**Cucumber mosaic virus and turnip mosaic virus occurrence in garlic mustard in Ukraine**

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**Abstract**

Garlic mustard (*Alliaria petiolata*) is an herbaceous biennial plant native to Europe. In Ukraine, in addition to becoming a serious invader, garlic mustard can serve as a host to several viruses, which may affect agricultural crops. In view of this, the purpose of the study was to identify the virome of garlic mustard growing in Ukraine. Plant samples collected in Kyiv regions were tested for the presence of cucumber mosaic virus (CMV), turnip mosaic virus (TuMV), turnip yellow mosaic virus (TYMV), watermelon mosaic virus II (WMV), and turnip crinkle virus (TCV) by serological and/or molecular methods. According to the results found in the present study, symptomatic *A. petiolata* obtained in 2021 were infected with CMV (60%), TuMV (20%), or co-infected with CMV + TuMV (20%). TYMV, WMV II, and TCV were not detected in any of the collected samples. The cDNA fragments encoded the coat protein (CP) gene of CMV and TuMV were sequenced and named as CMV-Ap and TuMV-Ap, respectively. In phylogenetic analysis, the CMV-Ap closely resembled the German isolate MW582807 (*Sarracenia* sp.), with 99.8% nucleotide identity and belongs to subgroup II of CMV. In the phylogenetic tree, TuMV-Ap clustered with isolates AP017803, AP017764, AP017791, and JQ073722, and represented the highest identity (98.6%) to Iranian isolate IRNTRa9 (AP017803) from *Rapistrum rugosum* and Turkish isolate TUR49 (AP017872) from *Raphanus raphanistrum*. The sequences of CMV-Ap and TuMV-Ap were deposited in the GenBank under Accession Numbers MZ540213 and OM799323, respectively. The results obtained in the study indicate the important role of infected garlic mustard as alternative host and natural reservoir of CMV and TuMV from which these economically important viruses can spread to other wild and cultivated plants. This is the first molecular evidence of TuMV infection in *A. petiolata* from Ukraine.

**Keywords**: Alliaria petiolata, mixed infection, plant virus reservoirs.

**Introduction**

*Alliaria petiolata* (*Alliaria petiolata*, (M. Bieb.) Cavara & Grande, named also *Alliaria officinalis* Andr. ex. Bieb.), known as “Alliaria” or garlic mustard is a biennial herbaceous plant belonging to the mustard family (*Brassicaceae*). The genus *Alliaria*, in addition to garlic mustard includes two other species – *Alliaria auriculata* Kom. and *Alliaria brachycarpa* M. Bieb. (http://www.theplantlist.org/tpl1.1/search?q=Alliaria). *A. petiolata* is received 4 September 2022, last revision 2 March 2023, accepted 6 March 2023.

Abbreviations: ACP-ELISA - antigen coated plate ELISA; CMV - cucumber mosaic virus; CP - coat protein; CMV-Ap - CMV isolate from *Alliaria petiolata*; DAS-ELISA - double-antibody sandwich ELISA; ELISA - enzyme-linked immunosorbent assays; NJ - neighbor-joining; RT-PCR - reverse transcription polymerase chain reaction; TCV - turnip crinkle virus; TuMV - turnip mosaic virus; TuMV-Ap - TuMV isolate from *Alliaria petiolata*; TYMV - turnip yellow mosaic virus; WMV - watermelon mosaic virus.

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Conflict of interest: The authors declare no conflict of interest.
the only species of the genus *Alliaria* widespread in Ukraine. Plants grow throughout the country in forest habitats, forest edges, along roadsides and disturbed lands as well as in parks, orchards, and in field margins, often surrounding croplands (Vasilchenko and Pidotti 1975, Dobrochaeva et al. 1987).

