

## ORNAMENTAL *PRUNUS* SPECIES AS NEW NATURAL HOSTS OF *PLUM POX VIRUS* AND THEIR IMPORTANCE IN THE SPREAD OF THE VIRUS IN HUNGARY

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### SUMMARY

To determine the natural incidence of *Plum pox virus* (PPV) in ornamental *Prunus* species, surveys of 120 species/cultivars were conducted in Hungary between 2002 and 2007. Fifty-nine PPV-infected trees were detected by ELISA and/or PCR. Twenty-five were *P. cerasifera* cv. Nigra with dark red leaves, which accounts for a significant proportion of street-grown *Prunus* trees in Hungary. Molecular assays showed a prevalence of strain PPV-D (22) over PPV-M (2) and mixed infection of strains M and D (1). Infection was identified in: \**Persica x davidiopersica* cv. Atropurpurea, *P. cerasifera*, \**P. cerasifera* (Blue-fruited), \**P. cerasifera* (Light-pink-fruited), \**P. cerasifera* cv. Nigra, \**P. cerasifera* cv. Pendula, \**P. cerasifera* cv. Pissardii, \**P. cerasifera* cv. Woodii, *P. glandulosa*, \**P. glandulosa* cv. Alba Plena, \**P. japonica*, \**Prunus x blireana*, \**Prunus x blireana* cv. Moseri. On cultivars with dark-red leaves, symptoms could not be recognized even if the virus concentration was high. It can be assumed that in Hungary the latently infected red-leaved cultivars can play an important role in the wide distribution of PPV. Therefore it is a must to implement the certification scheme for propagating materials also considering that a number of *Prunus* species and cultivars (marked by an asterisk above) have been identified as previously unreported natural hosts of PPV in Hungary.

**Key words:** *Prunus*, PPV, natural hosts, new PPV records, ELISA, RT-PCR, indexing.

### INTRODUCTION

*Plum pox virus* (PPV) is widespread on stone fruit species in Hungary, where it causes heavy yield losses (Nemeth, 1986). In the last decades natural occurrence

of this virus has been recorded also in some ornamental and wild *Prunus* species in Hungary and in other countries (Nemeth, 1986; James and Thompson, 2006).

To provide scientific data to support the requirements of the certification scheme for the production of virus-free planting material, the present paper reports the results of a six-year survey conducted by the Plant Protection and the Soil Conservation Service in order to determine the rate of PPV infection of ornamental *Prunus* species in Hungary.

### MATERIALS AND METHODS

**Survey.** Surveys and sampling in propagation sites, such as nuclear stocks, propagation blocks, nurseries, arboretums, public areas including parks and street-grown trees, were carried out by plant pathologists and inspectors of the county Services in at least one location of each county.

**ELISA.** Samples from the south-eastern part of Hungary were tested by ELISA at the Virus Laboratory of the Plant Protection and Soil Conservation Service (PPSCS) of county Csongrád using broad spectrum PPV kits from Loewe Biochemica (Germany). ELISA on samples collected in other parts of Hungary in 2002-2006 and molecular testing were done in the Central Laboratory for Pest Diagnosis of the Central Service for Plant Protection and Soil Conservation (PPSCS), Budapest. In 2007 ELISA testing of all samples was done in the PPSCS Virus Laboratory of county Fejér, Velence. Samples were always tested in duplicate. In the latter two laboratories universal (5B) and specific monoclonal antibodies raised either to PPV-D (Cambra *et al.*, 1994) (Durviz, Spain), or PPV-M (Boscia *et al.*, 1997) (Agritest, Italy) were used. PPV-infected trees were repeatedly tested in ELISA with the universal monoclonal antibody 5B throughout the duration of the survey.

**RT-PCR assays.** ELISA-positive samples were further assayed by RT-PCR. RNeasy Plant Mini Kits (Qiagen, Germany) were used for RNA extraction. General PPV detection was conducted with P1/P2 primers (Wetzel *et*

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tenhegyi ut 29, 1125 Budapest Hungary.

*al.*, 1991). For strain-specific identification, P1/PD and P1/PM primers were used, according to Olmos *et al.* (1997).

**Woody indexing.** Indexing of trees, selected among the ELISA-positive ones in 2002 and 2003, was done by chip budding onto GF 305 peach seedlings in 5 replicates in the experimental nursery of PPSCS of county Fejér. As controls, samples were collected from inoculated indicator plants and tested by RT-PCR.

