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Original scientific paper

Plum pox virus infection level in *Prunus* species growing along roadsides or in backyards in Vojvodina province

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SUMMARY

The present study was conducted on 106 leaf samples of *Prunus* species (44 plum, 47 myrobalan plum, 10 apricot, 3 peach, 1 blackthorn, and 1 sour cherry) collected from the Vojvodina province of Serbia and assessed for the presence of Plum pox virus (PPV) with the aim of establishing whether trees growing along roadsides and in backyards could be sources of PPV infection in commercial orchards. Analyses confirmed PPV infection in 68.2%, 38.6%, and 20% of plum, myrobalan plum and apricot samples, respectively. The infection level varied considerably across locations, with 80% noted in Uljma, followed by 69% in Sremska Kamenica, 60% in Bečej, 50% in Temerin, 50% in Senta, 40% in Petrovaradin, 31% in Novi Sad, 30% in Susek, and 20% in Subotica. As no difference in infection rates was noted between young and older plum trees, the infections seemed to have occurred in their early life stage. Trees growing along roadsides were infected to a higher extent (89.6%) than those in backyards (66.6%). In some cases, symptomless trees were also proven to be PPV positive, indicating that they are a potentially important source of further virus infections. Due to the widespread presence of the virus, tolerant or hypersensitive cultivars are recommended for planting new orchards.

Keywords: plum pox virus, *Prunus* trees, virus infection, Vojvodina

INTRODUCTION

Sharka caused by Plum pox virus (PPV) is one of the most devastating diseases affecting stone fruits worldwide, causing particularly severe losses in plum orchards (Barba et al., 2011). Yield quality and quantity, as well as the longevity of infected apricot and peach trees are also significantly reduced. Other cultivated host species of PPV are almond, cherry, sour cherry, and ornamental *Prunus* species. Among the wild *Prunus* species, myrobalan plum and blackthorn are the most widespread PPV hosts. PPV was detected for the first time in Bulgaria in 1917 (Atanasoff, 1932), and it is currently considered a typical plant pathogen for the Balkan peninsula, but it is also an important common or quarantine pathogen worldwide (Melika & Krizbai, 2021). According to Jevremović and Paunović (2014), PPV infection has reached 80% in some southern localities in Serbia. As some of the autochthonous and widely-grown plum cultivars (such as Požegača) are very susceptible to PPV, plum production in Serbia has sustained a considerable decline. This is highly problematic, given that plum production has a long tradition and plum is considered to be one of the most famous fruit species in Serbia. Available records indicate that plum was an important Serbian export product back in the 18th century (Demeter, 2016).

Typical Sharka-disease symptoms include yellow circles and mosaics on infected leaves and fruits. The appearance of symptoms and their type are variable and depend on the host, virus strain, and environmental conditions (Bagi et al., 2016). Severe fruit infection manifests as deformation and premature fruit drop, and a significant decline occurs in the parameters of fruit technological quality (Maejima et al., 2020).

PPV has several strains, and PPV-D (Dideron), PPV-M (Marcus) and PPV-Rec (recombinant) are the most important and widespread (James et al., 2013). Currently, strains are determined by molecular techniques, and their pathogenicity and spreading potential via different aphid species are most important for practical purposes (Serçe et al., 2009).

Sources of virus infection are host species of the genus *Prunus*. The virus can spread very efficiently by different aphid species in a non-persistent manner. Vector control typically involves the use of insecticides, but its efficacy is low due to the non-persistent manner of virus transmission (Levy et al., 2000). Aphid control on already infected neighboring (cultivated or wild-grown) trees is usually not possible.

Since the use of genetically modified plants is prohibited in many countries (including Serbia), planting of virus-tested healthy *Prunus* trees and use of tolerant or hypersensitive cultivars are the most effective preventive measures that are currently available. As a result of these measures, stable yield can be achieved even under high infection pressure, but yield quality and quantity provided by healthy trees would be higher. In addition, securing a sufficient geographic distance between infected trees and newly planted orchards is another viable virus-control measure. That distance should be several kilometers, since migratory aphids carried by wind can traverse considerable distances (Mihaljfi et al., 2021).

Abandoned old orchards, *Prunus* trees growing by roadsides, and those cultivated in small gardens or used for garden decoration can be important sources of virus infection. Therefore, the aim of this research was to assess the presence of virus infection in *Prunus* species growing along roadsides and in backyards, and so provide important information for fruit growers planning to expand their stone-fruit production capacities by planting new orchards. Testing of different myrobalan plum varieties and blackthorn is also significant because they are often used as rootstocks for grafting, and grafting is widely known as an efficient means of virus transmission.

