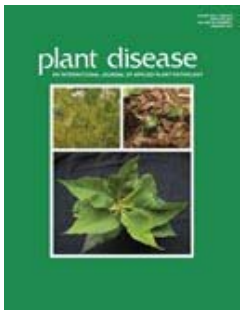

plant disease

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Disease Notes

First Report of *Tomato spotted wilt virus* on *Gerbera hybrida* in Serbia

I. Stanković, **A. Bulajić**, **A. Vučurović**, and **D. Ristić**, Institute of Plant Protection, Department of Phytopathology, University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia; **J. Jović**, Department of Plant Pests, Institute for Plant Protection and Environment, Banatska 33, 11080 Zemun, Serbia; and **B. Krstić**, Institute of Plant Protection, Department of Phytopathology, University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia

In May 2009, approximately 30% of plants within a greenhouse-grown *Gerbera hybrida* crop in Vranjska Banja (Pčinj District) in Serbia displayed chlorotic oak-leaf patterns followed by necrosis and distortion of leaves. Symptoms on naturally infected gerbera plants and local necrotic spots on *Petunia* × *hybrida* mechanically inoculated with infected gerbera sap using chilled 0.05 M phosphate buffer (pH 7) containing 1 mM Na-EDTA, 5 mM Na-DIECA, and 5 mM Na-thioglycolate (4) suggested the presence of a *Tospovirus*. Symptomatic leaves were tested for the presence of *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), and *Chrysanthemum stem necrosis virus* (CSNV) by commercial double-antibody sandwich (DAS)-ELISA diagnostic kits (Loewe Biochemica, Sauerlach, Germany). Commercial positive and negative controls and extract from healthy gerbera tissue were included in each ELISA. All 20 tested plants were negative for INSV and CSNV. TSWV was detected serologically in 18 of 20 gerbera samples. The presence of TSWV in ELISA-positive symptomatic gerbera plants was further confirmed by conventional reverse transcription (RT)-PCR. Total RNAs were extracted with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and RT-PCR was conducted with the OneStep RT-PCR Kit (Qiagen) using Serbian tobacco TSWV isolate (GQ279731) and RNA extract from healthy gerbera as positive and negative controls, respectively. Two different sets of TSWV-specific primers, L1 TSWVR/L2 TSWVF (2) and M962/M66 (3), for a 276-bp fragment of the RNA-dependent RNA polymerase (RdRp) gene and a 897-bp fragment of the NSm gene, respectively, were used for both amplification and sequencing. RT-PCR analyses of each tested plant detected the presence of amplification fragments of expected size. The amplified products corresponding to part of the RdRp and NSm genes derived from the isolate 158-Gerb were purified (QIAquick PCR Purification Kit, Qiagen) and sequenced in both directions (GenBank Accession Nos. HQ246452 and HQ246453, respectively). Sequence analysis of the partial RdRp gene, conducted using MEGA4 software, revealed 91.1 to 98% nt identity (95.1 to 98.8% amino acid [aa] identities) with corresponding sequences of TSWV L RNA deposited in GenBank. The highest identity was found with an isolate from globe artichoke (AM940436) in Greece, and isolates from tomato (GQ279732), impatiens (GQ132190), and tobacco isolates (GQ279731, FJ189392, and FJ189393) found within Serbia. Analysis of the NSm sequence of isolate 158-Gerb demonstrated nucleotide identities varying between 90.6 and 99.6% (80.9 and 99.6% aa identities) with those of previously reported TSWV isolates. The

highest identity was with tobacco isolate GQ373174 from Serbia. Therefore, while gerbera is one of the principal ornamental hosts of TSWV in the EPPO region (1), to our knowledge, this is the first report infecting gerbera in Serbia, which may have a devastating influence on its production.

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