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Investigation on the Incidence of *Plum Pox Virus* in Fruit Nurseries of the Czech Republic

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Abstract

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Nine different visual evaluations of *Plum pox virus* (PPV) presence were carried out in four nurseries during 2012–2015. Results of visual evaluation were verified by ELISA. The presence of PPV was confirmed by ELISA in all the trees showing PPV symptoms. PPV has never been detected by ELISA in trees without PPV symptoms on leaves. A very low occurrence of PPV was proved in plums in all the four nurseries. There were two cases of higher occurrence of PPV in plum cultivars, where PPV originated from infected grafts. The PPV occurrence was 0.126 and 1.59%. The average occurrence of PPV in plums was 0.075%, 93 trees out of 123 630 inspected were PPV-infected. All the PPV-infected trees were destroyed immediately. No new PPV infection was proved later in season (August–September). In comparison with the situation in the 60s of the last century, the PPV occurrence in plums was 2.48% in one nursery in 1963 and there were nurseries with PPV occurrence exceeding 5%. All growing plants were destroyed in this case. The occurrence of PPV in the Czech fruit nurseries today is more than hundred times lower in comparison with the situation fifty years ago. The incidental occurrence of PPV in nurseries cannot be excluded in countries and areas with the endemic presence of PPV (in the Czech Republic and in most European countries). Visual inspection of PPV symptoms in nurseries confirmed by ELISA testing is sufficient. Infected plants must be removed immediately.

Keywords: sharka disease; plum; apricot; peach; incidence; nurseries

Plum pox virus (PPV) is the causal agent of sharka disease in stone fruit trees. It is the most devastating virus for stone fruit production worldwide. The disease appeared in Bulgaria in 1917 on *Prunus domestica* cv. Kjustendil and was first described by Atanasoff (1933). The presence of sharka disease in Bohemia was recorded in the 1930s (Blattný 1930).

Sharka disease has progressively spread from the South-East Europe to the West and was recorded in the most western part of Europe, in Spain and Portugal in 1984 (Llácer *et al*. 1985; Louro & Corvo 1985). It has also been found by the end of the 20th century in South America – Chile (Acuña 1993), Argentina (Dal Zotto *et al*. 2006), and in North America – USA (Levy *et al*. 2000), Canada (Thompson *et al*. 2001). The presence of PPV was proved around the Mediterranean basin in North Africa and Near and Middle East Asia (Dunéz 1987; Roy & Smith 1994). PPV has spread via Pakistan, India and China to the East. The last country where the presence of PPV was proved is Japan (Maejima *et al*. 2010). The presence of PPV has not been proved in Australia, New Zealand, and South Africa up to the present time.

Cambra *et al*. (2006) supposed that illegal traffic and insufficient controlled exchanges of infected propagative plant material have been the main pathway of plum pox spread over long distance. There is no report on the role of nurseries in spreading of PPV over middle distance. Once sharka has become established in an orchard, aphid species transmit the disease locally. Not a single scientific paper has so far been published on the occurrence of PPV in

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fruit nurseries. According to the European Union (EU) rules, in the case of some occurrence of PPV in a nursery, a two-year prohibition on selling *Prunus* species should be applied. This rule is very problematic for countries or areas with endemic presence of PPV. PPV is endemically present in most European countries. The endemic occurrence has appeared after 5–10 years of PPV presence in the area. Epidemic of PPV culminates after approximately thirty years of presence in the area or country. PPV epidemic in the Czech regions (Bohemia, Moravia) culminated in the 60s of the last century. Sharka disease was widely distributed in plum trees in the lowlands of Central Bohemia and Moravia up to the 300 m a.s.l. PPV spread also in apricot and peach orchards. Later on, PPV shifted from lowland to hilly areas (300–450 m a.s.l.) of North-East Bohemia (Polák 1964), and fruit nurseries played a significant role in this spreading. There were four nurseries in the district of Jíčín, two of them in lowland (Kopidlno, Jičín – 250–300 m a.s.l.), one at the foot of the hilly area (Hořice – 320 m), and the last in hills (Vidochov near Nová Paka – 470 m). A high occurrence of PPV was proved in nurseries Kopidlno and Jičín in 1960. Totally 52 000 of plum, apricot, and peach trees were cut down in Jičín nursery and 30 000 of stone fruit trees in Kopidlno nursery. Growing of plums, apricots, and peaches was forbidden in these two nurseries in 1961–1965. The mentioned stone fruits were grown only in nurseries Hořice and Vidochov. The presence of PPV was not proved in a very small nursery Vidochov situated in the PPV-free area in 1963, while in nursery Hořice the presence of PPV was proved in plums, apricots, and peaches (Polák 1964). Nurserymen obtained rootstocks and grafts of cultivars from different producers from Central Bohemia with the endemic presence of PPV. Sharka disease was not present in the northern part of the district Jičín (Hořice–Nová Paka–Vidochov) at that time. Trees infected with PPV were cut down in nursery Hořice in 1963, but the infection had been distributed to northern parts of the district in preceding years. Nurserymen were not able to recognise PPV symptoms at that time, and governmental control was insufficient. PPV was distributed from nurseries to the areas without the presence of this quarantine disease.

