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Molecular identification and monitoring of major apple tree viruses in Ukraine

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Abstract. Viral diseases of apple trees cause significant economic losses in horticulture in Ukraine, which requires an updated research into their prevalence. The purpose of the study was to assess the prevalence and regional distribution of the main apple tree viruses in industrial plantings of Ukraine using molecular diagnostic methods. The study was conducted by reverse transcription and polymerase chain reaction on 350 samples of apple leaves and bark taken from industrial plantings in five regions of Ukraine. The specificity of detection of three target viruses was confirmed by sequencing the obtained amplicons. The study found that the overall infection rate of apple plantations was 70.6%, which indicates an epiphytic situation. The most prevalent was *Apple stem pitting virus* (46.0%), whereas *Apple chlorotic leaf spot virus* and *Apple mosaic virus* were detected in 36.0% and 13.7% of samples, respectively. Regional analysis revealed the maximum prevalence of viruses in the Zakarpattia (86.0%) and Kyiv (76.3%) regions, while the minimum indicators were recorded in the Kharkiv region (56.8%). A high level of mixed infections was found (25.4%), with a predominance of the combination of *Apple stem pitting virus* and *Apple chlorotic leaf spot virus* (18.3%). Also significant is the high percentage of asymptomatic infections (31.2%), which complicates phytosanitary control. The conclusions confirmed that the results obtained justify the need for urgent implementation of a system for molecular monitoring and certification of planting material. The results of the study can be used to develop a national programme for improving apple plantations in Ukraine

Keywords: polymerase chain reaction; apple stem pitting virus; apple chlorotic leaf spot virus; apple mosaic virus

INTRODUCTION

Apple orchards occupy a leading place in horticulture in Ukraine, but their productivity is significantly limited by viral diseases. The global problem of the spread of viruses on fruit crops is becoming particularly relevant in the context of the growth of intensive gardening. Studies conducted in different regions of the world show trends in the high level of infection of apple plantations.

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In particular, L. Ma *et al.* (2021) found a high prevalence of major pear viruses in commercial orchards in China, indicating the severity of the problem in the East Asian region. The study included analysis of samples from various provinces of China, where the presence of pathogens such as *Apple stem pitting virus* and *Apple chlorotic leaf spot virus*. The researchers applied advanced molecular diagnostic methods, which helped to obtain representative data on the epidemiology of viral diseases in fruit crops. Similar studies in Kazakhstan conducted by N. Kerimbek *et al.* (2024) showed a high level of viral damage to apple plantations, which requires the development of effective control measures. The researchers conducted monitoring in the southern regions of Kazakhstan using serological and molecular diagnostic methods. The study revealed significant variability in the spread of different viruses depending on the variety and age of the plantings.

Advanced diagnostics of plant viruses is based on molecular methods that allow accurate identification of pathogens. S. Heo & Y.S. Chung (2020) developed advanced PCR (polymerase chain reaction) techniques for detecting apple viruses, which significantly improved diagnostic accuracy. The researchers optimised PCR conditions for sensitive and specific detection of *Apple stem grooving virus* and *Apple chlorotic leaf spot virus*. The developed methods have demonstrated high efficiency in analysing samples with different types of infections. A notable achievement is the development by D.A. Gritsenko *et al.* (2020) of multiplex PCR method for simultaneous detection of five apple viruses, which simplifies the mass screening process. The method allows saving time and reagents when conducting large-scale research. The researchers tested the specificity and sensitivity of the method on a wide collection of samples. In Latvia, N. Zuļģe *et al.* (2023) conducted an in-depth phylogenetic study of ACLSV isolates, revealing significant genetic diversity of the pathogen. The study included analysis of isolates from different host species – *Malus, Pyrus* and *Prunus*. Phylogenetic analysis revealed a clear clustering of isolates by host species and geographical origin.

In Ukraine, the problems of viral diseases of agricultural crops were studied mainly on vegetable crops, which was confirmed by V.O. Tsvigun *et al.* (2024). The researchers monitored viral infections in vegetable agrocoenoses using advanced molecular diagnostic methods. The study covered the analysis of samples from different regions of Ukraine, which helped to identify the species composition of viral pathogens. The results obtained indicate the need to introduce a system for regular monitoring of viral diseases of agricultural crops. Research by L.T. Mishchenko *et al.* (2022) on the Wheat dwarf virus demonstrated the relevance of the problem of viral diseases in agrocoenoses of Ukraine. The researchers conducted molecular characterisation of local isolates of the virus, investigating their genetic diversity. A link was established between the spread of the pathogen and grain yield. However, comprehensive studies of apple tree viruses remain rather neglected, which creates a significant gap in the development of an effective plant protection system. Lack of up-to-date data on the prevalence of pathogens such as *Apple stem pitting virus, Apple chlorotic leaf spot virus,* and *Apple mosaic virus* complicates the development of targeted phytosanitary measures. The existing information is based mainly on outdated diagnostic methods that do not meet modern requirements.