Garlic mustard has a three-stage life cycle: seeds, rosettes, and adults. Seedlings of the plant appear in early spring and develop into rosettes during the first growing season, typically by mid-summer. Rosettes overwinter and then become adult plants in the spring. Adult plants flower, set seed, and subsequently die in midsummer. Once mature plants have produced fruit they senesce and die (Pardini et al. 2009). A single plant produces hundreds of seeds, most of which fall nearby but may be carried further by wind, water, wildlife, and people. Despite the fact that garlic mustard contains ascorbic acid and carotenoid, flavonoids, nitrogen-containing compounds, higher fatty acids, and oil (about 30% in seeds), plants has been little used in herbal medicine (Stuart 1990, Kornievsky et al. 2009). In some countries (USA, North America, and Canada) *A. petiolata* is considered as one of the most notorious invasive plants that actively displace local plants and, forming dense monocultures, reduce the biodiversity and aesthetic value of natural areas. The impact of plants on ecosystems is long-lasting, as plants produce secondary metabolites that can exert negative effects on native plants, insects, and microbes causing changes in species composition and ecosystem processes (Wolfe et al. 2008). In addition, the environmental impact of the species is determined of its ability to serve as a host plant to several viruses – cucumber mosaic virus (CMV, *Potyviridae*; *Cucumovirus* (Brčák and Polák 1963, Boehm and Nameth 2000, Jerković-Mujkić et al. 2017), turnip mosaic virus (TuMV, *Potyviridae*; *Potyvirus*) (Horvath et al. 1975, Lisa and Lovisolo 1976, Stobbs and Van Schagen 1987, Lockhart 2012), white clover mosaic virus (WCIMV, *Alphaflexiviridae*; *Potexvirus*) (Polák 1985, Zhao et al. 2016), turnip vein-clearing virus (TVCV, *Virgaviridae*; *Tobamovirus*) (Lockhart et al. 2014, Tóth et al. 2019), turnip crinkle virus (TVC, *Tobamoviridae*; *Betacarmovirus*) (Gaafar et al. 2019), and turnip yellow mosaic virus (TYMV, *Tymoviridae*; *Tymovirus*) (Pelikánová 1990, Pelikánová et al. 1990), which may affect horticultural plants and a wide range of agricultural crops.

Since the role of *A. petiolata* as virus reservoir in Ukraine has not yet been studied, the purpose of this work was to identify the virome of garlic mustard growing in Ukraine. Turnip mosaic virus, white clover mosaic virus, and turnip vein-clearing virus are currently known to be found in garlic mustard in the USA, while cucumber mosaic virus, turnip crinkle virus, and turnip yellow mosaic virus have been found in Europe. In the present study, we have focused our attention on two cosmopolitan and important viruses infecting field-grown vegetable crops and weeds – cucumber mosaic virus and turnip mosaic virus. In addition, the plants have also been tested for TCV and TYMV, common on garlic mustard in Europe. Although watermelon mosaic virus II (WMV II, *Potyviridae*; *Potyvirus*) has not been reported to infect garlic mustard, samples were also tested for this virus, given that WMV II has a wide range of host plants, including more than 170 plant species from 27 different families (Hajizadeh and Mohammadi 2016), and is widely distributed in various crops growing in Ukraine (Shevchenko et al. 2021).

Materials and methods

**Sample source, data collection:** During a survey in May and June of 2020 - 2021 growing seasons, symptomatic plants of the weed *A. petiolata* showing vein banding, mosaic, and leaf deformation were sampled from ruderal sites of five Kyiv regions for subsequent virus screening (Fig. 1). In 2020, we sampled only one location (number 1 on the sampling map), while in 2021 we repeated a targeted sampling of plants from this location and also collected 5 samples (4 from plants showing virus-like symptoms and 1 from healthy, asymptomatic plants) from each of the additional 4 locations. Thus, in site 1, we sampled the same plants of the first and second years of vegetation (5 in 2020, and 5 in 2021). A total of 30 samples were collected in 2020 - 2021 from symptomatic and healthy plants. Sap from infected and healthy plants was extracted in 0.01 M potassium phosphate buffer (pH 7.0) and used for infectivity assays and electron microscopy.

