RELATIVE CONCENTRATION OF PLUM POX VIRUS IN LEAVES AND FLOWERS OF SOME *PRUNUS* SPECIES AND CULTIVARS

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Summary. – The relative concentration of plum pox virus (PPV) in leaves and flowers of plum, damson, myrobalan, blackthorn, apricot and peach trees was determined by enzyme-linked immunosorbent assay (ELISA) and expressed as the lowest dilution with positive reaction. Significant differences in relative PPV concentration were found in leaves among individual *Prunus* species naturally or artificially infected with the virus. The highest relative PPV concentration was found in blackthorns (7.81×10^{-4}), common plum and apricot (1.56×10^{-3} for the both latters). Wild growing PPV-infected plums and blackthorns can be considered equally important source of sharka infection as PPV-susceptible cultivars of plums, apricots and peaches. High PPV concentration in flowers is of diagnostical value. High variability of relative PPV concentration was observed inside the species among individual cultivars. Susceptible cultivars were characteristic by high relative PPV concentration, e.g. apricot cvs. Vegama (9.8×10^{-5}) and Velkopavlovická (1.95×10^{-4}), and peach cvs. Maria Emilia (7.81×10^{-4}) and Harbinger (1.56×10^{-3}). On the other hand, cultivars resistant to PPV were characteristic by very low relative PPV concentration, e.g. apricot cv. Stark Early Orange (5×10^{-2}) and peach cvs. Envoy (5×10^{-2}) and Favorita Morettini (2.5×10^{-2}). The highest relative PPV concentration was found in young trees newly infected with PPV.

Key words: plum pox virus; common plum; damson; myrobalan; blackthorn; apricot; peach; enzyme-linked immunosorbent assay; relative virus concentration; leaves; flowers

Introduction

Recently we succeeded in proving the correlation between relative PPV concentration in flowers and leaves of peaches (Polák, 1996) and apricots (Polák *et al.*, 1997), and quantitative PPV resistance including intensity of leaf symptoms of the disease. The relative concentration of PPV protein has been determined by ELISA (Albrechtová *et al.*, 1986; Polák, 1995). PPV has been easily and reliably detected in flowers of peaches in the course of flowering time, and in leaves in May. The relative PPV concentration was 2 times lower in young leaves of peaches in comparison with flowers at the beginning of May (Polák, 1995). Studies on the epidemiology of PPV in the Czech Republic showed wild growing plums, myrobalans and blackthorns as important natural source of PPV infection (Polák, 1997).

The PPV concentration in trees is in agreement with the abovementioned results supposed to be important from the point of view of the source of PPV infection. Therefore, in this study, we ascertained the relative virus concentration in leaves of different *Prunus* species (plums, damsons, myrobalans, blackthorns, apricots and peaches) and cultivars. It was also determined in flowers of several *Prunus* species and in peach and apricot cultivars with different level of resistance to PPV.

Materials and Methods

Plant material. Plum, damson, myrobalan, blackthorn, apricot and peach trees infected with PPV were used for determination of relative PPV concentration in their leaves and flowers. The PPV infection of the examined trees was proved by ELISA. Twelve flowers or six leaves were taken at random from different branches on the periphery of crown of each tree. Flowers of all the examined *Prunus* species did not exhibit any PPV symptoms. On the other hand, for determination of relative PPV concentration in leaves only those showing PPV symptoms were sampled. Besides trees infected with PPV for several years, also young ones infected for one year were examined. Flowers were sampled and investigated

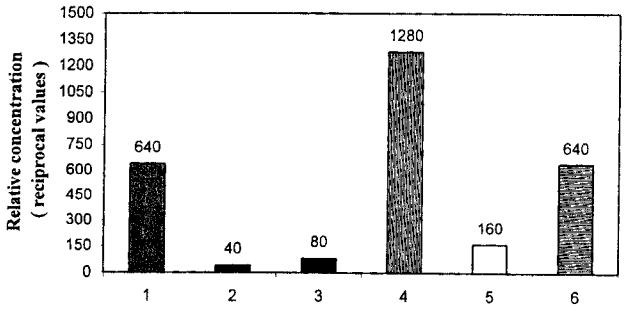

Fig. 1
Relative PPV concentration in leaves of different *Prunus* species
1 – *Prunus domestica* L. *ssp Oeconomica* (Borkh.) C.K. Schn , common plum; 2 – *Prunus domestica* L. *ssp. Insititia* (Yusl.) Schn., damson; 3 – *Prunus cerasifera* Ehrh. *ssp. myrobalana* (L.) Hegi, myrobalan; 4 – *Prunus spinosa* L., blackthorn; 5 – *Persica vulgaris* Mill. (*Prunus persica*), peach seedling; 6 – *Armeniaca vulgaris* L. (*Prunus armeniaca*), apricot.