MATERIAL AND METHODS

Sampling of *Prunus* material

Leaf samples from different *Prunus* species were collected from nine locations (Bečej, Temerin, Susek, Petrovaradin, Novi Sad, Senta, Sremska Kamenica, Subotica and Uljma) in three geographically remote areas: Bačka, Banat and Srem. During the sampling process, tree age and location (roadside or backyard) were recorded. Depending on age, trees were separated into three groups: (i) young (1-5 years old), (ii) mature (6-10 years), and (iii) older (more than 10 years old). GPS coordinates of each observed/analyzed tree were recorded in order: (i) to inform the tree owner of confirmed PPV infection, and (ii) to determine the virus strain using the infected samples.

To determine the level of disease incidence, samples were collected from randomly chosen symptomatic and asymptomatic trees (Figures 1 and 2).

Samples were collected on the 8th of July at Bečej, Temerin, Susek, and Petrovaradin locations, while locations in Novi Sad, Senta, Sremska Kamenica, Subotica and Uljma were visited on the 28th of July 2021. Among the 106 samples collected across all locations, 63 were from Bačka (Temerin – 20, Bečej – 10, Novi Sad – 13, Senta – 10, Subotica – 10), 33 originated from Srem (Susek – 10, Petrovaradin – 10, Sremska Kamenica – 13), and 10 from Banat (Uljma). In terms of plant species, we collected 44 plum, 47 myrobalan plum, 10 apricot, 3 peach, 1 blackthorn, and 1 sour cherry sample. All samples were kept at 4 °C for 24 hours before proceeding with analyses.

Figure 1. Backyard plum tree with severe symptoms

Figure 2. Untended myrobalan plum tree located on a roadside near Temerin village

Serological analysis

Leaves closer to the tree trunk were collected and the leaf part just next to the stem was separated and used for sample preparation. A total of 0.25 g of leaf tissue per sample was added to an extraction bag and macerated with 5 ml of sample buffer (Clark & Adams, 1977). Samples were subjected to the DAS ELISA (Double Antibody Sandwich *ELISA*) serological test, using Loewe Biochemica GmbH chemicals and protocols. In the first step, antigen-specific coating-antibody (IgG) was diluted at a 1:200 ratio in the coating buffer and was added to a microtiter plate. The plate was covered with sealing tape and was incubated at 37 °C for 4 hours, after which four wash cycles were performed in an automated washer BioTech 50TS (BioTek Instruments, Winooski, VT, USA) using wash buffer. In the next step, samples were added to a microtiter plate covered with sealing tape and incubated at 4 °C overnight. After incubation, plates were washed four times using a wash buffer. Next, the 1:200 diluted enzyme-labeled antibody-AP-conjugate in conjugate buffer was added to a plate covered with sealing tape and incubated at 37 °C for 4 hours, followed by four washing cycles. In the final step, 1 mg/ml 4-nitrophenylphosphate-di-Na-salt in substrate buffer was added to each plate well. Alkaline phosphatase (AP) reacts with the substrate 4-nitrophenylphosphate in an enzymatic reaction, resulting in yellow-colored 4-nitrophenol as a product. After 60 and 120 minutes of substrate incubation, color appearance was measured using the Epoch Microplate Spectrophotometer (BioTek Instruments, Winooski, VT, USA) at $\lambda = 405$ nm. Samples for which the recorded values were ≥ 2× OD of that in the negative control were considered as PPV infected (EPPO, 2015).

RESULTS AND DISCUSSION

The results of Plum pox virus testing revealed a very high infection level among the tested *Prunus* trees growing by roadsides or in backyards. Plum trees were most severely affected (68.2% infection rate), followed by myrobalan plum (38.6%), and apricot (20%), as shown in Table 1 and Figure 3. In the case of peach (33.3%), blackthorn (100%), and sour cherry (0%), the number of samples was too small for a clear conclusion.

Table 1. Plum pox virus-infected samples from different locations analysed based on *Prunus* species, age and growth location

