UDK 578
DOI 10.17721/1728.2748.2022.90.26-29

T. Shevchenko, Dr. Sci.,
R. Bederak, Stud.,
K. Taher, Stud.,
H. Snihur, PhD,
O. Shevchenko, PhD
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

MIXED VIRAL INFECTIONS IN VEGETABLES IN UKRAINE

Aim. Screening of vegetable crops for mixed viral infections caused by 8 viruses, and evaluation of relative share for different combinations of pathogens using serological methods for plant virus diagnostics.Methods.Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed for detection of viral antigens.Results.For this study, the samples from plants with virus-like symptoms were collected from the agroecosystems in 4 regions of Ukraine during three vegetative seasons in 2019–2021. Plant samples were tested for presence of the following viruses: cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV2), zucchini yellow mosaic virus (ZYMV), tomato mosaic virus (ToMV), tobacco rattle virus (TRV), potato virus Y (PVY), potato virus X (PVX),and tomatospottedwiltvirus (TSWV).Among the screened plants with virus-like symptoms, three viruses were the most common: CMV, WMV2 and ZYMV, with the incidence rate of 32 %,33 % and 29 %, respectively. Apart from monoinfected plants, several patterns of mixed infections were shown typically induced by two and less commonly by three viruses (CMV+WMV2+ZYMV). From these patterns of mixed infections, five groups of pathogens were registered. Conclusions. In this work, we have analyzed cucurbit and solanaceous vegetable crops with virus-like symptoms for viral infections caused by 8 pathogens, and established virus combinations inducing mixed infections in the field. Five groups of pathogens were demonstrated as such combinations: CMV+ToMV, CMV+PVY, CMV+WMV2, CMV+ZYMV, andWMV2+ZYMV, with CMV+ToMV being the most common for tested plants. Also, there was one group of pathogens formed by three viruses CMV+WMV2+ZYMV. CMV has been shown present in every group of mixed viral infections in plants from both families, CucurbitaceaeandSolanaceae. The incidence rate for these combinations differed from 4 to 8 %. The presented results are important in the context of ecology and epidemiology of viral diseases of vegetables.

Keywords: viruses, mixed infections, vegetable crops, Ukraine.

Introduction. Viral plant diseases can be caused by either monoinfection or mixed infection involving two or more viruses. Simultaneous infection caused by different viruses or different strains of the same virus is not uncommon in nature [1, 2]. In plants, simultaneous infection with different viruses can lead to a phenomenon described as neutrality, when viruses do not interfere with replication, accumulation and transmission of each other [3]. Sometimes this can also lead to antagonism when one virus reduces the infection or accumulation of another virus [4], or synergism when a mixed infection leads to increased symptoms and/or elevated virus content. Synergistic interactions between plant viruses can lead to increased disease rate in crops that are susceptible to different viral combinations [5,6]. In addition, viral synergies can lead to resistance or limited spread of another virus. A significant increase in symptoms' severity in case of dual infection as compared to a single infection has been observed in several studies involving a combination of potyviruses and viruses belonging to other genera [7].

In nature, synergistic interactions affect the level of vector transmission, which can lead to changes in the ecology and epidemiology of the pathogens involved. Studies show that the level of vector transmission often positively correlates with mixed infections, due to increased titers of viruses in the plant [8], or to changes in tropism, and in some cases, due to the fact that plants with mixed infections are more attractive to insect vectors (probably due to the weakening of phloem transport and the accumulation of sugars in the areas affected by the two viruses).

Infections of cucurbit species induced by such viruses as ZYMV, WMV-2 or CMV are very common and cause significant damage worldwide in the form of severe epidemics, either in single or double infection. Synergism is often observed between WMV-2 and other viruses infecting cucurbits, which can be expressed either by higher rate of virus replication or more severe symptoms. This was demonstrated, in particular for mixed infection caused by WMV-2 and poleroviruses, such as cucumber aphid-born virus (CABYV) [9].

The interaction between WMV-2 and ZYMV, two closely related viruses from *Potyviridae* family, is of particular interest because these genetically distinct viral species belong to the same subgroup and occupy similar ecological niches, suggesting that competition between these viruses may be important factor influencing their evolution. WMV-2 and ZYMV have similar host ranges (primarily cucurbits), cause similar symptoms and are mostly transmitted by the same aphid species, although with a different efficiency. Mixed infection of WMV-2 and ZYMV in the same host populations and in individual plants is equally common for both cultured and wild host species. Such mixed infection, however, does not cause such severe synergistic effects as reported for double infections induced by potyviruses and viruses from other genera like cucumoviruses (*Bromoviridae* family) or potexviruses (*Alphaflexiviridae* family) [10].

