Distribution of *Plum pox virus* in the Czech Republic

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Abstract

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Plum pox virus (PPV) is widely distributed in plums and myrobalans in western, central and eastern Bohemia, in north-western, central and north-eastern Moravia of the Czech Republic. In southern Bohemia and partly also in southern Moravia there is only a low and sporadic incidence. Naturally growing plums and myrobalans, and plums growing along roads were found to be the main sources and reservoirs of PPV infection. This high incidence in naturally growing plum and myrobalan trees makes it impossible to grow plum cultivars that are susceptible to PPV; only resistant cultivars can be grown in this country. In blackthorns the occurrence of PPV is limited to the regions with high and long-term presence of the virus. Therefore, we can conclude that blackthorn is not the primary, but a secondary source of PPV. On the other hand, sweet and sour cherries at localities of central and western Bohemia, and of southern Moravia are PPV-free. Till now the presence of strain PPV-C was not proved in the Czech Republic. Strain PPV-M was proved only in two plum and one damson trees. It was also found in one apricot and one peach orchard planted with imported nursery material. Strain PPV-M appears to have been introduced recently and is absent from or has a very low incidence in spontaneous PPV hosts, while the widespread and long-term dissemination of strain PPV-D may indicate that it originated in the Czech Republic.

Keywords: *plum pox virus*; distribution; natural sources; PPV strains

Sharka disease appeared in Bulgaria in the widely grown plum variety Kjustendil in 1917. The disease has spread from the Balkans to central and western Europe. It is caused by *Plum pox virus* – PPV, the economically most important member of the genus *Potyvirus*. Sharka symptoms were first observed on plums in the district Chrudim (Central Bohemia) in the 40ies. *Plum pox virus* was first detected in the Bohemian part of Czechoslovakia in 1952 (SMOLÁK & NOVÁK 1956). The disease reached Spain and Portugal in 1984. In the last decade of the 20th century it was reported in Chile, India and Azores, in 1999 in the U.S.A. (Pennsylvania) and in 2000 in Canada (Ontario, Nova Scotia). The major sources of PPV are diseased *Prunus* species from which the virus spreads by grafting or by aphid vectors. Wild *Prunus* species or other host plants can be considered as potential reservoirs of the virus. Four distinct serotypes of PPV strains have been identified: PPV-D, PPV-M, PPV-C and PPV-El Amar. PPV-El Amar infects mainly apricots and was detected only in Egypt. PPV-C infects sweet and sour cherries, and was detected only in Italy, Moldova, Bulgaria and Hungary. PPV-D and PPV-M strains are prevalent, infecting plums, apricots and peaches in most countries where PPV is distributed. PPV-M strains originated from Greece where they infect peaches (KERLAN & DUNEZ 1979) and are more pathogenic, especially for peaches and apricots. Hence rises the impression of European virologists that PPV-M strains are prevalent in Eastern and Central Europe.

For the gradual elimination of PPV it is necessary to know the epidemiology of the disease, particularly the distribution of natural sources of virus infection and the occurrence of PPV strains in spontaneous hosts. PPV and its strains can by transmitted nonpersistently by different species of aphids colonizing fruit-trees.

An investigation into the diversity and distribution of natural sources of PPV in the Czech Republic began in 1996. Partial results were published by POLÁK (1997), and by POLÁK and PÍVALOVÁ (2001) for the years 1999 to 2001. PPV isolates from spontaneous hosts were analysed for the serotypes they belonged to. Some intensive apricot and peach orchards were also tested for the presence of PPV strains. The objective of this work is to study the present status of PPV occurrence in the Czech Re-

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public. In addition, the monitoring of diversity and distribution of natural sources of PPV throughout the country continues.

MATERIAL AND METHODS

Plant material

Naturally growing plum trees, myrobalan and blackthorn were evaluated for the presence of PPV infection in different regions and localities of the Czech Republic. Naturally growing plants were trees or shrubs that originated from seeds or suckers, usually in uncultivated stands (Fig. 1). Old plum trees growing along roads were also tested.

Naturally growing sweet cherry and sour cherry trees as well as trees growing in old uncultivated orchards in areas of central and western Bohemia and southern Moravia with a high incidence of sharka on plums were tested for the presence of PPV. Especially trees showing diffuse spots or rings in leaves were tested.

