–	–	–	–	–	–	–
64.	*P. avium*	'Lapins'	Konjuh	–	–	–	–	–	–	–
65.	*P. cerasus*	'Keleris 16'	Milutovac	–	–	–	–	–	–	–
66.	*P. avium*	'Burlat'	Milutovac	–	–	–	–	–	–	–
67.	*P. persica*	'Springtime'	Milutovac	–	–	–	–	–	–	–
68.	*P. domestica*	'Bluefree'	Milutovac	–	–	–	–	–	+	–
69.	*P. domestica*	'Opal'	Lazarevac	–	–	–	–	–	+	–
70.	*P. armeniaca*	'Novosadska rodna'	Lazarevac	–	–	–	–	–	–	–
71.	*P. domestica*	'Victoria'	Lazarevac	–	–	–	–	–	–	–
72.	*P. avium*	'Kordia'	Lazarevac	–	–	–	–	–	–	–
73.	*P. cerasus*	'Oblačinska'	Lazarevac	–	–	–	–	–	–	–
74.	*P. armeniaca*	'Mađarska najbolja'	Lazarevac	–	–	–	–	–	+	–
75.	*P. persica*	'Redtop'	Konjuh	–	–	–	–	–	–	–
76.	*P. persica*	'Redhaven'	Konjuh	–	–	–	–	–	–	–
77.	*P. armeniaca*	'Polumela'	Konjuh	–	–	–	–	–	–	–
78.	*P. cerasus*	'Montmorensy'	Konjuh	–	–	–	–	–	–	–
79.	*P. domestica*	'Stenley'	Konjuh	–	–	–	–	–	–	–
80.	*P. cerasus*	'Oblačinska'	Krajkovac	–	–	–	–	–	–	–
81.	*P. cerasus*	'Oblačinska'	Krajkovac	–	–	–	–	–	–	–
82.	*P. cerasus*	'Oblačinska'	Baličevac	–	–	–	–	–	–	–
83.	*P. cerasus*	'Oblačinska'	Donja Rasovača	–	–	–	–	–	–	–
84.	*P. cerasus*	'Oblačinska'	Čokot	–	–	–	–	–	–	–
85.	*P. avium*	'Burlat'	Rimski Šančevi	–	–	–	–	–	–	–
86.	*P. avium*	'Kordia'	Rimski Šančevi	–	–	–	–	–	–	–
87.	*P. avium*	'Lapins'	Rimski Šančevi	–	–	–	–	–	–	–
88.	*P. avium*	'Summit'	Rimski Šančevi	–	–	–	–	–	–	–
89.	*P. avium*	'Regina'	Rimski Šančevi	–	–	–	–	–	–	–
90.	*P. avium*	'Early Star'	Kać	–	–	–	–	–	–	–
91.	*P. avium*	'Primavera'	Kać	–	–	–	–	–	–	–
92.	*P. avium*	'Droganova'	Kać	–	–	–	–	–	–	–
93.	*P. cerasus*	'Ujfehertoi Furtos'	Kać	–	–	–	–	–	–	–
94.	*P. cerasus*	'Erdi Botermo'	Kać	–	–	–	–	–	–	–
95.	*P. avium*	N/A	Topola	–	–	–	–	–	–	–
96.	*P. domestica*	N/A	Topola	–	–	–	–	–	–	–
97.	*P. armeniaca*	N/A	Topola	–	–	–	–	–	+	–
98.	*P. cerasus*	N/A	Topola	–	–	–	–	–	–	–
99.	*P. cerasus*	N/A	Topola	–	–	–	–	–	–	–
100.	*P. avium*	N/A	Topola	–	–	–	–	–	–	–
101.	*P. domestica*	N/A	Topola	–	–	–	–	–	–	–
102.	*P. armeniaca*	N/A	Topola	–	–	–	–	–	–	–
103.	*P. avium*	'Early Lyons'	Valjevo	–	–	–	–	–	–	–
104.	*P. avium* x *P. cerasus*	'Spanish Marela'	Valjevo	–	–	–	–	–	–	–
105.	*P. domestica*	'Čačanska Lepotica'	Valjevo	–	–	–	–	–	–	–
106.	*P. armeniaca*	'Ambrosia'	Valjevo	–	–	–	–	–	–	–
107.	*P. cerasus*	'Keleris'	Valjevo	–	–	–	–	–	–	–

