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THE SENSITIVITY OF BUCKWHEAT PLANTS TO PATHOGENS UNDER CONDITIONS OF MIXED VIRAL AND MYCOPLASMAL INFECTION

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Abstract. *It was investigated, that mixed infection of buckwheat distributed in various ecological regions of Ukraine, the pathogens that belong to different taxonomic groups have different and peculiar morphological and structural properties. First was shown that buckwheat is affected by buckwheat burn virus (BBV) together with Mycoplasma. Mixed infection leads to proliferation of sepals, clarification of the flowers petals, reduction and greening of petals, hypoplasia or hypodevelopment of stamens and pistils: all of this can lead to partial or complete sterility.*

Keywords*: buckwheat, buckwheat burn virus, Mycoplasma, mixed infection*

It is known that phytopathogens are widespread in nature and in conditions conducive to their development cause significant damage to crops. The study of buckwheat diseases in scientific institutions in different regions of this crop growing does not lose its relevance in our time, because these studies are an integral

component of obtaining high and stable yields. It is known that rhabdovirus infection affecting different varieties of buckwheat and damage both plants and seeds [1]. Research of rhabdoviruses, that are malicious for buckwheat, in particular the buckwheat burn virus (BBV) as well as various bacterial and viral diseases carried out in different institutes, but in recent years more attention has been paid to the study of mixed infections caused by pathogens of different taxonomic groups [2, 3, 4].

Mycoplasma disease of plants is widespread in the world. Nowadays it is known a large number of phytoplasmas that relate to more than 30 groups and are pathogens that strike more than 1000 species of plants. It was thought for a long time that viruses cause these diseases, although no pathogens were isolated or visualized. In 1967 Japanese scientists Y.M. Doi [5] and others in ultrathin sections of plant phloem of aster with jaundice revealed structures morphologically similar to animal pathogens – mycoplasmas. These new plant pathogens were identified as mycoplasma-liked organisms (MLO) and classified as *Mollicutes,* bacteria without cell walls [6].

In the early 1990s it has been shown on the base of phylogenetic analysis of DNA sequences of these organisms that they are a great monophyletic (derived from a single ancestor) group within the class *Mollicutes*, so they were named "phytoplasmas". Other members of this class were mycoplasma, aholeplasma and spiroplasma, they are close to such bacteria as *Bacillus, Clostridium, Streptococcus*. Current trivial name "phytoplasma" was officially adopted in 1994 at the 10^{th} Congress of the International Organization of mycoplasmology, replacing the term "mycoplasma-liked organisms" [7]. Currently phytoplasma isolated in a separate genus "*Candidates Phytoplasma*". Category *Candidates* was used to describe organisms that cannot be grown *in vitro*. The first 20 *Candidates* species were assigned to this genus on the basis of identity of the nucleotide sequence of DNA 16S rRNA gene, that was not less than 97,5% [8]. These pathogens are well identified in the transmissive and scanning electron microscopy. Phytoplasma of wheat and hops using homogenates and ultra-thin sections of cells were studied [9].

Phytoplasma of buckwheat belongs to the poorly understood disease and was first described by Sidorova S.F. in 1965. It was made an assumption about disease of buckwheat plants, but there was no visualization of the pathogen [10].

The aim of the work was to determine the prevalence of mixed infections of buckwheat caused by pathogens of different taxonomic groups, to investigate the existence of mono BBV and mixed BBV and Mycoplasma infection, and also to conduct visualization of the causative agent of buckwheat mycoplasmosis.

Research methods. Plants of tobacco (*Nicotiana rustica*) were used for the accumulation of the BBV. The research was carried out in vegetation compartments of the D.K. Zabolotny Institute of microbiology and virology of NAS of Ukraine and experimental fields of Podolsky State Agrarian Technical University.

Conventional detection methods of pathogen investigation were used to study the mixed infection in buckwheat plants (mycoplasma, fungi, viruses, bacteria) [11]. To identify buckwheat phytoplasma investigation of crops and stationary experiments of buckwheat were conducted. Harmfulness of buckwheat mycoplasmosis was determined by comparing the elements of morphobiometrical indicators of sick and healthy plants, and in particular: general condition of buckwheat habitus, plant height (cm); branching (pc); number of grains (pc); seed weight (g).

In order to prove the pathogenicity of the buckwheat phytoplasma causative agent visual diagnostic, method of diseased plants vaccinations on the plant-indicator (pink periwinkle (*Catharanthus roseus* G. Don)) was used. Infection was carried out mechanically by vaccinations into split. The observations were carried out during one month. Electron microscope study was also used, as different methods of microscope studies were previously used to identify and confirm the presence of phytoplasma in the plant and to study the localization of the pathogen [12].

