

VIRUSES OF SWEET AND SOUR CHERRY IN SERBIA

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SUMMARY

One hundred twenty-five trees (each of a different cultivar) of sour and sweet cherry from two large varietal collections in Serbia were visually inspected for virus symptoms and tested for the presence of cherry viruses by ELISA, herbaceous host assays, graft-indexing on *P. serrulata* cv. Kwanzan, and RT-PCR. All samples were tested by ELISA for *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), and *Plum pox virus* (PPV). The overall detection of PDV, PNRSV, and ACLSV was 63%. Additional ELISA tests were done on 80 trees for *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Strawberry latent ring spot virus* (SLRSV), *Petunia asteroid mosaic virus* (PetAMV), *Raspberry ringspot virus* (RpRSV), *Tomato black ring virus* (TBRV), *Tobacco mosaic virus* (TMV), and *Tomato ringspot virus* (ToRSV). In these tests, one tree tested positive for PetAMV. RT-PCR testing of 44 trees detected another five viruses: *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry virus A* (CVA), European rusty mottle associated virus (ERMaV) and Plum bark necrosis and stem pitting-associated virus (PBNSPaV), but not *Cherry mottle leaf virus* (CMLV). In graft-indexing tests on Kwanzan with all 125 trees, samples from 38 trees induced symptoms of necrotic crook disease (causal agent unknown). Viruses reported for the first time in Serbia were CGRMV, CNRMV, CVA, ERMaV, PBNSPaV, and PetAMV.

Key words: Cherry, survey, ELISA, RT-PCR, indexing.

INTRODUCTION

The stone fruit and nursery industries are traditionally important sectors of agriculture in Serbia. The European plum (*Prunus domestica*) is the commonest cultivated

species represented by an estimated 48 million trees. The sour (*P. cerasus*) and sweet cherry [*P. avium* (L.)] account for 13 million trees, producing 50,000 and 17,000 tons of fruit, respectively (Anonymous, 2003).

In addition to the use of cherry nursery stock for domestic orchards, there is a large market in cherry trees for shipment to neighbouring countries. *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) have been found in commercial cherry orchards in Serbia (Jordović, 1955; 1958). As cherry trees are known to be hosts for more than 30 viruses and virus diseases (Németh, 1986; Myrta and Savino, 2007), we conducted a virus survey in two cherry variety collections and report our findings.

MATERIALS AND METHODS

Orchard surveys. Two variety collections with 125 cultivars of sweet and sour cherry were inspected for symptoms of virus infection. Samples were collected from one plant of each variety.

ELISA. The entire collection was tested by using DAS-ELISA (Clark and Adams, 1977) for PNRSV, PDV, *Plum pox virus* (PPV) and *Apple mosaic virus* (ApMV), and by DAS-simultaneous ELISA (Flegg and Clark, 1979) for *Apple chlorotic leaf spot virus* (ACLSV). Also, 80 trees were further tested by ELISA for *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Strawberry latent ringspot virus* (SLRSV), *Petunia asteroid mosaic virus* (PetAMV), *Raspberry ringspot virus* (RpRSV), *Tomato black ring virus* (TBRV), *Tobacco mosaic virus* (TMV), and *Tomato ringspot virus* (ToRSV). Absorbance values three times or more than those given by healthy controls were considered to indicate infection. Serological reactants were from commercially purchased kits (Loewe, Germany).

Mechanical inoculation of herbaceous test plants. Sap extracts from 45 trees were inoculated to *Nicotiana occidentalis*, *N. benthamiana*, *Cucumis sativus*, *Chenopodium quinoa*, and *C. amaranticolor*. Leaves were ground in the presence of a solution containing 0.1 M phospho-

te buffer, pH 7.2 and 2.5% nicotine in a 1.5/1 ratio (v/v). Inoculated plants were kept in a temperature-controlled glasshouse at 24-26°C for a month.