The aim of the study. Screening of vegetable crops for mixed viral infections caused by 8 viruses, and evaluation of relative share for different combinations of pathogens using serological methods for plant virus diagnostics.

Materials and Methods. Samples of vegetable plants from *Cucurbitacea* and *Solanaceae* families were collected basing on visual screening of crops for virus-like symptoms in several parts of Ukraine: Vinnytsia, Kyiv, Poltava, and Cherkasy regions.

Plant samples were tested for presence of the following viruses: cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV2),zucchini yellow mosaic virus (ZYMV), tomato mosaic virus (ToMV), tobacco rattle virus (TRV), potato virus Y (PVY), potato virus X (PVX), and tomato spotted wilt virus (TSWV). For virus diagnostics, a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used [11, 12] with specific diagnostic kits from Loewe Biochemica GmbH (Germany) and following the manufacturer's recommendations. Absorbance values at 405 nm were measured using a BioTek μQuant microtiter plate reader (USA). Absorbance values greater than three times those of the negative controls, were considered positive. Mathematical processing of experimental data (mean values, standard deviation) was carried out using Origin 9.1 software. Variation statistics methods were used for statistical analysis of the obtained results. Significance of differences between the values was determined by Student's t-test at p = 0.05.

ISSN 1728-3817

Results and Discussion. For this study, the samples from plants with virus-like symptoms were collected from the agroecosystems in 4 regions of Ukraine during three vegetative seasons in 2019–2021. The screening included cucurbit and solanaceous plants with the symptoms of dark green mosaic along the leaf veins, yellow-green interveinal mosaic of leaf blades, deformation of leaf blades and fruits (**Fig. 1**). The manifestation of symptoms was different, from a barely noticeable mosaic to a severe deformation in the form of filamentous leaves. Taking into account the available data [2, 7], such a variety of symptoms could be the result of a mixed viral infection. Thus, collected experimental samples were tested by DAS-ELISA to determine the species composition of viruses that could cause such symptoms. In total, 161 plant samples were tested for eight viruses characterized either by a wide (CMV, PVY, TRV, PVX, TSWV, ToMV) or narrow host range (ZYMV, WMV2).

Fig. 1. Virus-like symptoms on leaves o fpumpkin (a), custard squash (b), squash (c), melon (d), sweet pepper (e), and tomato (f)

Among the total number of 161 samples of vegetable plants analyzed for virus infections, 135 samples were collected from plants belonging to *Cucurbitaceae* family and 26 samples – from plants of *Solanaceae* family. Given the different number of samples by family and the different species composition of viruses for which different plants were analyzed, it is not the number of positive samples that is considered informative, but their percentage values. Thus, among plants with virus-like symptoms, the three most common viruses were CMV, WMV2 and ZYMV, which were detected in 32 %, 33 % and 29 % of the samples, respectively (**Table 1**).

***Table* 1. Virus detection in vegetable crops by DAS-ELISA**

Virus	CMV	PVX	PVY	ToMV	TRV	TSWV	WMV2	ZYMV
Numberofpositivesamples	52	1	3	7	2	1	44	39
Percentofpositivesamples, %	32	4	12	27	8	4	33	29
Number of samples	161	26	26	26	26	26	135	135

Apart from monoinfected plants, several patterns of mixed infections were shown for tested samples. Such mixed infections were induced by two and less commonly by three viruses (CMV+WMV2+ZYMV). From these patterns of mixed infections, five groups of double infections were registered: CMV+ToMV, CMV+PVY, CMV+WMV2, CMV+ZYMV, andWMV2+ZYMV. The incidence rate for these virus combinations in plants from both families, *Cucurbitaceae* and*Solanaceae*, differed from 4 to 8 % (**Fig. 2**).

CMV has been shown present in all but one group of mixed viral infections. These data can be explained by the fact that CMV belongs to viruses with an extremely wide host range and therefore may persist in the same agriecosystem despite the crop rotation and other measures. CMV was found in mixed infection among the representatives of both *Cucurbitaceae* and *Solanaceae* families (**Fig. 2**).

