

DURABLE RESISTANCE OF APRICOT CULTIVARS HARLAYNE AND BETINKA TO SIX DIFFERENT STRAINS OF *PLUM POX VIRUS*

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SUMMARY

Results of a six-year evaluation carried out in the Czech Republic (CR) for resistance of apricot cvs Harlayne and Betinka to six different strains and isolates of PPV are presented. Two-year-old trees of both cultivars were inoculated by chip budding with PPV-M (type strain), PPV-D (type strain), PPV-Rec (originally recovered in the CR as PPV-W), PPV-M (isolated in the CR from peach), PPV-D (isolated in the CR from *Prunus insititia*), and PPV-Rec (isolated in the CR from *P. insititia*). PPV susceptible apricot cvs Karola and Velkopavlovická were used as controls and were chip-bud inoculated with PPV strains. No symptoms appeared in the leaves and fruits of cv. Betinka during six years of observation following inoculation with PPV-D (type), PPV-D (*P. insititia*), PPV-Rec (PPV-W), PPV-M (peach) and PPV-Rec strains, and in two trees inoculated with PPV-M (type) strain. Likewise, ELISA did not detect the presence of PPV in the leaves of both cultivars. RT-PCR gave a slight positive reaction from the leaves of a symptomless tree of cv. Harlayne inoculated with PPV-Rec (PPV-W) and from leaves of a symptomless tree of cv. Betinka inoculated with PPV-D (*P. insititia*). The presence of PPV in these trees is under investigation. Mild diffuse rings and spots appeared occasionally in the leaves and fruits of one tree of cv. Betinka after inoculation of PPV-M (type). The presence of PPV was confirmed by ELISA and RT-PCR. The relative concentration of PPV-M (type) protein in the leaves of a tree of cv. Betinka determined by ELISA was low (2.5×10^{-1}) in comparison with the control (cv. Karola, 1.95×10^{-3}). Severe symptoms appeared in the leaves and fruits of susceptible control cvs Karola and Velkopavlovická. The presence of different PPV strains in control cultivars was confirmed by ELISA and RT-PCR. Thus, cvs Harlayne and Betinka displayed durable resistance to the six different strains and isolates of PPV over the six year trial.

Key words: Apricot, resistance, PPV, diagnosis, ELISA, RT-PCR.

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INTRODUCTION

Many apricot cultivars and hybrids have been tested for resistance to the D and M strains of *Plum pox virus* by Dosba *et al.* (1992), who reported that cv. Harlayne is immune to the virus. The immunity of this cultivar was later confirmed by grafting it onto five-year-old trees of apricot cv. Vegama infected with PPV-M (Polák *et al.*, 1997). One of 149 apricot hybrids, (LE-3276) obtained in the Faculty of Horticulture, Lednice, Czech Republic, and later registered as cv. Betinka, was also shown to be immune to PPV-M.

Since resistance or immunity of cultivars to plant viruses can be strain specific, a field trial was set up to assess the behaviour of cvs Harlayne and Betinka against six different strains and isolates of PPV. Preliminary results of a three-year evaluation by serological testing and symptom observation have already been published (Polák *et al.*, 2005). Now we report the results of six-year evaluation based on ELISA, RT-PCR, and symptom observation on the leaves and fruits.

MATERIALS AND METHODS

Plant material and inoculation with PPV strains.

Virus-free, two-year-old trees of cvs Harlayne and Betinka and the PPV-susceptible control cvs Karola and Velkopavlovická were inoculated by chip budding in 2001 with the following strains and isolates of PPV: (i) PPV-M type, original strain from France; (ii) PPV-D type, original strain from France; (iii) PPV-Rec (recombinant PPV-M x PPV-D), isolated from *Prunus insititia* in the Czech Republic (Poncarová and Komínek, 1998; Glasa *et al.*, 2004); (iv) PPV-M, isolated from peach, cv. Catherina in the CR; (v) PPV-D, isolated from *Prunus insititia* in the CR; (vi) PPV-Rec, originally recovered in CR as PPV-W and re-classified by Komínek as PPV-Rec.