Woody indexing. The entire collection was chip-bud grafted using three replicates to flowering cherry *P. serotulata* Lindl. cv. Kwanzan (Boscia *et al.*, 1999). The indicator trees had been propagated *in vitro* and, after grafting, were maintained and observed for 6 months in a temperature-controlled glasshouse.

RT-PCR assay. This procedure was used to assay for infection by *Cherry green ring mottle virus* (CGRMV), *Cherry mottle leaf virus* (CMLV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry virus A* (CVA), Plum bark necrosis and stem pitting-associated virus (PBN-SPaV), and European rusty mottle-associated virus (ERMaV). Total nucleic acid (TNA) extraction and complementary DNA synthesis were done as described by Rott and Jelkmann (2001). PCR primers and their expected products are shown in Table 1.

Table 1. List of used primers for virus detection by RT-PCR.

Primers	Amplified fragment	PCR conditions
		94°C 2min
GRM8316 5'(CCTATA GCC AGT CTT CAT ATT ATG)3' antisense GRM7950 5'(GCA GCC TTT GAC TTT TTT GAG)3' sense Rott and Jelkmann (2001)	CGRMV (366 bp)	94°C 45 s 52°C 45 s 72°C 45 S 35
		72°C 5 min
		94°C 2min
NEG1U 5'(AGT TCG CAG CYT TTG AYT TYT TTG)3' antisense NEG1L 5'(GAK GGR WTT GCG RGG TTT ATCA)3' sense Rott and Jelkmann (2001)	CNRMV (400 bp)	94°C 45 s 52°C 45 s 72°C 1min 35
		72°C 5min
		94°C 2min
ERMUP 5'(GCG CTT TTG ATT TCT TTG AG)3' antisense ERMLO 5'(GGA CAG GCC CAC TTA TTT ACT)3' sense Rott and Jelkmann (2001)	ERMaV (341 bp)	94°C 45 s 55°C 45 s 72°C 1min 35
		72°C 5min
		95°C 5min
CML-26R 5'(AGA TCC TCT TTC CCT TCT AAA ATG)3' antisense PM16AFF 5'(CAA ACA TGG CTT TCA CCT TCT GCA)3' sense James and Upton (1999)	CMLV (705 bp)	95°C 30 s 60°C 45 s 72°C 1min 35
		72°C 7min
		94°C 2min
CVA11L 5'(CAG GAT TCT TGC ACT CTA GC)3' antisense CVA11R 5'(AGG TGA TCG CCT TTA TTG TA)3' sense Al Rwahnih, unpublished	CVA (497 bp)	94°C 45 s 55°C 45 s 72°C 1min 35
		72°C 7min
		94°C 2min
ASP2 5' (GTA GTC CGC TGG TAC GCT ACA AG)3' antisense ASP1 5' (CGG TAG GGC TGT GAC TAC CG)3' sense Abou Ghanem-Sabanadzovic <i>et al.</i> (2001)	PBN-SPaV (290 bp)	94°C 30 s 40°C 30 s 72°C 1 min 35
		72°C 7min
		94°C 1,5min
ASPn2 5' (AGG CAC TAC TGA CCT GTA GG)3' antisense ASPn1 5' (ACG AAT CCG AGT TTC GTC GC)3' sense Amenduni <i>et al.</i> (2005)	PBN-SPaV (190 bp)	94°C 20 s 55°C 30 s 72°C 45 s 30
		72°C 5min

RESULTS

Field symptoms. Virus symptoms were frequently seen in most of the cultivars and consisted of leaves with chlorotic spots and shot-holes. Occasionally, we observed leaf enations in sweet cherries and leaf yellowing in sour cherries. Removal of trunk bark revealed stem-pitting in 20% of the sweet and sour cherry trees.

ELISA. Seventy-nine trees (63% of 125 samples) tested positive for one or combinations of viruses, i.e. PNRSV, PDV, and/or ACLSV. Thirty one trees were found to be infected by more than one virus (39 %) (Table 2). PPV and ApMV were absent. Extended ELISA testing of 80 trees for ArMV, CLRV, SLRSV, PetAMV, RpRSV, TBRV, TMV and ToRSV identified infection of one sour cherry (cv. Marasca di Verona) with PetAMV; the remainder were negative.

