Viruses of Blackthorn and Road-Bordering Trees of Plum, Myrobalan, Sweet and Sour Cherries in the Czech Republic

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Abstract

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The distribution of *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Apple chlorotic ringspot virus* (ACLSV) and *Apple mosaic virus* (ApMV) in naturally growing shrubs of blackthorn and road-bordering trees of plum and myrobalan, and of PPV, PDV, PNRSV and *Cherry leafroll virus* (CLRV) in sweet and sour cherry trees were investigated. The most widely distributed viruses were PPV in plums (74% of the investigated trees were infected); PPV, PDV, and PNRSV in myrobalans (26%, 11% and 18%, respectively), PDV in blackthorns (27%), and PDV and PNRSV in cherries (25% and 22%). PPV was not detected in sweet and sour cherries. The incidence of ACLSV and ApMV was negligible in individually growing trees of the genus *Prunus* in the Czech Republic.

Keywords: blackthorn; myrobalan**;** plum; sweet and sour cherry; stone fruit viruses; distribution; sources of infection

Plum pox virus (PPV), causal agens of sharka disease in plums, apricots and peaches, is the most harmful virus of stone fruits in the Czech Republic (CR). The distribution of natural sources of PPV was investigated over the last several years (Polák 1997, 2002; Polák & Pívalová 2001). Results from those studies indicated that it would be useful to similarly investigate other economically important viruses in naturally growing shrubs of blackthorn (*Prunus spinosa* L.) and trees of myrobalan (*Prunus cerasifera* Ehrh.), plum, sweet and sour cherries growing individually along roads. Thus, the distribution of PPV, *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Apple chlorotic leafspot virus* (ACLSV), and *Apple mosaic virus* (ApMV) in trees of plum and myrobalan and shrubs of blackthorn, and of PPV, PDV, PNRSV and *Cherry leafroll virus* (CLRV) in sweet and sour cherries was investigated from 2004 to 2006.

The distribution of stone fruit viruses in the Czech Republic, in former Czechoslovakia respectively, has not been studied. Old contributions on the presence of these viruses in the territory of Czechoslovakia, including partial data of their distribution, came from Blattný (1958), Králiková (1959, 1962) and Paulechová and Baumgartnerová (1970). Studies on the presence and distribution of viruses in stone fruits were carried out in Spain (Llácer *et al.* 1986) and France (Dunez 1986). More detailed data were obtained for the sanitary status of stone fruits in the Mediterranean area during the 1990s. Results of

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research carried out in Albania, Jordan, Lebanon, Malta, Palestine, Syria and Tunisia were collected by Myrta *et al.* (2003). Additional complex data on the distribution of individual viruses in stone fruits were obtained e.g. in Lebanon (Choueiri *et al.* 2003) and in Turkey (Caglayan & Ulabas 2003). Published results proved that PPV, PNRSV, PDV, ApMV and ACLSV were the important viruses of stone fruits. Other viruses, with marginal importance, were also present in some countries, mainly of southern Europe.

The aim of our study was to determine the distribution of the most important viruses and to identify infection sources in individually growing trees of stone fruits in the Czech Republic.

MATERIAL AND METHODS

Sampling. Naturally growing shrubs of blackthorn and old trees of plum and myrobalan planted along roads in different regions and localities of Bohemia and Moravia, namely in Middle Bohemia (Kladensko, Berounsko, Příbramsko, Boleslavsko), West Bohemia (Chomutovsko, Rokycansko, Plzeňsko, Stříbrsko, Domažlicko, Klatovsko), East Bohemia (Královéhradecko, Rychnovsko), North Bohemia (Českolipsko, Liberecko, Frýdlantsko), North Moravia (Šumpersko, Jesenicko, Osoblažsko), and South Moravia (Třebíčsko, Moravské Krumlovsko, Mikulovsko) were evaluated. Samples of leaves or flowers were collected in the period from April to June of 2004 to 2006; they were taken randomly from four places of the periphery of the tree canopy, without symptom observation.

Serological detection by ELISA. A mixture of the four samples was used for virus detection by DAS-ELISA (Clark & Adams 1977). Polyclonal antibodies of Bioreba, Switzerland, were used for detection of PPV, PDV, PNRSV, ACLSV, ApMV and CLRV.

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 in a ratio of 1:20. The procedure recommended by the producer of antibodies was used for detection of viruses by DAS-ELISA. Results of DAS-ELISA were evaluated by reader MR 5000 (Dynatech) at 405 nm. Samples with $A_{405} > 0.10$ were considered as positive, samples with $A_{405} < 0.03$ as negative.

RESULTS AND DISCUSSION

Samples from 162 trees of myrobalan (Figure 1) were tested; PPV was found in 42 trees (26%), PDV in 18 trees (11%), PNRSV in 29 trees (18%), ACLSV in 3 trees (1.8%) and ApMV in one tree (0.6%). Eighty-six trees of plum were tested; PPV was detected in 64 trees (74%), PDV in 2 trees (2%), PNRSV in 5 trees (27%), ACLSV and ApMV in one tree each (1%). Fifty-six shrubs of blackthorn were tested (Figure 2); PPV was proved in 3 shrubs (5%), PDV in 15 shrubs (27%), PNRSV in 2 shrubs (4%), while ACLSV and ApMV were not found in blackthorns. Fifty-five trees of sweet and sour cherries were tested (Figures 3 and 4); PPV was not detected, PDV was proved in 14 trees of cherry (25%), PNRSV in 12 trees (22%) and CLRV in one tree (1.8%).

Figure 1. Fruits of myrobalan with very mild PPV symptoms

Figure 2. Leaves of blackthorn with severe diffuse spots and oak mosaic symptoms of PPV

Figure 3. Leaf of sweet cherry with red spot symptoms. The presence of PDV was proved in this sample

Figure 4. Leaves of sweet cherry with chlorotic spots and ringspots. The presence of PNRSV was proved in this sample

The first results of the investigation of the distribution of economically important viruses in *Prunus* species suggest a high distribution of PPV in plum and myrobalan, considerable distribution of PDV in blackthorn, cherry and myrobalan, considerable distribution of PNRSV in myrobalan and cherry, and only sporadic incidence of ACLSV, ApMV and CLRV in stone fruit species.

The high incidence of PPV and low frequency of other viruses found in the Czech Republic differs from results obtained in Mediterranean countries (Myrta et *al*. 2003). In that region, in plum trees the incidence of PPV infection was 35.7% (in the CR 74%), PNRSV 27.1% (CR 5.8%), PDV 15.1% (CR 2.3%), ACLSV 9.4% (CR 1.2%) and ApMV infection 1.1% (CR 1.2%). However, the correlation among the distribution of individual viruses was similar.

In cherries, a higher incidence of PDV and considerable one of PNRSV was found in the Czech Republic, while in the Mediterranean region the incidence of PDV dominated (PDV 67.2%, PNRSV only 5.9%). ACLSV and ApMV were not found in cherries in the Czech Republic (in the Mediterranean 10.6% and 3.3%, respectively). The incidence of ApMV in stone fruits is negligible both in the Mediterranean countries and the Czech Republic. The distribution of viruses in myrobalan and blackthorn was not investigated in the Mediterranean region. However, our results are preliminary when considering the number of tested trees. Therefore, the investigation of the distribution of viruses in stone fruits will be continued in the next years.

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