Distribution of *Plum Pox Virus* Strains in Natural Sources in the Czech Republic

Jaroslav POLÁK and Petr KOMÍNEK

Department of Virology, Division of Plant Health, Crop Research Institute, Prague-Ruzyně, Czech Republic

Abstract

Polák J., Komínek P. (2009): **Distribution of *Plum pox virus* strains in natural sources in the Czech Republic**. Plant Protect. Sci., **45**: 144–147.

The distribution of *Plum pox virus* (PPV) strains, PPV-D, PPV-M and PPV-Rec, was investigated in the Czech Republic in 2005–2008. Fifty-two to ninety-four samples of flowers or leaves of plum, myrobalan and blackthorn trees from different regions were tested in individual years. The presence of PPV was detected by DAS-ELISA with serotype-specific polyclonal antibodies. PPV-M was proved by DASI-ELISA with serotype-specific monoclonal antibodies; PPV-D, PPV-M and PPV-Rec were detected by RT-PCR in leaf samples from PPV infected trees. The presence of PPV-D ranged from 94.6% to 100%, the presence of PPV-M from 0.0% to 2.3% and the presence of PPV-Rec from 0.0% to 3.1% during 2005–2008. More than 95% of analysed samples of PPV were infected with PPV-D and less than 2.5% of analysed samples of PPV were infected with PPV-M or PPV-Rec. The presence of PPV-C was not proved in sweet cherry and sour cherry trees. The presence of PPV-EA was not proved in apricot trees.

Keywords: *Plum pox virus*; blackthorn; myrobalan; plum; sweet cherry; sour cherry; apricot; PPV-D; PPV-M; PPV-Rec; PPV-C; PPV-EA

Plum pox virus was first detected in plum trees in the Bohemian part of the Czechoslovakia in 1952 (Smolák & Novák 1956). Sharka symptoms were first observed on plums in Central Bohemia already in 1925 (Smolák 1926). Investigations into the diversity and distribution of natural sources of PPV in the Czech Republic began in 1996. "Natural sources" are taken to mean wild trees and shrubs of plum, myrobalan, blackthorn, sweet (*Prunus avium* L.) and sour cherries, and very old *Prunus* trees growing along roads outside the intensive orchards. These PPV infected *Prunus* trees are sources of infection both for intensive orchards and private gardens. Partial results were published by Polák (1997, 2002, 2007), Polák and Pívalová (2001). PPV-Marcus (PPV-M) was typed in natural sources of plum, myrobalan and blackthorn from 1999 to 2004. PPV-M was detected in 5.88% of the investigated plum trees, 7.41% of myrobalan trees and 4.0% of blackthorn shrubs (Polák & Pívalová 2005).

The distribution of PPV-Dideron (PPV-D), PPV-M and PPV-Recombinant (PPV-Rec) strains was investigated in the Czech Republic in 2005–2008. The presence of PPV-Cherry (PPV-C) in sweet and sour cherries (PPV-C is adapted only to cherries) and PPV-El Amar (PPV-EA) in apricots (PPV-EA is adapted only to apricot) was also checked. The aim of our study is to determine the distribution of PPV strains present in natural conditions of the Czech Republic.

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE 0002700604.

MATERIAL AND METHODS

Sampling. Naturally growing trees of plum and myrobalan (Figure 1), shrubs of blackthorn (Figure 2), naturally growing cherry trees, and sweet and sour cherry trees growing in old orchards (PPV-C could probably be present in such orchards) in different regions and localities of Bohemia and Moravia were tested. Apricot trees in selected orchards in Southern Moravia where PPV-D and PPV-M are present were tested for the presence of PPV-EA. Trees were selected randomly at the given locality. Samples of leaves or flowers were collected in the period from April to August of 2005 to 2008. Samples were taken randomly at four places of the tree canopy periphery, without symptom observation.