Location and *Prunus* species	Number of samples	Infected	Healthy	0-5 years old infected	0-5 years old healthy	6-10 years old infected	6-10 years old healthy	> 10 years old infected	> 10 years old healthy	Back--yard infected	Back--yard healthy	Road--side infected	Road--side healthy
Bačka	***63***	***27***	***36***	***6***	***7***	***12***	***16***	***9***	***13***	***3***	***1***	***26***	***33***
Temerin	**20**	**10**	**10**	**2**	**2**	**5**	**5**	**3**	**3**	**2**	**1**	**10**	**7**
Plum	5	5	0	1	/	4	/	/	/	1	/	4	/
Apricot	3	1	2	/	1	/	1	1	/	1	/	2	/
Myrobalan plum	11	4	7	1	1	1	4	2	2	/	/	4	7
Peach	1	0	1	/	/	/	/	/	1	/	1	/	/
Bečej	**10**	**6**	**4**	**1**	**/**	**5**	**4**	**/**	**/**	**/**	**/**	**6**	**4**
Plum	1	1	0	1	/	/	/	/	/	/	/	1	/
Myrobalan plum	9	5	4	/	/	5	4	/	/	/	/	5	4
Novi Sad	**13**	**4**	**9**	**/**	**/**	**/**	**/**	**4**	**9**	**1**	**/**	**3**	**9**
Myrobalan plum	13	4	9	/	/	/	/	4	9	1	/	3	9
Senta	**10**	**5**	**5**	**3**	**3**	**2**	**2**	**/**	**/**	**/**	**/**	**4**	**6**
Plum	7	4	3	2	2	2	1	/	/	/	/	4	3
Apricot	1	1	0	1	/	/	/	/	/	/	/	/	1
Myrobalan plum	2	0	2	/	1	/	1	/	/	/	/	/	2
Subotica	**10**	**2**	**8**	**/**	**2**	**/**	**5**	**2**	**1**	**/**	**/**	**3**	**7**
Plum	5	2	3	/	/	/	3	2	/	/	/	3	2
Myrobalan plum	4	0	4	/	2	/	2	/	/	/	/	/	4
Apricot	1	0	1	/	/	/	/	/	1	/	/	/	1
Srem	***33***	***16***	***17***	***6***	***6***	***5***	***5***	***5***	***6***	***4***	***2***	***12***	***15***
Susek	**10**	**3**	**7**	**/**	**/**	**1**	**3**	**2**	**4**	**/**	**/**	**3**	**7**
Plum	5	2	3	/	/	1	2	1	1	/	/	2	3
Apricot	3	0	3	/	/	/	1	/	2	/	/	/	3
Myrobalan plum	2	1	1	/	/	/	/	1	1	/	/	1	1
Petrovaradin	**10**	**4**	**6**	**4**	**4**	**/**	**2**	**/**	**/**	**/**	**/**	**4**	**6**
Plum	9	4	5	4	4	/	1	/	/	/	/	4	5
Sour cherry	1	0	1	/	/	/	1	/	/	/	/	/	1
Sremska Kamenica	**13**	**9**	**4**	**2**	**2**	**4**	**/**	**3**	**2**	**4**	**2**	**5**	**2**
Plum	6	6	0	1	/	2	/	3	/	3	/	3	/
Myrobalan plum	4	2	2	1	1	1	/	/	1	/	/	2	2
Black thorn	1	1	0	/	/	1	/	/	/	1	/	/	/
Apricot	1	0	1	/	/	/	/	/	1	/	1	/	/
Peach	1	0	1	/	1	/	/	/	/	/	1	/	/
Banat	***10***	***8***	***2***	***1***	***/***	***/***	***1***	***2***	***6***	***3***	***2***	***5***	***/***
Uljma	**10**	**8**	**2**	**1**	**/**	**/**	**1**	**2**	**6**	**3**	**2**	**5**	**/**
Plum	6	6	0	1	/	/	/	/	5	2	/	4	/
Myrobalan plum	2	1	1	/	/	/	/	1	1	/	1	1	/
Apricot	1	0	1	/	/	/	1	/	/	/	1	/	/
Peach	1	1	0	/	/	/	/	1	/	1	/	/	/

Considering the origin of the tested samples (location), analyses revealed that the infection level was the lowest (20%) in Subotica and the highest (80%) in Uljma, while 69% was recorded in Sremska Kamenica, 60% in Bečej, 50% in Temerin, 50% in Senta, 40% in Petrovaradin, 31% in Novi Sad, and 30% in Susek.

