OCCURRENCE OF SEVERAL VIRUSES INFECTING WILD GROWING STONE FRUIT TREES IN CENTRAL BOHEMIA*

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The phytosanitary status of wild growing stone fruit trees and shrubs was examined in surveys conducted in 2013 and 2014 in the region of Central Bohemia, Czech Republic. A total of 159 leaf samples were collected (42 cherries, 77 bird cherries, 10 cherry plums, 13 blackthorns, 2 round plums, 15 plums) and tested for the presence of *Plum pox virus* (PPV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV), *Cherry virus A* (CVA), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry green ring mottle virus* (CGRMV), and *Cherry leafroll virus* (CLRV) using reverse transcription-polymerase chain reaction (RT-PCR). Totally 28.3% of the investigated trees and shrubs were infected by at least one monitored virus. Mixed infection occurred in 5 out of 159 trees (3.1%). PPV was the most widespread virus (13.2% of samples), followed by PDV (11.3%). Contrary to these two most relevant viral pathogens, the incidence of CLRV and CVA was negligible in individually growing trees of the genus *Prunus* and ApMV, ACLSV, CGRMV, and CNRMV were not detected at all.

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INTRODUCTION

The wide diversity of the genus *Prunus* is reflected in the large number of viruses that are known to infect these species (Uyemoto, Scott, 1992), among others *Plum pox virus* (PPV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV), *Cherry virus A* (CVA), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry green ring mottle virus* (CGRMV), and *Cherry leafroll virus* (CLRV).

All the above mentioned viruses are distributed worldwide and their presence was confirmed in the Czech Republic, too, with the exception of CGRMV and CNRMV. In detail, PPV was detected in several stone fruit species including peaches, apricots, plums, cherries, and blackthorns in this region (Polak, 1997, 2002, 2007; Polak, Pivalova, 2001), PNRSV and PDV were confirmed on sweet and sour cherries (Sucha, Svobodova, 2010) and on plums and blackthorns (Polak, 2007). CVA was detected on cherries and plums (Grimova et al., 2010; Safarova et al., 2013) and CLRV was found on cherries and birches (Polak, 1995, 2007). ACLSV was detected in wild plums and cherry plums and ApMV on plum and myrobalan (Polak, 1995, 2007; Polak, Zieglerova, 1997; Polak et al., 1997; Polak, Svoboda, 2006; Svoboda, Polak, 2010).

Because all of these viruses are graft-transmitted pathogens, their uncontrolled distribution could be considerable in commercial orchards, plantings, and nurseries and should be regularly monitored (Barksdale, 1959; Wadley, Nyland, 1976; Cole et al., 1982; Jones, 1986; Nemeth, 1986). Some of them are also spread by vectors (PPV is transmitted by aphids, PNRSV by thrips, and CLRV potentially by nematodes), transmitted by pollen (CLRV, PNRSV, PDV), and by seeds (PNRSV, PDV, CLRV) (George, Davidson, 1963; Kunze, Krczal, 1971; Wang et al., 2002). Thanks to this fact, the presence of viral pathogens infecting not only the commercially planted fruit trees but also wild growing woody plants situated in their vicinity, can be threatening to them, since these ones can be source of virus infection. In spite of this there are not many attempts to check the health status of wild fruit trees. Few exceptions are e.g. reports of Polak

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Table 1. Sets of oligonucleotide primers used for polymerase chain reaction (PCR) detection of stone fruit-infecting viruses

