Difference in reactions of apricot and peach cultivars to *Plum pox virus*: serological and symptomatological evaluation

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ABSTRACT: Differences in reactions to infection and different development of Plum pox virus (PPV) symptoms were observed in leaves and fruits of one hundred sixty-five apricot and seventy-nine peach cultivars and hybrids. A very broad spectrum of reactions from high susceptibility to high resistance and immunity was proved in apricot cultivars and hybrids and the results were published (POLÁK et al. 1997). A much narrower spectrum of reactions was proved in peach cultivars. Relative concentrations of PPV by ELISA in flowers, intensity of virus symptoms in leaves and fruits of peach cultivars were evaluated. The relative concentration of PPV coat protein (PPV-CP) in flowers was found to be positively correlated with the intensity of leaf and fruit symptoms in most cultivars. On the basis of obtained results cultivars of peaches were divided into four groups and classified as medium resistant, tolerant, medium susceptible and susceptible to PPV. None of the investigated cultivars was immune, highly resistant or resistant to PPV. Eighteen peach cultivars were classified to be medium resistant to PPV. Nine peach cultivars were characterized as tolerant to PPV, with high relative concentration of PPV-CP protein in flowers and mild symptoms in leaves and fruits. Twenty-seven peach cultivars were characterized as medium susceptible to PPV. Twenty-five peach cultivars were rated as susceptible to PPV. It is recommended to grow medium resistant peach cultivars in the areas where PPV is widely distributed.

Keywords: *Plum pox virus*; apricot; peach; cultivars and hybrids; virus symptoms; virus relative concentration; ELISA; resistance; susceptibility

Studies on resistance of apricots to PPV started in Greece (SYRGIANIDIS 1979). Two apricot cultivars Stark Early Orange and Stella were proved to be resistant to PPV. KARAYIANNIS (1988) discovered other cultivars of American origin as resistant to PPV. The evaluation of resistance to PPV was done in Greece by observation of symptoms in leaves and fruits from naturally infected trees. DOSBA et al. (1992) tested many apricot cultivars and hybrids for resistance in France. Trees were inoculated with PPV using either chip budding or aphid transmission. Symptoms of PPV infection, ELISA values, and biological indicator GF 305 were used as criteria (AUDERGON et al. 1995).

Attempts to evaluate the resistance of peach cultivars to PPV started in Europe in the nineties. The first results were based mostly on observations of the intensity of PPV symptoms (MAINOU, SYRGIANIDIS 1992; BALAN et al. 1995). The methods for reliable detection of PPV in peach trees have improved in comparison with methods of PPV detection in plums and apricots. DOSBA et al. (1986) showed differences in PPV detection in peach trees at different growth stages. POLÁK (1989) detected PPV by ELISA in symptomless peach trees. ALBRECHTOVÁ (1990) studied the distribution of PPV in naturally infected peach trees and found that the detection of PPV in flowers and fruits was more reliable than that in leaves. POLÁK (1995) found the time period with the highest concentration of PPV in leaves and flowers of infected peach trees.

The virological programme to evaluate resistance in apricot and peaches started in the Czech Republic in 1991 (POLÁK et al. 1995). The first procedure for the evaluation of resistance of apricot and peach cultivars to PPV was drawn up. After that crosses between apricots described abroad as PPV resistant and local first-rate susceptible cultivars were carried out at the Faculty of Horticulture at Lednice. Resistance of apricot cultivars and hybrids was rated by own procedure developed after five years of evaluation (POLÁK et al. 1997). The evaluation of resistance to PPV in peach cultivars was based both on determination of the relative concentration of PPV-CP protein in flowers of infected trees and

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evaluation of symptoms in leaves and fruits. The evaluation of resistance in peach cultivars spontaneously or artificially infected with PPV was always carried out minimally in the course of three vegetation periods. Altogether 82 peach cultivars were rated gradually (POLÁK 1998, 1999; POLÁK et al. 2003).

In the course of evaluation differences in reactions of apricots and peaches to PPV infection were ascertained. Obtained results are presented in our contribution summarily.

MATERIAL AND METHODS

The procedure for evaluation of apricot resistance to PPV

Apricot cultivars and hybrids were grafted onto 5-years-old apricot trees cv. Vegama, naturally infected with PPV showing severe symptoms in leaves. PPV isolate was characterized as serotype M (KOMÍNEK et al. 1996). Infected plant material was evaluated in the course of 1993 to 1996. Four different methods were used to evaluate the resistance of apricots to PPV:

1. Visual evaluation of the intensity of leaf and fruit symptoms.
2. Determination of relative concentration of PPV-CP protein in leaves by ELISA.
3. Grafting of a biological indicator (*Prunus tomentosa* or plum cultivar Čačanska rodna) onto resistant cultivars and hybrids.
4. Detection of PPV in resistant cultivars and hybrids by IC-PCR.

