

Short Communication: First Report Of Aphid-Borne Viruses Infecting Raspberry (*Rubus Idaeus* L.) In Bosnia And Herzegovina

Dejana Stanić^{1*}, Mihajlo Voruna^{2*}, Anđa Radonjić³, Stefan Bojić¹, Duška Delić^{2**}

¹University of East Sarajevo, Faculty of Agriculture
Vuka Karadžića 30, 71123 East Sarajevo, Bosnia and Herzegovina

²University of Banjaluka, Faculty of Agriculture
Bulevar vojvode Petra Bojovica 1a, 78000 Banjaluka, Bosnia and Herzegovina

³University of Belgrade, Faculty of Agriculture
Nemanjina 6, 11000 Belgrade, Republic of Serbia

*All authors equally participated in the work

**Corresponding author: duska.delic@agro.unibl.org

Abstract

During 2024, surveys were conducted after sampling aphids and plants that bore virus-like symptoms in six locations in the most important raspberry production area in Bosnia and Herzegovina (BiH). The identity of the caught aphids was checked using morphological characteristics of adults and employing molecular methods such as PCR on COI region and sequence analyses. Moreover, plant samples were also molecularly checked for the presence of the aphid-borne viruses. All analyses confirmed the presence of *Amphorophora idaei* and *Aphis idaei* in four and two the surveyed raspberry orchards, respectively and rubus yellow net virus (RYNV) in two locations (four orchards). According to the available data this is first report of the presence and distribution of raspberry aphids in BiH. This will contribute to a better understanding of virus diseases and their vectors in raspberry production in BiH.

Keywords: vector, RMD, morphological identification, PCR, COI, sequence analyses

Zusammenfassung

Kurzmitteilung: Erster Nachweis von blattlausübertragenen Viren an Himbeere (*Rubus idaeus* L.) in Bosnien und Herzegowina. Im Jahr 2024 wurden in sechs Standorten im wichtigsten Himbeeranbaugebiet in Bosnien und Herzegowina (BiH) Erhebungen nach Probenahme von Blattläusen sowie von virusähnlich symptomatischen Pflanzen durchgeführt. Die Identität der gefangenen Blattläuse wurde anhand morphologischer Merkmale adulter Individuen sowie mittels molekularbiologischer Methoden überprüft, darunter PCR der COI-Region und Sequenzanalysen. Zudem wurden Pflanzenproben molekularbiologisch auf das Vorkommen von durch Blattläuse übertragenen Viren untersucht. Sämtliche Analysen bestätigten das Vorhandensein von *Amphorophora idaei* und *Aphis idaei* in vier bzw. zwei der untersuchten Himbeeranlagen sowie des *Rubus yellow net virus* (RYNV) an zwei Standorten (insgesamt vier Anlagen). Nach den vorliegenden Daten handelt es sich hierbei um den ersten Nachweis des Vorkommens und der Verbreitung von Himbeerblattläusen in BiH. Dies trägt zu einem besseren Verständnis von Viruskrankheiten und deren Vektoren im Himbeeranbau in BiH bei.

Schlagwörter: Vektor, RMD, morphologische Identifikation, PCR, COI, Sequenzanalysen

