Agronomy Research **19**(X), xxx–ccc, 2021
https://doi.org/10.15159/AR.20.238

Detection of sour and sweet cherry viruses in Ukraine

L. Pavliuk*, K. Udovychenko, I. Riaba and M. Bublyk

National Academy of Agrarian Sciences of Ukraine, Institute of Horticulture, Sadova Str., 23, UA03027 Novosilky, Kyiv region, Ukraine
*Correspondence: pavliukl.92@ukr.net

Received: October 14th, 2020; Accepted: November 20th, 2020; Published: December 10th, 2020

Abstract. One of the main obstacles on the way to successful cultivation of orchards and planting material of sour and sweet cherry is infection with viral pathogens. They can adversely affect yields, reduce plant resistance to abiotic factors and cause losses in the nursery. Therefore, it is important to determine the spread of viruses in plantations and the selection of virus-free plants for the establishment of nuclear stock collections. In order to study phytovirological state of the orchards, tests for the presence of five viral pathogens were performed: Tomato black ring nepovirus (TBRV), Cherry leaf roll nepovirus (CLRV), Apple chlorotic leaf spot virus (ACLSV), Petunia asteroid mosaic tombusvirus (PeAMV), Plum pox virus (PPV). The level of infection with these pathogens reaches 19.2% for sour cherry, 5.8% - for sweet cherry, and 7.4% - for rootstocks. PPV prevailed in sour cherry material - 7%, in sweet cherry material - ACLSV (3.6%), while the rootstock samples were most infected by PeAMV - 5.6%. Until now, this virus has not been reported in Ukraine. TBRV and ACLSV viruses have been known in other crops so far, while not yet been detected in sour and sweet cherry. It was determined that PPV isolates (Mahaleb 1, Mahaleb 2) which were detected in sour cherry, belong to strain D.

Key words: ELISA, TBRV, CLRV, ACLSV, PeAMV, PPV, phylogenetic.

INTRODUCTION

The modern horticultural industry involves the use of planting material that is free of viral pathogens and meets current standards. Growing planting material of cherry in Ukraine today is guided by EPPO PM 4/29 (1) standard, which includes 15 viral pathogens. The distribution of many viruses in domestic plantations is still unclear. Survey of propagation stock orchards of sour cherry, sweet cherry and their clonal rootstocks, carried out in 2018, has not detected the presence of TBRV, CLRV, ACLSV, ApMV and PPV (Pavliuk et al., 2019). Although it is previously known that all these viruses, except for PeAMV, were found in Ukraine in other types of sour and sweet cherry plantations, or in other crops (Gospodaryk et al., 2005; Tryapitsyna & Vasiuta, 2010; Tryapitsyna et al., 2013; Kutsenko et al., 2019).

In addition, new Plum pox virus (PPV) strains have recently been reported in neighboring countries, infecting sour and sweet cherries, including PPV-CR (Cherry Russian) and PPV-CV (Cherry Volga) strains (Chirkov et al., 2017, 2018). Therefore, there is a need to test plant material for this virus. Typical symptoms of PPV are

appearance of chlorotic spots, or slightly pigmented yellow rings or lines. The fruits deform and abscise prematurely (Dehkordi et al., 2017). Virus of the C strain can also affect yield and fruit quality. The fruits do not ripen, and necrosis is observed on sweet cherry branches (Nemchinov et al., 1996). In general, PPV is considered to be one of the most destructive pathogens of *Prunus* species, crop losses can reach 30-40%, and in some years on sensitive varieties - 70–90% (Yusko, 2009). In 2006, losses caused by PPV worldwide were estimated at around € 10 billion in total over the last 30 years (Cambra et al., 2006).

Therefore, we included PPV to the list of tested pathogens. As of today, it has been identified in 10 regions of Ukraine (Odessa, Cherkasy, Kyiv, Kharkiv, Ivano-Frankivsk, Vinnytsia, Lviv, Mykolaiv, Ternopil, Chernivtsi regions) and the Autonomous Republic of Crimea. Private, collection, selection, and propagation stock orchards of certain stone fruit crops were checked for this pathogen. Crops such as plum, myrobalan plum, apricot, and peach were mainly tested. Sour and sweet cherry were tested selectively (Kondratenko & Udovichenko, 2006; Tryapitsyna, 2016; Kutsenko et al., 2019).

Thus, the aim of our research is to determine the spread of uncommon viral pathogens in sour and sweet cherry and their rootstocks.