Methods 3 and 4 were used for the evaluation of resistant cultivars and hybrids of apricots to verify the presence of immunity in 1996 to 1998.

Details of the procedure for the evaluation of apricot resistance to PPV were published by POLÁK et al. (1997).

List of selected apricot cultivars evaluated for resistance to PPV

Fifteen cultivars presented in the literature as resistant or tolerant (Dacia, Goldrich, Harcot, Harlayne, Harval, Chuang Zhi Hong, Krymskij Amur, Leronda, Mai Chua Sin, Marii de Cenad, Pentagonála, San Castrese, Stark Early Orange, Sundrop and Vestar) and two susceptible (control) Czech and Slovak cultivars Velkopavlovická and Vegama were evaluated.

Apricot hybrids evaluated for resistance to PPV

Hundred forty-eight Czech apricot hybrids originating from Lednice (Prof. Vachůn) and Valtice (Mr. Oukropec) and one from INRA (France), crosses between immune or resistant apricot cultivars and susceptible ones (type Ungarn) with high quality of fruits, were evaluated for resistance to PPV. Only 31 hybrids did not show any leaf symptoms in the year of grafting (1993), and were evaluated for resistance in the years 1994 to 1998 by the same procedure as selected apricot cultivars.

The procedure for evaluation of peach resistance to PPV

Fifty-five peach cultivars (POLÁK 1998, 1999) naturally infected with PPV and twenty-eight peach cultivars (POLÁK et al. 2003) artificially infected with PPV-D by aphids and by chip-budding were evaluated. Infected plant material was evaluated at least for three years during the period from 1995 to 2002. Two different methods were used:

1. Determination of relative concentration of PPV-CP protein in flower petals by ELISA.
2. Visual evaluation of the intensity of leaf and fruit symptoms.

Preparation of samples for serological evaluation

Serological evaluation of peaches was performed during a flowering time in April, evaluation of apricots (leaves) during June. Flowers or leaves were ground in polyethylene bags using a manual homogenizer. Five flowers or leaves showing symptoms from every tree of PPV infected cultivars (3–5 trees) were sampled for the determination of relative concentration of PPV-CP. Flower petals or leaves were ground at a 1:20 ratio of plant material to extraction buffer (phosphate buffered saline), pH 7.2 with 0.05% Tween 20, 0.2% polyvinylpyrrolidone and 0.2% egg albumin.

Immunoenzymatic assay

PPV antibodies (our own, prepared by Prof. E. Fuchs, Germany, University Halle-Wittenberg, or commercial from Bioreba) were used in a double antibody sandwich method (ADAMS 1978). PPV IgG were adjusted to the concentration l mg/1 ml. PPV IgG was used in a 1:1,000 dilution and two wells were used for each sample.0.2 ml of the sample was pipetted into one well of an ELISA plate. PPV IgG conjugated with alkaline phosphatase was used in a 1:1,000 dilution. Absorbance values were measured with photometer MR5000 (DYNEX, GFR) at 405 nm. The relative concentration of PPV was established by the determination of the lowest dilution that gave a positive reaction (ALBRECHTOVÁ et al. 1986). The titer of PPV in a sample was established as the dilution of sap with the minimum absorbance value 0.04. The absorbance values of negative controls were 0.01 or less. Tests were repeated twice.

RESULTS AND DISCUSSION

The evaluation of PPV symptoms in leaves of apricot cultivars and hybrids and peach cultivars showed distinct reactions of these stone fruits to PPV infection. Apricots manifested a broad variety of symptoms

