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SPREAD AND MORPHOLOGICAL-STRUCTURAL PROPERTIES OF PLANT RHABDOVIRUSES AND SIMILAR PHATHOGENS IN *BASIDIOMYCETES*

Long-term studies of spread of rhabdoviruses which indicated their harmfulness in different plant species under conditions of environmental factors were first discussed. Their harmfulness to different plant species under environmental conditions was shown. A comparative description of rhabdoviruses with similar pathogens of the mushrooms is carried out. Thus the main focus was on the morphology and structure of the pathogens. These data are extremely important in the study of distribution of the rhabdovirus on crops in different regions.

Key words: rhabdoviruses, plants, fungi.

For the current period of the changes in ecological balance on our Planet the viruses of different taxonomic groups acquire great harmfulness [1-6]. Therewith, the plants that are producers of food for the population are extremely sensitive to these pathogens. Edible and medicinal mushrooms (*Basidiomycetes*) of natural biocenoses and those which are grown under conditions of different levels of complexity in biotechnological processes are no exception in this situation [3, 4]. Under these conditions researchers and industrial professionals are often not aware that they are working with infectious material of plants and fungi.

The main objective of our work was to study the distribution of rhabdoviruses on the agricultural crops in different regions and to investigate their morphological and structural features, their localization in the cells of infected organisms. The results of the study of the pathogens with bacillar morphology on mushrooms are also discussed in the experiments.

Materials and Methods. An inspection of plants and fungi was performed with the use of visual approaches, electron microscopy methods, ELISA, Uhterloni precipitation reaction in agar, plants-indicators. The purification of the virus is mainly carried out by differential centrifugation with phosphate buffer (1/15 M pH 7.2) with additional purification in the sucrose density gradient. Preparations for transmission electron microscopy were prepared by the known methods. For contrasting of the preparations the researchers used 2% solution of uranilacetate and phosphoric-tungstate acid pH 6.5-7.2. Viral preparations and ultrathin sections of cells were studied at instrumental magnification in the range of 25000-40000 (EM-125 and JEM 1200 EX). Ultrathin sections were prepared from plant material and were examined by conventional methods. In this case the young leaves were selected for fixation and pouring.

For degradation of some viruses their viral suspensions were sonicated with a frequency of 22 kHz and exposition for 10-15 minutes.

With the aim of studying fungi pathogens we have developed the rapid method for making the preparations for electron microscopy. The essence of this methodological approach is that the contrasting of the object was performed directly in a cell of the pileus which was prepared for the introduction of contrasting fluid followed by applying of the homogenate on the lining and by drying the sample under vacuum or in a sterile Petri dish [4].

In some experiments the fish rhabdovirus (carp rubella virus) was used as a standard to compare the morphological and structural characteristics of the studied viruses.

All the studied environmental agrocenoses for rhabdoviruses isolation were divided according to the importance of growing the agricultural crops.

The analysis of fungi (*Basidiomycetes*) grown both in terms of biotechnological process and in natural biocenoses of Ukraine and other parts of Europe was conducted. The analysis of the following fungi was mainly carried out: mushroom bisporus (*Agaricus bisporus* (J. Lange) Imbach); oyster mushroom (*Pleurotus ostreatus* Kumm.); tinder fungus lacquered (*Reishi mushroom*) (*Ganoderma lucidum* (Curtis: Fr.) P. Karst); common puffball (*Lycoperdon perlatum* Pers.); meadow mushroom (*Agaricus campestri* Fr.); jersey cow mushroom (*Suillus bovinus,* (Pres.) Rousse).

Results and Discussion. An analysis of the study of morphological and structural properties of plant rhabdoviruses showed that pathogens of potato (Fig. 1-4), sunflower (Fig. 5 c) and buckwheat (Fig. 5 d) in its structure are similar to mammalian rabies virus and carp rubella virus. These viruses have helical symmetry type, matrix protein and supercapsid membrane. One side often is rounded and the other has "clipped" ribbed section. The agents differ from wheat, sugar beet, hop rhabdovirus in size and other parameters (Fig. 6 a). It is important that sunflower rhabdovirus can affect plants together with bacilliform pathogen – Lucerne mosaic virus (*Alfamovirus*), polymorphic tospovirus (*Tospovirus*) and spherical virus – cucumber mosaic virus (*Cucumovirus*). It is noted that bacilliform sunflower virus that contaminates also soybean and alfalfa (lucerne) is not serologically relative to the pathogen of meadow mushroom. The meadow mushroom and oyster mushroom are affected with the oblong bacilliform virus (Fig. 6 b) which is found in *Basidiomycetes* with other pathogens.

Table 1.