MATERIALS AND METHODS

During the vegetation period of 2018–2020, 291 samples were tested, including 138 - of sweet cherry, 99 - of sour cherry, 54 - of clonal rootstocks. Samples were collected from 10 regions of Ukraine (Ivano-Frankivsk, Kyiv, Zaporizhzhya, Ternopil, Donetsk, Kharkiv, Kherson, Transcarpathian, Dnepropetrovsk, Cherkasy regions). A total of 17 sweet cherry cultivars, 13 sour cherry cultivars and 4 types of rootstocks were tested. Testing was performed by Enzyme-linked immunosorbent assay (ELISA) method using Loewe Biochemica GmbH (Germany) and Bioreba AG (Switzerland) commercial test kits. ELISA was performed according to standard methods of M. Clark and A. Adams (1977). Test tissue was taken from the basal part of the leaf, then it was homogenized with the addition of conjugate buffer with 1:20 ratio. Test results were read on a microplate spectrometer ImmunoChem-2100 Microplate Reader (USA), at a wavelength of 405 nm. Analysis of variance of the research data was performed using STATISTICA software, at confidence level $p < 0.05$. Positive PPV samples were further tested by reverse transcription polymerase chain reaction (RT-PCR) method to confirm the presence of the virus in the plant and to establish its strain affiliation. Fresh plant tissues were used for total RNA extraction, and isolation was performed using a commercial RNeasy Plant Mini kit (Qiagen, UK) according to the manufacturer's instructions. A commercial Verso 1-Step RT-PCR Kit ReddyMix (Thermo Scientific, USA) was used according to the manufacturer's recommendations to set up the reaction. The following components were used to carry out one reaction with a volume of 20 µl: 2X 1 Step PCR ReddyMix - 10 µl, Verso Enzyme Mix - 0.4 µl, RT Enhancer - 1 µl, Primer forward - 0.4 µl (10 mmol), Primer reverse - 0.4 µl (10 mmol), H_2O - 6.6 µl, RNA - 50 ng per reaction. The following primers were used for molecular identification - P1: 5'-ACC GAG ACC ACT ACA CTC CC-3' and P2: 5'-CAG ACT ACA GCC TCG CCA GA-3', proposed by Wetzel et al. (1991). The expected amplification product is 243 bp. Primers to the *Nad-5* mitochondrial dehydrogenase gene, with an expected 181 bp fragment, were used to control reverse transcription (Menzel et al., 2002).

Amplification was performed in a programming thermostat 'Ependorf Mastercycler Personal' (Ependorf AG, Germany) according to the following parameters: 55 °C - 15 min, 95 °C - 2 min, 40 amplification (95 °C - 20 s, 60 °C - 30 s, 72 °C - 1 min), final elongation at 72 °C - 5 min.

The presence of amplification fragments was checked by separating the PCR products in 2% agarose gel, with TBE buffer with the addition of ethidium bromide.

The resulting amplicons were sequenced. Nucleotide sequences were compared with those deposited in the Genbank (www.ncbi.nlm.nih.gov) PPV isolates using BLAST software. Sequence alignment was performed using CLUSTAL W option in MEGA X software (Kumar et al., 2018). The construction of the phylodendrogram was performed by Neighbour-Joining method (NJ) (Saitou & Nei, 1987), using bootstrap 500 analysis (Felsenstein, 1985).

RESULTS AND DISCUSSION

Enzyme-linked immunosorbent assay

Serological testing of the material of sour cherry, sweet cherry, and rootstocks showed that the general level of infection of sour cherry was 19.2% (± 0.08), while sweet cherry and rootstocks were infected only by 5.8% (± 0.04) and 7.4% (± 0.07), respectively.

In general, all viruses for which plant material was tested were detected in sour and sweet cherry cultivar samples (Table 1). In sour cherry samples, PPV prevailed - 7%, while in sweet cherry it was 1.4%, and in rootstock samples - 1.9%. Monitoring data from foreign colleagues indicates the absence of PPV in the collection plantations of sour and sweet cherry in Serbia (Mandic et al., 2007) and in stone crop orchards of Isparta province (Turkey) (Çevik et al., 2011). In Bosnia and Herzegovina, on the other hand, in the orchards of the same type of stone crops (including sour and sweet cherry), the virus was present in 47% of the tested material (Matić et al., 2008).

Table 1. Distribution of infection to different viruses, % (2018-2020)

Tested plants	Number of samples tested (n)	Viruses, %				
		PPV	PeAMV	ACLSV	TBRV	CLRV
Prunus avium	138	1.4 ± 0.02	1.4 ± 0.02	3.6 ± 0.03	0.7 ± 0.01	0.7 ± 0.01
Prunus cerasus	99	7 ± 0.05	1 ± 0.02	3 ± 0.03	5 ± 0.05	2 ± 0.03
Rootstocks	54	1.9 ± 0.04	5.6 ± 0.06	1.9 ± 0.04	-	-

We detected PeAMV for the first time in Ukraine. Until now, there were no reports of its discovery, and it was considered absent in sour and sweet cherry orchards. The highest level of infection was observed in rootstock samples - 5.6%, in sweet and sour cherry samples - 1.4 and 1%, respectively. In general, this virus has been reported in stone fruit crops in Canada, Germany, Switzerland, Latvia, and Serbia (Koenig & Kunze, 1982; Mandic et al., 2007; Gospodaryk et al., 2013), however, there is no monitoring data on the level of infection of the orchards with this pathogen. In Latvia, infection with this virus was found on 'Kubanskaya Kometa' and 'Skoroplidna' plum cultivars. These varieties are popular in Ukraine and are used in home gardens and industrial orchards.