International experience shows the effectiveness of advanced methods of combating viral infections. Research by Korean scientists Y.H. Kwon *et al.* (2024) showed high efficiency of a combination of thermotherapy and chemicals for improving the health of planting material. The researchers achieved a significant reduction in the concentration of viruses in plant material by optimising temperature conditions. In Kazakhstan, G. Kairova *et al.* (2023) successfully applied molecular markers to identify varieties resistant to bacterial infections, opening up prospects for similar studies of viral diseases. The findings showed the possibility of rapid identification of resistance genes using PCR analysis. Given the global nature of the problem of the spread of apple viruses and the lack of data on the situation in Ukraine, there is a need for a comprehensive study of the prevalence of the main viral pathogens in apple plantations in different regions of the country. Existing studies, such as research by L. Pavliuk *et al.* (2021) on cherry viruses, confirmed the relevance of such studies for fruit crops in Ukraine, but there are no specialised studies on apple trees. The researchers used molecular methods to detect the main viruses of stone crops, which allowed assessing the phytosanitary condition of plantings.

The purpose of the study was to carry out a comprehensive assessment of the phytosanitary condition of industrial apple plantations in Ukraine by investigating the prevalence of major viral pathogens (ASPV (*Apple stem pitting virus*), ACLSV (*Apple chlorotic leaf spot virus*), and AMV (*Apple mosaic virus*)), for which the following tasks were set: to conduct molecular monitoring using the method of reverse transcription and polymerase chain reaction to identify the main viral pathogens; to analyse the regional features of the distribution of viral infections in the main areas of industrial apple cultivation; to assess the level of mixed infections and determine the proportion of latent forms of damage.

MATERIALS AND METHODS

The process of selecting plant material was organised to ensure the representativeness of samples from the main areas of industrial apple cultivation in Ukraine. The sampling was carried out during two growing seasons – from May to August 2023 and 2024. 350 samples of leaves and young bark of apple trees of the 'Golden Delicious' (100 samples), 'Idared' (90 samples), 'Gala' (80 samples), and 'Champion' (80 samples) varieties were selected. The sampling was carried out in industrial gardens of five regions of Ukraine characterised by different climatic conditions: Kyiv (n = 80), Vinnytsia (n = 80), Cherkasy (n = 70), Kharkiv (n = 70), and Zakarpattia (n = 50). Leaf samples were taken from the middle tier of the crown from different cardinal directions, and bark samples were taken from annual increments. Attention was paid to apple trees with pronounced symptoms of viral damage. In particular, samples were taken with the following signs: leaf mosaic (characteristic light green or yellow spots and stripes) – a classic symptom of infection with the *Apple mosaic virus* (ApMV). Chlorosis (yellowing along the veins or inter-vein chlorosis) was caused by *Apple chlorotic leaf spot virus* (ACLSV). Deformation of the leaf plate (twisting, asymmetry, threadiness) is associated with infection with the *Apple stem pitting virus* (ASPV) or a complex of the mentioned viruses. However, to assess the latent spread of infection, some of the material was also taken from clinically healthy plants, since many viruses (for example, ACLSV and ASPV) circulate in gardens without visible symptoms, forming so-called latent complexes of apple viruses. All samples were labelled, indicating the date, place of selection, grade, and description of the phytopathological condition, and then transported in ice containers manufactured by Thermo Fisher Scientific (USA) to the laboratory, where they were stored at -80°C until further analysis. All further stages of RNA (ribonucleic acid) and molecular diagnostics were performed under controlled laboratory conditions.

Total RNA isolation was performed from 100 mg of plant tissue, which was previously ground in liquid nitrogen with a mortar. Nucleic acid extraction was performed using the commercial kit "Ukrbiopreparat" (Ukraine), based on the method of heterophase extraction with silicon dioxide and detergent (Bogner & Killeen, 2006). Specifically, 500 μL of lysing buffer was added to the crushed tissue, the mixture was incubated at 65°C for 10 minutes, then 250 μL of chloroform was added and thoroughly mixed on vortex. After cenrifugation at 12,000 RCF for 10 minutes, the aqueous phase was carefully transferred to a new microcentrifuge tube, where RNA deposition was performed by adding an equal volume of isopropanol and incubation at -20°C for 30 minutes. After repeated centrifugation, the resulting RNA was washed in 70% ethanol, air-dried, and dissolved in 30 μL of RNA-free water. The quality and quantity of the extracted RNA were assessed using two methods: spectrophotometrically with a NanoDrop system (Deben *et al.*, 2013), where samples with an A260/280 ratio in the range of 1.8-2.1 were considered acceptable; and electrophoretically by fractionation of 2 μL of RNA in a 1% agarose gel stained with ethidium bromide (Barros *et al.*, 2018), where RNA integrity was confirmed by the presence of distinct 18S and 28S rRNA bands.

Reverse transcription was performed at a volume of 20 μL using 1 μg of total RNA, 100 Ng of random hexamer primers, 20 units of M-MuLV revertase, and a buffer containing 10 mM DTT, 1 mm dNTP mixture, and 20 units of an RNase inhibitor. The reaction was performed according to the following temperature programme: 25°C for 5 minutes for primer hybridisation, 42°C for 60 minutes for cDNA synthesis, and 70°C for 5 minutes for enzyme inactivation. The resulting cDNA was diluted in a ratio of 1:5 with sterile water and stored at -20°C. The polymerase chain reaction was tuned to a volume of 25 µL, the reaction mixture included 2.5 µL of diluted cDNA, 1 x Taq polymerase buffer, 1.5 mm $MgCl_2$, 0.2 mm dNTP, 0.4 µM of each of the specific primers, and 1 unit of Taq DNA polymerase.