Mechanical inoculations. Extracts from 45 trees (30 sweet and 15 sour) were mechanically inoculated to herbaceous plants. Five (3 sweet and 2 sour) were infectious and were shown by ELISA to contain PDV or PNRSV. As expected, sap-transmission was less efficient than ELISA, and it can be considered only an additional detection method.

Woody indexing. The entire collection (83 sweet and 42 sour cherry cultivars) were graft-indexed on plantlets of *P. serrulata* cv. Kwanzan. After 2-3 months incubation,

leaf deformation and epinasty, thought to be symptoms of CGRMV infection were induced by eight samples (6 sweet and 2 sour). In addition, a quick decline (Necrotic Crook) (Fig. 1) was elicited by 37 samples (Table 3). Indicator plants affected by necrotic crook rapidly succumbed when shoots were 10-15 cm long. No pathogenic fungi or bacteria were isolated from the diseased plants.

RT-PCR. Forty-four samples (27 sweet and 17 sour) were tested by RT-PCR for CGRMV, CNRMV, CMLV, CVA, PBNSPaV and ERMaV. The viruses detected were: CGRMV (Figure 2), CNRMV (Fig. 3), CVA (not shown) and ERMaV (not shown). Of the 44 trees, CGRMV was identified in 12 (27%), CNRMV in 13 (30%), ERMaV in 5 (11%); CVA was found in 11 (84%) of the 13 trees tested. Mixed virus infections were frequently found (Table 4). PBNSPaV was detected in a few trees with stem pitting. CMLV was not detected.

DISCUSSION

To our knowledge, this is the first extensive survey for cherry viruses in Serbia. The high rates of virus infection found in two cherry variety collections were similar to results reported from neighbouring Albania (Digiario *et al.*, 1994) and Bosnia and Herzegovina (Matić *et al.*, 2006). The most abundant viruses were PDV in sweet cherry and PNRSV in sour cherry; mixed infections were common. Cherry trees with mixed infections have

