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Virus diseases of plants in agrocenosis and forest ecosystems: diagnostics and prevention

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Goal. To analyze the already known results of virological tests and to investigate virus diseases in agricultural lands and forest ecosystems. **Methods.** Visual examination, enzyme-linked immunosorbent assay in various modifications, allocation of total RNA from plant material, purification of cDNA, electrophoresis of nucleic acids, CT-PCR, and method of statistical data processing. **Results.** They analyzed the results of domestic and foreign researches on phytovirusology. Joint research was conducted in the Institute of Agroecology and environmental management of NAAS and the National University of life and environmental Sciences of Ukraine regarding the prevention of viral infections of plants at the use of the composition of basidiomycetes fungi. For the recovery of plants from viral diseases, they used a process of simulated microgravity. **Conclusions.** Taking into account scientific, environmental and social importance of biological security in agriculture as an important condition of balanced development of Ukraine and the production of quality food products, agriculture and forestry should be based primarily on the use of quality certified disease-free seed of resistant varieties. So pressing is the problem of the creation of the Depositary of the main viruses affecting agricultural and forest crops, and scientific center «Virus», which was initiated by the Bureau of the Presidium of the National Academy of agrarian sciences of Ukraine on 18 September 2019.

Key words: *phytovirology, biosafety, crops, yield, varieties, planting material, symptoms.*
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According to FAO, at the dawn of the twenty-first century, plant virus epiphytotics has led to significant economic losses in forestry and agriculture, exacerbating the world's food security problem. In the context of accelerating scientific and technological progress with the development of new molecular methods and capabilities of biotechnology, biological security becomes an integral part of environmental security, and therefore of national security as a whole. However, the importance and necessity of plant virology for the agricultural production of Ukraine remains is still being ignored, namely ignored the spread, accumulation, emergence of new species and resistant forms of viruses in agrocenoses and forest ecosystems [1]. Unfortunately, the monitoring and control of viral infections receives far less attention than it deserves [2].

Export and import of seed and planting material, change in the number of insect vectors as well as the emergence of new mixed infections all contribute to altering the pathogenicity of viruses.

The International Committee on Taxonomy of Viruses (ICTV) has already registered 1,100 species of plant viruses. Given the economic and environmental importance of agricultural products and forest ecosystems for the economy and in the future to reduce the negative effects of climate change, it is necessary to expand the study of plant viruses [3]. Average crop losses in Ukraine from viral infections can reach: for cereals from cereal yellow dwarf virus – 60-96%, for wheat from wheat streak mosaic virus – 20-63%; for legumes from soybean mosaic virus – 26-67%, for soybean from soybean dwarf virus – 11-46%. Vegetable yields are reduced by 3-80% due to cucumber mosaic virus infection and by 39-57% by tobacco mosaic virus infection; potato yield due to potato virus Y infection is reduced by 4-80% and 40- 52% due to potato virus X infection. Fruits are infected by plum pox virus (PPV), which is a quarantine species in Ukraine and crop losses from PPV account for 30-53%, whereas from cherry ringspot virus are 25-44%, and from grapevine fanleaf virus (GFLV) are 20-41% [4]. It is worth noting that plant and mushroom populations of forest ecosystems of Ukraine also experience significant viral damage, namely the infectivity of some stands of silver birch is 16-23%, and 10-12% of aspen trees and 6-8% of common hazel trees are infected with carlaviruses. Up to 63% of basidiomycetes are infected by isometric, filamentous, and rod-shaped viruses. These problems are exacerbated by climate change (Fig. 1).

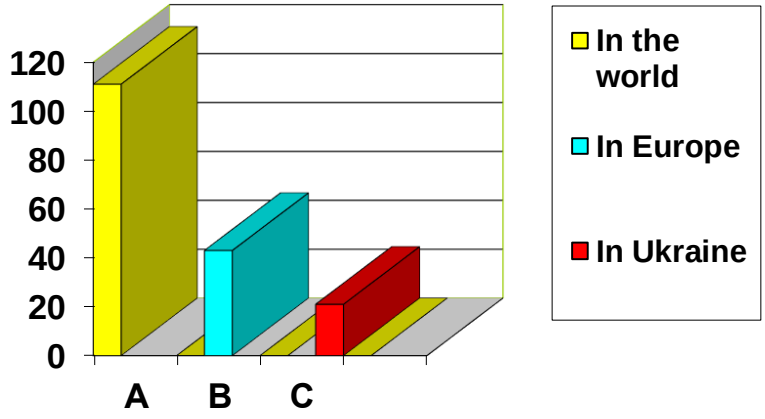

Fig. 1. Identified species of viruses of agricultural and forest crops

In this context, an important task is to create new varieties of plants resistant to viral infections and to develop environmentally friendly methods of protecting agrophytocenoses from viral diseases (viruses) [5].