Virus	Genus	Annealing temperature (°C)	Amplicon length (bp)	Primer sequence (from 5′- 3′)	Reference
PPV	*Potyvirus*	51	345	F: GGAATGTGGGTGATGATGG R: CTCTTCTTGTGTTCCGACGTTTC	Varga, James (2005)
ApMV	*Ilarvirus*	58	464	F: TCCTGAGCAGTCGAGAAGTG R: CGTTATCACGTACAAATCCCTC	Petrzik (unpublished)
PDV		50	610	F: CCGGTATGATATCTCGTACCGAG R: TAGTGCAGGTTAACCAAAAGGAT	Rowhani et al. (1998)
PNRSV		50	346	F: GAACCTCCTTCCGATTTAG R: GTCTCCCTAACGGGGCATCCAC	Sanchez-Navarro et al. (2005)
ACLSV	*Trichovirus*	57	432	F: TCGCGAACATAGCGATACAG R: ACGACATTTTCGCCTCATTC	Watpade et al. (2012)
CGRMV	*unassigned*	52	366	F: GCAGCTTTGACTTTTTTGAG R: CCTATAGCCAGTCTTCATATTATG	Rott, Jelkmann (2001)
CNRMV		52	186	F: TAAACCTCTGCAAACCCAATC R: CTCTCGTAGAAAACTGAAGGA	Isogai et al. (2004)
CVA	*Capillovirus*	58	425	F: CAAGAATCCAGGGGCCTACT R: ACCTTTGGAACAAACGATGC	Grimova et al. (2010)
CLRV	*Nepovirus*	55	283	F: GTTACTTTTACCTCCTCATTGTCCATGGTTG R: GACTATCGTACGGTCTACAAGCGTGTGGCGTC	Kumari (2009)

PPV = *Plum pox virus*, ApMV = *Apple mosaic virus*, PDV = *Prune dwarf virus*, PNRSV = *Prunus necrotic ringspot virus*, ACLSV = *Apple chlorotic leaf spot virus*, CGRMV = *Cherry green ring mottle virus*, CNRMV = *Cherry necrotic rusty mottle virus*, CVA = *Cherry virus A*, CLRV = *Cherry leafroll virus*

(2007) and Ilbagi et al. (2008). Moreover, they are mainly concentrated onto plums whereas cherries and especially bird cherries are quite neglected in this respect. Therefore, the aim of our study was focused on monitoring selected stone fruit tree viruses in different wild growing hosts to determine their natural extension in the Central Bohemian region.

MATERIAL AND METHODS

Relatively small area of trees growing naturally along the nature reserve Tiché údolí (Silent Valley) on the northwestern border of Prague (Central Bohemia) at the altitude of approximately 250–270 m a.s.l. was investigated to determine the incidence of several stone fruit viruses. Beside stone fruit trees there are other broad leaf trees as ashes, birches, oaks, rowans as well as wild apples and pears. Leaves from 159 symptomatic and asymptomatic diversely old *Prunus* trees from various sites in the concerned area (Fig. 1) were randomly collected during years 2013 and 2014 and used for the virus detection. The set of the tested species included 15 plums (*Prunus domestica*), 42 cherries (*P. avium*), 13 blackthorns (*P. spinosa*), 77 bird cherries (*P. padus*), 10 cherry plums (*P. cerasifera*), and 2 round plums (*P. domestica* ssp. *italica*). These numbers approximately correspond with the proportion of individual species on stands.

As a testing biological material fully expanded leaves were collected from each tree from late May to early July (6–8 leaves taken randomly from four places of the periphery of the tree canopy represented

Fig. 1. Typical virus-triggered symptoms of chlorotic mottling and mosaic on leaves of cherry plum

Table 2. Numbers and proportions of cherry, bird cherry, cherry plum, blackthorn, round plum, and plum samples tested and shown to contain PNRSV, PDV, PPV, CLRV, and CVA as indicated by reverse transcription-polymerase chain reaction

Host	Samples		Infection rate	PPV		PDV		PNRSV		CLRV		CVA	
	tested	infected											
	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cherry	42	6	14.3	–	–	5	11.9	2	4.8	2	4.8	–	–
Bird cherry	77	14	21.2	–	–	13	19.7	1	1.3	–	–	–	–
Cherry plum	10	5	50	5	50	–	–	–	–	–	–	–	–
Blackthorn	13	3	23.7	1	7.7	–	–	2	15.4	–	–	–	–
Round plum	2	2	100	–	–	–	–	1	50	–	–	1	50
Plum	15	15	100	15	100	–	–	2	13.3	–	–	1	6.7
Total	159	45	28.3	21	13.2	18	11.3	8	5	2	1.3	2	1.3

PPV = Plum pox virus, PDV = Prune dwarf virus, PNRSV = Prunus necrotic ringspot virus, CLRV = Cherry leafroll virus, CVA = Cherry virus A

one sample) and were immediately analyzed or stored at –21°C until use.

