To define biological properties of the pathogens, we conducted a host assay using indicator plants. Indicator plants were inoculated with sap obtained from cactus plants demonstrating virus-like symptoms. Necrotic local lesions observed on *Chenopodium murale* and *Gomphrena globosa* were typical for Cactus virus X (CVX) [5,6]. In indirect ELISA we observed positive reactions with sap *Mamillaria microhelia, Sulcorebutia sp., Bolivicereus samupatanus, Opuntia sp., Ferocactus sp., Mamillaria magnimamma, Opuntia sp., Cereus sp., Astrophytum capricorne, Astrophytum myriostigma* and *Ritterocereus pruinosus* with antiserum to Potato virus X (PVX, which is serologically related to Cactus virus X).

Comparing the results of bioassay and ELISA tests we further focused on samples of *Mamillaria microhelia, Sulcorebutia sp., Bolivicereus samupatanus, Opuntia sp., Ferocactus sp., Mamillaria magnimamma, Opuntia sp., Cereus sp., Astrophytum capricorne, Astrophytum myriostigma* from collection of Nikita Botanical Gardenand *Ritterocereus pruinosus* from the collection of Karazin' Botanic Garden of Kharkiv National University. These plants were probably infected with CVX. To confirm our assumption about CVX infection and to study the morphology of the pathogen we carried out transmission electron microscopy. In sap of all plants we registered filamentous virions with size 580×13 ± 2 nm (Fig.3), which is typical for *Cactus virus X.*

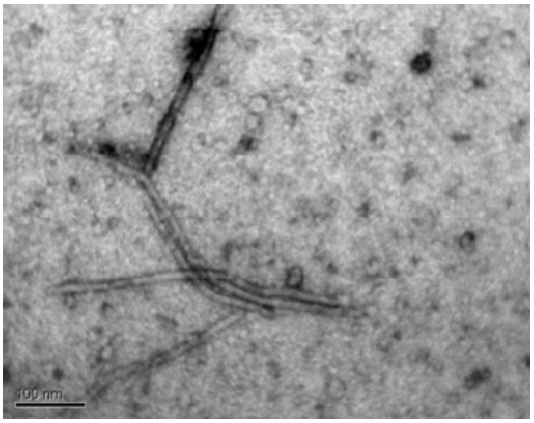

Fig.3. Electron micrograph of flexible particles from plant material from *Ferocactus* sp.

Summarizing the obtained results it is possible to assert that collectionsof Nikitsky Botanical Garden and Botanical garden of Karasin's Kharkiv National University were contaminated by Cactus virus X. Exchange collection material without testing on virusinfection, can increases chances of uncontrolled distributionof viral infections. Some infections are symptomless. In this case the asymptomatic virus infectionin cactus makes a danger because these plants can become thesource of distribution pathogens in collections. In addition,cactuses could support reproduction of viruses other typesof plants and, thus, be the reservoirs of plant virus infections.A timely exposure and permanent control of the stateof population of these cultural plants is the obligatory link ofthe system of their protecting from this group of pathogens.

1. M. Chessin, D. Lesemann. Distribution of Cactus Viruses in Wild Plants// Phytopatology. – 1972. – Vol.62. – P.97-99. 2. L.M.L. Duarte, M.A.V. Alexandre, E.B. Rivas, R. Harakava, S.R. Galleti and M.M. Barradas POTEXVIRUS DIVERSITY IN CACTACEAE FROM SÃO PAULO STATE IN BRAZIL Journal of Plant Pathology (2008), 90 (3), 545-551. 3. Virus Taxonomy Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses Editors Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz International Union of Microbiological Societies Virology Division Elsevier academic press. 4. R. Koenig, C.W.A. Pleij, S. Loss, W. Burgermeister, H. Aust, J. Schiemann. Molecular characterization of potexviruses isolated from three different genera in the family Cactceae // Arch. Virol. – 2004. – №149. – P.903-914. 5. J.R. Lastra, Daisy Gaskin, y R.C. de Uzkątegui. Virus x del cactus en Venezuela//Agronomia Tropical.1976. – №26. – P.303-310. 6. Liou MR, Hung CL, Liou RF (2001) First report of Cactus virus X on Hylocereus undatus(Cactaceae) in Taiwan. Plant Dis 85: 229. 7. G.M. Milbrath, M.R. Nelson and R.E. Wheeler. The Distribution and Electron Microscopy of Viruses of Cacti in Southern Arizona// Phytopatology. – 1973. – №63. – P.1133-1139.