In Ukraine, the first large-scale virological studies began in 1962, when the first Department of Virology (also the only one at that time in the territory of the former Soviet Union) was established at Taras Shevchenko National University of Kyiv. Highly qualified teachers and famous scientists have made a significant contribution to the development of the department at different times: Kornyushenko N.P., Topchii M.K., Baisheva V.G., Sidorenko O.V., Dulevich Zh.A., Taikova N.V., Moskovets S.M., Boyko A.L., Polishchuk V.P., Budzanivska I.G., Mishchenko L.T.

Academician A.L. Boyko headed the department for 26 years, founded a scientific school of studies of the structure and function of viruses under various environmental conditions. Currently, he continues to actively work on the school "Structure and Function of Viruses in Various Environmental Situations".

As a result of this works, Ukrainian virologists have gained a lot of valuable information about the properties of a number of new viruses of sunflower, hops, wheat, oil rose and other crops.

In recent years, molecular research methods have become widespread, enabling us to expand our capabilities and deepen our knowledge in the field of ecology of plant viruses.

Molecular ecology makes possible monitoring the distribution of viruses in soil, water, air environments and various ecosystems, as well as forecasting epiphytotics viral diseases [5].

Ukraine does not have a nationwide program on biosecurity in agroindustrial complex and monitoring of plant viruses, and virological research is not coordinated, conducted haphazardly in scientific institutions of the NAS of Ukraine, NAAS and institutions of higher education, therefore, it is difficult to carry out consolidated virological studies.

Virological studies are performed under the programs of the NAAS for 2016-2020: "Agroecology" - Institute of Agroecology and Environmental Management of NAAS, "Agricultural Microbiology" - Institute of Agricultural Microbiology and Agroindustrial Production of NAAS, "Fruit and Ornamental Horticulture" - Institute of Horticulture of NAAS, “Potato Research” - Institute for Potato Research of NAAS, “Viticulture and winemaking” - NSC «V.Ye. Tairov Institute of Viticulture and Winemaking» of NAAS, “Plant Protection” - Institute of Plant Protection of NAAS, “Sugar Beet” - Institute of Bioenergy Crops and Sugar Beet of NAAS.

Viral diseases cause a number of symptoms on plants: mosaics, necrosis, chlorosis, dwarfism, various deformities.

Viral infection has a negative effect on the course of physiological and biochemical processes in plants. In particular, bushing productivity in infected cereals is 72-95% lower than in healthy ones, decreases the content of chlorophyll and carotenoids, the process of grain formation is disturbed and its quality deteriorates, in particular the number of grains in the ear and their weight are reduced by 29.2-54.5%. In leguminous plants the ability to nitrogen fixation decreases, heat resistance of plants decreases. Sugar content of beet root crops infected by beet mosaic virus is on average lower by 1.7-2%. The potato virus x reduces the starch content of potatoes by up to 2%. Forest plant viruses cause symptoms of tree bark cracking, mottle mosaic on leaves. These viruses are included: Potex-, Tobamo-, Poty-, Nepo-, and Ilar-groups. Many viruses have also been identified in flowering forest plants. These pathogens are a potential threat, namely, they reduce the productivity of forest stands, which damages the forestry economy as a whole [6].

Plant viruses have the ability to spread rapidly in biocenoses. They can be spread due to the mechanical contact of plants, by vegetative propagation through tubers, cuttings, bulbs, by vectors (ticks, insects, nematodes, fungi).

Seed transmission can occur in several ways, namely the virus may be inside the seeds (for example, tobacco ringspot virus, barley stripe mosaic virus, cucumber mosaic virus) or the virus may be on the surface of the seed skin (for example, tobacco mosaic virus).

The transmission of viruses in the process of vegetative propagation of plants can be carried out by means of tubers (eg, potato virus X), bulbs and cuttings. The most environmentally friendly way to control viral diseases is to use virus-resistant varieties [7].

