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Sporadic distribution of *Plum pox virus* M strain in natural sources in the Czech Republic

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ABSTRACT: The presence and distribution of M strain of *Plum pox virus* (PPV-M) were investigated in natural hosts of Sharka, plums, myrobalans and blackthorns in the Czech Republic. Leaves or flowers of trees were evaluated for the presence of PPV by specific polyclonal antibodies at first. PPV infected samples were investigated for the presence of PPV-M by strain specific monoclonal antibodies. 102 PPV isolates from plum, 81 from myrobalan and 25 from blackthorn were typed. PPV-M was detected in six plum trees, six myrobalan trees and in one shrub of blackthorn. Sporadic incidence of PPV-M was proved in all investigated areas of the Czech Republic. Molecular and serological typing of different PPV strains in natural hosts, plum, apricot, and peach orchards was proposed to realize in Central Europe.

Keywords: *Plum pox virus*; PPV-M strain; detection; plum; myrobalan; blackthorn; monoclonal antibodies

Plum pox virus (PPV) is the causal agent of Sharka disease in stone fruit trees. Sharka was reported for the first time from Bulgaria in 1917. The disease has spread from Eastern Europe to many European and Mediterranean countries. In the Czech Republic, Sharka was detected for the first time in 1952 (Smolák, Novák 1956). The disease reached Spain and Portugal in 1984. In nineties it was reported from Chile, India and Azorean Islands. In 1999 PPV was proved in the USA, and in 2000 in Canada. Five distinct serotypes of PPV were identified, PPV-D, PPV-M, PPV-C, PPV-EA, and recently (Glasa et al. 2004) PPV-recombinant, PPV-DxM (PPV-Rec).

PPV-M strain was described by Kerlan and Dunez (1979) from Greece. PPV-M is more pathogenic especially to plums and peaches, and more epidemic for peaches and apricots. Hence the impression of plant virologists from Western Europe (especially France, Italy) arises that PPV-M strains are prevalent in Eastern and Central Europe. On the other hand, nobody has investigated the distribution of natural sources of PPV-M in the Eastern or Central part of Europe until now.

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), Polák and Pívalová (2001), and Polák (2002). The results of distribution of natural sources of PPV provided a good background for the typing of PPV strains in the Czech Republic. The first attempts to characterize PPV isolates in the Czech Republic were done by Komínek et al. (1998), Navrátil et al. (1998), Poncarová and Komínek (1998). Mainly PPV isolates obtained from abroad or isolated in the Czech Republic and maintained in research institutes were used. Most PPV isolates were classified as PPV-D and a limited number of them as PPV-M (Polák et al. 2003).

PPV strains were identified in natural hosts, plums, myrobalans and blackthorns by monoclonal antibodies from 1999. PPV-M monoclonal antibodies enabled to obtain reliable results whereas reactions with PPV-D monoclonal antibodies were weak and the results were not reliable. Therefore only PPV-M monoclonal antibodies have been used in studies since 2003. Results of the distribution of PPV-M in natural sources obtained in 1999–2004 are presented in this contribution.

MATERIALS AND METHODS

Plant material

Naturally growing plums (*Prunus domestica* L.), myrobalans (*Prunus cerasifera* Ehrh.) and blackthorns (*Prunus spinosa* L.) were evaluated for the

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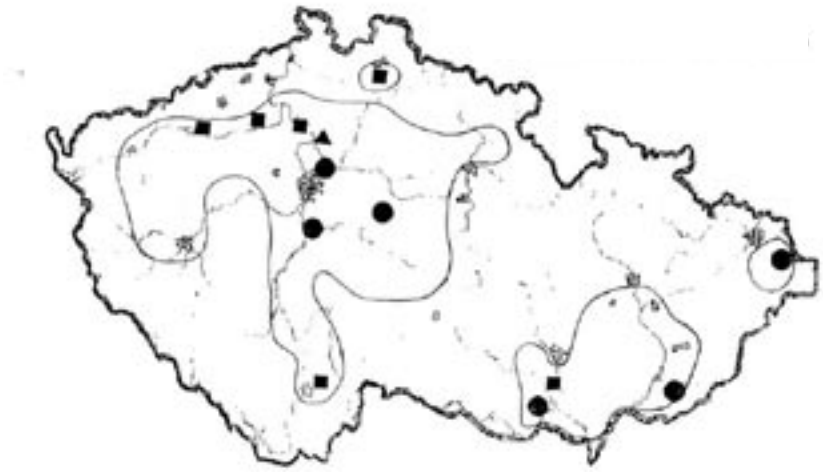

Fig. 1. Localization of proved incidence of PPV-M strain in natural sources, plum, myrobalan and blackthorn trees in the territory of the Czech Republic
● PPV-M infected tree of plum
■ PPV-M infected tree of myrobalan
▲ PPV-M infected shrub of blackthorn
⬭ areas of investigated natural hosts of PPV

presence of PPV-M strain infection in different regions and localities of Bohemian and Moravian parts of the Czech Republic (Fig. l). Collections included trees and shrubs, originating from seeds or root suckers, growing in uncultivated stands. Old plum trees planted along roads were also rated.

