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Phyto-virologic state of parent plantings of cherry and mazzard cherry in Ukraine

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The purpose. To select pure clones of female plants and to clarify spread in domestic plantings of cherry, mazzard cherry and their stocks of virus pathogens which were not widely study. **Methods**. Visual diagnostic study of plantings, immunoenzymatic analysis, statistical methods for data processing. **Results.** During visual diagnostic study of plantings of asymptomatic plants they selected 90 samples of mazzard cherry, 63 samples of cherry and 33 — of clonal stocks. Their inspection on presence of 11 viruses proved that the level contamination of mazzard cherry (16,7%) was considerably above, than that of cherry (9,5%). In the probed samples of varieties presence of viruses only from two taxonomic groups (of stems Ilarvirus and Nepovirus), namely VKS (8,7%), VNKP (11,8), VMR (6,7), VLKPS (6,7), VKPM (1,1%), and also their complex infection contaminations was revealed. In stocks VSL-2, Kolt and Studenykivska viruses were not revealed. It is determined that the most spread pathogen in plantings of both crops was VNKP (11,8%). It goes into all the identified complexes from 2 and more viruses. Among 11 tested varieties of mazzard cherry the most infected were Valerii Chkalov and Kazka (50%). Free from viruses were samples of varieties Donetskii ugoliok, Donetskaya krasavitsa, Nizhnist, Melitopolska chorna, and Udivitelnaya. At the same time samples of 3 varieties of cherry — Vidrodzhennia and Solidarnist (33%), Ksenia (12%) — were infected. **Conclusions**. Spread of virus pathogens to plantings of cherry and mazzard cherry in 6 regions of Ukraine is fixed. Pure plants of all tested varieties are selected for the further inspection on presence of virus pathogens by OT-PCR method with the purpose to takeoff plants in initial clones which can be used for reproduction according to standard schemes of certification of these crops. The state of execution of technology requirements on saving virus-free status of parent plantings in equipments of different patterns of ownership is analyzed and measures on prevention of spread of viruses in parent and industrial plantings are offered.

Key words: *viruses, cherry, mazzard cherry, IFA, PCR.*

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Sour and sweet cherry are valuable fruit crops. According to the State Statistics Service, Ukraine produced more than 300 thousand tons of cherries and sweet cherries in 2018, the areas of these crops orchards is about 33 thousand hectares. Most of the sour cherry plantations are located in the Dnipropetrovs'k (2.1 thousand hectares) and L'viv (1.9 thousand hectares) regions and those of sweet cherry are mostly in the Zaporizhzya (2.9 thousand hectares) and Dnipropetrovs'k (2.0 thousand hectares) regions. In the recent years, there is a tendency to increase the areas occupied by these crops, so there exists an obvious demand for planting stock. The major method of the horticulture intensification is to use high quality virus-free planting stock because the viral diseases can bring about significant yield losses and shortening the orchards exploitation terms [0].

Viruses are dangerous intracellular pathogens and important limiting factor in increasing the stone fruits total yield. Due to the latent nature of the disease course, they spread widely and quickly with the infected planting stock, in the contact way, when carrying out agrotechnical measures. In addition, many viruses can be transmitted in the natural way too: by vector organisms, pollen, aquatic nutrition, and the like.

The first stage in the production of virus-free planting stock is the selection of mother plants that meet the standards of the cultivar and are free from viral and other diseases. The orchards' monitoring, in order

to isolate such clones, also makes it possible in time detection of viral pathogens that is an important control measure to prevent from the introduction of viral infections into the stone fruit crops plantations in Ukraine.

"The sectorial program of the horticulture development in Ukraine for the period until 2025"[0] stipulates that the planting stock production in nursery farms of Ukraine in the future will be carried out exclusively on the virus-free basis. The production and circulation of the planting stock is regulated by the Law of Ukraine No.411-IV (December 26, 2002) “On seeds and planting stock” with edits [0]. Besides, this material is to meet to the modern international requirements, in particular, the EPPO standard PM (4) 29 (1) [0], which stipulates the control over the absence of 15 viral pathogens. Purpose is to identify pure maternal plants and make out the viral pathogens spread in the inland plantations of cherry and sweet cherry and their rootstocks. Those viruses were not studied widely yet.