Vectors of viruses, that cause diseases of the most economically important crops, include: mites, aphids, ticks, whiteflies and beetles, which are the main vectors of plant viruses and spread throughout Ukraine. With the help of vectors, viruses from diseased plants are spread over long distances and propagated in healthy plants [8].

Viral diseases can also be transmitted through soil. Viral particles for a long time from 10 to 40 days can be found in dead plant remains. For example, as shown in the diagram: cucumber mosaic virus (CMV) - persists for 29 days, potato virus X (PVX) - 25 days, tobacco mosaic virus (TMV) - 39 days (Fig. 2).

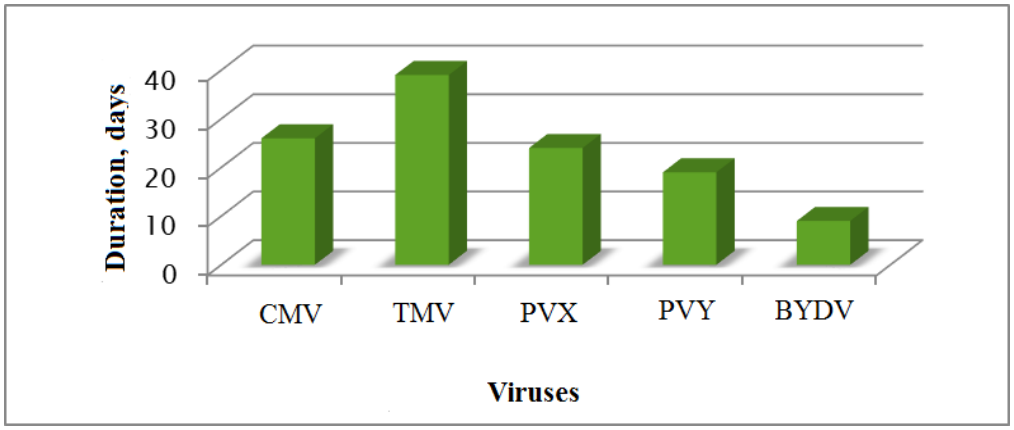

Fig. 2. Localization of some viruses in the soil
CMV – Cucumber mosaic virus, TMV – Tobacco mosaic virus, PVX – Potato virus X, PVY – Potato virus Y, BYDV – Barley yellow dwarf virus.

Now for the diagnosis of viral infections use the following methods: visual diagnostics, bioindication, electron microscopy, serological methods, enzyme-linked immuno sorbent assay, polymerase chain reaction, nucleic acid sequencing and phylogenetic analysis [9].

In recent years, molecular methods of research have been widely used and, as a result, empower and deepen knowledge in the field of plant virus ecology [10]. Knowledge of virus characteristics (virus identification) as well as its infectious process are the first steps to develop appropriate phytosanitary strategies for the cultivation of virus-free plants and improving productivity of the agricultural and forestry sector [11].

Monitoring the spread of viral infections and researching the genetic variability of plant viruses allows us to predict evolutionary relationships, virulence, possible ways of distribution of viruses and forecasting the emergence of new pathogenic strains that are capable of infecting plants and localizing in soil and aquatic environments.

The purpose of the research is to analyze the already known results of virological studies and to research viral diseases in agrocenoses and forest ecosystems.

Materials and methods of research. Plant specimens were selected by visual examination of plants, namely selected were plants with typical virus-like symptoms (mosaic, necrosis, chlorosis, deformation of the leaf plate and fruits, etc.). Identification of viruses was performed by an enzyme-linked immunosorbent assay using commercial test systems (LOEWE, Germany) according to the manufacturer's recommendations. The results were measured by reader (Termo Labsystems Opsis MR, USA) with Dynex Revelation Quicklink software at the wavelength of 405 nm. The index E405 was taken as positive and it was three times bigger for control index. Average (arithmetic mean) was used to compare results. Total RNA was extracted from the samples using kits from Invitrogen (USA), Promega (USA), Qiagen (United Kingdom), Omega (USA). One-step-RT-PCR kit (Qiagen, UK) was used to detect viruses by RT-PCR. The reaction was carried out according to the manufacturer's recommendations. The analysis of the amplification products was carried out by electrophoresis in 1.5% agarose gel using Gene Ruller 100 bp DNA Ladder plus (Fermentas, Latvia) as a size marker. These studies were conducted at the Department of Virology of Taras Shevchenko National University of Kyiv. Bioorganic composition “Bioecofunge-1” was investigated as a means of preventing viral diseases in buckwheat, soybean, sunflower, tomato, etc. These studies, as well as the development of the composition itself, were carried out by National University of Life and Environmental Sciences of Ukraine. For the healing of plants, with infected different viruses were used clinostating using clinostat "Ekoloh". In this way, for example, was cured tobacco, which infected TMV. Data processing was performed using Microsoft Excel 2010. Statistical significance was evaluated by paired Student's t-test.