Here it occurred as a complex of infections togsether with ArMV, TBRV and SLRV (Gospodaryk et al., 2013). In our research, PeAMV was found on rootstock samples, 'Bigarreau Burlat' sweet cherry cultivar and 'Shalun'ia' sour cherry cultivar.

ACLSV is characterized by cosmopolitan distribution, making it one of the most economically important pathogens. In addition to severe deformation of leaves and fruits, cracking of the bark, incompatibility of scion-rootstock combinations (Desvignes & Boye, 1989; Pasquini et al., 1998), infected plants become more susceptible to bacterial and fungal diseases (Zawadzka, 1989). The level of ACLSV infection in investigated plantations of sweet cherry (3.6%), sour cherry (3%) and rootstocks (1.9%) was significantly lower than in fruiting orchards of sweet cherry in Spain, where the infection with this virus reached 16% (Sánchez et al., 2015). In the Mediterranean region, the rate of ACLSV infection for sour and sweet cherry was 14% (Myrta & Savino, 2008), in Bosnia and Herzegovina in plantations of stone fruit crops, virus was present in 4% of the tested material (Matić et al., 2008), the same result (4%) was obtained when testing sweet and sour cherry cultivars in Serbia (Mandic et al., 2007). The lowest level of infection with this pathogen was found in Isparta province (Turkey), where only 1% of samples of sour and sweet cherry were infected with this virus (Çevik et al., 2011). Despite the wide spread of ACLSV, it was not detected in sour cherry orchards in Algeria (Rouag et al., 2008). In sour cherry orchards of Palestine, the infection rate reached 25%, but PPV, TBRV and CLRV viruses were not detected there (Jarrar et al., 2001).

Tomato black ring nepovirus was identified only in sweet (0.7%) and sour cherry (5%) orchards. Tested rootstock samples were not infected with this pathogen. In other types of sour and sweet cherry plantations, the virus was not previously identified. However, it is widespread in Ukraine in raspberry plantations - 29.6% (Tryapitsyna et al., 2013). Typical symptoms may include chlorotic spots, mosaic, necrotic lesions, ring spots or patterns, and deformation of the fruit (Šneideris & Staniulis, 2014). In Poland, this pathogen has been identified on elderberry (*Sambucus nigra)* (Pospieszny et al., 2004), in Lithuania - on tomato (*Lycopersicon esculentum*), rhubarb (*Rheum rhabarbarum* L), strawberry (*Fragaria vesca* L) (Šneideris & Staniulis, 2014). As one can see, the range of host plants of this virus is quite wide, so many plants can be reservoirs for its accumulation, which in turn increases the likelihood of infection of the main host plants.

According to the preliminary data, the collection plantations of sweet cherry in Ukraine are infected with CLRV by 21.63%, while sour cherry - by 16.19% (Tryapitsyna & Vasiuta, 2010). In Russia, the virus was detected in plantations of sour, sweet cherry and their rootstocks in 8.9-28.6% of the samples (Upadyshev et al., 2017). Data from our research indicate the presence of the pathogen in 0.7% of sweet cherry and 2% of sour cherry. On infected cherry trees, virus causes deformation and twisting of leaves, mosaic, as well as growth retardation and death of individual branches or the whole tree (Tryapitsyna & Vasiuta, 2010).

Complex pathogen infection was also identified: PPV + ACLSV was detected in samples of sour cherry (1% ± 0.02) and rootstocks (1.9% ± 0.03), while sweet cherry had a complex infection with PPV + TBRV (0.5% ± 0.01). As these viral pathogens do not have common vectors of carriers, it is obvious that infection occurred due to non-compliance with the requirements of care and agronomic measures.

Territorial distribution of pathogens

Analysing the geographical distribution of viral pathogens, one see that all studied viruses were found in Donetsk region, except for TBRV. According to the State Statistics Service, sour and sweet cherry plantations in this region occupy about 1,300 ha. This region is one of the largest regions for growing sour and sweet cherry.

The spread of some viruses may depend on the variety of strains and isolates that may infect plants selectively, focusing on the host plant rather than the range (Rebenstorf et al., 2006). For example, the CLRV strain that infects walnut (*Juglans regia* L.) is not transmitted to sour, sweet cherry and birch (Langer et al., 2009). According to some studies, in Ukraine the Polissya (the north-western part of the country) area is the most vulnerable to the spread of this pathogen, as it hosts the largest number of plants that can be reservoirs of pathogen accumulation and source of its spread (Tryapitsina & Vasiuta, 2010). However, in our research, CLRV was identified only in Donetsk and Kharkiv regions, thus refuting previous assumptions.