The aim of our present research was to determine the extent of the current occurrence of PPV in the Czech nurseries and compare it with the situation fifty years ago.

MATERIAL AND METHODS

Fruit nurseries. The first evaluation of PPV occurrence was realised in the nursery Hořice in 1963. PPV was not detected in Hořice and its vicinity at that time. To obtain relevant data on the current presence of PPV in nurseries, four different anonymous nurseries situated in North-East Bohemia in areas with a severe endemic presence of PPV (Figure 1) were chosen. One of them was the Hořice nursery. Plant material was evaluated in one nursery during 2012–2015, in the second nursery in 2013–2015, and in the two remaining nurseries in 2015. At the present, there is only one nursery in the Jičín district and it was enrolled in our survey. The severe endemic presence of PPV has been recorded in the vicinity of all the four selected nurseries. The nearest source of PPV infection was detected ca. 200 m from the nearest trees grown in the nursery.

Plant material. Stone fruit trees susceptible to PPV (plums, apricots, peaches) are grown in all the four selected nurseries, along with apples, pears, sweet and sour cherries. Different cultivars are grafted on rootstocks in March or April (mainly cherries), or budded on rootstocks in August. Grafts of fruit cultivars originated in space isolates. Mother plants in space isolates must be regularly tested for the presence of PPV, and must be PPV-free not only for the production of virus-free plants, but also for the production of Conformitas Agraria Communitatis material (CAC-material). One-year-old rootstocks are produced in specialised agricultural enterprises and must be also PPV-free for both categories of gardening products. The health state of mother plants for graft production and production of rootstocks is under the regular control of the Central Agricultural Control and Testing Institute. One- or two-year-old

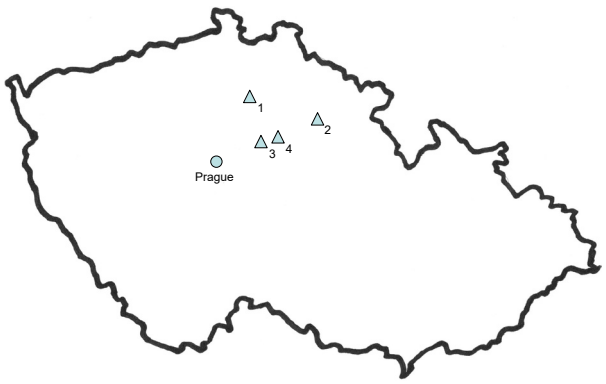

Figure 1. Locations of four nurseries situated in the area with severe endemic presence of PPV in the Czech Republic

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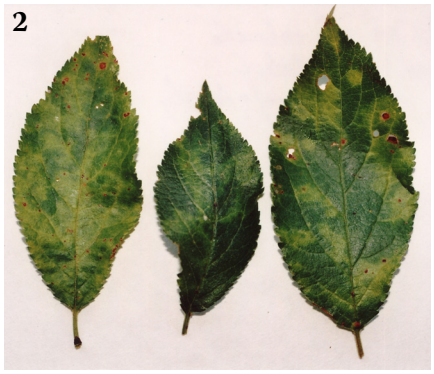

Figure 2. Severe diffuse and yellow spots in leaves of plum infected with PPV
Figure 3. Spots and rings in leaves of apricot infected with PPV
Figure 4. Diffuse rings and spots in leaves of peach infected with PPV

trees (exceptionally three-year-old) are distributed from nurseries to customers.