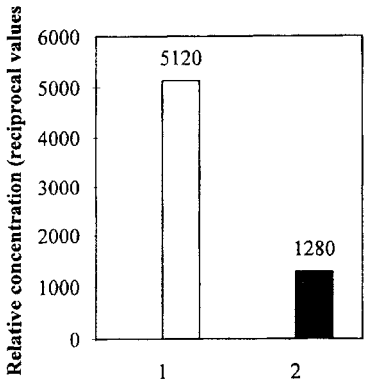

Fig. 3
Relative PPV concentration in leaves of young and old infected plum trees
1 – young tree, first year after PPV infection, second year after planting; 2 – old tree (more than 40 years) many years after PPV infection.

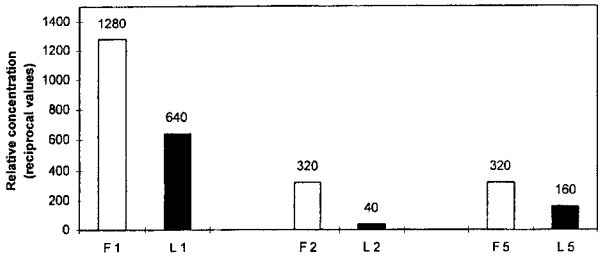

Fig. 2
Relative PPV concentration in flowers and leaves of different *Prunus* species
F – flowers; L – leaves; 1 – *Prunus domestica ssp. oeconomica*; 2 – *Prunus domestica ssp. insititia*; 3 – *Prunus persica.*

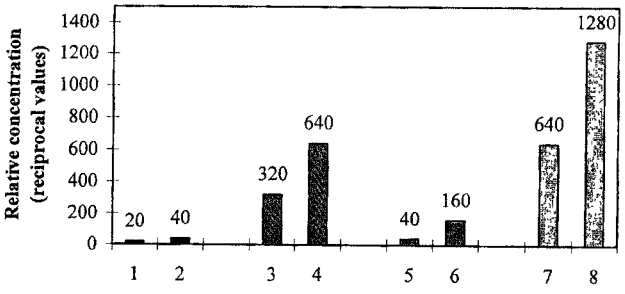

Fig. 4
Relative PPV concentration in flowers of peach cultivars with different resistance to the virus
1 – Envoy; 2 – Favorita Morettini; 3 – Canadian Harmony; 4 – Harken; 5 – Adriatica; 6 – Harson; 7 – Harbinger; 8 – Maria Emilia (nectarine).

in the course of flowering time, while leaves in the period from the middle of May to the middle of June.

Sample preparation. A pooled sample was prepared both from flowers (petals) and leaves by homogenizing 0.6 g of tissues in an extraction buffer (phosphate-buffered saline pH 7.2 with 0.05% Tween 20.2% polyvinylpyrrolidone and 0.2% ovalbumin) in ratio of 1:20 in polyethylene bag by manual homogenizer.After centrifugation, an 0.2 ml aliquot of the obtained sap was used per well in ELISA. Samples were assayed in duplicate.

ELISA. Relative concentration of PPV was determined in tissue samples from plant tissues by double-antibody sandwich ELISA. A commercial antiserum (Bioreba) and an antiserum prepared by us were used in the assay (Adams, 1978). Absorbance values were measured by photometer MR 5000 (Dynatech). The relative concentration of PPV (protein) was established by determination of the lowest dilution with positive reaction (Albrechtová *et al.*, 1986). The titre of PPV in a sample (sap) was expressed as reciprocal value of the sample dilution and the minimum absorbance value was 0.04. The absorbance value of negative controls was 0.01 or less.

Results and Discussion

Significant differences in relative PPV concentration were ascertained among individual *Prunus* species (Fig. 1) naturally or artificially infected with the virus. The lowest PPV concentration was found in leaves of damson and myrobalan, while the highest one in leaves of blackthorn, common plum, and apricot trees. Whereas the relative virus concentration in blackthorn was 7.81×10^{-4}, in common plum and apricot about 1.56×10^{-3}, and in myrobalan and damson only 1.25×10^{-2} and 2.5×10^{-2}, respectively.

In comparing relative PPV concentration in flowers and leaves (Fig. 2), it was found in all cases 2 – 4 times higher in flower petals than in leaves. The increased PPV concentration in flowers was proved for the first time in peach (Polák, 1995) and now also in common plum and damson trees.

