

To the distribution of PPV-M strain in selected orchards of apricots and peaches in the Czech Republic

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ABSTRACT: The presence and distribution of PPV-M strain of *Plum pox virus* were investigated in selected orchards of apricots and peaches in the Czech Republic. PPV-M was found to be distributed in apricot and peach orchards planted with nursery material imported from abroad. The presence of PPV-M was not proved in orchards planted with trees from local nurseries. The absence of PPV-M in a majority of spontaneous PPV hosts was proved previously. PPV-M strain appears to have been introduced recently.

Keywords: apricot; peach; orchards; *Plum pox virus*; PPV-M strain; distribution; ELISA; polyclonal and monoclonal antibodies

Symptoms of sharka disease were first observed on plums in Chrudim district, Central Bohemia, in former Czechoslovakia in the forties of the last century. *Plum pox virus* (PPV) was first detected in the Bohemian part of Czechoslovakia in 1952 (SMOLÁK, NOVÁK 1956). An investigation into the diversity and distribution of natural sources of PPV in the Czech Republic began in 1995. Partial results were published by POLÁK (1997) and POLÁK and PÍVALOVÁ (2001) for the years 1999–2001. The results of distribution of PPV in naturally growing plum trees, myrobalans and blackthorns were presented completely in 2002 (POLÁK 2002).

A small percentage of isolates of the PPV-M serotype in the Czech Republic was found by NAVRÁTIL et al. (1997). They identified it from different, not in greater detail characterised hosts, genetic resources, and experimental plants of institutions. The occurrence of PPV-M strain was investigated in our laboratory from 1999 to 2001 at various localities in naturally growing plums, myrobalans and blackthorns. 78 plum and damson trees, 32 myrobalans, and 8 blackthorns were investigated. By means of Mabs, PPV-M was detected only in two plum trees and one damson tree (POLÁK 2002). Furthermore, the presence of PPV-M strain was investigated in selected peach and apricot orchards.

MATERIALS AND METHODS

Plant material

The incidence and distribution of PPV-M strain were investigated in two selected intensive orchards of apricots and peaches in Southern Moravia and Central Bohemia. Large orchards (more than 20 hectares) were fifteen to twenty years old. The first orchard of apricots, Velké Pavlovice locality, was planted with trees from Kyjov nursery (Southern Moravia). In the second

apricot orchard, Hrušky locality in Southern Moravia, prevalently trees of cv. Vegama imported from Slovakia were planted. The first orchard of peaches, Pasohlávky locality, Southern Moravia, was partly planted with nursery material imported from Hungary. The second peach orchard, a locality near Slaný, Central Bohemia, was planted with trees from nurseries in the environs of Slaný. In both selected apricot orchards, and in the peach orchard in Pasohlávky locality PPV is widely distributed and about 50% of trees or more are infected. In the second peach orchard (Slaný) PPV is only randomly distributed, less than 10% of trees are infected.

Visual evaluation, sampling and testing of individual trees for the presence of PPV and PPV-M strain

Selected orchards of apricots and peaches were visually evaluated for the presence of PPV symptoms in leaves during spring and summer. In every orchard twenty trees showing distinct PPV symptoms in leaves were randomly selected for immunoenzymatic testing for the presence of PPV. Leaves with PPV symptoms were sampled from four different branches of the tree for the detection of PPV by polyclonal and monoclonal antibodies in the years 2000 and 2002.

Serological testing by ELISA

PPV polyclonal antibodies were used in DAS-ELISA (CLARK, ADAMS 1977) for detection of PPV presence in trees of apricots and peaches from selected orchards. Samples for ELISA were prepared by grinding 0.2 g of leaf tissue in phosphate buffered saline, pH 7.4, with 2% polyvinylpyrrolidone and 0.2% of egg albumin at the ratio 1:20. Indirect DAS-ELISA for the detection of PPV-M strain with PPV-M specific Mabs was per-

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Table 1. The presence of PPV-M strain in representative selected orchards of apricots and peaches in the Czech Republic