Results and Discussion. Institute of Agroecology and Environmental Management of NAAS, Department of Virology, NSC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, National University of Life and Environmental Sciences of Ukraine, D.K. Zabolotny Institute of Microbiology and Virology of the NASU and Institute of Agricultural Microbiology and Agroindustrial Production of NAAS have conducted a series of studies of importance for the agro-industrial complex of Ukraine.

Results of joint research by Institute of Agroecology and Environmental Management of NAAS and National University of Life and Environmental Sciences of Ukraine on the use of "Bioekofunge-1" as a means of preventing viral plant infections were positive (Fig. 3). This composition has been tested on various plants (buckwheat, soybean, sunflower, tomatoes) at Skvyra Research Station of Organic Production of the Institute of Agroecology and Environmental Management of NAAS [13].

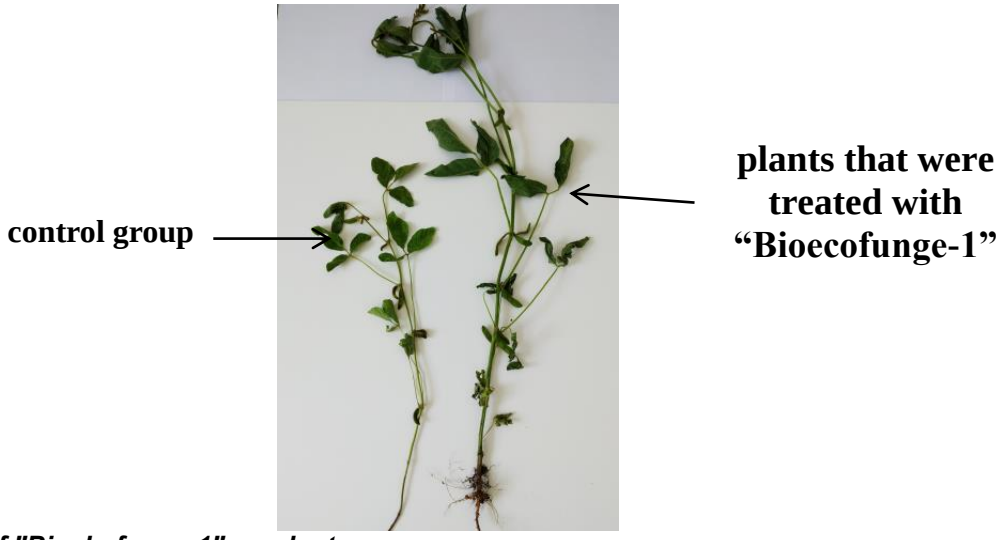

Fig. 3. Effect of "Bioekofunge-1" on plants

We used clinostating for healing plants, with infected different species of viruses [14]. Ground-based facility (clinostat) "Ekoloh", with was used in these studies, was designed by NSC "Institute of Agricultural Engineering and Electrification" of NAAS to order a Laboratory of ecology of viruses and biosafety of Institute of Agroecology and Environmental Management of NAAS (Fig. 4).

Fig. 4. General view of clinostat "Ekoloh"

Our research has shown that clinostating allows the healing of plants, with was infected viruses, in the seedling stage. Under the influence of the clinostating, the content of viral antigens in diseased plants is reduced by 1.5-2.3 times.

An important step in preventing the development and spread of viral infections is timely diagnostics using polymerase chain reaction methods.

The Institute of Agroecology and Environmental Management of NAAS applies diagnostic technologies to detect the following viruses: Watermelon mosaic virus 2, Zucchini yellow mosaic virus and Cucumber mosaic virus.