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ELECTRON MICROSCOPY AND BIOLOGICAL PROPERTIES OF THE PATHOGEN AFFECTING *PLATANTHERA BIFOLIA*

Проведено обследование больных растений Platanthera bifolia, отобранных в Канивском природном заповеднике. Была доказана инфекционность сока, а также возможность механической передачи вируса. Методом трансмиссионной электронной микроскопии в образцах пораженных растений были выявлены сферические вирусоподобные частицы, диаметром около 50 нм. Согласно морфологическим особенностям, вирус, выделенный из растений Platanthera bifolia, может принадлежать к семейству Caulimoviridae.

The plants of Platanthera bifolia collected in Kaniv National Reserve were observed. The infectivity of plant sap and viral capability to transmit in mechanical manner were confirmed. The spherical particles with diameter about 50 nm were observed in preparations from symptomatic leaf tissue in transmission electron microscope. According to viral morphology the pathogen of Platanthera bifolia may be a member of Caulimoviridae.

Introduction.*Orchidaceae* Juss. is one of the biggest families of flowering plants which includes about 35 000 species. They can be found all over the world excluding deserts and Polar Region [2]. Viral diseases of orchids are known from the middle of XX century [4]. Until now more over 30 viruses of orchids have been described [8, 10]. The majority of these viruses were detected as pathogens of tropical orchids which are cultivated *in situ*. On the other hand the viruses of terrestrial orchids of temperate zone are studied not enough. The infection of *Cypripedium* sp., *Orchis sp.*, *Ophrys* sp. by T*obacco rattle* (TRV) and T*urnip mosaic virus* (TuMV) were described [5]. Also the antigens

to A*rabis mosaic virus* (ArMV)*, Bean yellow mosaic virus* (BYMV)*,Tobacco mosaic virus* (TMV),*Tomato aspermy virus* (TAV), TRV and TuMV were detected in orchids of natural Ukrainian flora [1].

Materials and methods. Plant material was collected in Kaniv Natural Reserve (Snakes' Islands). For further investigation we used plants of *Platanthera bifolia* with typical viral symptoms (fig. 1).

Figure 1. Necrotic lesions on the leaves of *Platanthera bifolia*

Virus identification was carried out using standard indirect ELISA and DAS-ELISA with polyclonal antiserums to *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Potato virus Y* (PVY), BYMV, TAV, TMV, TRV, TuMV and *Cauliflower mosaic virus* (CaMV).

Biological properties of viruses were studied using following test-plants: *Chenopodium murale, Gomphrena globosa, Lycopersicon esculentum, Nicotiana alata, N. benthamiana, N. rustica, N. tabacum, Petunia hybrida* and *Zinnia elegans.* The test-plants were inoculated in early stages of growth by mechanical sap transmission, applying carborundum as an abrasive. The inoculum was prepared by homogenizing plant tissue with 0.1 M phosphate buffer (pH 7.4) and followed by centrifugation (5000 rpm for 20 min).

The morphology of virions were studied in leaf dip preparations negatively stained with 2% uranyl acetate EM using a JEM-1400 electron microscope, magnification 60 000.

Results and discussion. The ELISA testing revealed no antigens of BYMV, CMV, TAV, TMV, TRV, TSWV, TuMV and PVY in samples of infected *P. bifolia.*

The sap of necrotic leaves from *P. bifolia* were experimentally inoculated to the range of test-plants, common for many polyhostal viruses [3, 7]. All inoculated plants show no local reaction but *L. esculentum, N. benthamiana, N. rustica* and *N. tabacum* reacted systemically. The systemic symptoms of viral infection appeared two months after inoculation. No visual symptoms were observed on *C. murale, G. globosa,P. hybrida* and *Z. elegans* (table 1).