Analysis of the distribution and morphological characteristics of plant rhabdoviruses and similar pathogens in fungi

Object of affection	Symptoms	% of affection	Shapes	Notes
Potato	Curliness and chlorosis	9,0-35,0	53×165 75×239-278	In all agrocenosis are present 2 groups of virions
Raspberries	Chlorotic mosaic	3,0-15,0	65×175 70-90×345	In the south regions are allocated 2 groups of virions
Buckwheat	Chlorosis, plants deformation and shallowness of the leaves	1,5-62,3	75×230-270	The different range of affection on the plant varieties
Wheat	Mosaic	1,5-18,0	65×168 62×240-330	Is determined in the south and northern regions, 2 groups of a virus
Hop	Mosaic, deformation of the leaves	0,5-5,0	53×178	Affects separate plant varieties
Sunflower	Deformation	6,0-12,0	80×175-180	Rhabdoviruses
Sunflower	Mosaic	8,0-29,0	30×90	Bacilliform virus – Lucerne mosaic virus (*Alfamovirus*)
Mushroom *A. bisporus*	Black and brown spots	2,0-5,5	24×45	Bacilliform virus. Not serologically relative to Lucerne mosaic virus (*Alfalfa mosaic virus*)
Oyster mushroom	Brown spots	3,0-11,5	24×45	Bacilliform virus
Mushroom *A. bisporus***	spots of different colors, deformation of fruiting bodies	2,5-6,0	20×108	Bacilliform pathogen, is different from rhabdoviruses in morphology, is registered for the first time
Carp (standard)	Rubella (red spots, necrosis)	3,0-26,0	80-195	Rhabdovirus – in the artificial ponds

*In our research in the European region rhabdoviruses affect about 60 species of plants.

**Affection under the terms of biotechnological processes and natural biocenoses.

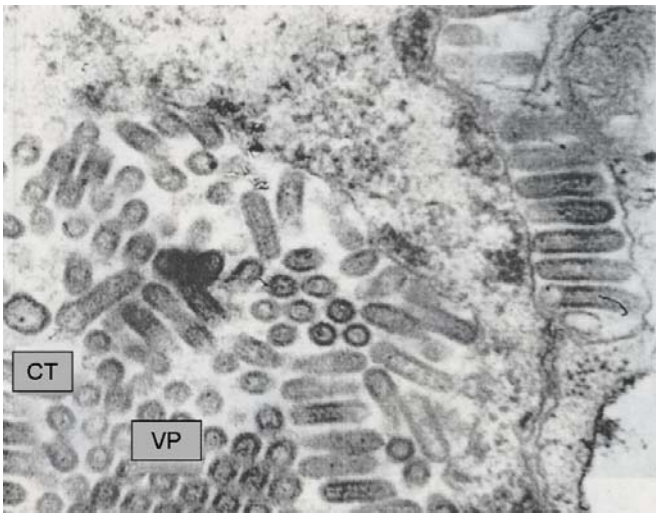


Fig. 1. Ultrathin sections of tobacco *Nicotiana rustica* L., leaf infected CPDV. VP – virus particles, CT – mace-like thickening (×40000)

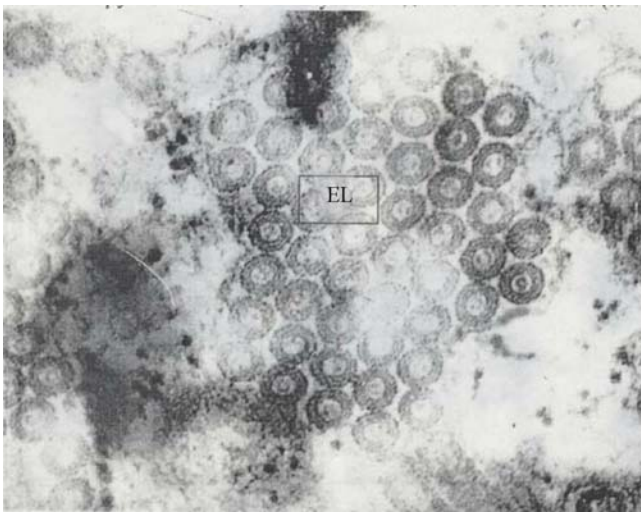


Fig. 2. CPDV in leaves of tobacco *N.rustica* L. ultrathin cross section. EL – electron-densitive layer (× 94000)