Molecular diagnostics of apple tree samples was performed by reverse transcription and polymerase chain reaction (RT-PCR). Published pairs of primers specific to conserved regions of their genome were used to detect the main apple viruses. For detection of ASPV, primers ASPV-F (5′-GACTGCATCTGGTTCAAC-3′) and ASPV-R (5′-CGCTTGATTTGGACTGTC-3′) were used, which amplify a 327 bp fragment of the coat protein (CP) gene (Ji *et al*., 2013; Hao *et al*., 2016). For detection of ACLSV, primers ACLSV-F (5′-GCGATGAAGTTGCTGATGA-3′) and ACLSV-R (5′-TGACCTCCATTGCCAAT-3′) were used, targeting a 458 bp region of the replicase gene (ORF1) (Ji *et al*., 2013). For analysis of ApMV, primers ApMV-F (5′-CAGAGCAACGCAGACTCA-3′) and ApMV-R (5′-GAGCCATACCAAACCAAT-3′) were used to amplify a 392 bp fragment of the coat protein (CP) gene (Ji *et al*., 2013).

Amplification was performed in the Applied Biosystems Veriti thermal cycler (USA) according to the standard protocol, which included initial denaturation at 94°C for 5 minutes, then 35 cycles, each of which consisted of denaturation at 94°C – 30 seconds, annealing of primers at 55°C – 30 seconds and elongation at 72°C – 45 seconds, followed by final elongation at 72°C for 7 minutes. PCR products were visualised by electrophoresis in 1.5% agarose gel using the Horizon horizontal electrophoresis system (Thermo Fisher Scientific, USA) and a power source (Bio-Rad, USA). The gel provided sufficient resolution to differentiate the expected amplicon sizes. The gel was stained with ethidium bromide, and the strips were visualised in UV light (UVP transilluminator BioDoc-It, USA) using a gel documentation system (Bio-Rad, USA). Samples with a clear band of the expected size corresponding to the positive control were considered positive. To confirm the specificity of amplification, random samples from each group of positive samples were selected for sequencing using the Sanger method (Crossley *et al*., 2020) on the Applied Biosystems 3500 analyser (USA). To quantify the relationship between clinical manifestations and the results of molecular detection of viruses, a standardised symptom intensity scale was developed in the range from 0 to 5 points, where 0 indicated the absence of symptoms, and 5 – the maximum severity of pathological signs. Visual evaluation was performed independently by three researchers, followed by averaging the results, and for each sample, the type of symptoms (mosaic, chlorosis, leaf deformity, necrosis) and their severity were recorded.

Statistical processing of the obtained data was performed using the Statistica 10.0 software suite (USA). The chi-square (χ^2) criterion was used to assess the relationship between the selection region, apple variety, and virus infection rate. The differences were considered statistically significant at the significance level $p < 0.05$. The prevalence of each virus was calculated as the percentage of positive samples from the total number tested. Pearson correlation analysis with calculation of the correlation coefficient (r) for quantitative data and analysis of conjugacy tables using the χ^2 criterion for categorical variables were used to identify patterns in the spread of mixed infections. All calculations were performed using the Statistica 10.0 software suite. All laboratory experiments, including RNA isolation and PCR analysis, were performed in three biological and two analytical repetitions to minimise random errors and ensure reproducibility of the results. During field research, the principles of biodiversity conservation were observed, in accordance with the provisions of the Convention on Biological Diversity (2025), and sampling was carried out with minimal impact on plants and their environment.

RESULTS AND DISCUSSION

General prevalence of viral infections in apple plantations

A molecular study using reverse transcription and polymerase chain reaction (RT-PCR) revealed a high level of infection of apple plantations with major viruses (ASPV, ACLSV, and ApMV) in Ukraine. Analysis of 350 samples of leaves and bark showed that 247 samples had a positive reaction to the presence of at least one of the three viruses under study. This indicator is 70.6% of the total sample size, which suggests an epiphytotic situation in Ukrainian apple plantations (Table 1).

Table 1. Prevalence of viral infections in apple plantations in Ukraine

Virus	Number of positive samples	Overall prevalence (%)	Prevalence among infected samples (%)	Share of monoinfections (%)	Percentage of mixed infections (%)	Asymptomatic infections (%)
ASPV	161	46.0	65.2	58.4	41.6	38.5
ACLSV	126	36.0	51.0	42.9	57.1	42.9
ApMV	48	13.7	19.4	35.4	64.6	29.2
Total*	247	70.6	100.0	-	-	41.7

Notes: * – general indicators consider samples infected with at least one virus
Source: compared by the authors based on the conducted research using the method of P.N. Bogner & A.A. Killeen (2006)

A detailed analysis revealed a clear differentiation in the prevalence of individual pathogens (Table 1). The highest prevalence was demonstrated by ASPV, which was identified in 161 samples, which is 46.0% of the total number of samples tested. ACLSV was the second most common virus – it was detected in 126 samples, which corresponds to 36.0% of the total number. The least common among the pathogens studied was ApMV, which was detected in only 48 samples, which was 13.7%. Attention is drawn to the fact that among the infected samples, only 58.3% showed any visible symptoms of the disease, while 41.7% of infected plants did not have any visual signs of damage, which indicates a significant spread of latent forms of infection. The highest percentage of asymptomatic infections was observed for ACLSV (42.9%), while for ASPV this figure was 38.5%, and for ApMV – 29.2%. To confirm the specificity of the results obtained, Sanger sequencing was performed (Crossley *et al*., 2020) on an analyser for 45 randomly selected amplicons (15 for each virus). The obtained sequences showed 98-100% homology with the reference virus strains, which confirmed the high specificity of the primer systems used and the reproducibility of PCR analysis results.