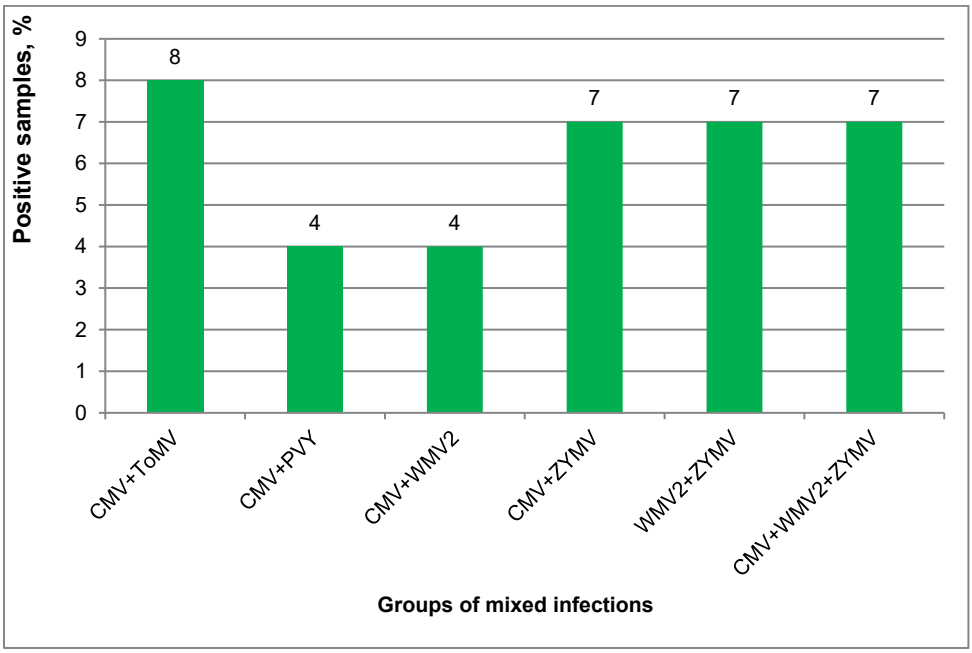

Fig. 2. Mixed infections found in solanaceous (CMV+ToMVandCMV+PVY) and cucurbit (CMV+WMV2, CMV+ZYMV, WMV2+ZYMV, andCMV+WMV2+ZYMV) crops

In general, mixed CMV and ZYMV infections characterized with more severe symptoms. According to the literature, the co-infection of CMV and ZYMV is an example of synergism, as it leads to a stronger manifestation of symptoms [2, 6].

The occurrence of mixed infection caused by CMV and PVY on tomatoes is confirmed by literature data, which leads to an increase in the concentration of both viruses in the plant and is important for the epidemiology of these viruses [13].

Despite the fact that CMV and ToMV have wide host ranges and in our studies they were detected with a incidence of 32 % and 27 %, respectively, the studies on mixed infections caused by these viruses on tomato and pepper have been published only recently. Particularly, when these crops were cultivated in one of the provinces in Turkey, the percentage of mixed infection on tomato and pepper was 5.31 % and 1.11 %, correspondingly, which is lower than in our studies [14].

The interaction of WMV and ZYMV is significantly different from that of ZYMV and CMV, because the former are the members of the same genus and have a similar ecological niche. This suggests that competition between viruses may be an important factor influencing their evolution. WMV and ZYMV have overlapping host ranges, cause similar symptoms on the plants, and are transmitted non-persistently mostly by the same aphid species, although with different efficiency. As already mentioned, WMV and ZYMV are often found in the same populations of host plants and in individual plants, which is characteristic of both cultivated and wild plants, and leads to more severe symptoms [15].

ZYMV isolates replicate at a similar rate at mono- and mixed infection, whereas WMV isolates accumulate to much lower levels in presence of ZYMV. In addition, ZYMV induces changes in leaf coloration and the production of volatile substances enhancing the attraction of aphids to infected plants. In contrast, WMV does not have a strong effect on the plant-aphid interactions. However, WMV can take advantage of a mixed infection and be easily transmitted to other plants in such case [16].

Plants affected by three viruses (CMV, WMV-2, and ZYMV) were also found. The incidence of such plants was 7 %. Dark green mosaic, leaf blade deformation, and blistering were observed on melons and pumpkins, some of them were fruitless.