When analysing the distribution by region, one can observe a sporadic spread of viral pathogens, rather than some specific pattern. Most likely, this distribution is a consequence of the human factor - non-compliance with sanitary requirements for agronomic measures and the spread of infected planting material.

Infection of tested cultivars and types of rootstocks

In total, 17 cultivars of sweet cherry, 13 cultivars of sour cherry, and four types of rootstocks were tested. Among the sweet cherry material, 'Bigarreau Burlat', 'Donetskyi Uhol`ok', 'Donetska Krasavitsa', 'Nizhnist' and 'Krupnoplidna' cultivars were infected (Table 2). The most infected samples were found in Bigarreau Burlat - 11.8%. This cultivar is of French origin, dating from 1915. Since then, planting material has spread to Central Europe, and later the cultivar became popular in Ukraine. Apparently, over the years of its spread there was an uncontrolled reproduction of planting material, which caused a high level of infection.

Table 2. Infection of cultivars of the tested material

Cultivar	Number of samples tested (n)	Infected samples, %
Bigarreau Burlat	17	11.8 ± 0.15
Donetskyi Uhol'ok	11	9 ± 0.17
Donetska Krasavitsa	13	7.7 ± 0.14
Nizhnist'	17	5.9 ± 0.11
Krupnoplidna	21	4.8 ± 0.09
Shalun'ia	14	28.6 ± 0.24
Bohuslavka	15	20 ± 0.20
Kseniia	31	3.2 ± 0.06
P.mahaleb	18	22.2 ± 0.19

'Donetskyi Uhol`ok' and 'Donetska Krasavitsa' cultivars, infection level of which was 9 and 7.7%, respectively, are cultivars of selection of Bakhmut Resarch Station of Horticulture, in the vicinity of which the largest localization of pathogens was detected. 'Valery Chkalov', 'Kitaivska Chorna', 'Melitopolska Chorna', 'Talisman' and 'Udivitelna' cultivars were free from the tested pathogens.

In contrast to sweet cherry, in tested sour cherry samples three infected cultivars were found - 'Shalun'ia' (28.6%), 'Boguslavka' (20%) and 'Kseniia' (3.2%), while 'Vstrecha' was virus-free. CLRV predominated in the samples of 'Shalun'ia' cultivar, while PPV prevailed in 'Boguslavka' samples.

Today, both seedling (*P. mahaleb*) and clonal (VSL-2, Colt, Studenykivska) rootstocks are used in industrial sweet and sour cherry orchards in Europe (Jänes & Pae,

2004; Maas et al., 2014; Vasyuta & Ryabyi, 2017; Csihon et al., 2018). Analysed Colt, VSL-2 and Studenykivska rootstock samples were free of the tested pathogens, while *P. mahaleb* smaples were infected. These samples were dominated by PeAMV. The ability of these viruses to be transmitted by seeds has not been reliably confirmed, but their particles were isolated from the seeds of sour and sweet cherry plants (Pfeilstetter et al., 1992).

Strain identification of Plum pox virus

Strain affiliation of PPV, which circulates in Ukraine and infects sour and sweet cherries, has not been previously studied. Of the nine known strains, only C, CR and CV are thought to be able to infect sour and sweet cherry (Chirkov et al., 2017, 2018; James et al., 2013; García et al., 2014). CV strain can cause typical chlorotic spots on sour cherry (Chirkov et al., 2017, 2018). In Germany, the same symptoms were observed on leaves of sour cherry infected with C strain, as well as leaf deformation and dark rings on the fruits (Jelkmann et al., 2018).

A potential vector of the pathogen is aphids. It is believed that virus can be transmitted by roughly 20 species of aphids, but the most effective vectors are *Aphis craccivora, A. spiraecola, A. cardui, Brachycaudus helichrysi, B. cardui, Hyalopterus pruni, Myzus persicae, Phorodon humuli* (Gaborjanyi & Basky 1995; Labbone et al., 2001). It is known that some of them are common in Ukraine and other European countries (Yusko et al., 2008; Latinović et al., 2017; Musa et al., 2020). These insects can easily be the carriers of the pathogen, because virus particles together with the sap of infected trees can be stored for about 4 hours in the food apparatus of the insect, while the insect can change the plant it feeds on during that period, thus spreading infection in the orchards (Jordovic, 1968). However, the effectiveness of this way of virus transmission depends on the strain, cultivar and species of the host, aphid species, and season (Levy et al., 2000).

