UDC 578.3+582.974

S. Pavlova, PhD stud.,
Experimental station of grapes and fruit crops quarantine
Institute of Plant Protection National academy of agrarian sciences of Ukraine, Odessa, Ukraine,
O. Stakhurska, PhD stud., I. Budzanivska, DSc. Biol, Prof.,
V. Polischuk, DSc., Biol, Prof.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

GISTECHNOLOGY FOR THE MONITORING OF SHARKA DISEASE IN THE ODESSA REGION

Plant virus causes many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world, and consequently, agronomists and plant pathologists have devoted considerable effort toward controlling virus diseases. One the most important virus on many Prunus species, causing great economic losses is Plum pox virus (PPV),casual agent of Sharka disease. Since its discovery, Sharka has been considered as a calamity in stone orchards. The virus has been detected in almost every country where any significant commercial stone fruit cultivation occurs [1]. The virus is entered into the list of regulated pests common in Ukraine. In Ukraine, the total area of PPV spread totals 4013,2764 ha. In Odessa region, 18.5 ha districts are in PPV quarantine. Six hotbeds of PPV infection totalling 28 hectares were found in Odessa region. For the first time in Odessa region, PPV was found on cherry trees. Peach and plum trees are hit equally. In this study, we use geographic information systems technology to identify potential locations in a Odessa region for controlling the spread of Plum pox virus. To our knowledge, this is the first attempt to employ GIS technology for controlling plant diseases in Ukraine. Provided it is properly maintained, the geospatial data, and the ability to generate detailed maps with it, is key to the success of PPV containment. Information management will be a key to improving for controlling the spread of Plum pox virus.

Keywords: Plumpoxpotyvirus, Sharkadisease, plantdiseasemonitoring, hotbeds, GIS technology.

Introduction. Plant viruses are group of pathogens that cause important loses in different fruit croups and they have great economic importance. This is especially true for diseases associated with vectored pathogens such as PPV, an aphid transmitted virus.

Plum pox virus(PPV) is one of the most important pathogens causing destructive viral diseases in stone fruit trees such as peach, plum, apricot and cherry. Sharka was first reported in plum trees in Bulgaria in 1917–1918 and was recognized as a viral disease by Atanasoff (1932) [2]. Since then, the virus has spread progressively to most of Europe, around the Mediterranean basin and the Near and Middle East. It has also spread to South and North America and Asia [3].

To understand the scope of PPV infection, regular surveys are necessary to determine the presence of the disease. PPV symptoms may appear on leaves, shoots, bark, petals, fruits and even stones (Fig. 1) They are usually distinct on leaves early in the growing season and include mild light-green discoloration, chlorotic spots, bands or rings, vein clearing or yellowing and leaf deformation. Flower symptoms can occur on petals (discoloration) of some cultivars. Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape, and may develop brown or necrotic areas under the discolouredrings[4].

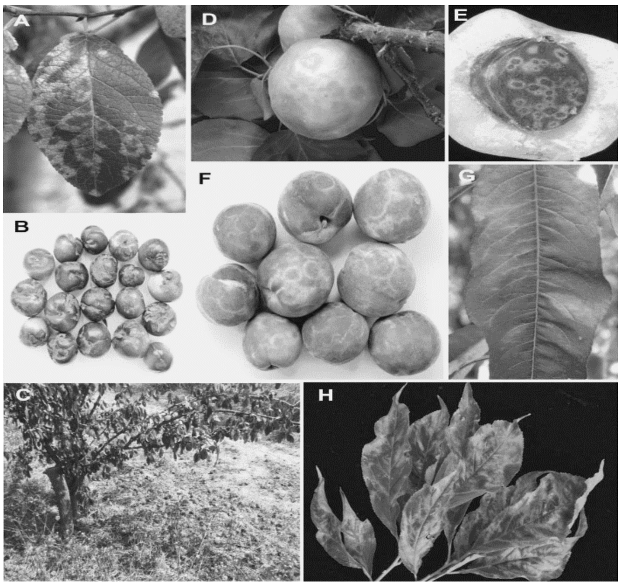

Figure. 1. Typical symptoms induced by *Plum pox virus* on a domestic plum leaf (A), domestic plum fruits (B), premature domestic plum fruit drop (C), an apricot fruit (D), an apricot stone (E), peach fruits (F), a peach leaf (G) and Japanese plum leaves (H) [5]

Visual inspection of trees is not a reliable detection method because of the variability in the expression of symptoms and many years may pass before an infected host manifests symptoms. To avoid PPV spread over long distances by the movement of plant material, reliable detection methods are needed for the accurate detection of the virus in symptomless nursery plants and propagative material. Two official and validated international protocols for the detection and characterization of PPV strains have been developed [6,7].