**Transmission electron microscopy (TEM):** Droplet of sap from infected *A. petiolata* was mounted on formvar coated electron microscope grid (200 mesh), stabilized with evaporated carbon film for 30 s. After washing, the grids were negatively stained with 1% (m/v) uranyl acetate and observed under the electron microscope Jeol JEM 1010. (Fig. 1). In 2020, we sampled only one location (number 1 on the sampling map), while in 2021 we repeated a targeted sampling of plants from this location and also collected 5 samples (4 from plants showing virus-like symptoms and 1 from healthy, asymptomatic plants) from each of the additional 4 locations.
acetate and examined with an electron microscope (JEM 1400, JEOL, Tokyo, Japan) (Milne 1993). Transmission electron microscopy studies were conducted at the Center for Collective Use of Scientific Equipment of National Academy of Sciences of Ukraine.

Experimental host range assays: For host-range studies, inoculated leaf extract was inoculated mechanically to different plant species, namely Cucumis sativus, Cucurbita pepo, Capsicum annuum, Brassica oleracea var. capitata, Brassica oleracea var. botrytis (cauliflower cv. Early Harvest), B. napus cv. Redstone, Nicotiana tabacum cv. Samsun NN, Nicotiana rustica, Phaseolus vulgaris cv. Top Crop, Chenopodium amaranticolor, and Chenopodium quinoa. Eight plants from each plant species were inoculated and kept under greenhouse conditions (at 22°C, natural daylight). Plants were regularly inspected for symptom development for up to 30 d post inoculation.

Enzyme-linked immunosorbent assays (ELISA): The preliminary screening was performed by antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA), using wide-spectrum monoclonal antibodies as described by Liu et al. (2017). ACP-ELISA kits (RT-0573/1, DSMZ, Braunschweig, Germany) were used for the genetic serological detection of potyviruses (73 species of the genus Potyvirus) in infected plant tissues. Leaf samples were also tested by double-antibody sandwich ELISA (DAS-ELISA) for CMV, TYMV, TuMV, and WMV II infection using the phytodiagnostic ELISA kits (Loewe Biochemica, Sauerlach, Germany). ACP- and DAS-ELISA were performed according to the manufacturers' guidelines as described by Clark and Adams (1977), Ward et al. (2004), and Liu et al. (2017). Absorbance at 405 nm were measured using a Termo Labsystems Opxis MR microtitre plate reader (Thermo Fisher Scientific, Waltham, MA) with Dynex Revelation Quicklink software (DYNEX, Zelenopole, USA). Absorbance values, greater than three times those of the negative controls were considered positive. The samples scored as positive by DAS-ELISA used for the subsequent analysis by reverse transcription polymerase chain reaction (RT-PCR).

RT-PCR and sequencing: Nucleic acid extracts were tested by RT-PCR for the presence of virus RNA using the three sets of universal primers for detection of CMV (CMV-F/R; 5'-TATGATAAGAAGCTTGTTTCCGCA-3'; 5'-TTTAGCGTAAAGCCTGGATGGCAACC-3') (Bariana et al. 1994), TCV (HZ632/HZ633 5'-AAAGGCCAATAFCTGGGTGGA-3'; 5'-TAAGGTTT GCCGGCTAG65-3') (Gaffor et al. 2019), and TuMV (CP-F/R; 5'-TATACACGGCGGAGGAAGC-3'; 5'-CGCAGTCTGTGCTTTCAT-3') (Lockhart 2012). Total RNA was extracted from 0.5 g of symptomatic or healthy leaf tissues using AmpliSens Ribo-Sorb DNA/RNA extraction kit (AmpliSens, Moscow, Russia). The RT was performed with AmpliSens Reverta-L-100 (AmpliSens), according to the manufacturer’s instruction. For PCR, 10 - 50 ng of the template genomic DNA was amplified in total volume of 20 mm³ containing 1× reaction buffer with 1.5 mM MgCl₂, 5 pmol of each primer, 0.2 mM dNTPs, 0.5 U Taq polymerase, and nuclease-free water. Reactions were performed under the following conditions: initial denaturation at 95°C for 3 min, thermal cycling for 35 cycles (1 min at 94°C, 1 min at temperature optimal for each primer pairs, and 1 min 30 sec at 72°C), ending with the final extension at 72°C for 5 min. PCR products of amplification were visualized in 1.7% (m/v) agarose gels with Tris-borate-EDTA (TBE) buffer and ethidium bromide (0.5 mg cm⁻³). The resulting PCR products of CMV and TuMV (500 bp and 463 bp fragments, respectively) were sequenced by Sanger dideoxy sequencing method using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Waltham, Massachusetts, USA). The samples were run on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