The occurrence of strain PPV-M was investigated from 1999 to 2001 at various localities in naturally growing plums, myrobalans and blackthorns. The presence of this strain was also tested in two older intensive peach and apricot orchards.

Visual evaluation, sampling and testing of PPV isolates

Visual evaluation and description of PPV symptoms in leaves of plums, myrobalans (Fig 2), blackthorns and cherries were carried out in May and June of the years 1996 to 2001. Flowers and or leaves from investigated trees were sampled from at least four different branches of the tree for the detection of PPV by DAS-ELISA. PPV isolates obtained from plums, myrobalans and blackthorns in 1999–2001 were tested with monoclonal antibodies specifically recognizing strain PPV-M (Agritest, Italy). Trees for evaluation were chosen randomly without consideration of the presence of PPV symptoms.

Serological testing by ELISA

PPV polyclonal antibodies were used in DAS-ELISA (CLARK & ADAMS 1977) for the detection of PPV in natural hosts, plums, myrobalan, blackthorn or the suspected potential natural hosts sweet and sour cherry. Samples for ELISA were prepared by grinding 0.2 g of leaf tissue or flowers in phosphate buffered saline, pH 7.4, with 2% polyvinylpyrrolidone and 0.2% of egg albumin in the ratio 1:20. Indirect DAS-ELISA for the detection of PPV-M isolates with PPV-M specific Mabs was performed by the procedure of Agritest, Italy. Czech isolates PPV-Vegama and PPV-Slivoň, characterized by KOMÍNEK *et al*. (1998) and PONCAROVÁ and KOMÍNEK (1998) as PPV-M serotypes, and the PPV-M strain provided by INRA Bordeaux, France, were used as positive controls. The PPV-W isolate characterized as PPV-D serotype and the PPV-D strain provided by INRA Bordeaux, France, were used as negative controls in the characterization of PPV isolates from natural sources of infection. Two wells for each PPV isolate, the positive PPV-M controls (PPV-M, PPV-Vegama), negative PPV-D controls (PPV-D, PPV-W), negative virus free controls (PPV-free blackthorns and *Nicotiana benthamiana*) and buffer control per ELISA microplate were used.

Microplates were rated using a MR 5000 (Dynatech) reader at 405 nm. The reading of A_{405} was performed

Fig. 1. Group of plums naturally growing from runners, infected with PPV (Central Bohemia, locality Černý Vůl)

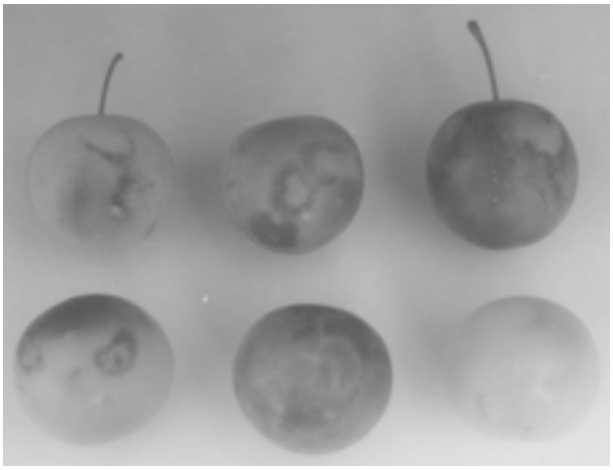

Fig. 2. Fruits of an infected myrobalan with rings and spots as symptoms of PPV

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

Wild growing plums that had multiplied by runners, or plums growing along roads, and wild growing myrobalans multiplied by runners and seed dissemination were found to be the main and primary natural sources of PPV infection in the Czech Republic. Out of 343 plum trees tested by ELISA, 174 were found to be PPV infected (Fig. 3). Virus infection is characterized by distinct symptoms on leaves and fruits. PPV infection of plums and myrobalans was found mainly in central, western and eastern Bohemia, in north-western, central and eastern Moravia. It is surprising that plums are infected even in upland and mountain areas (Jeseníky, Beskydy) of Moravia. In myrobalan, PPV was detected in 140 out of 280 trees tested by ELISA (Fig. 4). A high variability of intensity of symptoms in leaves and fruits was observed. There were medium severe PPV symptoms on myrobalan fruits, diffuse spots and rings without deformations. The area with this spontaneous source of infection corresponds with the occurrence of PPV in plums, even though myrobalans are not frequent in the open countryside.