After ELISA testing, samples positive with PPV were further tested by RT-PCR. The analysis confirmed the presence of this virus in all tested samples which was confirmed by the presence of the expected fragment of amplification. Two samples of *P. mahaleb* rootstocks from Zaporizhzhia region were chosen for sequencing.

After sequencing, the resulting fragments of Mahaleb 1 (MW055900) and Mahaleb 2 isolates were equal to 243 bp, which reffered the position 9313-9555 in the viral genome. According to the results of comparison of our isolates with known ones, its affiliation to D strain was established. To date, it has been investigated that D strain of PPV can cause epidemics in peach, apricot and plum orchards (Gottwald et al., 1995; Dallot et al., 1998; Polák & Komínek, 2009). In contrast to the samples affected by cherry strains, which were described by other authors (Chirkov et al., 2017, Jelkmann et al., 2018), our infected samples did not show symptoms of virus infection on the leaves.

Direct analysis of all Ukrainian isolates of this virus present in the GenBank (www.ncbi.nlm.nih.gov) has indicated that 83% of them belong to D strain. The other 17% of isolates belong to strains W, M and Rec. The host plants are mainly peach, apricot and plum. Mahaleb 1 and Mahaleb 2 isolates are the first PPV isolates of D strain isolated from *P. mahaleb* in Ukraine.

For phylogenetic analysis, all known isolates of PPV circulating in Ukraine, as well as representatives of cherry strains C, CR, CV were selected.

Since this segment is highly conservative, at least 272 known isolates from different countries and crops have been found to be 100% identical to our isolates. Mahaleb 1 and Mahaleb 2 isolates showed a high level of identity between themselves - 100%. When comparing all Ukrainian isolates, variability within the group was found, identity ranged between 89.5–100%, and the overall mean identity was 97%. Since some isolates were distant by nucleotide sequences, they were included in a separate group (IV) on the phylogenetic tree (Fig. 1). Thus, MK209075 (Kharkiv region, apricot) and MK209074 (Odessa region, plum) isolates turned out to be distant. By nucleotide sequences, the identity of our isolates between them was 94 and 89.5%, respectively.

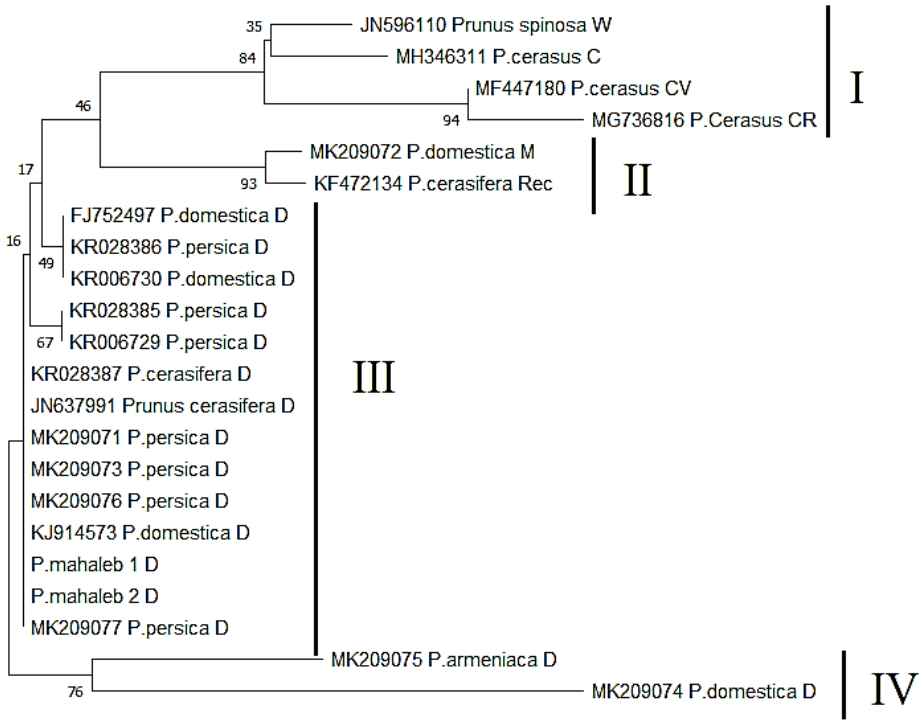

Figure 1. Phylogenetic tree of Plum pox virus isolates reconstructed using a 149-bp nucleotide fragment polyprotein gene (position of 9369-9517) constructed by Neighbour-Joining method.

Mahaleb isolates were classified in group III, together with the most identical (100%) isolates of this strain: plum isolate KJ914573, peach isolates MK209077, MK209076, MK209073, MK209071, and myrobalan plum isolates JN637991 and KR028386.

Representatives of cherry strains combined a separate group, together with isolate JN596110, which belongs to strain W. Mahaleb isolates are identical by 90% to isolate MG736816 (CR), by 92.5% to isolate MF447180 (CV), and by 93.3% to MH346311 (C).

