

Health status of pome and stone fruit planting material imported to Serbia

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Abstract

We summarize results of the analysis of pome and stone fruit planting material to be imported to Serbia for the presence of quarantine and economically important viruses and phytoplasmas. The analysis was conducted 2004 – 2009 whereby, in compliance with the phytosanitary law regulations of the Republic of Serbia, officially inspected samples were subjected to the examination. During the period, a total of 325 samples were analyzed, i.e. 89 rootstock samples, 215 samples of different pome and stone fruit varieties, and 21 samples of stone fruit seed. The obtained results reveal that 5 samples were infected with viruses. *Apple chlorotic leafspot virus* was found in one sample of apple budwood, *Prune dwarf virus* was detected in *Prunus avium* L. seedlings, *Plum pox virus* was confirmed in 2 plum budwood samples, and *Apple mosaic virus* was also found in one sample of apple budwood.

Keywords: Pome fruits, stone fruits, planting material, seed, viruses, phytoplasmas.

Introduction

The exchange in fruit propagation material between Republic of Serbia and other countries, mostly European ones, is very dynamic. However, the exchange poses a risk of spreading economically important pathogens through infected propagation material (rootstocks, budwoods or entire plants). The establishment of the National Reference Laboratory, that will take over the responsibility for this type of sample analysis, is currently in progress. All samples of fruit planting material intended for import to Serbia are analyzed for the presence of viruses and phytoplasmas in the virology laboratory of Fruit Research Institute, Čačak. Phytosanitary service and certified laboratories act to prevent the import of infected material into the country. The import procedure includes regular inspection of planting material, both visual and in laboratory, insuring that data written in the phytosanitary certificate are correct. All tests are performed according to plant protection law regulations of the Republic of Serbia. The analysis includes examination for the presence of harmful pathogens, in compliance with Bylaw on the declaration of quarantine list of detrimental organisms (Official Gazette of RS 11/08), Bylaw on health inspections of plant consignments in traffic across the border (Official Gazette of RS, 42/08) and Regulation on sanitary inspection of growing plants and structures intended for the production of seed, replantation and planting material, and sanitary control of seed, replantation and planting material (Official Gazette of SCG 13/03; Official gazette of RS 39/06, 59/06, 115/06 and 119/07).

Material and methods

By the decision of Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, virology laboratory of Fruit Research Institute - Čačak has been officially authorized to analyze fruit propagation material imported to Serbia. Border phytosanitary inspectors are the official representatives, that perform sampling at border crossings, and forward samples to the laboratory for examination. During the six-year period (2004 – 2009), 325 samples of fruit propagation material were analyzed. A total of 89 rootstock samples (*Malus domestica*, *Pyrus communis*, *Cydonia oblonga*, *Prunus cerasifera*, *P. persica*, *P. armeniaca*, *P. avium* and *P. mahaleb*), 215 samples of apple, pear, plum, peach and nectarine, apricot, sweet and sour cherry varieties, and 21 samples of seed (*P. cerasifera*, *P. persica*, *P. armeniaca*, *P. avium*, *P. mahaleb* and *P. amygdalus*) were laboratory inspected (Tables 1, 2, 3 and 4).

Border inspection of samples need to be rapid, and employed methods sufficiently sensitive. ELISA test is used for routine detection of viruses, whereas PCR test is the most appropriate technique for the detection of phytoplasmas in imported material. The results are reliable and available within 2 – 3 days. Additionally, for all imported stone fruits, one-year supervision is prescribed at the production site.

Depending on fruit species, type of sample, vegetation season, category of planting material and country of origin, laboratory tests were performed for respective viruses (*Plum pox virus* -PPV, *Prune dwarf virus* -PDV, *Prunus necrotic ringspot virus* -PNRSV, *Cherry leaf roll virus* -CLRV, *Arabidopsis mosaic virus* -ArMV, *Strawberry latent ringspot virus* -SLRSV, *Raspberry ringspot virus* -RpRSV, *Tobacco black ring virus* -TBRV, *Tomato ringspot virus* -ToRSV, *Apple mosaic virus* -ApMV, *Apple stem pitting virus* -ASPV, *Apple stem grooving virus* -ASGV and *Apple chlorotic leafspot virus* -ACLSV) (Tables 1, 2, 3 and 4). The viruses were detected by ELISA test (Clark and Adams, 1977) with reagents supplied by BIOREBA AG, Switzerland and LOEWE, Germany. The tests were performed immediately upon the arrival of samples. All the tests were done according to the manufacturers' recommendations. OD values were recorded

on the Multiskan MCC340 ELISA plate reader. On request of a phytosanitary inspector, 4 apple and 5 pear samples were tested for the presence of 'Candidatus *Phytoplasma mali*' and 'Candidatus *Phytoplasma pyri*' respectively. The analyses were done by nested-PCR test. Specific primers for phytoplasmas belonging to the Apple proliferation group were also included in the analyses (Schneider et al., 1995; Lee et al., 1995).