We had used enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) remain the preferred lab tests to screen for and confirm the presence of PPV in collected samples.

The climate in Odessa region is influenced by the vicinity of the Black Sea and fluctuates from year to year but typically is characterized as having higher summer temperatures, warmer winters and relatively rare rainfalls. Here, warm spring normally begins in mid-April followed by dry and hot summer months. Therefore, monitoring of stone fruit orchards in Odessa region is conducted in spring and autumn, when the symptoms are more obvious and plant tissues contain more virus.

This work was aimed at conducting Sharka disease monitoring of planted stone fruit crops in Odessa region using traditional visual, serological and molecular methods, and subsequent coupling of obtained data on virus spread with geographical information system (GIS) technology for further use by plant quarantine services, virologists and producers

Materials and methods. For the 3 years (2014-2016), plant disease monitoring of 185 hectares of stone fruit orchards in Odessa region was conducted, including 68 hectares in Belyaev and 43 hectares in Ovidiopol districts confirmed as the areas under PPV quarantine.

Visual diagnostics of PPV-specific symptoms was followed by serological analysis in the laboratory. Collected samples were tested for PPV by double antibody sandwich enzyme-linkedimmunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977) [8], using specific polyclonal antibodies purchased from Bioreba (Switzerland) following the manufacturer's recommendations. RT-PCR was mainly used as a confirmatory test for selected samples using probes specific to PPV-D and PPV-M strains.

Portable navigation system Garmin GPS 60 was used for accurate recording of geographical coordinates of the sample collection spots and locations where the Sharka symptoms were evident.

Results and discussion. In 2014-2015, for the first time in Odessa region, PPV was found on cherry trees (Fig. 2) in Khlebodarskoye village of Ovidiopol district (area 2,5 ha). Strain PPV M identified in 2015. Prior to this, surveys of cherry orchards have not been conducted due to the fact the PPV has never been detected there.

Figure. 2. Symptoms of PPV on cherry trees of Ovidiopol district, Odessa region

Also, new PPV hotbeds were found in Doslidnoe village (plum orchard, area 2,3 ha) and in Mirnoje village (peach orchard, area of 5 hectares; the total area of 80 ha) in Belyaev district. As of today, we have confirmed six hotbeds of Sharka disease totaling to 28 hectares in Odessa region(Fig. 3).

Therefore, the results of 3-year PPV monitoring in the affected areas of Odessa region revealed new hotbeds of virus spread.

Figure. 3. Pilot GIS-aided map showing plotted quarantine areas for PPV in Odessa region

In turn, these results underline the need for a) regular screening of stone fruit orchards (commercial, suspect/endangered, and quarantined ones as minimum) for PPV using EPPO-approved serological and/or molecular techniques, and b) a database system combining virus monitoring, crop, vector, climate and map data.

Plant disease management practices can be improved by putting epidemiological information in the same format as other plant pathologistsinformation using a geographic information system (GIS). The integration of GIS provides a mean for the refined analysis of traditional and contemporary biological/ecological information on plant diseases. GIS has been applied in agriculture for the spatial analysis of insect pests, weed infestations, and plant diseases, which is particularly true for Sharka disease[8].

To our knowledge, this is the first attempt to employ GIS technology for controlling plant diseases in Ukraine. Provided it is properly maintained, the geospatial data, and the ability to generate detailed maps with it, is key to the success of PPV containment allowing for the ease of navigation to known sites each survey season but, most importantly, for decision making regarding the:

1) Efficient development of buffer zones and quarantine boundaries when a PPV-positive tree was identified;

2) Calculation of acreage to predict number of samples and prepare sample labels before field survey;

3) Calculations and verification of acreage for destruction of diseased trees;

Another advantage of interest to Odessa region is that such approach may also combine climate and vector data – both are of importance for PPV. Altogether, use of GIS technology for controlling PPV spread allows better planning of activities, personnel and limited funds

Conclusions.