Isolates of M and Rec strains also formed a separate group. When comparing Mahaleb isolates with isolates belonging to these strains, an identity level of 95% was observed.

CONCLUSIONS

This research demonstrates the presence of previously undetected sour and sweet cherry viruses in Ukraine. The presence of a panel of viral pathogens identified by EPPO PM 4/29 (1) indicates the need to test plant material and comply with sanitary requirements when maintaining orchards and when propagating material to prevent the spread of pathogens with planting material. Detection of viruses that have not been previously reported in Ukraine (PeAMV) and in sour cherry orchards (TBRV, ACLSV) once again confirms the need for testing of plant material and further investigation of their molecular characteristics.

Since PPV isolates identified by us belong to D strain, it is necessary to further determine its ability to be transmitted to plants that are more susceptible to the virus, as well as to investigate its harmfulness in sour and sweet cherry orchards.

REFERENCES

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.

Çevik, B., Yardimci, N. & Çulal-Kiliç, H. 2011. Detection of viruses infecting stone fruits in western Mediterranean region of Turkey. *Plant Pathology Journal* **27**, 44–52. https://doi.org/10.5423/PPJ.2011.27.1.044

Chirkov, S., Ivanov, P., Sheveleva, A., Zakubanskiy, A. & Osipov, G. 2017. New highly divergent Plum pox virus isolates infecting sour cherry in Russia. *Virology* **502**, 56–62.

Chirkov, S., Sheveleva, A., Ivanov, P. & Zakubanskiy, A. 2018. Analysis of genetic diversity of Russian sour cherry Plum pox virus isolates provides evidence of a new strain. *Plant Disease* **102**, 569–575. https://doi.org/10.1094/PDIS-07-17-1104-RE

Clark, M.F. & Adams, A.N. 1977. Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses. *Journal of General Virology* **34**(3), 475–483. https://doi.org/10.1099/0022-1317-34-3-475

Csihon, Á., Bicskei, D.K., Dremák, P. & Gonda, I. 2018. Performance of sweet cherry cultivars grafted on Colt rootstock. *International Journal of Horticultural Science* **24**(1–2), 7–10. https://doi.org/10.31421/IJHS/24/1-2./1540

Dallot, S., Labonne, G., Boeglin, M., Quiot-Douine, L., Quiot, J.B. & Candresse, T. 1998. Peculiar plum pox potyvirus D – populations are epidemic in peach trees. *Acta Horticulturae* **472**, 355–365. https://doi.org/10.17660/ActaHortic.1998.472.41

Dehkordi, A.N., Babaeian, N., Karimpour, S., Martínez-Gómez, P., Rubio, M. & Bagheri, N. 2017. Sharka (Plum pox virus): A forgotten disease in Iran. *International Journal of Horticultural Science and Technology* **4**(2), 183–191. https://doi.org/10.22059/IJHST.2017.241714.205

Desvignes, J.C. & Boye, R. 1989. Different diseases caused by chlorotic leaf spot virus on the fruit trees. *Acta Horticulturae* **235**, 31–38. https://doi.org/10.17660/ActaHortic.1989.235.3

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791. https://doi.org/ 10.2307/2408678

Gaborjanyi, R. & Basky, S. 1995. Correlation between migration of aphid vectors and natural spread of plum pox virus. *Acta Horticulturae* **386**, 201–206. https://doi.org/10.17660/ActaHortic.1995.386.26

García, J.A., Glasa, M., Cambra, M. & Candresse, T. 2014. Plum pox virus and sharka: a model potyvirus and a major disease. *Molecular Plant Pathology* **15**(3), 226–241. https://doi.org/10.1111/mpp.12083

Gospodaryk, A., Moroèko-Bièevska, I., Pûpola, N. & Kâle, A. 2013. Occurrence of stone fruit viruses in plum orchards in Latvia. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences* **67**(2), 116–123. https://doi.org/10.2478/prolas-2013-0018

Gospodaryk, A.V., Udovychenko, K.M. & Polishchuk, V. 2005. Diagnosis of fruit crop viruses in the conditions of Ukraine. *Scientific notes of NUKMA. Biology and ecology* **43**, 51–53 (in Ukrainian).

Gottwald, T.R., Avinent, L., Llácer, G., Hermoso de Mendoza, A. & Cambra, M. 1995. Analysis of the spatial spread of sharka (plum pox virus) in apricot and peach orchards in eastern Spain. *Plant Disease* **79**, 266–278. https://doi.org/10.1094/PD-79-0266

James, D., Varga, A. & Sanderson, D. 2013. Genetic diversity of Plum pox virus: strains, disease and related challenges for control. *Canadian Journal of Plant Pathology* **35**(4), 431–441. https://doi.org/10.1080/07060661.2013.828100

Jänes, H. & Pae, A. 2004. Evaluation of nine sweet cherry clonal rootstocks and one seedling rootstock. *Agronomy research* **2**(1), 23–27.