Table 1. Results of evaluation of apricot cultivars for resistance to PPV

Cultivar	Symptoms in leaves and fruits	Relative concentration by ELISA	Biological indicator	IC PCR	Characterization of cultivars
Harlayne	ns	0	ns	–	immune
Leronda	ns	0	+	+	highly resistant
Harval SEO* Marii de Cenad	very mild in leaves, only close to the place of grafting, none in fruits, or very occasionally	0 Harval and SEO low concentration only in the first year after grafting	+	+	resistant
Harcot Sundrop	mild chlorotic spots	6.25×10^{-3} only close to the point of graft	+	+	medium resistant
Mai Chua Sin Goldrich Dacia	mild oak mosaic in leaves, rings and spots in fruits	6.25×10^{-3} to 7.8×10^{-4}	+	+	medium susceptible
Chuang Zhi Hong Pentagonála San Castrese Vestar Velkopavlovická Vegama	mild to severe oak mosaic in leaves, rings and spots in fruits	1.6×10^{-3} to 9.8×10^{-4}	+	+	susceptible
Krymskij Amur	severe oak mosaic in leaves, rings, spots and malformation of fruits	9.8×10^{-4}	+	+	highly susceptible

ns – no symptoms
0 – no virus detected by ELISA
+ PPV proved (by biological indicator, by IC PCR)
– no PPV detected by IC PCR
SEO* – Stark Early Orange

ranging from very moderate thickening of veins, mosaic and diffuse spots to severe oak-leaf mosaic and ringspot. Symptoms in leaves appear immediately after their development (in May) and persist till the late vegetation period (minimally till August). By means of ELISA the virus is detectable in symptomatic leaf tissues even in September. Resistant cultivars (Stark Early Orange, Harval, Leronda, Marii de Cenad) reveal a perspicuous tendency to escape virus infection. Mild symptoms of PPV appeared in leaves (frequently in a few leaves only) close to the point of grafting on diseased cultivar Vegama frequently in the second year after grafting. Mild symptoms sometimes appeared even in the third year but in subsequent years no symptoms in leaves and fruits were observed. In leaves carrying mild symptoms the virus was detected by ELISA with difficulties while in symptom-free leaves detection failed at all. PPV in these cultivars was detectable by means of IC-PCR only (POLÁK et al. 1997).

Peaches infected with PPV react by the clearing and thickening of veins of the first leaves that appear under the environmental conditions of the Czech Republic in early May. The leaves are thickened and leathery. The highest concentration of the virus in leaves appears in May, in June it drops to a half or a quarter of the original value and in August detection of the virus by ELISA as a rule fails. In more susceptible cultivars PPV symptoms develop in the second, the third, contingently in the fourth and further leaves of the growing branch. Those leaves show marked or severe oak-leaf mosaic, sometimes also diffuse spots and rings. Leathery leaves with thickened veins during June get yellow and drop off. That is why no symptoms can be observed by the end of June especially in cultivars more resistant to PPV. In July symptoms can be seen only in lower leaves on branches of susceptible or highly susceptible cultivars in the cases when symptoms develop in the third, the fourth or the fifth leaf of the branch. Under the conditions of the Czech Republic as a rule no symptoms develop in the seventh and next leaves of the branches.

The results of evaluation of resistance of fifteen apricot cultivars described in the literature as immune, resistant or tolerant to PPV and two control susceptible cultivars (Vegama, Velkopavlovická) are summarized in Table 1. Cultivar Harlayne was proved as immune to PPV-M and cultivar Leronda as highly resistant. Also cultivars Stark Early Orange, Harval and Marii de Cenad were proved as resistant but very mild PPV symptoms were observed in leaves close to the point of grafting in the first year. In these leaves the virus was

Table 2. Evaluation of selected apricot hybrids for resistance to PPV

Hybrid	Symptoms	Relative concentration	Biological indicator	IC PCR	Characterization of hybrids
LE-3276	no symptoms	0	ns	–	immune
LE-833	no symptoms	0	+	+	highly resistant
LE-806	occasionally mild rings	0	+	+	
LE-3184	mild mosaic, vein banding	0	+	+	
VA-N3	occasionally oak mosaic	$2.5 \times 10^{-2*}$	+	+	
VA-F1	rarely diffuse spots	0	+	+	
VA-E3	very mild spots in 10% of fruits	0	+	+	resistant
LE-3216	very mild mosaic and spots in 10% of fruits	0	+	+	
LE-3662	mild oak mosaic, mild spots in 10% of fruits	0	+	+	
LE-3187	spots in 10% of fruits	0	+	+	
LE-2913	rings in 10% of fruits	0	+	+	
VA-L1	vein clearing, ringspots, very mild spots in 10–15% of fruits	0	+	+	
LE-2935	very mild mosaic, rings and spots in fruits	0	+	+	
INRA 804 669255a	mild mosaic	1.6×10^{-3}	+	+	medium resistant
LE-3232	mild oak mosaic, rings, spots in fruits	from 7.8×10^{-4} to 0 5.0×10^{-2}	nt	nt	medium susceptible
LE- 3195	mild mosaic, rings in fruits	1.25×10^{-2}	nt	nt	
LE- 3194	oak mosaic, rings, spots in fruits		nt	nt	
131 hybrids	medium to severe oak mosaic in leaves, rings and spots in fruits malformations of fruits	10^{-3} to 10^{-5}	nt	nt	susceptible and highly susceptible