## RESULTS AND DISCUSSION

**Field symptoms.** Between 2002 and 2007, 120 ornamental *Prunus* species and cultivars in arboretums/botanical gardens (288 samples), propagation sites (870 samples) and public areas including parks and streets (574 samples) were surveyed and sampled (Table 1). Symptoms affecting the leaf colour were observed only in a few trees of 10 ornamental *Prunus* species/cultivars. Among the green-leaved plants, the most conspicuous symptoms were shown by the leaves of *P. glandulosa*, i.e.

whitish or light yellow rings of different size, speckles, line pattern or light green vein banding. On *P. glandulosa* cv. Alba Plena the symptoms were pale and blurred, and similarly light were the green discoloured areas along the midribs and the sparse blotches on the leaves of *P. japonica*. Round blotches associated with the veins or long light green discoloured areas were present on *P. cerasifera* cv. Pendula. *P. cerasifera* cv. Pissardii leaves turned dark-red or purple, mainly along the main and secondary veins, with a higher intensity on the lower surface. The lower surface of *Prunus x blireana* leaves exhibited similar symptoms along the main, secondary and tertiary veins. On dark-red-leaved trees such as *P. cerasifera* cv. Nigra and *Prunus x davidopersica* cv. Atropurpurea symptoms were not visible.

**ELISA.** Fifty-nine of 1732 samples collected from 93 locations of 15 counties were ELISA-positive. PPV-infected trees were repeatedly tested in successive years with consistent positive results. Reactions of the 5B monoclonal were convincing both in controls and positive samples throughout the six-year survey. Extinction values ranged

**Table 1.** Results of surveys of ornamental *Prunus* species/cultivars for natural PPV infection in Hungary (2002-2007).

Year	Origin of samples				PPV-infected ornamental <i>Prunus</i> (number of samples)
	From arboretums and botanical garden	From propagation sites <sup>(a)</sup>	From public areas	Total	
2002	2/90 <sup>(b)</sup>	8/294	5/131	15/515	<i>P. x blireana</i> (6) <i>P. x blireana</i> cv. Moseri (1) <i>P. cerasifera</i> cv. Nigra (6) <i>P. glandulosa</i> (1) <i>P. japonica</i> (1)
2003	4/46	0/26	0/37	4/109	<i>Persica x davidopersica</i> cv. Atropurpurea (1) <i>P. cerasifera</i> (1) cv. Nigra (1) cv. Pendula (1)
2004	0/67	2/191	11/245	13/503	<i>P. cerasifera</i> (Blue-fruited) (1) (Light-pink-fruited) (1) cv. Nigra (9) cv. Pissardii (2)
2005	0/7	-	6/68	6/75	<i>P. cerasifera</i> cv. Nigra (6)
2006	8/78	2/243	8/93	18/414	<i>P. cerasifera</i> (3) cv. Nigra (2) cv. Pissardii (4) cv. Woodii (4) <i>P. glandulosa</i> (1) cv. Alba Plena (2) <i>P. x blireana</i> (2)
2007	-	3/116	-	3/116	<i>P. cerasifera</i> cv. Woodii (3)
Total	14/288	15/870	30/574	59/1732	

<sup>(a)</sup> propagation sites: nuclear stocks, propagation blocks, nurseries.

<sup>(b)</sup> number of positive samples/Number of tested samples.

between 0.400 and 3,000. Reactions of control PPV-M and PPV-D strains with the strain-specific monoclonals were generally slightly weaker but always convincing. With strain-specific ELISA testing, strain PPV-M was not detected at all, PPV-D was identified only in 4 of the 59 samples that had previously tested ELISA-positive using

the 5B monoclonal (Table 3). Further PPV strain-specific serological investigations are planned.

**Woody indexing.** PPV symptoms were observed on indicator plants of 10 of the 15 ELISA-positive trees selected for indexing in 2002 and 2003 (Table 3).

**Table 2.** List of ornamental *Prunus* species, cultivars and botanical variety surveyed for natural PPV infection in Hungary (2002-2007).