According to the results of the study, CMv was the most common virus found in collected samples of vegetable crops of *Cucurbitaceae* and *Solanaceae* families. This may be due to the fact that, unlike ZYMV and WMV-2, CMV can be transmitted not only by aphids but also by dodder and seed. To date, it has been confirmed that various species of wild flora may be the source of CMV in Ukraine [17], serving as additional risk factor for agricultural plants. In addition, there is evidence of CMV interaction with members of *Potyvirus* family. According to it, when found in mixed infection in some plants, CMV titer will increase compared to monoinfection, while the titer of the second pathogen may remain unchanged, which increases the efficiency of vector transmission of CMV. Wide spread of ZYMV in the screened

regions can be explained by the fact that despite a rather narrow host range, the virus does not cause severe symptoms on plants that do not belong to *Cucurbitaceae* family. Therefore, this virus can survive in natural reservoirs unnoticed. Given the wide host range of WMV-2, it may be preferable for it to switch to other hosts rather than competing with ZYMV, which has a limited ability to reproduce in plants outside *Cucurbitaceae* family and hence is forced to maintain its niche more aggressively.

Conclusion. In this work, we have analyzed cucurbit and solanaceous vegetable crops with virus-like symptoms for viral infections caused by 8 pathogens, and established virus combinations inducing mixed infections in the field. Five groups of pathogens were demonstrated as double combinations: CMV+ToMV, CMV+PVY, CMV+WMV2, CMV+ZYMV, andWMV2+ZYMV, with CMV+ToMV being the most common for tested plants. Also, there was one group of pathogens formed by three viruses CMV+WMV2+ZYMV. CMV has been shown present in every group of mixed viral infections in plants from both families, *Cucurbitaceae* and *Solanaceae*. The incidence rate for these combinations differed from 4 to 8 %. The presented results are important in the context of ecology and epidemiology of viral diseases of vegetables.

Acknowledgments. This work was partly funded by the Ministry of Education and Science of Ukraine (project 0121U109863).

References

1. Moreno A. B. & López-Moya J. J. When Viruses Play Team Sports: Mixed Infectionsin Plants. Phytopathology. 2020;110(1):29–48. https://doi.org/10.1094/PHYTO-07-19-0250-FI

2. Faiza A. Fattouh. Double Infection of a Cucurbit Host by Zucchini Yellow Mosaic Virus and Cucumber Mosaic Virus. Plant Pathology Journal. 2003; 2:85-90.DOI:10.3923/ppj.2003.85.90 URL:https://scialert.net/abstract/?doi=ppj.2003.85.90

3. Singhal P., Nabi S.U., Yadav M.K. et al. Mixed infection of plant viruses: diagnostics, interactions and impact on host. J PlantDisProt. 2021;128:353–368. https://doi.org/10.1007/s41348-020-00384-0

4. Cho JD. Ultra structural a spects of water melon necrosis disease caused by mixed infection with water melon mosaic potyvirus and cucumber green mottle mosaic tobamo virus. A thes is for the degree of Master of Science. Seoul National University: Korea; 1998.

5. Zeng R., Liao Q., Feng J., Li D. & Chen J. Synergy between cucumber mosaic virus and zucchini yellow mosaic virus on Cucurbitaceae hosts tested by real-time reverse transcription-polymerase chain reaction. Acta biochimica et biophysica Sinica. 2007;39(6):431–437. https://doi.org/10.1111/j.1745-7270.2007.00292.x

6. Wang, Y, Gaba V, Yang J, Palukaitis P, Gal-On A. Characterization of synergy between Cucumber mosaic virus and potyviruses in cucurbithosts. Phytopathology. 2002;92:51–58.

7. Wang Y., Lee, K., Gaba, V. *et al.* Breakage of resistance to *Cucumber mosaic virus* by co-infection with *Zucchini yellow mosaic virus*: enhancement of CMV accumulation independent of symptom expression. ArchVirol. 2004;149:379–396.https://doi.org/10.1007/s00705-003-0240-4

8. Wintermantel W. M., Cortez A. A., Anchieta A. G., Gulati-Sakhuja A. & Hladky L. L. Co-infection by two crini viruses alters accumulation of each virus in a host-specific manner and influence sefficiency of virus transmission. Phytopathology. 2008;98(12): 1340–1345. https://doi.org/10.1094/PHYTO-98-12-1340

9. Bourdin D. and Lecoq H. Increase in cucurbit aphid-borne yellows virus concentration byco-infection with sap-transmissible viruses does not increase its aphid transmissibility. Phytopathology. 1994;141: 143-152.

10. Brian WJ, Mahy, Marc HV van Regenmortel. Desk Encyclopedia of Plant and Fungal Virology. Academic Press; 2009:632.

11. Crowther J *R.* ELISA. Theory and practice. Humana Press; 1995: 223.

12. Clark M. F., & Adams A. N. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. The Journal of general virology. 1977;34(3):475–483.