Introduction

Raspberry (*Rubus idaeus* L.) is an economically important perennial crop for Bosnia and Herzegovina (BiH) with the annual production of 16,833 tons in 2023 (Anonymous, 2023), ranking it 9th in world raspberry production by country. Raspberry and other *Rubus* spp. are particularly susceptible to many pathogens including viruses (Wilson, 2014). Viruses infecting raspberry are frequently transmitted through vectors, and propagation material (Matthew and Hull, 2002). Aphids are a well known vector of various plant viruses including raspberry viruses (Martin et al., 2013). Raspberry is infected with multiple viruses among which aphid transmitted viruses are responsible for most of the decline, especially black raspberry necrosis virus (BRNV), raspberry leaf mottle virus (RLMV) and rubus yellow net virus (RYNV) all of which cause raspberry mosaic disease (RMD) (Alford, 2007; Converse et al., 1987). RMD is used to describe a range of diseases caused by the various combinations of viruses transmitted by aphids (Wilson, 2014). The viruses of RMD complex are transmitted by *Amphorophora* (*Am.*) *agathonica* Hottes in North America and *Am. idaei* Börner in Europe (Martin et al., 2013). Beside these viruses, raspberry vein chlorosis virus (RVCV), which is transmitted by *Aphis* (*Ap.*) *idaei* van der Goot (Martin et al. 2013), is another important aphid borne virus. In BiH so far, the main causal agents of RMD and raspberry leaf blotch virus (RLBV), responsible for leaf blotch disease, have been identified throughout the country on raspberries (Delic et al., 2020; Radulovic et al., 2021; Voruna et al., 2023a). Vector borne viruses have a close relationship with their vector, which is their primary means of transmission to a new host in the orchard (Lightle and Lee, 2014). However, there is a lack of any reports about presence and distribution of raspberry aphids in BiH, so there is a big gap in the RMD epidemiology and in disease management, consequently. Thus, a better virus management strategy is necessary to propagate and cultivate healthy raspberries in BiH. Having this in mind, the main objective of the study was to perform a survey on the occurrence

and distribution of aphids as potential vectors of RMD (*Am. idaei* and *Ap. idaei*) in selected areas of BiH.

Material and Methods

Surveys were carried out between June and July 2024, in different raspberry growing sites of BiH mainly located in a region called Podrinje, which is the main raspberry growing area in the country (Tab. 1). Raspberry plants ("Miker" and "Willamette" varieties) were visually inspected for presence of virus symptoms, while shoots with leaves that had aphids (Fig. 2-3) were collected. Aphids were collected from six raspberry orchards (Tab. 1). The presence of aphids was determined by a detailed examination of the aerial parts of the plants, primarily the tops and abaxial surfaces, apical shoots and stems. The following accessories for collecting aphids were used: transparent plastic boxes of different sizes with an opening for air to enter, plastic bags, a pocket magnifier and scissors. Samples were taken by cutting off part of the plant and carefully placing them in plastic boxes. The aphids collected in the raspberry orchards were brought to the laboratory alive, along with the part of the plant they were on, so that the larvae would develop into adults. The samples consisted of larvae, and wingless and winged adults. The aphids were carefully examined under a stereo microscope (ZOOM mikroskop Leica EZ4HD) using 30x magnification and identified using keys for identification of apterae aphids (wingless morphs) (Blackman and Eastop, 2000) and alatae aphids (winged morphs) (Remaudière and Seco Fernandes, 1990). The total DNA of identified single aphid specimens (eight in total) was isolated using the DNeasy® Blood & Tissue Kit (QUIAGEN, Hilden, Germany), according to manufacturer's instructions. For the PCR analyses primers LCO1490/HCO2198 amplifying the mtCOI fragment were employed (Folmer et al., 1994). Further identification was done by Sanger sequencing of

obtained PCR products in MacroGen Europe BV. All sequences were blasted at BLAST: Basic Local Alignment Search Tool, using the megablast algorithm and default search parameters (Altschul et al., 1990). 20 symptomatic leaves were collected from each raspberry sampling site and pooled into one sample for further laboratory analyses. Leaf samples taken from each raspberry production site were submitted to the total nucleic acids (TNA) extraction according to Poudel et al. (2013) and reverse transcribed using random primers and the M-MLV Reverse Transcriptase (Sigma-Aldrich). cDNA quality was evaluated using the NADH dehydrogenase ND-2 subunit (*ndhB* gene) transcript as an internal control (Tzanetakis et al. 2007).

The presence of BRNV was checked according to Radulovic et al. (2021), RLMV following Tzanetakis et al. (2007) procedure and RVCV using primers described in McGavin et al. (2011) in RT-PCR, while PCR amplifying conserved region of the reverse transcriptase and ribonuclease H genes (Diaz-Lara et al., 2015) was employed for RYNV detection.

Results and Discussion

In the observed raspberry orchards symptoms such as leaf chlorosis and leaf blotching (Fig. 1) were commonly encountered.



Fig. 1: Virus symptoms on raspberry (orig.)



Fig. 2: *Aphis idaei* (orig.)