Material and methods

The researches were conducted in the Department of Virology, Sanitation and Reproduction of Fruit and Small Fruit crops of the Institute of Horticulture of NAAS, in the vegetation period of 2018. Samples of cherry and sweet cherry were collected in the mother plant orchards of farms of various property forms on the territory of 6 regions of Ukraine (Kyiv, Zaporizhya, Cherkasy, Ternopil’, Donet’sk, Ivano-Frankiv’sk).

According to the results of the visual inspection samples were collected from the asymptomatic plants with the confirmed varietal identity. The detection of viruses (table 1) was carried out using the ELISA by means of the commercial test sets manufactured by Loewe Biochemica GmbH (Germany) and Bioreba AG (Switzerland) in accordance with the sets standard methods proposed in 1977 by M.Clarke and A.Adams [0]. The results were recorded by the ImmunoChem – 2100 Microplate Reader microplate spectrometer was used for the the program Exel statistical data processing.

Table 1. Viruses of sweet and sour cherry

	Ukrainian name	International name	Family	Genus
	Вірус мозаїки яблуні (ВМЯ)	*Apple mosaic ilarvirus (ApMV)*	*Bromoviridae*	*Ilarvirus*
	Вірус некротичної кільцевої плямистості кісточкових культур (ВНКП)	*Prunus necrotic ringspot ilarvirus (PNRV)*	*Bromoviridae*	*Ilarvirus*
	Вірус карликовості сливи (ВКС)	*Prune dwarf ilarvirus (PDV)*	*Bromoviridae*	*Ilarvirus*
	Вірус мозаїки резухи (ВМР)	*Arabis mosaic nepovirus (ArMV)*	*Secoviridae*	*Nepovirus*
	Вірус скручування листя черешні (ВСЛЧ)	*Cherry leaf roll nepovirus (CLRV)*	*Secoviridae*	*Nepovirus*
	Вірус чорної кільцевої плямистості томату (ВЧКТ)	*Tomato black ring nepovirus (TBRV)*	*Secoviridae*	*Nepovirus*
	Вірус кільцевої плямистості малини (ВКПМ)	*Raspberry ringspot nepovirus (RpRSV)*	*Secoviridae*	*Nepovirus*
	Вірус латентної кільцевої плямистості суниці (ВЛКПС)	*Strawberry latent ringspot nepovirus (SLRSV)*	*Secoviridae*	*Nepovirus*
	Вірус шарки сливи (ВШС)	*Plum pox virus (PPV)*	*Potyviridae*	*Potyvirus*
	Вірус хлоротичної плямистості листя яблуні (ВХПЛЯ)	*Apple chlorotic leaf spot virus (ACLSV)*	*Betaflexiviridae*	*Trichovirus*
	Вірус зіркоподібної мозаїки петунії (ВЗМП)	*Petunia asteroid mosaic tombusvirus (PeAMV)*	*Tombusviridae*	*Tombusvirus*

Results and discussion

The selected plant material was checked for the presence of 11 viral pathogens. 186 samples were tested in all, among them 90 of sweet cherry, 63 of sour cherry, 33 clonal rootstocks.

The immune diagnostics of these two crops variety samples showed that the sweet cherry infection level (16.7%) was much higher than that of sour cherry (9.5%) (fig.1).