Preparations for electron microscopy were prepared by the conventional method. Formvar film, which used 0.2% solution of polyvinyl formaldehyde in chloroform were prepared. Next slide were immersed in a formvar solution for 10s, removed and dried a little for 40s. The formed film was clipped with blade; glass was dipped in distilled water at a 45 º angle. Grids were superimposed to the film and

removed on a clean glass slide. 2% uranyl acetate in distilled water, pH 7.0 was used to contrast the preparation. A drop of drug was deposited into the film, covered by filter paper and dried for 1 min in the air, and then a drop of uranyl acetate was applied and left for 60 s. Preparations were investigated using electron microscope JEM-100 mark ("JEOL", Japan) with instrumental increase in 5-60 thousand times and acceleration voltage of 80 kV [12]. Fluorescent microscopy was used, the the preparations were prepared on the basis of fixation in 1.5 % trichloracetic acid and staining with a solution of fluorochrome with acridine orange (1:10000) [13].

Results and discussion. Years of research make it possible to state that the buckwheat plants often have a complex infection, which is caused by viruses, bacteria and microscopic fungi. Modern methodological approaches using – ELISA, electron and fluorescent microscopy, plants – indicators, selection of culture media for bacteria and microscopic fungi gave an opportunity, as shown on the diagram (Fig. 1), to conclude that BBV rhabdovirus is able to destroy this plant in the various infection combinations.