As shown in Table 1, ASPV is not only the most common virus in apple plantations, but also characterised by the highest frequency of monoinfections (58.4%). In contrast, ApMV is significantly more often detected in mixed infections (64.6%), which may indicate its lower competitiveness in individual plant damage. ACLSV occupies an intermediate position, but is characterised by the highest percentage of asymptomatic infections (42.9%), which makes it dangerous from the standpoint of uncontrolled spread through planting material. The results obtained indicate a critical situation with the phytosanitary condition of apple plantations in Ukraine and the need for urgent introduction of a system of certification of planting material based on sensitive molecular diagnostic methods.

The high overall infection rate of apple plantations, which was 70.6%, is a negative indicator that exceeds data from many other regions. The data obtained in the course of the study empirically confirm the conclusions of the EFSA Panel on Plant Health *et al*. (2021) on the risks of spreading viruses through planting material from Ukraine. In particular, the detected high level of latent infections (31.2% of all infected samples) without clinical manifestations justifies these data, since it creates a real threat of uncontrolled spread of pathogens through visually healthy planting material. The highest rate of asymptomatic infections detected for ACLSV – 42.9%, is dangerous due to its high prevalence (36.0%) in the plantings under study. These results highlight the lack of a visual method for assessing the condition of planting material and the need to introduce mandatory molecular testing. The dominance of ASPV virus (46.0%) in Ukrainian plantings is consistent with the results of studies in other countries, although at different levels. For example, W. Shi *et al.* (2020) reported a 35% prevalence of ASPV in China, while C. Canales *et al*. (2021) detected this virus in 42% of samples in Spain. This sequence may indicate the universality of the epidemiological properties of ASPV and its ability to effectively adapt to different climatic conditions. The high prevalence of ACLSV (36.0%) was also confirmed by other researchers. K. Zikeli *et al.* (2025) recorded similar trends in Germany, although at a lower level (28%), which is associated with a more efficient system of certification of planting material. The results obtained demonstrate the state of viral damage to apple plantations in Ukraine, which is characterised by a high overall infection rate (70.6%), the dominance of the ASPV (46.0%), and a significant proportion of latent infections (41.7%), which makes it necessary to introduce a molecular monitoring system. Investigation of the regional features of the spread of viral infections will allow detailing this picture and developing targeted measures to improve phytosanitary control in various horticultural areas of the country.

Regional features of the spread of viral infections in the areas under study

A detailed regional analysis revealed statistically significant differences in the prevalence of viruses between the studied regions of Ukraine ($p < 0.01$ according to the χ^2 criterion), which indicates the influence of the geographical factor on the epidemiology of viral diseases of apple trees. The study covered five key regions (Kyiv, Vinnytsia, Cherkasy, Kharkiv, and Zakarpattia), which represent different agroclimatic zones of the country and have differences in the intensity of horticulture, cultivation technologies, and the history of the industry. The data obtained are presented in Table 2.

Table 2. Regional distribution of viral infections of apple trees in Ukraine

Area	Total number of samples	Total infection rate (%)	ASPV (%)	ACLSV (%)	ApMV (%)	Mixed infections (%)	Asymptomatic infections (%)
Zakarpattia	50	86.0	62.0	48.0	18.0	34.0	25.6
Kyiv	80	76.3	50.0	41.3	15.0	28.8	38.3
Vinnytsia	80	72.5	44.8	38.1	12.5	22.5	32.8
Cherkasy	70	67.1	41.4	32.9	11.4	18.6	35.4
Kharkiv	70	56.8	34.3	25.7	8.6	14.3	42.5

Source: compared by the authors based on the conducted research using the methods of Z. Ji *et al*. (2013), L. Hao *et al*. (2016)