Visual evaluation of PPV symptoms. All trees of plum, apricot, and peach grown in nurseries were evaluated by visual inspection for the presence of PPV symptoms in early season at the end of May or at the beginning of June. There is no and/or very low activity of vectors at this time (regular reports of the Central Control and Testing Institute of the CR). Aphid species *Myzus persicae*, *Phorodon humuli*, and *Brachycaudus helichrysi* are the main vectors of PPV in the Czech Republic (Polák & Komínek 2014). The presence of PPV in symptomatic trees was checked by ELISA. Trees infected with PPV were immediately removed. The second control of PPV symptoms was carried out in August and September. PPV symptoms on leaves of plum (Figure 2) and apricot (Figure 3) trees are diffuse spots or rings of different intensity. Exceptionally necrotic rings can appear on the leaves of infected trees. Peach trees infected with PPV response by the clearing and thickening of veins of the first leaves in May. The leaves are thickened and leathery. Leathery leaves with thickened veins get yellow during June and drop off. That is why no symptoms can be observed by the end of June especially in cultivars more resistant to PPV (Polák *et al*. 2003). In more susceptible peach cultivars PPV symptoms develop in the third, contingently in the fourth and further leaves of growing branch. Those leaves show marked or severe oak-leaf mosaic, sometimes also diffuse spots and rings (Figure 4). Symptoms are observable still in July.

Serological detection of PPV. ELISA testing of the leaves with symptoms was performed just after the visual evaluation of trees in nurseries. In case that more plants of one cultivar showed PPV symptoms, twenty asymptomatic trees were tested by ELISA later in July. For the ELISA, 1 g of leaf samples were homogenised in 20 ml of a buffer based on phosphate-buffered saline, containing polyvinylpyrrolidone (20 g/l) and bovine serum albumin (0.5%). Spanish antibodies (IVIA/AMR; Lab Consultants, Valencia, Spain) were used in DAS-ELISA (Clark & Adams 1977). Rabbit-PPV specific polyclonal antibodies were used for coating and mixture of monoclonal antibodies 5B-IVIA/AMR conjugated with alkaline phosphatase was used for the detection of PPV.

RESULTS

Results of visual evaluation of the PPV presence in the fruit nurseries in 1963 and in 2012–2015 are presented in Tables 1–3. PPV was present in most of plum cultivars, and in several apricot and peach cultivars in nursery Hořice in 1963 (Table 1). Sharka disease was not present in Hořice and its vicinity at that time. 2.48% of plum trees, 2.47% of apricot trees, and 4.38% of peach trees were PPV-infected. All infected trees were removed, but in previous years some other infected trees were probably sold, because the nurseryman was not able to recognise PPV symptoms. Knowledge of sharka disease was poor at that time. Serological diagnosis did not exist. The disease could be recognised only by the evaluation of symptoms. The occurrence of PPV in fruit nurseries Kopidlno and Jičín was over 5% in 1960, all plum, apricot, and peach trees were cut down and it was not possible to grow designated plants in nurseries Kopidlno and Jičín for five years.

Nine different visual evaluations were carried out in the early vegetative season in four nurseries during

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Table 1. Occurrence of *Plum pox virus* in Hořice nursery in 1963

Plant species	Number of plants	PPV infected	PPV infection (%)	PPV infection in cultivars
(1) plum	4673	119	2.48	Domácí velkoplodá, Esslingenská, Wangenheimova, Althanova r., Oullinská r., Ananasová r., Zelená r.
(2) apricot	567	14	2.47	Bredská, Rakovského
(3) peach	320	14	4.38	South Haven
(1) + (2) + (3)	5560	147	2.64	

2012–2015. Results of visual evaluation were verified by ELISA. The presence of PPV was confirmed by ELISA in all the trees showing PPV symptoms. Absorbance values of ELISA were very high, over 2.5 in samples from leaves of plum and apricot trees with PPV symptoms in years 2012–2015. Only in nursery 1 in the year 2013 the absorbance values fluctuated from 0.96 to 1.24 in samples from PPV infected leaves of cvs Zimmerova and Oulinská. PPV was never detected by ELISA in trees without PPV symptoms on leaves. Absorbance values of ELISA were always under 0.01. The presence of PPV was never proved in peaches. PPV was proved in one nursery in one year only in one cultivar of apricot. A very low occurrence of PPV in plums was proved in all four nurseries. This PPV presence was usually limited to one cultivar (Tables 2 and 3). This very low occurrence originated mostly in rootstocks, or by incidental transmission by aphids from distant natural sources, which cannot be excluded. PPV symptoms in plants adjacent to those infected and removed were never found during the second visual inspection of trees in August or September. Results of ELISA testing of representative samples were negative, too. All the plum, apricot, and peach trees were PPV-free in the four investigated nurseries.