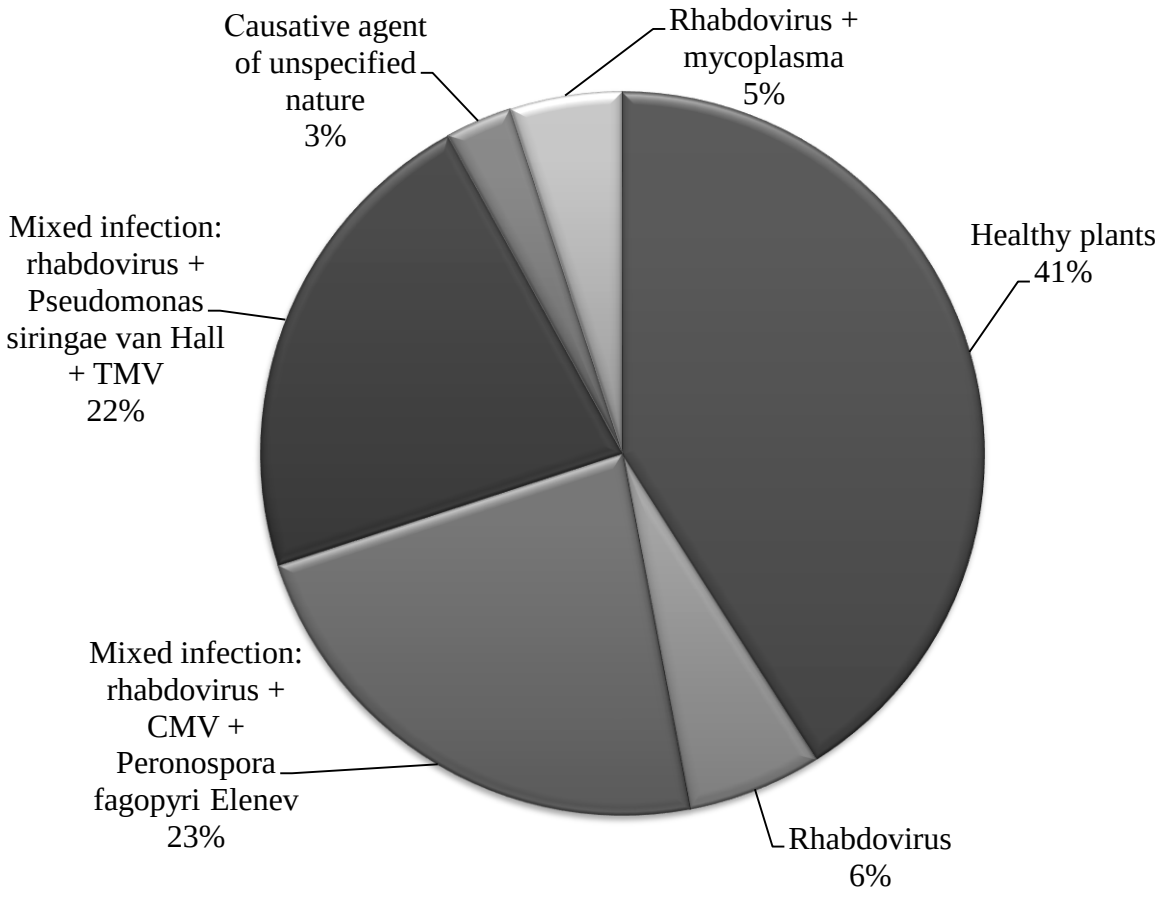

Fig. 1. Chart of BBV distribution in cases of joint infection with other pathogens

Applying modern methods for the study of pathogens it was noted that "spherical" virus of buckwheat should be attributed to CMV (Fig. 2). As shown by electron microscopic studies of CMV, which was diagnosed by the method of plants – indicators on *Chenopodium album*, by ELISA and Ouchterlony reaction, had different particle size of the isometric form. Given that this pathogen is based on few fragments of RNA, its protein coat has original layout. As shown by the variation curve of virions, native particles are often meeting in the size of 25-38 nm (250 – 380 A°). In the field of view of the electron microscope the virus sometimes also met in the size of 50-80 nm (500-800 A°), indicating spontaneous aggregation, and the particles in the size of 5-20 nm (50-200 A°) were "isolated" protein fragments of the virus. CMV is rare for buckwheat, but occurs in plants in combination with TMV and rhabdoviruses, inducing much severe symptoms of leaf roll.

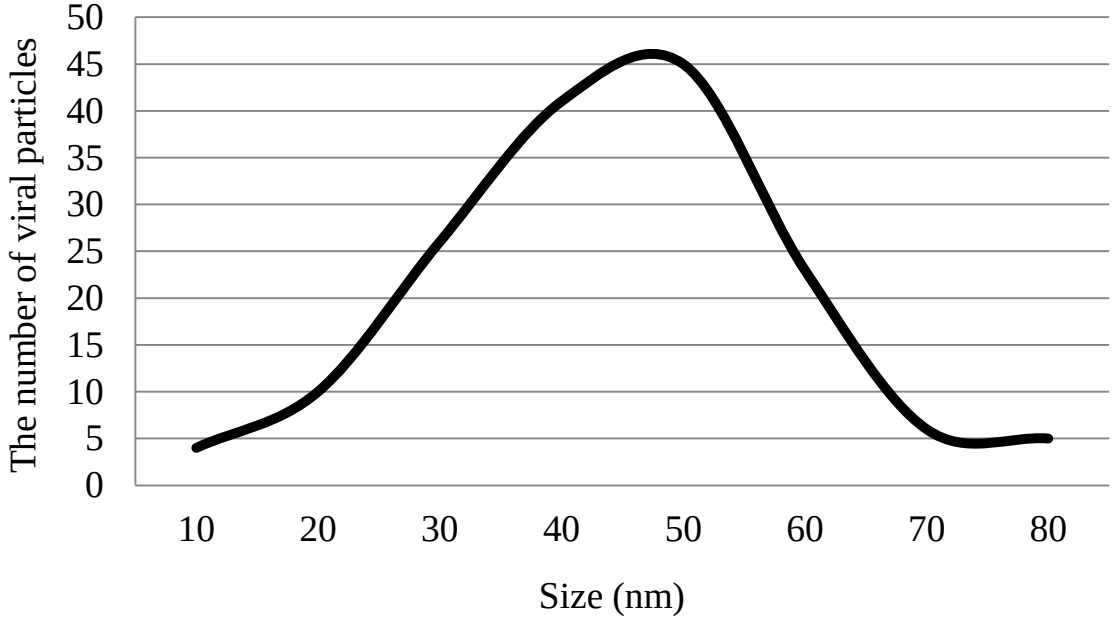

Fig. 2 Variation curve of the size of the CMV, which infects plants of buckwheat in the Forest-steppe zone of Ukraine (the results of electron microscopy).

An isolate from plants of buckwheat caused small necroses on plants (*Datura Stramonium*) and had a serological affinity with TMV in the Ouchterlony reaction. Most often its dimensions by variational calculation had dimensions 275-315 nm (2750 – 3150 A°), indicating its morphological affinity with the TMV.

