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Подано експериментальні дані про особливості будови, розвитку і функціонування рослинних і тваринних організмів, флору і фауну України, одержані на основі досліджень, що проводяться науковцями біологічного факультету в галузях фізіології рослин і тварин, генетики, ботаніки, зоології, мікробіології, вірусології. Викладено також нові дані стосовно біохімічних і біофізичних основ регуляції у клітинах і органах у нормі й після впливу різноманітних фізико-хімічних факторів, наведено результати нових методичних розробок.

Для наукових співробітників, викладачів, аспірантів і студентів.

Collection of articles written by the scientists of biological faculty contains data on research in molecular biology, physiology, genetics, microbiology, virology, botanics, zoology concerning the structure, development and function of the plant and animal organisms, flora and fauna of Ukraine. Results of newly developed biophysical methods of biological research, biochemical data regarding metabolic regulation under the influence of different factors are presented.

For scientists, professors, aspirants and students.

ЗМІСТ

CONTENTS

УДК 632.38:635.1/7

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VIRAL DISEASES OF TOMATOES AND CUCUMBERS IN GREENHOUSES OF BELARUS

В статті наведено інформацію про випадки вірусних захворювань томатів та огірків, що вирощуються в умовах закритого ґрунту. Показано, що при інтенсивному розвитку вірусних захворювань огірків, їх шкодочинність становить 14,7-46,2 %, в залежності від гібриду. На основі біологічного тестування та ІФА ідентифіковані віруси ВТМ, ВОМ, ВМТ, ВЗКМО. Доведена можливість використання біологічно активних речовин для обмеження експресії ВЗКМО на ранніх стадіях розвитку огірків.

In the article the information on virus diseases incidence in tomato and cucumber cultivated under protected ground conditions is presented. It is shown that at the intensive virus diseases development in cucumber plants their harmfulness makes 14,7-46,2% depending on hybrid. Based on biological test and ELISA-test TMV, CMV, ToMV and CGMMV viruses are identified. A possibility of biologically active substances application for restriction the CGMMV expression and development at the early stage of cucumber plant development is determined.

Virus diseases are widely spread in vegetables and are one of the reasons of yield and production quality decrease. According to literary data more than 100 viruses of different taxonomic belonging differing by biological, serological, ecological peculiarities and ways of its transference are registered [6]. The most spread and harmful in tomato are: *Tobacco mosaic virus* (*TMV), Cucumber mosaic virus* (*CMV*), *Tomato mosaic virus* (*ToMV*), *Tomato aspermy virus* (*TAV*), *Tomato spotted wilt virus* (*TSWV*); in cucumber – *Cucumber green mottle mosaic virus* (*CGMMV*) and *Cucumber mosaic virus* (*CMV*) [3, 4, 9, 10].

The most actual problem of virus diseases concerns tomato and cucumber which are the most important protected ground crops. At present in greenhouses small-volume technologies allowing to decrease the material expenses essentially are widely used. However, the characteristic feature of tomato and cucumber cultivation using such technologies is the dominance of seed-born pathogens and the ones transmitted by contact and aerogenic means.

The objective of our researches was to study the phytoviral situation of greenhouses tomato and cucumber crops. The virus diseases monitoring was carried out in 2009-2010 by way of itinerary inspections of vegetable farms of different regions of the Republic. During budding-blossoming and fruit-bearing 100 plants from every tomato and cucumber hybrid were under visual inspection and leaf samples for laboratory studies were selected.

Plants with chlorosis, mosaic spot and leaf deformation symptoms were the object of studies. To determine virus etiology of diseases and their agents identification the selected tomato and cucumber plant samples were analyzed by plant-indicator and enzyme-linked immunosorbent assay (ELISA-test). *Nicotiana tabacum* L. (cv. Samsun), *N. glutinosa* L., *N. debneyi* Domin., *Datura stramonium* L., *Capsicum annum* L.*, Lycopersicon esculentum* Mill., *Tetragonia expansa* L., *Cucumis sativus* L. were used as test plants. ELISA delivery was accomplished with the test set use the Sediag (France) and ADGEN (Scotland).

By inspecting the greenhouse tomato and cucumber plantings it was determined that some hybrids are rather heavy infected by virus diseases, the incidence of which varied depending on hybrid and cultivation conditions. So, 40-90% of plants of tomato hybrids Alkasar, Barselona, Admira, Bomax have got the symptoms of mosaic and leaf deformation and the plants of cucumber hybrids Raiss, Kurazh, Evergreen, Tristan, Mirabel, Sharzh, Ceroz have a got – y 45-100% (Table 1).

Table 1. **Distribution of virus diseases in greenhouses (2009-2010)**

Tomato		**Cucumber**	
Hybrids (F1)	Distribution,%	Hybrids (F1)	Distribution,%
Admira	50-90,0	Ceros	65-75,0
Alkasar	45,0	Estafeta	32,0
Bomax	30-35,0	Evergreen	26-80,0
Barselona	40,0	German	25-35,0
Bolduin	30,0	Gladiator	21,0
Emoushen	15,0	Kolanell	75,0
Gayana	18,0	Kurazh	20-80,0
Greis	15-35,0	Malkum	20,0
Grodena	20,0	Marsel	25,0
Makarena	21,0	Meva	0
Pablo	0	Mirabell	20-70,0
Persei	0	Raiss	40-100,0
Raissa	25-30,0	Rafael	12-21,0
Siluet	10,0	Pacto	15-55,0
Starbuck	20,0	Pikas	24,0
Zhyronimo	15-30,0	Tristan	21-100,0
Zuko	30,0	Sharzh	85,5

The virus diseases damage brings vegetable crop yield decrease and trade production quality worsening. The evaluation of viral diseases influence on cucumber plant productivity has shown that they decrease yield and fruit trade qualities. So, in cucumber Kurazh F1 the intensive infection symptoms were developed during blossoming and active plant fruit-bearing. There was a delay in growth and bright mosaic accompanied by rugosity was discovered. The fruits have got unevenness and mosaic spot, as a result, 30% turned out to be non-standard. As a result, the disease harmfulness in the hybride Kurazh F1 has made 46,2% (Table 2).

Table 2. **Virus infection influence on cucumber productivity**

Hybrids (F1)	Plants	R, %	Ovaries number, pcs/plant	Fruit yield, kg/m^2	P, %
Rafael	with symptoms	15,6	13	1,0	14,7
	without symptoms	-	17	1,5	-
LSD05			1,5	0,11	
Kurazh	with symptoms	42,5	15,0	0,6	46,2
	without symptoms	-	19,3	1,1	-
LSD05			2,31	0,14	
Sharzh	with symptoms	33,1	17,3	1,1	26,1
	without symptoms	-	21,0	1,4	-
LSD05			1,76	0,23	

Note – R – disease development, %; P – harmfulness, %.

The analysis of the infected tomato plant samples by ELISA method and plant –indicator methods has shown that the main disease agents are *Tomato aspermy virus* (*TAV*) и *Tomato mosaic virus* (*ToMV*), cucumber – *Cucumber green mottle mosaic virus* (*CGMMV*) (Figure 1).

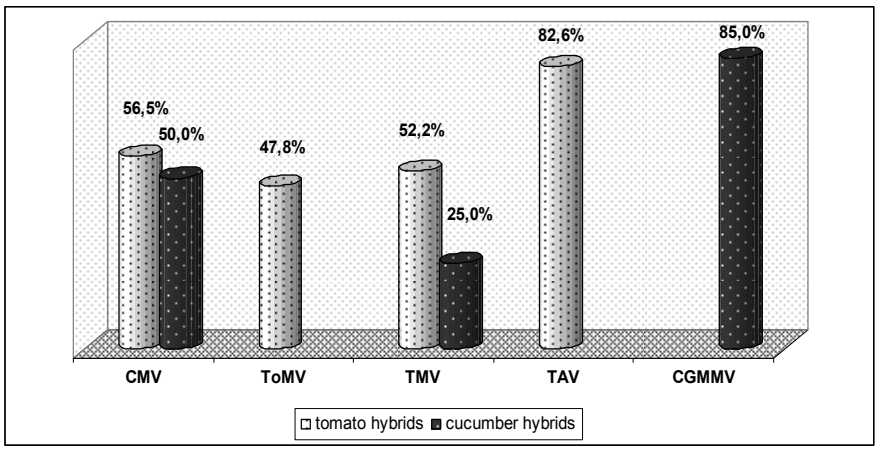

Figure 1. Frequency of virus incidence in studied tomato and cucumber hybrids (2009-2010 ELISA-data)

According to literary data the most effective antiviral measure against *CGMMV* is seed dressing in 15% trisodium phosphate solution [7]. At present in the technologies of vegetable crops growing the biological active substances (BAS) are more frequently used. It is determined that these combinations are able to raise both agricultural crop yield and essentially decrease the negative influence of abiotic and biotic factors through the hormonal exchange and plant immunity stimulation raising their disease resistance. Brassinosteroids showing high growth-stimulating are of interest and can be a basis for the effective and ecologically safe application [8]. The active biological stimulators are the preparations containing a complex of amino acids , micro and macro elements. The essential role in raising plant resistance to phytopathogens play the preparations the main active ingredient of which are the proteins- the proteolytic ferment inhibitors rendering the protective action [2, 5].

We studied a possibility of *CGMMV* inhibition by way treatment of cucumber see phytoregulators epine, s. and tubelak, WSP which are included into the "State register of plant protection products pesticides and fertilizers allowed for application on the territory of Belarus" A range of active concentrations of preparations and their influence on *CGMMV* expression and development at pre-sowing seed dressing is determined.

The results of researches have shown that pre-sowing cucumber seed treatment (Raiss F1, Evergreen F1) by growth regulators promoted seedlings emergence, cucumber plant growth and development of studied hybrids. At the same time seed retting in growth-regulating substances solutions held in cucumber plant symptoms appearance and development induced by *CGMMV* at an early stage of plant ontogenesis. Based on ELISA-test it is determined that the virus concentration in cucumber plants grown from seeds treated by the above-indicated preparations was 1,5-2 times less in comparison with the control (Figure 2).

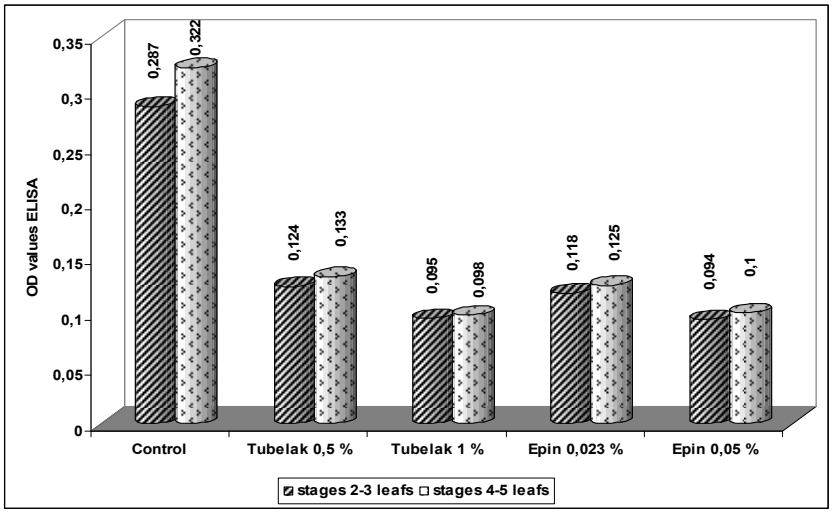

Figure 2. The inhibition of *CGMMV* by the treatment of seeds of cucumber (Evergreen F1) with plant growth regulators tubelak, WSP and epin, s (laboratory experiments 2009-2010)

So, the inspection of vegetable farms of the republic carried out in 2009-2010 has shown a wide spread of viral diseases in greenhouses tomato and cucumber plantings. It is determined that at the intensive viral diseases development in cucumber crop their productivity depending on hybrid is decreased for 21,0-45,4% and harmfulness index makes 14,7-46,2%. Based on biological test and ELISA-test *TMV, CMV, ToMV* and *CGMMV* viral diseases agents are identified. The prevalent ones are *Tomato aspermy virus* (*TAV*) и *Cucumber green mottle mosaic virus* (*CGMMV*). A possibility of biologically active substances application (epin, s., tubelak, WSP) to reduce *CGMMV* development at early cucumber plant ontogenesis stage is established.

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CHARACTERISTICS OF THE INTERACTION BETWEEN VIRUS AND SPECIFIC ANTIBODIES IN CELL HOMOGENATES

Розглянуто підхід до детекції методом поверхневого плазмонного резонансу з використанням формування комплексу антиген-антитіло під час преінкубації замість послідовного нанесення. На прикладі Nicotiana tabacum за допомогою запропонованого підходу досліджено вірусну інфекцію. Досягнуто узгодження між методами ППР та ІФА. Розглянуто також кінетику взаємодії.

Surface plasmon resonance approach using a detection of complex formed by viral antigen and antiviral antibody during the preincubation step instead of their consecutive application is discussed. With the example of Nicotiana tabacum the development of the viral infection in the plant was studied by the mentioned approach. The agreement between SPR and ELISA methods was achieved. Kinetics of the interaction is discussed.

Detection of the specific interactions against the background of the unspecific components of the sample is the one of the important tasks of the modern analytical biochemistry. The new ways to overcome the matrix effect are especially essential for the methods based on surface plasmon resonance (SPR) technique, particularly those which could be used for the express-diagnostics of viruses.The main advantage of the SPR technique is possibility of direct registration of surface interactions without any additional procedures or labels. Real time response registration enables the investigation of interaction kinetics and, thereby, tracking of a system's evolution.

There exist many approaches for detection of virus specific proteins and nucleic acids, but only few of them can detect intact virus particles. Enzyme-linked immunosorbent assay (ELISA), the traditional technique for detection of virus specific antigens, enables one to obtain exact and reliable information concerning presence of virus infection using interaction between the virus particle elements and corresponding antibodies. This technique, however, has certain constraints; most essential of them is the lack of kinetic information regarding the reaction under consideration.

Among the optoelectronic methods the surface plasmon resonance technique is widely used when studying virus specific macromolecules. However most works in this field deal with individual components of viruses rather than entire virus particles [8]. Although virus peptides, recombinant and refined virus proteins can serve as convenient model to investigate antibody affinity, their structure, conformation and microsurroundings of subunits, both separate and incorporated into virus particle, may essentially differ. That is why investigations of interaction between

virus and specific antibody under conditions which are as much as possible close to *in vivo* are of great importance [5]. It is evident, that viral particle is a complex mixture of antigens, whose diversity is determined by the number of virus specific proteins, which usually contain a number of antigen determinants depending on the spatial structure of molecule and located in different sectors of a polypeptide chain. That is why one should use viruses with small number of antigen determinants as model when developing novel approaches to direct methods of detection of intact virus particles. From these considerations tobacco mosaic virus (TMV) seems to be the most convenient model to refine approaches for direct detection of virions of plant viruses, because of its' small size and presence of the only one envelope protein.

The peculiarities of surface excitations at the conditions of surface plasmon resonance lead to some limitations when applying this technique in virology. In particular, if it is used for investigation of intact virus particles, then some problems appear concerning adequate interpretation of the results of measurements for subjects whose characteristic length L is over several tens of nm. The plasmon–polariton wave, which decays exponentially with moving off the surface, has penetration depth approximately equal to half of the incident wavelength λ, so the SPR excitation angle depends on environmental optical parameters adjacent to the metal layer. So the size of the receptor–analyte complex must not be over 20-30 nm for linear relation between angle shift and total mass of materials on the surface [4]. Thus, surface plasmon resonance technique is quite applicable for investigation of plant viruses whose sizes are small [2, 7]. At the same time up to now surface plasmon resonance based approaches were not used for quantitative detection of intact viruses. In accordance with this, the objective of our work is developing of the surface plasmon resonance based approach for quantitative determination of viruses using TMV as a model system.

For investigations using the surface plasmon resonance technique the traditional approach is that at which a receptor is immobilized at the sensor surface, while an analyte is in solution. In this case the angle shift depends on the effective thickness of the analyte layer specifically bound to the immobilized receptor layer, the densities of both layers being constant. This means that angle shift change is due to variation of parameters of the molecular ensemble of interacting molecules in the vertical plane. However, the shift of the SPR angle depends not only on the layer thickness but on the refractive index variation within the interfacial layer. Therefore the molecular layer compactness will also affect the response due to refractive index variation. If, at the same time, the surface structure thickness can be fixed (due to specificity of the interaction process and constant form of the interacting components), then the shift of the angle will be a one-valued function of the biomolecular ensemble compactness. The present work is focused on these issues of functional sensing architectures based on the variation of refractive index of interfacial layer.

To realize the approach described above following procedure has been used. Initially the protein A *Staphylococcus aureus* (which is specific towards Fc fragment of immunoglobulin) has been immobilized on the transducer surface. Then, the previously preincubated specific IgG-virus complexes were applied to the sensor surface. Depending on the relative concentration of viral particles and specific antibodies, the virion–*n*· IgG complexes have different numbers (*n*) of antibodies per virion. This determines compactness variations within the complexes, as well as in their packing at the surface. At that the average thickness of the whole complex will remain approximately the same, due to statistical character of interaction between the antivirus immunoglobulins and epitopes at the virus surface (about 800 for TMV). So, in this case the shift of the SPR angle depends on the layer parameters in the horizontal plane, e.g. layer density.

In this work the development of the viral infection in the tobacco (*Nicotiana tabacum*) infected by tobaco mosaic virus (TMV) (Fig.1) was investigated by the SPR and ELISA methods.

Plants were inoculated mechanically using carborundum abrasive with the virus concentration 300 mkg/ml in phosphate buffer saline (PBS). The samples of infected leaves were collected weekly and stored frozen. Then they were homogenized mechanically in the carbonate buffer and centrifuged at 4000 rpm. Precipitate-free solution was used for further investigations.

a b

Fig.1. *Nicotiana tabacum*: (a) control, (b) infected by TMV

Investigations with the surface plasmon resonance were undertaken using a "PLASMON" spectrometer developed at the V. Lashkaryov Institute of Semiconductor Physics, National Academy of Sciences, Ukraine [1]. A GaAs laser served as source of excitation (λ = 670 nm). The glass plates (refractive index n = 1.61), with as-deposited (through an intermediate Cr adhesion layer 1–1.5 nm thick) gold layer (50 nm), were pretreated with the "piranha" mixture (H_2O_2–H_2SO_4 in the ratio 1:3) to remove organic contaminations. After this plates were fixed at a supporting prism (n = 1.61); optical contact was realized using immersion oil (polyphenyl ether, n = 1.61).Water solution of potassium thiocyanate, KNCS (concentration of 10^{-2} M) was prepared immediately before the experiment. The glycine (pH 2.2) and carbonate (pH 9.6) buffers were prepared using the standard procedure. The protein A *Staphylococcus aureus* (Sigma) was used in a water solution (concentration of 50 mg/ml).

Indirect ELISA was used in the work for detection of virus antigen in algae. ELISA plates ("NUNC", Denmark) were coated for 2 hours at 37°C with the antigen (TMV or algae samples) at 1 μg/ml (in case of TMV) in 50 mM sodium carbonate buffer pH 9.6. Uncoated sites on each microtiter well were blocked with 1% bovine serum albumin (BSA) in phosphate buffer saline (PBS) for 2 hours at 37°C. Then 100 μL of primary rabbit polyclonal antibody (was obtained from Virology Dpt., Taras Shevchenko' Kyiv national university) diluted with (1:10000) was added to the wells, and the plate was incubated overnight at 4°C. 100 μL of goat anti-rabbit IgG peroxidase cojugate ("Sigma") diluted 1:6000 in PBS-0.05% Tween 20-1% BSA was added, and the plates were incubated for 2 hours at 37 °C. Plates were washed three times with PBS-0,2% Tween 20 for 5 min between each of the following incubation steps. After the final plate wash, the color reaction was initiated with 100 μL of substrate solution with chromogen added (O-phenylene diamine ("Sigma")) followed by 50 μL of 2M sulfuric acid to stop the reaction 20-30 min later. The absorbance was measured at 492 nm using an PE-2 ELISA plate reader ("PE-2 Sumal", Germany). A492 results were assumed to be positive in case they're twofold comparing with A492 of negative control well (sap of healthy plant).

The above mentioned approach [3] based on exposure of the complex containing specific IgG and virus-containing material onto the sensor surface modified by NCS^- and protein A *St. aureus* was used. Detection of the virus level in homogenates of *Nicotiana tabacum* taken at the different stages of the infection has been done simultaneously by SPR and ELISA (Fig.2). Characteristics of sensor response constant were obtained by fitting routines using the program Origin of MicroCal Software, Inc. (Northamption, NJ, USA).