* close to the point of grafting only
0 – no virus detected by ELISA
+ PPV proved
– no PPV detected by IC PCR
nt – not tested

detected by ELISA. Cultivars Harcot and Sundrop were characterized as medium resistant with mild virus symptoms in leaves and fruits and low concentration of PPV-CP. On the other hand, cultivars Goldrich, Dacia and Mai Chua Sin were rated as medium susceptible with medium concentration of PPV-CP in leaves. Cultivars Chuang Zhi Hong, Pentagonála, San Castrese and Vestar were PPV susceptible and cv. Krymskij Amur even highly susceptible with severe PPV symptoms in leaves and fruits, malformations of fruits and high concentration of the virus in leaves.

In the course of four years of evaluation 148 of apricot hybrids crossed between resistant cultivars and susceptible ones of the type Ungarn were investigated. Out of them only fourteen resistant hybrids were identified. The rest of hybrids was susceptible to PPV, some of them were even highly susceptible with malformed fruits. Evaluation of the fourteen resistant apricot hybrids was finished in 1998 and is quoted in Table 2. Apricot hybrid LE-3276 was proved as PPV immune, hybrids LE-806, LE-833, LE-3184, VA-N3 and VA-F1 as highly resistant and resistant at the level of Leronda or Stark Early Orange cultivars.

On the basis of determination of the relative concentration of PPV-CP in flower petals and evaluation of PPV leaf and fruit symptoms, peach cultivars were grouped into four categories: medium resistant, tolerant, medium susceptible and highly susceptible. None of the investigated peach cultivars was immune to PPV, or highly resistant, based on leaf or fruit symptoms.

Cultivars Camden, Candor, Cotender, Envoy, Favorita Morettini, Flamencrest, Flame Prince, Harcrest, Harmony, Jefferson, Jersey Queen, Maycrest, Newhaven, Ruby Prince, Spring Lady, Sun Prince, Triestina and Velvet were characterized as medium resistant to PPV. Vein clearing, thickening and brittleness or no symptoms appeared in the first and second leaves of branches. Most fruits of these cultivars were without visible symptoms, but very mild diffuse spots appeared in a limited number of fruits. Relative concentration of PPV-CP in flower petals was very low (0 to 2.5×10^{-2}).

Cultivars Blaze Prince, Canadian Harmony, Harken, June Prince, Legend, Loring, Rosired 1, Springcrest and Suncrest were rated to be tolerant to PPV. These cultivars showed vein clearing in the first two, three, or even fourth leaves of branches. Very mild diffuse spots or rings appeared in the limited number of fruits. Relative concentration of PPV-CP in flower petals was high (6.25×10^{-3} to 3.91×10^{-4}), comparable with that of highly susceptible cultivars.

Cultivars Adriatica, Anderson, Biscos, Carogem, Carolina Belle, Crest Haven, Ellerbe, Fire Prince, Golden Red, Harbelle, Harrow Diamond, Harson, Harvester, Madison, Maria Serena, NJC 102, O'Henry, Quachita Gold, Redglobe, Rosired 3, Sentry, Springbrite, Sunhigh, Ta-Tiou-Pao, Veteran, Vivid and Weinberger were characterized as medium susceptible to PPV. Vein clearing, mosaic, yellowing, thickening and brittleness were found usually in the first three leaves of branches. Mild to medium severe diffuse spots, and/or rings appeared in the limited number of fruits. Relative concentration of PPV-CP in flower petals fluctuated from 6.25×10^{-3} to 1.56×10^{-3} and usually was lower in comparison with tolerant cultivars.

Cultivars Ambergold, Bounty, Catherina, Croce del Sud, Dixired, Flavorcrest, Fortuna, Gala, Gold Prince, Harbinger, Harbrite, June Lady, Kisinec, Lamone, Maria Luisa, NJC 106, Pusistyj Rannij, Record aus Alfter, Redhaven, Rubired, Somervee, Spotlight, Summer Prince, Sunbrite and Sunhaven were rated as highly susceptible to PPV. Symptoms in leaves were distinct. Yellowing, mosaic and vein clearing appeared in the first, second, third and sometimes in the fourth leaves of branches. Severe or medium severe diffuse spots and rings appeared in most fruits. Relative concentration of PPV protein in flower petals was very high (1.56×10^{-3} to 1.95×10^{-4}).