The ApMV, ACLSV, PPV, and CVA isolates, used in this study as positive controls, were obtained from the virus collection of the Department of Crop Protection (Czech University of Life Sciences Prague). The PNRSV-, PDV-, and CLRV-infected cherry leaves were kindly provided by Dr. Suchá from the Czech Research and Breeding Institute of Pomology Holovousy, and the CGRMV and CNRMV isolates by Dr. Komorowska from the Research Institute of Horticulture in Skierniewice (Poland).

Total RNA was extracted from 100 mg leaf tissue from all species using the modified silica-capture method (Rott, Jelkmann, 2001) as described Grimova et al. (2015).

Reverse transcription-polymerase chain reaction (RT-PCR) tests were done using specific primers for PPV, PNRSV, PDV, ApMV, ACLSV, CGRMV, CNRMV, CVA, and CLRV detection (for all primers used in the study see Table 1). The cycling parameters were as follows: 35 cycles of denaturation at 94°C for 30 s, annealing for 30 s at different temperatures set for each virus pathogen (see Table 1), and extension at 72°C for 45 s, with a final extension at 72°C for 7 min.

The PCR-amplified fragments of 10 µl of the reaction mixture were visualized after electrophoresis in ethidium bromide stained 1% agarose gels.

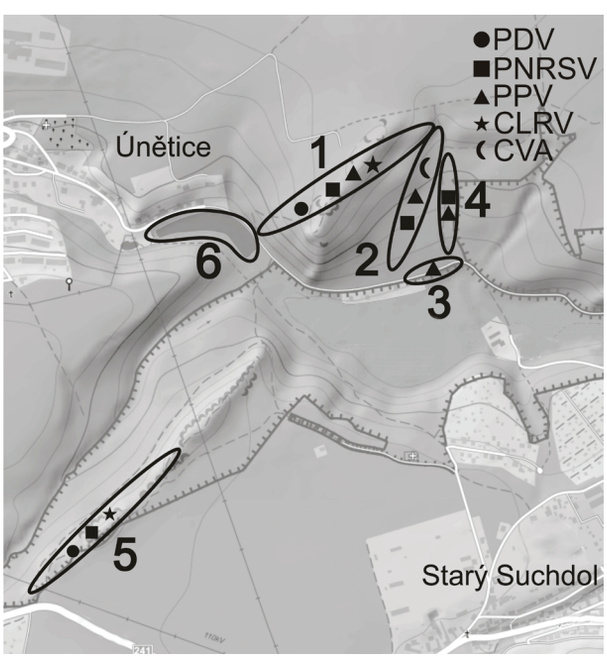

Fig. 2. Geographic localities throughout the region of Central Bohemia, where the stone fruit virus monitoring has been carried out. The occurrence of selected viruses is marked with various symbols

RESULTS

All of the 159 tested trees of plums, cherries, blackthorns, bird cherries, cherry plums, and round plums were individually inspected.

Some trees showed symptoms which indicated virus infection. All plums and round plums exhibited distinct symptoms – mosaic and ring spots of different severity. Other infected species did not show symptoms so readily. Only two out of five infected cherry plums and four out of six cherries had typical virus symptoms (Fig. 1). However, only few bird cherries bore symptoms which could moreover hardly be attributed to some virus disease (almost white dots and some leaf deformation).