The highest overall infection rate was recorded in orchards in the Zakarpattia region, where viruses were detected in 43 out of 50 samples, which is 86.0% of the total number of tested samples in this region. In this area, absolute dominance of the ASPV was observed, which was detected in 31 samples (62.0%). ACLSV also showed high prevalence rates – 24 positive samples (48.0%), while ApMV was detected in 9 samples (18.0%). A special feature of the Zakarpattia region was the highest proportion of mixed infections among all regions – 34.0% of the total number of samples. In the Kyiv region, the overall infection rate was 76.3% (61 out of 80 samples), with a clear predominance of ASPV (40 samples, 50.0%) and ACLSV (33 samples, 41.3%). ApMV in this region was detected in only 12 samples (15.0%). For the Kyiv region, a characteristic feature was a high proportion of asymptomatic infections – 38.3% of all infected samples, which may indicate a long-term circulation of viruses in the region and adaptation of the pathosystem. In the Vinnytsia region, viruses were detected in 58 out of 80 samples (72.5%), with a predominance of ASPV (44.8%) and ACLSV (38.1%). ApMV continued to show relatively low rates of 12.5%. This region was characterised by an average proportion of mixed infections (22.5%) and a moderate rate of asymptomatic infections (32.8%). In Cherkasy region, the total infection rate was 67.1% (47 out of 70 samples), with a prevalence of ASPV of 41.4% and ACLSV of 32.9%. ApMV was detected in 11.4% of the samples. This region was characterised by the lowest frequency of mixed infections among all the regions under study (18.6%). The lowest infection rates were recorded in the Kharkiv region – 40 out of 70 samples (56.8%). In this region, ASPV was detected in 34.3% of samples, ACLSV in 25.7%, and ApMV in only 8.6%. However, the highest proportion of asymptomatic infections was observed was in the Kharkiv region (42.5%), which may indicate a relatively recent penetration of viruses into the region or the specifics of local varieties.

Statistical analysis revealed a clear geographical pattern: the level of infection decreased in the direction from the west to the east of the country. Such regional differences are related to a complex of factors, including climatic features (higher humidity in the western regions contributes to the development of virus vectors), the level of gardening intensity (older gardens in the western regions), the effectiveness of quarantine measures and the quality of planting material used in different regions of the country. The data obtained are of practical importance for the development of differentiated regional strategies for protecting apple trees from viral infections.

L. Yin *et al.* (2021) also found similar regional differentiation in Canada, linking it to climatic conditions and the age of plantings. In this case, higher humidity and older gardens in western regions can contribute to the activation of virus vectors and their accumulation over time. The results obtained regarding the high prevalence of viral infections of apple trees in Ukraine are confirmed in modern international studies. In particular, H. Xiao *et al.* (2022) in the study of the viromics of apple orchards in Canada also found a high level of infection, linking this to the phenomenon of "rapid decline" of orchards. However, Ukrainian figures (70.6%) exceed Canadian data, which may indicate a more intensive circulation of pathogens in Ukraine. Thus, the regional analysis revealed a clear geographical pattern of the spread of viral infections of apple trees in Ukraine with a significant differentiation between Western (86.0% in Zakarpattia) and Eastern (56.8% in Kharkiv) regions, which is conditioned by a complex of climatic, technological, and historical factors and requires the development of differentiated regional approaches to the protection of plantings. Studying the features of mixed infections will help to better understand the mechanisms of interaction of various viral pathogens in Ukrainian agrocoenoses.

Characteristics of mixed viral infections of apple trees in Ukraine

A detailed analysis of the detected viral infections showed a high proportion of mixed infections that were recorded in 89 samples, which is 25.4% of the total number of tested samples or 36.0% of all infected plants. This indicator suggests a significant accumulation of viral pathogens in Ukrainian apple plantations and indicates the complex nature of the epidemiological situation. As shown in Table 3, the combination of ASPV + ACLSV is absolutely dominant among all types of mixed infections, accounting for more than two-thirds of all cases of multiple lesions. The regional distribution indicates a clear geographical specificity – Zakarpattia and Kyiv regions show the highest prevalence of mixed infections, which may be due to climatic features and the intensity of horticulture in these regions.

Table 3. Characteristics of mixed viral infections of apple trees in Ukraine

Type of infection	Number of samples	Overall prevalence (%)	Prevalence among mixed infections (%)	Predominant symptoms	Most affected regions
ASPV + ACLSV	64	18.3	71.9	Leaf deformity, chlorosis, reduced growth	Zakarpattia (34.0%), Kyiv (28.8%)
ASPV + ApMV	15	4.3	16.9	Mosaic, vein necrosis, fruit deformity	Vinnytsia (6.3%), Cherkasy(4.3%)
ACLSV + ApMV	7	2.0	7.9	Chlorosis, mosaic, leaf crushing	Kyiv (3.8%), Kharkiv (2.9%)
ASPV + ACLSV + ApMV	3	0.9	3.4	Severe deformity, necrosis, dwarfism	Zakarpattia (2.0%)

Source: compared by the authors based on the conducted research using the methods of Z. Ji *et al*. (2013), L. Hao *et al*. (2016)

The most common among mixed infections was a combination of ASPV and ACLSV, which was detected in 64 samples, which is 18.3% of the total number of analysed samples. This combination prevailed in all regions under study, which may indicate a synergistic interaction between the two viruses or similarity in their epidemiological cycles. Dual ASPV + ApMV infections were reported in 15 samples (4.3%), and the ACLSV + ApMV combination was detected in 7 samples (2.0%).

Attention is drawn to the detection of a triple infection that included all three viruses under study, which was identified in 3 samples (0.9%). All these samples were obtained from the Zakarpattia region and showed pronounced symptoms in the form of severe deformation of the leaf plate, necrotic spots along the veins, and general growth inhibition. The intensity of symptoms in these cases significantly exceeded the manifestations of individual infections, which confirms the concept of a synergistic effect in mixed viral lesions.