Phylogenetic analyses: The sequence homology was checked by NCBI BLASTn tool (http://www.ncbi.nlm.nih.gov/) with CMV and TuMV sequences available in GeneBank. Isolates/strains associated with subgroup I (A/B) and II of CMV were also used for differentiating of CMV isolate. Pairwise sequence comparisons along with nucleotide identities of selected sequences were calculated by CLUSTAL W (Thompson et al. 1994). The phylograms were constructed in the MEGA X software package (Kumar et al. 2018) using the Jukes-Cantor model for calculation of evolutionary distances and the Neighbor-joining (NJ) method with 1 000 bootstrap replicates.

Results
In the year 2020 severe virus-like mosaic symptoms – vein banding, mosaic, and leaf deformation were observed on several A. petiolata plants growing along road-side in one of the districts of Kyiv (location 1 on the sampling map, Fig. 1). During 2021 growing season, field surveys were conducted at this and an additional four ruderal sites of Kyiv regions, and symptomatic A. petiolata plants showing conspicuous virus-like symptoms were collected. Leaf tissue extracts obtained from individual diseased plants (n = 4 from location 1 in 2020 and n = 20 from locations 1 - 5 in 2021) as well as from asymptomatic ones (n = 1 from location 1 in 2020 and n = 5 from locations 1 - 5 in 2021) were used for subsequent virus screening. Sampling sites are numbered in the article from 1 to 5; sampling site 1 was the same in 2020 and in 2021 (Table 1).

The plants collected in the surveyed sites in 2021 differed in the symptoms of the disease; therefore, they were divided into two groups. Plants of one group collected in four of the five study areas, exhibited symptoms of intercellular chlorosis, mosaic, bleaching, and green vein-banding. No crinkling symptoms were observed and no strong deformity, although the leaf blistering and waving of the edge were in some leaves very pronounced (Fig. 2A-D). While the plants of the other group showed symptoms of the leaf blistering, leaf malformation, and rugosity (Fig. 2E,F). The stems of second-year infected plants of both groups were small, with short internodes,
and in general the plants looked noticeably stunted. Whereas the symptoms of the disease differ in plants of two groups, it can be assumed that garlic mustard infection is caused by different virus types.

Preliminary screening was conducted by ACP-ELISA using wide spectrum monoclonal antibodies for serological detection of potyviruses. It was shown that 40% of the samples collected in 2021 and all samples collected in 2020 were potyvirus-positive (Table 1). After prescreening with ACP-ELISA, these samples were tested by DAS-ELISA specifically for TuMV and WMV II. All specimens were also tested by DAS-ELISA for CMV/TYMV and by RT-PCR for TCV. According to the results of serological tests, all potyvirus-positive samples of *A. petiolata* collected in years 2020 and 2021 were infected by TuMV whereas watermelon mosaic virus II and turnip yellow mosaic virus were not identified (Table 1).

Results of PCR and ELISA obtained in 2021 indicated that out of 20 symptomatic *A. petiolata*, 12 (60%) were CMV positive, whereas 4 (20%) were TuMV positive. Co-infection of CMV and TuMV was diagnosed in 20% of samples. Moreover, co-infected mustard plants showed an enhanced accumulation of CMV in DAS-ELISA-treated samples: the absorbance value (A\textsubscript{405}) was higher in these plants compared to plants infected only with CMV. Thus, CMV was detected in 80% of the analyzed samples, 60% of which were monoinfected. Asymptomatic garlic mustard plants were negative in ELISA for CMV, TuMV, TYMV, WMV II, and TCV viruses.

By a transmission electron microscopy, icosahedral virions of 28 - 30 nm in diameter, characteristic of viral particles belonging to *Cucumovirus*, *Betacarmovirus*, and *Tymovirus* genera were observed in crude plant sap preparations obtained from garlic mustard of the first group (growing in four out of five sampling sites) (the data not provided) (King *et al*. 2012). Plants of this group, as noted above, were scored as positive in DAS-ELISA using CMV-specific antibodies and negative for TYMV infection. Turnip crinkle virus was not detected in examined samples by RT-PCR using a pair of primers HZ632/HZ633 (Gaafar *et al*. 2019). Flexuous rod-shaped virus particles about 750 nm in length typical of the family *Potyviridae* were observed in negatively stained infected tissue of the second plant group (growing in location 1 and 5).