<i>Persica x davidiopersica</i> cv. Atropurpurea*	<i>P. nipponica</i> cv. Brillant
<i>Prunus</i> cv. Accolade	<i>P. padus</i>
<i>Prunus</i> cv. Hally Jolivette	<i>P. padus</i> cv. Coloratus, cv. Schloss Tiefurt
<i>Prunus</i> cv. Okame	<i>Prunus pendula</i> (= <i>Prunus subhirtella</i> cv. Pendula)
<i>Prunus</i> cv. Pandora	<i>P. pensylvanica</i>
<i>Prunus</i> cv. Rubin	<i>P. persica</i>
<i>Prunus</i> cv. Shosar	<i>P. persica</i> cv. Bonanza
<i>Prunus</i> cv. Spire	<i>P. pseudocerasus</i>
<i>Prunus</i> cv. Umineko	<i>P. salicina</i>
<i>P. americana</i>	<i>P. sargentii</i>
<i>P. armeniaca</i> var. <i>Ansu</i>	<i>P. serotina</i>
<i>P. avium</i>	<i>P. serrulata</i>
<i>P. avium</i> cv. Pendula, cv. Plena	<i>P. serrulata</i> cv. Albo-plena, cv. Amanogawa
<i>P. cerasifera</i> *	<i>P. serrulata</i> cv. Edo-zakura, cv. Fukurokuju
<i>P. cerasifera</i> (Blue-fruited)*	<i>P. serrulata</i> cv. Hisakura, cv. Hokusai
<i>P. cerasifera</i> (Light-pink-fruited)*	<i>P. serrulata</i> cv. Kanzan, cv. Kiku-shidare-zakura
<i>P. cerasifera</i> cv. Hessei, <i>Prunus</i> cv. Trailblazer (= <i>P. cerasifera</i> cv. Hollywood)	<i>P. serrulata</i> cv. Mikuruma-gaeshi
<i>P. cerasifera</i> cv. Nigra*	<i>P. serrulata</i> cv. Oyochin, cv. Pink Perfection
<i>P. cerasifera</i> cv. Pendula*	<i>P. serrulata</i> cv. Royal Burgundy, cv. Shirotae
<i>P. cerasifera</i> cv. Pissardii* (= <i>P. cerasifera</i> cv. Atropurpurea)	<i>P. serrulata</i> cv. Taihaku, cv. Ukon
<i>P. cerasifera</i> cv. Woodii*	<i>P. serrulata</i> cv. Yae-murasaki
<i>P. cerasus</i> cv. Pendula, cv. Semperflorens	<i>P. sibirica</i>
<i>P. curdica</i>	<i>P. spinosa</i>
<i>P. davidiana</i>	<i>P. spinosa</i> cv. Rosea, cv. Rubra
<i>P. dulcis</i> ( <i>P. amygdalus</i> )	<i>P. sibirica</i>
<i>P. dulcis</i> cv. Balaton, cv. Rosea Plena, cv. Spring Glow	<i>P. subhirtella</i>
<i>P. fruticosa</i>	<i>P. subhirtella</i> cv. Autumnalis, cv. Autumnalis Rosea
<i>P. glandulosa</i> *	<i>P. subhirtella</i> cv. Fukubana, cv. Dahlem (= <i>P. subhirtella</i> cv. Plena)
<i>P. glandulosa</i> cv. Alba Plena*	<i>P. tenella</i>
<i>P. glandulosa</i> cv. Rosea Plena	<i>P. tenella</i> cv. Fire Hill, cv. Kati
<i>P. grayana</i>	<i>P. triloba</i>
<i>P. humilis</i>	<i>P. triloba</i> cv. Multiplex, cv. Rosenmund
<i>P. japonica</i> *	<i>P. virginiana</i>
<i>P. kansuensis</i>	<i>P. virginiana</i> cv. Canada Red, cv. Schubert
<i>P. laurocerasus</i>	<i>Prunus x blireana</i> *
<i>P. laurocerasus</i> cv. Baumgartner, cv. Cherry Brandy	<i>Prunus x blireana</i> cv. Moseri*
<i>P. laurocerasus</i> cv. Grüner Teppich, cv. Herbergii, cv. Klári	<i>Prunus x cistena</i>

<i>P. laurocerasus</i> cv. Magnoliifolia, cv. Marbled White	<i>Prunus</i> × <i>eminens</i> cv. Umbraculifera (= <i>P. fruticosa</i> cv. Globosa)
<i>P. laurocerasus</i> cv. Mari, cv. Otto Luyken, cv. Piri, cv. Schipkaensis	<i>Prunus</i> × <i>mohacsyana</i>
<i>P. laurocerasus</i> cv. Van Nes, cv. Zabeliana	<i>Prunus</i> × <i>persicoides</i> (= <i>Prunus</i> × <i>amygdalopersica</i> )
<i>P. lusitanica</i>	<i>Prunus</i> × <i>persicoides</i> cv. Spring Glow (= <i>P.</i> × <i>amygdalopersica</i> cv. Spring Glow)
<i>P. maackii</i>	<i>Prunus</i> × <i>schmittii</i>
<i>P. mahaleb</i>	<i>Prunus</i> × <i>yedoënsis</i>
<i>P. mandshurica</i>	<i>Prunus</i> × <i>yedoënsis</i> cv. Moerheimii cv. Shidare-yoshino
<i>P. mume</i>	
<i>P. nipponica</i> (= <i>P. nipponica</i> var. <i>kurilensis</i> )	

\* Natural PPV host in Hungary

**Table 3.** PPV-infected ornamental *Prunus* species and cultivars detected by ELISA, woody indexing and/or PCR in Hungary (2002-2007).