N/A– no data / *nema podatka*

* not detected / *nije utvrđeno prisustvo virusa*

** detected / *potvrđeno prisustvo virusa*

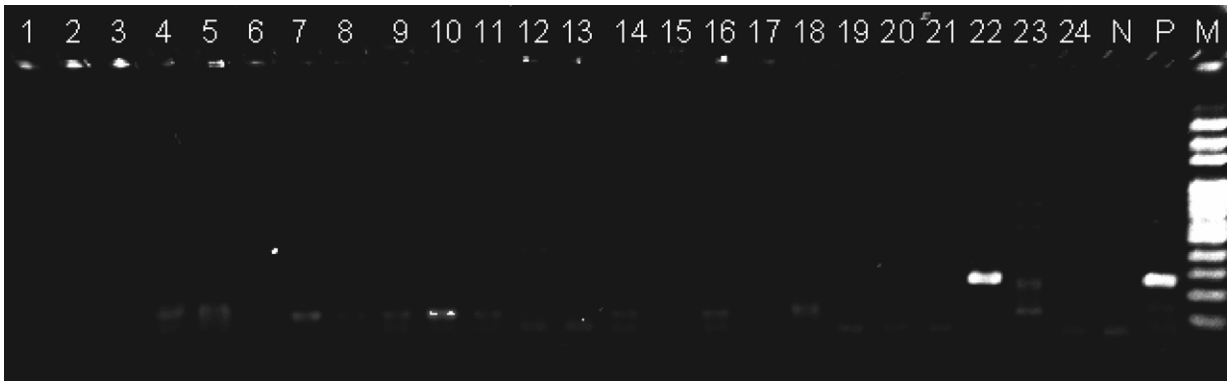

Fig. 1. RT–PCR detection of 255 bp fragment of CNRMV genome. Lines 1–24: laboratory numbers of analyzed samples, N: healthy control, P: positive control, M: marker, 100 bp DNA Ladder (Solis BioDyne, Estonia)
Sl. 1. RT–PCR detekcija 255 bp fragmenta CNRMV genoma. Linije 1–24: laboratorijski brojevi analiziranih uzoraka, N: negativna kontrola, P: pozitivna kontrola, M: marker, 100 bp DNK ledder (Solis BioDyne, Estonija)

ToRSV is distributed worldwide on numerous crops, including *Prunus* species. This virus has restricted distribution in EPPO region and it is not present in Serbia (EPPO, 2021b). In previous surveys it was not detected in any of analyzed samples (Jevremović, unpublished results).

TBRV infects a wide range of plant species, including *Prunus* species (peach). Most of infected species do not exhibit any symptoms of infection. TBRV is transmitted by nematodes over short distances and main mode of its transmission over long distances is through contaminated plant material.

The RT-PCR analysis on CNRMV presence revealed that an expected 255 bp fragment was obtained in six samples (Fig. 1). CNRMV was confirmed in one sour cherry, one sweet cherry, two apricots and two plums (Tab. 1).

CNRMV is present in North and South America, Asia, Australia and Europe (EPPO, 2021d). It infects peach, apricot, sweet and sour cherry. Infected plants develop brown, angular, necrotic spots, rusty chlorotic, and shot hole symptoms on leaves. Sometimes blisters, gum pockets, and bark necrosis appear (Rott & Jelkmann, 2001). CNRMV was detected in Serbia for the first time in 2007 in sweet and sour cherries from two collection orchards (Mandić et al., 2007). During survey in 2016–2017, CNRMV was detected in about 8% of analyzed sweet and sour cherry trees (Paunović & Jevremović, 2018).

CRLV was not detected in any of the analyzed samples. It is only present in North America and Asia (China), and the main pathway for CRLV transmission is via infected planting material (EPPO, 2021c). CRLV is only transmitted by nematodes, but slowly. According to EU categorization CRLV is A1 quarantine pest (Annex II A).

Conclusion

This survey confirmed that monitored viruses, except CNRMV, are not presented in Serbian mother blocks and nurseries and do not pose a threat to the stone fruit production in the country.

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Poseban nadzor nad virusima koštičavih voćaka u Srbiji

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Rezime

U ovom radu predstavljeni su rezultati istraživanja prisustva sedam virusa (virus nekrotičnog rđastog šarenila trešnje/cherry necrotic rusty mottle virus, virus uvijenosti lišća trešnje/cherry leaf roll virus, virus nazubljenosti lišća trešnje/cherry rasp leaf virus, virus crne prstenaste pegavosti paradajza/tomato black ring virus, virus prstenaste pegavosti paradajza/tomato ringspot virus, virus nekrotične prstenaste pegavosti prunusa/prunus necrotic ringspot virus i virus mozaika jabuke/apple mosaic virus) koji zaražavaju koštičave vrste voćaka. Prikupljeno je i analizirano ukupno 107 uzoraka iz matičnih zasada i rastila iz 30 lokaliteta. Uzorci su analizirani ELISA i RT–PCR metodom. Prisustvo virusa nekrotičnog rđastog šarenila trešnje potvrđeno je u šest uzoraka (po jedan trešnje i višnje, i po dva šljive i kajsije). Prisustvo drugih virusa nije potvrđeno ni u jednom od analiziranih uzoraka.

Ključne reči: *Prunus* sp., virusi, ELISA, RT–PCR