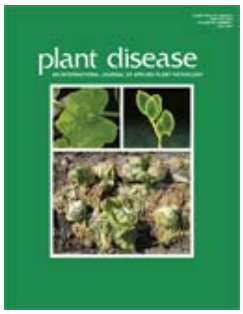

plant disease

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Disease Notes

First Report of the Occurrence of *Cucurbit aphid-borne yellows virus* on Oilseed Pumpkin in Serbia

A. Vučurović, **A. Bulajić**, **I. Stanković**, and **D. Ristić**, Institute of Plant Protection, Department of Phytopathology, University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia; **J. Berenji**, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia; **J. Jović**, Department of Plant Pests, Institute for Plant Protection and Environment, Banatska 33, 11080 Belgrade, Serbia; and **B. Krstić**, Institute of Plant Protection, Department of Phytopathology, University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia.
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In July 2008, field-grown oilseed pumpkins (*Cucurbita pepo* L. 'Olinka') showing severe yellowing and thickening of older leaves were observed in the Kisač locality of Vojvodina Province, Serbia. Symptomatic plants were found only near the borders of the field. Leaf samples collected from 15 symptomatic plants were tested for the presence of four viruses causing the cucurbit yellowing disorder. Total RNAs were extracted from deep frozen plant materials with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and reverse transcription (RT)-PCR was conducted with the OneStep RT-PCR Kit (Qiagen) following the manufacturer's instructions. RNA extracted from healthy *C. pepo* and molecular-grade water were included as negative controls in each PCR reaction. Species-specific primers (1,2) failed to detect the presence of three viruses causing the cucurbit yellowing disorder, *Cucumber vein yellowing virus*, *Cucumber yellow stunting disorder virus*, and *Beet pseudo-yellows virus*, in symptomatic samples. When two different sets of CABYV-specific primer pairs, CABYVup/CABYVdown (2) and CE9/CE10 (3), for a 484-bp and a 600-bp fragment of the CP gene of *Cucurbit aphid-borne yellows virus* (CABYV), respectively, were used for amplification, the former amplified fragments of the expected size from all symptomatic samples, whereas the latter successfully amplified a 600-bp fragment from only 7 of 15 samples. The 600-bp amplified product derived from isolate 145-08 was purified (QIAquick PCR Purification Kit, Qiagen), sequenced in both directions, deposited in GenBank (Accession No. HQ202745), and subjected to sequence analysis by MEGA4 software. Sequence comparisons revealed a high nucleotide identity of 99.8% (100 and 99.5% amino acid identities for the CP and the overlapping MP genes, respectively) with Czech CABYV isolates from *C. pepo* 'Ovifera' (HM771271-73). A neighbor-joining tree obtained on a 545-bp CP fragment of CABYV isolates available in GenBank database revealed that Serbian CABYV isolate 145-08 was clustered with isolates from Spain, Italy, France, and Tunisia in the Mediterranean subgroup denoted previously (4). In a persistent type transmission test, which was carried out using *Aphis gossypii* Glover, the aphids were allowed to feed on

leaves of the collected sample (145-08) for an acquisition access period of 2 days and then 10 aphids were transferred to each of 20 *C. pepo* 'Olinka' plants for a 5 day inoculation access period. Transmission was successful in 6 of 20 plants as assessed by the development of a mild yellowing symptom 2 weeks after transmission and confirmed by RT-PCR with the CABYVup/CABYVdown primers. To our knowledge, this is the first record of the occurrence of CABYV in Serbia. The discovery of CABYV on oilseed pumpkin should prompt more detailed surveys and subsequent testing of other cucurbits cultivated in Serbia to establish the distribution and incidence of CABYV in Serbia.

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