Table* 1. Reaction of-test plants inoculated with sap from *Platanthera bifolia

Test-plant	Symptoms
Chenopodium murale	-/-*
Gomphrena globosa	-/-
Lycopersicon esculentum	Chlorotic lesions, leaf deformation
Nicotiana alata	-/-
Nicotiana benthamiana	Mosaic
Nicotiana rustica	Mosaic
Nicotiana tabacum	Chlorosis
Petunia hybrida	-/-
Zinnia elegans	-/-

*"-/-" – no visual symptoms

EM examination of infected plants tissue revealed the presence of spherical virus-like particles about 50 nm in diameter (fig. 2). Such morphology are typical for representatives of *Caulimoviridae*: *Caulimovirus, Cavemovirus, Petuvirus, Soymovirus* [9].

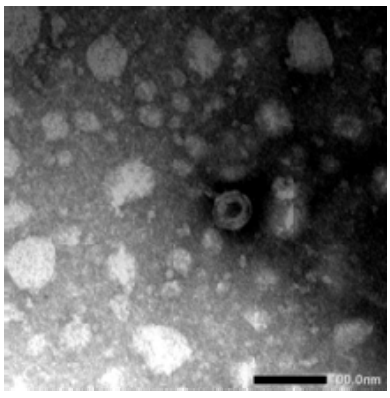

Figure 2. Electron micrograph of virus-like particles in the sap of infected plants

The majority of viruses belonging to the listed genera can be transmitted by mechanical inoculation. The reaction of test-plants eliminates such viruses as *Blueberry red ringspot virus*, *Cestrum leaf curling virus*, *Petunia vein clearing virus* and *Tobacco vein clearing virus* which are not transmitted in mechanical manner [3, 6].

For further identification of virus DAS-ELISA test were used. No positive reaction with antiserum to CaMV was obtained. According to results of DAS-ELISA we can exclude infection of *P. bifolia* by CaMV and serologically related viruses: *Carnation etched ring virus*, *Dahlia mosaic virus*, *Horseradish latent virus* and *Strawberry vein banding virus* [3].

In conclusion, host assay of sap samples extracted from infected plants of *P. bifolia* approved its infectivity. EM studies confirmed the presence of virus-like particles in infected plant tissues. The isometric morphology and size range allows to suggest that the viral pathogen of *P. bifolia* can be a representative of family *Caulimoviridae*. The cases of similar virus infection of orchids are not described in literature data. However, there are not much investigation about spreading viruses in natural ecosystems. Further work will be focused on identification of the virus infecting *P. bifolia*.

1. Коротєєва Г. В.,Поліщук В. П. ВірусизозулинцевихприродноїфлориУкраїни // Мікробіол. Журн. – 2004. – Т. 66 – № 2. – С. 74-80. 2. Черевченко Т.М. Тропические и субтропические орхидеи/ К.: Наукова думка. – 1993. – 253 с. 3. Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) (1996 onwards). 'Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996. 4. Jensen D.D. Mosaic of Cymbidium orchid // Phytopathology. – 1950. – Vol. 40. – P. 966-967. 5. Lesemann, D.E., Vetten, H.J. The occurrence of tobacco rattle and turnip mosaic virus in *Orchis ssp*., and of an unidentified potyvirus of *Cypripedium calceolus* // Acta Hortic. – 1985. – Vol.64. – P.45-54. 6. Lockhart B. E., Menke J., Dahal G., Olszewski N. E. Characterization and genomic analysis of tobacco vein-clearing virus, a plant pararetrovirus that is transmitted vertically and related to sequences integrated in the host genome. // Journal of General Virology – 2000. – Vol. 81. – P.: 1579–1585. 7. Navalinskienė M., Samuitienė M. Dekoratyvinių augalų virusinės ligos ir jų sukėlėjai Lietuvoje. Monografia. – Kaunas:Lutute, 2006, – 256 p. 8. Orchid biotechnology II // eds. Chen H.-W. and Chen H.-H. – World Scientific Publishing Co Pte Ltd, Singapore. – 2011. – 380 p. 9. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses / King A. M. Q., Adams M. J., Carstens E.B., Lefkowitzl E.J. – NewYork: Elselver Academic Press., 2012. 10. Zettler F.W., Wistler G.C., Elliot M.S., Ko N.-J. Viruses of orchids and their control // Plant. Disease. – 1990. – Vol. 74. – P. 621-626.