1. For today six pestholes of PPV, total area of twenty-eight hectares, is found in Odessa region. Most of infected trees have peaches and plums.

2. For the first time in Odessa region, PPV was found on cherry trees: all the pestholes in plum and peach orchards the Odessa region identified strain PPV – D; in the pesthole in sweet cherry orchard the Odessa region identified strain PPV – M.

3. Modern technology phytosanitary monitoring of viral diseases, including PPV should be based on the integrated use of methods that allow you to get information about the spread of viral diseases in Ukraine and to determine the general trend of development of the pathological process, to inform the selection institutions of new invasive races of pathogens to develop reliable forecast for the development of the disease.

References

1. James D. Perspectives on strategies for controlling the spread of Plum poxvirus, causalagent of sharka/plum pox disease // Acta Horticulturae – 2016.-in press.

2. Atanasoff D. Plumpox. A new virus disease //Annals of the University of Sofia, Faculty of Agriculture and Silviculture. – 1932. – V.11. – P.49–69.

3. Barba M., Hadidi A., CandresseT.,Cambra M. Plum pox virus. In: Virus and Virus-like Disease of Pome and Stone Fruits (Hadidi A., Barba M., Candresse T., Jelkmann W., eds) St. Paul, Minnesota: APS Press. – 2011. – P.185–197.

4. Glasa M., Palkovics L., Komínek P., Labonne G., Pittnerova S., Kudela O., Candresse T., Šubr Z. Geographically and temporally distant natural recombinant isolates of Plum pox virus (PPV) are genetically very similar and form a unique PPV subgroup // J. Gen. Virol. – 2004. – V.85. – P. 2671–2681.

5. Cambra M., Boscia D., Gil M., Bertolini E.,Olmos A. Immunology and immunological as say sapplied to the detection, diagnosis and control off ruit tree viruses. In: Virusand Virus-like Disease of Home and Stone Fruits (Hadidi A., Barba M., Candresse T., Jelkmann W., eds) // St. Paul, Minnesota:APS Press. – 2011. – P. 303–313.

6. EPPO. Diagnostic protocol for regulated pests. Plumpoxpotyvirus // EPPO Bull. – 2004. – V.34. – P.247–256.

7. IPPC-FAO. International standards for phytosanitary measures: diagnostic protocols: Plum pox virus // ISPM 27, Annex 2 (DP2). – 2012.

8. Clark M.F., Adams A.M. Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses // The Journal of General Virology. – 1977. – V.34. – P.475–483.

9. Nelson M.R., Felix-Gastelum R., Orum T.V., Stowell L.J., Myers D.E. Geographic in for mation systems and geostatistics in the design and validation of regional plant virus management programs // Phytopathology. – 1994. – V.84. – P.898-905.

References (Scopus)

1. James D. Perspectives on strategies for controlling the spreadof Plum pox virus, causal agent of sharka/plum pox disease. Acta Horticulturae. 2016; inpress.

2. Atanasoff D. Plumpox. A new virus disease. Annals of the University of Sofia, Faculty of Agriculture and Silviculture. 1932; 11:49–69.

3. Barba M, Hadidi A, Candresse T,Cambra M. Plum pox virus. In: Virus and Virus-like Disease of Pome and Stone Fruits (Hadidi A, Barba, Candresse T, Jelkmann W, eds) St. Paul, Minnesota: APS Press. 2011; 185–197.

4. Glasa M, Palkovics L, Komínek P, Labonne G, Pittnerova S, Kudela O, Candresse T, Šubr Z. Geographically and temporally distant natural recombinant isolates of Plum pox virus (PPV) are genetically very similar and form a unique PPV subgroup. J. Gen. Virol. 2004; 85:2671–2681.

5. Cambra M, Boscia D, Gil M, Bertolini E, Olmos A. Immunology and immunological as say sapplied to the detection, diagnosis and control off ruit tree viruses. In: Virusand Virus-like Disease of Home and Stone Fruits (Hadidi A, Barba M, Candresse T, Jelkmann W,eds). St. Paul, Minnesota:APS Press. 2011; 303–313.