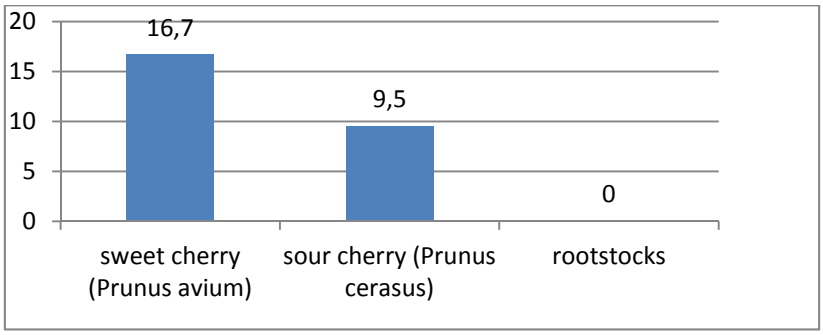

Fig.1. Infection of the tested samples from the sour and sweet cherry mother gardens and their rootstocks with viral pathogens (2018)

It should be noted that despite the rather wide list of the tested viruses, only few of them were detected. It is the Ilarviruses – PNRV and PDV that appeared the most spread both in the sour cherry and sweet cherry material. In particular only the Ilarviruses PDV (3.2%) and PNRV (6.3%) were detected in the sour cherry tested material. In the sweet cherry samples the infection level by their viruses was 5.5% each. PDV and PNRV are the most spread viral pathogens of the sour and sweet cherry plants not only in Ukraine [0]. Losses, which are caused by these two viruses in stone fruit plantations, achieve 30-50% [0, 0, 0]. The investigations conducted in different countries give us the following statistics as concerns the distribution of these pathogens in different types of plantations. In the Iberian Peninsula the infection of the sour and sweet cherry commercial orchards with PNRV was 46%, with PDV 32%, and their complex infections occurred in 15% of the cases among the tested trees [0]. In the orchards of the same type in Spain it is PDV that prevailed (62%) while PNRV spread was 13% only [0]. In Serbia PDV was detected in 16.8% of the tested samples selected in the collection plantations whereas PNRV – in 18.4%, the complex infection was 16% [0]. According to the data of Russian virologists, these viruses are the most spread in the Central region of Russia too. The sour cherry orchards affection by PDV was 37%, by PNRV 40%. The PDV and PNRV spread in the sweet cherry with plantations was almost the same – 33% and 39% respectively. The infection with these two pathogens complexes was detected in 10% of the tested material [0]. In the mothergardens of sour and sweet cherry in Czech Republic the infection with these viruses has the lowest level as compared to the other European countries: PDV – 10.2%, PNRV – 1.8% and only their complex affections is 0.3%. The presented data show that even in mothergardens where according to the technological requirements the transfer of these viruses with pollen is not possible during pollination, the PNRV and PDV are nevertheless present and poor quality material can get into production without quality control over such plantations.

Contrary to sour cherry in addition to Ilarvirus, Nepoviruses were also detected – ArMV (6.7%), SLRV (6.7%) and RRSV (1.1%) in the sweet cherry samples. The complex virus infection in various combinations: PNRV+ArMV, PNRV+SLRV, PDV+PNRV+SLRV, PDV+PNRV+SLRV+ArMV+RRSV was observed in some of this fruit crop tested samples. The above presented data show that PNRV is present in all the combinations, that indicates the high level of its spread in the orchards of the studied crops (Table 2).

Table 2. Level of the infection of mother gardens of sour, sweet cherry and their rootstocks by certain viruses, 2018

Object of researches	Infection, %								
	Mono viral infections, % on the total amount of the tested material					Complex infections			
	PDV	PNRV	ArMV	SLRV	RRSV	PNRV ArMV	PDV PNRV SLRV	PDV PNRV SLRV ArMV RRSV	PDV
Sweet cherry *(Prunus avium)*	5,5	5,5	6,7	6,7	1,1	1,1	1,1	1,1	1,1
Sour cherry *(Prunus cerasus)*	3,2	6,3	-	-		-	-	-	-
Rootstock	-	-	-	-		-	-	-	-
Total	8,7	11,8	6,7	6,7	1,1	1,1	1,1	1,1	1,1

We failed to detect CLRV, TBRV, ACLSV, PeAMV, PPV in the plant material. From the entire tested material we managed to single out virus-free plants as candidates for nuclear stock (which meet the requirements of the above mentioned standard EPPO PM (4) 29 (1) [0]) for five sweet cherry cultivars (Donets'ky Ugolyok, Donets'ka Krasavytsya, Nizhnist', Melitopol's'ka Chorna, Udivityel'na) and six of sour cherry cultivars (Alpha, Bogyslavka, Vyshnya Magalebska, Vstryecha, Lyubava, Shalunya) (Table 3).