The occurrence of PPV-M strain was investigated from 1999 to 2004. Flowers from inspected trees and/or leaves showing PPV symptoms were sampled in spring, flower petals in the time of flowering, leaves in May and June.

Testing of PPV isolates by polyclonal and monoclonal antibodies and visual evaluation

Samples of leaves or flowers were employed for virus detection by DAS (double antibody sandwich)-ELISA. Commercial PPV specific polyclonal antibodies Bioreba, Switzerland, or antibodies prepared at the University Halle-Wittenberg, Germany, were used. Samples for DAS-ELISA (Clark, Adams 1977) were prepared by grinding 0.2 g of leaf tissue or flower petals in phosphate buffered saline, pH 7.4 with 2% polyvinylpyrrolidone and 0.2% of egg albumin at the ratio 1:20. Leaves of trees with positive reactions in DAS-ELISA (PPV polyclonal antibodies) were evaluated visually for the presence of PPV symptoms.

PPV samples in which the virus was proved by DAS-ELISA and PPV symptoms were present on the leaves were investigated for the presence of PPV-M strain. DASI (double antibody sandwich indirect)-ELISA (Cambra et al. 1994) for the detection of PPV-M was carried out with the identified PPV isolates from plums, myrobalans and blackthorns, tested against Mab AL (Agritest, Italy), recognized as specific to PPV-M (Boscia et al. 1997). Czech isolates PPV-Vegama and PPV-Slivoň, characterized by Komínek et al. (1998) and Poncarová and Komínek (1998) as PPV-M serotypes using RT-PCR, 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), PPV-D controls (PPV-D, PPV-W), negative virus-free controls (PPV-free plum or myrobalan, or blackthorn and *Nicotiana benthamiana*) and buffer control per ELISA microplate were used.

Microplates were rated using an MR 5000 (Dynatech) reader at 405 nm. The reading of OD_{405} was performed after one-hour incubation of the substrate at room temperature. Samples with $OD_{405} > 0.10$ were considered as positive, and samples with $OD_{405} < 0.03$ were rated as negative. No samples with OD_{405} 0.03–0.10 occurred.

RESULTS AND DISCUSSION

Hundred and two PPV isolates from the same number of plum trees were investigated by monoclonal antibodies for the presence of PPV-M strain. PPV-M was detected in six plum trees. PPV-M was present in 5.88% of investigated plum trees naturally infected with PPV.

Eighty-one PPV isolates from the same number of myrobalan trees were investigated by monoclonal antibodies for the presence of PPV-M strain. PPV-M was detected in six myrobalan trees. PPV-M was present in 7.41% of investigated myrobalan trees naturally infected with PPV.

Twenty-five PPV isolates from the same number of blackthorn shrubs were investigated by monoclonal

antibodies for the presence of PPV-M strain. PPV-M was detected only in one blackthorn shrub. PPV-M was present in 4.0% of investigated blackthorn shrubs naturally infected with PPV.

Areas where the presence of PPV was investigated and localities where PPV-M was proved in plums, myrobalans and blackthorns are presented in Fig. 1. Sporadic presence of PPV-M has been proved in Central, Northern and Southern Bohemia, Southern and North-Eastern Moravia so far. Some regions and areas of the Czech Republic were not investigated. PPV-M was proved in plum trees in the localities Pasohlávky and Uherský Brod (Southern Moravia), Třinec (North-Eastern Moravia), Kostelec nad Labem, Pikovice, Ždánice (Central Bohemia). PPV-M was detected in myrobalan trees in the localities Bratčice (Southern Moravia), Liberec (Northern Bohemia), Račice, Kusín, Hrušovany (North-Western Bohemia) and Líšov (Southern Bohemia). PPV-M was found in one shrub of blackthorn in Liběchov locality (Central Bohemia).

Ninety-six plum trees, seventy-five myrobalan trees, and twenty-four blackthorn shrubs were proved to be infected with PPV in the inspected areas of the Czech Republic, but the presence of PPV-M was not confirmed by PPV-M monoclonal antibodies. Investigated trees were probably infected with PPV-D. The presence of PPV-C and PPV-EA has not been proved in natural sources until now (Polák et al. 2003 and unpublished results from 2004).