Fig. 3: *Amphorophora idaei* (orig.)

A total of 126 of aphid individuals were collected (Tab. 1). During surveys, following morphological identification keys (size and color of body, siphunculi, cauda, antennae) two aphid species were determined, the small raspberry aphid (*Aphis idaei*) (Fig. 2) and large raspberry aphid

(*Amphorophora idaei*) (Fig. 3) on both sampled varieties. The highest number of aphids of both species were determined at the end of June and the beginning of July. In that period, winged and wingless aphids, as well as their larvae, were found (Tab. 1).

Tab. 1: Number of aphids collected at each location

Numb.	Location	Collection date	Species	Number of aphids
1.	Bratunac/Kravica (44°12.427' N, 019°12.885' E)	21.6.2023.	<i>Amphorophora idaei</i>	4 adults
2.	"Willamette" varieties	28.6.2023.	<i>Amphorophora idaei</i>	20 (12 adults, 8 larvae)
3.	Bratunac/Krasan polje (44°14.646' N, 019°20.012' E) "Miker" varieties	7.7.2023.	<i>Amphorophora idaei</i>	32 (22 adults, 10 larvae)
4.	Bratunac/Bjelovac (44°09.139' N, 019°24.662' E) "Willamette" varieties	12.7.2023.	<i>Amphorophora idaei</i>	15 (10 adults, 5 larvae)
5.	Miljevina (43° 30' 59.99" N, 18° 38' 59.99" E)	27.6.2023.	<i>Aphis idaei</i>	27 (21 adults, 6 larvae)
6.	"Willamette" varieties	04.7.2023.	<i>Aphis idaei</i>	17 (12 adults, 5 larvae)
7.	Ustikolina (43° 35' 0.17" N, 18° 46' 53.62 E) "Miker" varieties	27.6.2023.	<i>Amphorophora idaei</i>	9 (6 adults, 3 larvae)
8.	Vojkovići (43° 47' 26.99" N, 18° 20' 22.20 E) Willamette" varieties	7.7.2023.	<i>Aphis idaei</i>	2 adults

The amplification of the COI gene provided fragments with a size of 730 bp in seven tested aphid samples. The COI sequences from all the amplified samples are available in GenBank. The BLAST analyses of the sequences with Acc. Nos. PQ471321- PQ471324; PQ471327 revealed that they were 99,72% identical with the *Am. idaei* specimen collected from Belarus (GenBank Acc.No. JF340095) and sequences with Acc. No. PQ471325 and PQ4713262 showed highest identity of 100% between themselves and with *Ap. idaei* specimen from France (GenBank Acc.No. KF638947). Only one virus, RYNV was detected in four raspberry orchards in Bratunac and Miljevina both on "Willamette" variety. For the first-time, this study provided evidence for the presence of *Am. idaei* and *Ap. idaei* in BiH raspberry orchards. The former, *Am. idaei*, is responsible for transmitting

BRNV, RLMV and RYNV. All three viruses were found in BiH in raspberries during previous surveys in which RYNV was found to be the most prevalent (data not shown). *Ap. idaei*, the confirmed vector of RVCV, was also confirmed, although the virus presence has not been detected so far. *Ap. idaei* is spread throughout most of Europe, western Siberia, New Zealand and North America (Gordon et al., 1997). It is widespread in raspberry orchards in Croatia (Gotlin Čuljak et al., 2012), Montenegro (Hrnčić and Radonjić, 2012) and Serbia (Petrović-Obradović, 2003). Aphids are of light green color, 1.2 - 1.8 mm length and are covered with wax powder (Blackman and Eastop, 2000). This species develops numerous colonies on the tips of the shoots, causing deformation and twisting of the leaves (Gotlin Čuljak et al., 2012; Stanić and Spasić, 2024), which is in accordance with the results of