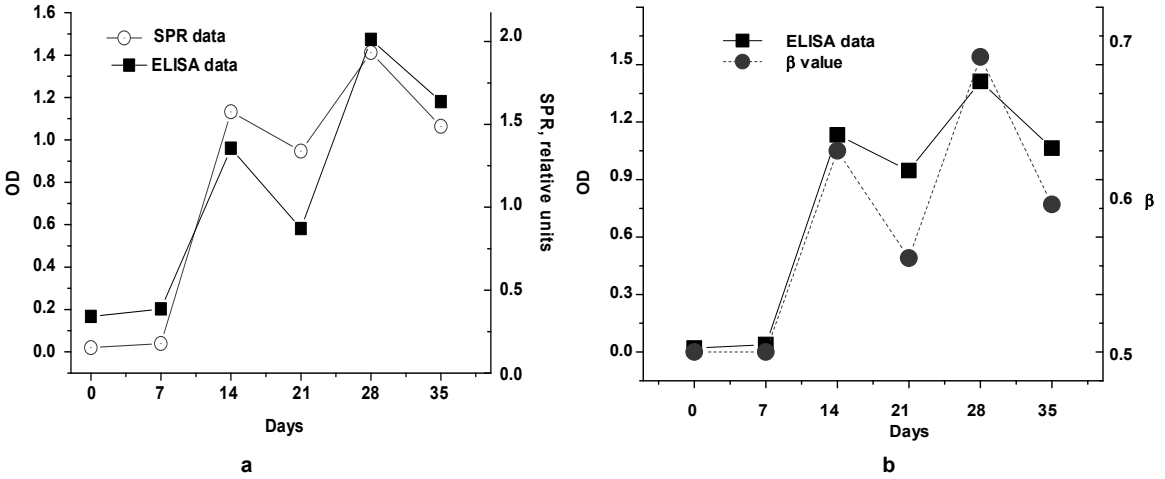

Fig.2. a – Comparison of the results of virus detection in homogenates of Nicotiana tabacum leaves taken at the different stages of the infection obtained by SPR and ELISA (OD – optical density). b – Parameter β obtained by the approximation the SPR kinetic curves by the equation of the stretched exponential function (~exp(-(t/τ)^β)

It was shown, that the ratio between data obtained by ELISA and SPR carries linear character (Fig.3) in the region specific for the development of the viral infection.

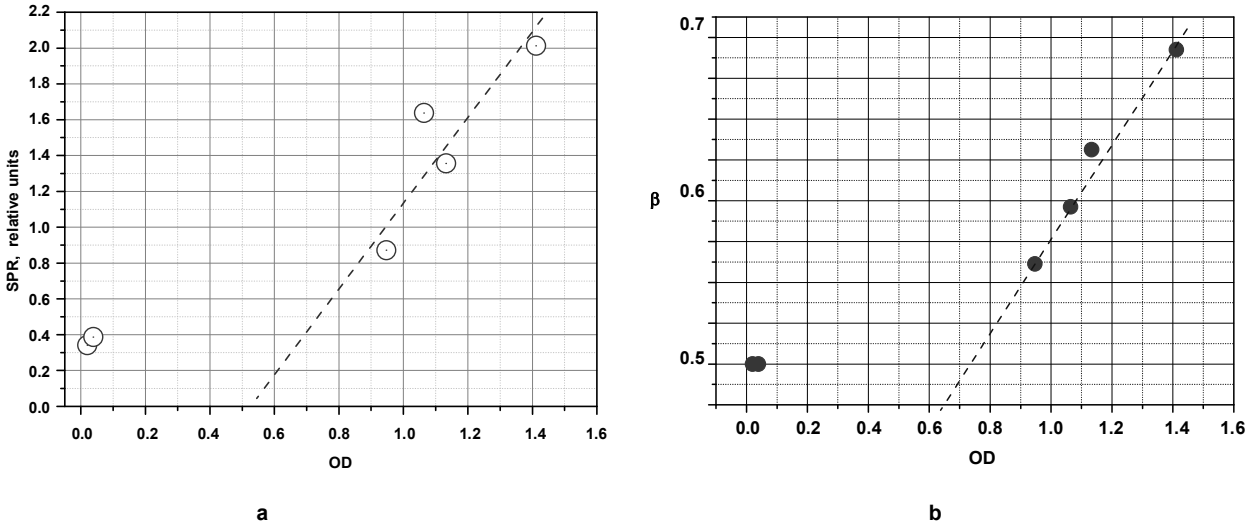

Fig.3. Correlation between a – SPR and ELISA data, b –β value and ELISA data

Kinetics of the interaction between modified by protein A SPR transducer and IgG-virion complexex can be described by the equation of the stretched exponential function (~exp(-(t/τ)^β) or Kohlrausch transient response function KWW, which indicates the big amount of the parallel adsorption processes mainly unspecific by nature[6]. Analysis of the kinetic dependences allowed concluding that the β value as well correlates with the virus concentra-

tion obtained by the ELISA. It means that the kinetic parameters of stretched exponential low have hidden intrinsic properties of the underlying interfacial reactions that are dependent on the concentration of specific component in the complex multicomponent mixture.

Stretched exponential regression to equilibrium is a frequently observed phenomenon in relaxation of non-equilibrium states. The coupling constant parameter β provides a measure of the width of the distribution of relaxation times. When β is equal to 1 then the KWW equation describes the single relaxation time of a simple exponential decay. As β decreases the breadth of the distribution of relaxation times increases. Non exponential relaxation in complex multicomponent systems may be the consequence of dispersedness giving rise to different free-energy barriers for different molecules [8]. A common view is that the two states may be split into a large number of substates that have a different exponential kinetics for the transition. The observed kinetics is then constructed by averaging over an ensemble of molecules with different exponential relaxation rates. The dispersedness is, thus represented by a probability distribution for the transition rates.

Further investigations are needed for the more precise determination of kinetic parameters, which should make possible a proper characterization of the stretched response function; their optimal combination can give opportunity to obtain new information regarding reaction under investigation. It gives a good basis for approaching a more specific microscopic model governing this exceptional physical phenomenon in complex protein mixtures.

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O. Boyko, assis., M. Melnychuk, prof., I. Grygoryuk, prof., V. Dubrovin, prof.

THE ANTIPATHOGENIC BIOPREPARATIONS BASED ON MUSHROOMS' COMPONENTS AND CARRIES OF PLANTS

В результаті проведених досліджень був розроблений продукт з високою біологічною активністю "Biocofunge"-1. До складу даного препарату входять компоненти грибів та інших речовин, що містяться в зернах хмелю. Препарат активізує ріст та розвиток рослин за зазначених умов.

The product with high biological activity "Bioecofunge"-1 was developed as a result of experiments. This preparation consists of fungi components and other substances, carriering of lupulin grains. It intensify growth and development of plants under appointed conditions.

Introduction. There are significant changes because of anthropogenic impact on the biosphere structure and function of living systems' chains at present period can be supported in viable state by modern biotechnology. The development of processes of biologically active substances of different directions for the needs of the population remains very important in spite of significant scientific achievement in agricultural and medical engineering [1-8].

The main tasks of our study were researches based on the development of complex biopreparation [7] that stimulated the growth and development of crops while reducing their infection process caused by bacteria, viruses and microscopic fungi [2, 7, 8].

Materials and methods. The basis of experiments was a part of our working out technology based on the compositions of several families of plants, trace elements and volcanic rocks [3, 4]. The components of hop Humulus lupulus L. – bitter substances, which includes humulon ($S_{21}N_{30}O_5$) and other valuable substances, carriering of lupulin grains were used. The selection of these and others compounds was carried out from productive plants which were grown on healthy donors basis. The lasts were previously analyzed on virus-keeping by using electron microscopy, ELISA, PCR. Bacterial hop diseases and those that caused by microscopic fungi were determined visually and using well-known microbiological techniques [7]. The composition of hop components was supplemented with fractions of fungi (Basidiomycetes), selected from natural ecological niches and transformed environment served. Virological, microbiological and mycological control of fungi were performed in order to obtain healthy material. There were used 12 kinds of mushrooms (Basidiomycetes) that formed biological bank for selection of promising materials for the creation of biopreparations. More than 10 important components were tested during screening experiments.

There were used Agaricus bisporus (J. Lge) Imbach and Pleurotus ostreatus Kumm in this work. 0,68-0,72 kg of grinding fruit bodies was performed in frozen state with a gradual addition of 1l phosphate buffer 1/15 M, at pH 7,0-7,2. Thus, fungi homogenate and its fractions, as shown by research, can be prepared from fresh raw and dried materials using stepwise centrifugating of solutions for creating active compositions, with adding some microelements in some versions.

The dry weight of hops was grinded to dusty condition: received components were mixed in the way of adding 2,8-3,2 g of hop fraction to one liter of filtered solution from mushrooms.

For creation the working concentration of preparation "Bioecofunge-1" 1liter of H_2O was added to15-18ml of received composition. The expenditure of working solution were 100-180 litters for 1 ha [3, 4].

Such crops as hops, tomato, tobacco, sugar beet, corn, beans, sunflower, have been involved in the laboratory and production experiments. Vegetation laboratory experiences were performed in 10 replicate.

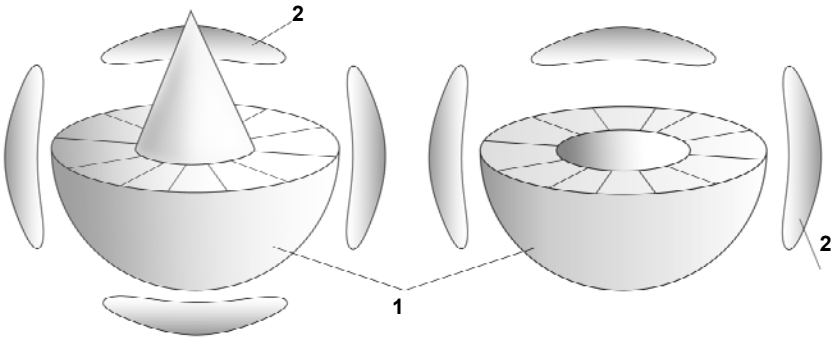

Fig. 1. The diagram of biochemical agents composition for productivity stimulation and crops diseases protection: 1 – lupulin grains, nutrients and antipathogenic hop (Humulus lupulus L.) components, 2 – nutrients and antipathogenic mushrooms (Basidiomycetes) components

Chenopodium amaranticolor (Coste & A. Reyn.) Coste & Reyn, Nicotiana glutinosa L. and Cucurbita pepo L. served as indicative plants for virological control.

Results of research and discussion. As the screening tests of preparation showed that "Bioecofunge – 1" reveals as a plants growth and development stimulator and shows antypathogen effect on crops during their ontogenesis. The best time for processing of plants by the preparation: sugar beet – on the stage of 3-4 leaves, wheat – in spring before returning to the tube, corn – on the stage of 5-6 leaves; beans – on the stage of 3-4 of real leaves, hop – after winding stems 0,8-1m height; tomato – 5-6 leaves; tobacco – 5-6 leaves. The better time to repeate the processing is after 14 days from the first case (spraying). These conditions help more intensive growth and development of processed by preparation "Bioecofunge"-1 plants in comparison with the control ones.

It is noted a significant reduction in the biological titer of virus genomic RNA: TMV, karlohrupy and cucumber mosaic vegetation during specialized studies. Thus, the hop plants, tomato, tobacco were infectioned after two-single processing by working solution "Bioecofunge – 1".

So, according to research, it was important to find the optimal proportion between fungi fractions and components of hop for forming the composition "Bioecofunge – 1". In this engineering process the improvement of products with high biological activity are very perspective for the further.

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COMPARISON OF RUSSIAN AND UKRAINIAN ISOLATES OF *POTATO SPINDLE TUBER VIROID* (PSTVD)

З 21 зразка картоплі відібраних на Україні, в тому числі 4 проростки, отримані в умовах in vitro, 13 ізолятів були ідентифіковані як позитивні в результаті дот-блот аналізу. Продукти ПЛР отримані з шести ізолятів пізніше були просіквіновані, два ізоляти сіквінувалися двічі. Було встановлено, що чотири ізоляти є ідентичними до російських ізолятів, а один – до польського ізоляту. Нуклеотидні послідовності трьох українських ізолятів ВВБК раніше не були описані.

Of 21 potato samples including 4 in vitro potato plantlets collected in the Ukraine, 13 isolates were identified as positive in dot-blot analysis. Six isolates gave PCR products suitable for sequencing that were made then, two isolates were sequence twice. Four isolates were identical in sequence to Russian isolates, and one to Poland isolate. Sequences of three Ukrainian PSTVd isolates were not described previously.

Introduction. The first report of a 'spindle tuber' disease affecting potatoes growing in the USA appeared in 1922 (5). In the former USSR, the 'gothic' disease of potatoes was first reported and described in Ukrainian Republic in 1937 (10, 11). Later, Leontyeva (9) proposed that the causal agents of these two diseases were identical. In that same year, Diener (1) showed that the agent responsible for spindle tuber disease belonged to a new class of pathogens – small, unencapsidated, autonomously replicating RNA molecules that he termed 'viroids', Finally, Mozhaeva (8) used sedimentation analysis, mobility in PAGE electrophoresis, and infectivity analyses to demonstrate that the 'gothic' disease was caused by a viroid.

The first plant pathogen to have the complete nucleotide sequence of its genome determined (2), known isolates of *Potato spindle tuber viroid* (PSTVd) range in size from 341–364 nucleotides. Infection can be symptomless, or, alternatively, symptoms may range from mild to 'lethal'. Three groups of PSTVd strains (i.e., mild, intermediate, and severe) have been distinguished based on the symptoms observed on certain sensitive cultivars of tomato. In potato, typical symptoms induced the intermediate strain include:

i) stunting and an upright growth habit; ii) leaf distortion (curling and epinasty), iii) a reduction in tuber size and number; iv) small, elongated (spindly) tubers, sometimes with cracks.

In the 1980's and 1990's, a severe epidemic of PSTVd disease was observed in the Central region of European Russia (7). A lack of special potato seed certification services that could test for the presence of PSTVd led to distribution of PSTVd-infected seed potatoes to farmers producing potatoes. Similar problems were associated with virus-free potato plantlets produced by meristem culture and/or genetic engineering. Corrective measures were put in place some years ago, and several institutions in Russia now have laboratories testing potatoes for PSTVd presence. Nevertheless, the number of such laboratories is too small for such a large country as Russia and, from time to time, new outbreaks remind us about the danger of PSTVd infection.

In the 1990s, the Virology Group at the Russian Research Institute of Phytopathology began to assemble a collection of PSTVd isolates present in Russia. Some of these isolates were maintained by annually planting infected potato tubers in field, and others were stored as RNA solutions at low temperature. Funding provided by ISTC Project #3468 has allowed sequence analysis of, first, four isolates (3, 4), next, more than 30 additional isolates (6), and, finally, more than 100 PSTVd isolates from the State Russian Collection of Phytopathogenic Organisms.

Materials and methods. Total RNA was isolated from frozen leaf tissue using Trizol *RNA* Prep 100 reagent as recommended by the manufacturer (Lab Isogene, Moscow). Full-length double-stranded PSTVd cDNAs were synthesized by RT-PCR using primers PSTVd 179 (5'-AAACCCTGTTTCGGCGGGAATTAC-3', complementary to positions 179-156) and PSTVd 180 (5'-TCACCCTTCCTTTCTTCGGGTGTC-3', homologous to nucleotides 180-203). SmarTaq DNA polymerase (Dialat Ltd., Moscow) was used for all PCR amplifications, and an overlapping cDNA was synthesized using primers PSTVd 112 (5'-ACTGGCAAAAAAGGACGGTGGGGA-3', homologous to positions 112-135) and PSTVd 359 (5'-AGGAA CCAACTGCGGTTCCAAGGG-3', complementary to positions 359-336) to confirm the sequence corresponding to the first pair of primers. Nucleotide sequences of uncloned PSTVd cDNAs were determined by automated sequence analysis using an ABI Prism Genetic Analyzer 3100.

Results. In 2008, 17 samples of potato tubers showing symptoms of PSTVd infection and four samples of *in vitro* potato plantlets collected in the Ukraine were received for analysis. A majority of these samples (including the *in vitro* plantlets) originated from Chernigov, and others were purchased in markets in Odessa or, occasionally, other locations. All samples were first analyzed for the presence/absence of PSTVd by dot-blot hybridization; positive samples were then used for synthesis of complementary DNA and PCR amplification using two pair of primers to generate both full-length and shorter, overlapping RT-PCR products.

As shown in Table 1, dot-blot hybridization and/or PCR analysis revealed that two *in vitro* plantlets and 11 potato plants grown from tubers were PSTVd-infected. Nucleotide sequences of eight isolates were determined, and seven isolates were also bioassayed on Rutgers tomato to determine strain type. Sequences of the Ukrainian isolates were compared with that of the type strain from the USA (PSTVd.018, GenBank V01465) which produces symptoms of intermediate severity. Differences are summarized in Table 2 where the numbering system corresponding to that of PSTVd.018, and deletions (e.g., -A121) and insertions (e.g., +U313a) are denoted by '–' or '+' signs, respectively.

Sequences of three Ukrainian isolates (Obrit/82B, Priekul'ski/86B and Rodich/88B) were identical to that of Russian isolate Bugry-97 previously deposited in GenBank (EF044303). Isolate Sokol'ski-08/80B_09 was identical to a previously reported Polish isolate (X76844). When re-sequenced in 2010 after one additional passage, its primary structure contained one additional mutation. Isolate Odessa-08/83B was identical to Russian isolate VNIIKH_07/42M. Isolate Panda/87B was sequenced twice, with slightly different results; in each case, the nucleotide sequences had not been described previously. Like most Russian isolates of PSTVd, all Ukrainian isolates lack an A residue at position 121 and contain an A120U/C substitution when compared to the Intermediate/type strain. The latter A120C change is characteristic exclusively of Russian isolates as is the C256U substitution found in isolate Ch-Panda/87B. Thus, the majority of Ukranian PSTVd isolates sequenced to date are closely related to isolates circulating in Russia.

Table 1. **Detection of PSTVd in Ukrainian potatoes**

Item #	Symbols	Place of origin	Pototo variety	Dot-blot*	PCR*
1	67B	Lugansk	Unknown	0/1	0/1
2**	m69B	Chernigov	Pekurovski	+	
3**	m70B	Chernigov	Pekurovski	+	
4**	m71B	Chernigov	Priekul'ski rannii	–	
5**	m72B	Chernigov	Priekul'ski rannii	–	
6	73B	Odessa	Unknown	0/3	0/3
7	74B	Poltava	Cardinal	1/1	1/1
8	75B	Kilikia	Belonevskii	0/1	0/1
9	76B	Vinnitsa	Unknown	1/1	0/1
10	77B	Odessa	Belonevskii	0/3	0/1
11	78B	Odessa	Unknown	1/1	?
12	79B	Chernigov	Dobrochin	2/3	1/1
13	80B	Chernigov	Sokol'skii	1/1	1/1
14	81B	Chernigov	Lugovskoi	0/4	0/1
15	82B	Chernigov	Obrit	2/2	1/1
16	83B	Chernigov	Bylina	1/2	?
17	84B	Chernigov	Bimonda	0/2	0/2
18	85B	Chernigov	King Edward	1/4	0/0
19	86B	Chernigov	Priekul'skii rannii	2/2	1/1
20	87B	Chernigov	Panda	4/4	2/2
21	88B	Chernigov	Rodich	1/3	1/1

* number of samples positive/number of samples analyzed.
** *in vitro* plantlets.

Table 2. **Sequence changes in Ukrainian isolates of PSTVd**

Item #	Isolate name	Sequence Changes *	Identity	Symptom severity (tomato)
1	Odessa-07/73B	A120C, –A121, C256A, A310U, +U313a	VNIIKH-07/42M (EU879921) Russia	Mild
2	Sokol'skii-08/80B_09	A120U, –A121 A310C, +U313a	PSTVd.021 (X76844) Poland	Mild
3	Sokol'skii-08/80B_10	A120U, –A121 -A126, A310U, +U313a	Unique	Mild
4	Obrit-08/82B	A120C, –A121	PSTVd.125 (Bugry-97) Russia	Intermediate
5	Priekul'ski-08/86B	A120C, -A121	PSTVd.125 (Bugry-97)	Intermediate
6	Panda-08/87B_09	A120U, –A121, C256U, +U101a, A310U, +U313a	Unique	?
7	Panda-08/87B_10	A120U, –A121, C256A, A310U, +U313a	Unique	Mild
8	Rodich-08/88B	A120C, –A121	PSTVd.125 (Bugry-97)	Intermediate

Compared to PSTVd-Intermediate strain (V01465).

Discussion. Russian isolates of PSTVd appear to form two groups/populations: one population can be considered to be derivatives of Russian isolate Bugry-97 (PSTVd.123 = EF044304); a second population contains derivatives of Russian isolate Onega-Premier-94 (PSTVd.125 = EF044303). Both populations differ from the type strain (PSTVd.018 = V01465) by i) the absence of A121 and ii) the presence of a single A120U or A120C substitution. All eight Ukrainian isolates sequenced in this study are members of these two populations. Five isolates belong to the PSTVd.125 population containing an A120C substitution found exclusively in Russian isolates. Isolates Panda-09/87B_09 and its variant Panda-09/87B_10 from Chernigov also contain a second substitution (i.e., C256U/A) that is present only in Russian isolates. Symptoms induced by PSTVd.123 and 125 on Rutgers tomato were very similar to those of the type strain. Several other Ukrainian isolates, in contrast, induced only mild symptoms. Thus, Ukrainian isolates of PSTVd resemble Russian isolates in both nucleotide sequence and biological properties.

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ETIOLOGICAL PROGNOSTICATION OF INFLUENZA EPIDEMICS IN UKRAINE

Етіологічне прогнозування спалахів грипу використовується для передбачення епідемій, розрахунку очікуваних рівніе захворюваності та смертності, а також для вибору стратегії профілактики. Оскільки на Україні вакцини не виробляються, особливу увагу слід приділяти ідентифікації основних інфекційних агентів. В статті наведено аналіз етіологічних прогнозів епідемій грипу в Україні за останні 12 років.

An etiological prognostication influenza outbreak is used for epidemic forecast, calculating expected morbidity and mortality and choosing preventive strategies. As influenza vaccines aren't produced in Ukraine, attention should be paid on identification leading infectious agent. It was analyzed etiological prognoses of influenza epidemics in Ukraine through last 12 years.

