**Disease Note**

# FIRST REPORT OF BLUEBERRY MOSAIC-ASSOCIATED VIRUS IN HIGHBUSH BLUEBERRY IN SERBIA

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From 2012 to 2014 symptoms of yellow to yellowish green, white and pink mosaic patterns were observed on the leaves of highbush blueberry (*Vaccinium corymbosum*) plants in two locations of Serbia. These symptoms resembled those of blueberry mosaic disease. After several unsuccessful attempts to identify the agent of the disease, Thekke-Veetil *et al.* (2014) detected the presence of a virus in the samples with mosaic symptoms and named it Blueberry mosaic-associated virus (BlMaV). A total of 13 samples were collected and tested by RT-PCR for the presence of BlMaV. Total nucleic acids were extracted from fresh and frozen leaves with CTAB method (Li *et al*., 2008). RT-PCR reactions were carried out with specific BlMaV primers designed to amplify a fragment from the 3' part of RNA 1 of the BlMaV genome (Thekke-Veetil *et al*., 2014). The expected 756 bp PCR fragment was obtained from all the nine samples with mosaic symptoms from cvs Bluecrop, Duke and Goldtraub and in one sample from an asymptomatic bush of cv. Bluecrop. DNA fragments from five isolates were purified, custom-sequenced (Macrogen, The Netherlands) and the sequences were deposited in GenBank under accession Nos KP188570-KP188574. Sequences of Serbian isolates were aligned with the BlMaV sequence of the Arkansas isolate of this virus from the USA (accession No. KJ704366). The nucleotide and amino acid sequence identity among the analyzed isolates ranged from 88.0-100.0% and 94.8-100%, respectively. To our knowledge, this is the first report of BlMaV in Serbia.

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**Disease Note**

# FIRST REPORT OF *CUCUMBER MOSAIC VIRUS* IN *ANEMONE* sp. IN GREECE

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Virus-like symptoms, such as severe stunting and mosaic, were observed in a greenhouse-grown *Anemone* sp. (cv. Jerusalem), in Pella (northern Greece) in January 2014. Foliar samples were collected from 20 symptomatic and five symptomless plants and subjected to three initial tests: (i) DAS-ELISA to check for the presence of *Cucumber mosaic virus* (CMV), (ii) RT-PCR using generic primers for the detection of potyviruses (Pappu *et al*., 1993) and (iii) PCR for phytoplasmas (Gundersen and Lee, 1996). CMV was detected in all symptomatic plants but in none of the asymptomatic ones, whereas PCR-based tests for potyviruses or phytoplasmas resulted negative. In order to verify the results of serological tests, a nested RT-PCR was performed on total RNA extracts from all 25 samples. Degenerate primers CMVup624a (5′- ATGGACAAATCTGRATC-3′) and CMVdo1244a (5′- TGRTGCTCRAYGTCKACATGA-3′), used in the first round of PCR were followed by the set CMVup624b (5′- GGACAAATCTGRATCTCCCAATGC-3′) and CMVdo1244b (5′- TGCTCRAYGTCRACATGAAG-3′), designed to amplify a 622 nt-long fragment of the viral coat protein gene. Amplicons of the expected size were obtained from all the symptomatic samples but not from the asymptomatic ones. Sequencing of two randomly chosen PCR products (GenBank accession Nos LN810056, LN810057) showed 99% nucleotide identity with CMV isolates from various hosts (KC763473, EU191027). Phylogenetic analyses showed that studied CMV isolates from *Anemone* sp. belong to subGroup II. Finally, CMV was mechanically transmitted from the original symptomatic plants onto *Cucurbita pepo* and *Nicotiana benthamiana*, and its presence was confirmed by DAS-ELISA. To our knowledge this is the first report of CMV in *Anemone* sp. in Greece.

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