Table 1. Serological tests of *Alliaria petiolata* samples with virus-like symptoms (Mo - mosaic, Chl - chlorosis, LB - leaf blistering, GVB - green vein-banding; NCS - no crinkling symptoms, LM - leaf malformation. Sampling site 1 in 2020 is the same as in 2021.)

<table>
<thead>
<tr>
<th>Year/site</th>
<th>Samples examined</th>
<th>Symptoms</th>
<th>Positive by ACP-ELISA for Potyvirus genus</th>
<th>Positive by DAS-ELISA for TuMV</th>
<th>WMV II</th>
<th>CMV</th>
<th>TYMV</th>
</tr>
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<tr>
<td>2020/1</td>
<td>4</td>
<td>Mo, Chl, LB, GVB</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2021/1</td>
<td>4</td>
<td>Mo, Chl, LB, GVB</td>
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<td>4</td>
<td>0</td>
</tr>
<tr>
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<td>4</td>
<td>Mo, Chl, GVB, NCS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2021/3</td>
<td>4</td>
<td>Mo, Chl, GVB, NCS</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>2021/4</td>
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<td>Mo, Chl, GVB, NCS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2021/5</td>
<td>4</td>
<td>LB, LM</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2021/total</td>
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<td></td>
<td>8 (40%)</td>
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<td>0</td>
<td>16 (80%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Disease symptoms on *Alliaria petiolata* (A-G) and *Nicotiana benthamiana* (H) plants used in this study: A,B - mixed infection of CMV+TuMV (A - first-year plants observed in 2020, B - second-year plants observed in 2021); C,D - first-year plants infected by CMV; E,F - first-year plants infected by TuMV; G - leaf of symptomless plant; H - symptomless *Nicotiana benthamiana*, artificially infected by CMV.
VIRUS OCCURRENCE IN GARLIC MUSTARD

Discussion

Cucumber mosaic virus is one of the most widespread plant viruses. Besides a large number of horticultural crops, CMV infects many weed species. In Ukraine, CMV has been detected on a various host plants, and as our previous study showed, the virus can infect naturally garlic mustard as well (Krychenko et al. 2022). The natural occurrence of TuMV infection in *A. petiolata* has been confirmed in Europe (Horvath et al. 1975, Lisa and Lovisolo 1976) and Canada (Stobbs and Van Schagen 1987) since 1975. In Ukraine researches exploring the occurrence of viruses in wild plants and, in particular, in garlic mustard, have not been conducted. According to the results of biological assay, serological, and molecular detection presented here (Table 1, Fig. 3) CMV is widespread in central Ukraine (Kyiv region) with 80% incidence among symptomatic

![Image](image_url)
plants surveyed in the study. This is consistent with our previous result on the detection of CMV in A. petiolata in Ukraine (Kyrychenko et al. 2022). It is noteworthy that 20% of CMV-positive samples were co-infected with TuMV. The incidence of TuMV among the studied samples was 40%, and half of these TuMV-positive samples were also infected with CMV. To our knowledge, this is the first report of TuMV isolate naturally infecting garlic mustard in Ukraine. None of the other analyzed viruses (TYMV, WMV, TCV) was detected in any sample under study.

CMV and TuMV are naturally transmitted by aphids (Hemiptera, Aphidoidea) in a non-persistent manner. At least 89 species of aphids are efficient vectors for TuMV and 80 species for CMV (Sevik 2017, Lu et al. 2022). TuMV is transmitted at a higher rate both by green peach aphid (Myzus persicae) and cabbage aphid (Brevicoryne brassicaceae). The main transmission mediators of CMV are Myzus persicae and cotton aphid Aphis gossypii although Brevicoryne brassicaceae can also be an active vector of the virus (Palukaitis and García-Arenal 2003, Garzo et al. 2004, Sevik 2017). It has been shown that aphids of two species, Myzus persicae and Aphis gossypii, can transmit either a single virus (TuMV or CMV) or both viruses (TuMV and CMV) simultaneously (Fujisawa 1985). All three species (green peach, cotton, and cabbage aphids) are widespread throughout the world, including Ukraine, and can be effective vectors of these viruses in the field.