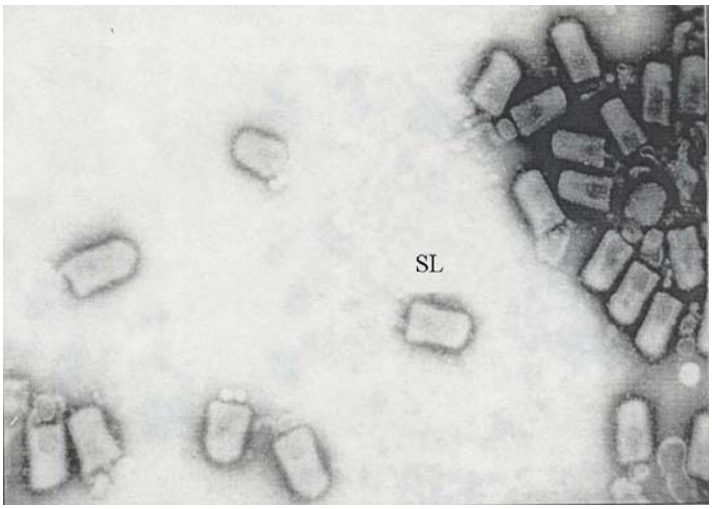


Fig. 3. The purified preparation CPDV: SL – grainy layer (× 95000)

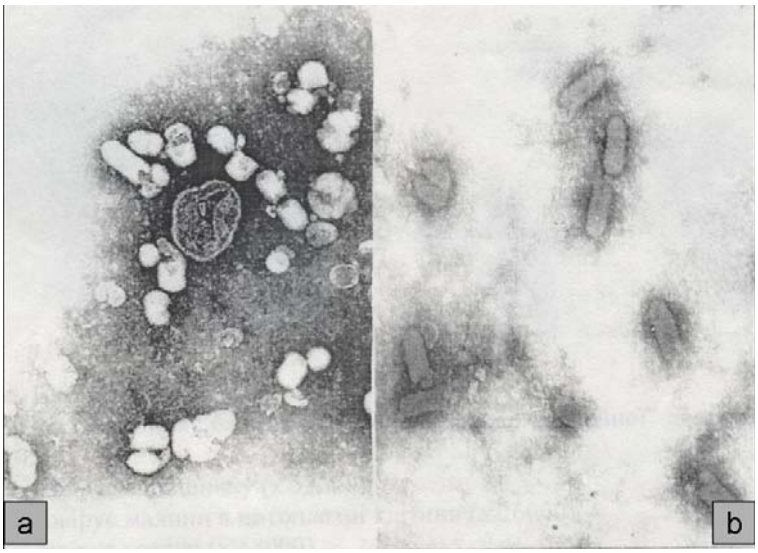
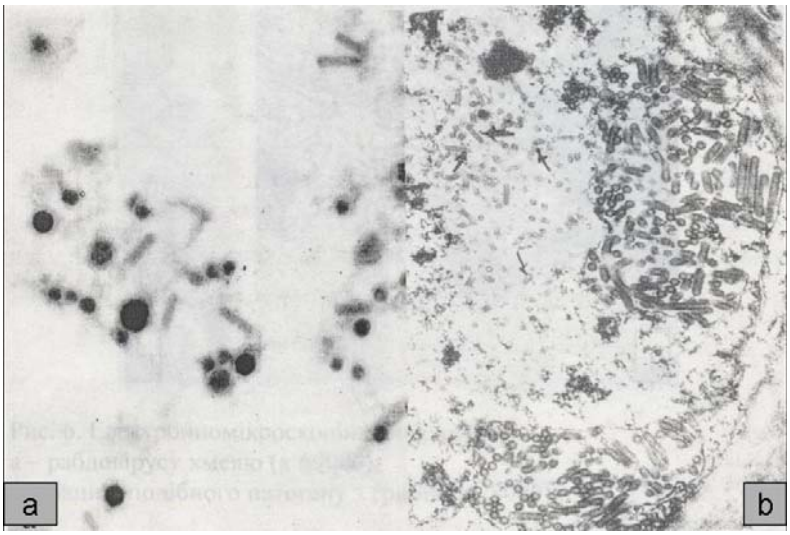


Fig. 4. Preparation CPDV processed with ultrasound frequency of 22 kHz for 15 minutes: a – treated, b – control (×56000)