The incidence of selected stone fruit viruses in the concerned area is in detail reported in Table 2 and geographically localized in Fig. 2. According to the PCR results, of the total 159 tested trees 45 samples (28.3%) were infected with at least one monitored virus. Namely, PPV was detected in the highest number of tested trees (13.2%), followed by PDV (11.3%), PNRSV (5%), CLRV (1.3%), and CVA (1.3%). Mixed infections were found in 5 out of 159 trees (3.1%) – two

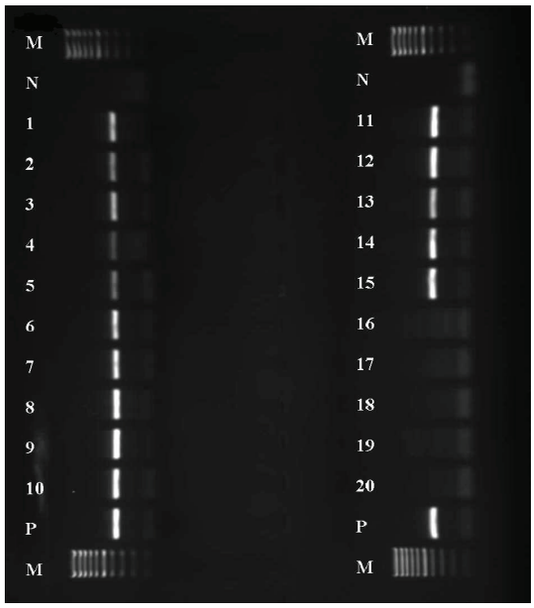

Fig. 3. Representative results for the detection of Plum pox virus (PPV) in several Prunus species by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers (Varga, James, 2005)

Lane M: MassRuler Low Range DNA Ladder (ThermoFisher Scientific); lane N: RT-PCR product of healthy control; 1–15: RT-PCR products of PPV-infected samples; 16–20: RT-PCR product of non-infected plant samples; P: RT-PCR product of positive control agarose gels stained by ethidium bromide

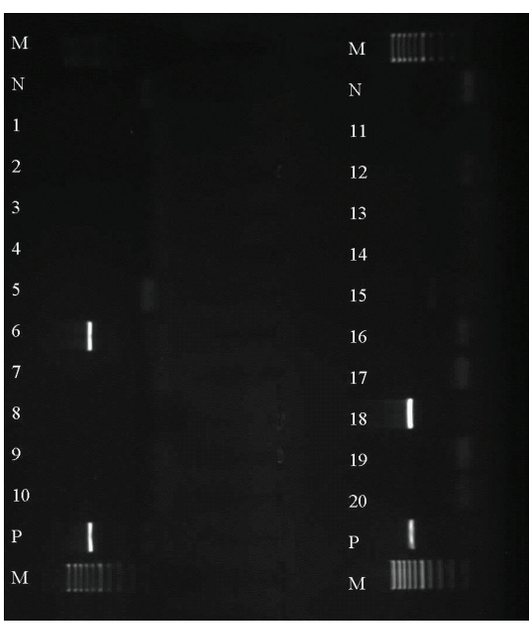

Fig. 4. Representative results for the detection of Prune dwarf virus (PDV) in several Prunus species by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers (Rowhani et al., 1998)

lane M: MassRuler Low Range DNA Ladder (ThermoFisher Scientific); lane N: RT-PCR product of healthy control; 6, 18: RT-PCR products of PDV-infected samples; 1–5, 7–17, 19–20: RT-PCR product of non-infected plant samples; P: RT-PCR product of positive control agarose gels stained by ethidium bromide