Influenza is a highly contagious acute viral infection of the respiratory tract that can affect nasal and mucous, bronchi and even alveoli. This infection might present an endemic, epidemic or pandemic behavior [4]. Etiological prognostication influenza outbreaks is used for epidemic forecast, calculating expected morbidity and mortality, choosing the most effective preventive strategy, planning of controlling and prophylactic measures.

Prognostication influenza epidemics requires for many conditions and only comprehensive derivative information as the final link of annual epidemiological surveillance in Ukraine. Annual outbreaks in different countries are provoked by high variability and transmission influenza viruses, especially for North hemisphere region [2]. It should be noted, periodic shift of influenza viruses cause pandemics with the high morbidity and mortality level. New pandemic influenza A(H1N1) virus, that emerged in the April 2009, have been differed significantly from previous epidemics. This fact has given rise to new problems and tasks [5].

Composition of etiological prognosis is important for predicting annual epidemics. More over, immunity against influenza viruses is strain specific. This means, that only relevant vaccines should be used for influenza prophylactic. An influenza vaccine does not produce in Ukraine. In this reason, we should pay more attention to leading infectious agent of next season for vaccines purchase.

The main aim of our work was to analyze the etiological prognostication of influenza epidemics in Ukraine during last 12 years.

Materials and methods. Materials: nasopharyngeal swabs and autopsy materials from patients, received from the different areas of Ukraine, field isolates of influenza viruses 1998-2010 seasons, strain specific serum for HIA identification.

Methods: hemagglutinin inhibition assay with using chicken and guinea pig red blood cells; real-time RT-PCR analyses with using CDC primers and adopted protocols [1].

Results and discussion. We determined that for relevant etiological prognostication influenza outbreaks at strain level is necessary to obtain the information concerning:

1) The etiological structure of influenza virus populations during previous 5 years in our country;

2) Influenza and ILI (influenza-like illnesses) morbidity level for previous years;

3) Dominated influenza viruses in the world during previous 5 years;

4) New viruses emerged in the world;

5) Intensity of epidemic process in the world for previous years.

Our prognoses are based on statistically-calculated values with including fundamental epidemiology basis and experience of previous pandemics which defined by WHO experts.

We analyzed influenza epidemic situation in Ukraine during previous 12 years (systematic virological surveillance) and have found the clear epidemiological and virological regularity of this disease, which can be a very reliable basis for predicting future epidemics.

During 1998-1999 epidemic season the new virus A/Sidney/5/97 (H3N2) was predicted as a leading infectious agent of epidemic in Ukraine, that was entirely approved. That virus strain was isolated in Volynska and Cherkasska regions. More over, another virus strain (A/Yohannezburh/33/94 (H3N2)) was isolated in Odessa. The diversity among influenza virus populations in Odessa can be explained by the special geographical location of this city and presence of large seaport. These circumstances increase the ability of coming agent from other areas.

At 1999-2000 epidemic season influenza virus type B was prognosed as major infectious agent in Ukraine. However, most isolated viruses belonged to A/Panama/2007/99(H3N2) strain, which were isolated in Kiev and Kiev region., Like previous season difference between populations of influenza viruses observed in Odessa. There have been isolated A(H1N1)/Bern/7/95-like influenza viruses. Thus, the etiological prognosis wasn't confirmed for this influenza season.

Influenza virus type B also was expected as a dominant agent in the next 2000-2001 epidemic season in Ukraine. However, the prognosis wasn't approved again. The leading infectious agent of that outbreake was A(H1N1)/New Kaledoniya/20/99-like influenza viruses. Moreover, the amount of isolated viruses was considerable during the epidemic – 56 isolates in 9 regions. It has provided representativeness of virological data and more exact completion of etiological prognostication for the next epidemic season.

We prognosed B/Sychuan/379/99 influenza virus as a leading infectious agent during 2001-2002 epidemic season. Prognosis was completely confirmed, despite the low intensity of the epidemic process during that season. Such viruses were isolated in three regions of Ukraine.

Next year B/Sychuan/379/99 strain have been prognosed as a leading infection agent of epidemic again. Perhaps, the level of intensity the influenza outbreak hasn't enabled this strain realize fully in previous season. However, A(H1N1)/New Caledoniya/20/99 strain has been dominated during the epidemic So, prognosis was not confirmed [3].

A/Fudzyan/411/02 (H3N2) strain was predicted as the leading infection agent in 2003-2004 epidemic season. Despite of the low intensity the influenza epidemic, the greatest number of isolated viruses belonged to subtype A(H3N2), which is related to both A/Panama/2007/99 strain and A/Fudzyan/411/02 strain, as prognosed.

During the 2005-2006 epidemic season in Ukraine the new virus A/Californiya/7/2004 (H3N2) was predicted as a leading agent. Circulated viruses were similar to the strain A/Californiya/7/2004. Therefore the etiological prognosis was approved.

The majority (45,9 %) of isolated viruses in 2007-2008 were related to the vaccine virus A/Brisbane/59/07 (H1N1). A small part of influenza viruses (28,8 %) were related to the B/Floryda/4/06 strain of B/Yamahata/16/99 genetic branch.

The higher proportion of isolates during the 2008-2009 outbreak were related to A/Brizben/10/07 (H3N2) strain (83 %). It was only 16 % of type B influenza viruses that were similar to the B/Brisbane/60/08 strain, which belonged to the V/Viktoriya/2/87 genetic branch. And only 2 isolates (1 %) were related to A (H1N1) subtype.

Although, B/Brysben/60/2008 strain had been predicted as the main infection agent, the leading agent of 2009-2010 pandemic season were A/California/07/2009-like viruses.

Referencing of predicted and actual infection agent of influenza outbreaks in Ukraine are shown in Table 1.

Table 1. **Conformability between predicted and actual infectious agent of influenza epidemic in Ukraine for 12 epidemic seasons**

Season	Predicted agent	Actual leading agent	Correspondence
1998-1999	A/Sydney/7/95 (H3N2)	A/Sydney/7/95 (H3N2)	Yes
1999-2000	B	A/Panama/2007/99 (H3N2)	No
2000-2001	B	A/New Caledonia/20/99 (H1N1)	No
2001-2002	B/Sichuan/379/99	B/Sichuan/379/99	Yes
2002-2003	B	A/New Caledonia/20/99 (H1N1)	No
2003-2004	A/Fudzyan/411/02 (H3N2)	A/ Panama/2007/99 – like (H3N2)	serotype – yes
2004-2005	A/Fudzyan/411/02 (H3N2) B/Shanghai/361/02	B/Dzhanhsu/10/03 (B/Shanghai/361/02-like)	Partly – yes
2005-2006	A/California/7/04 (H3N2)	A/California/7/04 (H3N2)	Yes
2006-2007	A/Wisconsin/67/05	A/Wisconsin/67/05	Yes
2007-2008	A/Brisbane/59/07 (H1N1)	A/Brisbane/59/07 (H1N1)	Yes
2008-2009	A/Brisbane/10/07 (H3N2)	A/Brisbane/10/07 (H3N2)	Yes
2009-2010	B/Brisbane/60/08 A/California/07/09	A/California/07/09	Partly – yes

Conclusions. We have concluded etiological prognoses of influenza epidemics in Ukraine through last 12 years. Data toward accordance of prognoses and actually leading strain of influenza viruses in country has shown that prognoses had been confirmed entirely in 50%. In 25% of cases prognoses were confirmed partly. And in 25 % of cases prognoses were not justified.

The demand of annual etiological prognostication is determined by the strain-dependent immune response to influenza viruses. It means that only actual vaccines must be used for the prophylactic of flu. An influenza vaccine does not produce in Ukraine. In this reason, we should pay more attention to leading infectious agent of next season for vaccines purchase.

Thereby, 12 epidemics have happened during surveillance influenza viruses. Our etiological prognoses were completely or partly approved in 75 % of cases.

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THE CHERRY LEAF ROLL VIRUS (CLRV) ON WALNUT IN THE REPUBLIC OF MOLDOVA

Проведено тестування бруньок 6 сортів волоського горіху, відібраних на комерційних плантаціях, на наявність вірусу скручування листя черешні (родина Secoviridaea, рід Nepovirus). Для детекції CLRV у зразках використовували специфічну антисироватку при проведенні ІФА, в модифікації DAS, та ISEM. Загальна кількість протестованих зразків становила 315. Вірус був ідентифікований у 14 зразках рослин сорту Когалнічану. Проведено механічне зараження рослин Nicotiana occidentalis 37B, потім вірус перенесено на рослини Chenopodium quinoa Willd, які використовували для отримання очищених препаратів. Була отримана поліклональна антисироватка до CLRV з титром 1:4000.

Bud samples from 6 varieties of walnut from commercial plantation were collected and tested for the presence of cherry leafroll virus (family of Secoviridae, genus of Nepovirus). The specific antisera was used for detection CLRV in samples by DAS-ELISA as well as by ISEM. There were tested 315 samples in all. The virus was found in 14 samples of var. Kogalnichanu. The virus was transmited mechanicaly on the tobacco plants Nicotiana occidentalis 37B and then passed on Chenopodium quinoa Willd that was used for obtaining the purified preparation. There was obtained the policlonal antiserum to the selected isolate CLRV with the titre 1:4000.

Introduction. The Republic of Moldova has a favorable natural conditions and ancient traditions to promote a culture of walnut. Early last century, Moldova exported 10 000 tonnes of fruit and nut plant material to neighboring countries, grown exclusively by seed. Lack of theoretical and practical skills of vegetative propagation has led to irretrievable loss of many valuable forms of culture. Since 60-ies 19th century in Moldova was selected varieties according to certain criteria. So, in 1980 were legitimized five varieties, and in 2009 there were already assortment of 14 items. At the same time, scientists of Moldova are developing technology of vegetative propagation of walnut, which makes it possible to produce annually up to 250,000 grafted plants per year. Production of such a large number of planting material without proper fitosanitary control is a threat of widespread destructive virus in this culture of cherry leaf roll virus (CLRV).

This virus was first described in 1976 in Italy (Savino et al., 1976). Subsequent studies have identified three isolates of the virus causing yellow mosaic disease, ring spot and black line on union zone. The first two of the disease got its name based on the symptoms they cause on the herbaceous indicators. Isolate black line is associated with the incompatibility of sorts with some rootstock and is the cause of mass mortality of plants in the traditional walnut-growing this crop (Mirchetich et al., 1982; Cooper, 1980; Quacquarelli and Savino, 1977; Nemeth et al., 1982; Delbos et al., 1983; Kolber et al., 1983). Established that CLRV transmitted vegetatively and pollen. According to Kolber and Nemeth (Kolber and Nemeth, 1983) the percentage of infected seeds sampled from diseased trees may reach 92.8%. Same number of infected seedlings grown from seeds of infected trees, reaching more than 4% (Quacquarelli and Savino, 1977). As regards the transfer CLRV pollen, found that during the pollination of about 19% of the trees can be infected with the virus under investigation. Based on the above and taking into consideration the annual growth of walnut plants produced in the country, we have initiated a study on the infected trees in mother plantation.

Materials and methods. The material of study was axillary buds annual shoots of mother walnut trees. Diagnosis CLRV was performed by ELISA (Clark and Adams, 1977) using commercial diagnostic kits firms "LOEWE" and immunosorbent electron microscopy (ISEM). Primary mechanical transfer of virus to herbaceous indicator Nicotiana occidentalis 37B was performed by rubbing leaves an indicator of buds extracts in phosphate buffer, pH 7.4. The same buffer is used to transfer CLRV from infected tobacco plants to Chenopodium quinoa Willd. to obtain of purified virus particles. Purification of the virus was carried out using standard stages of homogenization, clarification of leaf extract, the concentration of particles by high-speed centrifugation and fractionation in sucrose density gradient. To obtain the antiserum short schema of immunization is used with subsequent double reimmunization experimental animals at 60 and 70 from the beginning of the cycle of immunization. Antibody activity was determined by ISEM with the use of extracts from systemically infected leaves indicator Ch.quinoa.

Resalts and discussion. The study of virological situation in the plantations of walnut were started by us with visual inspection of high-quality mother plantation at specialized commercial nursery "AMG KERNEL" for the production of walnut trees. From each tree, regardless of the presence or absence of their symptoms, we selected

samples that were tested by ELISA and ISEM for infection CLRV. As the source of infection were used tissues of axillary buds of annual shoots. A total of 315 trees were tested of six varieties of this crop, 14 trees were infected CLRV (Fig. 1).Axillary buds of infected samples were subsequently used for the mechanical transfer of CLRV to tobacco plants N.occidentalis 37B. For 6-7 days after infection, some plants of this indicator stood in growth. After verification by ISEM tissues of new growing tobacco leaves on CLRV infection, the virus was passaged to the indicator Ch.quinoa, inoculated leaves showed chlorotic spotting in 4-5 days after infection. Purification was carried out from systemically infected leaves, sampled at 10-12 days after infection as follows – 100 gr. infected leaves were homogenized in 0.05 M pH 7.2 phosphate buffer in the presence of 0.02 M 2 mercaptoethanol, followed by filtration through 3 layers of gauze. The resulting plant extract was centrifuged 12 min at 8 000g in the Al 35 BECKMAN rotor. Supernatant was clarified by a magnetic stirrer at +4 ° C by adding butyl alcohol final concentration of 9%. After 30 min the suspension centrifuged for 15 min at 8 000g in the same rotor. In the clarified liquid is added polyethyleneglycol (6000) at a final concentration of 9% + 1.15% NaCl. After 60 minutes the resulting suspension was centrifuged for 10 min at 15 000g. The resulting pellet was suspended in 0.05 M phosphate buffer overnight and centrifuged for 150 min at 83 000g in a Ti 70 BECKMAN rotor. The pellet was suspended and layered on sucrose gradient (10-40%). Viral band was diluted 5 times with phosphate buffer and centrifuged for 150 min at 83 000g. The purity of virus preparations tested on a spectrophotometer SPECOL 1500. A ratio of 260/280 = 1.62, which corresponds to published data. Verification of the preparate in the electron microscope showed the presence of particles which morphology was not disrupted (Fig. 2). Shinshillas rabbits aged 1,5-2 years were used for immunization. The scheme consisted of subcutaneous ibjuction with complete Fraund's Adjuvant, intramuscular – with incomplete Adjuvant and intravenous injections at weekly intervals. During the rise of specific antibodies in the blood of immunized animals they took blood starting from 10 days from the last injection. Blood was taken from the ear vein every 7 days to 120 days from the start of immunization. In 10 days after the last injection antiserum titer was 1 / 4000 and stayed at that level for 7 weeks. Activity of antibodies in the blood of animals increased again to 1 / 4000 after 2 cycles of reimmunization. Antiserum stored at -18 ° C and is widely used in detection of CLRV in infected trees not only walnuts, but other fruit crops.

According to long-term plan by 2020 the area under the walnut in Moldova should be increased to 14 thousand ha., It is necessary to produce 2 million plants. This, in turn, will require new seed and varieties mother plantations. On the other hand as a result of studies in plantations of walnut identified one of the most dangerous viruses. Of the 315 trees tested, 4.4% were infected with CLRV. Given that the virus spreads vegetatively and pollen in the selection of mother trees for nut and sorts plantings will need to comply with internationally accepted phytosanitary standards prior to testing the purity of CLRV. Prepared antiserum will be used to diagnose the virus by ELISA and ISEM, which will provide a new mother plantation free from CLRV trees.

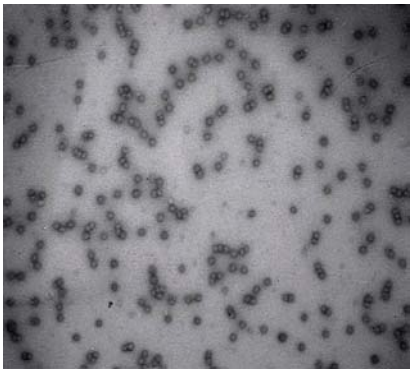

Fig. 1. CLRV particles in the product prepared

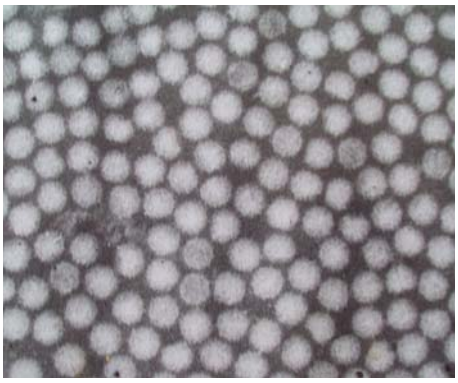

Fig. 2. Electron micrograph of purified preparation CLRV. from axillary buds walnut by ISEM

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MOLECULAR CHARACTERIZATION OF AN IRIDOVIRUS ISOLATED FROM MOSQUITO *AEDES FLAVESCENS*

В роботі представлені результати вивчення молекулярних властивостей іридовіруса комара Aedes flavescens методами електронної мікроскопії, електрофорезу білків, рестрикційного аналізу вірусної ДНК та ПЛР. Молекулярна вага головного капсидного білку іридовірусу A. flavescens, розмір вірусної ДНК та специфічна ПЛР-ампліфікація свідчать, що даний ізолят належить до родини Iridoviridae. Натомість за білковим та рестрикційним профілями іридовірус комара A. flavescens відрізняється від іншого іридовіруса комара Aedes taeniorhynchus, типового представника роду Chloriridovirus.

An iridovirus, isolated from mosquito Aedes flavescens in Kiev region, was investigated by the methods of electron microscopy, analysis of viral protein in gradient SDS polyacrylamide gel electrophoresis, restriction of viral DNA and amplification of a segment of viral DNA in polymerase chain reaction. All approaches yielded results consistent with the suggestion that mosquito iridovirus Ae. flavescens was a member of the family Iridoviridae. Moreover RFLP analysis of viral DNA, number of virus proteins and molecular weight of major capsid protein distinguished iridovirus Ae. flavescens from another mosquito iridovirus Ae. taeniorhynchus, the type species of the genus Chloriridovirus.

Introduction. Iridoviruses are large DNA viruses that replicate in the cytoplasm of infected cells. Iridovirus genomes are circularly permuted and terminally redundant, and range in size from 105 to 212 kbp. The family *Iridoviridae* is currently subdivided into five genera: *Chloriridovirus, Iridovirus, Lymphocystivirus, Megalocytivirus*, and *Ranavirus*. Iridoviruses have been found to infect invertebrates and poikilothermic vertebrates, including amphibians, reptiles, and fish [1].

Invertebrate iridescent viruses (IIVs) are icosahedral particles of approximately 120–200nm in diameter that infect invertebrates, mostly insects, in damp or aquatic habitats. These viruses cause two types of disease: one patent and the other covert (inapparent). An abundance of virus particles in the cells of patently infected insects causes them to develop an obvious iridescent color that typically ranges from violet, blue, green, or orange [2].

Iridoviruses that infect mosquitoes and midges belong to the genus *Chloriridovirus.* The taxonomic situation is even more unsatisfactory for the genus *Chloriridovirus* , which, despite numerous records of infections in many species of mosquitoes and midges of medical and veterinary importance, consists of a single species, *Invertebrate iridescent virus 3* (IIV-3) from the saltmarsh mosquito, *Ochlerotatus* (*Aedes*) *taeniorhynchus*. That is why the newly isolated mosquito iridovirus *Ae. flavescens* was investigated with the purpose to understand its relatedness within family *Iridoviridae.*

Materials and methods. Virus propagation. An iridovirus, isolated from mosquito *Ae. flavescens* in natural reservoirs of the Kyiv region*,* was propagated in multihost wax-moth larvae *Galleria mellonella.* The wax-moth larvaes were infected by intraperetonal injection and incubated at temperature 20-22 ^{0}C. In 14 days after injection the mosquito iridovirus was purified from wax-moth larvae *G. mellonella*.

Virus purification. The mosquito iridovirus *Ae. flavescens* was purified from the infected wax-moth larvae *G. mellonella* by the differential centrifugation method. Briefly, after homogenization of infected larvae *G. mellonella*, cell debris was separated by centrifugation at 3000 × *g* for 5 min at 10°C. The pellet was discarded and the supernatant centrifuged in ultracentrifuge Beckman L5-50B in a rotor SW-40 for 40 min at 70500 × *g* at 4°C. Characteristic blue pellet confirmed the presence of mosquito iridovirus virions. The virus pellet was suspended in TNE (50 mM Tris-HCl, 150 mM NaCl, 1 mM disodium ethylene diaminetetracetic acid [EDTA], pH 7.5) and centrifuged at 1100 × *g* for 5 min at 10°C. The suspension was layered onto a 10 to 50% (w/w) linear sucrose gradient in TNE. After centrifugation at 70500 × *g* for 40 min at 4°C, 1 visible band near the bottom of the tube were collected, diluted in fresh TNE and centrifuged at 70500 × *g* for 40 min at 4°C. The virus pellet was placed into TNE at a final protein concentration of 1,7 mg ml^{-1}.

Electron-microscopy. For electron-microscopy investigation the viral suspension was stained with 2% uranyl acetate and observed in microscopy EM-125 (SEO).

DNA extraction. Viral genomic DNA was extracted using a 25:24:1 phenol:chloroform:isoamyl alcohol (IAA) mixture, followed by ethanol precipitation [3]. Briefly, purified virus was incubated with Proteinase K in the presence of lysis buffer for 3 h at 56°C and then treated with IAA. A 0.1 volume of 3 M sodium acetate was added to the aqueous phase following IAA extraction followed by 2.5 volumes of cold 95% ethanol. The mixture was incubated overnight at –20°C, followed by centrifugation at 12 500 × *g* to precipitate the DNA. The supernatant was removed and the pellet was washed with cold 70% ethanol and then air-dried. TE buffer was added and the DNA pellet was incubated for 5 min at 37°C to dissolve the DNA. DNA concentrations were determined by spectrophotometer (APEL PD-303 UV) and the samples were stored at –20°C.