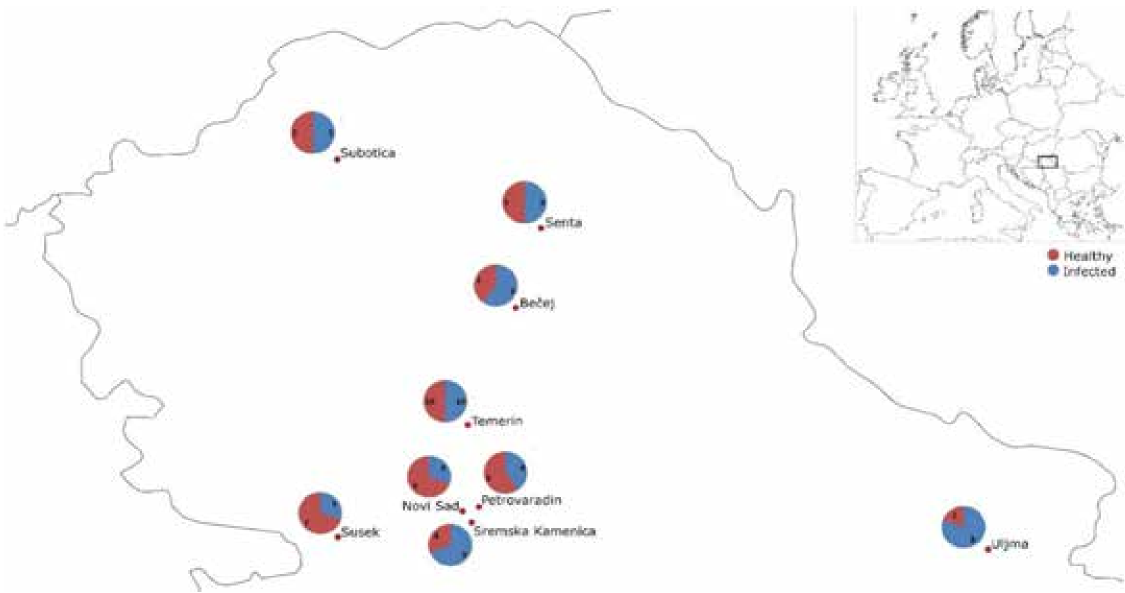

Figure 3. Distribution of Plum pox virus on *Prunus* species in Vojvodina

The infection rate among the samples originating from the Bačka region (42.8%) was slightly below that noted for the Srem region (48.9%). Although 80% infection rate was recorded in Banat, this finding was based on a much lower number of samples. Nonetheless, these results indicate that infections occur regularly in the early stage of tree life, given that 62.5% of young plum trees, 56.2% of mature trees, and 50% older trees were infected. Those results do not seem logical because once infected, trees will remain infected for a lifetime. Thus, we attribute these findings to higher virus concentration in younger plum trees. Similar differences in virus concentration in plum tissues were observed by Marini et al. (2015). In the case of myrobalan plum, the results are much clearer, as 28.6%, 38.9%, and 36.7% infection rates were obtained for young, mature and old trees, respectively.

Roadside trees were infected at a higher rate (89.6%) than those growing in backyards and gardens (66.6%) but both categories of location should be considered as an important infection source.

PPV was detected in some samples originating from asymptomatic trees. In the case of myrobalan plum, virus was found in 47 collected samples, 44 of which were symptomless. All apricot, peach, blackthorn and sour cherry samples were asymptomatic. Conversely, 25 of the 30 positive plum samples exhibited clearly visible yellow circles and mosaics on leaves. In two plum samples with visible symptoms, virus presence was not confirmed by the ELISA test. Five plum samples were asymptomatic but positive for PPV presence (Figures 4-7).

Figure 4. Severe Plum pox virus infection on a roadside plum tree in Temerin

Figure 5. Typical Plum pox virus symptoms on an infected plum leaf

Figure 6. Symptomless infected blackthorn in Sremska Kamenica

Figure 7. Symptomless infected myrobalan plum in Sremska Kamenica

According to information provided by the Serbian Statistical Institute, plum is grown on about 73,000 ha, apricot on 5,700 ha, and peach on 5,100 ha in the Republic of Serbia. These orchards are increasing in number and size owing to their higher profitability relative to field crop production, and potentials for stone fruit exports. However, quality-oriented production is needed for maintaining stable export capacity, which stresses the importance of effective preventive plant protection measures.

The results yielded by the present study are in line with those reported by Jevremović and Paunović (2014), who noted a widespread presence of PPV-infected *Prunus* trees along roads and in small gardens. Even though different eradication measures can be applied to mitigate the effects of all possible infection sources, most such measures need high financial investments and support from government institutions, and must also be very well organized and synchronized. A good example of such strategy is Brazil, where 4 million trees deemed a potential infection source had been destroyed over a short period in order to combat the Citrus tristeza virus, and citrus production was subsequently raised from the ashes (Fadel et al. 2018). Similar effort was made in Puglia Province in Italy (Boscia, personal communication). Similarly, about 125 million dollars was spent in Canada on virus eradication (Wang et al. 2006). In Serbia, the project "Creating conditions for the establishment of a safe zone free of sharka virus for the production of planting material of stone fruits on the territory of Lazarevac municipality", carried out by Paunović et al. and funded by the Serbian Ministry of Agriculture, Forestry and Water Management, achieved respectable results around the Lazarevac county.

