

Evaluation of susceptibility to *Plum pox virus* and ‘*Candidatus Phytoplasma prunorum*’ in Japanese plum and pluot cultivars

JAROSLAV SALAVA ^{*}, JANA BROŽOVÁ

Division of Crop Protection and Plant Health, Crop Research Institute, Prague, Czech Republic

^{*}Corresponding author: salava@vurv.cz

Citation: Salava J., Brožová J. (2024): Evaluation of susceptibility to *Plum pox virus* and European stone fruit yellows phytoplasma in Japanese plum and pluot cultivars. Hort. Sci. (Prague), 51: 238–243.

Abstract: Resistance to *Plum pox virus* (PPV) and ‘*Candidatus Phytoplasma prunorum*’ has been evaluated for Japanese plum (‘Aphrodite’, ‘Black Amber’, ‘Crimson Glo’, ‘Santa Rosa’) and pluot (‘Dapple Supreme’, ‘Flav Queen’, ‘Flav Supreme’) cultivars. Each cultivar was grafted by chip-budding on GF 305 peach rootstocks and infected with the virulent PPV-Rec or ‘*Candidatus P. prunorum*’ strain. After bud break, cultivar evaluation consisted of observing the presence or absence of symptoms on leaves and noting the intensity of symptoms on leaves and on the whole plant. Plants were studied under controlled conditions in a sealed screen-house for three consecutive growth periods. DAS-ELISA and RFLP-PCR analyses were also employed to verify the presence or absence of PPV, respectively ‘*Ca. P. prunorum*’. Results obtained in the context of this very severe biological test against PPV and ‘*Ca. P. prunorum*’ confirm the high level of susceptibility of Japanese plum and pluot cultivars.

Keywords: *Prunus salicina*; *Prunus armeniaca*; interspecific hybrids; PPV; sharka; ESFY; ‘*Ca. P. prunorum*’; resistance

Ongoing climate change constrains fruit growers to seek ways to stabilize yields and ensure economically profitable production. In addition to this, it is necessary to take into account the demands of the market and the societal emphasis placed on sustainable fruit production (Nečas et al. 2021).

Plum trees belonging to the *Rosaceae* family are a widespread stone fruit worldwide, and together with apple and pear trees, belong to the most important species cultivated in the temperate zone (Zohary et al. 2012). Plums, including the Japanese plum, are high in vitamins, fiber, and potassium. They are

very high in plant compounds, which have antioxidant properties, this means they help prevent oxygen from reacting with other chemicals and causing damage to cells and tissues. They are especially rich in anthocyanins. Studies suggest plums are richer in these protective compounds than other fruits including peaches and nectarines (Traore et al. 2020).

Japanese plums (*Prunus salicina* Lindl.) originated in China but were introduced to the west, from Japan, only 150 years ago. Luther Burbank hybridized them with other plum species with the result that most modern cultivars are multi-species mix-

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QK1910137.

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<https://doi.org/10.17221/170/2023-HORTSCI>

tures (Topp et al. 2012). Pluots are interspecific hybrids of complex crosses of Japanese plum species and apricot species (*Prunus armeniaca* L.) with predominantly plum parentage (75% plum and 25% apricot). Thus, in the last decade a large number of cultivars with notable flavor characteristics, i.e. strong plum flavor, a blend of apricot and plum flavors, very sweet and high antioxidant capacity have been released (Wills et al. 1983; Tomas-Barbera et al. 2001).

So far, very few Japanese plum trees and their hybrids are planted in the Czech Republic, due to lower frost resistance, often worse taste quality and lack of market interest (Beneš 2013).

Sharka (causal agent '*Plum pox virus*', PPV) and European stone fruit yellows (ESFY, causal agent '*Candidatus Phytoplasma prunorum*', '*Ca. P. prunorum*') are a serious threat to stone fruit orchards not only in the Czech Republic (Cambra et al. 2006, Marcone et al. 2010). Among *Prunus* species, Japanese plums are the most susceptible to PPV and '*Ca. P. prunorum*' (Jarausch et al. 2000; Barba et al. 2011). PPV and '*Ca. P. prunorum*' appear to be endemic in the Czech Republic (Nečas, Krška 2005; Polák, Komínek 2016).