our research because the colonies were located on the mentioned plant organs of raspberries. *Am. idaei* is the most economically important aphid pest of commercially grown raspberry cultivars in the U.K. and Northern Europe (McMenemy, 2009). It is often present in raspberry orchards in Croatia (Gotlin Čuljak et al., 2012) and Serbia (Petrović-Obradović, 2003; Tanasković et al., 2012). The body length of adults is in the range 2.5–4.1 mm (Blackman and Eastop, 2000). This species does not form colonies, but individuals inhabit young shoots or raspberry leaves without causing leaf deformation (Borowiak-Sobkowiak, 2006; Stanić and Spasić, 2024). The literature is consistent with our research regarding finding individuals on the abaxial surfaces of raspberry leaves without forming colonies. In previously published studies RYNV has been found to be widely distributed in raspberry orchards including orchards from the surveyed locations in BiH and has mostly been found integrated rather than episomal (Voruna et al., 2023a; 2023b). RYNV, is one of the important

aphid borne viruses contributing to raspberry mosaic disease, in combination with BRNV and RLMV. Due to the small amount of the collected aphids in the present study, the presence of RYNV could not be checked so it cannot be used to claim the total absence of this virus in *Am. idaei*. The connection of the symptoms with the virus presence could not be made either because symptoms of blotching were probably connected with RLBV which is commonly found in the country (Delic et al., 2020).

Finally, this small-scale study presents the first step in the management of the RMD in BiH. Detailed aphid life cycles should be better studied in order to design proper control measures. However, infected planting material still represents the main threat for virus introduction and spread. Due to their limited mobility, aphids are primarily responsible for virus transmission at a local scale or within orchards. Further research should be dedicated to the detailed study of the identified aphids' life cycles and possible control measures.

Declarations

Ethical Approval: We hereby confirm that there are no potential conflicts of interest related to this work. No human or animal participants were involved in the research, and no issues related to informed consent arise.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Acknowledgments: This work has been partially conducted through funds of Ministry of Agriculture, Forestry and Water resources of Republika Srpska.

References

Alford, D.V. 2007: Pests of Fruit Crops – A Colour Handbook. U.K.: Manson Publishing

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. 1990: Basic local alignment search tool. *Journal of Molecular Biology* 215(3): 403-10. PMID: 2231712. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)

Anonymous 2023: World Raspberry Production by Country, <https://www.atlasbig.com/en-us/countries-raspberry-production>.

Blackman, R.L., Eastop, V.F. 2000: Aphids on the World's Crops: An Identification and Information Guide. U.K.: John Wiley & Sons Ltd