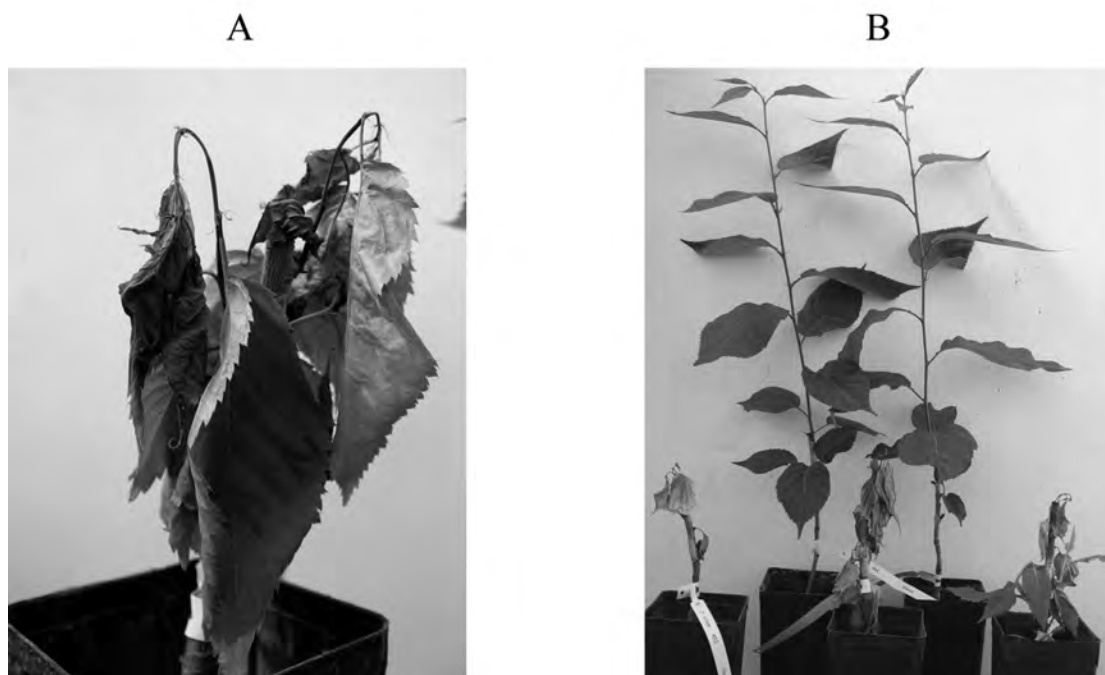


Fig. 1. Kwanzan flowering cherry showing symptoms of necrotic crook (A), and comparisons of healthy (inner two) and necrotic crook-infected (outer three) plants (B).

Table 2. Viruses detected in varietal collections by ELISA*.

Species	Cultivars		Infection rate (%)	Single infections					Mixed infection			
	Tested	Infected		PNRSV	PDV	ACLSV	PPV	ApMV	PNRSV + PDV	PNRSV + ACLSV	PDV + ACLSV	PNRSV + PDV + ACLSV
Sweet cherry	83	52	62.6	8	17	4	0	0	14	3	2	4
Sour cherry	42	27	64.3	15	4	0	0	0	6	2	0	0
Total	125	79	63.2	23	21	4	0	0	20	5	2	4

* ELISA for PPV, PNRSV, PDV, ApMV and ACLSV.

Table 3. Results of cherry indexing on *P. serrulata* cv. Kwanzan.

Species	Cultivar	Epinasty (E)	Necrotic crook (NC)	E + NC
Sweet cherry	83	6	32	-
Sour cherry	42	2	5	1
Total	125	8	37	1

been reported in other regions, such as Japan (Isogai *et al.*, 2004) and California (Sabanadzovic *et al.*, 2005). In addition to infection by the commonly found cherry viruses (PDV, PNRSV and ACLSV) (Jordović, 1955, 1958; Ranković, 1976), infections of cherry by six viruses: CGRMV, CNRMV, CVA, ERMaV, PBNSPaV, and PetAMV are here reported in Serbia for the first time.

Comparing molecular detection data with the results of woody indexing, seven of eight trees that induced leaf deformation and epinasty in tests on Kwanzan indicators tested positive for CGRMV and CNRMV by RT-

PCR. This indicates the reliability of the PCR procedures used as compared to the gold standard of inoculation to woody indicators. Mixed virus infections of CGRMV and CNRMV prevailed over single virus infections. Both viruses were detected in eleven samples, whereas, only two single infections by CNRMV and one by CGRMV were found. In all probability, symptoms of epinasty induced by CGRMV were masked by rapid necrosis of the indicators in five instances. There was no clear association between infection by ilarviruses (PDV and PNRSV) and the necrotic crook syndrome.

The relatively high incidence of viruses in cherry reported here is in line with a recent report from Japan (Isogai *et al.*, 2004) of incidences in sweet cherry of 14, 49, and 92% for CGRMV, CNRMV, and CVA respectively.

Even though our surveys were limited to two cherry variety collections, our results probably reflect the situation in Serbia's cherry industry. This is because the collections tested are being used to provide mother trees for propagation of nursery stocks.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