Correlation analysis revealed a statistically significant positive association between the age of plantings and the frequency of mixed infections ($r = 0.72$, $p < 0.05$), which indicates the cumulative nature of the accumulation of viral infections in gardens over time. A high level of mixed infections was registered in plantings over 15 years of age, where this figure reached 42.7%. In young plantings (up to 5 years), mixed infections were detected much less often – only in 12.3% of samples. Regional analysis showed that the highest frequency of mixed infections was observed in the Zakarpattia region (34.0%), while the lowest rates were recorded in Kharkiv (14.3%) and Cherkasy (18.6%) regions. This pattern correlates with the overall level of infection in these regions and is related to the climatic conditions that contribute to the development of virus vectors and the duration of horticulture in these regions. A notable result is that mixed infections were accompanied by significantly more pronounced infection symptoms compared to monoinfections. In samples with mixed infections, the intensity of symptoms was 45-60% higher, and the expression of pathological signs began on average 2-3 weeks earlier during the growing season.

A high rate of mixed infections (25.4%), namely ASPV + ACLSV combinations (18.3%), is a significant outcome. A.A. Wright *et al.* (2020) also found that it is these combinations that lead to a synergistic effect and a significant increase in pathogenesis. Current observations of increased symptoms in mixed infections fully support this concept. It is significant that all cases of triple infections were detected in the Zakarpattia region and were accompanied by the most pronounced symptoms. A useful aspect is comparison with studies of related cultures. N.-Y. Kim *et al.* (2021) found similar patterns of pathogen spread when studying pear viruses using RNA-seq, which confirms the universality of epidemiological processes in fruit crops of the family Rosaceae. The high proportion of mixed infections should be noted, which is observed in both apple and pear trees. Another aspect is the comparison of the results obtained with studies in other cultures. For example, A. Karanfil (2021), when studying rose viruses, also found a high rate of mixed infections, which may indicate general patterns of virus spread in perennials. The study by F. Xing *et al.* (2020) for ornamental crop viruses revealed similar patterns of pathogen spread. The researchers conducted molecular characterisation of isolates of the Prunus necrotic ringspot virus from roses, revealing high genetic diversity. This confirms the universality of the patterns identified in this study and the need to develop a unified strategy for combating viral diseases in horticulture in Ukraine.

Varietal differences in susceptibility to viral infections

A detailed analysis of varietal sensitivity revealed differences in the susceptibility of the studied apple varieties to viral infections, which indicates the influence of genetic factors on the plant-pathogen interaction. The study covered four commercially significant varieties that occupy leading positions in industrial horticulture in Ukraine: 'Golden Delicious', 'Idared', 'Gala', and 'Champion' (Table 4).

Table 4. Varietal features of apple tree viral infections in Ukraine

Variety	Total infection rate (%)	ASPV infection rate (%)	ACLSV infection rate (%)	ApMV infection rate (%)	Frequency of mixed infections (%)	Preferred type of infection
'Golden Delicious' (n = 100)	78.4	54.3	41.6	15.2	32.1	ASPV + ACLSV (22.4%)
'Idared' (n = 90)	74.2	48.7	41.6	12.9	27.3	ASPV + ACLSV (18.9%)

Table 4. Continued

Variety	Total infection rate (%)	ASPV infection rate (%)	ACLSV infection rate (%)	ApMV infection rate (%)	Frequency of mixed infections (%)	Preferred type of infection
'Gala' (n = 80)	67.8	42.1	35.2	11.4	23.8	Uniform distribution
'Champion' (n = 80)	63.5	36.8	32.1	9.8	18.9	ASPV monoinfection (42.1%)

Source: compared by the authors based on the conducted research using the methods of Z. Ji *et al*. (2013), L. Hao *et al*. (2016)

The highest overall susceptibility to viral infections was demonstrated by the 'Golden Delicious' variety, in which the infection rate was 78.4% of all tested samples. This indicator significantly exceeded the average values for the entire sample. The second most affected variety was 'Idared' with an infection rate of 74.2%. Significantly lower rates were observed in the varieties 'Gala' (67.8%) and 'Champion' (63.5%), which indicates their relatively higher resistance to viral pathogens.

Statistical analysis using the χ^2 criterion confirmed the significance of varietal differences in susceptibility to ASPV at a significance level of $p < 0.05$. The 'Golden Delicious' variety showed the highest sensitivity to ASPV (54.3% of infected samples), while the 'Champion' variety showed only 36.8%. For ACLSV and ApMV, varietal differences did not reach statistical significance, although the trend towards higher susceptibility of 'Golden Delicious' and 'Idared' varieties persisted.

Analysis of the structure of infections in different varieties is of interest. The 'Golden Delicious' variety was characterised by a high frequency of mixed infections, which accounted for 32.1% of all infected samples of this variety. ASPV + ACLSV combinations prevailed (22.4%), while triple infections occurred in 3.7% of cases. In contrast, monoinfections prevailed for the 'Champion' variety (71.4% of all infected samples of the variety), with ASPV monoinfection being the most common (42.1%). The 'Idared' variety showed intermediate characteristics with a frequency of mixed infections of 27.3%, but with a high susceptibility to ACLSV (41.6%). The 'Gala' variety was characterised by the most equal distribution of infection types and a moderate frequency of mixed lesions (23.8%).