Before new species and cultivars of fruit trees are cultivated, it is necessary to test, in addition to their pomological and agronomic traits, their resistance to important diseases and pests. The aim of the study described in this paper was to evaluate, in controlled conditions, the degree of resistance to PPV and '*Ca. P. prunorum*' of several Japanese plum and pluot cultivars that are considered for cultivation in the Czech Republic.

MATERIAL AND METHODS

Plant material. Plant material assayed included 4 Japanese plums (*Prunus salicina* Lindl) and two European plums (*P. domestica* L.), as susceptible controls. We also included three inter-specific hybrids (*P. salicina* × *P. armeniaca* L.) × *P. salicina* (pluots). The cultivars to be evaluated were grafted onto peach GF 305 rootstocks.

Plants were grown in 5.0 L pots in an insect-proof screen-house. Before winter (frost season), the plants were transferred to a cold chamber (7 °C, darkness) periodically, and successive evaluations were conducted. At the beginning of each growth period, pruning was performed to induce vigorous

new shoots for symptom scoring. Plants were studied for three consecutive growth periods.

PPV strain. The strain assayed was a PPV-Rec type strain (Slivoň) originally isolated from a plum in central Bohemia (Glasa et al. 2004). This isolate is kept at the Crop Research Institute, Prague, Czech Republic. Each plant was inoculated by chip-budding with three infected buds. The buds came from a plum infected with the strain and showing typical sharka symptoms on leaves.

Resistance evaluation procedure. Evaluation experiments were carried out in controlled conditions. Ten replications of each cultivar were grafted onto peach GF 305 rootstocks in September 2019. In summer 2020, 4 plants were inoculated with PPV and 4 with '*Ca. P. prunorum*'. Two plants were left like a healthy control. Plants without sprouting inoculum buds (and without symptoms) were re-inoculated in summer 2021 (cycle 1). In each cycle, the presence of sharka and ESFY symptoms in leaves of the rootstock and cultivar was scored according to different scales of 0 (no symptoms) to 4 (maximum intensity of symptoms) for both diseases in June and August, respectively.

DAS-ELISA analysis. After observation of PPV symptoms, in the second and third cycle of study, to ascertain the presence or absence of PPV, a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark, Adams 1977) with polyclonal antibody from Bioreba AG (Reinach, Switzerland) was applied to the leaves. The optical densities (OD) were recorded at 405 nm after 60 min. All ELISA samples were performed in duplicates and a reaction was considered positive when the mean absorbance at 405 nm was at least double that of the negative control. The PPV strain V 10.32 PPV D was used as a positive control. A healthy plum tree served as a negative control.

'Ca. P. prunorum' strain. The strain Saldcot3 was used as the phytoplasma inoculum source. This strain is an A6-P2-I34-S2 haplotype from the Faculty of Horticulture, Mendel University, Brno, Czech Republic (Kiss, personal information). Each plant was inoculated by chip-budding with three infected buds. The buds came from an apricot infected with the strain and showing strong ESFY symptoms on leaves.

RFLP-PCR analysis. Total DNA was isolated from approximately 0.1 g of frozen (–20 °C) leaf petiole and midrib tissues using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Direct PCR with fAT/

rPRUS primer pair (Smart et al. 1996) and nested amplification with primers R16F2/R2 and R16F1/R1 (Lee et al. 1995) were performed on plant samples. The '*Ca. P. prunorum*' strain VURV-V 40.1 was employed as a positive control. Corresponding negative controls were made using non-infected plants. Amplicons obtained with R16F2/R2 and R16F1/R1 primer pairs were subjected to restriction fragment length polymorphism (RFLP) analysis with *RsaI* restriction enzyme to verify phytoplasma identity (Marccone et al. 1996).

RESULTS AND DISCUSSION

Susceptibility of Japanese plum pluot cultivars to PPV. The reaction of the 4 Japanese plum and 3 pluot cultivars to PPV inoculation is summarized in Table 1. After three years of study, all of the cultivars assayed behaved as susceptible, showing symptoms of sharka (confirmed by DAS-ELISA), although the level of susceptibility was different for each cultivar. Starting with the most susceptible, the mean symptom intensity of 'Dapple Supreme' and 'Santa Rosa' ranged from 1.7 to 2.0, and were DAS-ELISA positive. 'Aphrodite', 'Black Amber', 'Crimson Glo', 'Flav Queen' and 'Flav Supreme' were

also susceptible to PPV, with mean symptom intensity between 1.1 and 1.5, and were DAS-ELISA positive. The control European plum cultivars 'Top Hit' and 'Chrudimská' showed lower (1.8), respectively higher (2.6) mean symptom intensity, and were DAS-ELISA positive. Healthy, non-inoculated plants performed as controls never showed symptoms and were DAS-ELISA negative. For most cultivars, the mean intensity of symptoms in the second and third year was higher than in the first year, although in 'Chrudimská' and 'Top Hit' it was lower, respectively similar.