Analysis of virion polypeptides. Purified virus (1,7 mg protein ml^{-1}) in TNE was mixed 1:1 with 2× sample application buffer, heated to 100°C for 5 min. Virion polypeptides were separated by SDS-PAGE in 5-20% gradient gel according to the system of Laemmli [4]. Molecular weight standards (Fermentas) were included in gel. After electrophoresis, the gels were stained with Coomassie blue R-250 and the approximate molecular weight of the virion polypeptides was estimated by their mobility relative to the molecular weight standards.

Restriction fragment length polymorphism (RFLP). For RFLP analysis, 1 to 2 μg of viral DNA from purified preparations of iridovius *Ae. flavescens* was incubated with 10 U of *Eco*RI, *Bam*HI, *Xba*I, *Hind*III, *Hpa*II and *Msp*I endonuclease (Fermentas) for 16 h at 37°C. DNA fragments were separated by electrophoresis on 0.5% agarose gels and observed after staining with 1% ethidium bromide.

Primer selection and oligonucleotide synthesis. We designed oligonucleotide primers to amplify DNA of iridovirus *Ae. flavescens*. Using nucleotide sequences of all iridoviruses a set of oligonucleotide primers was designed for ribonucleoside reductase small subunit (RNRS) gene [5]. Selected oligonucleotides were tested for possible secondary structure and self-complementarity using Vector NTI 11 software (INVITROGEN). The forward primer (MIV_RNRS_F) sequence was 5' – CTC CAC GAC CAG AGT GCT AAA G – 3' and the reverse primer (MIV_RNRS_R) sequence was 5' – TGC TAC GAC AAG TGG GAT ACG C – 3'. Oligonucleotides were synthesized commercially (Metabion). The predicted location of the 350 base-pair (bp) amplified product within the ribonucleoside reductase small subunit (RNRS) gene was based on the

sequence of Invertebrate iridescent viruses 3 (IIV-3; GenBank Accession No. DQ643392) [6].

Polymerase chain reaction (PCR) assay. The reagents used for PCR amplification were from Fermentas. Each 25 µl reaction mixture (prepared on ice) contained the sample DNA, 2,5 µl of 10× PCR buffer, 2 mM $MgCl_2$, 2.5 µl of dNTP mixture, 10 pmol of each primer, 0.75 U of *Taq* polymerase and distilled water up to total volume. A MasterCycler (Eppendorf) was used for the PCR as follows, Cycle 1: 94°C for 2 min; Cycles 2 to 30: 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min; Cycle 31: 72°C for 5 min. Control received the PCR mixture containing no DNA template (reagent control). After the PCR, products were transferred to a 1.0% agarose gel, electrophoresed, and DNA was visualized by ethidium bromide staining.

Results and Discussion. In this study, we described the isolation and characterization of a potentially novel iridescent virus from mosquito *Ae. flavescens* . Our data from electron microscopy reveal that iridescent virus *Ae. flavescens* exhibits the major morphological characteristics of an iridovirus. A sixfold symmetry of virus particles can be observed in negatively stained material. With sizes of 200 ± 5 nm, the particles exhibit dimensions ranging between the reported typical sizes of large (genus *Chloriridovirus*: 180–200 nm) invertebrate iridoviruses [1].

The ultrastructural investigations of viral particles show an electron-dense core surrounded by an internal lipid bilayer and an outer electron-dense shell. This is in accordance with structural data of other members of the family *Iridoviridae* [7]. The nucleoprotein core of iridoviruses is surrounded by an internal lipid membrane and a protein shell forming the icosahedral particle.

Gradient SDS-PAGE of purified virions revealed the presence of 12 polypeptides ranging from 14,5 to 122 kDa. The molecular weight of a major capsid protein (MCP) was a 48 kDa. Interestingly that number of proteins of another mosquito iridovirus *Ae. taeniorhynchus* is 9 ranging from 15,5 to 98 kDa and molecular weight of MCP is approximately 55 kDa (Tab. 1). These data have the characteristics of those of iridoviruses [1].

Table 1. **The molecular weight of proteins of mosquito iridoviruses isolated from *Ae. flavescens* and *Ae. Taeniorhynchus***

№	Molecular weight of proteins, Da	
	Aedes flavescens iridescent virus	*Aedes taeniorhynchus iridescent virus*
1	14 500	15 500
2	18 000	19 000
3	34 000	30 000
4	48 000	37 000
5	53 000	54 000
6	57 500	68 500
7	60 000	84 000
8	63 000	94 000
9	80 500	98 000
10	108 500	
11	114 000	
12	122 000	

Restriction enzyme analysis of isolated iridovirus *Ae. flavescens* DNA with the endonucleases *Eco*RI, *Bam*HI, *Hin*dIII, *Xba*I, *Msp*I, and *Hpa*II has revealed that the restriction fragment patterns of iridovirus *Ae. flavescens* differ from those obtained from experiments with iridovirus of mosquito *Ae. taeniorhynchus* (Tab. 2) and iridoviruses of lower vertebrates, such as Rana esculenta iridovirus (REIR) or *Frog virus 3* (FV-3). The genome of FV-3 does not show cleavage sites for *Eco*RI, but it exhibites numerous cleavage sites for *Bam*HI (Ahne *et al.,* 1998) [8]. In contrast to invertebrate iridoviruses, the genome of vertebrate iridoviruses has been shown to be methylated by a virus-specific methyltransferase. The methylation of viral DNA at cytosine residues is a characteristic stage of the replication cycle of these viruses, protecting newly synthesized virus DNA from digestion by vertebrate cytoplasmic endonucleases. Methylated viral DNA can be distinguished from unmethylated forms by digestion with the endonucleases *Msp*I and *Hpa*II. These restriction enzymes have the same cleavage site (CCGG). However, whereas *Msp*I is able to cleave viral DNA in the presence of methylcytosine, *Hpa*II is not. DNA of Iridovirus *Ae. flavescens* is degraded by both endonucleases. Thus, it must be unmethylated and exhibits the expected characteristics of invertebrate iridovirus DNA.

Table 2. **Comparative restriction fragment length polymorphisms of the viral DNA from iridoviruses *Ae. flavescens* and *Ae. taeniorhynchus* ***

Restriction endonuclease	*Aedes taeniorhynchus iridescent virus*		*Aedes flavescens iridescent virus*	
	Number of fragments	Fragment length, kbp	Number of fragments	Fragment length, kbp
*Bam*HI	24	0,323-23,9	11	5,3-24,1
*Eco*RI	11	0,238-55,753	23	2,1-24,3
*Hind*III	45	0,08-23,969	25	1,3-13,0
*Xba*I	19	0,021-31,765	18	3,1-24,3
*Hpa*II	560	0,004-6,272	19	1,5-11,9
*Msp*I	560	0,004-6,272	19	1,5-11,9

* – RFLP analysis of *Aedes taeniorhynchus iridescent virus* was made by usage the Vector NTI11 software

Using nucleotide sequences of all iridoviruses a set of oligonucleotide primers was designed for RNRS gene. After 30 cycles of amplification of mosquito iridovirus *Ae. flavescens* genomic DNA, PCR products of about 350 bp were visible on agarose gels stained with ethidium bromide.

Knell *et al.* (2006) and Tang *et al.* (2007) have recently reported the isolation of an iridescent viruses from mopane warm *Imbrasia* (*Gonimbrasia*) *belina* and sergestid shrimp *Acetes erythraeus* [9, 10]. We have described an invertebrate DNA virus from *Ae. flavescens,* termed *Aedes flavescens* iridescent virus (AfIV), which can be

classified as a member of the genus *Chloriridovirus.* The impact of this virus in the natural environment is unknown. Although, it is not known whether or not this iridovirus can infect any other mosquito species, many members of the *Iridoviridae*, such as IIV-6 and ranaviruses, are known to have large host ranges.

Interest in invertebrate iridoviruses has been limited by the perception that they have little potential as biological control agents against insect pests. However, there is now growing awareness of the potential impact of sublethal IIV disease on the dynamics of insect populations, including insect vectors of medical importance worldwide. Studies with insects indicate that sublethal iridovirus infections can also seriously impact host fitness through reduction in reproductive capacity, body size, and longevity [7]. The mosquito iridovirus *Ae. flavescens* can be promising biological control agent against insect pests, as it causes covert disease and reduces mosquito stocks.

Therefore, future experiments are required to establish whether AfIV is a new species within the genus *Iridovirus* or just an isolate of a previously known virus species. In addition, it would be worthwhile to investigate the susceptibility of AfIV to other species of *Aedes* and to explore the prevalence of these viruses in wild stocks.

Conclusion. Electron microscopy and molecular approaches yielded results consistent with the suggestion that mosquito iridovirus *Ae. flavescens* was a member of the family *Iridoviridae*. Moreover RFLP analysis of viral DNA, number of virus proteins and molecular weight of MCP distinguished iridovirus *Ae. flavescens* from another mosquito iridovirus *Ae. taeniorhynchus*, the type species of the genus *Chloriridovirus*. Presently, a large numberof mosquito iridoviruses, that have been reported in literature, are insufficiently studied and consequently they are not classified. Our observation may help to understand the relatedness of mosquito iridovirus *Ae. flavescens* within family *Iridoviridae.*

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DETECTION AND IDENTIFICATION OF VIRUSES AFFECTING IRISES IN LITHUANIA AND UKRAINE

Робота виконана згідно зі спільною Литовсько-Українською програмою співробітниками кафедри вірусології Київського національного університету імені Т. Шевченка та лабораторії вірусів рослин інституту ботаніки в рамках проекту "Моніторинг складу вірусних патогенів декоративно-квіткових культур в Україні та Литві". Мета даної роботи – дослідження вірусних захворювань півників (Iris L.) ботанічних садів Литви та України; ідентифікацію збудників проводили за допомогою класичних та новітніх, молекулярно-біологічних, методів. Вірус помірної мозаїки півників (IMMV) був виявлений в усіх досліджених зразках як Литви так і України. Методами рослин індикаторів, електронної мікроскопії, імуноферментного аналізу, в модифікації DAS, та зворотно-транскрипційної полімеразної ланцюгової реакції (RT-PLR) з деяких литовських зразків були ізольовані та ідентифіковані віруси тютюнової мозаїки (TMV), кільцевої плямистості тютюну (TRSV) та кільцевої плямистості томатів (ToRSV).

According to the joint Lithuanian–Ukrainian program the research workers of Department of Virology T. Shevchenko Kyiv National University and Plant Virus Laboratory of Nature Research Center Institute of Botany are fulfilling research project "Monitoring of diversity of virus pathogens infecting ornamental plants in Ukraine and Lithuania". The aim of this work was to investigate viral diseases affecting irises (Iris L.) grown in Botanical gardens in Lithuania and Ukraine; to identify their agents applying the classical and modern, molecular biology methods. Iris mild mosaic virus (IMMV) was detected in all investigated Lithuanian and Ukrainian samples; Tobacco mosaic (TMV), Tobacco ringspot (TRSV), and Tomato ringspot (ToRSV) viruses have been isolated and identified in some investigated Lithuanian samples by the methods of test–plants, electron microscopy, double–antibody sandwich enzyme–linked immunosorbent assay (DAS–ELISA), and reverse transcription polymerase chain reaction (RT–PCR).

Introduction. Irises have been used for centuries both as ornamentals and as a source of perfumes and medicine. This wide-ranging genus of more than 200 species is native to the temperate regions of the Northern hemisphere. There are many hybrids. Irises are divided into main two groups: rhizomatous and bulbous.

Spread of viruses in irises remains a serious problem. Until now 15 viruses affecting irises have been described in literature: *Iris mild mosaic virus*, *Narcissus latent virus*, *Iris severe mosaic virus*, *Bean yellow mosaic virus*, *Tomato spotted wilt, Impatiens necrotic spot virus*, *Turnip yellow mosaic virus* [1], *Tobacco ringspot virus, Tobacco mosaic virus, Tobacco rattle virus*, *Iris fulva mosaic virus*, *Cucumber mosaic virus, Broad bean wilt virus*, *Lilac chlorotic leafspot virus*, *Iris germanica virus*.

According to the joint Lithuanian–Ukrainian program the research workers of Department of Virology T. Shevchenko Kyiv National University and Plant Virus Laboratory of Nature Research Center Institute of Botany are fulfilling joint research project "Monitoring of diversity of virus pathogens infecting ornamental plants in Ukraine and Lithuania". The aim of this work was to investigate viral diseases affecting *Iris L.* grown in Botanical gardens in Lithuania and Ukraine; to identify their agents applying the classical and modern, molecular biology methods.

Materials and methods. Plant material was collected in Botanical Garden of Vilnius University, Experimental Station of Field Floriculture and collection of iris breader O. Griniuviene in Lithuania; Botanical garden of Kiyv T. Shevchenko university, and N. N. Griško Kyiv National Botanical Garden in Ukraine.

Viruses have been identified by the methods of test–plants [2], electron microscopy (EM), double–antibody sandwich enzyme–linked immunosorbent assay (DAS–ELISA) and reverse transcription polymerase chain reaction (RT–PCR) [3, 4, 5, 6].

The test–plants were inoculated in early stages of growth by mechanical sap transmission, applying carborundum as an abrasive. The inocula were prepared by homogenizing infected plant tissue in 0.1 M phosphate buffer pH 7.0, containing 1 % nicotine acide as virus–stabilizing additive.

Virus particles were examined in leaf dip EM preparations negatively stained with 3 % uranyl acetate using a JEOL–100S electron microscope, at magnification 25000.

DAS–ELISA was carried out using commercial kits (DSMZ Plant Virus Collection, Germany), according to standard procedure. Immunoglobulins (IgGs) and IgG alkaline phosphatase conjugates were used at a dilution of 1/1000. 50 mg of sample leaf tissue was extracted in 1 ml of sample buffer. 0.1 % p–nitrophenylphosphate was used as substrate. The reactions were measured after 90 min incubation with substrate photometrically at 405 nm (Labsystems Multiskan RC).

RT–PCR was accomplished using the TMV specific primers: F (5'-GAC CTG ACA AAA ATG GAG AAG ATC T-3') and R (5'-GAA AGC GGA CAG AAA CCC GCT G -3') [4].

RT–PCR for TRSV identification has been carried out using forward primer 5'– CTT GCG GCC CAA ATC TAT AA–3' and reverse primer 5'–ACT TGT GCC CAG GAG AGC TA–3' which anneal to the conserved region in coat protein gene. The reaction produced an amplification product of 348 bp [5].

Primers for ToRSV identification by RT–PCR included U1, 5'– GACGAAGTTATCAATGGCAGC–3' (nt 1,078 to 1,098) and D1, 5'–TCCGTCCAATCACGCGTAATA–3' (nt 1,506 to 1,527) of putative viral polymerase gene, resulting in a 449 bp amplification product [6].

Total RNA was extracted from symptomatic plant material stored frozen at –20^{o}C using Plant RNA Purification TRIzol reagent (Carlsbad, CA. USA). Extraction procedure was carried out according to the manufacturer's instructions.

All PCR procedures were carried out in Biometra Tgradient Thermocycler. RNA denaturation mixture (for each sample) of 9 µl RNA and 1 µl of 20 pM reverse primer was incubated 5 min at 70 ^{0}C and 5 min at 4 ^{0}C.

Reverse transcription (RT, cDNA synthesis) reaction mixture (for one sample): 4 µl 5x PCR buffer; 1 µl RNasin; 2 µl 10 mM dNTPs; 1 µl 200 U/µl MulRev Transcriptase and 11 µl of denatured RNA mix. Reaction was performed incubating at 42 ^{0}C for 60 min, at 70 ^{0}C for 10 min and at 4 ^{0}C for 5 min.

TMV PCR mixture contained (for one sample): 10 µl cDNA; 34.75 µl PCR water, 4 µl 2 mM dNTPs, 1 µl 20 pM primer F, 1 µl 20 pM primer R, 5 µl 10xPCR buffer, 3 µl $MgCl_2$, 0.25 µl 5 U/µl *Taq* DNA polymerase. Reaction mixtures were incubated at 94 ^{0}C for 2 min (for first step), 35 cycles of 94 ^{0}C for 30 sec, 62 ^{0}C for 45 min, 72 ^{0}C for 1 min, and at 72 ^{0}C for 5 min (final step).

TRSV PCR reaction mixtures contained (for one sample): 10 µl cDNA; 34.75 µl PCR water; 1 µl 20 pM forward primer; 1 µl 20pM reverse primer; 4 µl 2mM dNTPs; 5 µl 10xPCR buffer; 3 µl $MgCl_2$; 0.25 µl 5 U/ µl *Taq* DNA polymerase. Reaction was performed incubating at 94 ^{0}C for 4 min (for first step), 40 cycles of 94 ^{0}C for 1 min, 52 ^{0}C for 2 min, 72 ^{0}C for 2 min, and at 72 ^{0}C for 10 min (final step).

ToRSV PCR reaction mixture contained (for one sample): 10 µl cDNA; 34.75 µl PCR water; 1 µl 20 pM forward primer; 1 µl 20pM reverse primer; 4 µl 2mM dNTPs; 5 µl 10xPCR buffer; 3 µl $MgCl_2$; 0.25 µl 5 U/ µl *Taq* DNA polymerase. Reaction was performed incubating at 94 ^{0}C for 4 min (for first step), 40 cycles of 94 ^{0}C for 1 min, 53 ^{0}C for 2 min, 72 ^{0}C for 2 min, and at 72 ^{0}C for 10 min (final step).

Resulting PCR products were analyzed by electrophoresis through 1 % agarose gel, stained with ethidium bromide. DNA bands visualized and documented using Bio-Rad Gel Doc XR.

Results. Samples for investigations have been collected from irises bearing symptoms of chlorotic and necrotic streaks; various shape spots, including ringspots on leaves and flower bud sheaths; flower breaking. For investigations 47 samples of symptomatic iris have been collected in Lithuania and 10 in Ukraine.

EM investigation of negatively stained dip preparations from naturally infected plants revealed the presence of flexuous filamentous particles, 750 nm long and isometric particles 30 nm in diameter. Filamentous particles have been detected in all investigated Lithuanian and Ukrainian samples.

Symptomatic host plants and inoculated test–plants were tested in DAS–ELISA using the IgGs and IgG alkaline phosphatase conjugates specific for ArMV, CMV, TMV, TRSV, ToRSV. Reaction was considered positive when absorbance at 405 nm was twice and higher the mean of healthy plants (negative controls). All collected Ukrainian samples gave negative reaction to tested viruses. TMV, CMV, ToRSV infection was reliably confirmed for one, ArMV, TRSV for two tested Lithuanian iris samples.

***Iris mild mosaic virus* (IMMV)** was detected in all investigated Lithuanian and Ukrainian iris plants. **IMMV c**auses mosaic in the leaves consisting of pale-green and yellowish green stripes. Flowers show a breaking pattern as stripes or spots (Fig. 1).

Fig. 1. Flower breaking symptoms induced by IMMV on iris flower

The test–plants were inoculated by mechanical sap inoculation. Virus infected only *Chenopodium quinoa* causing chlorotic local lesions. EM investigation of negatively stained dip preparations from naturally infected plants and inoculated *C. quinoa* plants revealed the presence of flexuous filamentous particles, 750 nm long (Fig. 2).

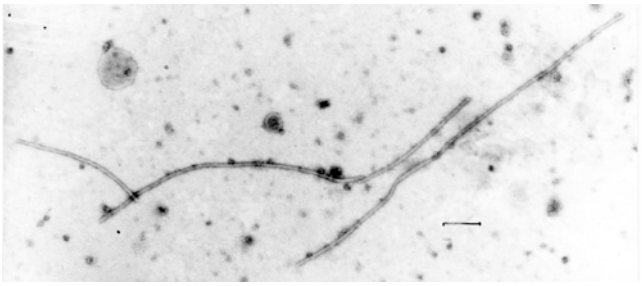

Fig. 2. IMMV particles. Bar represents 100 nm

IMMV is a member of *Potyvirus* genus. Virus is distributed worldwide, most important cultivars are totally infected [1]. Virions filamentous; usually flexuous with modal length of 750 nm. Transmissible in non persistent manner by *Aphis gossipi, Macrosiphum euphorbiae*, and *Myzus persicae* [2].

***Tobacco mosaic virus* (TMV) w**as isolated from irises bearing symptoms of chlorotic turning to necrotic mottling and streaks on leaves (Fig. 3).

Fig. 3. Symptoms of TMV on iris leaves

The test–plants were inoculated by mechanical sap inoculation. The most specific reaction to this virus was induced on *Nicotiana glutinosa* expressed by local dark necrotic ringspots, which appeared in 2–3 days after inoculation. Local (L) lesions were induced by virus infection on following test-plants: *Amaranthus caudatus, A. paniculatus, Chenopodium amaranticolor, C. foetidum, C. quinoa, C. urbicum*; local necrotic spotting on *Datura metel, Datura stramonium, Gomphrena globosa, Nicandra physalodes; Petunia hybrida, Physalis floridana, Tetragonia expansa* reacted expressing grey necrotic spots and leaf necrosis. *Nicotiana debneyi* and *N. tabacum* 'Samsun' showed local necrotic spotting, followed by systemic (S) mosaic and leaf distortion. *N. rustica* developed local necrotic spots; systemic reaction was expressed by vein and plant top necrosis. EM investigation of negatively stained dip preparations from naturally infected plants and inoculated test-plants revealed the presence of rod-shaped particles, 300 nm long. Symptomatic host plant and inoculated test–plants were tested in DAS–ELISA. All tested plants gave clearly expressed positive reaction confirming TMV infection.