6. EPPO. Diagnostic protocol for regulated pests. Plum pox poty virus. EPPO Bull. 2004; 34:247–256.

7. IPPC-FAO. International standards for phyto sanitary measures: diagnostic protocols: Plum pox virus. ISPM 27, Annex 2 (DP2). 2012.

8.Clark MF, Adams AM. Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. The Journal of General Virology.1977; 34:475–483.

9. Nelson MR, Felix-Gastelum R, Orum TV,Stowell LJ, Myers DE. Geographic in for mation systems and geostatistics in the design and validation of regional plant virus management programs.Phytopathology. 1994; 84:898-905.

Received to editorial board 06.12.16

С. Павлова, асп.
Опытная станция карантина винограда и плодовых культур
Института защиты растений национальной аграрной академии наук Украины, Одесса, Украина,
О. Стахурская, асп.
И. Будзанивская, д-р биол. наук, проф., В. Полищук д-р биол. наук, проф.
Киевскоий национальный университет имени Тараса Шевченко, Киев, Украина

ПРИМЕНЕНИЕ ГИС-ТЕХНОЛОГИИ ДЛЯ МОНИТОРИНГА ШАРКИ СЛИВЫ НА ТЕРРИТОРИИ ОДЕССКОЙ ОБЛАСТИ

Вирусы растений вызывают много различных заболеваний растений, что приводит к большим потям и качества урожая во всем мире, и поэтому, агрономы и фитопатологии посвящают значительные усилия для контроля за вирусными заболеваниями. Одиним из самых важных вирусов вызывающих большие экономические потери у рода Prunus это Plumpoxvirus (PPV), возбудитель шарки сливы. С момента своего открытия шарка является настоящим бедствием для косточковых садов. Этот вирус есть в каждой стране, в которой есть промышленное выращивание косточковых деревьев[1]. Вирус относится к списку регулируемых вредных организмов Украины. На территории Украины зараженные вирусом сады занимают площадь в 4013,2764 га. В Одесской области эта площадь карантинных очагов составляет 18,5 га. За последнее время здесь было найдено 6 новых очагов общей площадью 28га. Впервые в Одесской области вирус PPV обнаружен на деревьях черешни. Персиковые и сливовые деревья поражаются в одинаковой степени. На сколько нам известно, мы впервые использовали ГИС-технологии для контроля вирусных болезней растений в Украине. Правильного использование геопространственных данных является ключом к успеху контроля распространения PPV.

Ключевые слова: вирус шарки сливы, фитосанитаный мониторинг, карантинный очаг, ГИС технологии.

С. Павлова асп.
Дослідна станція карантину винограду і плодових культур
Інституту захисту рослин національної аграрної академії наук України, Одеса, Україна,
О. Стахурська, асп.
І. Будзанівська, д-р біол. наук, проф., В. Поліщук, д-р біол. наук, проф.
Київський національный університет імені Тараса Шевченка, Київ, Україна

ЗАСТОСУВАННЯ ГІС-ТЕХНОЛОГІЇ ДЛЯ МОНІТОРИНГУ ШАРКИ СЛИВИ НА ТЕРИТОРІЇ ОДЕСЬКОЇ ОБЛАСТІ

Віруси рослин викликають багато важливих хвороби рослин і несуть відповідальність за великі втрати і якість врожаю у всьому світі, і тому, агрономи і фітопатологи докладають значних зусилль для контролю за вірусними захворюваннями. Одиним з найбільш поширених вірусів, який викликає значні економічні втрати у роду Prunus це Plumpoxvirus (PPV), збудник шарки сливи. З моменту свого відкриття шарка є справжнім лихом для кісточкових садів Цей вірус присутній в кожній країні, в якій є промислове вирощування кісточкових дерев[1]. Вірусвідноситься до перелікурегульованихшкідливихорганізмівУкраїни. На територіїУкраїнизараженівірусом сади займаютьплощу в 4013,2764 га. В Одеськійобластіплощакарантиннихвогнищ становить 18,5 га. За останній час тут булознайдено 6 новихвогнищзагальноюплощею 28 га. Вперше в Одеськійобластівірус PPV був виявлений на деревах черешні. Персикові і сливові дерева уражаютьсяоднаковоюмірою. На скільки нам відомо, ми впершевикористали ГІС-технологии для контролю за вірусними хворобамирослин в Україні. Правильневикористаннягеопросторовихданих є ключем до успіху контролю поширення PPV.