The Institute of Agroecology and Environmental Management of NAAS together with Department of Virology of Taras Shevchenko National University of Kyiv conducted monitoring these viruses in agrocenoses in 8 regions of Ukraine to compare the properties of these viruses with already known. As a result, genomic sequences of Ukrainian isolates Watermelon mosaic virus 2, Zucchini yellow mosaic virus, Cucumber mosaic virus and Tobacco mosaic virus were first registered in GenBank [15].

Screening of forest plants for the presence of viral antigens by enzyme-linked immunosorbent assay was conducted.

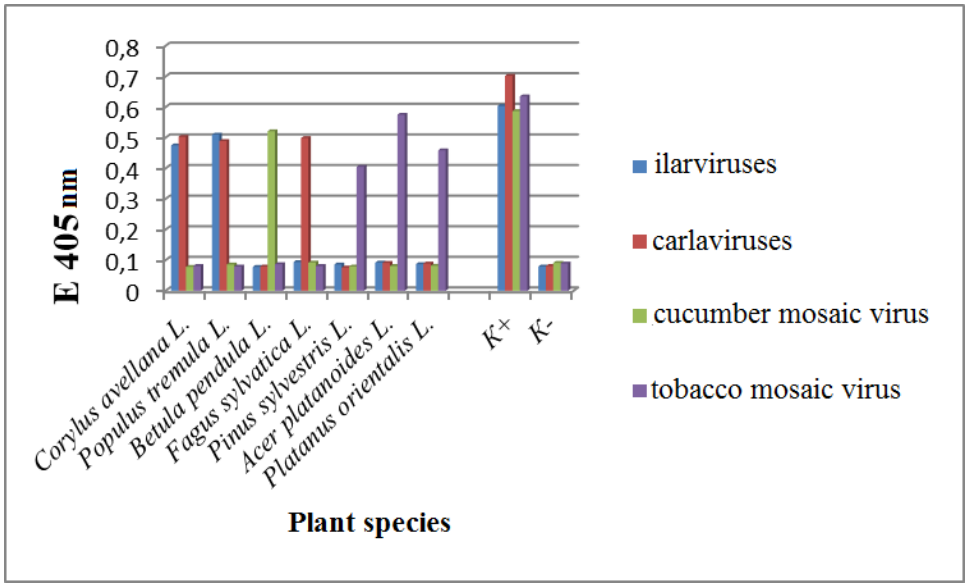

Fig. 5. Diagnosis of viral diseases by enzyme-linked immunosorbent assay in forest ecosystem plants

The tobacco mosaic virus was first discovered in Norway maple (Acer platanoides L.) and Oriental plane (Platanus orientalis L.). The cucumber mosaic virus has been diagnosed in silver birch (Betula pendula Roth) (Fig. 5).

Thus, timely diagnostics, carrying out preventive measures for the detection of reservators and vectors of plant viruses, cultivation of virus-resistant varieties of plants, using of virus-free planting and seed materials is the key to the effectiveness of plant viral disease control measures. Knowledge of molecular mechanisms of virus-host interaction is a requirement for the development of means of preventing and treating viral infections. Monitoring the spread of viral infections and researching the genetic variability of plant viruses allows us to predict evolutionary relationships, virulence, possible ways of distribution of viruses and forecasting the emergence of new pathogenic strains that are capable of infecting plants and localizing in soil and aquatic environments.

Conclusions

Now in Ukraine there is no national program for biosafety in agriculture and has no general data on the spread of plant viruses, and therefore, plant virological research needs consolidation. Thus, creating a depository of viruses that infect agricultural and forest plants as well as the creating a Research Center "VIRUS" that will perform such tasks is essential. A Research Center "VIRUS" can be established on the basis of Institute of Agroecology and Environmental Management of NAAS, Institute of Horticulture of NAAS, Institute of Agricultural Microbiology and Agroindustrial Production of NAAS and other institutions of NAAS. A Research Center "VIRUS" will coordinate virological research of different institutions of NAAS and will ensure rational use of available instruments, reagents and materials that required for different research. In addition, this research center will be able to involve specialists of different institutions of NAAS to undertake comprehensive research as well as will be able to purchase high-priced instruments, reagents and materials and provide their effective use. In the future, this center will be able to develop and implement approaches of monitoring, prevention and treatment of viral diseases of plants as well as will be able to train professionals in plant virology and to implement the latest virological achievements in an agricultural manufacturing.

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