Total RNA for performing RT–PCR was extracted from frozen leaf tissue of naturally infected iris plant and inoculated *C. quinoa* (L), *N. glutinosa* (S) and *N. rustica* (S) test-plants. Leaf tissue from healthy *N. glutinosa* was used as negative control (K–). TMV isolate from *Petunia hybrida* was used as positive control (K+). Specific for TMV PCR products were obtained with all investigated samples and positive control, but not with negative control. Specific bands in agarose gel of analyzed products after electrophoresis at a position corresponding to the expected size of amplification product of 422 bp were obtained, confirming TMV identity (Fig. 4).

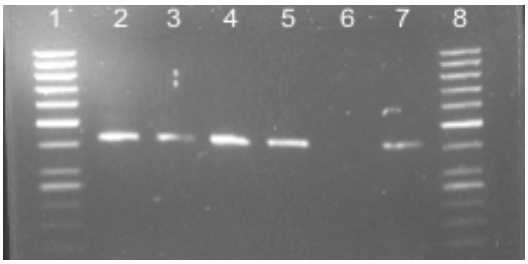

Fig. 4. RT–PCR products of amplified DNA fragments from TMV (EF 1 % agarose gel).
1, 8 – DNA size standard SMO371,
fragment sizes: 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, 50 bp.;
2 – iris (host-plant); 3 – *C. quinoa* (L);
4 – *N. glutinosa* (S); 5 – *N. rustica* (S); 6 – K–; 7 – K+.
Size of TMV specific product 422 bp

On the basis of particle morphology data, symptom expression on inoculated test–plants, positive reaction in DAS–ELISA test, RT–PCR results, and in accordance with published descriptions of TMV [2], the virus with rod-shaped particles isolated from irises was identified as TMV.

Tobacco ringspot virus **(TRSV)** was isolated from irises expressing symptoms of chlorotic streaks and ringspots (Fig. 5).

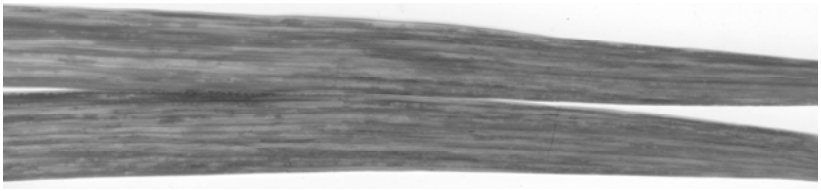

Fig. 5. Symptoms of TRSV on iris leaves

Virus has been identified basing on positive reaction in DAS–ELISA, EM data and test–plant inoculation results. Specific for TRSV local symptoms have been observed on *Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Petunia hybrida, Tetragonia expansa*, systemic symptoms on *Cucumis sativus, Datura stramonium, Nicandra physalodes, Nicotiana tabacum, Phaseolus vulgaris.* The inoculated test–plants have been tested in DAS–ELISA. Both tested plants gave clearly expressed positive reaction confirming TRSV infection. EM investigation revealed isometric particles 30 nm in diameter in symptomatic test–plants.

RT–PCR was carried out with tissues from two naturally infected plants. TRSV isolate from *Echinacea purpurea* was used as positive control (K+), leaf tissue from healthy *N. glutinosa* as negative control (K–). Specific for TRSV bands in agarose gel of analyzed products after electrophoresis in 1 % agarose gel at a position corresponding to the expected size of the amplification product of 348 bp have been obtained with both iris samples and positive control, but not with negative control (Fig. 6).

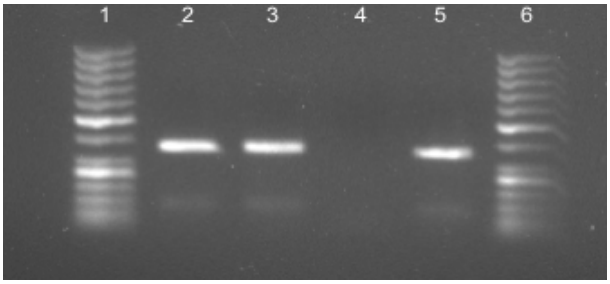

Fig. 6. RT–PCR products of amplified DNA fragments from TRSV (EF 1 % agarose gel).
1, 6 – DNA size standard SMO371;
2, 3 – irises (host-plants); 4 – K–; 5 – K+.
Size of TRSV specific product 348 bp

On the basis of test–plant reaction, morphology of virus particles, DAS–ELISA, RT–PCR results and according to literature data [2] it was established, that virus with isometric particles affecting iris plants was TRSV.

TRSV is a type member of *Nepovirus* genus. It is transmitted by nematodes *Xiphinema americanum* and other closely related *Xiphinema* spp.*, Thrips tabaci*, some aphid and mite species are reported as vectors of this virus. Reports of natural spread are largely confined to North America but the virus has been disseminated to many countries in infected planting material. It causes serious disease problems in those regions where the nematode vectors also occur. Particles are 30 nm in diameter sedimenting as three components and with a bipartite RNA genome. The virus is readily transmitted by sap inoculation and has a wide host range, including woody and herbaceous plants, annuals and perennials [2]. Ornamental hosts including *Anemone* L.*, Begonia* L.*, Crocus* L.*, Gladiolus* L.*, Hyacinthus* L.*, Hydrangea* L.*, Impatiens* L.*, Iris* L.*, Lilium* L.*, Narcissus* L.*, Rosa* L.*, Tulipa* L. have been reported as host plants of TRSV. TRSV has been isolated and identified from *Dicentra* Bernh.*, Gladiolus* L.*, Echinaceae* Moench. and *Tulipa* L. in Lithuania [7].

Tomato ringspot virus **(ToRSV)** was isolated from irises bearing viral symptoms, similar to symptoms induced by TRSV, chlorotic streaks and ringspots on leaves.

EM investigation of negatively stained dip preparations from naturally infected plants revealed the presence of isometric particles, 30 nm in diameter. The test–plants were inoculated by mechanical sap inoculation. Virus induced local and systemic reaction in inoculated test–plants except *Nicotiana glutinosa*, *N. rustica* and *Physalis floridana*, which reaction was only local. *Celosia argentea, Gomphrena globosa* and *Chenopodium quinoa* developed the most conspicuous and specific for ToRSV reactions. Inoculated leaves of *C. argentea* showed dark brown ringspots, which later extended along veins and became necrotic. Systemic reaction was expressed by malformation of leaves, vein chlorosis, small brown spots and lines located mainly on the base of leaf lamina. *G. globosa* developed local necrotic spots. Systemic reaction was expressed by mild leaf distortion and light green spots on leaves. Inoculated *C. quinoa* leaves showed local chlorotic spots, necrosis, distortion, systemic reaction was expressed by distortion of young leaves, chlorotic and necrotic spots on the base of leaf lamina. Tip of plant was distorted with necrosis and turned downwards.

EM investigation of negatively stained dip preparations from infected test–plants revealed the presence of isometric particles, 30 nm in diameter.

Symptomatic host plants and inoculated test–plants were tested in DAS–ELISA. All tested plants gave clearly expressed positive reaction confirming ToRSV infection.

RT–PCR was carried out with leaf tissue from naturally infected host–plant and inoculated *C. quinoa* (S). ToRSV isolate from pelargonium was used as positive control (K+), leaf tissue from healthy *Nicotiana glutinosa* as negative control (K–). Specific for ToRSV bands of analyzed products after electrophoresis in 1 % agarose gel at a position corresponding to the expected size of the amplification product of 449 bp have been obtained with both samples (Fig. 7).

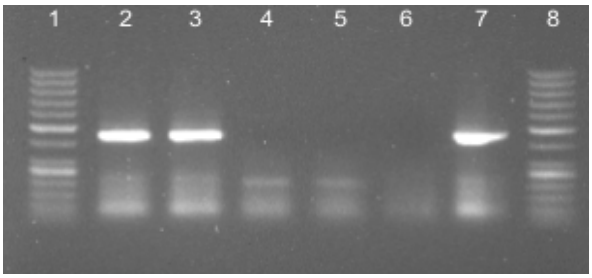

Fig. 7. RT–PCR products of amplified DNA fragments from ToRSV (EF 1 % agarose gel).
1, 8 – DNA size standard SMO371;
2 – iris (host-plant); 3 – *C. quinoa* (S); 6 – K–; 7 – K+.
Size of ToRSV specific product 449 bp

On the basis of test–plant reaction, morphology of virus particles, DAS-ELISA, RT-PCR results and according to literature data [2] it was concluded, that investigated iris plant was infected by ToRSV.

ToRSV is a member of genus *Nepovirus* and causes economically important diseases in a wide range of crops. It is readily transmissible by inoculation of sap and has a wide host range, including both woody and herbaceous plants. It is transmitted by the nematode *Xiphinema* spp. Seed transmission has been reported in several crops. The infected plants show distinctive symptoms as a shock reaction. Chronically infected plants usually exhibit no obvious symptoms but show a general decline in productivity [2]. The virus occurs in nature mostly in perennial crops. Ornamental hosts including *Anemone* L.*, Gladiolus* L.*, Hydrangea* L*, Iris* L.*, Narcissus* L.*, Pelargonium* L'Her.*, Petunia* Juss. sp. have been found naturally infected by ToRSV. The range of ToRSV host–plants in Lithuania includs 15 ornamental plant species belonging to 10 botanical families [8].

Identification of ArMV and CMV viruses by the RT-PCR have been described earlier [9, 10].

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SPREAD AND PHYLOGENETIC RELATIONSHIPS OF *WHEAT DWARF VIRUS* ISOLATED IN UKRAINE AND HUNGARY

Вірус карликовості пшениці (ВКП) є типовим патогеном злакових культур та викликає значні втрати врожаю. У даній роботі досліджувалося поширення ВКП в агроекосистемах України. Результати свідчать про високу гомологію українських ізолятів з європейськими представниками ВКП та про наявність унікального ячмінного ізоляту, здатного до інфікування рослин пшениці.

Wheat dwarf virus (WDV) is ubiquitous virus in cereals causing huge losses. This paper analyses spread of WDV in Ukrainian agriecosystems. The results show high homology of Ukrainian isolates to European representatives of WDV and presence of unique barley isolate capable of infecting wheat.

Introduction. During the last decade *Wheat dwarf virus* (WDV) became the most frequently isolated and most ubiquitous cereal-infecting virus in Hungary and now it becomes a serious problem in Ukraine [19, 25]. WDV is a frequent causal agent of dwarfing, mottling, yellowing or reddening in cereals and suppressed heading and root growth in infected plants can drastically reduce yield. WDV was first described by Vacke (1961) [29] in the former Czechoslovakia and later it has been found in Sweden [18], Bulgaria [24], Hungary [3], France [16], Germany [7], Poland [8], Finland [15], Romania [9], Spain [1], Tunisia [20], Turkey [12], Zambia [11], Ukraine (for the first time approximately in 1975, then in 2007) and China [30]. WDV is transmitted by the leafhopper *Psammotettix alienus* [29]

in a circulative, non-propagative manner [17] therefore the occurrence of diseased plants in the field depends on the presence of the vector.

WDV belongs to the genus *Mastrevirus* (family *Geminiviridae*) infecting monocotyledonous plants. Mastreviruses have a monopartite single-stranded genome of circular DNA and the genome encodes four different proteins: movement protein (MP) and coat protein (CP) on the viral-sense strand, while two replication-associated proteins (Rep and RepA) on the complementary-sense strand [4]. The presence of an intron in the Rep gene makes it possible for WDV to produce two different forms of the replication protein. The non-coding long intergenic region (LIR) and short intergenic region (SIR) contain sequence elements necessary for viral replication and transcription. The LIR comprises the origin of rolling circle replication of the virus [6]. The SIR contains polyadenylation signals, and a region to which a short complementary primer for the second strand synthesis binds [10].

Two different forms of WDV exist: a wheat-adapted form (WDV wheat strain) and a barley-adapted form (WDV barley strain) [2, 14, 17]. Both strains infect plants in the family Poaceae [17]. There are, however, contradictory reports as to whether the wheat strain can infect barley, and barley strain can infect wheat [13, 17, 22, 27]. According to Kundu et al. (2009) [13], barley strain is restricted to the barley host, while the wheat strain is present in both wheat and barley plants.

The genomes of the barley and wheat strains of WDV share an average of 85% identity, whereas the isolates within wheat strain show high degree of homology (>98%), when the isolates of barley strain are more variable (>94 %). As the demarcation criterion for mastrevirus species has been set to 75% nucleotide sequence identity by the International Committee for Taxonomy of Viruses, both strains are currently considered to belong to the same species. Recently Schubert et al. (2007) [22] surveyed cereal samples and based on DNA sequence differences they proposed the new mastrevirus species *Barley dwarf virus* (BDV) and *Oat dwarf virus* (ODV*)*. ODV was accepted as a new tentative mastrevirus species sharing 70% genome-wide nucleotide sequence identity with the wheat and barley strains of WDV.

The aim of this study was to make molecular characterisation of WDV isolates from Hungary and Ukraine and compare them with the available sequences of WDV.

Materials and Methods. *Virus isolates.* The symptoms of viral infection were found during spring observations carried out in wheat crops in Martonvasar (Middle Hungary), Pula (Southern Hungary) and Mironivka (Middle Ukraine). Plants manifesting yellowing of leaves or dwarfing were placed in an insect-proof greenhouse and were tested for WDV with ELISA using a WDV kit (Bio-Rad No. 31202) or rabbit polyclonal WDV-specific antisera (Loewe, Germany). The collected WDV-infected plants were replanted into clay pots and placed in an insect-proof isolation net. For use as a vector organism, thirty individuals of virus-free *Psammotettix alienus* Dahlb. were placed underneath each net. One week later the leafhoppers were transferred to young seedlings (10 cm long) of wheat. Six weeks later the plants were tested again for WDV with ELISA. Three isolates WDV-HU-2Marton:08 (collected in 2008 from Martonvasar), WDV-HU:Pula:07 (collected in 2007 from Pula) and WDV-Uk:Mironivka:09 (collected in 2009 from Mironivka and maintained in our greenhouse by subsequent transmission) were selected for further studies. Ten wheat samples from Odessa region (South Ukraine) and one from Glevakha (Central North Ukraine) were initially tested by PCR. Two samples (WDV-Uk:Odessa:09 and WDV-Ukg:08 collected from Odessa and Glevakha respectively) were selected for molecular characterization.

Isolation of virus DNA, cloning and sequence analysis of the WDV isolates. DNA extraction and amplification were done according to Shepherd et al. (2008) [23]. A small leaf sample (approximately 40 mg) from WDV-positive plants was placed into an Eppendorf tube covered with 50 µl extraction solution (Extract-n-Amp^{TM} Plant PCR kit, Sigma) and heated at 95^{o}C for 10 min. Lastly 50 µl of dilution solution (also supplied with the kit) was added and the sample was then stored at -20^{o}C or used directly as a template for rolling circle amplification (RCA) of the WDV genome [5]. One microliter of the final Extract-n-Amp DNA solution was mixed with 4 µl of Templi Phi^{TM} sample buffer (TempliPhi^{TM}, Amersham Biosciences), heated for 2 min at 94 ^{o}C, and then brought to room temperature. Five µl of reaction buffer and 0.2 µl of enzyme mix were added to the cooled mixture and the Templi Phi^{TM} extension reaction was run at 30 ^{o}C for 18 – 20 h. WDV genome concatemers generated during Phi29 DNA polymerase amplification were digested with *Hind*III to release unit-length genomes. After digestion genomic DNA was separated in 1 % agarose gels and extracted by a DNA purification kit (Fermentas DNA Extraction Kit). The WDV genome was inserted into a *Hind*III digested pBSK+ plasmid (Stratagene). The recombinant plasmids were transformed into *Escherichia coli* DH5α [21].

Clones containing inserts with the expected size of 2.7 kb were sequenced with the DyeDeoxyTerminator Kit (Applied Biosystems) using reverse, universal (-20) and internal primers. Sequence analysis was performed using University of Wisconsin Genetics Computer Groups (GCG) sequence analysis software package version 9.1.

In order to determine the phylogenetic relationships between different isolates complete genomes were analysed. Sequence alignment, tree formation, and bootstrap analysis were done with the help of the software Clustal X 1.83.

Results. *WDV spread in Ukraine*. Large scale survey of agriecosystems where grain cereals are regularly cultivated in Ukraine has been conducted for WDV diagnostics. In particular, fields in Kyiv (North), Kharkiv (East), Cherkassy (Center), Khmelnytsk (West), Kherson and Odessa (South) regions have been screened giving rather large cover of the country. Winter wheat and winter barley plants were sampled in each region. In addition, in Kyiv, Kherson and Odessa regions wild cereals have also been identified with typical WDV symptoms or sampled symptomless. These plants have further been put into *Deschampsia sp.* genus. In total, more than 500 samples have been collected and subsequently analyzed serologically, and also some of them via TEM. It should be said that apart from symptomatic plants sampled in Kharkiv and Kyiv region we have also detected vectors for this virus – leafhoppers *Psammotettix alienus*. Their occurrence is an indirect proof for significant spread of WDV in Ukrainian agriecosystems and also points on changes in climatic conditions towards an increase in average annual temperature.

Further, WDV diagnostics have been carried out for taken samples by DAS ELISA (LOEWE kits, Germany). Most of the samples were shown WDV-positive. Electronic microscopy of some of WDV-positive samples confirmed presence (however, in very low quantity) of typical geminivirus particles of about 22-24 nm in diameter.

Level of WDV spread of Ukraine has been established and tentative list of cultivars/lines of wheat and barley (cultivated in surveyed regions) which are susceptible or insusceptible/tolerant/resistant has been proposed. Statistical data shows that average level of WDV infection spread in cultivated cereals was 6-9% in 2009, comparing to maximum 5% in 2006. Coupled with other outcomes, it highlights the tendency towards positive dynamics of WDV spread in Ukrainian ecosystems. WDV has probably become the second biggest virus threat to cereals in Ukraine, with BYDV being the first.

Virus isolates. The collected wheat plants were WDV infected as confirmed by ELISA tests and PCR. The virus was transmitted by *P. alienus* and maintained on wheat plants. Nucleic acids were isolated from plants infected with WDV-HU-2Marton:08, WDV-HU:Pula:07, WDV-Uk-g:08 and WDV-Uk:Odessa:09, WDV-Uk-g:08 and WDV-Uk:Mironivka:09.

Virus DNA isolation, cloning and sequence analysis of the WDV strains. The Extraction-n-Amp DNA extraction method was very rapid and simple in order to isolate the full length WDV genomes. Combined with the RCA method, a vast number of virus genome concameters were produced, which were digested with *Hind*III and released as unit-length genomes (Fig. 1) with sticky ends. The size of the full length genome prepared from WDV-HU-2Marton:08 and WDV-HU:Pula:07 were 2750 nucleotides, WDV-Uk-g:08 and WDV-Uk:Mironivka:09 were 2749 nt, and WDV-Uk:Odessa:09 was exactly 2734 nucleotides. The very special properties of this isolate that it has originated from wheat plant. In the literature there is no data that barley strain of WDV could be isolated from naturally infected wheat plant. The genome contained all four expressed mastrevirus ORFs (MP, CP, Rep, RepA), and the intergenic regions LIR and SIR.

The nucleotide sequences were deposited into GenBank as FN806783 (WDV-Uk-g), FN806784 (WDVUk-Miron), FN806785 (WDV-HU-2Marton), FN806786 (WDV-HU-Pula) and FN806787 (WDV-Uk-Odessa), and were further compared to previously characterized WDV isolates [26, 27, 28].

The sequence analysis of the full genome revealed high levels of identity among wheat strains and high level of diversity among barley strains. The sequences' identities between isolates of the wheat strain from different geographical origin are very similar (>98.7 %). Inside the movement protein (MP) and coat protein (CP) we observed high sequence identity (>98.8 %) at amino acid level, in some cases MP were even identical (between Hungarian and Swedish isolates), or CP (between Hungarian isolates originating from different part of the country). In the short intergenic region (SIR) and large intergenic region (LIR) we observed higher variability (97 % and 96.6 % respectively) (data not shown). Regarding the diversity of WDV isolates of barley strain we observed higher variability (96.3 % – 99.4%). Interestingly, MP and CP at amino acid level revealed high level of identity among barley strain isolates originating from different geographical region (98.5 % – 100 %). Barley strain isolates showed greater variability also in the LIR and SIR. Molecular characterization of Ukrainian and Hungarian WDV isolates was followed by phylogenetic analysis in order to compare the relationships with previously characterized wheat and barley isolates from the GenBank (Fig. 1). The phylogenetic analysis of WDV isolates showed that they are clearly distinguishable, both barley and wheat strains form two clades. Clade 1 could be subdivided into two groups formed by wheat isolates from Hungary and Sweden in one and wheat isolates from China in the other group. In Clade 2 WDV-Uk-Miron and WDV-Swe-Enk2 clusterized in one subgroup and the other Ukrainian isolate WDV-UK-g grouped with isolates from Czeh Republic and Germany. Similar situation can be observed with barley isolates forming two clades, which could be subdivided into more subgroups. Both clades represent a pool a divergent isolates. Clade 3 could be devided into 3 or 4 subgroups formed by German and Czech isolates.

Clade 4 could be grouped into three subgroups, the first is formed by Hungarian barley isolates, Ukrainian and Bulgarian isolates belong to subgroup two and Barley isolate from Turkey form the third subgroup. Within this clade subgroups correspond to the geographical origin of isolates of WDV barley strain.