Species/cultivar	ELISA	Indexing	RT-PCR	Strain-specific PCR
<i>P. cerasifera</i> <sup>(a)</sup>	4/4 <sup>(b)</sup> (1 PPV-D)	1/1	2/2	1/2 (1 PPV-D)
<i>P. cerasifera</i> (Blue-fruited) <sup>(a)</sup>	1/1	NT <sup>(c)</sup>	1/1	1/1 (PPV-D)
<i>P. cerasifera</i> (Light-pink-fruited) <sup>(a)</sup>	1/1	NT	1/1	1/1 (PPV-D)
<i>P. cerasifera</i> cv. Nigra	24/24 (1 PPV-D)	2/3	7/8	7/7 (PPV-D)
<i>P. cerasifera</i> cv. Pendula <sup>(a)</sup>	1/1 (1 PPV-D)	1/1	1/1	1/1 (PPV-D)
<i>P. cerasifera</i> cv. Pissardii <sup>(a)</sup>	6/6	NT	1/2	1/1 (PPV-D)
<i>P. cerasifera</i> cv. Woodii	7/7	0	3/3	3/3 (PPV-D)
<i>P. glandulosa</i> <sup>(a)</sup>	2/2	1/1	1/1	1/1 (PPV-M)
<i>P. glandulosa</i> cv. Alba Plena <sup>(a)</sup>	2/2	NT	1/1	1/1 (PPV-D)
<i>P. japonica</i> <sup>(a)</sup>	1/1	1/0	1/1	1/1 (PPV-D)
<i>Prunus</i> × <i>blireana</i> <sup>(a)</sup>	8/8	4/6	5/6	3/5 (3 PPV-D, 1 PPM-M, 1 PPV D+M)
<i>Prunus</i> × <i>blireana</i> cv. Moseri <sup>(a)</sup>	1/1	1/1	1/1	1/1 (PPV-D)
<i>Persica</i> × <i>davidiopersica</i> cv. Atropurpurea	1/1 (PPV-D)	NT	1/1	1/1 (PPV-D)

<sup>(a)</sup> PPV-infected; <sup>(b)</sup> Number of positive samples/total number of tested sample; <sup>(c)</sup> not tested.

**RT-PCR.** To verify the indexing results of the 15 ELISA-positive trees, molecular tests were positive with all the inoculated and symptomatic indicators of the 10 ELISA-positive trees. In the case of five ELISA positives, PPV was detected at least in one of the symptomless indicators. Twenty-five samples were identified as PPV-D, two as PPV-M and, in one case, a mixed infection (PPV-D and PPV-M) was found out of the 28 samples selected for strain-specific identification (Table 3). Further molecular studies of PPV isolates have been initiated and their characterization is in progress. Comparison is planned of the new findings with earlier results by Myrta *et al.* (2001), Salamon and Palkovics (2002) on other Hungarian PPV isolates, and with the classification of PPV strains reported by James and Glasa (2006).

Based on the six-year study, consisting of surveys for new host species and laboratory analyses (serological and molecular assays), it can be concluded that natural PPV infection was found in 13 of the 120 ornamental *Prunus* species/cultivars tested. Although PPV was not detected during this survey in *P. spinosa* plants, infected blackthorn plants were often found in the course of woody indexing performed in the 60s' in the frame of the Hungarian certification programme (M. Nemeth, unpublished information). Due to these indexing results, *P. spinosa* was included in the regulations for certification in the 70s. The presence of PPV in symptomatic and symptomless *P. spinosa* was confirmed by Salamon and Palkovics (2002) and identified as a new subgroup of M strain in Hungary.

In the present study, 11 of 13 species/cultivars were identified as new natural PPV hosts for Hungary and also internationally. Only 10 of the 13 naturally PPV-infected species/cultivars/botanical varieties were symptomatic. On infected light red-leaved cultivars symptoms are clearly visible on the lower surface of the leaves. This is important for phytosanitary inspectors to bear in mind. On dark red-leaved ornamental *Prunus* cultivars (such as *P. cerasifera* cv. Nigra), PPV symptoms cannot be recognized even if the virus concentration is high. It can be assumed that in Hungary these latently infected dark red-leaved cultivars can play an important role in the wide distribution of PPV. Such trees in public areas are permanent virus sources. Symptomless infected trees in the vicinity of nuclear stocks and nurseries constitute a high risk. Therefore, it is a must for the future to enforce the requirements of the certification scheme for the production of propagating materials also to the natural PPV hosts newly identified in this study. Considering the high frequency of latent infections, the use of reliable laboratory methods in regular screening is essential.

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