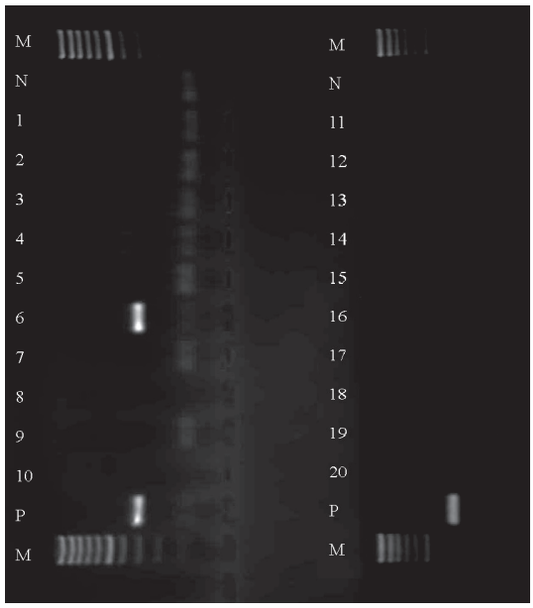

Fig. 5. Representative results for the detection of Cherry leafroll virus (CLRV) in several Prunus species by RT-PCR using the specific primers (Kumari, 2009).

Lane M: MassRuler Low Range DNA Ladder (ThermoFischer Scientific);lane N: RT-PCR product of healthy control; 6: RT-PCR products of CLRV infected samples; 1–5, 7–20: RT-PCR product of non-infected plant samples; P: RT-PCR product of positive control agarose gels stained by ethidium bromide

cherries and three plums. Representative results for the detection of PPV, PDV, and CLRV are demonstrated in electrophoreograms (Figs. 3–5). Other tested viruses, namely ApMV, ACLSV, CGRMV, and CNRMV, were not detected in any tree.

Presence of PPV was confirmed on plums, cherry plums, and blackthorn. All 15 tested plums were infected by PPV (100%). In two cases PPV was detected together with PNRSV and in one case it was found together with CVA. Presence of PPV was confirmed also in 5 out of 10 tested cherry plums (50%) as well as in 1 out of 13 tested blackthorns (7.7%).

Infection of the second most prevalent virus, PDV, was confirmed on 5 out of 42 tested cherries (11.9%) and in 13 out of 77 tested bird cherries (19.7%). In cherry trees, PDV was found in mixed infection together with PNRSV in one case and in the second case PDV, PNRSV, and CLRV were found together.

PNRSV was detected on plums, blackthorns, cherries, round plum, and bird cherry. Two of 13 blackthorns (15.4%), 2 of 15 tested plums (13.3%), 1 of 2 tested round plums (50%), 1 of 77 bird cherries (1.3%), and 2 of 42 cherry trees (4.8%) were positive to this virus in the executed study.

Two of 42 tested samples of cherries (4.8%) showed presence of CLRV, in one case together with PNRSV and PDV.

Finally, CVA was present in 1 of 15 tested plums (6.7%) together with PPV and in 1 of 2 tested round plums (50%).

DISCUSSION

Even though this survey covers only a relatively small area, it may be considered as rather important, because there do not exist any other reports on the sanitary status of wild and especially bird cherries.

Five of nine European most important stone fruit viruses were detected in several wild growing trees in the region of Central Bohemia, Czech Republic. These results indicate rather high prevalence of stone fruit viruses in the uncultivated *Prunus* species in the region.