Table 3. Level of the infection of the tested material of cvs of sour and sweet cherry and their rootstocks, 2018

Crop	Cultivar	Infection level, %
Sweet cherry *(Prunus avium)*	Anons	8
	Bigarreau Burlat	20
	Valery' Chkalyov	50
	Donets'ky Ugolyok	-
	Donets'ka Krasavytsya	-
	Krupnoplidna	12
	Nizhnist'	-
	Melitopol's'ka Chorna	-
	Kazka	50
	Talisman	33
	Udivityel'na	-
Sour cherry *(Prunus cerasus)*	Al'fa	-
	Boguslavka	-
	Vidrodzhennya	33
	Vyshnya Mahaleb	-
	Vstryecha	-
	Ksyeniya	12
	Lyubava	-
	Solidarnist'	33
	Shalunya	-
Rootstock	VSL-2	-
	Colt	-
	Studenykivs'ka	-

The infected samples were detected among 6 varieties of sweet cherry and 3 ones of sour cherry. Among the sweet cherry cultivars the highest infection level was 50% for samples of Valery Chkalov and Kazka. It should be noted that the largest complex of infections (5 viruses) was present in Valery Chkalov samples. The third part of the cherry varieties Vidrodzhennya and Solidarnist' tested material was also infected.

In general the members of only two viral genus – Nepovirus (ArMV, SLRV, RRSV) and Ilarvirus (PNRV and PDV) were identified in the cherry and sweet cherry orchards. The ways of the transmission of the representatives of these genera are somewhat different. For the Ilarviruses (PDV, PNRV) main ways of transmission are by pollen, vegetative propagation, mechanical inoculation [0, 0].

The detected Nepoviruses (ArMV, RRSV, SLRV) are transmitted mechanically, by nematodes of the families *Longidorus* and *Xiphinema diversicaudatum*. The viral particles that enter the vector body remain active for the long period (from 9 weeks for the *Longidorus* family up to 4 years for *Xiphinema*) [0].

Some of the viral pathogens we are investigating are transmitted through pollen (PDV, PNRV). But taking into consideration that the samples were collected in the motherplant orchards where tree flowering is not permitted this method of transmission can be excluded. Therefore in the farms where the material was collected, a special attention should be paid to their mechanically and vector-mediated transmission, in particular, nematodes.

Taking into account the type of the investigated plantations and methods of the viruses transmission, the following measures should be stressed to prevent pathogens` spread:

in the mother gardens to avoid the plants flowering that can bring about the infection with pathogens that are spread by means of pollen;

for mechanically transmitted pathogens to disinfect the instruments while agrotechnical measures are carried out;

to conduct the soil inspection for the presence of the nematodes, which may be vectors of the Nepoviruses and if they are detected, to disinfect the soil during the calendar year;

in order to prevent the virus transmission by insects it is necessary to use insecticide in time;

to avoid the plantations weeding since weeds can be an intermediate link between a tree and a pathogen.

Conclusions the carried out diagnostic made it possible to detect the infection of the tested mother plant orchards of the stone fruit crops achieved 11.2%. That indicates the actuality of the orchards monitoring, in order to identify and eliminate outbreaks of viral diseases and to prevent their spread.

Plants were selected for the further testing using the RT-PCR method in order to isolate the plants candidates for initial clones, which can be used as candidates for nuclear stock for the reproduction in stages according to the standards of these crops certification.

The vegetative rootstocks have appeared free from viral pathogens. That confirms the high level of the compliance with all the requirements when establishing orchards of this type, namely: the selection of virus-free planting material and appropriate level of the agrotechnical measures directed to preventing from the virus reinfection.

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