Results

The results of the analysis reveal that plant viruses were detected in 5 samples of tested planting material intended to be imported to Serbia (1.54% of the total number of analyzed samples). One rootstock sample out of 89 tested was found positive in ELISA test. Seedlings of *Prunus avium* L. originating from Hungary were found to be infected with *Prune dwarf virus* (Table 1). *Prune dwarf virus*, spread worldwide, is economically important pathogen of stone fruits. It is a pollen- and seed borne virus, and transmission through infected seed in wild cherry is up to 50% (Desvignes, 1999). As proposed by the EPPO, all categories of plum, peach, apricot and almond planting material are required to be free of this virus (OEPP/EPPO, 2001).

Tab. 1 Number of samples tested and found positive of pome- and stone rootstocks intended to be imported to Serbia, and viruses and phytoplasmas whose presence was tested

Species affiliation of tested rootstock	Viruses and phytoplasmas whose presence were tested and number of tested samples										Total No of tests	No of positive samples
	PPV	PDV	PNRSV	ToRSV	ApMV	ASPV	ASGV	ACLSV	AP	PD		
<i>Malus domestica</i>				4	19	27	27	27	2		34	0
<i>Pyrus communis</i>						4	4	4		4	7	0
<i>Cydonia oblonga</i>				2		2	3	4			9	0
<i>Prunus cerasifera</i>	14	5	6					3			14	0
<i>Prunus persica</i>	2	1									2	0
<i>Prunus armeniaca</i>	1										1	0
<i>Prunus avium</i>	8	5	6		1			2			9	1 PDV
<i>Prunus mahaleb</i>	9	11	12					6			13	0

Out of 46 analyzed samples of different *Prunus domestica* L. varieties, 2 plum samples (budwood) originating from Hungary were found to be infected with *Plum pox virus* (Table 2). *Plum pox virus* is the most detrimental virus of stone fruits causing great losses in sensitive plum, peach and apricot varieties. It is included in the EPPO A2 list (<http://www.eppo.org/>) and A2 quarantine list of Serbia (Official Gazette of RS 11/08). As stated above for the *Prune dwarf virus*, fruit planting material is required to be free of this virus (EPPO, 2001).

Tab. 2 Number of samples of stone fruit cultivars intended to be imported to Serbia, which were tested and found positive, and viruses whose presence was tested

Species affiliation of cultivars	Viruses whose presence was tested and number of tested samples										Total No of tests	No. of positive samples
	PPV	PDV	PNRSV	CLRV	ArMV	SLRSV	RpRSV	TBRV	ACLSV			
<i>Prunus domestica</i>	46	6	15						4		46	2 PPV
<i>Prunus persica</i>	34	2	1			1					34	0
<i>Prunus armeniaca</i>	20	8	8								20	0
<i>Prunus avium</i>	12	14	14	2	1	1	1	1			17	0
<i>Prunus cerasus</i>	13	3	3	1							14	0

Sixty six samples of apple (*Malus domestica* L.) varieties were analyzed for the presence of viruses. *Apple mosaic virus* was found in one sample (budwood) imported from Belgium (Table 3). The imported material was characterized as certified. *Apple mosaic virus* causes mosaic symptoms on apple leaves, and yield losses in sensitive cultivars may be up

to 30% (Šutic et al., 1999). Out of 9 samples tested for the presence of phytoplasmas none was found to be infected (Table 1 and 3).

One apple sample (budwood) originating from Italy was infected with *Apple chlorotic leafspot virus* (Table 3). Infection with ACLSV is symptomless in most of commercial apple varieties, occurring frequently with the other apple latent viruses, i.e. *Apple stem pitting virus* and *Apple stem grooving virus*. It is present worldwide and listed in the EPPO certification schemes (EPPO, 2001).

Tab. 3 Number of samples intended to be imported to Serbia which were tested and found positive of pome fruit cultivars (according to species affiliation), and viruses and phytoplasmas whose presence was tested

Species	Viruses and phytoplasmas whose presence was tested, and number of tested samples						Total No. of tested samples	No. of positive samples
	ToRSV	ApMV	ASPV	ASGV	ACLSV	AP		
<i>Malus domestica</i>	10	35	40	40	51	2	66	1 ApMV, 1 ACLSV
<i>Pyrus communis</i>			7	6	13		18	0

Presence of viruses was not detected in any of the tested samples of stone fruit seeds (Table 4).

Tab. 4 Number of samples tested and found positive of seeds intended to be imported to Serbia, and viruses whose presence was tested

Species of the tested seed	Viruses whose presence was Tested, and number of tested samples			Total No. of tested samples	No. of positive samples
	PDV	PNRSV	ToRSV		
<i>Prunus cerasifera</i>	3	4		4	0
<i>Prunus persica</i>	5	6	2	8	0
<i>Prunus armeniaca</i>		1		1	0
<i>Prunus avium</i>	1	2		2	0
<i>Prunus mahaleb</i>	3	4		4	0
<i>Prunus amygdalus</i>	1	2		2	0

Discussion

According to the laboratory tests, the great majority of the material imported from European countries (98.46%) was free of quarantine and economically important viruses and phytoplasmas. Similarly, performed laboratory tests and results clearly suggest that Phytosanitary service of the Republic of Serbia and certified laboratory are fully capable of identifying whether certified material fulfills strict requirements set by EPPO and National law regulations.

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