Jarrar, S., Myrta, A., Di Terlizzi, B. & Savino, V. 2001. Viruses of stone fruits in Palestine. *Acta Horticulturae* **550**, 245–248. https://doi.org/10.17660/actahortic.2001.550.34

Jelkmann, W., Sanderson, D., Berwarth, C. & James, D. 2018. First detection and complete genome characterization of a Cherry (C) strain isolate of plum pox virus from sour cherry (Prunus cerasus) in Germany. *Journal of Plant Diseases and Protection* **125**(3), 267–272. https://doi.org/10.1007/s41348-018-0155-7

Jordovic, M. 1968. The effects of sources of infection on the epidemiology of plum pox disease. *Tagunsber* **97**, 301–308.

Koenig, R. & Kunze, L. 1982. Identification of Tombusvirus isolates from cherry in southern Germany as Petunia asteroid mosaic-virus. *Phytopathol. Z.* **103**, 361–368. https://doi.org/10.1111/j.1439-0434.1982.tb01760.x

Kondratenko, P. & Udovichenko, V. 2006. Plum pox virus (PPV) in Ukraine. *EPPO Bulletin* **36**(2), 217. https://doi.org/10.1111/j.1365-2338.2006.00974.x

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549. https://doi.org/10.1093/molbev/msy096

Kutsenko, O.V., Budzanivska, I.G. & Shevchenko, O.V. 2019. Genetic diversity of Plum pox virus in Ukraine. *Biopolymers and Cell* **35**, 476–485. https://doi.org/10.7124/bc.000A1A

Labbone, G. & Quiot, J.B. 2001. Aphids can acquire Plum pox virus from infected fruits. *Acta Horticulturae* **550**, 79–82. https://doi.org/10.17660/ActaHortic.2001.550.8

Langer, J., von Bargen, S. & Büttner, C. 2009. Epidemiological investigations on Cherry leaf roll virus. *Crop Plant Resistance to Biotic and Abiotic Factors: Current Potential and Future Demands*: Proceedings of the 3rd International Symposium on Plant Protection and Plant Health in Europe held at the Julus Kühn-Institut (Berlin-Dahlem, Germany, 14–16 May 2009), pp. 568.

Latinović, N., Karamaouna, F. & Kavallieratos, N.G. 2017. First record of Aphis craccivora Koch (Hemiptera: Aphididae) on aronia crop in Montenegro. *Hellenic Plant Protection Journal* **10**, 67–69. https://doi.org/10.1515/hppj-2017-0007

Levy, L., Damsteegt, V., Scorza, R. & Kolber, M. 2000. Plum Pox Potyvirus Disease of Stone Fruits. *APSnet Feature Articles.* https://doi.org/10.1094/apsnetfeature-2000-0300

Maas, F.M., Balkhoven-Baart, J. & Van Der Steeg, P.A.H. 2014. Evaluation of Krymsk-5 (VSL-2) and Krymsk-6 (LC-52) as rootstocks for sweet cherry 'Kordia'. *Acta Horticulturae* **1058**, 531–536. https://doi.org/10.17660/actahortic.2014.1058.66

Mandic, B., Matić, S., Al Rwahnih, M. & Myrta, A. 2007. Viruses of sweet and sour cherry in Serbia. *Journal of Plant Pathology* **89**, 103–108.

Matić, S., Rwahnih, M., Myrta, A. & Đurić, G. 2008. Viruses of stone fruits in Bosnia and Herzegovina. *Acta horticulturae* **781**, 71–74.

Menzel, W., Jelkmann, W. & Maiss, E. 2002. Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. *Journal of Virological Methods* **99**(1–2), 81–92. https://doi.org/10.1016/s0166-0934(01)00381-0

Musa, F., Krasniqi, D. & Musa, S. 2020. Aphid complex associated with potato in a gro-climatic conditions of Kosovo. *Agronomy Research* **18**(1), 206–215. https://doi.org/10.15159/AR.20.074

Myrta, A. & Savino, V. 2008. Virus and virus-like diseases of cherry in the Mediterranean region. *Acta Horticulturae* **795**, 891–896. https://doi.org/10.17660/ActaHortic.2008.795.144

Nemchinov, L., Hadidi, A., Maiss, E., Cambra, M., Candresse, T. & Damsteegt, V. 1996. Sour cherry strain of plum pox potyvirus (PPV): Molecular and serological evidence for a new subgroup of PPV strains. *Phytopathology* **86**, 1215–1221.