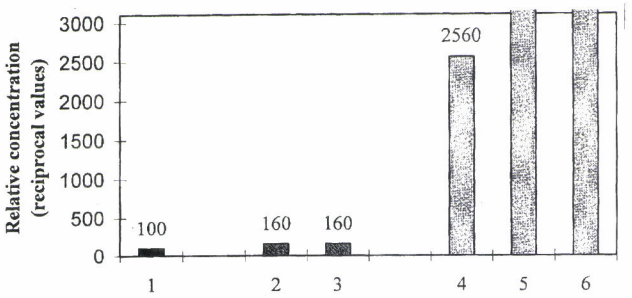

Fig. 5
Relative PPV concentration in leaves of apricot cultivars with different resistance to the virus
1 – Stark Early Orange; 2 – Harcort; 3 – Sundrop; 4 – Velkopavlovická; 5 – Vegama; 6 – Krymskij Amur.

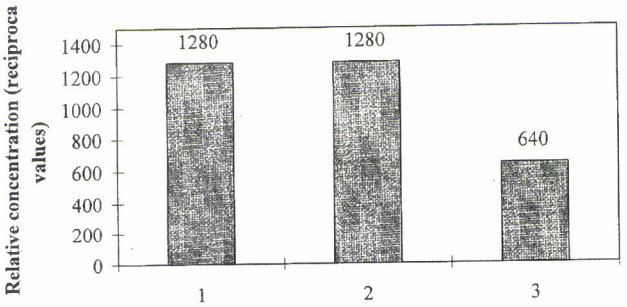

Fig. 6
Relative PPV concentration in leaves of wild growing blackthorns
1, 2, 3 – various infected blackthorn shrubs.

Fig. 7
Leaves of PPV-infected blackthorn
Severe oak mosaic, rings and spots with high concentration of the virus.

Relative PPV concentration in leaves of a young, newly infected plum tree was compared with that in more than 40-year-old and for many years infected one (Fig. 3). Relative PPV concentration in leaves of a young common plum tree in the first year after infection and in the second year after planting was 4 times higher than that in an old tree.

Examples of relative PPV concentration in flowers of peach cultivars with different resistance to the virus are given in Fig. 4. The susceptibility of 34 peach cultivars to PPV was evaluated according to the symptoms developed on leaves and fruits and assayed for PPV by ELISA (Polák, 1998). Medium resistant cultivars were characteristic by a very low relative PPV concentration values (Envoy – 5 x 10^{-2}, Favorita Morettini – 2.5 x 10^{-2}), tolerant cultivars by high values (Canadian Harmony – 3.12 x 10^{-3}, Harken – 1.56 x 10^{-3}), medium susceptible cultivars by medium values (Adriatica – 2.5 x 10^{-2}, Harson – 6.25 x 10^{-3}), and very susceptible cultivars by high values (Harbinger – 1.56 x 10^{-3}, Maria Emilia – 7.81 x 10^{-4}).

High variability of relative PPV concentration in leaves was observed among various cultivars inside the apricot species, too (Fig. 5). Resistant cultivars were charecteristic by low values (Stark Early Orange – 5 x 10^{-2}, medium resistant cultivars by medium values (Harcot and Sundrop, both 1.56 x 10^{-3}) and cultivars susceptible to PPV by high values (Velkopavlovická – 1.95 x 10^{-4}, Vegama and Krymskyj Amur both 9.8 x 10^{-5}).

Relative PPV concentration in leaves of wild growing blackthorn was compared among individual shrubs (Fig. 6). The values were high (from 1.56 x 10^{-3} to 7.81 x 10^{-4}) and the differences were negligible

PPV-infected blackthorns with high virus concentration (Fig. 7) are supposed to be an important natural source of infection. Wild growing PPV-infected plums and blackthorns can be considered an equally important source of sharka infection as PPV-susceptible cultivars of plums, apricots, or peaches. PPV-infected apricots could be also an important source of infection, but aphid vectors suck on them only rarely. We suppose a low efficiency of PPV transmission from infected myrobalans and damsons.

High PPV concentration in flowers is of practical value as it enables reliable diagnosis of PPV in peach trees as well as evaluation of peach cultivars for resistance to the virus (Polák, 1995). Very high concentration of PPV in young, newly infected plum trees corresponds with the fact that this dangerous virus spreads in young orchards much faster that in older ones. The presented results just stimulate our detailed research on PPV epidemiology and resistance in different *Prunus* species.

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