The results reported in this work also highlight the importance of producing virus-tested plant propagation materials according to the EPPO (European Plant

Protection Organisation) certification schemes, but they also point to a necessity of applying all available preventive measures. As asymptomatic plants can also be a source of infection, destruction of infected plants, as well as adequate geographic distancing from all wild *Prunus* species should be applied. In some cases, creating a "buffer zone" around stone-fruit orchards with non-host species, such as apple, pear or walnut trees, can be effective since migrating aphids lose their infecting capacity during feeding on non-host species.

In the present situation of high infection pressure and no feasible eradication method on disposal, while some infected trees are asymptomatic, plant breeding remains the most important control measure. Due to the multigene nature of plant resistance to viruses (Levy et al. 2000), the conventional plant breeding goal has been to produce tolerant or hypersensitive stone-fruit cultivars (Neumüller et al. 2010). Tolerant cultivars secure stable yield even in case of infection, while hypersensitivity allows the infection place to be localized in plant tissue by the sensitive reaction of plant cultivar, thus preventing the spread to other plant tissues. By growing tolerant cultivars, we are "making a compromise" and simply "living together" with plant pathogens, since those cultivars still represent a source of virus infection. In some countries, virus control is based on GMO stone-fruit cultivars that incorporate the virus coat protein coding genes in the plant genome (Malinowski et al., 2006) but that type of breeding is prohibited by the Serbian law.

Several tolerant stone-fruit cultivars are available on the Serbian market, mostly the breeding products of the Fruit Research Institute, Čačak, or the Faculty of Agriculture, University of Novi Sad. They include the tolerant plum cultivars 'Čačanska najbolja', 'Čačanska rana', 'Čačanska lepotica', 'Valjevka', 'Timočanka', 'Boranka', 'Krina', and 'Čačanska pozna' (Milenković et al., 2006), but the cultivars 'Stanley', 'President', 'Ruth Gerstetter', and some others are also available on the market. 'Jojo' is one of the hypersensitive plum cultivars. Breeding in Novi Sad has resulted in the tolerant apricot cultivars 'NS-4', 'NS-6', 'Novosadska rodna' and some others.

Besides further investigation of PPV strains, future research endeavors can also be directed towards estimating the differences in myrobalan plum susceptibility. According to Horvath et al. (2008), the genetic diversity within this species is extremely high. Thus, it would be greatly valuable to understand better the interaction between myrobalan plum varieties and different PPV strains.

CONCLUSION

According to the results obtained in this research, Plum pox virus is highly prevalent among trees growing on roadsides and in backyards, thus posing a constant infection threat to commercial stone-fruit production. Consequently, in addition to applying all available preventive control measures, focus should be placed on planting virus-tested tolerant or hypersensitive stone-fruit cultivars.

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Nivo zaraze *Prunus* vrsta koje rastu pored puteva i u okućnicama u Vojvodini virusom šarke šljive

REZIME

U cilju provere prisustva virusa šarke šljive, Plum pox virus (PPV), na stabalima pored puteva i u okućnicama i njihove uloge kao izvora zaraze za komercijalne voćnjake, istraživanje je sprovedeno na 106 uzoraka listova *Prunus* vrsta (44 šljiva, 47 džanarike, 10 kajsije, 3 breskve, 1 crnog trna 1 višnje) sakupljenih na teritoriji Vojvodine. PPV je analizom utvrđen u 68,2%, 38,6% i 20% uzorka šljive, džanarike i kajsije, respektivno. Procenat zaraze je varirao u zavisnosti od lokaliteta od 80% (Uljma), 69% (Sremska Kamenica), 60% (Bečej), 50% (Temerin), 50% (Senta), 40% (Petrovaradin), 31% (Novi Sad), 30% (Susek) i 20% (Subotica). Nije utvrđena razlika u infekciji između mladih i starijih stabala šljive, što ukazuje da se infekcije javljaju u ranoj fazi razvoja stabla. Stabla pored puteva su bila zaražena u većem procentu (89,6%), u odnosu na stabla u okućnicama (66,6%). U nekim slučajevima je dokazano da su stabla bez simptoma takođe bila pozitivna na PPV, što ukazuje da su potencijalno važan izvor daljih infekcija ovim virusom. Zbog rasprostranjenosti virusa, za sadnju novih zasada preporučuju se tolerantne ili hipersenzitivne sorte.

Ključne reči: virus šarke šljive, *Prunus* stabla, virusna zaraza, Vojvodina