Fig. 4. Phylogenetic trees based on nucleotide sequences of CP domain of CMV (A) and TuMV (B) from Ukraine along with reported CMV or TuMV isolates, respectively. The analyses were conducted using the MEGAX software, the Jukes-Cantor evolutionary model and the NJ method with 1 000 bootstrap replicates.

Fig. 5. Phylogenetic relationships of partial CP sequences of CMV-Ap isolate with 15 RNA 3 sequences of CMV isolates, determined by MEGAX software package using the Jukes-Cantor evolutionary model and the NJ method with 1 000 bootstrap replicates.
Despite the fact that previous reports on the detection and characterization of the CMV showed the prevalence in Ukraine viruses of subgroup I (Shevchenko et al. 2015, Sovinska et al. 2022), the present study showed that the CMV-Ap belongs to subgroup II strains. Thus, the CMV isolates distributed in Ukraine belong to subgroups that have a worldwide distribution (IA or II) as well as to subgroup restricted mainly to Asia (IB) (Miftakhurohmah and Wahyuno 2017).

In Ukraine, TuMV has been detected in Brassica oleracea var. capitata, Raphanus sativus, Sinapis alba, Brassica juncea, Camelina sativa, and Bifuris orientalis (Shevchenko et al. 2016, 2018) and this study shows for the first time the occurrence of TuMV in A. petiolata. Based on phylogenetic analyses, TuMV-Ap isolate was clustered along with isolates AP017803, P017764, AP017791, and JQ073722 from Iran. At the same time, Ukrainian isolates LC537585, LC537586, LC537587, LC537588, LC537589, and LC537590 out TuMV fell into distinct clade. Ukrainian isolate UKR9, previously reported in the Kyiv region (Shevchenko et al. 2018), have relation to Turkish isolates, while the TuMV-Ap is very closely related to Iranian isolates. UKR9 biologically was classified into BR host type, isolates of which infect both Brassica and Raphanus plants systemically with mosaic symptoms (Nguyen et al. 2013). Unfortunately, we have not established the host infecting type of TuMV-Ap although the question of the genetic diversity of TuMV populations in Ukraine is very important for understanding evolutionary history of this virus. Nevertheless, our results may provide evidence that more than one TuMV lineage circulates in Ukraine.

Mixed infection of CMV and potyviruses has been studied by many scientists (Wang et al. 2002, Fattouh 2003, Mascia et al. 2010, Fukuzawa et al. 2010, Barbosa et al. 2016, Arogundade et al. 2018, Salehzadeh 2018). As a rule, researchers reported about synergistic effect on different hosts. Accumulating of CMV to a considerably higher amount and reduction of potyvirus accumulation in mixed infection has also been reported by other authors (Poolpol and Inouye 1986, Sano and Kojima 1989). The results obtained in the study also showed an increase in CMV accumulation in doubly infected mustard plants. Obviously, the synergistic interactions of potyviruses with CMV as well as with viruses belonging to other genera in Alliaria sp. are the subject of a more detailed study.

Turnip crinkle virus is not widespread in the world. Based on the information available in the literature, the virus is distributed in the former Yugoslavia, Germany (on garlic mustard), and the United Kingdom (https://www.cabi.org/isc/datasheet/54292). To our knowledge, a survey of distribution of TCV in Ukraine has not been conducted by the time. Though we did not find the virus in garlic mustard, it is obvious that the monitoring of the virus should be carried out in both cultivated plants and weeds, including garlic mustard.

Thus, the results presented in this paper indicate that biennial Alliaria petiolata plants are affected by CMV and TuMV in mono- and co-infection and therefore can play an important role in survival of these viruses acting as potential pathogen reservoirs for virus transmission onto crop fields by vectors. Since these plants can be important sources of primary virus inoculum, management of the CMV and TuMV diseases may be feasible through weed control.

References

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