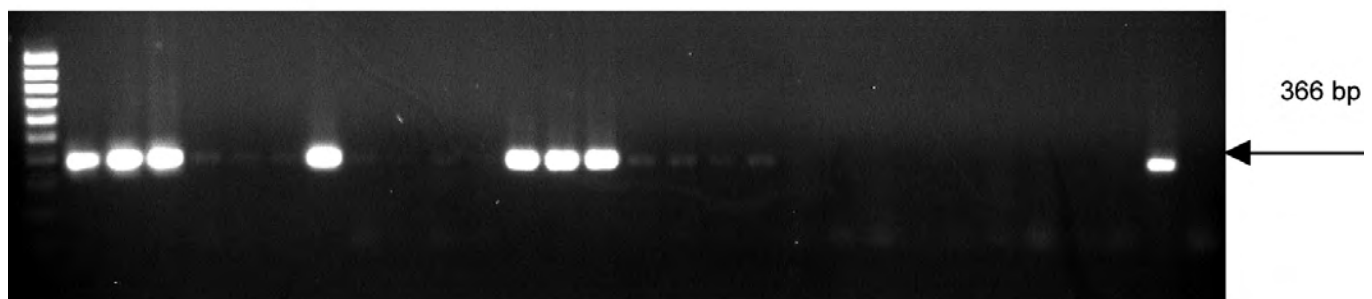


Fig. 2. Agarose gel analysis of RT-PCR products for CGRMV: amplification with GRM7950/GRM8316 primers, product size 366 bp. Lane M = marker 100 bp (Roche); lanes 1, 2, 3, 7, 12, 13, 14 are positive samples; lane 29 - healthy control and lane 27 - water control; lane 28 is positive control CGRMV - CGRM 141/00 von 53198, Baum Schule Q1 Gr.6 PL2. Dossenheim, Germany.

Table 4. RT-PCR, ELISA and indexing results from sweet and sour cherry cultivars.

No	Stone fruit species	Cultivar	Symptoms on Kwanzan	Tested viruses*						
				RT-PCR				ELISA		
				CVA	CGRMV	CNRMV	ERMaV	PDV	PNRSV	ACLSV
1	<i>P. avium</i>	Burlat	NC	n.t.	-	-	-	+	-	+
2	<i>P. avium</i>	Bianca di Verona	NC	n.t.	-	-	-	+	-	-
3	<i>P. avium</i>	Dnjeprovka	NC	n.t.	+	+	-	-	+	-
4	<i>P. avium</i>	Sam	NC	n.t.	+	-	-	+	+	-
5	<i>P. avium</i>	Snajderova	NC	n.t.	-	+	+	+	+	-
6	<i>P. avium</i>	Vežorsova	NC	n.t.	+	+	-	+	+	-
7	<i>P. avium</i>	Emperor Francis	NC	n.t.	+	+	+	-	+	-
8	<i>P. cerasus</i>	Geroj Ranij	NC	+	-	-	-	-	+	-
9	<i>P. avium</i>	K. Rana	NC	n.t.	-	-	-	+	-	-
10	<i>P. avium</i>	Moser	NC	n.t.	-	-	-	-	-	-
11	<i>P. avium</i>	Seneca	NC	n.t.	+	+	-	+	-	-
12	<i>P. avium</i>	Inga	NC	n.t.	-	-	-	-	+	-
13	<i>P. avium</i>	Merpet	NC	n.t.	-	-	-	-	-	-
14	<i>P. cerasus</i>	Mond Late	NC	n.t.	-	-	+	-	+	-
15	<i>P. avium</i>	Kordia	NC	n.t.	-	-	+	+	-	-
16	<i>P. cerasus</i>	Pandy Iveg Megi	NC	+	-	-	-	-	+	+
17	<i>P. avium</i>	Lambert	EP	+	+	+	-	-	-	+
18	<i>P. avium</i>	Burbank	EP	-	+	+	-	+	+	-
19	<i>P. avium</i>	Imperial	EP	n.t.	+	+	-	+	-	-
20	<i>P. avium</i>	Knaufts	EP	n.t.	+	+	-	+	-	+
21	<i>P. avium</i>	Noir de Guben	EP	+	+	+	-	+	-	-
22	<i>P. avium</i>	Nord Wonder	EP	n.t.	-	-	-	-	-	-
23	<i>P. avium</i>	Eureka	EP	n.t.	+	+	+	+	-	-
24	<i>P. cerasus</i>	Richmorency	EP+NC	n.t.	+	+	-	+	+	-
25	<i>P. cerasus</i>	Hajmanov Rubin	NS	+	-	-	-	+	-	+
26	<i>P. avium</i>	Vipavka	NS	n.t.	-	-	-	+	-	-
27	<i>P. cerasus</i>	_a_anski Rubin	NS	n.t.	-	-	-	-	-	-
28	<i>P. cerasus</i>	Kereska	NS	+	-	-	-	-	+	+
29	<i>P. cerasus</i>	Hajmanova Konzervna	NS	n.t.	-	-	-	-	-	-
30	<i>P. avium</i>	Summit	NS	+	-	-	-	-	-	-
31	<i>P. avium</i>	Ljana	NS	n.t.	-	-	-	-	-	-
32	<i>P. avium</i>	Vista	NS	n.t.	-	-	-	-	-	-
33	<i>P. cerasus</i>	Erdi Botermo	NS	n.t.	-	-	-	+	+	-
34	<i>P. cerasus</i>	Stevnsbar	NS	n.t.	-	-	-	-	-	-
35	<i>P. avium</i>	Bing	NS	n.t.	-	-	-	+	-	-
36	<i>P. avium</i>	Strarking Hardy Giant	NS	+	-	+	-	-	-	-
37	<i>P. avium</i>	Lapins	NS	n.t.	-	-	-	-	-	-
38	<i>P. cerasus</i>	Maraska iz Zadra	NS	+	-	-	-	-	-	-
39	<i>P. cerasus</i>	Saten Morelo	NS	-	-	-	-	+	-	-
40	<i>P. cerasus</i>	Mont Mamut	NS	n.t.	-	-	-	-	+	-
41	<i>P. cerasus</i>	Gorsemska	NS	+	-	-	-	-	+	-
42	<i>P. cerasus</i>	Kelleris 14	NS	n.t.	-	-	-	-	+	-
43	<i>P. cerasus</i>	Dropia	NS	+	-	-	-	-	-	-
44	<i>P. cerasus</i>	Kelleris 16	NS	n.t.	-	-	-	-	-	-