Ключові слова: Вірус віспи сливи, шарка сливи, фітосанітарний моніторинг, карантинне вогнище, ГІС технології.

UDC 578.01

O. Shevchenko, PhD., S. Petrenko, lead ing., O. Iutynska, PhD, A. Bysov, PhD, T. Shevchenko, PhD, Ass.Prof.
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

SPREAD OF *TURNIP MOSAIC VIRUS* IN SUSCEPTIBLE CROPS IS STRONGLY EFFECTED BY DIFFERENT CULTIVATION PRACTICES

Samples of plants showing symptoms of Turnip mosaic virus (TuMV) were collected from fields planted to Brassicaceae crops in Kyiv region and different locations in the city of Kyiv. TuMV was detected in the main brassica-crop fields, private gardens and urban locations of Ukraine, with a high overall incidence of 50%. This paper describes the effects of different cultivation approaches on the incidence rate of viral infection in susceptible crops and confirms the importance of preventive measures for disease control.

Key words: Turnip mosaic virus, cultivation practices.

Introduction. *Turnip mosaic virus* (TuMV) is a member of *Potyvirus* genus belonging to the largest *Potyviridae* family of plant viruses [1]. As many potyviruses, TuMV has an extremely wide host range but infects mostly plant species from the *Brassicaceae* family and induces persistent symptoms (mosaics, mottling, chlorotic lesions, etc.). For domesticated *Brassica* plants, TuMV is considered one of the most damaging and economically important viruses [2]. TuMV is mainly transmitted by many aphid species non-persistently as well as mechanically from plant to plant. TuMV probably occurs worldwide and has been found in both temperate and subtropical regions of Africa, Asia, Europe, Oceania and North and South America. In Europe, TuMV was reported from the UK, Spain, Italy, Greece, Germany, The Netherlands, Czech Republic, Hungary, Bulgaria, Poland, and Russia [3-9]. Despite Ukraine's geographical location and wide cultivation of different *Brassica* crops for centuries, it's only recently that the authors have registered TuMV in our country (unpublished data). In the study reported here, we describe the importance of preventive measures for the control of wide-spread and damaging pathogen of brassicas.

Materials and methods. Sampling was restricted to crop-producing areas in Kyiv region and different locations in the city of Kyiv where Brassicaceae plants were growing/cultivated. In Kyiv, sampling locations included two botanical gardens, the city center, Museum of Folk Architecture and Life of Ukraine (open-air location w/o agricultural activity), and private gardens where different brassica plants were regularly cultivated. Several large fields in Luka and Gorenychi villages used for commercial cabbage cultivation were chosen for sampling in Kyiv region. Brassica plants were visually examined, samples were collected from plants with TuMV-like symptoms typically including mosaics, mottling, vein banding and/or leaf deformation.

Collected samples were tested for TuMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977) [10], using specific polyclonal antibodies purchased from Loewe (Germany). Briefly, 0,5 g leaf tissue was ground to a powder with a mortar and pestle in 10 mL phosphate-buffered saline, pH 7,4, containing 0,05% Tween 20, 2,0% polyvinylpyrrolidone (MW 40 000) and 0,2% bovine serum albumin. In the meantime, microtitre plates (Maxisorb, NUNC, Denmark) were coated with TuMV-specific broad-spectrum polyclonal antibodies (1:200) in carbonate buffer according to the manufacturer's instructions. Leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of TuMV in the samples was detected in 200 µL homogenate by TuMV-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Multiscan-334 microtitre plate reader (Labsystem, Finland). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

Results and discussion. A total of 54 plant samples with TuMV-like mosaic and mottling symptoms were collected in different districts of the city of Kyiv and Kyiv region. Sampling areas included both agricultural sites (two cabbage producing fields and private gardens) and urban locations where no agricultural activity was carried out (different sites in the City of Kyiv, two botanical gardens and open-air Museum of Folk Architecture and Life of Ukraine).

Using ELISA, TuMV was detected in samples from cabbage, red radish, mustard, radish, white mustard, gold of pleasure, weed species (hill mustard), etc. (Table 1).