Buds from apricot, peach GF-305 and *P. insititia* sources infected with different PPV isolates were used as inoculum. Three trees per cultivar were inoculated each with two buds infected by each viral strain or isolate. Bud growth and symptoms development were checked in the vegetation period that followed.

Visual inspection and evaluation. From May to September 2002 through 2007, inoculated trees of the four cultivars under study were inspected monthly for the presence of PPV symptoms in the leaves. Symptoms in the fruits and stones were evaluated at the time of ripening in 2004 through 2007.

ELISA. Samples of cvs Harlayne and Betinka leaves with or without PPV symptoms were tested serologically every June. A polyclonal antiserum (Bioreba, Switzerland) was used for virus detection by DAS-ELISA (Clark and Adams, 1977). Samples were prepared by grinding 0.2 g of leaves in phosphate buffered saline, pH 7.4 with 2% polyvinylpyrrolidone and 0.2% of egg albumine in the ratio 1:20. Microplates were read with a MR 5000 reader (Dynex, USA) at 405 nm after 1 h incubation of the substrate at room temperature. Samples with $A_{405} > 0.10$ were considered as positive, and samples with $A_{405} < 0.03$ were rated as negative.

RT-PCR. Total RNA was isolated from flowers and leaves of apricots using a RNeasy Plant Mini Kit (Qia-

gen, Germany). Primers for PPV detection were P1 and P2 (Candresse *et al.*, 1995), which amplify a 243 bp long fragment of a coat protein gene of PPV. One step RT-PCR kit (Qiagen, Germany) was used for RT-PCR detection. Reactions were done in a PTC200 thermocycler (MJ Research, USA). Reverse transcription was for 60 min at 45°C and the reaction was finished by denaturation at 95°C for 15 min. PCR was run for 40 cycles; each consisting of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C for 45 sec. PCR products were separated by electrophoresis on 2% agarose gel and visualised under UV light.

RESULTS AND DISCUSSION

Apricot cv. Betinka was shown to be highly resistant to PPV. No symptoms appeared in the leaves after inoculation with PPV-D (type), PPV-Rec (*P. insititia*), PPV-M (peach), PPV-D (*P. insititia*), and PPV-Rec (PPV-W) for the whole duration of the trial (2002-2007). The first fruits were produced in 2004 and, up to now, have