PPV detection, PPV strain detection and typing using ELISA. A mixture of four partial samples was used for PPV detection by polyclonal specific antibodies (Bioreba, Switzerland) in DAS-ELISA (Clark & Adams 1977). Only the samples where the presence of PPV was detected by polyclonal specific antibodies were taken for PPV strain detection. PPV-M specific Mabs of Agritest, Italy were applied in indirect DASI-ELISA for the detection of both PPV-M and PPV-Rec strains in samples with positive reaction to PPV polyclonal antibodies. Cherry trees were tested both by polyclonal and PPV-C specific (Agritest, Italy) monoclonal antibodies. The same procedure and PPV-EA specific (Agritest, Italy) monoclonal antibodies were used for the detection of PPV-EA in apricot trees.

Samples of 0.2 g of leaves or flowers were homogenised in PBS buffer, pH 7.4 with 2% of polyvinylpyrrolidone, and 0.2% of egg albumin at a ratio 1:20. Average sample was prepared from partial samples and 0.2 g of leaves or flowers. The procedure recommended by the producer of antibodies was used for the detection of PPV and PPV strains. Results of ELISA were evaluated with an MR 5000 reader (Dynatech) at 405 nm. Samples with $A_{405} > 0.10$ were considered as positive, samples with $A_{405} < 0.03$ as negative.

PPV strain detection and typing using RT-PCR. The same sample used for ELISA was also employed for PPV strain detection by RT-PCR. Average sample was prepared from partial samples and 0.1 g of leaves or flowers. The isolation of RNA from leaves or flowers was done using the RNeasy Plant Mini Kit (Qiagen). Reverse transcription was performed for 50 min. at 42°C using Superscript II enzyme (Invitrogen) with oligo dT primer. In each sample, three subsequent PCR reactions were conducted, separately for PPV-D, PPV-M and PPV-Rec strains. Primers and reaction conditions were used according to Šubr *et al.* (2004). Taq polymerase (Promega) was used for PCR reactions, which were run in a MJ Research PTC200 thermo cycler (GMI Inc., Ramsey, USA). Products of PCR were separated by electrophoresis on a 1.5% agarose gel and visualised by UV light.

RESULTS AND DISCUSSION

Two hundred one plum trees growing in different regions of the Czech Republic were investigated for the presence of PPV, of them 134 trees were found to be infected. The presence of PPV was proved in 66.7% of the investigated plum trees. In these trees PPV strains were typed. PPV-M strain was detected in plum trees by DASI-ELISA: the presence of PPV-M was confirmed by RT-PCR with

Figure 1. Older trees of myrobalan, naturally growing close to a local road

Figure 2. Flowering blackthorn shrubs near a forest

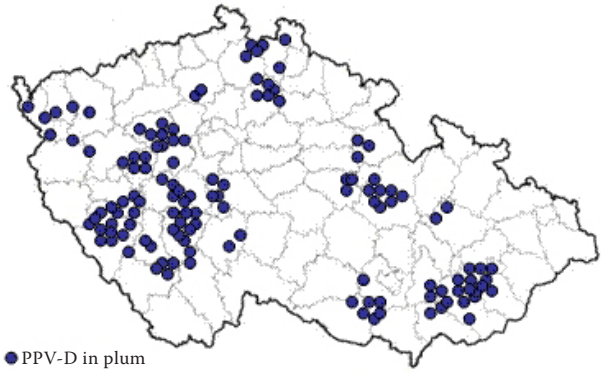

Figure 3. The presence of PPV-D in plum trees tested in the Czech Republic in 2005–2008

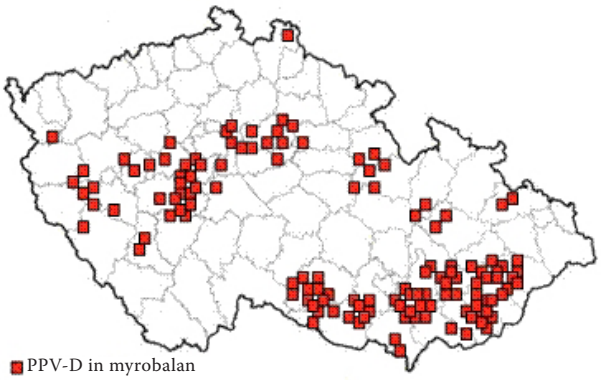

Figure 4. The presence of PPV-D in myrobalan trees tested in the Czech Republic in 2005–2008

strain specific primers in 2 plum trees (1.5%) while the PPV-Rec strain was not proved by RT-PCR with strain specific primers in any plum tree. PPV-D strain was proved in 98.5% of plum trees by RT-PCR with strain specific primers (Figure 3).