*all samples were negative to CMLV; NC: necrotic crook; EP: epinasty; NS: No symptoms; n.t. not tested.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

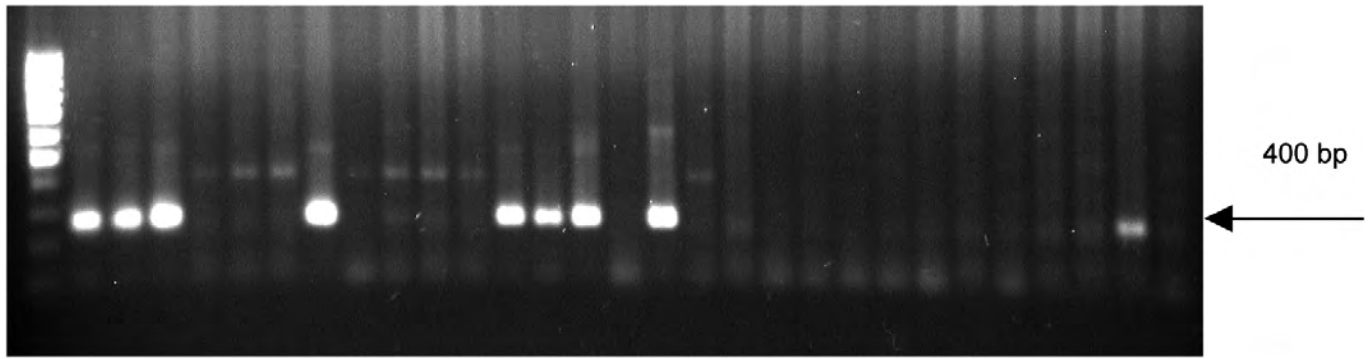


Fig. 3. Agarose gel analysis of RT-PCR products for CNRMV: amplification with NEG1L/ NEG/1U primers, product size 400 bp. Lane M, marker 100bp; lanes 1, 2, 3, 7, 12, 13, 14, 16 are positive samples; lane 29 - healthy control and lane 27 - water control, lane 28 positive control CNRMV - NRMV 120/86 Baum Schule A5 Gr.8 Baum1, Dossenheim, Germany.

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