- Borowiak-Sobkowiak, B.** 2005: Bionomy and ecology of *Aphis idaei* v.d. Goot on raspberry. *Aphis and Other Hemipterous Insects* 11: 5–16.
- Borowiak-Sobkowiak, B.** 2006: Bionomy and ecology of *Amphorophora idaeis* (BÖRN.) on raspberry. *Journal of plant protection research* 46 (2): 169-180.
- Converse, R. H.** 1987: *Virus Diseases of Small Fruits*: United States Department of Agriculture Handbook No. 631. Washington, District of Columbia: U.S. Government Printing Office
- Delić, D., Radulović, M., Vakić, M., Sunulahpašić, A., Villamor, D.E.V., Tzanetakis, I.E.** 2020: Raspberry leaf blotch emaravirus in Bosnia and Herzegovina: population structure and systemic movement. *Molecular Biology Reports* 47(6): 4891-4896. doi: 10.1007/s11033-020-05560-x.
- Diaz-Lara, A., Mosier, N.J., Keller, K.E., Martin, R.R.** 2015: A variant of *Rubus* yellow net virus with altered genomic organization. *Virus Genes* 50 (1): 104–110. <https://doi.org/10.1007/s11262-014-1149-6>.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R.** 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology* 3: 294–299. <https://pdfs.semanticscholar.org/943d/38b9d96f8222e883604822bcafb7930ca6da.pdf>.
- Gordon, S.C., Woodford, J.A.T., Birch, A.N.E.** 1997: Arthropod pests of *Rubus* in Europe: pest status, current and future control strategies. *The Journal of Horticultural Science and Biotechnology* 72: 831–862.
- Gotlin Čuljak, T., Grubišić, D., Mešić, A., Juran, I.** 2012: List of aphids (Homoptera: Aphidoidea) and their host plants in Croatia. *Natura Croatica* 21 (1): 191-221.
- Hrnčić, S., Radonjić, S.** 2012: A survey of raspberry pest in Montenegro. *ISHS Acta Horticulturae* 946: X International *Rubus* and *Ribes* Symposium. doi: 10.17660/ActaHortic.2012.946.38.
- Lightle, D., Lee, J.** 2014: Raspberry viruses affect the behavior and performance of *Amphorophora agathonica* in single and mixed infections. *Entomologia Experimentalis et Applicata* 151 (1): 57–64. <https://doi.org/10.1111/eea.12170>.
- Martin, R.R., MacFarlane, S., Sabanadzovic, S., Quit, D., Poudel, B., Tzanetakis, I.E.** 2013: Viruses and virus diseases of *Rubus*. *Plant Disease* 97 (2): 168-182. doi: 10.1094/PDIS-04-12-0362-FE
- Matthews R.E.F., Hull, R.** 2002: *Matthews' plant virology*. San Diego: Academic Press, 4th Ed.
- McGavin, W. J., Cock, P. J. A., MacFarlane, S. A.** 2011: Partial sequence and RT-PCR diagnostic test for the plant rhabdovirus Raspberry vein chlorosis virus. *Plant Pathology* 60: 462-467. DOI:10.1111/j.1365-3059.2010.02387.x
- Nault, L. R.** 1997: Arthropod Transmission of Plant Viruses: A New Synthesis. *Ann. Entomol. Soc. Am.* 90 (5): 521–541.
- Petrović-Obradović, O.** 2003: *The aphids (Homoptera: Aphididae) of Serbia*. Belgrade: Faculty of Agriculture, University of Belgrade
- Poudel, B., Wintermantel, W.M., Cortez, A.A., Ho, T., Khadgi, A., Tzanetakis, I.E.** 2013: Epidemiology of Blackberry yellow vein associated virus. *Plant Disease* 97(10): 1352–1357. <https://doi.org/10.1094/PDIS-01-13-0018-RE>.
- Radulovic, M., Mavrič-Pleško, I., Maclot, F., Delić, D., Massart, S.** 2021: First report of black raspberry necrosis virus and raspberry leaf mottle virus in Bosnia and Herzegovina. *Journal of Plant Pathology* 103: 371. <https://doi.org/10.1007/s42161-020-00697-x>.
- Remaudière, G., Seco Fernandez, M.V.** 1990: Claves para ayudar al reconocimiento de alados depulgonos trampeados en la region mediterranea (Hom. Aphidoidea). León, Spain: Universidad De León.
- Stace-Smith, R.** 1955: *Studies on Rubus Virus Diseases in British Columbia: II. Black raspberry necrosis*. *Canadian Journal of Botany* 33(4): 314-322.

Stanić, D., Spasić, R. 2024: Pests of fruit trees and vines and their control. Sarajevo: University of East Sarajevo, Faculty of Agriculture

Tanasković, S., Sretenović, D., Inđić, D., Vuković, S., Gvozdenac, S. 2012: Economically significant pests in raspberry and blackberry plantations. *Plant Doctor* 40 (5): 391-399.

Tzanetakis, I.E., Halgren, A., Mosier, N., Martin, R.R. 2007: Identification and characterization of raspberry mottle virus, a novel member of the Closteroviridae. *Virus Research* 127: 26–33. <https://doi.org/10.1016/j.virusres.2007.03.010>.

Voruna, M., Mihaljica, M., Vakić, M., Elbeaino, T., Delić, D. 2023a: First report of Rubus yellow net virus in Rubus idaeus in Bosnia and Herzegovina. *Journal of Plant Pathology* 105: 1217. <https://doi.org/10.1007/s42161-023-01428-8>

Voruna, M., Mihaljica, M., Vakić, M., Elbeaino, T., Delić, D. 2023b: Molecular analysis of rubus yellow net virus on raspberry from Bosnia and Herzegovina. Book of Abstracts of 25th International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops July 09-13, 2023, Wageningen, The Netherlands, pp. 48.

Wilson, C.R. 2014: Applied plant virology. Oxfordshire, United Kingdom: CAB International

Received July 4th, 2025