The European plum 'Top Hit' was susceptible, contrary to the weak susceptibility/slight tolerance described by Neumüller (2010). The contradictory results observed could be attributable to the use of different isolates, evaluation and environmental conditions and authenticity of plant material. The susceptibility of 'Chrudimská' has also been reported previously by Neumüller (2010).

Our results confirm the high susceptibility of the Japanese plum cultivars previously described in this species (Dragoiski et al. 2002, Pascal et al. 2002, Cambra et al. 2004; Rubio et al. 2011). The evaluation of 'Black Amber' and 'Santa Rosa' disagrees with Glowacka et al. (2021), who observed no symptoms in these cultivars. The distinct results were most like-

Table 1. Evaluation of the resistance to *Plum pox virus* of 4 *Prunus salicina*, 3 pluot and 2 *Prunus domestica* cultivars. Number of plants evaluated, symptoms (mean intensity) and ELISA positive (mean optical intensity) during three years of study

Cultivar	<i>n</i>	2021	2022		2023		Total	
		symptoms ¹	symptoms ¹	ELISA ²	symptoms ¹	ELISA ²	symptoms ³	ELISA ²
<i>Prunus salicina</i>								
Aphrodite	4	1.0	1.7	0.133	1.8	0.119	1.5 ± 0.4	0.126
Black Amber	4	0.6	1.5	0.153	1.3	0.177	1.1 ± 0.6	0.165
Crimson Glo	4	0.8	1.0	0.043	1.9	0.141	1.3 ± 0.6	0.092
Santa Rosa	4	0.9	1.8	0.162	3.3	0.438	2.0 ± 1.1	0.300
<i>Pluot</i>								
Dapple Supreme	4	0.9	1.8	0.204	2.4	0.173	1.7 ± 0.6	0.189
Flav Queen	4	1.0	1.9	0.081	1.0	0.010	1.3 ± 0.5	0.046
Flav Supreme	4	0.7	1.6	0.035	1.5	0.066	1.2 ± 0.5	0.051
<i>Prunus domestica</i>								
Chrudimská	4	2.8	2.1	0.308	2.8	0.417	2.6 ± 0.5	0.363
Top Hit	4	2.3	1.9	0.086	1.2	0.464	1.8 ± 0.7	0.275

¹Intensity of symptoms evaluated from 0 (no symptoms) to 4 (maximum intensity).

²Optical density of ELISA at 405 nm in 60 min (optical density of healthy plum control; minimum = 0.001; maximum = 0.010).

³Intensity of symptoms ± standard deviation (SD)

<https://doi.org/10.17221/170/2023-HORTSCI>

ly obtained due to the different methodology (natural inoculation conditions) used by Polish researchers.

The interspecific hybrids *P. salicina* × *P. armeniaca* (pluots) ‘Dapple Supreme’, ‘Flav Queen’ and ‘Flav Supreme’ were also susceptible. Regarding PPV phenotyping in pluots, there are a very few studies that deal with PPV resistance. One precedent of Karayiannis and Ledbetter (2009) found that a pluot was highly susceptible to PPV. Rubio et al. (2011) obtained similar results, finding that the French selection ‘J300’ and the American ‘Flav Supreme’ were susceptible to PPV.

In any case, the present work seems to be the first evaluation of PPV resistance in ‘Aphrodite’, ‘Crimson Glo’, ‘Dapple Supreme’ and ‘Flav Queen’.