Obtained results showed only sporadic incidence of PPV-M in natural hosts of Sharka in the Czech Republic. Moreover, PPV-M was proved only in one orchard of apricots and one orchard of peaches (Polák 2004). Both orchards were planted with nursery material imported from abroad.

Very similar results were obtained recently also in other countries of Central Europe. Malinowski (2004) proved that a majority of the investigated PPV isolates from plum, peach and apricot found in locations covering a major part of the area of Poland were PPV-D type. Only a few isolates from plum trees found in three isolated locations reacted with PPV-M specific monoclonal antibodies. A total of 181 PPV isolates from *Prunus armeniaca*, *P. spinosa*, *P. cerasifera, P. persica* and *P. domestica* were typed by serological and molecular techniques (Laimer et al. 2003). The vast majority, 159 Austrian PPV isolates, belongs to PPV-D, and only 16 isolates 10.0% were assigned with certainty to PPV-M.

Recent discovery of a new PPV-Rec subgroup (Glasa et al. 2004) complicated the typing of PPV strains in Central Europe only a little. Published results indicate that a vast majority, 90–95% of PPV isolates in Central Europe, belongs to PPV-D subgroup of strains. According to the results of Glasa et al. (2004) approximately 50% of PPV-M isolates belongs in fact to PPV-Rec subgroup. As a consequence only 3–5% of PPV isolates in Central Europe belongs to PPV-M or PPV-Rec subgroup. PPV-M and PPV-Rec strains are more pathogenic in comparison with PPV-D strains (e.g. Polák et al. 2004). Therefore quarantine measures could be limited to pathogenic PPV strains, namely PPV-M, PPV-Rec, PPV-C, and PPV-EA in Central Europe, where the presence of PPV is endemic. Some conclusions presented by Glasa et al. (2004) are very speculative, based on a wrong selection of PPV field isolates used for typing. PPV recombinants accounted for about one third of isolates analyzed by Glasa and his coworkers, but in the PPV field population of the Czech Republic, Austria or Poland PPV-Rec does not probably represent more than 2% to 5%. The statement that recombinants have unwittingly been spread through the movement of tolerant plum varieties from Yugoslavia is also speculative. The PPV isolate Horoměřice (PPV-Rec) obtained in 2002 originates from an old tree of *Prunus domestica* L. cv. Domácí, PPV infected already before the year 1968. This tree was about thirty years old in 1968. PPV infected Sharka tolerant plum cultivars from Yugoslavia were never grown close to or in the surroundings of this old plum tree.

Czech, Polish and Austrian results indicate that PPV-D subgroup is prevalent while the incidence of PPV-M is sporadic in Central Europe. The programme of PPV-M, PPV-D and PPV-Rec subgroup typing in natural hosts and fruit orchards is recommended to realize in countries of Central Europe. The combination of molecular and serological typing will allow to obtain reliable results. PPV subgroup typing in three genomic regions (CP, CI and P3-6K1) of each PPV isolate by RT-PCR followed by RFLP analysis and serological typing using specific PPV-M and PPV-D monoclonal antibodies performed by Glasa et al. (2004) are suitable methods for the detection of prevalent PPV subgroup in the Czech Republic and Central Europe.

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Sporadické rozšíření M kmenu viru šarky švestky v přírodních zdrojích v České republice

ABSTRAKT: V přírodních hostitelích šarky – ve švestkách, myrobalánech a trnkách – v České republice byla zkoumána přítomnost a rozšíření M kmenu viru šarky švestky (PPV-M). Nejprve byly na přítomnost PPV vyhodnoceny listy a květy stromů pomocí polyklonálních protilátek. Vzorky infikované PPV byly zkoumány na přítomnost kmenu PPV-M pomocí kmenově specifických monoklonálních protilátek. Testovalo se sto dva izolátů PPV ze švestky, 81 z myrobalánu a 25 z trnky. PPV-M byl zjištěn v šesti stromech švestky, v šesti stromech myrobalánu a v jednom keři trnky. Sporadický výskyt PPV-M byl prokázán ve všech zkoumaných oblastech České republiky. Bylo navrženo realizovat molekulární a sérologickou typizaci různých kmenů PPV v přírodních hostitelích a v sadech slivoní, meruněk a broskvoní ve střední Evropě.

Klíčová slova: virus šarky švestky; kmen PPV-M; detekce; švestka; myrobalán; trnka; monoklonální protilátky

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