Two cases of higher occurrence of PPV in plum cultivar were identified. In plum cv. Hamanova 42 trees were PPV-infected in nursery 2 in 2015, and in plum cv. Valor twenty trees were PPV-infected in nursery 1 in 2014. The occurrence of PPV was not incidentally distributed in this case, but infected trees were in one row; PPV originated in infected grafts. Twenty other trees of cvs Hamanova and Valor without PPV symptoms growing close to the infected trees were tested in August by ELISA. Results of testing of all forty trees were negative.

The average occurrence of PPV in peaches in nine evaluations in four nurseries during four years (2012 to 2015) was zero, not a single tree from the 44 163 trees inspected was infected. The occurrence of PPV in apricots was 0.003%, two trees of the 62 666 inspected were infected. The occurrence of PPV in plums was 0.075%, 93 trees of the 123 630 inspected were infected. We may conclude that the PPV occurrence in the Czech fruit nurseries today is by more than hundred times lower compared to the situation fifty years ago.

DISCUSSION

Some European countries have proposed testing of individual trees in nurseries. They probably have problems with the occurrence of PPV in nurseries. The incidental occurrence of PPV in nurseries cannot be excluded in countries and areas with endemic presence of this virus. To our knowledge, the occurrence of PPV in fruit nurseries has not been reported in literature up to the present time. We propose to conduct similar research studies on the presence of PPV in nurseries of the EU countries. Nine different evaluations in three nurseries during three years will be sufficient. Early visual inspection of PPV symptoms in nurseries verified by ELISA will be sufficient in agreement with our experience. PPV infected trees must be removed immediately. Our study proved that the transmission of PPV to healthy plants is practically impossible in this case. Mass testing of individual trees in nurseries is very expensive, not effective, just a loss of money. Much more important is the existence of the governmental system of certification of planting material, especially annual visual inspections and testing of mother plants in screenhouses and space isolates. The education of nurserymen and governmental control are also very important.

There are several reasons for the high decrease of PPV occurrence in Czech fruit nurseries:

(1) Screenhouses (technical isolates) for plums were established in the 80s of the last century. The governmental system of certification of planting material of fruit trees and grapevines was established in 2001. In order to prevent PPV, all plants of plum,

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Table 2. Occurrence of *Plum pox virus* in nursery 1 (2012–2014) and in nursery 2 (2013 and 2014)

Plant species	2012				2013				2014			
	number of plants	PPV infected	PPV infection (%)	PPV infection in cultivars	number of plants	PPV infected	PPV infection (%)	PPV infection in cultivars	number of plants	PPV infected	PPV infection (%)	PPV infection in cultivars
Nurseŕy 1												
(1) plum	9 870	1	0.010	Domácí velkoplodá	11 300	4	0.035	Zimmerova, Oullinská r.	18 310	23	0.126	Valor, Domácí velkoplodá
(2) apricot	5 398	2	0.037	Karola	3 310	0	0		6 120	0	0	
(3) peach	5 500	0	0		2 880	0	0		3 300	0	0	
(1) + (2) + (3).	20 768	3	0.014		17 490	4	0.023		27 730	23	0.083	
Nursery 2												
(1) plum					2720	3	0.110	Domácí velkoplodá	3780	1	0.026	Domácí velkoplodá
(2) apricot					870	0	0		320	0	0	
(3) peach					1200	0	0		2393	0	0	
1) + (2) + (3)					4790	3	0.062		6493	1	0.015	

Table 3. The occurrence of *Plum pox virus* in nurseries 1–4 (2015)

Plant species	Nursery 1				Nursery 2				Nursery 3				Nursery 4			
	number of plants	PPV infected	PPV infection (%)	PPV infection in cultivars	number of plants	PPV infected	PPV infection (%)	PPV infection in cultivars	number of plants	PPV infected	PPV infection (%)	PPV infection in cultivars	number of plants	PPV infected	% of PPV infection	PPV infection in cultivars
(1) plum	34 760	14	0.040	Valor	2 640	42	1.590	Hamanova	18 000	1	0.006	Tegera	22 250	4	0.018	Katinka
(2) apricot	10 160	0	0		638	0	0		12 000	0	0		23 850	0	0	
(3) peach	6 460	0	0		2 050	0	0		8 000	0	0		12 380	0	0	
1) + (2) + (3)	51 380	14	0.027		5 328	42	0.788		38 000	1	0.003		58 480	4	0.007	

