

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.

UDC 632.38

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Y. Kalashyan, PhD, A. Chernets, PhD, V. Lukitsa, scientist,
L. Prodaniuk, scientist, N. Kalashyan, scientist

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 Secoviridae, 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 transmitted mechanically 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 polyclonal 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 phytosanitary 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*.

Results 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 AI 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

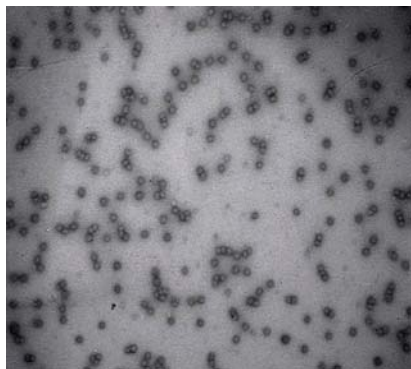


Fig. 1. CLRV particles in the product prepared

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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.

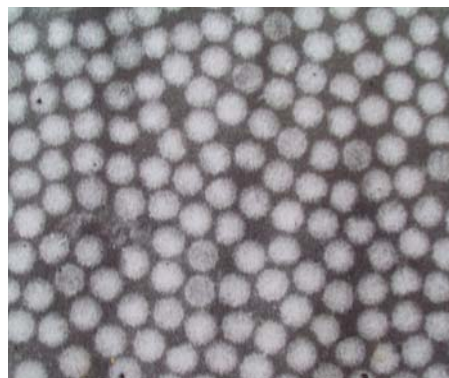


Fig. 2. Electron micrograph of purified preparation CLRV. from axillary buds walnut by ISEM

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