Screening of cherry leaf roll virus in Sambucus nigra plants in Ukraine

Goal. Cherry leaf roll virus (CLRV) infects naturally a wide range of herbaceous and woody plants, different species of trees, shrubs, horticultural, ornamental, weed plants and causes significant economic losses in many hosts and countries. CLRV on elderberry species was reported in Europe, USA, Iran and North America. Recently this virus has been detected in sour and sweet cherry fruit orchards in Ukraine. Testing of other hosts than Prunus was performed in Ukraine only recently. The aim of the study was to test the black elderberry plants (Sambucus nigra L.) for the presence of CLRV in the Poltava region and Kyiv regions.

Methods. Sampling was carried out in the summer-autumn period in 2019—2021 in the territories of the Poltava and Kyiv regions and in Kyiv city. Samples of healthy elderberry plants were also analyzed. Visual diagnostics, enzyme-linked immunosorbent assay in DAS-ELISA modification, total RNA extraction, RT-PCR with primers for a 412 bp fragment of the 3’ untranslated region of the CLRV genome, and statistical data analysis were used in this research. PCR products were separated on an 1.5% agarose gel. Commercial CLRV preparations were used for positive controls in DAS-ELISA.

Results. Thirty three elderberry samples with symptoms of leaf rolling and mosaics of varying degrees were selected in the territory of the Poltava and Kyiv regions and in Kyiv city in 2019 and 2021 and used in the study. ELISA and RT-PCR results showed that 82% of the tested black elderberry samples were infected by CLRV.

Conclusions. The presence of CLRV, its harmfulness for elderberry plants, and Sambucus potential to serve as a reservoir for the virus indicate the necessity of testing a wider range of plant species for cherry leaf roll virus in Ukraine.
mental, weed plants, and significant economic losses in many hosts and countries due to CLRV infection, testing of other hosts than Prunus was performed in Ukraine only recently [18]. So, the aim of the study was to test the black elderberry plants for the presence of CLRV in the Poltava and Kyiv regions an in Kyiv city.

Materials and methods. Sample collection and visual diagnostics. Sampling was carried out in the summer–autumn period in 2019—2022 in the territories of the Poltava and Kyiv regions and in Kyiv city. Visual diagnostics revealed symptoms of viral infection on leaves of wild-grown black elderberry plants (Sambucus nigra L.). Samples of elderberry with symptoms and visually healthy plants were selected.

Enzyme-linked immunosorbent assay. To determine the presence of viral antigens, the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used. The analysis was performed using commercial antibodies against CLRV manufactured by Loewe (Germany) in three replicates. Samples of healthy elderberry plants were used as negative controls. Commercial CLRV preparations (Loewe, Germany) were used for positive controls. The reaction results were recorded on a Thermo Labsystems Opsis MR (USA) reader with a Dynex Revelation Quicklink software at wavelength of 405 nm. Samples with absorbance values that exceeded the negative control at least three times were considered positive [19].

RNA extraction, RT-PCR. Total RNA was extracted from symptomatic leaves using GeneJet Plant RNA Purification Kit; cDNA was synthesized using RevertAid Reverse Transcriptase (Thermo Fisher Scientific, USA) following the manufacturer’s protocol. DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA) and specific oligonucleotide primers for amplifying a 412 bp fragment of the 3’ untranslated region (3’UTR) of the CLRV genome were used: forward CLRV-5 5’-TGGCGACCGTGTAACCGGCA-3’ and reverse CLRV-3 5’-GTGGAAAGATACGTAAAAGG-3’ [20]. Amplification steps using Dream Taq Green PCR Master Mix (Thermo Scientific, USA) were performed using a Genetic Research Instrumentation LTD thermocycler (UK). The amplification reactions were set up as follows: initial denaturation for 3 min at 95°C, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 45 s. The final extension was at 72°C for 5 min. The primers are expected to amplify a 412-bp DNA fragment of CLRV 3’UTR. PCR products were separated on an 1.5% agarose gel with DNA markers CSL-MDNA-100bp (Cleaver Scientific, UK), and visualized under UV light.