It was noted that varietal differences were manifested not only in the frequency of infection, but also in the nature of clinical manifestations. In the 'Golden Delicious' variety, even monoinfections were accompanied by pronounced symptoms – intense chlorosis and deformation of the leaf plate. In the 'Champion' variety, even mixed infections occurred with minimal manifestations, which indicates the presence of effective tolerance mechanisms. Regional analysis revealed no significant differences in varietal sensitivity between different regions of Ukraine, which confirms the stability of genetically determined susceptibility regardless of growing conditions.

Correlation between symptoms and molecular diagnostic results

Comparative analysis of the results of molecular diagnostics and phytopathological observations revealed a complex and multidimensional interaction between visual symptoms and the presence of specific viral infections. Among samples with severe leaf mosaic symptoms that were characterised by typical uneven leaf plate colour with alternating light green and yellow areas, ApMV was detected only in 58.3% of cases. This fact indicates the significant involvement of other pathogens in the development of similar symptoms, in particular, some strains of ASPV and individual phytoplasmas. In approximately 23.5% of cases, mosaicism was accompanied by simultaneous detection of two or more viruses, which makes it difficult to diagnose aetiology solely based on visual observations.

In samples with symptoms of chlorosis that manifested as inter-vein yellowing or lightening along the veins, ACLSV was detected in 72.4% of cases. This confirms the key aetiological role of this pathogen in the development of this symptom complex. However, it should be noted that the intensity of chlorosis correlated with the type of infection: in mixed ACLSV + ASPV infections, symptoms were significantly more pronounced and spread over a larger area of the leaf surface. The highest correlation between the presence of symptoms and virus detection was observed for ASPV. Among samples with characteristic leaf deformity, including twisting, leaf plate asymmetry, threadiness and wavy edges, this pathogen was detected in 84.6% of cases. This high specificity makes leaf deformity a relatively reliable diagnostic marker for preliminary identification of ASPV infection in the field.

Another result of the study is the detection of a significant proportion of asymptomatic infections – 31.2% of all infected samples did not show any visible symptoms of the disease. This confirms the widespread prevalence of latent forms of infection, which is particularly dangerous for the spread of viruses through planting material. The highest percentage of asymptomatic infections was observed for ACLSV (42.9%), which is associated with the long co-evolution of this virus with apple trees and the development of mechanisms for "mitigating" pathogenesis. For ASPV, this figure was 38.5%, and for ApMV – 29.2%.

Correlation analysis revealed a weak negative correlation between symptom intensity and altitude ($r = -0.38$, $p < 0.05$). In areas located at an altitude of more than 300 metres above sea level (foothill areas of Zakarpattia and Vinnytsia regions), the intensity of symptoms was on average 25-30% lower compared to flat areas, despite the same level of infection. This indicates the influence of climatic factors, in particular, lower night temperatures and high humidity, on the expression of viral diseases. Significant varietal differences in the severity of symptoms in similar viral infections were established. For example, in the 'Golden Delicious' variety, even ACLSV monoinfection caused intense chlorosis, while in the 'Champion' variety, even mixed infections were often almost asymptomatic. This indicates a significant influence of host genetic factors on the pathogenesis of viral infections.

The use of RT-PCR has demonstrated its effectiveness for mass screening, but recent studies show that the introduction of the latest diagnostic methods is promising. In particular, J. Jiao *et al.* (2020) proposed CRISPR/Cas12a, a system for visual virus detection that can significantly speed up and simplify the testing process. Similarly, L. Yin *et al.* (2021) described the prospects of CRISPR-Cas systems for diagnosing plant viruses. The introduction of such methods in Ukrainian practice could significantly increase the effectiveness of phytosanitary control. Methodological aspects of the study indicate the need to introduce advanced approaches to the diagnosis of apple tree viruses in Ukraine. The results obtained, in particular, the high level of asymptomatic infections (31.2%) and regional features of the spread of pathogens, justify the feasibility of developing rapid test systems based on the experience of S. Franco Ortega *et al*. (2020), who developed innovative molecular methods for rapid detection of apple scab using isothermal amplification.

CRISPR/Cas methods described by S. Srivastava *et al.* (2020) demonstrated high sensitivity in detecting various plant viruses. Remote sensing technologies by Y.M. Wang *et al.* (2022) proposed an innovative approach to detecting viral infections using spectral analysis. The need for a combined diagnostic approach was emphasised by A. Kanapiya *et al.* (2024), supported by current data on the high frequency of mixed infections (25.4%). A notable aspect is the genetic characteristics of viruses. M.S. Noorani *et al.* (2023) emphasised the importance of full-genome sequencing for accurate identification of strains, which is important for Ukrainian isolates due to their high genetic diversity. The emergence of new viral pathogens, such as *Apple necrotic mosaic virus*, detected by S.U. Nabi *et al.* (2020) in India, pointed out the need for continuous monitoring of the viral spectrum in Ukrainian plantings. Viral disease management strategies proposed by A. Mandal *et al.* (2024) can be adapted to Ukrainian conditions. An important aspect is the implementation of LAMP methods similar to those developed by H.J. Lee & R.D. Jeong (2022) for ASPV detection. The economic aspect of viral infections requires a separate study, as evidenced by N. Mohammadi *et al*. (2023) on the commercial value of processed apple products.