Altogether 280 blackthorns were investigated for PPV-infection by means of ELISA. The virus was detected in 41 bushes (Fig. 5). PPV symptoms in form of diffuse spots and rings on leaves could usually be observed. They were mainly mild, but sometimes it was possible to find severe mosaic spots and rings on leaves of infected shrubs. No distinct symptoms or mild discolorations were observed on fruits of blackthorns infected with PPV. Infected blackthorns were found mainly in the lowlands of central, western and eastern Bohemia, central Moravia (areas of western, northern and eastern Moravia have not yet been investigated), and in areas with severe incidence of PPV in plums and myrobalans. Blackthorns growing in forests, in marginal areas with low incidence of PPV in plums and myrobalans were proved to be PPV-free.

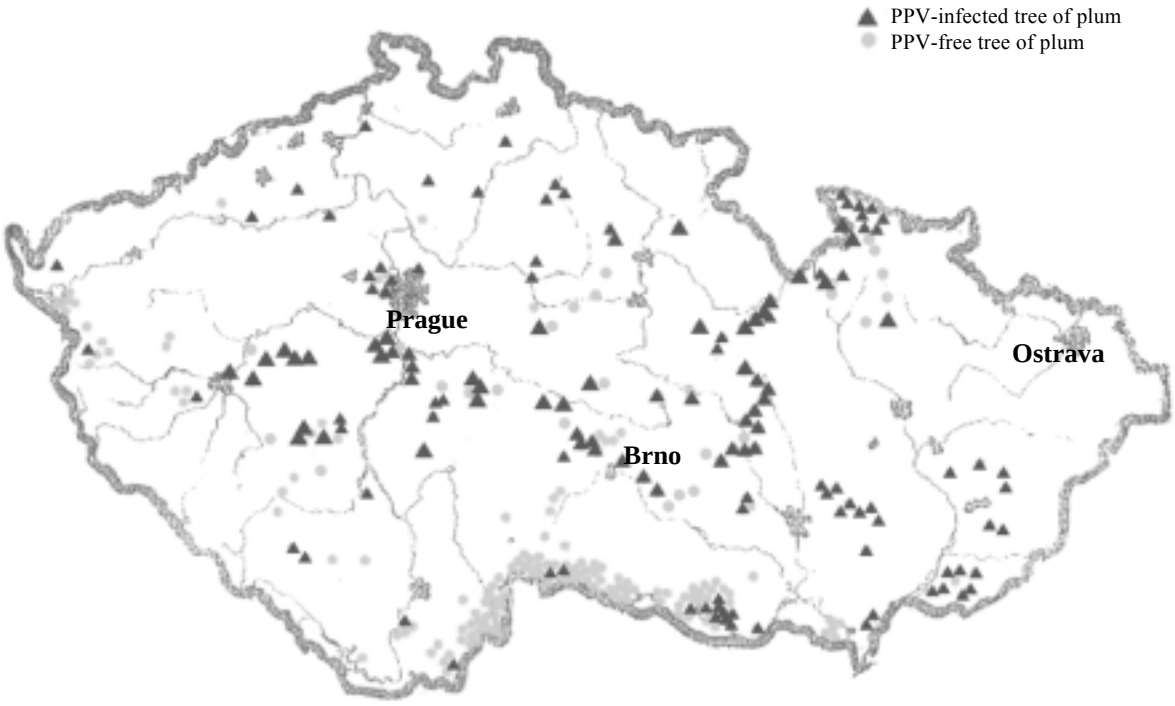

Fig. 3. PPV detection in trees of plum achieved in the years 1996–2001

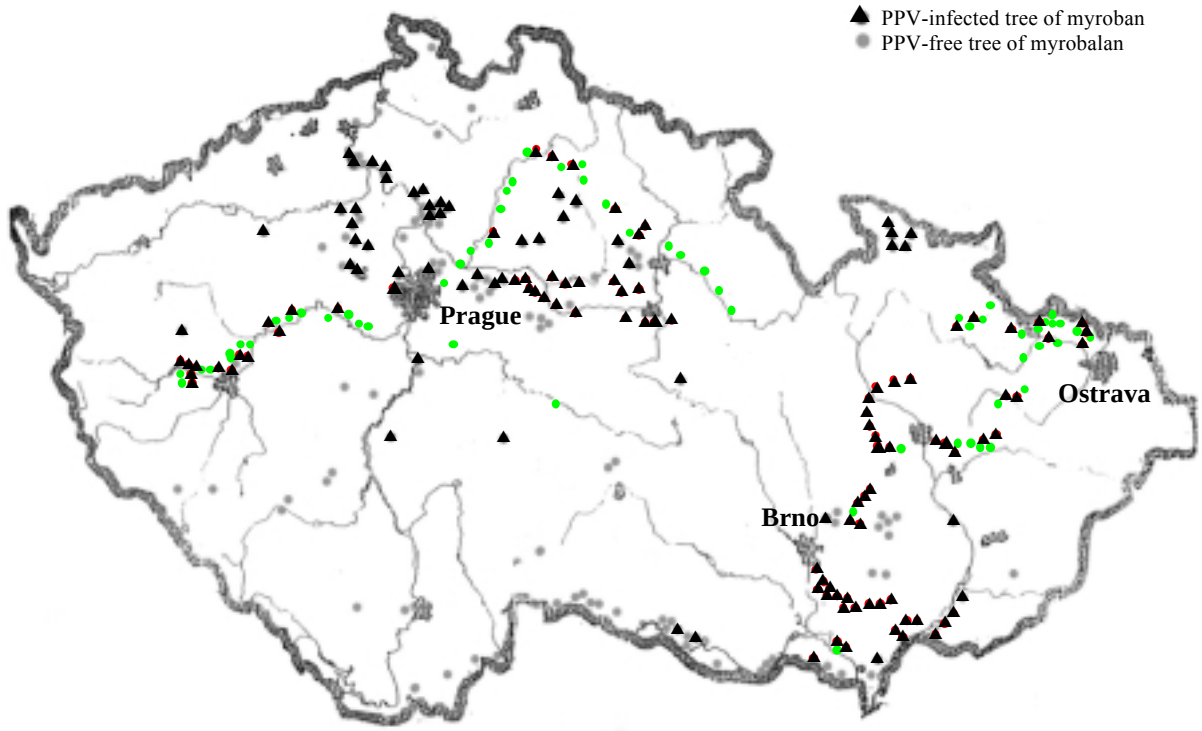

Fig. 4. PPV detection in trees of myrobalan achieved in the years 1996–2002

Sweet and sour cherries investigated for the presence of PPV in localities of central and western Bohemia, and southern Moravia were PPV-free. Until now only trees showing diffuse spots and rings in leaves were investigated by ELISA, these were 45 sweet cherry and 12 sour cherry trees. Trees showing mosaic symptoms in leaves