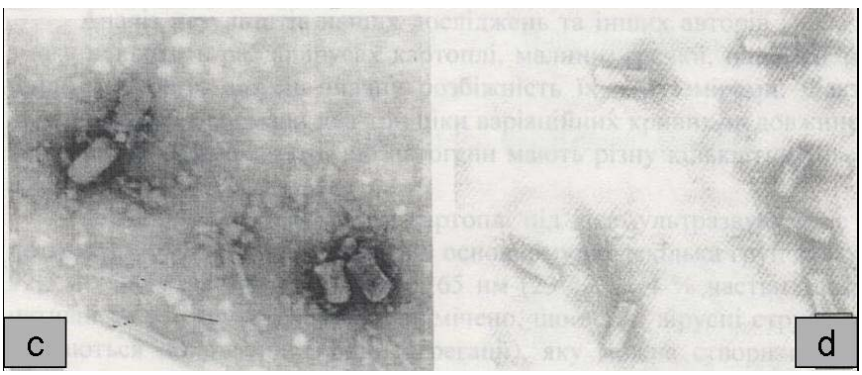


Fig. 5. Electron microscope images of viruses:
a – Sunflower viruses – bacilliform and those of "spherical" shape with two sizes (× 28000),
b – Raspberries rhabdovirus in the cell cytoplasm (× 26000) (photo of Taranuho M.P.),
c – Sunflower rhabdovirus (×53000), d – Buckwheat rhabdovirus (× 48000)

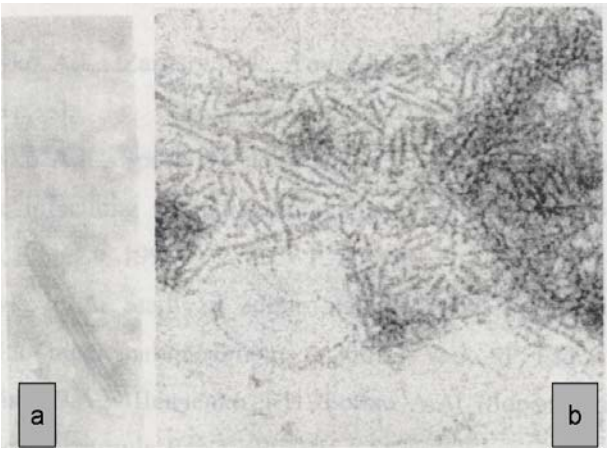


Fig. 6. Electron microscope images:
a) – hop rhabdovirus (×69000); b) – bacilliform pathogen from fungi *A. bisporus* (×28000).

The analysis of our and other authors' results [1, 2, 5, 6] makes it possible to detect a considerable diversity in size in potatoes, raspberries, buckwheat, wheat rhabdoviruses and some other similar viruses. In fact, these virions often had two or three peaks of the variation curves in length. Therefore, this makes it possible to predict that pathogens have different number of nucleotides in their genome.

It is important that virions of potato rhabdovirus under the action of ultrasound (Fig. 4 a) destructed into several groups according to size: 92.5 nm (45%), 140 nm (28%), 65 nm (23%) and 4% particles which have saved their nativity and structure. We observed that it is difficult for these viral structures to recollect (aggregate) under the action of various factors [1]: change of ionic strength, radiation, magnetic fields under the *in vitro* conditions.

Based on our recent analyses of agrocenosis we are able to draw some conclusions from the study. In the current period we have observed 60 species of plants that are affected with rhabdoviruses. Similar pathogens, but not identified as rhabdoviral ones, are isolated from certain types of mushrooms. Recently, the area of the spread of viruses with negative genomic RNA, including rhabdoviruses, has a "shift" of their spread from the south to the northern regions. A slight increase in percentage of the plants affected with rhabdoviruses and similar pathogens in fungi are marked in the regions with high radiation background.

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ПОШИРЕННЯ ТА МОРФОЛОГО-СТРУКТУРНІ ВЛАСТИВОСТІ РАБДОВІРУСІВ РОСЛИН ТА ПОДІБНИХ ПАТОГЕНІВ У *BASIDIOMYCETES*

Р е з ю м е

Подаються матеріали багаторічних досліджень поширення рабдовірусів, показана їх шкодочинність на різних видах рослин за умов факторів довкілля. Проводиться порівняльна характеристика їх з подібними патогенами на шапинкових грибах. При цьому головна увага була зосереджена на морфології та структурі патогенів.

Ключові слова: рабдовіруси, рослини, гриби.

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РАСПРОСТРАНЕНИЕ И МОРФОЛОГО-СТРУКТУРНЫЕ СВОЙСТВА РАБДОВИРУСОВ РАСТЕНИЙ И ПОХОЖИХ ПАТОГЕНОВ В *BASIDIOMYCETES*

Р е з ю м е

Представлены материалы многолетних исследований распространения рабдовирусов, показана их вредоносность на разных видах растений в условиях факторов окружающей среды. Проводится сравнительная характеристика их с подобными патогенами на шляпочных грибах. При этом главное внимание было сосредоточено на морфологии и структуре патогенов.

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К л ю ч е в ы е с л о в а: рабдовирусы, растения, грибы.

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