The phylogenetic analysis showed some isolates are out clades like WDV-Ge-SxA22, WDV-Ge-ScBB21, WDV-Cz19 and WDV-Swe-SE and ODV-Ge-SxA25. The last one is understandable since *Oat dwarf virus* has only about 70 % genome-wide nucleotide sequence identity with barley and wheat strain isolates of WDV.

Discussion. The research confirms rather wide spread of Wheat dwarf virus in Ukraine with positive tendency and infavorable forecast. There no WDV-insusceptible/resistant varieties of cereals cultivated in Ukraine. Unique virus vector has been repeatedly detected in Ukrainian ecosystems proving need for careful virus/vector monitoring and selection/growing of tolerant cultivars.

Five isolates (two Hungarian and three Ukrainian) originated from wheat plants were sequenced and compared with previously characterized barley and wheat strains of WDV. Four isolates belonged to wheat strain of WDV, which present a high degree of identity throughout the complete genome regardless that they are originated from different geographical origin. Ukrainian isolates were 2749 nucleotides long, while Hungarian isolates consists of 2750 nt.

WDV-Odessa isolate was a barley strain of WDV having 2734 nt long genome. This is the first data that barley isolate of WDV could infect wheat in natural conditions.

Wheat strain isolates present a low level of variability, while barley strain isolates showed higher level of diversity.

Phylogenetic analysis showed clear differences of barley and wheat strains of WDV isolates. Both strains could be divided into two clades and barley strain subdivided into more subgroups. Isolates grouped into the subgroups of barley clade 3 are in a good correlation with the geographic origin.

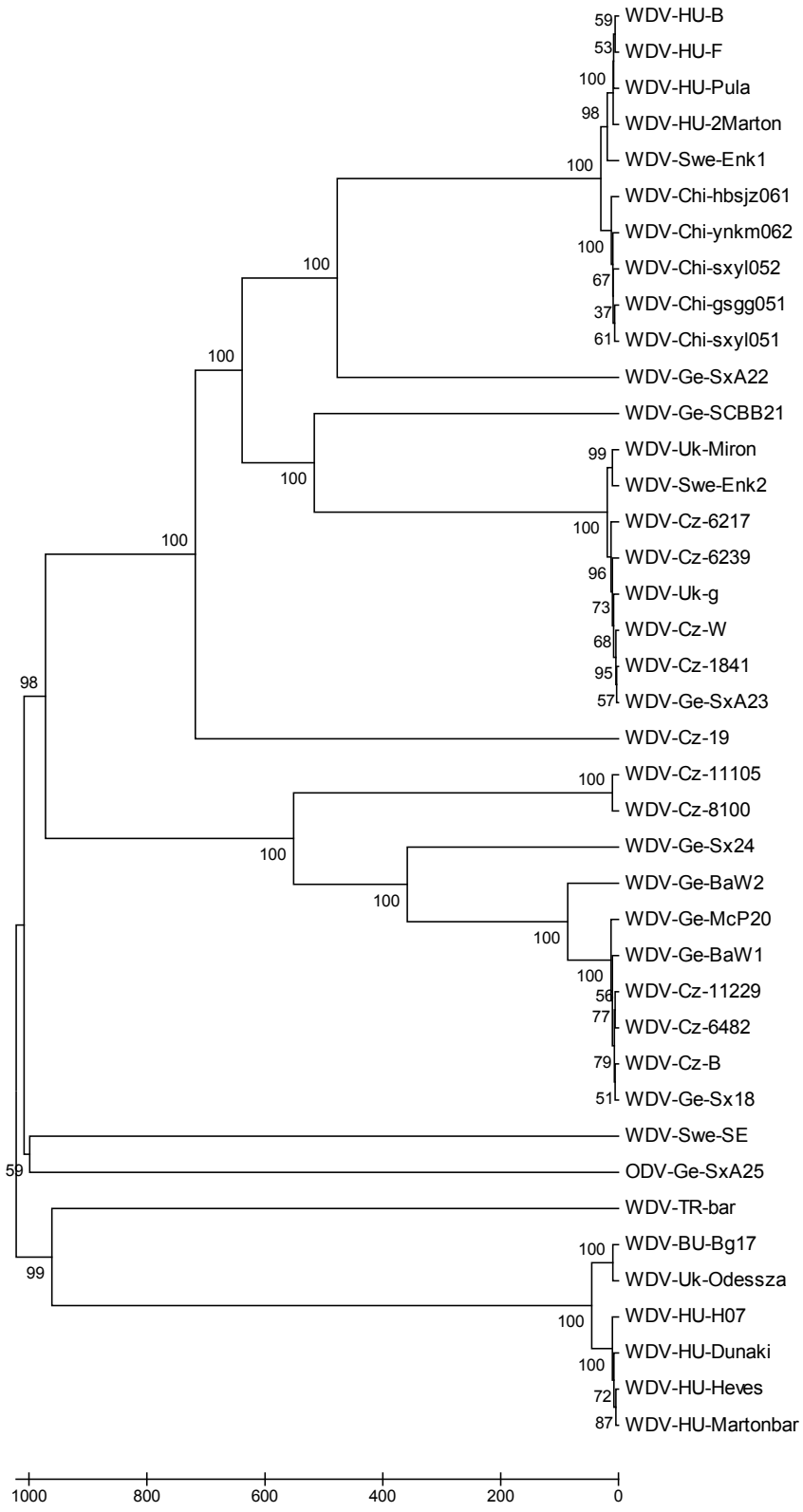

Figure 1. Phylogenetic tree constructed by UPGMA method for complete genome sequences of *Wheat dwarf virus* isolates (Bootsrap values are indicated)

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PHYTOVIROLOGICAL STATE OF PEAR VARIETIES AND ROOTSTOCK ORCHARDS

Визначено рівні інфікованості насаджень груші вірусами ACLSV, ASGC, ASPV та AMV. Зроблено статистичну оцінку спорідненості даних вірусних агентів до окремих хазяїв на основі перевірених типів підщеп груші.

Levels of viral infection caused by ACLSV, ASGC, ASPV and AMV in pear treas are detected. On the base of tested pear rootstocks statistical analysis of the viral pathogenes affinity to different hosts was made.

Introduction. The area of industrial plantations of pear – one of the main traditional fruit crops of Ukraine, which significantly declined in 90th years, now gradually increasing. Therefore, the need for virus free planting material of this culture is growing every year. Present time Ukraine takes the last but one place in production of pear fruits and leaves behind only Poland [1]. Among factors that cause decrease of pear areal in Ukraine are negative impact of environment, presence of pests and significant spread of causative agents of fungal, bacterial and viral diseases. These factors decline productivity of pear orchards and its profitability and as a consequence make no expediency to create new gardens.

In this context immunodiagnostics of varieties and rootstocks become very important. It gives possibility to conduct not only monitoring of viruses but also to select virus free samples and to create base of virus free mother plants of economically-valuable pear varieties and rootstocks.

During the last years on the base of Department of Virology and Propagation of Fruit and Berry Cultures of Institute of Horticulture inspections of pear orchards are regularly conducted in different regions of Ukraine. These surveys enable to reveal in time trees infected with the complex of latent viruses and to use in further gardening only virus free material.

Materials and methods. For detection of viral diseases by ELISA we collected samples in productive, collection and nursery orchards of pear in 2006-2008 yeas in period of intensive growth during May-July, when concentration of virus in plant tissue is the highest [4]. Altogether 224 samples of perspective cultivars were tested, which included 47 pear varieties. Also 271 samples of pear rootstocks which included 20 traditional and new breeding forms of clone rootstock types were investigated. Surveys were conducted in gardens of Institute of Horticulture UAAS, Crimean Research Station of UAAS and Podil Research Station of UAAS. Immunodiagnostics was carried out by classic ELISA and DAS-ELISA. Certified antibodies for ACLSV, ASGV, ASPV and ApMV produced by Loewe Phytodiagnostica, Germany and Bioreba AG, Switzerland, were used for investigation purposes. Results of analysis were registered by microplate spectrophotometer STAT FAX 2100, USA.

Percent of samples infected with virus *i* in orchards of certain type *k* was calculated according to equation:

$$Fik = \frac{N_{ik}}{N_k} \cdot 100\% \qquad (1)$$

where N_k – number of tested samples, N_{ik} – number of samples infected with virus *i* in orchards of type *k*.

The general infection level of virus *i* in all types of pear orchards (Figen) was calculated according to equation:

$$Figen = \sum_{1}^{k} Nik \cdot 100\% \Big/ \sum_{1}^{k} Nk \qquad (2)$$

For the estimation of phytovirological state of the plantations of each of pear clonal rootstock the null hypothesis was checked about the random distribution of each of the four analyzed viruses in those orchards with the use of 2x2 contingency table. The data about the distribution between the samples of certain type of rootstocks infected and non infected by an individual virus in the 2x2 contingency table were contrasted to the distribution between infected and non infected samples of the rest of the rootstocks concerning the same virus. The χ^2 criteria and contingency coefficient *Phi* were calculated. Such approach made it possible to determine the total resistance potential or, on the contrary, susceptibility of certain rootstocks to each virus as compared to the rest of the pear clonal rootstock orchards. The negative *Phi* coefficient values were considered as an indication of the certain resis-

tance potential, positive values were regarded to be the signs of the susceptibility to the virus infection.

For each type of rootstock odds ratio (OR) were calculated to assess the risk of disease distribution in nurseries orchards. The odds ratio is one of a range of statistics – a way of presenting probabilities. In our case the odds ratio is a relative measure of risk, telling us how much more likely it is certain rootstock will be infected by viruses under study comparing to the rest rootstocks analyzed. It was calculated also with 2x2 contingency table.

Results and discussion. Results of the test revealed high rate of pear viruses spread in different types of orchards in all regions where surveys were conducted. According to European scheme of certification, which estimates quality of planting material of pome cultures regarding to international normative documents of European Plant Protection Organization, among the viral agents that cause viral disease of fruit cultures the most harmful for pear and quince are three viruses of *Flexiviridae* family: *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem grooving virus* (ASGV) and *Apple stem pitting virus* (ASPV) [3, 5]. These viruses are rather widespread in *Rosaceae* family especially in apple, pear and quince orchards and have rather wide geographical distribution [6, 7]. Average rate of three viruses of pear plantings in Russia is 20% [2]. It is hard to detect infection in time because ACLSV, ASGV and ASPV belong to group of latent viruses and most of commercial varieties and rootstocks don't have any visual symptoms. *Apple mosaic virus* (ApMV) is another one virus frequently infecting pear trees.

In our study the most prevailing was *Apple stem pitting virus* which causes incompatibility of rootstock and scion and leads to low quality of planting material. So among tested samples of pear varieties rate of ASPV infection was 26%, when infection levels of other three viruses were significantly lower: ACLSV – 8,9%, ASGV – 2,7%, ApMV – 5,8% (Fig. 1).

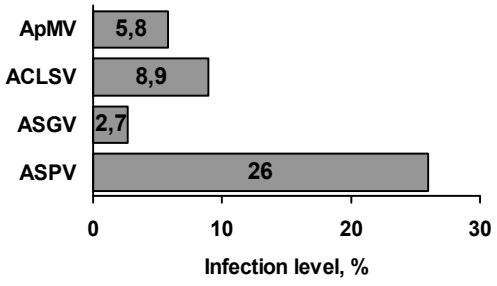

Fig. 1. Level of pear varieties (%) infected with viruses

New varieties of pear intensively propagated Ukraine in was rather frequently infected with the complex of viruses. In these cases the uninspected collection plantings become the primary source of infection. No samples free from virus infection were found in Zolota Osin', Jack Tel'ye and Dicolor varieties. High rates of infected samples had varieties Vyzhnitsya and Stryis'ka – 62,5 and 42,1% accordingly. Test results gave the possibility to choose virus free samples of 44 pear varieties, from which all samples of 21 varieties were not infected by any of viruses. Perhaps, we got such results because not long ago seedlings were used to cultivate on initially virus free seed rootstocks.

During recent years horticulture in Ukraine has started active use of clone rootstocks which are vegetative propagated and can be permanent source of viruses if phytosanitary control is not conducted. we should pay not the less attention to testing of pear rootstocks. That's why nursery orchards of pear clonal rootstocks are one of the main objects for the regular monitoring inspections conducted by the Department of Virology of Institute of Horticulture UAAS since demand on the pear virus free planting material grows every year. During 2004-2008 the orchards of 18 rootstock types were inspected for the presence of four viruses.

Diagnostics revealed general lower infection level – 10,0% comparing to 29,5% of variety material. Difference was observed also in infection levels of certain viruses. While cultivars were mainly infected with ASPV, rootstocks were frequently infected with ACLSV – 6,3 %. Infection rate of ASGV, ASPV and ApMV was – 1,1%, 3,5% 1,5% accordingly fig.2.

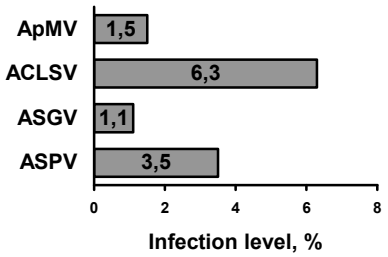

Fig. 2. Level of pear rootstocks (%) infected with viruses

The same as varieties, rootstocks were infected with the complex of two or three viruses. General lower level of infection can be explained by short period of use of these rootstock types in Ukraine and planting of their nurseries with tested certified material. In whole, virus free clones were selected for all analyzed rootstock types. But the phytovirological state of certain rootstock orchards varies widely reflecting both the technological level of their management as well as the quality of the primary stock used for the mother plantations establishment and the level of certain rootstock sensitivity to the virus infection.

For evaluation the sensitivity and resistance of certain rootstocks to plant viruses the contingency coefficient was calculated for 18 rootstock types. The rootstocks with the significant maximum deviation from the random virus diseases distribution are enumerated in the table 1.

Table 1. **Results of the data using the 2x2 contingency table (*Phi* is a contingency coefficient, *p* – probability level)**

№	Rootstock type	ACLSV		ASGV		ASPV		AMV	
		Phi	*p*	*Phi*	*P*	*Phi*	*p*	*Phi*	*p*
1	BA-29	-0,150	0,015	-	-	-0,153	0,015	-0,164	0,014
2	IS 2-10	-0,307	0,000	-	-	-	-	-	-
3	KA104	0,232	0,023	0,281	0,016	-	-	-	-
4	A422	0,424	0,000	-	-	-	-	-	-
5	OHF-40	0,275	0,000	-	-	-	-	0,240	0,034
6	A391	-	-	-	-	0,274	0,000	-	-

The application of such approach allowed to select among the 18 tested pear rootstocks two ones (BA-29 and IS 2-10). Their inland orchards possess the highest antiviral potential. As regards the rootstock BA-29 the value of the *Phi* coefficient appeared significantly negative concerning ACLSV (-0,150), ASPV (-0,153) and AMV (-0,307). Such state of those rootstock orchards may be explained by the successful combination of the proper level of their management technology with climatic and geographical conditions of growing adequate to their natural potential. Four rootstocks (KA 104, A 422, A 391 and OHF-40) were included into the group the propagation of which today demands special attention, in particular supplementary measures as for the support of the virus-free status for instance sanitation *in vitro* culture, thermo- and chemotherapy etc.

According to our calculation the presenting probabilities of virus diseases distribution in nurseries orchards are the highest for such types of rootstocks as KA104 (26,222), OHF-40 (16,857), A391(8,393) and OHF-51(4,052) (Table 2). Generally the OHF group of rootstocks is more problematic for introduction in sense of resistance to virus infection in comparison to other groups. The most promising types of rootstocks are BA-29 and IS group. The group KA and Quince Cido were considered as group with intermediate probability of virus diseases distribution.

Table 2. **Results of the data using the 2x2 contingency table (OR is the odds ratio)**

№	Rootstock type	OR	95%confidence interval
1	A391	8,393	1,422±49,586
2	A422	0,000	0,000±10,346
3	Q.Cydo	2,277	0,741±7,070
4	BA-29	0,193	0,082±0,459
5	IS 2-10	0,000	0,000±2,259
6	IS 4-12	0,000	0,000±2,986
7	IS 4-15	1,612	0,383±6,991
8	IS 4-6	0,000	0,000±2,259
9	KA 53	0,000	0,000±7,727
10	KA 61	0,000	0,000±7,727
11	KA 86	0,000	0,000±7,727
12	KA 92	0,000	0,000±7,727
13	KA104	26,222	3,585±188,312
14	OHF-40	16,857	2,124±132,227
15	OHF-51	4,052	0,517±32,146
16	OHF-333	0,000	0,000±32,146
17	Pyrognom	2,750	0,608±12,636
18	UUPROS-6-1	2,750	0,608±12,636

Thus we can state the presence of virus infection in all tested orchards of pear. Viruses ACLSV, ASGV, ASPV and ApMV were detected both in cultivars and rootstocks and composed rather high infection level 18,8% (Fig. 3).

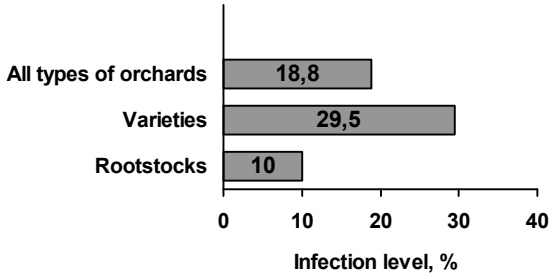

Fig. 3. General phytovirology condition of pear orchards

The results of the tests allowed selecting 64 virus free cultivars and rootstock types which will be biologically tested on woody indicators and complete fond of virus free pear clones. Tested clones that revealed the presence of infection gave us possibility to continue the investigation of Ukrainian strains of pear viruses. So our further researches will be directed on molecular-biological and phylogenetic analysis of these viruses.

Conclusion. The regular monitoring inspections conducted along last 6 years permited

1 – to chose virus-free clones of pear varieties and pear rootstocks for creation base of virus free material;

2 – to reveal the virus incidence in different types of pear orchards;

3 – to evaluate the sensitivity and resistance of different rootstocks to virus diseases in nurseries;

4 – to assess the risk of virus distribution in nurseries orchards of different types of rootstocks.

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HETEROGENEITY OF *ENTEROBACTER AEROGENES* BACTERIOPHAGE POPULATION IN SEWAGE

Бактеріофаги, які уражують Enterobacter aerogenes були детектовані у стічній воді за допомогою спот-тесту та методу подвійних агарових шарів. Шляхом багаторазових пасажів поодиноких колоній були були отримані чисті лінії чотирьох ізолятів фагів, виділених з води. Виділені ізоляти бактеріофагів відрізнялись за типом негативних колоній і морфологією віріонів. Таким чином, популяція фагів Enterobacter aerogenes в стічних водах є гетерогенною і представлена щонайменше чотирма видами вірусів.

Bacteriophages specific to Enterobacter aerogenes were detected in sewage using spot-titering assay and agar overlay method. Four phages were isolated from water and purified by serial propagation of single plaques. Isolated viruses deffered for plaque type and virion morphology. It was concluded that population of E. aerogenes bacteriophage in sewage are heterogenic and consists of at least four virus species.

Introduction. Many publications suggest that phages that inhabit Earth's oceans play a huge role in the development of microbial populations. But no less interesting may be diversity of phages in freshwater ecosystems and fully synthetic ecosystems, such as sewage facilities. Due to its characteristics, sewage can inhabit a variety of types of bacteria, which in a normal environment occupy different ecological niches and have different life strategies. This is especially true for countries where there is differentiation of sewage and they all mingle on the aeration station. From the viewpoint of ecology, effluents represent a unique biocaenosis with dozens of species of micro- and macroorganisms. Moreover, the microbial population of sewage is very dynamic and overestimate the quality of the water that comes to aeration station. Species diversity of bacteria and bacteriophages respectively depends on the biological loading of wastewater.

Characteristics of bacterial and phage populations depend on where they live in water treatment systems. Water, that comes to water treatment structure directly from the sewage loaded with organics and is a nutrient substrate for microorganisms .

Our scientific work is devoted to the study of phages that inhabit water treatment systems in Kiev. Set of papers by various authors describe different phages *E. coli*, however *E. coli* is not the only microorganism that inhabits wastewater. *Enterobacter aerogenes*, a close relative of *E. coli* are also found in bulk water purification systems. As *E. coli*, this bacterium is conditionally pathogenic to humans and does not cause disease in healthy people. But unlike *Escherihia coli*, phages *Enterobacter aerogenes* studied very poorly. So we decided to explore the *Enterobacter aerogenes* phages in sewage that arrives at the Bortnichi aeration station.

Materials and methods. Bacterial strains and culture conditions

Laboratory strain of Enterobacter aerogenes was taken from the culture collection of Microbiology department (Taras Shevchenko Nashional University of Kiev, Biological faculty). Bacteria were cultivated on plate count agar or in PC-broth. Bacteria were stored on PC-agar slants at 4^{O}C. Incubation temperature was 37 ^{O}C.

Sample collection

The samples of sewage were collected from ***Bortnichi aeration station. Aqueous samples were centrifuged and filtered*** through a bacterial filter to remove the bacterial cells.

Spot-titer assay

Plates seeded with test culture were spot tested using 10 µl per spot from a series of 10 fold dilutions of the sewage samples. Following 30 min at room temperature the plates were inverted and incubated at 37^{O}C. After 18 h of incubation the plates were analyzed for the presence of phages. Results were recorded as the reciprocal of the highest dilution at which clearing of the lawn was evident [2].