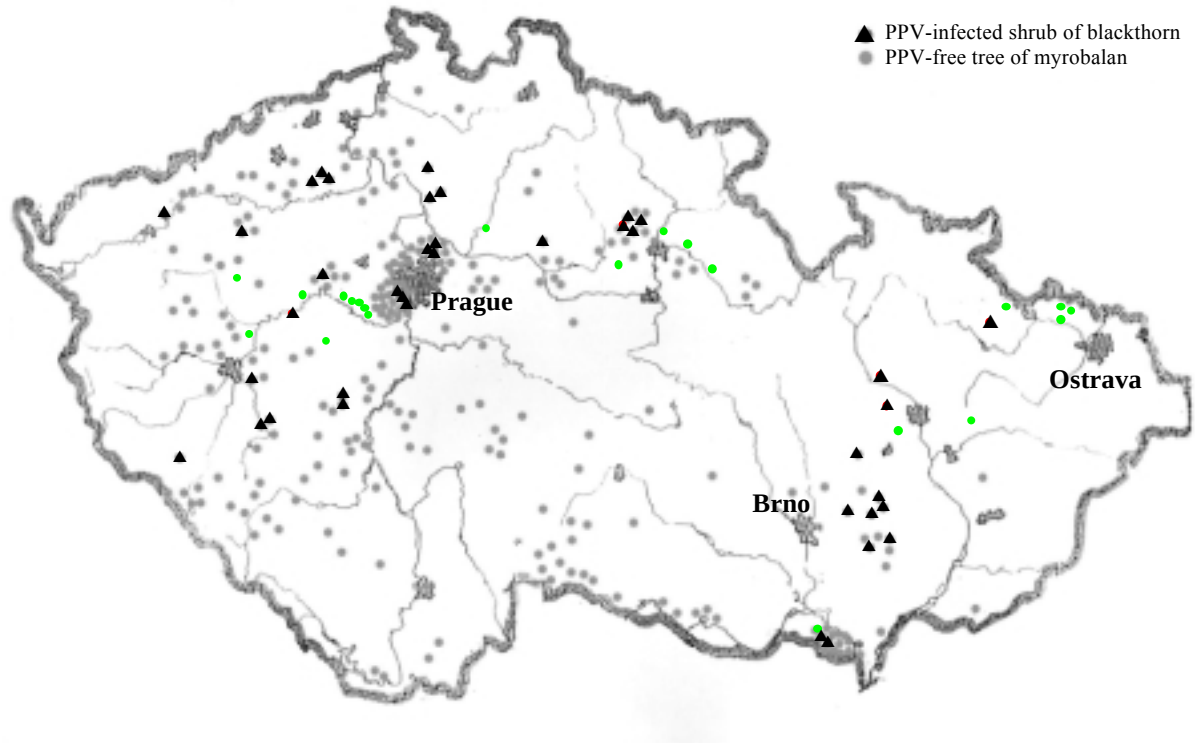

Fig. 5. PPV detection in shrubs of blackthorn achieved in the years 1996–2001

were usually infected with *Prunus necrotic ringspot virus* (PNRSV).

Occurrence of strain PPV-M was investigated from 1999 to 2001 at various localities in 78 plum and damson trees, 32 myrobalans and in 8 blackthorns. PPV-M was detected only in two plum trees and one damson tree. Till now PPV-M was not detected in myrobalans and blackthorns. Strain PPV-M was found to be distributed in one older intensive peach orchard in southern Moravia; it was proved with monoclonal antibodies by indirect ELISA. The nursery material to plant this orchard had been imported from abroad. PPV-M was also proved in two peach trees of the variety Lesiberian in the orchard of the Faculty of Horticulture, Lednice, southern Moravia. Strain PPV-M was not found in one intensive peach orchard in central Bohemia (near Slaný). This strain was detected in an apricot orchard at Hrušky, southern Moravia. Again, the apricots for planting had been imported from abroad. On the other hand, strain PPV-M was not detected in an apricot orchard at Velké Pavlovice, southern Moravia. The trees planted at this locality had been grown in a Moravian nursery. Part of the trees in this orchard had become infected with PPV-D 20 years ago.

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Souhrn

POLÁK J. (2002): **Rozšíření viru šarky švestky v České republice.** Plant Protect. Sci., **38**: 98–102.

Virus šarky švestky, *Plum pox virus* (PPV), je široce rozšířen na švestkách a myrobalánech v západních, středních a východních Čechách a na severozápadní, střední, severovýchodní a jihozápadní Moravě. Nízký a sporadický výskyt PPV je pouze v jižních Čechách a částečně také na jižní Moravě. Jako hlavní zdroje a rezervoáry infekce PPV v České republice byly zjištěny přirozeně rostoucí švestky a myrobalány a švestky rostoucí podél silnic. Vysoký výskyt PPV v přirozeně rostoucích stromech švestky a myrobalánu znemožňuje v ČR pěstování odrůd švestky náchylných k PPV. Jediná možnost je pěstovat odrůdy rezistentní k PPV. Výskyt PPV na trnkách je omezen na oblasti se silným a dlouhodobým rozšířením viru. Proto docházíme k závěru, že trnka není primárním, ale pouze sekundárním zdrojem PPV. Třešně a višně na lokalitách středních a západních Čech a na jižní Moravě jsou šarky prosté. Přítomnost kmenu PPV-C nebyla dosud na území ČR prokázána. Přítomnost kmenu PPV-M byla prokázána ve dvou stromech švestky a v jednom stromu slívy. Kromě toho byla přítomnost PPV-M prokázána v jednom sadu meruněk a v jednom sadu broskvoní vysazených z importovaného školkařského materiálu. Absence nebo velmi nízký výskyt kmenu PPV-M ve spontánních hostitelích PPV indikuje původ a dlouhodobé rozšíření kmenu PPV-D na území ČR, zatímco PPV-M byl introdukován nedávno.

Klíčová slova: virus šarky švestky; rozšíření; přírodní zdroje; kmeny PPV

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