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apricot, and peach originate from virus-free mother plants in technical isolates. Not only virus-free, but also CAC plants must be PPV-free. Mother plants in space isolates are derived from mother plants in a technical isolate. Nurseries obtain grafts from space isolates where mother trees are also visually evaluated and tested for the presence of PPV by ELISA in agreement with governmental regulation.

(2) Nurserymen are regularly educated in recognising PPV symptoms. They can recognise PPV symptoms already at the end of May. Leaf symptoms of PPV have appeared not only in PPV susceptible cultivars, but also in those resistant to PPV (Polák *et al*. 1997). PPV symptoms slowly disappear in resistant cultivars in the following 3–4 years. Trees are grown in fruit nurseries usually for one or two years.

(3) Nurserymen remove PPV infected trees immediately. There are no PPV infected trees at the time of governmental inspection. The transmission of PPV from infected to healthy plants by aphids is practically impossible. There is usually no aphid activity by the end of May or at the beginning of June in the climate conditions of the Czech Republic.

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References

Acuña R. (1993): Outbreaks of Plum pox virus in Chile. European & Mediterranean Plant Protection Organization (EPPO) Conference Plum pox virus, Aug 5–8, 1993, Bordeaux, France. Paris, EPPO.

Atanasoff D. (1933): Plum Pox. A New Virus Disease. Faculty of Agriculture and Forestry, University of Sofia Yearbook (1932–1933), Vol. 11: 49–69.

Blattný C. (1930): Poznámky o virových a příbuzných chorobách rostlin I. Ochrana Rostlin, 10: 130–138.

Cambra M., Capote N., Myrta A., Llacer G. (2006): *Plum pox virus* and the estimated costs associated with sharka disease. Bulletin OEPP/EPPO Bulletin, 36: 202–204.

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.

Dal Zotto A., Ortego J.M., Raigón J.M., Caloggero S., Rossini M., Ducasse C.A. (2006): First report in Argentina of *Plum pox virus* causing sharka disease in *Prunus*. Plant Disease, 90: 523.

Dunéz J (1987): Plum pox disease of stone fruit trees. Rome, FAO.

Levy L., Damsteegt V., Welliver R. (2000): First report of *Plum pox virus* (sharka disease) in *Prunus persica* in the United States. Plant Disease, 84: 202

Llácer G., Cambra M., Lavina A. (1985): Detection of plum pox virus in Spain. Bulletin OEPP/EPPO Bulletin, 15: 325–329.

Louro D., Corvo L.M. (1985): Occurrence of sharka in Portugal. Acta Horticulturae, 193: 183

Maejima K., Hoshi H., Hashimoto M., Himeno M., Kawanishi T., Komatsu K., Yamaji Y., Hamamoto H., Namba S. (2010): First report of plum pox virus infecting Japanese apricot (*Prunus mume* Sieb. et Zucc.) in Japan. Journal of General Plant Pathology, 76: 229–231.

Polák J. (1964): Výskyt virových chorob v ovocných školkách okresu Jičín a možnosti ochrany. [Diploma Work.] Prague, Czech University of Agriculture.

Polák J., Oukropec I., Komínek P., Krška B., Bittóová M. (1997): Detection and evaluation of resistance of apricots and peaches to plum pox virus. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 104: 466–473.

Polák J., Oukropec I., Krška B., Pívalová J., Miller W.R. (2003): Difference in reactions of apricot and peach cultivars to *Plum pox virus*: serological and symptomatological evaluation. Horticultural Science (Prague), 30: 129–134.

Polák J., Komínek P. (2014): Evaluation of rootstocks of stone fruits for resistance to natural *Plum pox virus* infection. Canadian Journal of Plant Pathology, 36: 116–120.

Roy A.S., Smith I.M. (1994): Plum pox situation in Europe. Bulletin OEPP/EPPO Bulletin, 24: 515–523.

Thompson D., McCann M., MacLeod M., Lye D., Green M., James D. (2001): First report of *Plum pox potyvirus* in Ontario, Canada. Plant Disease, 85: 97.

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