Agar overlay method

Double-layer agar method described by Adams was used to separate phages in water samples [1]. Briefly, 0.2 ml of overnight bacterial culture (10^8 c.f.u./ml) was mixed with 2,5 ml of 0,7% soft agar equilibrated at 46-49^{o} C, then 0.5 ml of environmental sample was added. The resulting mix was overlain over bottom nutrient agar (1,4%). As shown by spot-titer assay, the concentration of viral particles in the water was more than 1000/1ml, so we diluted the samples to the third degree to get separate plaques. Following 15 min at room temperature the plates were inverted and incubated at 37^{O}C. After 18 h of incubation all resulting plaques were enumerated. Separate phage plagues were than picked. Isolated bacteriophages were purified by serial propagation of single plaques and amplified.

Electron microscopy.

Virion morphology was studied employing electron microscopy. Formvar films placed on 400-mesh copper grids were dipped into sample for 2 min and contrasted in 2% uranyl acetate. The preparations were dried and viewed under an electron microscope at an instrumental magnification of 90,000.

Results. A preliminary spot-test was performed to reveal the availability of bacteriophages in sewage collected from wastewater treatment station. According to obtained results bacteriophages specific to E. aerogenes are present in effluents in relatively high concentrations (fig. 1). Titers obtained from the spot-test method were 10^3 p.f.u/ml.

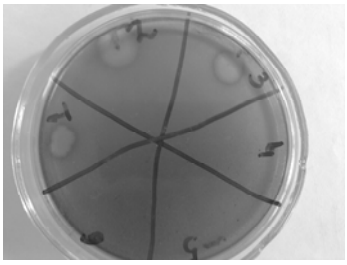

Fig. 1. The spot-titer of E. aerogenes specific bacteriophages in sewage

Spot-titer assay does not allow evaluating the morphology and type of bacteriophage plaques, taking into consideration this fact water samples were diluted to third degree and seeded by agar overlay method. As a result of our experiments various types of plaque morphologies were obtained for a given host organism, suggesting that population of *E.aerogenes* bacteriophages in sewage are heterogenic (fig 2).

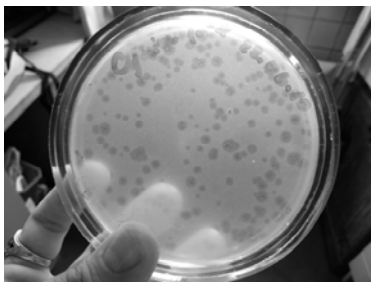

Fig. 2. The plaques cased by bacteriophages isolated from sewage on a lawn of *E. aerogenes.*

The most frequently we observed four types of negative colonies: small, d=1-2 mm (isolate №1), clear middle plagues with diameter 3-4 mm (isolate №2), middle size turbid plaques, d=3 mm (isolate №3), large plaques with halo, d=5-7 mm (isolate №4, fig. 2).

Four *E. aerogenes* bacteriophages were isolated from sewage and purified. Titres of phages after accumulation were following: isolate №1 – 98×10^9, №2 – 27×10^8, №3 – 71×10^7, №4 – 64×10^9.

Transmission electron microscopy after negative staining has revealed that isolate №1 was a member of family Siphoviridae. It had a long flexible tail and icosahedral head. Isolate N2 is a member of Myoviridae family and correspond to morphotype A1. This bacteriophage has an isometric capsid, tail and baseplate with fibers. Isolate №4 is a spherical phage 46-48 nm in diameter. This phage is sensitive to chlorophorm treatment. Morphologically isolate №4 seems to be like more archeal phages than enterophages (fig. 3).

Discussion. Thus it was shown that population of *E. aerogenes* phages in sewage is heterogenic. This indicates a significant concentration of bacteria in the effluent. Obviously, the conditions of sanitation are favorable for the replication of phages, as evidenced by their high concentration in the primary treated water.

In our work we investigated lytic phages only, but we do not know how many of lysogenic phages might be seeded on bacterial lawn at the primary infection. We have not performed the treatment of bacterial colonies with the ultraviolet irradiation, which can activate the prophage. So it is difficult to establish the true number of viruses in water. Phages replicate in bacteria at high levels so bacteriophage concentration in sewage expected to be high. Some studies demonstrated that the bacteriophage titer can be up to 2.5×10^8 virus particles per millilitre in natural waters [3]. From another hand bacteriophages can be inactivated during anaerobic degradation, which occurs during different water cleaning stages. According to our results concebrations of *E. aerogenes* phages in wast water is about 10^3 p.f.u/ml.

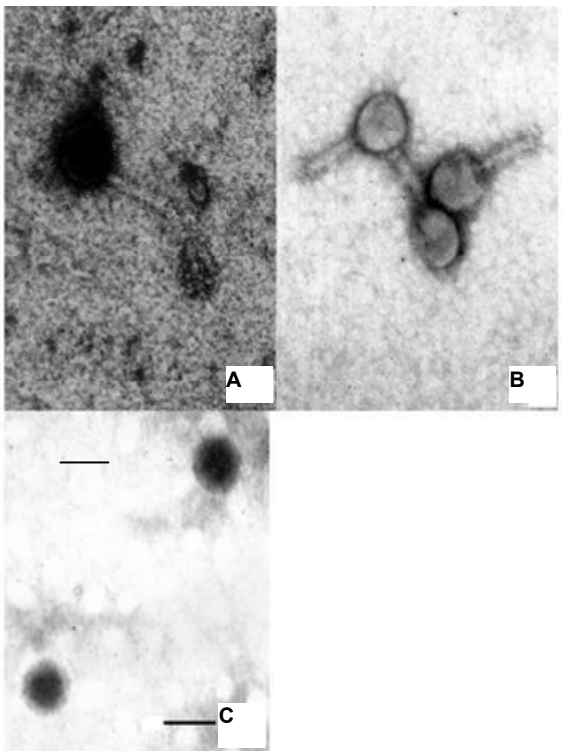

Fig. 3. Electron micrograph of bacteriophages isolated from sewage. The bars are 50 nm.
A.Isolate N1; B. Isolate N2; C. Isolate N4.

Isolated bacteriopages vary for their morphological characteristics. Recent research of another authors show that the most frequent phages in sewage belong to three families: Siphoviridae, Myoviridae and Podoviridae [4, 5]. Sometimes these three families are called "the great ecological triad" of phages. In terms of ecology, their mass distribution is easy to explain: they infect a large number of the most common bacteria in nature and their protein capsids are resistant to environmental conditions over a wide pH range, so these viruses are able to maintain its infectivity in nature long time. Our results coincide with those published by other authors. We isolated phages that represent the family Siphoviridae and Myoviridae. The only puzzle is phage number 4, which under the electron microscope has a spherical shape with an electron-permeable membrane. Perhaps, this phage has superkapsid, as evidenced by its sensitivity to chloroform. The answer to this question may provide further research.

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ANALYSIS OF WATER SAMPLES FOR VIRUSES INFECTING FRESHWATER ALGAE FROM *CHLOROPHYTA* DIVISION

У статті розглядаються основні підходи детекції вірусів прісноводних водоростей у прісних водоймах. Для детекції використовувалися методи біотестування і електронної мікроскопії з попереднім диференційним центрифугуванням.

In this article main approaches to detection of viruses infecting freshwater algae are discussed. For detection we used bioassay and transmission electron microscopy with previous differential centrifugation of water samples.

Introduction. Studies on ecology of algae viruses are important for both fundamental and applied science. Nowadays the issues of food shortage and energy deficit are regularly raised, and microalgae can provide solutions to both these problems as they are considered as perspective source of nutrients and biofuel. In turn, viruses of microalgae are viewed as the major cause of loss of basic raw material and hence as the primary reason for commercial losses [3]. That is why significant attention of researches has been drawn to algae viruses.

Today, the nomenclature of viruses invading single cell microalgae is a fast-developing branch of taxonomy. Basing on the first reports approved by the International Committee on Taxonomy of Viruses (ICTV), this group of viruses included about 60 representatives divided into four genera comprising the only *Phycodnaviridae* family. During the recent years many new algae viruses have been described [1; 4] and the family is currently under revision with several new genera being introduced. RNA-containing algae viruses have been described and consequently allotted into separate *Marnaviridae* family which in turn has been ascribed to the order *Picornavirales* [2].

Despite recent review, complexification and advances in the taxonomy structure of algae viruses, it is still rather questionable as establishing a new RNA virus family did not help with 'artificial' allotment of all DNA-containing viruses into single family. As of today the taxonomy structure of *Phycodnaviridae* family is deemed obsolete and unreasonable (for instance, only 9 homologous genes have been found for large full genomic sequences of three different viruses belonging to this family [8]). Phylogenetic analysis of family representatives based on full sequences of DNA polymerase gene also points on its extreme heterogeneity (Fig.1).

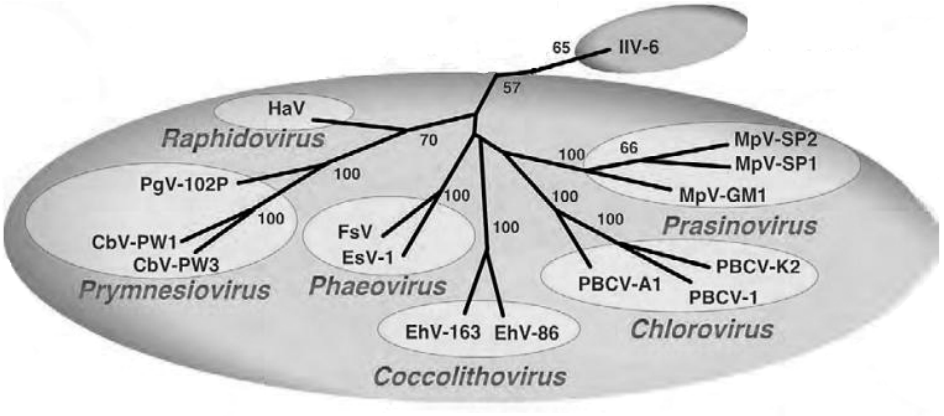

Fig.1. Phylogenetic tree showing evolutionary relationships among representatives of different genera of *Phycodnaviridae* family based on DNA polymerase gene [7]

Such attention to algae viruses is not surprising as they were demonstrated to take an active part in global biological processes of the oceans. The primary reason for studying some algae viruses lies with their crucial role in controlling the abundance of phytoplankton in water ecosystems. Ability of algae viruses to induce lysis of more than 25% of phytoplankton blooming [3; 1] is highly important from economic point of view as this phenomenon may be practically employed for such control eliminating the unavoidable water contamination with toxins and metabolic products. This would favor preservation and growth of aquatic fauna. From the other side, the algae (and especially microalgae) form the base of pyramid of numbers in water reservoirs and are the major primary producers in water food chains [1]. Hence the tentative applied use of algae viruses was another incentive for their fundamental research.

However we should note that recent advances in our knowledge of biological, morphological, genetic and molecular characteristics of these viruses are owing to research of marine microalgae. Few representatives of 'properly' described and systematized viruses infecting green freshwater algae (*Chlorophyta* division) belong to *Chlorovirus* genus. Several dozens of recent reports on isolation and description of viruses or virus-like particles from freshwater algae have not been evaluated and approved by the ICTV yet [8].

Starting from the lack of available sound information on viruses of freshwater algae, we focused on the identification of viruses able invading species of *Chlorophyta* microalgae.

Materials and Methods. Test (indicator) cultures of the following microalgae have been generously provided by the colleagues from the Department of Botany (Taras

Shevchenko' Kyiv National University): *Planophila sp., Chlorella sp., Carteria crucifera, Chlorococcum sp., Radiosphaera sp., Chlamydomonos heterogama, Scenodesmus sp., Borodinella sp.,* and *Oocystis sp*.

Approximately 40 water samples have been collected from various water reservoirs in different regions of Ukraine for the initial virus screening. The reservoirs were characterized with different level of anthropogenic pressures varying from negligibly low to moderate (city water ponds/lakes). To remove all cells from the samples these have been filtered via bacterial filter (millex GV 0,22).

Bioassay (microalgae indicator cultures) and transmission electronic microscopy after differential centrifugation of the samples were used for virus indication [5].

Algae cultures were grown following standard recommendations [7] in liquid and agar Bold's Basal Medium (BBM) medium. 2ml of algae culture suspension and 2ml of the medium were added in the tube. The active growth of the culture is normally expected in 5-7 days. Fresh suspension of algae cells then may be used again for subcultivation (in this case 2 ml of algae culture suspension and 2-4 ml of the medium are mixed). However the cultures rarely survive more than 10-15 days when employing such approach for algae cultivation.

We have also revealed that test cultures grew poorly in the flasks of relatively large volume (0.25-0.50l). After 2-3 days of active growth the algae cells either formed sediments at the bottom of such flask or became concentrated at the surface. The described effects probably reflect poor aeration of the medium in the flasks of large volumes highlighting the need for occasional mixing of the content with culture suspension for its better aeration.

Despite this modified technique was shown adequate for accumulation of algae cultures, it was also demonstrated to be inefficient for long term 'maintenance' of the cultures in active state. For this, the cultures needed to be cultivated on solid agar medium. Samples of 5-7 days' old algae cultures were loop-plated onto slants with 1.5% solid agar BBM. Normally the culture growth could be seen in 3-4 days. In such state the cultures could be maintained active for 3-4 weeks without additional passage or addition of fresh medium.

It should be said that algae viruses infect only those algae cultures which are at active growth stage or at a certain stage of the life cycle [3]. Keeping this in mind, the microalgae cultures required preliminary 'preparation' before commencing biotesting assay. Lawn scrapes of test cultures (previously cultivated on solid agar medium for 10-14 days) were deposited into plastic tubes with 1ml of BBM medium. Afterwards the tubes were kept in the dark for 12 hours for culture's synchronization and then cultivated under normal light conditions after adding equal portion of fresh medium. Occurrence of monad forms typical for active growth stage of the culture was monitored via light microscopy. The outcomes of biotesting assays have been registered by 7-10 days of cultivation [5].

Using biotesting we attained sufficient volumes of cultures' lysates for subsequent isolation and concentration of virus isolates. Concentration of virus isolated have been performed via differential centrifugation (low speed centrifugation stage at 5000 rpm for 30 min, then high speed centrifugation stage at 32000 rpm for 3 hours, and low speed centrifugation stage at 5000 rpm for 30 min) [5].

Virus morphology was studied employing transmission electronic microscopy (TEM) with positive contrasting technique (2% of sodium uranyl acetate (UA)). TEM grid with previously applied virus preparation was deposited into a drop of 2% UA and incubated for 1-10 min.

For biotesting, 2ml of collected water samples (filtered via bacterial filter) and 2ml of previously 'prepared' microalgae cultures (*Planophila sp., Chlorella sp., Carteria crucifera, Chlorococcum sp., Chlamydomonos heterogama, Scenodesmus sp., Borodinella sp., Oocystis sp.*) were added into tubes. According to literature sources [3, 5], algae viruses are normally abundant in natural habitats of microalgae and hence the lysis of algae culture may be expected by 7-10 days [5]. We have also conducted the biotesting assay on solid agar medium. 1ml of algae culture was plate into Petri dish and spread with sterile glass rod over the surface of the medium. After the uniform lawn is made, the bottom of the dish is divided into 10 sectors and 0.1ml of filtered water samples are separately plated on each of them [5].

Results and Discussion. Sample biotesting on solid medium provided no clear results, probably because of small volume of water samples applied onto the lawn of test cultures.

However, biotesting assay in liquid medium has shown that the samples collected in Kyiv from Opechen lakes of Obolon region (Verbne lake (No.1) and Velykyi Opechen lake (No.2)) and from Rylskyi Park' lakes (No.6) were capable of lysing test cultures of *Planophila sp*. and *Chlamydomonos heterogama*. In turn, water samples taken from the reservoir near the city of Slavutych and from Siverka River near Vita-Pochtova village lysed test cultures of *Chlorella sp*. and *Radiosphaera sp*. (Table 1). All samples capable of inducing lysis of test cultures were subsequently subcultivated. For this, 2ml of synchronized cultures and 1ml of respective lysat were added into a tube. The recurring effect of lysis was typical only for water samples collected from the reservoir near the city of Slavutych.

Table 1. **Results of biotesting assay for water samples on test algae cultures**

	Planophila sp.	Chlorella sp.	Carteria crucifera	Chlorococcum sp.	Radiosphaera sp.	Chlamydomonos heterogama	Scenodesmus sp.	Borodinella sp.	Oocystis sp.	Control
Verbne lake	-	+	+	+	+	-	+	+	+	-
Velykyi Opechen lake	-	+	+	+	+	-	+	+	+	-
Rylskyi Park' lakes	-	+	+	+	+	-	+	+	+	-
Siverka river near Vita-Pochtova village	+	+	+	+	-	+	+	+	+	-
Water reservoir near city of Slavutych	+	-	+	+	+	+	+	+	+	-
Control	+	+	+	+	+	+	+	+	+	

Notes: '-' lysis of test culture (no growth);
'+' normal growth of test culture.

Test cultures of *Carteria crucifera* and *Chlorococcum sp*. have demonstrated spontaneous lysis by 3 weeks of cultivation when no water samples were added.

Further, the water samples capable of inducing lysis of algae test cultures and culture samples demonstrating autolysis were subjected to TEM studies. In the autolysis

sample of *Chlorococcum sp*. culture we have identified spherical virus (or virus-like) particles of 50±2nm in diameter (Fig.2). However we have been able to demonstrate presence of virus particles in the lysates of other algae cultures. We deem that this is connected with low concentration of the particles in the sample not allowing their easy isolation and concentration.

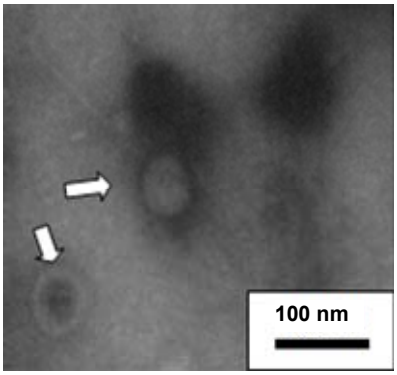

Fig.2. TEM image of virus-like particles in the autolysate sample of *Chlorococcum sp.* algae culture

Further studies will involve analysis of new water samples, as well as isolation and concentration of identified virus isolates.

In conclusion, biotesting assay of water samples collected in Verbne and Velykyi Opechen lakes, and from Rylskyi Park' lakes (Kyiv) were able to lyse *Planophila sp*. and *Chlamydomonos heterogama* algae test cultures. Respectively, water samples collected from the reservoir near the city of Slavutych and from Siverka River near Vita-Pochtova village lysed algae test cultures of *Chlorella sp*. and *Radiosphaera sp*. Using differential centrifugation of the samples, we purified and concentrated virus isolate(s) from the samples of autolysis of *Carteria crucifera* and *Chlorococcum sp*. algae cultures. TEM studies of such virus isolate(s) confirmed presence of spherical virus (or virus-like) particles of 50±2nm in diameter. Further work will be focused on studying molecular properties of the virus(es).

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SOIL-BORNE VIRUSES DETECTED IN SUGAR BEET IN LITHUANIA AND UKRAINE

Проведено обстеження зразків цукрового буряку отриманих на території Литви та України на наявність вірусу некротичного пожовтіння жилок буряку (ВНПЖБ), ґрунтового вірусу буряку (ҐВБ) та Q вірусу буряку (ВБQ). Тестування проводили методами ІФА, в модифікації DAS і TAS, та мультиплексною ЗТ-ПЛР (мЗТ-ПЛР), що дозволяє одночасно ідентифікувати ВНПЖП, ҐВБ та ВБQ. Зразки коренеплодів цукрового буряку, з симптомами ризоманії, відбирали на плантаціях Литви та України та тестували на наявність ВНПЖБ за допомогою DAS-ELISA. Для детекції ҐВБ, методом TAS-ELISA, використовували лише ті зразки, що позитивно прореагували з антисироваткою до ВНПЖБ. У литовських зразках цукрового буряку не було відмічено випадків змішаної інфекції ВНПЖБ і ҐВБ або ВПQ. На противагу в українських зразках (відібраних в Рівненській, Львівській, Івано-Франківській та Хмельницькій областях) крім ВНПЖБ була доведена наявність ҐВБ. Ідентифікацію ҐВБ підтверджували за допомогою мЗТ-ПЛР, в усіх випадках були отримані специфічні продукти для ВНПЖБ (545 п.о.) та ҐВБ (399 п.о.). ВПQ не було виявлено в жодному з досліджених зразків.

Tests for detection Beet necrotic yellow vein virus (BNYVV), Beet soil-borne virus (BSBV) and Beet virus Q (BVQ) in sugar beet samples from Lithuania and Ukraine were conducted using direct DAS-ELISA, direct TAS-ELISA and multiple RT-PCR (mRT-PCR), allowing simultaneous identification BNYVV, BSBV and BVQ. Sugar beet root samples with suspicious symptoms of rhizomania were collected in Lithuanian and Ukrainian sugar beet growing areas and analyzed by DAS-ELISA for the presence of BNYVV. For BSBV detection by TAS-ELISA only samples with positive reaction to BNYVV were used. Mixed infections of BNYVV and BSBV or BVQ in sugar beet samples in Lithuania were not detected. Meanwhile, in some Ukrainian sugar beet samples (from Rivno, Lviv, Ivano-Frankivsk and Khmelnytskyi regions) along with BNYVV, presence of BSBV was detected. Identification of BSBV was confirmed by mRT-PCR. In all cases specific BNYVV (545 bp) and BSBV (399 bp) products were obtained. BVQ was not found in any of investigated samples.