Like in the case of PPV symptoms in leaves different reactions of apricot and peach cultivars to infection with the virus were observed. In the case of apricots broader population biodiversity in susceptibility or resistance to PPV was found. Apricot cultivars and hybrids can be classified into seven groups: immune, highly resistant, resistant, medium resistant, medium susceptible, susceptible and highly susceptible. On the one hand, there are apricot cultivars and hybrids that cannot be infected with PPV, reveal immunity to the virus, or can be infected but the virus in symptomless plants does not practically reproduce and therefore cannot be detected by ELISA. It can be detected either by very sensitive procedures such as PCR or by grafting of a biological indicator (i.e. cultivar highly resistant or resistant) which, after infection, shows very mild virus symptoms in several leaves close to the point of grafting on a susceptible cultivar. Low concentration of PPV is then detectable by ELISA but plants gradually eliminate the virus, escape infection and after a couple of years PPV can be proved again by very sensitive methods only. On the other hand, there is a number of apricot cultivars with fruits frequently of high or outstanding quality which are susceptible to highly susceptible to PPV infection and the virus reaches a high concentration in their tissues. In such highly susceptible apricot cultivars and hybrids, besides usual severe leaf and fruit symptoms fruit malformations can be observed that are very severe in some cases.

A much narrower spectrum of reactions to PPV was proved in peach cultivars. None of the investigated cultivars was immune, highly resistant or resistant to PPV. On the basis of the obtained results cultivars of peaches were divided into four groups and classified as medium resistant, tolerant, medium susceptible and susceptible (very susceptible) to PPV.

In peaches a group of PPV tolerant cultivars with high relative virus concentration in flowers and leaves (corresponding to the level of susceptible cultivars) was found. They exhibit very mild symptoms in fruits while 80% of them or more remain free from symptoms. We did not identify any apricot cultivars tolerant to PPV. In regions where PPV is severely spread the growing of medium resistant peach cultivars is recommended. They show only very mild symptoms in fruits and 85% to 90% of them remain symptomless. In such regions we do not recommend to grow tolerant cultivars because they become significant sources of PPV infection spread by aphids.

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Rozdíly v reakci kultivarů meruňky a broskvoně na virus šarky švestky: sérologické a symptomatologické vyhodnocení

ABSTRAKT: Byly pozorovány rozdíly v reakci na infekci a odlišný vývoj příznaků viru šarky švestky (*Plum pox virus)* na listech a plodech sto padesáti šesti kultivarů a hybridů meruňky a sedmdesáti devíti kultivarů broskvoně. Bylo prokázáno široké spektrum reakcí kultivarů a hybridů meruňky od vysoké náchylnosti po vysokou rezistenci a imunitu; získané výsledky byly publikovány (POLÁK et al. 1997). V kultivarech broskvoně bylo prokázáno mnohem užší spektrum reakcí. Pomocí ELISA byla u kultivarů broskvoně vyhodnocena relativní koncentrace PPV v květech a intenzita příznaků na listech a plodech. Bylo zjištěno, že relativní koncentrace obalového proteinu PPV v květech je u většiny kultivarů v pozitivní korelaci s intenzitou příznaků na listech a plodech. Na základě získaných výsledků byly kultivary broskvoně rozděleny do čtyř skupin a klasifikovány jako středně rezistentní, tolerantní, středně náchylné a náchylné k PPV. Žádný ze zkoumaných kultivarů nebyl imunní, velmi rezistentní nebo rezistentní k PPV. Osmnáct kultivarů broskvoně bylo klasifikováno jako středně rezistentních k PPV. Devět kultivarů broskvoně bylo charakterizováno jako tolerantní k PPV s vysokou relativní koncentrací PPV proteinu v květech a mírnými příznaky na listech a plodech. Dvacet sedm kultivarů broskvoně bylo charakterizováno jako středně náchylné k PPV. Dvacet pět kultivarů broskvoně bylo vyhodnoceno jako náchylné k PPV. V oblastech, kde je virus šarky švestky široce rozšířen, je doporučováno pěstovat středně rezistentní kultivary broskvoně. V České republice to jsou registrované odrůdy Favorita Morettini a Envoy.

Klíčová slova: virus šarky švestky; meruňka; broskvoň; kultivary a hybridy; příznaky viru; relativní koncentrace viru; ELISA; rezistence, náchylnost

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