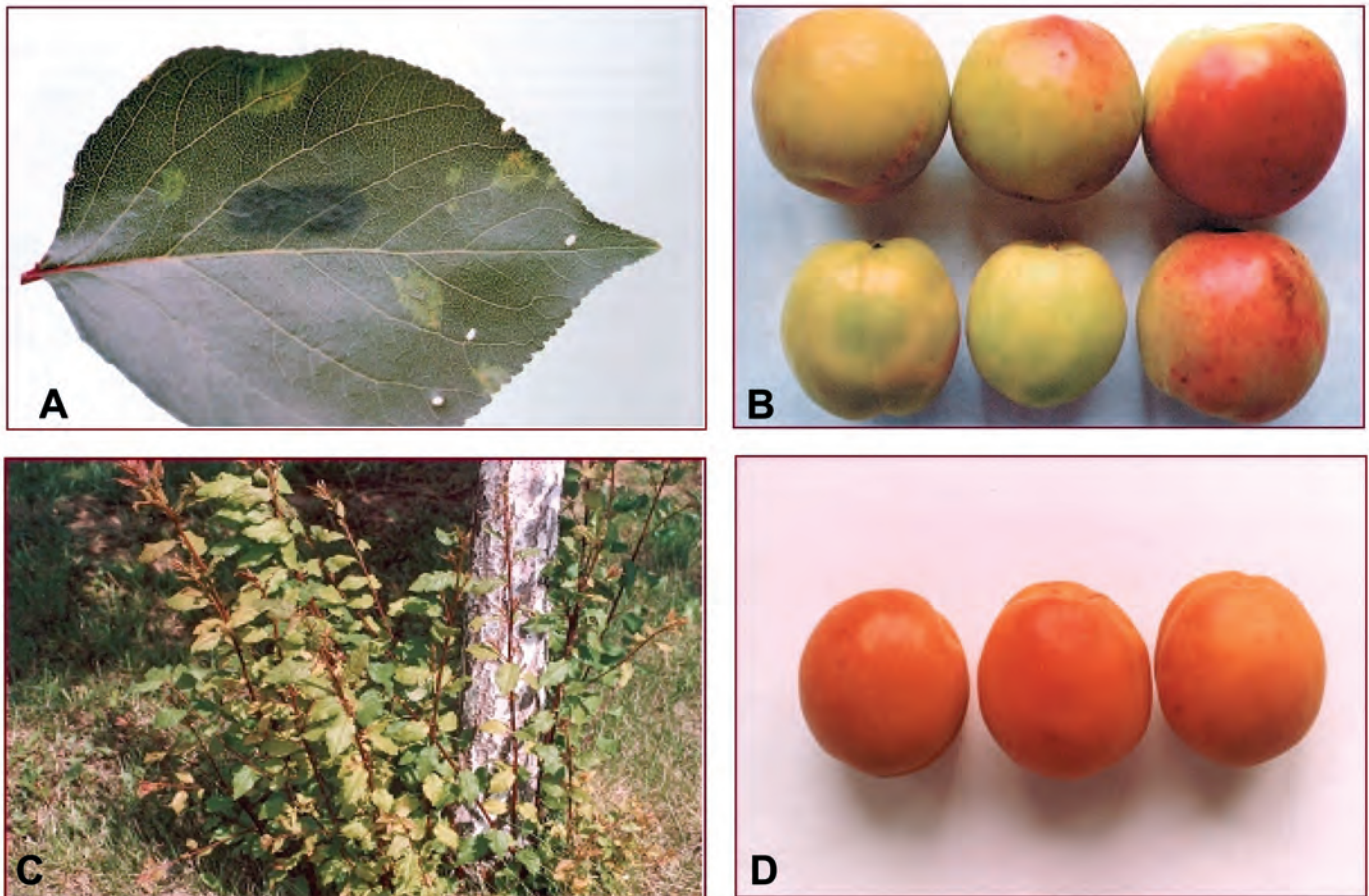


Fig. 1. A. Rings and spots in the leaf of a cv. Betinka apricot infected with PPV-M (type). B. Mild diffuse rings and spots occasionally present in the fruits of a cv. Betinka apricot inoculated with PPV-M (type). C. Healthy looking suckers of myrobalan under a cv. Harlayne apricot inoculated with PPV-Rec (PPV-W). D. Fruits of a cv. Harlayne apricot harvested in 2007 from a tree inoculated with PPV-M (Catherina). RT-PCR reaction was positive but no PPV symptoms are shown.

shown no symptoms, also on the stones. None of the five PPV strains was detected in the leaves by ELISA. Mild diffuse rings and spots appeared occasionally in the third year of evaluation (2004) and in the following years in the leaves of a single tree inoculated with PPV-M (type) (Fig. 1A). ELISA and RT-PCR confirmed the presence of PPV. Light spots also appeared in the fruits (Fig. 1B) and their stones. The infected tree started to decline during summer 2007. One tree inoculated with PPV-D (*P. insititia*) was found PPV-positive by RT-PCR in 2006 and 2007. However no symptoms were seen in