Three hundred sixty nine myrobalan trees were investigated for the presence of PPV, of them 130 trees were proved to be infected. The presence of PPV was detected in 35.2% of the investigated myrobalan trees. PPV-M strain was detected in 7 myrobalan trees by DASI-ELISA: the presence of PPV-M was confirmed by RT-PCR with strain specific primers in 3 myrobalan trees (2.3%), and PPV-Rec strain was proved by RT-PCR with strain specific primers in 4 myrobalan trees (3.1%). PPV-D strain was proved in 94.6% of myrobalan trees by RT-PCR with strain specific primers (Figure 4).

One hundred six blackthorn shrubs were investigated for the presence of PPV, of them 11 trees were proved to be infected. The presence of PPV was proved in 10.4% of the investigated blackthorn shrubs. PPV-M strain was detected in none of the blackthorn shrubs by DASI-ELISA. PPV-D strain was proved in all the investigated blackthorn shrubs (100%) by RT-PCR with strain specific primers (Figure 5).

The distribution of both PPV-M and PPV-Rec strains in plum and myrobalan trees is presented in Figure 6. PPV-M and PPV-Rec strains were not detected in blackthorns in the years 2005–2008. Sporadic presence of PPV-M and PPV-Rec strains was proved only in Southern Moravia (Figure 6).

The presence of PPV-C and PPV-EA strains was not proved in naturally growing trees and in selected orchards of cherries and apricots in the Czech Republic.

Results obtained in the years 2005–2008 are in agreement with our previously published results (Polák & Pívalová 2005) and with the fact that the PPV-D strain is strongly prevalent in natural sources of *Prunus* sp. in the Czech Republic, while the incidence of PPV-M is sporadic (Polák 2005) similarly like in Poland (Malinowski 2004) and

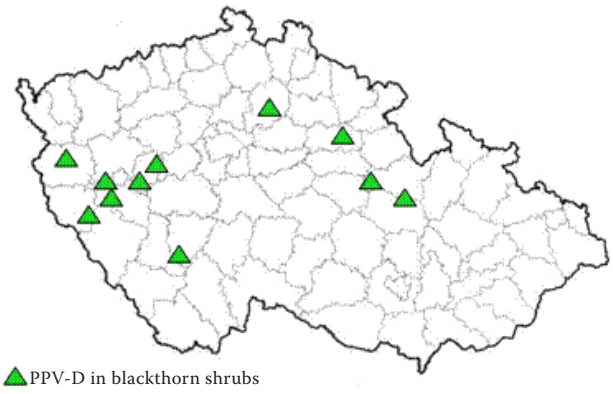

Figure 5. The presence of PPV-D in blackthorn shrubs tested in the Czech Republic in 2005–2008

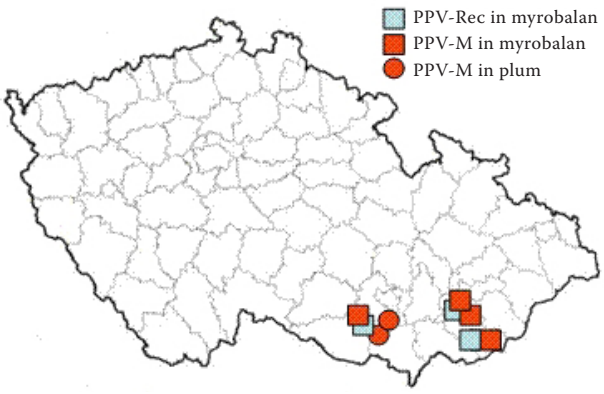

Figure 6. The presence of PPV-M and PPV-Rec in plum and myrobalan trees tested in the Czech Republic in 2005–2008

Austria (Laimer *et al*. 2003). The group of PPV-D strains strongly prevails in Central Europe.