According to literature data, there are over 200 known species of plants from 59 families that are affected by mycoplasma. Phytoplasmas are mainly spread in areas with temperate and warm climate, which has a positive effect on the existence of insects with piercing-sucking mouthparts – vectors of phytoplasma. Phytoplasma carried from plant to plant by insects of the *Hemiptera* class, which feed on the juice of cells phloem. *Cicadelloidea*, *Fulgoroidea* and *Psylloidea* insect families are the most commonly found among them. Some insect species can spread several types of phytoplasma, but for many phytoplasmas vectors are still not defined [6]. The following types of cicadas are noted in Russia as phytoplasmas vectors: *Hyalesthes obsoletus*, *Macrosteles laevis*, *Phylaenus spumarius*, *Cicadella viridis*, *Pentastridius leporinus*, *Aphrodes bicinctus* [7, 8].

The variety of symptoms most often associated with damage of the plants by phytoplasma in combination with other pathogens (viruses, bacteria), as well as with the effect of abiotic factors. So, the symptoms of a disease called jaundice, are similar in plants infected with viruses and phytoplasma. Symptoms appearance on plants depends on the pathogen concentration in conductive tissues, destructive changes in cellular structures and disturbance of the balance of plant hormones. During the primary infection of the plants the appearance of symptoms may occur through one or even several months, depending on species and varieties of cultivated plants, weather conditions and other factors.

Under mycoplasmas damage of buckwheat plant disturbance of regulation processes of plants, changing habit of plants, reduced leaf size, delayed plant growth, morphological changes in generative organs, leading to infertility of plants, are observed. Petals of flowers take the form of leaves, flowering stops. An increase of pistils and stamens, greening flowers are observed (Fig. 3). Forms of symptoms can be conditionally divided into several types: jaundice, "witches brooms", dwarfism, wilting (wilt) [9]. For the reproductive organs of plants, that are infected with phytoplasma, typical symptoms are: proliferation of sepals, clarification of the flowers petals (phyllode), reduction and greening of petals (virescence), hypoplasia

or hypodevelopment of stamens and pistils: all of this can lead to partial or complete sterility of plants [8, 10, 11, 12].

In order to prove the pathogenicity artificial infection of indicator plants *Catarantus roseus*, using inoculation method, was conducted. Observations of infected plant was conducted after inoculation of cuttings sick of infected buckwheat plants on the plant of periwinkle pink.

As a result it was found the deformation of flowers, greening of *Catarantus roseus* petals (Fig. 4).


a)


b)

Fig. 3. Mycoplasmosis of buckwheat plants: a) healthy plant; b) a plant affected by Mycoplasma.


a)


b)

Fig. 4. Mycoplasmosis of Catarantus roseus plants: a) healthy plant; b) a plant affected by Mycoplasma by artificial method.

The result was obtained by electron microscopic image of Mycoplasma (Fig. 5). Phytoplasma, like viruses, is characterized by small size (from 200 to 1000 nm) and has a genome size from 530 to 1350 kb with low content of GC pairs in DNA. It is the smallest known genome among organisms with the ability of self-replication [13, 14, 15]. Unlike bacteria, phytoplasma do not have a cell wall and surrounded by a trilaminar cytoplasmic membrane. Phytoplasma is pleomorphic, spherical or filamentous in structure, the diameter is less than 1 μm that can be observed in the phloem of infected plants under electron microscope.

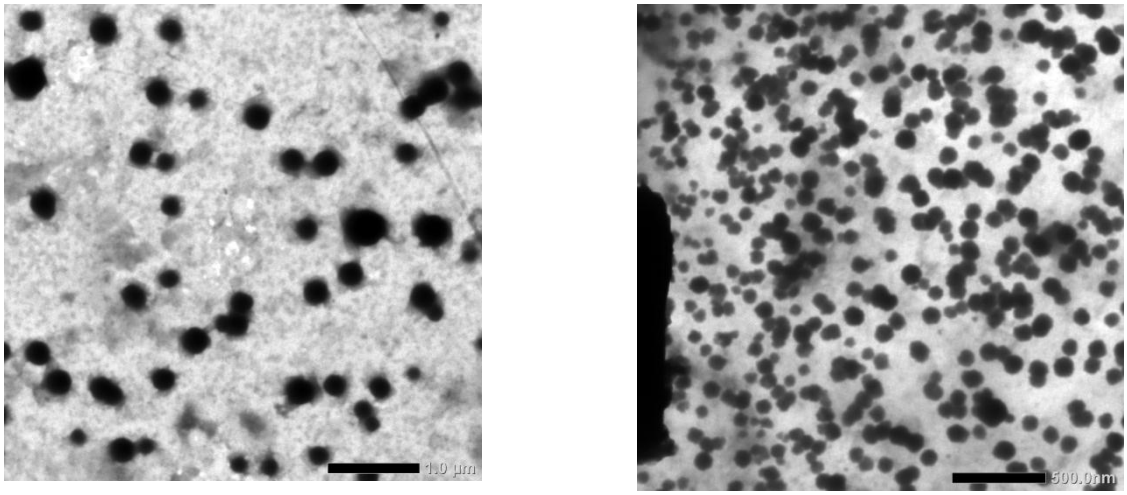

Fig. 5. Electron microscope images of phytoplasma, prepared using contrasting with uranyl acetate