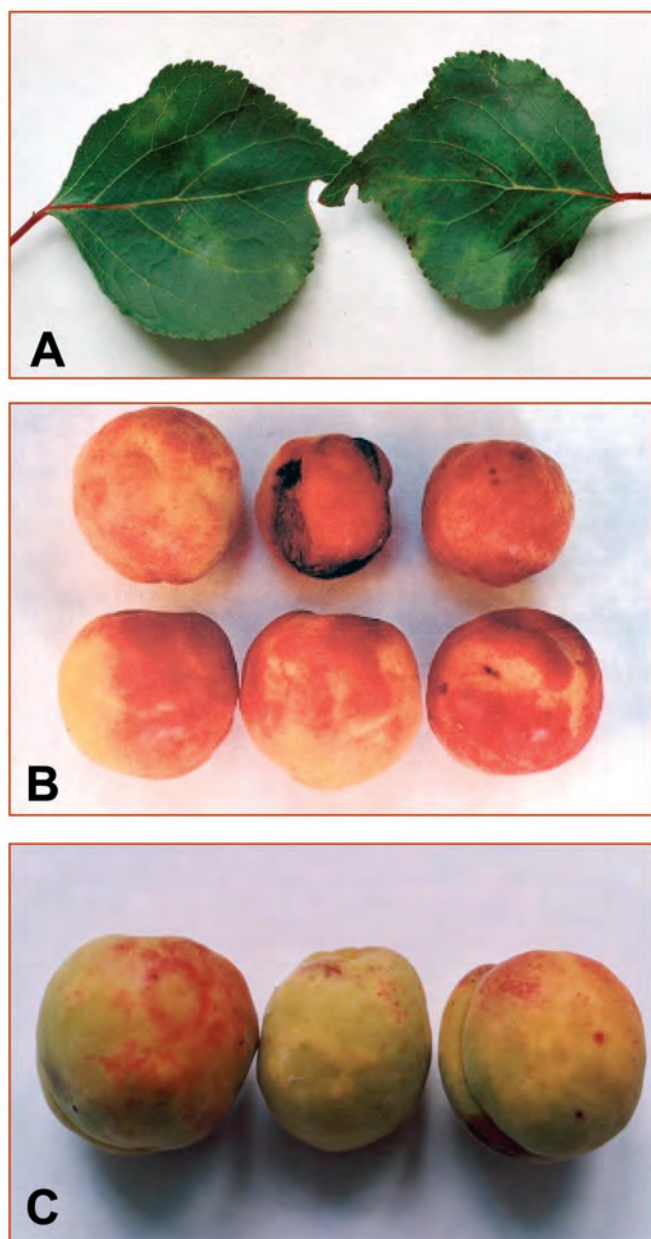


Fig. 2. A. Rings and diffuse spots in the leaves of a cv Karola apricot infected with PPV. B. Malformations and spots in the fruits of a cv. Karola apricot of infected with PPV. C. Rings, spots and mild malformation in the fruits of a cv. Velkopavlovická apricot infected with PPV-M (Catherina), harvested in 2007.

the leaves and fruits during the period 2002-2007, thus the presence of PPV in this tree is still under scrutiny.

Also apricot cv. Harlayne was confirmed as highly resistant to PPV. It did not show symptoms in the leaves during the six year trial after inoculation with PPV-M (type), PPV-D (type), PPV-Rec (*P. insititia*), PPV-M (peach), PPV-D (*P. insititia*), and PPV-Rec (PPV-W). The first fruits appeared in 2004 and remained symptomless for the whole duration of the trial. None of the six different viral strains and isolates was detected in the leaves of inoculated trees by ELISA. Although a positive response was obtained by RT-PCR in 2006 from one apricot tree inoculated with PPV-Rec (PPV-W), the reaction was weak and PPV presence was not confirmed. Suckers of the rootstock *Prunus cerasifera* developed in 2005-2007 around cv. Harlayne trees but they were symptomless (Fig. 1C) and ELISA-negative. PPV was detected by RT-PCR in 2007 in one Harlayne tree inoculated with PPV-M (peach), but here again no symptoms developed in the leaves or fruits (Fig. 1D) during the period 2002-2007. The presence of PPV in this tree is under investigation.

In the PPV-susceptible cvs Karola and Velkopavlovická used as controls, symptoms appeared in the leaves of some inoculated trees in 2002, in most trees in 2003, and in all trees in 2004. Symptoms (Fig. 2A) were the same or almost so, regardless of the infecting PPV strains. ELISA and RT-PCR confirmed the presence of the virus in symptomatic leaves. Severe symptoms, were also shown by fruits, especially those of cv. Velkopavlovická (Fig. 2C) and cv. Karola (Fig. 2B).

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