No case of mixed infection has been detected, which is an important epidemiological result. We proved mixed infection for the first time during the investigation of samples in 2009 (unpublish).

No peach trees have been analysed because there are not any wild peach trees and natural sources of peach in the Czech Republic. Results of investigation on the distribution of PPV-M and PPV-D strains in selected orchards of apricots and peaches situated mainly in South Moravia were published several years ago (Polák 2004). Molecular variability of some Czech *Plum pox virus* isolates was studied by Navrátil *et al*. (1998), but in this paper it was not described how and where the isolates were sampled; it was only reported that some of them originated from our institute (RICP Prague), only one case of mixed infection was found, and PPV-D isolates prevailed. Recently, Gadiou *et al*. (2008) studied 24 Czech PPV isolates from five different plum orchards situated in Moravia. PPV-D isolates were identified in all orchards, PPV-Rec only in two of them. No natural sources of PPV were studied in the cited report. So the evaluation of natural sources of plum, myrobalan and blackthorn for the presence of PPV strains will continue to cover all regions and districts of the Czech Republic.

Acknowledgements. The authors are indebted to Mrs. Jitka Pívalová for perfect technical assistance and to Mrs. Miloslava Ducháčová for the preparation of figures.

References

Clark M.F., Adams A.N. (1977): Characteristic of the microplate method of enzyme linked immunosorbent assay for the detection of plant virus. Journal of General Virology, **34**: 51–57.

Gadiou S., Šafářová D., Navrátil M. (2008): Genetic variability of *Plum pox virus* isolates in the Czech Republic. European Journal of Plant Pathology, **121**: 513–517.

Leimer M., Mendonca D., Arthofer W., Hanzer V., Myrta A., Boscia D. (2003): Occurrence of different plum pox virus strains in several stone fruit species in Austria. Options Méditerranéennes, Serie B, **45**: 79–83.

Malinowski T. (2004): Serological characterisation of *Plum pox virus* (PPV) isolates found in Poland. In: European Meeting, 04 on *Plum pox*. Rogów-Skierniewice, Poland. Book of Abstracts: 51.

Navrátil M., Šimonová V., Fialová R., Válová P. (1998): Molecular variability of Czech *Plum pox virus* isolates. Acta Virologica, **42**: 254–256.

Polák J. (1997): On the epidemiology of plum pox virus in the Czech Republic. Ochrana rostlin, **33**: 81–88.

Polák J. (2002): Distribution of *Plum pox virus* in the Czech Republic. Plant Protection Science, **38**: 98–102.

Polák J. (2004): To the distribution of PPV-M strain in selected orchards of apricots and peaches in the Czech Republic. Horticultural Science (Prague), **31**: 44–46.

Polák J. (2007): Viruses of blackthorn and road-bordering trees of plum, myrobalan, sweet and sour cherries in the Czech Republic. Plant Protection Science, **43**: 1–4.

Polák J., Pívalová J. (2001): Towards diversity of natural sources of *Plum pox virus* and distribution of its strains in the Czech Republic. In: Proceeding 5th Congress of European Foundation for Plant Pathology, Taormina, Italy 2000: 25–28.

Polák J., Pívalová J. (2005): Sporadic distribution of *Plum pox virus* M strain in natural sources in the Czech Republic. Horticultural Science (Prague), **32**: 85–88.

Smolák J. (1926): Služba informační. Druhá výroční zpráva, stav pro choroby rostlin na Mělníce za rok 1925–1926: 90–95.

Smolák J., Novák J.B. (1956): Příspěvky k virologii ovocných plodin. Acta Universitatis Agriculturae, Praha, **1**: 101–118.

Šubr Z., Pittnerová S., Glasa M. (2004): A simplified RT-PCR-based detection of recombinant *Plum pox virus* isolates. Acta Virologica, **48**: 173–176.

Received for publication July 24, 2009
Accepted after corrections October 28, 2009

Corresponding author:

Doc. Ing. Jaroslav Polák, DrSc., Výzkumný ústav rostlinné výroby, v.v.i., odbor rostlinolékařství, oddělení virologie, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 315, fax: + 420 233 311 592, e-mail: polak@vurv.cz