The results of the investigation of the virus distribution suggest a high distribution of PPV in plums as all of the tested trees were infected and also symptomatic. Presence of PPV was confirmed on cherry plums and blackthorn as well. These results are analogous with previous research conducted in the Czech Republic by Polak (2007). Relatively higher rate of PPV contrary to the lower infection rate of other tested viruses could be caused by the fact that PPV is spread by aphids (Kunze, Krczal, 1971; Labonne et al., 1995). All infected cherry plums grow not far from plums (stands 1–4 in Fig. 1), whereas those on the opposite side of the valley with the absence of plums were completely healthy (stand 5 in Fig. 1). Concerning blackthorns, the situation is quite different. Although they grow in close vicinity of infected plums, the rate of infection by PPV is low and corresponds rather with results of Polak (2007) who found PPV only in 5% of 56 tested blackthorn shrubs than with those of Ilbagi et al. (2008) or even Salamon, Palkovics (2002) who reported PPV infection showing much higher frequency (24.1% and 100% of tested plants, respectively). Nevertheless, the later authors tested symptomatic shrubs only. PDV was mainly found on bird cherries and cherries. Concerning cherries, the situation corresponds with the data obtained by Polak (2007) and also Sucha, Svobodova (2010) who tested trees planted in alleys along ways or in orchards. We have not found any data about virus infection of bird cherries. In our survey they were rather frequently infected. Moreover, they did not have any typical symptoms and thus can easily escape from possible sanitation practices. PNRSV was detected in not so many samples (only 5.3% of infected trees), but it was present in all tested species, with the exception of cherry plums, which just confirmed its well-known broad host range within the genus *Prunus* (Nemeth, 1986). CLRV was recognized only in cherry trees during our survey, although the range of host plants of CLRV is significantly broad and many ways of pathogen spread are under investigation (seed, pollen, vectors). Although CVA was detected mainly in cultivated sweet and sour cherries in other countries in the past (James, Jelkmann, 1998; Kirby et al., 2001; Isogai et al., 2004; Komorovska, Cieslinska, 2004; Sabanadzovic et al., 2005), our survey revealed the presence of CVA only in one plum and one round plum in the region. On the contrary, according to our results none of the tested cherry or bird cherry trees was infected by CVA. The plums have already been confirmed as the host for CVA in the past (James, Jelkmann, 1998; Barone et al., 2008; Marais et al., 2008, 2012), and moreover also in the Czech Republic by Safarova et al. (2013). Nevertheless, this is probably the first report of CVA presence in wild trees. We have not found any ApMV or ACLSV infection. It corresponds with the results of Polak (2007) who found these viruses only very exceptionally in planted stone fruit trees. On the contrary, Ilbagi et al. (2008) proved their presence rather frequently in the blackthorn in Turkey.

Although some suspicions do exist, the presence of CGRMV and CNRMV has not yet been detected in the Czech Republic in orchards or germplasm collection. So, it is not surprising that they were not discovered in such a rather limited survey of wild stone fruit trees.

Nevertheless, it is necessary to point out the potential misinterpretation of our survey by the presence of false negative results. Even though all used oligonucleotides were found to be effective in detecting a wide range of stone fruit virus isolates in previous experiments (Rowhani et al., 1998; Rott, Jelkmann, 2001; Isogai et al., 2004; Sanchez-Navarro et al., 2005; Varga, James, 2005; Kumari, 2009; Grimova et al., 2010; Watpade et al., 2012), they might have failed to react with some of the Czech particular isolates, thereby misleading our conclusions. In order to avoid false negative results within virus monitoring, more than one diagnostic method should be used. However, this precaution substantially increases the expenses of the analysis and therefore poses a limiting factor of such a research.

Taken together, our area of interest lies in the centre of cultivated countryside, where especially cherries used to be intensively grown. Now, there are only very old orchards or individual trees in gardens remaining. Plums are grown in lower numbers in private gardens. Thus, there can be an intensive exchange of viruses between cultivated and wild trees. PPV seems to have the most efficient mode of transmission by aphids among plum and cherry plum trees as all tested trees growing within a reasonable distance of few hundred meters were infected. On the other hand, blackthorn seemed to be a much worse host of PPV (or aphid vectors), which just does not correspond with previous studies (Salamon, Palkovics, 2002; Ilbagi et al., 2008; García et al., 2014). Nevertheless, as noted above, the former authors tested symptomatic shrubs only, which just do not give direct evidence of the presence and the frequency of PPV in *P. spinosa*

population in the monitored area. Even though still present in a relatively high proportion of tested trees, PDV and PNRSV show that pollen and seed transmission is probably much less effective for virus spreading. There is a question whether bird cherries can serve as a source of these viruses for cultivated trees as the possibility of virus transmission by pollen among unrelated species has not yet been completely proved.

CONCLUSION

The presence of five plant viruses, namely PPV, PDV, PNRSV, CLRV, and CVA, was confirmed in many wild growing stone fruit trees during our monitoring in the region of Central Bohemia in 2013 and 2014. The presence of ApMV, ACLSV, CNRMV, and CGRMV was not detected. However, it should not be neglected that 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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