In winter phytoplasma can be stored in the body of the insects and in different perennial plants, which serve as a constant source of infection. Vegetative organs of annual plants (tubers) sometimes can also preserve the infection. Phytoplasma associated with natural foci, which always have wild plants [9]. The circulation of the pathogen happens by using insect vectors; cultivated plants are affected by them when growing in the nidus infection area. Among plant which reserves phytoplasma are many wild species, the most common are bindweed, endive, sow sonchus [13].

In analysis of infected buckwheat plants were determined and identified phytoplasma (mycoplasma) (Fig. 5), which facilitates the future fight against this pathogen. Protective actions against mycoplasma in situation of complete absence of

tolerant varieties are preventive. That is, the use of healthy planting material, struggle against insects that spread infection, spatial isolation of foci of infection, destruction of infected plants etc.

Conclusions. Thus it should be noted that buckwheat is affected by BBV together with other pathogens, particularly *Mycoplasma*. Mixed infection of buckwheat plants distributed in various ecological regions of Ukraine, the pathogens that trigger belong to different taxonomic groups have different and peculiar morphological and structural properties. It was shown that a mixed infection (BBV + mycoplasmosis) of buckwheat leads to proliferation of sepals, clarification of the flowers petals, reduction and greening of petals (virescence), hypoplasia or hypodevelopment of stamens and pistils: all of this can lead to partial or complete sterility, therefore, further study of mixed infections of agricultural plants and the search for drugs to prevent infection is an important goal of applied research.

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ЧУТЛИВІСТЬ РОСЛИН ГРЕЧКИ ДО ПАТОГЕНІВ ЗА УМОВ ЗМІШАНОЇ ВІРУСНОЇ ТА МІКОПЛАЗМЕННОЇ ІНФЕКЦІЇ

О. А. Демченко, В. К. Шевчук, Л. В. Юзвенко, О. А. Бойко, Л. П. Бабенко, Л. М. Лазаренко, А. В. Калініченко, А. Л. Бойко

Анотація. *Досліджено, що змішані інфекції гречки поширені в різних екологічних регіонах України. Інфікуючі агенти, що належать до різних таксономічних груп, мають своєрідні морфологічні та структурні властивості. Вперше було показано, що вірус опіку гречки (ВОГ) зустрічається у рослинах в комплексі із мікоплазмою. Змішана інфекція призводить до розростання чашолистків, посвітління пелюсток квітів, укорочення та позеленіння пелюсток, гіпоплазія чи гіперплазія тичинок і маточок: все це може призвести до часткової або повної стерильності рослини.*

Ключові слова: *гречка, вірус опіку гречки, мікоплазма, змішана інфекція*

ЧУВСТВИТЕЛЬНОСТЬ РАСТЕНИЙ ГРЕЧКИ К ПАТОГЕНАМ В УСЛОВИЯХ СМЕШАННОЙ ВИРУСНОЙ И МИКОПЛАЗМЕННОЙ ИНФЕКЦИИ

А. А. Демченко, В. К. Шевчук, Л. В. Юзвенко, О. А. Бойко, Л. П. Бабенко, Л. Н. Лазаренко, А. В. Калиниченко, А. Л. Бойко

Аннотация. *Доказано, что смешанные инфекции гречки распространены в различных экологических регионах Украины. Инфицирующие агенты, принадлежащие к различным таксономическим группам, имеют своеобразные морфологические и структурные свойства. Впервые было показано, что вирус ожога гречки (ВОГ) встречается в растениях в комплексе с микоплазмой. Смешанная инфекция приводит к разрастанию чашелистиков, осветлению лепестков цветов, укорочению и позеленению лепестков, гипоплазии или гиперплазии тычинок и пестиков: все это может привести к частичной или полной стерильности растения.*

Ключевые слова: *гречка, вирус ожога гречки, микоплазма, смешанная инфекция*