Results and discussion. Thirty three elderberry samples of with symptoms of leaf rolling and mosaics were selected in the territory of the Poltava and Kyiv regions and in Kyiv city in 2019 and 2021 and used in the study (Table 1, Fig. 1-3 a). In 2022, the especially high fruit yield was observed on healthy elderberry plants in comparison with virus-infected plants (Fig. 3, b, c). ELISA results showed that 27 elderberry samples contained CLRV antigens (Fig. 4).

The results of RT-PCR are consistent with the data obtained by DAS-ELISA and demonstrate the presence of CLRV in the studied samples of elderberry (Fig. 5).

Elderberry in Ukraine and not only in our country is a valuable medicinal plant and a widely used source of dietary supplements. Our previous studies with viral diseases of purple coneflower, valerian and ginseng have shown that viruses can significantly deteriorate the

<table>
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Fig. 1. Elderberry with CLRV symptoms, Poltava region 8th June 2019: a, d — healthy; b — leaf rolling; c — mosaics and leaf rolling; e — mosaics.
quality of medicinal raw materials by reducing the amount of biologically active substances in plants [21, 2]. On the other hand, there is a danger that elderberry plants can be reservoirs of the virus. It was revealed that the isolate derived from *S. nigra* could be transmitted by sap inoculation to peach and cherry [9]. Langer et al. conducted investigation with three CLRV isolates (elderberry isolate E603, walnut isolate E326, rhubarb isolate E395) from different phylogenetic groups, which were selected and mechanically inoculated on five natural woody host plants by stem slashing. They proved that one year after inoculation the CLRV isolate from black elderberry was able to infect four out of five plant species: (*Sambucus nigra*, *Sorbus aucuparia*, *Juglans regia*, *Betula pendula*, but not *Prunus avium*) [14]. This suggests

![Fig. 2. Elderberry with CLRV symptoms, Poltava region, 30th May 2021 (a—c) and 13th June 2021 (d, e): a — a branch with mosaic leaves and a reduced inflorescence; b — an inflorescence from a healthy plant; c — leaf mosaics; d — leaf mosaics and mild leaf rolling; e — healthy branch](image1)

![Fig. 3. Elderberry with CLRV symptoms: a — CLRV mild mosaic and leaf rolling symptoms on elderberry at the flowering stage, Kyiv region, 21st June, 2021; b — severe symptoms of leaf mosaics during ripening of elderberry clusters, c — healthy plants, 29th August, 2022, Poltava region](image2)

![Fig. 4. Detection of CLRV in elderberry samples by DAS-ELISA. The commercial preparation (Loewe, Germany) was used as a positive control and a healthy-looking plant was used as a negative control. Three technical replicates (individual plants) were performed. The figure shows the average values of optical density for each sample](image3)

![Fig. 5. Detection of CLRV in the infected leaves using RT-PCR. Gel electrophoresis shows 412 bp RT-PCR fragments amplified using RNA from infected elderberry leaves with primers for the viral 3’UTR (lanes 1-8). Lane 9 contains DNA markers CSL-MDNA-100bp (Cleaver Scientific, UK). RNA isolated from a healthy-looking plant was used as a negative control (lane 10)](image4)
that for this isolate host adaptation is not stringent and/or transmission barriers between host species are developed differentially.

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CONCLUSIONS
Thus, studies conducted in the period of 2019—2021 showed that 27 samples of elderberry plants in the Poltava and Kyiv regions were affected by CLRV. The other studied samples with symptoms of mosaics of different degree and leaf rolling could also be infected by viruses but not by CLRV. These cases require further virusologic research. The results indicate the importance of further research in this field, as the presence of CLRV, its harmfulness for elderberry plants, and Sambucus potential to serve as a reservoir for the virus, indicate the necessity of testing a wider range of plant species for this virus.

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Supplementary information to this article is available online.