Thus, the results obtained not only state the difficult situation with viral diseases of apple trees in Ukraine, but also lay the foundations for the introduction of advanced diagnostic methods and the development of an effective system of phytosanitary control, which forms the basis for further research and practical measures to improve the condition of apple plantations in Ukraine. The results of the study on mixed viral infections of apple trees in Ukraine demonstrate a complex structure of multiple infections with a predominance of the combination of ASPV + ACLSV (71.9% of all mixed infections), accompanied by pronounced regional differentiation and increased pathological symptoms. The revealed specificity of interaction of viral pathogens in mixed infections, in particular, the synergistic effect in the combination of ASPV + ACLSV, which is manifested by increased deformation of leaves and chloroses, makes it necessary to develop targeted treatment strategies for different regions of Ukraine, considering local characteristics of the pathogenic complex.

CONCLUSIONS

The conducted study established a high level of infection of apple plantations in Ukraine with the main viral pathogens, which is 70.6% of the total number of samples analysed. This situation indicates the epiphytotic nature of the spread of viral diseases and makes it necessary to improve the system of phytosanitary control. Among some types of viruses, a clear hierarchy of prevalence was found: the most common was *Apple stem pitting virus* with an indicator of 46.0%, followed by *Apple chlorotic leaf spot virus* – 36.0%, and *Apple mosaic virus* – 13.7%. This dynamic is fully consistent with global trends, which confirms the universality of the epidemiological properties of these pathogens.

The study also revealed regional differences in the prevalence of viral infections in Ukraine. The highest infection rates were recorded in Zakarpattia (86.0%) and Kyiv (76.3%) regions, while the lowest – in Kharkiv (56.8%). Such regional disparities are caused by a complex of factors, including climatic features, age structure of plantings, intensity of horticulture, and effectiveness of phytosanitary measures in different regions. Attention is drawn to the high rate of mixed infections, which accounted for 25.4% of the total number of infected samples. The predominant combination was ASPV + ACLSV (18.3%), and the presence of a synergistic effect in such mixed infections was proved, which is manifested in a significant increase in pathogenesis and more pronounced symptoms of the disease. In addition, significant varietal differences in susceptibility to viral infections were established, where the most sensitive varieties were ‘Golden Delicious’ (78.4%) and ‘Idared’ (74.2%), and the most persistent – ‘Gala’ (67.8%) and ‘Champion’ (63.5%).

The study found a high incidence of asymptomatic infections, accounting for 31.2% of all infected samples. The highest rates of asymptomatic course were observed for ACLSV (42.9%), which poses a threat to the uncontrolled spread of viruses through planting material and significantly complicates effective phytosanitary control. The method of molecular diagnostics based on RT-PCR developed and tested during the study demonstrated its effectiveness for mass screening and can be recommended for introduction into the practice of phytosanitary control. The specificity of the results obtained was confirmed by sequencing the selected isolates, which proves the reliability of the approach used. A limitation of this study was that it covered only three major apple tree viruses and did not include analysis of other potentially important pathogens, such as *Apple stem grooving virus* or *Apple necrotic mosaic virus*, which could have led to an incomplete assessment of the phytosanitary condition of the plantings. Prospects for further research include expanding the spectrum of detected viruses, investigating the genetic diversity of Ukrainian isolates, introducing advanced diagnostic methods, and developing a strategy for integrated protection against viral diseases.

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CONFLICT OF INTEREST

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Молекулярна ідентифікація та моніторинг основних вірусів яблуні в Україні

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Анотація. Вірусні захворювання яблуні спричиняють значні економічні втрати в садівництві України, що потребує сучасного дослідження їх поширеності. Метою роботи було оцінити поширеність та регіональний розподіл основних вірусів яблуні у промислових насадженнях України за допомогою молекулярних методів діагностики. Дослідження проводили методом зворотної транскрипції та полімеразної ланцюгової реакції на 350 зразках листя та кори яблуні, відібраних із промислових насаджень п'яти областей України. Специфічність детекції трьох цільових вірусів підтверджували секвенуванням отриманих ампліконів. В ході дослідження встановлено, що загальний рівень інфікованості яблуневих насаджень склав 70,6 %, що свідчить про епіфітотічну ситуацію. Найпоширенішим виявився вірус ямчастості деревини яблуні (46,0 %), тоді як вірус хлоротичної плямистості листя яблуні та вірус мозаїки яблуні виявлені у 36,0 % та 13,7 % зразків відповідно. Регіональний аналіз виявив максимальну поширеність вірусів у Закарпатській (86,0 %) та Київській (76,3 %) областях, тоді як мінімальні показники зафіксовані в Харківській області (56,8 %). Встановлено високий рівень змішаних інфекцій (25,4 %), з переважанням комбінації вірусу ямчастості деревини яблуні та вірусу хлоротичної плямистості листя яблуні (18,3 %). Також значущим є високий відсоток безсимптомних інфекцій (31,2 %), що ускладнює фітосанітарний контроль. Висновки підтвердили, що отримані результати обґрунтовують необхідність термінового впровадження системи молекулярного моніторингу та сертифікації посадкового матеріалу. Результати дослідження можуть бути використані для розробки національної програми оздоровлення яблуневих насаджень України

Ключові слова: полімеразна ланцюгова реакція; вірус ямчастості деревини яблуні; вірус хлоротичної плямистості листя яблуні; вірус мозаїки яблуні