Introduction. Several soil-borne viruses are known to infect sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) worldwide. Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV; genus *Benyvirus*) (Tamada and Baba, 1973), is one of the most destructive diseases of sugar beet. Losses of sugar beet root yield can reach 50-60 % (Henry, 1996). Rhizomania was originally described in Italy (Canova, 1959) but now it is present in sugar beet areas all over the world. BNYVV has a multipartite single-stranded RNA genome with all natural isolates containing four RNA species, although some isolates have a fifth RNA (Tamada et al., 1989). The larger RNA1 and RNA2 contain the housekeeping genes of the virus and are always required for infection, whereas the smaller RNAs are involved in pathogenicity and vector transmission. RNA5-containing isolates are restricted to Asia and some parts of Europe and these isolates tend to be more aggressive (Richards and Tamada, 1992; McGrann et al., 2009). BNYVV is transmitted and preserved in the soil by *Polymyxa betae* Keskin. Like BNYVV, the plasmodiophorid has a host range mainly restricted to the roots of *Chenopodiaceae* spp. (Barr and Asher, 1992) and is present in most sugar beet growing countries (Payne and Asher, 1990). The virus can survive in *P. betae* cystosori for more than 15 years (Abe and Tamada, 1986; McGrann et al., 2009). According to the alert list of European and Mediterranean Plant Protection Organization (EPPO), BNYVV has been detected in many European, Asian countries (Austria, Belgium, Bulgaria, China, Croatia, Czech Republic, Denmark, Federal Republic of Yugoslavia, France, Germany, Greece, Hungary, Iran,

Japan, Kazakhstan, Kyrgyzstan, Mongolia, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, The Netherlands, Turkey and the United Kingdom) (Kanzawa and Ui, 1972; Gao et al., 1983; Asher, 1993; Miyanishi et al., 1999; Lennefors et al., 2000; Nielsen et al., 2001; Mouhanna et al., 2002; Sohi and Maleki, 2004; OEPP/EPPO, 2005; Yilmaz et al., 2007), also in Morocco and North America (USA) (Al Musa and Mink, 1981; OEPP/EPPO, 2005), and is likely to continue its spread across the world (Rush et al., 2006; McGrann et al., 2009).

Other soil-borne viruses detected in sugar beet are *Beet soil-borne virus* (BSBV) and *Beet virus Q* (BVQ) that belong to the genus *Pomovirus* and have multipartite genomes consisting of three RNA species (Hutchinson et al., 1992; Kaufmann et al., 1992; Koenig et al., 1998). BVQ was originally considered to be a serologically distinct strain from BSBV, but finally Koenig (1998) proved its different RNA composition substantiating its separation from BSBV. BSBV was first found by Ivanovič and Macfarlane (1982) in England and further described by Henry et al. (1986). It is widespread in sugar beet growing areas all over the world. BSBV has been detected in Europe (United Kingdom, Germany, Sweden, The Netherlands, Spain, France, Italy, Finland, Belgium, Bulgaria, Hungary, Turkey, Slovakia, Denmark, Poland and China) (Henry and Jones, 1986; Lesemann et al., 1989; Lindsten, 1993; Bremer et al., 1990; Meunier et al., 2003; Šubíková, 1995; Danielsen et al., 1992; Borodynko et al., 2006; Wang et al., 2007), in the Middle East (Iran and Syria) (Farzadfar et al., 2002; Mouhanna et al., 2002) and also in the USA (Lindsten and Rush, 1994). BVQ has been detected in Belgium, Bulgaria, Czech Republic, France, Germany, Hungary, Italy, Poland, Sweden and The Netherlands (Meunier et al., 2003; Rysanek et al., 2006; Borodynko et al., 2009).

Harmfulness of these two pomoviruses is questionable, because they also occur in fields with no problems concerning sugar beet growing. In common with BNYVV, the two pomoviruses have a common vector (the soil-borne protist *P. betae*), similar host ranges and particle morphologies but differ in serological properties, genome structure and sequence (Koenig et al., 1998; Koenig and Lesemann, 2005). BSBV and BVQ often occur together in the same field and, not rarely, in rhizomania-affected sugar beet plants (Meunier et al., 2003), although their etiological role in the disease remains a matter of debate (Prillwitz and Schlosser, 1992).

Surveys to detect BNYVV have been regularly carried out since 1998 in Lithuania (Lithuanian State Plant Protection Service) and since 1997 in Ukraine (Institute for Sugar Beet Research of NAAS, Ukraine). In recent years rhizomania was identified in three areas of Lithuania (Jackeviciene et al., 2005; Žižytė, et al. 2006; Zizyte and Staniulis, 2007). From 1997 till 2003 rhizomania was also detected in 4 areas of Crimea and 17 regions of Ukraine (Nurmukhammedov, 2005).

The aim of this work was to detect mix infection of beet soil-borne viruses in Lithuanian and Ukrainian sugar beet growing areas.

Materials and methods. Sugar beets with suspicious symptoms of rhizomania were collected in different regions of Lithuania and Ukraine. Samples of rootlet tissue were used for ELISA analysis and total RNA extraction. BNYVV was tested by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) and BSBV was tested by triple antibody sandwich-ELISA (TAS-ELISA) using Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) immunological kits as described by Clark and Adams (1977). Absorbance (A_{405}) was recorded using a Multiskan RC microplate reader 1-2 h after adding the substrate (*p*-nitrophenylphosphate). The ELISA test was considered positive if absorbance of the investigated sample was greater than 3 times the absorbance of the negative (healthy plant) control. Total RNA extraction was performed using QuickPrep total RNA extraction kit for the direct isolation of total RNA from most eukaryotic tissues or cells (Amersham Bioscience) or TRIzol Reagent (Invitrogen) according to the kit protocol. Three pairs of primers (Meunier et al., 2003) were combined in the mRT-PCR for specific BNYVV, BSBV and BVQ products (545 bp, 399 bp and 291 bp) amplification. The primers used in mRT-PCR are listed as follows (5'-3'): BNYVV/for, ACATTTCTATCCTCCTCCAC and BNYVV/rev, ACCCCAACAAACTCTCTAAC for RNR2; BSBV/for, CTTACGCTGTTCACTTTTATGCC and BSBV/rev, GTCCGCACTCTTTTCAACTGTTC for RNR1; BVQ/for, GCTGGAGTATATCACCGATGAC and BVQ/rev, AAAATCTCGGATAGCATCCAAC for RNR2. For each RT reaction, 20 Mμl of each reverse primers were mixed with 3 μl of total RNA and 3 μl of diethyl pyrocarbonate (DEPC)-treated water. The mixture was incubated at 70° C for 5 min and chilled on ice prior to the addition of 1.5 μl of DEPC-treated water, 2 μl of dNTPs (10 mM), 4 μl of MMLV RT 5× buffer (MBI Fermentas), 0.5 μl Ribonuclease Inhibitor (40 U/ μl) (MBI Fermentas) and 1 μl of MMLV reverse transcriptase (200 U/ μl) (MBI Fermentas). For the PCR, 20 Mμl of each of the forward and reverse primers was added to 12 μl of DEPC-treated water, 4 μl of $MgCl_2$ (25 mM) (MBI Fermentas), 6 μl of *Taq* polymerase 10× buffer (MBI Fermentas), 1.5 μl of dNTPs (10 mM), 0.5 μl of *Taq* polymerase (5 U/ μl) (MBI Fermentas), and 4 μl of cDNA. Amplification cycles were as follows: a first denaturation for 3 min at 94° C and then 35 cycles of denaturation for 30 s at 94° C, annealing for 30 s at 63° C, and elongation for 2 min at 72° C. A final elongation for 7 min at 72° C was added. In PCR reactions healthy plant material and water controls were used. All PCR products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide and visualized under UV light.

Results and discussion. In 2009 sugar beet root samples with suspicious symptoms of rhizomania were collected in 14 Lithuanian (Kaunas, Kėdainiai, Marijampolė, Šakiai, Vilkaviškis regions) and 17 Ukrainian sugar beet growing fields (Rivno, Lviv, Ternopil, Ivano-Frankivsk, Chernivtsi, Khmelnytskyi, Vinnytsya and Zhytomyr regions) and analyzed by DAS-ELISA for the presence of BNYVV. For BSBV detection by TAS-ELISA only samples with positive reaction to BNYVV were used. BVQ detection was relied on mRT-PCR, allowing simultaneously identify BNYVV, BSBV and BVQ viral particles in the same sample (Meunier et al., 2003). According to the positive DAS-ELISA results BNYVV was proved not to be very widespread in Lithuanian sugar beet growing areas because it was present just in 1 region (central part of Lithuania, Kaunas region) (*data not shown*). In Ukraine rhizomania was confirmed in 10 sugar beet growing areas. Mixed infections of BNYVV and BSBV or BVQ were not detected in Lithuanian sugar beet samples (*data not shown*). Meanwhile, in some Ukrainian sugar beet samples mix infection of BNYVV and BSBV was found (Table 1). BSBV detection was carried out by both TAS-ELISA and mRT-PCR. Obtained ELISA results of BNYVV and BSBV mix infection are shown in Table 1. According to TAS-ELISA results BSBV was detected in ukr2, ukr4, ukr9, ukr10 and ukr11 (Rivno, Lviv, Ivano-Frankivsk and Khmelnytskyi regions) sugar beet samples (Table 1.). Identification of BSBV was also confirmed by mRT-PCR. Three specific PCR primer pairs (Meunier et al., 2003), amplificating 291 bp (BVQ), 399 bp (BSBV) and 545 bp (BNYVV) products were used for

mRT-PCR. In all cases specific BNYVV (545 bp) and BSBV (399 bp) products were obtained (Fig. 1.). mRT-PCR confirmed the ELISA results. BVQ was not found in any of investigated samples although it is known, that BVQ infection was previously reported in Ukrainian agrocenosis (Senchugova et al., 2007).

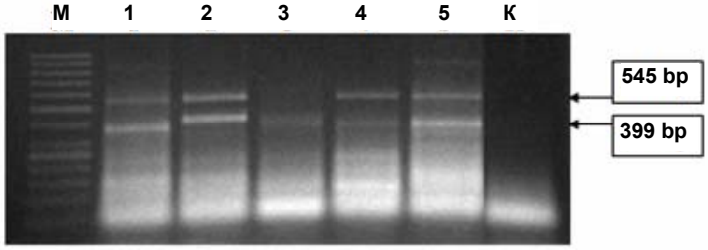

Fig. 1. mRT-PCR products of investigated mix infection of BNYVV and BSBV in 2% agarose gel: M – GeneRuler 50 bp DNA Ladder; 1 – ukr2 (Rivno); 2 – ukr4 (Lviv); 3 – ukr9 (Ivano-Frankivsk); 4 – ukr10 (Khmelnytskyi); 5 – ukr11 (Khmelnytskyi); K – water control

Table 1. **ELISA results of investigated mix infection of BNYVV and BSBV in Ukrainian sugar beet samples**

Location	Isolate	Virus infection	
		BNYVV	BSBV
		DAS-ELISA, absorbance, 405 nm	TAS-ELISA, absorbance, 405 nm
Rivno region, Radyvyliv area, Krupets village	Ukr2	0.586 +	0.458 +
Lviv region, Brody area, Brody	Ukr3	0.561 +	0.27 –
Lviv region, Radechiv area, Chmilno village	Ukr4	0.848 +	0.342 +
Lviv region, Radechiv area, Babychi village	Ukr5	1.011 +	0.249 –
Lviv region, Busk area, Ozhydiv village	Ukr6	0.452 +	0.253 –
Ternopil region, Chortkiv area, Oryshkivtsi village	Ukr7	0.451 +	0.278 –
Ternopil region, Zalischyky area, Torske village	Ukr8	0.466 +	0.288 –
Ivano-Frankivsk region, Gorodenka, Yaseniv-Pilnyi village	Ukr9	0.674 +	0.423 +
Khmelnytskyi region, Starokostyantyniv area, Starokostyantyniv	Ukr10	0.705 +	0.345 +
Khmelnytskyi region, Starokostyantyniv area, Ladygy village	Ukr11	0.843 +	0.512 +
K –		0.151	0.176
K +		1.65	2.408

"K–" – negative control; "K+" – positive control; "–" – negative result; "+" – positive result. The ELISA test was considered positive if absorbance of the investigated sample was greater than 3 times the absorbance of the negative (healthy plant) control (Clark and Adams, 1977).

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ELECTRON MICROSCOPY DETECTION OF VIRUSES IN THE CACTUS COLLECTION OF UKRAINE

Проведено скринінг колекцій кактусових у ботанічних садах України. У всіх обстежених колекціях кактуси були інфіковані вірусами. Виявлено вірус, який за морфологічними характеристиками подібний до вірусу кактусів 2.

Screening of Cactaceae plants on virus diseases in the collections of Ukrainian Botanical gardens have been conducted. Cactus plants in all collections are virus infected. Basing on morphological properties detected virus is related to Cactus virus 2.

Introduction. Cactus collections in Ukrainian botanical gardens are represented by hundreds various types of plants, located in the limited space greenhouses. This situation leads to rapid mechanical transmission of pathogens. Exchange collection material without testing on virus infection, can increases chances of uncontrolled distribution of viral infections. In case of asymptomatic virus infection in cactus uncontrolled import to Ukraine succulents makes a danger because these plants can become the source of distribution pathogens in collections. In addition, cactuses could support reproduction of viruses other types of plants and, thus, be the reservoirs of plant virus infections. A timely exposure and permanent control of the state of population of these cultural plants is the obligatory link of the system of their protecting from this group of pathogens.

Materials and methods. As biological material were used plants with the signs of affection: *Opuntia* sp., *Opuntia microdaysys* v. rufida, *Consolea rubenscens*, *Pereskia aculeata* v. godseffiana, *Echinocereus* sp., *Caralluma* sp., *Consolea rubescens, Pseudolobivia ancistrophora, Lophophora williamsii, Echinocereus penthalophus, Ferocactus echidne, Lophophora echinata, Rhipsalis* sp., *Rhipsalis pachyptera, Pseudolobivia cojasii* and crested graft plants *Chamaereus silvestrii*, *Echinopsis* sp., with virus-like symptoms from greenhouse collections of Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university (Kyiv), *Mamillaria centricirrha, Trichocereus bridgesii, Ritterocereus prinosus* – from the collection of Karazin' Botanic Garden of Kharkiv national university (Kharkiv)), *Ferocactus echidne, Gymnocalycium sp. Opuntia sp*. – from the collection of Botanical garden of Ivan Franko' Lviv national university (Lviv*)*), *Opuntia* sp., *Opuntia arechavelerae* – from the collection of the Donetsk botanical garden of the National academy of sciences of Ukraine (Donetsk) and *Opuntia* sp., *Opuntia microdaysys* v. *alba* – from the collection of Botanical garden of I.I. Mechnikov Odesa national university (Odesa).

Morphology of found viruses was determined by the method of transmission electron microscopy. Plants homogenized with addition of 0,1M phosphate buffered saline (pH 7,4). For besieging of cellular admixtures conducted low-speed centrifugation 7000 rpm during 20 minutes.

Preparations inflicted on copper string-bags from 0,2% formvar lining. The negative contrasting was conducted by 2% uranilacetat during 1,5 min. The revision of standards was conducted in an electron microscope EM–125 (Sumi, Ukraine), with an instrumental increase 30 000.

Results and discussion. Virus diseases of *Cactaceae* plants aren't enough studied. The most widespread viruses affecting plants of this family are *Cactus virus X* (genus *Potexvirus,* family *Alphaflexiviridae*): virions filamentous, not enveloped; usually flexuous with clear modal length 520 nm and 13 nm wide; *Zygocactus montana X virus* (genus *Potexvirus,* family *Alphaflexiviridae*): virions are flexuous with clear modal length 519 nm and 10 nm wide; *Cactus mild mottle virus* (genus *Tobamovirus*): virions long 320 nm and 18 nm in diameter, *Sammons' Opuntia virus* (genus *Tobamovirus*) virions rod-shaped, not enveloped, long 317 nm and 18 nm wide; *Saguaro cactus virus* (genus *Carmovirus,* family *Tombusviridae*): virions not enveloped, isometric nucleocapsid, 32 nm in diameter; *Virus cactus 2* (genus *Carlavirus,* family *Betaflexiviridae*): filamentous virions with normal length 650-655 nm and 11-13 nm wide [2, 3].

At the visual inspection of cactus collections we pay attention on plants with the changes of colouring, in particular by the inlaid and chlorotic spots. In cactus collection of Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university we found symptoms of necrosis, mosaic and chlorotic spots, wrinkling and atrophy of stems. The chlorotic colouring and atrophy of stems (symptoms of witches'-broom) were observed only on the *Opuntia* plants. These symptoms on *Opuntia* could be associated with phytoplasma [1]. Although we selected these plants to detect viruses as we proposed that affection of phytoplasma could reduced plant resistance to viruses. Furthermore in this situation activation of latent virus infection and following development of symptoms could took place. It should be noted that different cactus species had diverse symptoms. In collection of Karazin' Botanic Garden of Kharkiv national university we

registrated the symptoms of defeat on single plants: there were chlorotic spots on the plants *Trichocereus bridgesii* and *Ritterocereus prinosus*. At an inspection collection of the Donetsk botanical garden found out the symptoms of mosaic on the plants *Opuntia*. Only single plants in collection of Botanical garden of I.I. Mechnikov Odesa national university had symptoms of affecting. In cactus collection of Ivan Franko' Lviv national university chlorotic spots have been discovered on the plants *Gymnocalycium* sp. and *Ferocactus echidne*, and mosaic and deformation of stems heve been detected on the plants *Opuntia* sp.

As cactus plants demonstrated distinct symptoms of virus infection, we suggested that virus particles present in sap of unhealthy plants in high concentration. A threshold sensitiveness of electron microscopy is approximately 10^6, that is why for primary diagnostics and determination of morphology of exciter we applied an electron microscopy. In sap of all *Opuntia* plants, selected in collection of Karazin' Botanic Garden of Kharkiv national university, Botanical garden of I.I.Mechnikov Odesa national university, Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university and Botanical garden of Ivan Franko' Lviv national university, and also in *Chamaereus silvestrii*, *Echinopsis* sp. – from Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university we registered filamentous virions with size 650×12 ± 2 nm (Fig. 1a,b), which is typical for cactus virus 2.

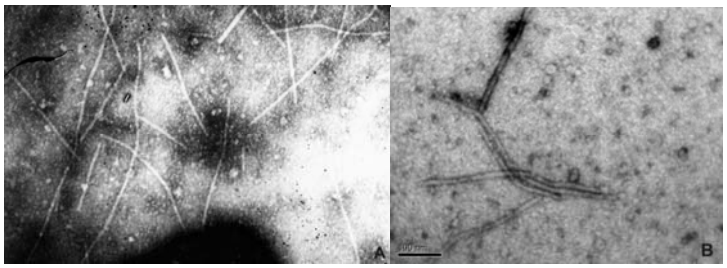

Fig.1. Electron micrograph of flexible particles from plant material from Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university *A.Opuntia sp* B. *Chamaereus silvestrii*

Our attention had been attracted by the high concentration of virus particles on electron micrograph. In our opinion this is the result of relatively high concentrations of viruses in the sap of all tested plants (Fig. 2). This is probably due to the fact that we tested plants that had clear visual symptoms. If a correlation between the concentration of virus in sap and visual manifestation of infection takes place, it is possible of wide distribution of viruses in investigated collections.

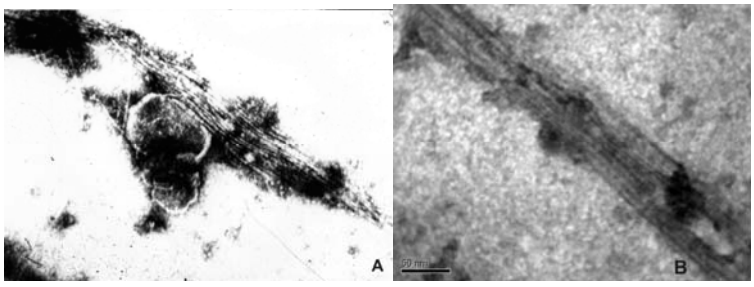

Fig.2. Electron micrograph viruses A.*Ritterocereus prinosus*, B. *Echinopsis* sp.

It should be marked that in plant samples of *Ritterocereus prinosus* and *Opuntia* sp. – from the Karazin' Botanic Garden of Kharkiv national university, *Opuntia* sp. – from the Botanical garden of Ivan Franko' Lviv national university and crested graft plants *Chamaereus silvestrii*, *Echinopsis* sp – from Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university were found out 2 types of virions filamentous and rod-shaped (Fig. 1A).

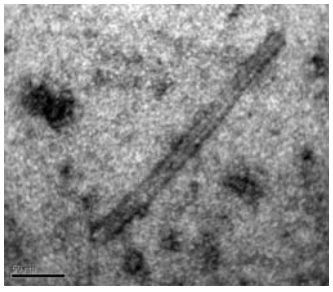

Fig.3. Electron micrograph rod-shaped virus from plant *Echinopsis* sp.

Discovered rod-shaped viral particles (Fig.3) belong virus from the genus *Tobamovirus*. In accordance with literature data [4] the *Sammons' Opuntia virus* infects only the plants genus *Opuntia*, though often an symptomless infection takes place. Virus particles have length 317 nm and diameter 18 nm. The *Cactus mild mottle virus* affecting different cactus species causes spotted and mosaics on plants, virions has length 320 nm and diameter 18 nm [3]. As plants cactus had the mixed infection, it is possible to assume that *Opuntia* sp. were infected by *Sammons' Opuntia virus* or *Cactus mild mottle virus* and other plant species were infected by *Cactus mild mottle virus* [3].

Thus *basing on morphological properties detected virus is related to Cactus virus 2.* Nature of rod-shaped virions is steel studied.

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