Susceptibility of Japanese plum and pluot cultivars to ‘*Ca. P. prunorum*’. All Japanese plum and pluot cultivars assayed showed the symptoms of ESFY and were PCR positive (Table 2). As results showed a gradient of susceptibility/resistance, we classified the cultivars into two groups: highly susceptible and susceptible. A first group of Japanese plums and pluots was classified as highly susceptible: ‘Aphrodite’, ‘Black Amber’, ‘Crimson Glo’, ‘Dapple Supreme’ and ‘Santa Rosa’. The mean symptoms intensity of these cultivars ranged from 1.5 to 1.8 with 100% of the evaluated plants with symptoms and PCR positive. A second group was classified as sus-

ceptible: ‘Flav Queen’ and ‘Flav Supreme’. The symptom intensity was lower; however, the percentage of infected replications was also 100% and they were PCR positive. The control European plum cultivars ‘Top Hit’ and ‘Chrudimská’ showed 1.3 mean symptom intensity and were positive with PCR. For most cultivars, the mean intensity of symptoms in the second and third year was higher than in the first year, although in some cultivars it was lower. None of the genotypes examined can be considered to be resistant. This conclusion is supported by the facts that no recovery was observed and colonization appears to be persistent.

The lower symptom intensity in European plum cultivars is in agreement with Landi et al. (2010), who ascribes it to their high degree of tolerance to ‘*Ca. P. prunorum*’.

The level of resistance to ‘*Ca. P. prunorum*’ of all Japanese plums assayed agrees with Landi et al. (2010), who found out a medium level of susceptibility of ‘Aphrodite’ in a field located in a severely ‘*Ca. P. prunorum*’ naturally infected area during a five years period.

Results demonstrate the susceptibility of the Japanese plum and pluot cultivars assayed. These findings agree with previous studies reporting the absence of resistance to ‘*Ca. P. prunorum*’ in Japanese plum (Duval 2004; Landi et al. 2010; Sabaté et al. 2015).

Table 2. Evaluation of the resistance to ‘*Candidatus* Phytoplasma prunorum’ of 4 *Prunus salicina*, 3 pluot and 2 *Prunus domestica* cultivars. Number of plants evaluated, symptoms (mean intensity) and PCR positive during three years of study

Cultivar	n	2021	2022		2023		Total	
		symptoms ¹	symptoms ¹	PCR ²	symptoms ¹	PCR ²	symptoms ³	PCR ²
<i>Prunus salicina</i>								
Aphrodite	4	1.3	1.8	+	1.8	+	1.6 ± 0.5	+
Black Amber	4	1.1	1.9	+	1.4	+	1.5 ± 0.7	+
Crimson Glo	4	1.3	2.1	+	1.9	+	1.8 ± 0.6	+
Santa Rosa	4	1.4	2.2	+	1.4	+	1.6 ± 0.5	+
Pluot								
Dapple Supreme	4	1.3	2.0	+	1.5	+	1.6 ± 0.4	+
Flav Queen	4	0.5	1.6	+	1.3	+	1.1 ± 0.6	+
Flav Supreme	3	0.4	0.9	+	1.1	+	0.8 ± 0.5	+
<i>Prunus domestica</i>								
Chrudimská	4	1.5	1.6	+	0.8	+	1.3 ± 0.6	+
Top Hit	4	1.6	1,2	+	1.0	+	1.3 ± 0.6	+

¹Intensity of symptoms evaluated from 0 (no symptoms) to 4 (maximum intensity).

²Positive (+) or negative (–) reaction

³Mean intensity of symptoms ± standard deviation (SD)

The European plum ‘Top Hit’ showed neither ESFY symptoms nor pathogen presence after five years in a field located in a severely ‘*Ca. P. prunorum*’ naturally infected area (Landi et al. 2010). The contradictory results could be due to differences in evaluation methods, the isolates used, or to the authenticity of the plant material.

As far as we know, the behavior of ‘Crimson Glo’, ‘Dapple Supreme’, ‘Flav Queen’, ‘Flav Supreme’, ‘Santa Rosa’, and ‘Chrudimská’ towards ‘*Ca. P. prunorum*’ has not been evaluated to date.

Based on the obtained results, we can only recommend growing susceptible Japanese plum and pluot cultivars in areas with low infection pressure of sharka and ESFY, where the diseases are absent and the presence of the vectors is very low (or absent). The use of high-quality, healthy and certified plant material is of basic importance.

ACKNOWLEDGEMENT

We are grateful to Dr. Tomáš Kiss from Faculty of Horticulture, Mendel University, Brno, for providing us with the ESFY phytoplasma inoculum used in this work. The authors sincerely thank Mrs. Marcela Komínková for performing PCR assays. The authors also wish to thank Dr. Jiří Svoboda for his expert technical assistance.

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Received: December 20, 2023

Accepted: April 5, 2024