Pasquini, G., Faggioli, F., Pilotti, M., Lumia, V. & Barba, M. 1998. Characterization of apple chlorotic leaf spot virus isolates from Italy. *Acta Horticulturae* **472**, 195–202. https://doi.org/10.17660/ActaHortic.1998.472.20

Pavliuk, L., Riaba, I., Udovychenko, K., Tryapitsyna, N. & Bublyk, M. 2019. Phyto-virologic state of parent plantings of cherry and mazzard cherry in Ukraine. *Bulletin of Agricultural Science* **97**(7), 20–26. https://doi.org/10.31073/agrovisnyk201907-03

Pfeilstetter, E., Zinkernagel, Y. & Kunze, L. 1992. Occurrence of petunia asteroid mosaic virus (PAMV) and carnation Italian ringspot (CIRV) viruses in cherry orchards in northern Bavaria. *Acta Horticulturae* **309**, 345–352. https://doi.org/10.17660/ActaHortic.1992.309.50

PM 4/29 (1) Certification scheme for cherry. *EPPO Bulletin* **31**, 2000. 447–462.

Polák, J. & Komínek, P. 2009. Distribution of Plum pox virus strains in natural sources in the Czech Republic. *Plant Protection Science* **45**, 144–147. https://doi.org/10.17221/31/2009-PPS

Pospieszny, H., Borodynko, N. & Jończyk, M. 2004. First report of Tomato black ring virus (TBRV) in the natural infection of Sambucus nigra in Poland. *Journal of Plant Protection Research* **44**, 373–376.

Rebenstorf, K.T., Candresse, T., Dulucq, M.J., Büttner, C. & Obermeier, C. 2006. Host Species-Dependent Population Structure of a Pollen-Borne Plant Virus, Cherry Leaf Roll Virus. *Journal of Virology* **80**(5), 2453–2462. https://doi.org/10.1128/JVI.80.5.2453-2462.2006

Rouag, N., Guechi, A., Matic, S. & Myrta, A. 2008. Viruses and viroids of stone fruits in Algeria. *Journal of Plant Pathology* **90**, 393–395. http://dx.doi.org/10.4454/jpp.v90i2.680

Saitou, N. & Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sánchez, R.P., Corts, R.M., Benavides, P.G. & Sánchez, M.Á.G. 2015. Main viruses in sweet cherry plantations of Central-Western Spain. *Scientia Agricola* **72**(1), 83–86. https://doi.org/10.1590/0103-9016-2014-0140

Šneideris, D. & Staniulis, J. 2014. Phylogenetic analysis of Lithuanian tomato black ring virus isolates. *Zemdirbyste-Agriculture* **101**(2), 193–198. https://doi.org/198.10.13080/z-a.2014.101.025

Tryapitsina, N.V. & Vasiuta, S.O. 2010. Regional features of the spread of nepovirus of sweet cherry leaf twisting in sour and sweet cherry orchards. *Agricultural microbiology* **12**, 130–139 (in Ukrainian).

Tryapitsyna, N.V. 2016. *Biotechnological basics of obtaining planting material of stone (Prunus spp.) and berry (Ribes spp., Rubus spp., Fragaria x ananassa) crops in Ukraine*. PhD Thesis. Kyiv, 390 pp. (in Ukrainian).

Tryapitsyna, N.V., Yareshchenko, O.M., Medvedeva, T.V., Udovychenko, K.M., Lushpigan, O.P. & Suprun, K.I. 2013. Dominant viruses in bushy small fruit crops patches. *Horticulture* **67**, 193–203 (in Ukrainian).

Upadyshev, M.T., Metlitskaya, K.V. & Petrova, A.D. 2017. Prevalence of virus diseases of fruit and berry crops. *Fruit growing and viticulture of South Russia* **44**(02), 5–16 (in Russian).

Vasyuta, S.O. & Ryabyi, V.Y. 2017. Peculiarities of using some clonal rootstocks of stone fruit crops for the sweet cherry (*Cerasus avium* Moench.) cultivation. *Horticulture* **72**, 65–73.

Wetzel, T., Candresse, T., Ravelonandro, M. & Dunez, J. 1991. A polymerase chain reaction assay adapted to plum pox potyvirus detection. *Journal of Virological Methods* **33**, 355–365.

Yusko, O.S. 2009. *Epidemiology of Plum pox virus in the Transcarpathian region*. PhD thesis summary in speciality 03.00.06 "Virology". Kyiv, 20 pp. (in Ukrainian).

Yusko, O.S., Chumak, V.O. & Snigur, G.O. 2008. Aphids are potential vectors of plum pox virus in Transcarpathian. *Scientific Bulletin of the Uzhhorod University. Series Biology* **24**, 96–99 (in Ukrainian).

Zawadzka, B. 1989. The influence of virus and mycoplasma diseases on frost damage of apple trees. *Acta Horticultulturae* **235**, 59–68. https://doi.org/10.17660/ActaHortic.1989.235.7