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J. Polák

BIOTECHNOLOGICAL (GM) CROPS IN THE WORLD AND EUROPE, PLUM HONEYSWEET RESISTANT TO PPV, RESEARCH IN THE CR: THE PAST, THE PRESENT AND THE FUTURE

Быстрое увеличение площадей посевов ГМ-культур, делает их наиболее высокоадаптивными посевными культурами в истории сельского хозяйства. Здесь представлены результаты девяти лет тестирования слив клона С5 (трансформирован капсидным белком вируса шарки сливы) при высокой и постоянной инфекционной нагрузке PPV-Rec, как самостоятельного агента, так и в сочетании с вирусами карликовости чернослива и кольцевой хлоротичности яблони.

Fast increasing hectarage of GM crops makes them the highly adopted crop technology in the history of agriculture. Results of nine years testing of plum clone C5 (transformed with the Plum pox virus coat protein) under the high and permanent infection pressure of PPV-Rec alone and in combinations with Prune dwarf virus, and Apple chlorotic leafspot virus both from graft inoculation and natural aphid vectors are presented.

Introduction. Research in genetic engineering started in late seventies (Colwell et al., 1985). The first genetically modified (GM), syn. biotechnological (Biotech) crops were commercialized in 1995 (cotton, company Monsanto; potato, company Syngenta). 1.7 million hectares of Biotech crops (cotton, corn, potato) were planted in 1996 already (USDA-APHIS Biotechnology Permits Database, available at htpp://www.nbiap.vt.edu/ (March 2001). Increase in hectarage to 160 million hectares in 2011 (Fig. 1) makes Biotech crops the fastest adopted crop technology in the history of agriculture. The International Service for the Acquisition of Agri-biotech Applications (ISAAA) is every year publishing "Global status of commerialized Biotech/GM crops". The last one was published as ISAAA Brief No. 43 in 2011 (James C., 2011). The objective of this Brief is to provide information and knowledge to the scientific community and society on Biotech/GM crops and its contribution to global food, feet, fiber and fuel security, to sustainable agriculture.

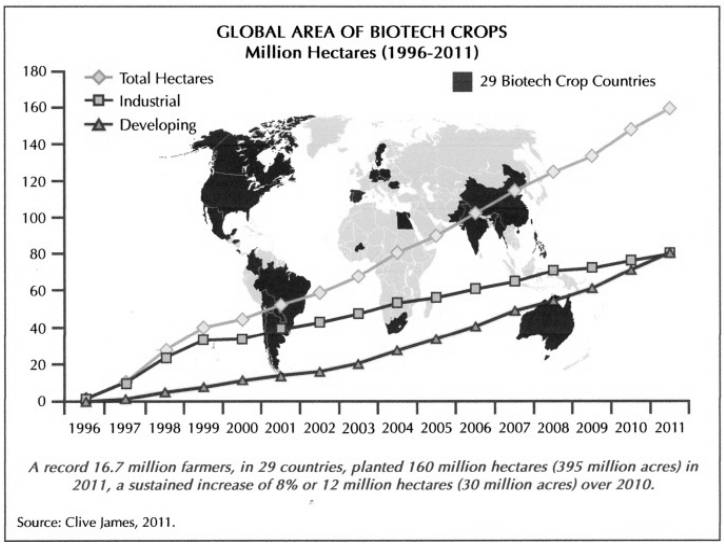

Fig. 1. Global area of biotech crops