13. Mascia T., Cill F., Fanelli V., Finetti-Sialer M. M., De S. A., Palukaitis P., and Gallitelli D. Characterization of the interactions between *Cucumber mosaic virus* and *Potato virus* Yinmixed infections in tomato. Mol. Plant-MicrobeInteract. 2010;23:1514-1524.

14. Akdura N, Kiliç HC. Genetic Diversity of CMV and To MV Isolates in Tomato and Pepper Production Areasin Hakkari, Turkey. Research Square; 2022; DOI: 10.21203/rs.3.rs-1451598/v1

15. Fletcher J. D., Wallace A. R. & Rogers B. T. Poty viruses in New Zealand butter cup squash (Cucurbitsmaxima Duch.): Yield and quality effects of ZYMV and WMV 2 virus infections.New Zealand Journal of Crop and Horticultural Science. 2000;28(1):17-26. DOI: 10.1080/01140671.2000.9514118

16. Salvaudon L, De Moraes CM, Mescher MC. Out comes of co-infection byt wopoty viruses: implications for the evolution of manipulative strategies. ProcBiolSci. 2013;280:20122959. Published 2013 Feb 13. doi:10.1098/rspb.2012.2959.

17. Kyrychenko A, Snihur H, Shevchenko T. First report of cucumber mosaic virus infecting Alliariapetiolata in Ukraine. J Plant Pathol. 2022; https://doi.org/10.1007/s42161-022-01107-0

Надійшла до редколегії 17.08.2022
Отримано виправлений варіант 19.09.2022
Підписано до друку 19.09.2022

Received in the editorial 17.08.2022
Received version on 19.09.2022
Signed in the press on 19.09.2022

Т. Шевченко, д-р біол. наук,
Р. Бедерак, студ.,
К. Тагер, студ.,
Г. Снігур, канд. біол. наук,
О. Шевченко, канд. біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

ЗМІШАНІ ІНФЕКЦІЇ ОВОЧЕВИХ КУЛЬТУР В УКРАЇНІ

Проаналізовано овочеві культури на змішані вірусні інфекції, що можуть бути спричинені вісьмома вірусами, і встановлення їх відсоткового значення для різних груп комбінації вірусів з використанням серологічних методів ідентифікації вірусів рослин. Детекцію вірусів у рослинах овочевих культур проводили з використанням імуноферментного аналізу у модифікації DAS-ELISA. Для досліджень відбирали зразки рослин з агроценозів із чотирьох областей протягом трьох вегетаційних сезонів у період із 2019 по 2021 рік включно тільки з вірусоподібними симптомами. Зразки рослин тестували на наявність таких вірусів: cucumbermosaicvirus (CMV), watermelonmosaic virus 2 (WMV2), zucchiniyellowmosaicvirus (ZYMV), tomatomosaicvirus (ToMV), tobaccorattlevirus (TRV), potatovirusY (PVY), potatovirusX (PVX) andtomatospottedwiltvirus (TSWV). Серед проаналізованих рослин з вірусоподібними симптомами найчастіше зустрічались три віруси: CMV, WMV2 та ZYMV, які детектувалися у 32, 33 та 29 %, відповідно, серед дослідних зразків. У протестованих зразках овочевих культур, окрім моноінфекції, зустрічалась змішана інфекція, викликана двома вірусами з переліку вірусів, на які тестували рослини, і навіть була одна група змішаних інфекцій, яка складалась із трьох вірусів, а саме CMV+WMV2+ZYMV. Серед змішаних інфекцій, які містили два віруси, було п'ять груп. У ході виконання досліджень було проаналізовано овочеві культури родин Cucurbitaceae та Solanaceae з вірусоподібними симптомами на наявність вірусних інфекцій, спричинених вісьмома вірусами, та ідентифіковано комбінації вірусів, що викликають змішані вірусні інфекції. Установлено, що серед змішаних інфекцій, які містили два віруси, було п'ять груп: CMV+ToMV, CMV+PVY, CMV+WMV2, CMV+ZYMV та WMV2+ZYMV. CMV зустрічався у вигляді змішаної інфекції серед представників двох родин: Cucurbitaceae та Solanaceae. Найбільший відсоток мала змішана інфекція, викликана CMV+ToMV. Одна група змішаних інфекцій складалась із трьох вірусів, а саме CMV+WMV2+ZYMV. Частота ідентифікації цих груп серед дослідних рослин овочевих культур родин Cucurbitaceae та Solanaceae була в діапазоні від 4 до 8 %. Результати цієї роботи є актуальними з еколого-епідеміологічного погляду.

Ключові слова: віруси, змішані інфекції, овочеві культури, Україна.