Species, cultivar	Locality	Number of tested trees	PPV positive trees in ELISA	
			polyclonal PPV antibodies	monoclonal PPV-M antibodies
Apricot, Vegama	Hrušky	20	20	20
Velkopavlovická	Velké Pavlovice	20	20	0
Peach, Redhaven	Pasohlávky	10	10	3
Sunhaven	Pasohlávky	10	10	6
Sunhaven	Vítov (Slaný)	20	17	0

formed by the procedure of Agritest, Italy. PPV-D Mabs obtained from Agritest, Italy, were also used, but we obtained only few positive results in samples with negative reaction with PPV-M specific Mabs. Czech isolate PPV-Vegama, described by KOMÍNEK et al. (1998) as PPV-M serotype, and PPV-M strain provided by INRA Bordeaux, France, were used as positive controls. The PPV-W isolate characterised as PPV-D serotype and PPV-D strain provided by INRA Bordeaux were used as negative controls for the characterisation of PPV isolates from selected orchards of apricots and peaches. Two wells for one leaf sample of apricot or peach, positive PPV-M controls, negative PPV-D controls, and negative virus-free controls (PPV-free sample of apricot or peach leaves), and buffer control per ELISA microplate were used.

Microplates were rated using an MR 5000 (Dynatech) reader at 405 nm. The reading of A_{405} was performed after 1-hour 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.

RESULTS AND DISCUSSION

The results of distribution of PPV-M strain in representative selected orchards of apricots and peaches are summarised in Table 1. PPV-M strain was found to be widely distributed in the apricot orchard in Hrušky locality, where trees for planting were imported from abroad. On the other hand, it was not detected in the orchard in Velké Pavlovice locality where the local planting material originating from Kyjov nursery was used. The distance between both orchards is not more than twenty kilometres. PPV-M was also often detected in the peach orchard in Pasohlávky locality. Most trees in this locality are infected with PPV. In a part of them PPV-M strain was proved. The peach orchard in Pasohlávky locality was partly planted with nursery material imported from abroad. The presence of PPV-M strain was not proved in the peach orchard near the town of Slaný, Central Bohemia. This orchard was planted with trees from local nurseries, and PPV is only randomly present there.

We tried to detect PPV-D in trees where PPV was proved with polyclonal antibodies, but PPV-M was not detected. We obtained only few positive results with PPV-D Mabs (Agritest, Italy) originating from Spain.

NAVRÁTIL et al. (1997) were able to detect mainly PPV-D strain while testing different hosts and genetic resources in the Czech Republic. They used three or four different PPV-D Mabs. These results indicate that until then probably no PPV-D specific Mabs detecting the whole spectrum of PPV-D serotype were produced. For the screening of PPV-D strain in cultural and natural hosts it is necessary to develop the whole spectrum of monoclonal antibodies. Until now we were able to detect PPV-M strain only in very few natural hosts of PPV (POLÁK 2002). The absence of PPV-M in spontaneous PPV hosts and in selected orchards of apricots and peaches planted with local nursery material fits to our hypothesis that the PPV-D strain originated in the Czech Republic and PPV-M strain appears to have been introduced recently.

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K rozšíření kmenu PPV-M ve vybraných sadech meruněk a broskvoní v České republice

ABSTRAKT: Ve vybraných sadech meruněk a broskvoní v České republice byla zjišťována přítomnost a rozšíření kmenu PPV-M viru šarky švestky. Bylo zjištěno, že PPV-M je rozšířen v sadech meruňky a broskvoně vysazených školkařským materiálem importovaným ze zahraničí. Přítomnost PPV-M nebyla prokázána v sadech osázených stromy z místních ovocných školek. Již dříve byla prokázána absence kmenu PPV-M ve většině spontánních hostitelů viru šarky švestky. Ukazuje se, že kmen PPV-M byl introdukován nedávno.

Klíčová slova: meruňka; broskvoň; sady; virus šarky švestky; kmen PPV-M; rozšíření; ELISA; polyklonální a monoklonální protilátky

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