

## Disease Notes (continued)

***Lamium maculatum* is a Natural Host for *Cucumber mosaic virus*.** R. Bešta-Gajević, A. Jerković-Mujkić, and S. Pilić, University of Sarajevo, Faculty of Science, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina; and I. Stanković, A. Vučurović, A. Bulajić, and B. Krstić, Institute of Plant Protection, Department of Phytopathology, University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia. This research was supported by grant III-43001 of the Ministry of Education and Science, Republic of Serbia. *Plant Dis.* 97:150, 2013; published online as <http://dx.doi.org/10.1094/PDIS-08-12-0717-PDN>. Accepted for publication 10 September 2012.

*Lamium maculatum* L. (spotted dead-nettle) is a flowering perennial ornamental that is commonly grown as a landscape plant for an effective ground cover. In June 2010, severe mosaic accompanied by reddish brown necrosis and leaf deformation was noticed on 80% of *L. maculatum* growing in shade under trees and shrubs in Sarajevo (Bosnia and Herzegovina). Leaves from 10 symptomatic *L. maculatum* plants were sampled and analyzed by double-antibody sandwich (DAS)-ELISA using commercial diagnostic kits (Bioreba AG, Reinach, Switzerland) against *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), and *Impatiens necrotic spot virus* (INSV), the most important viral pathogens of ornamental plants (1,2). Commercial positive and negative controls and extracts from healthy *L. maculatum* leaves were included in each assay. All samples tested negative for TSWV and INSV and positive for CMV. The virus was mechanically transmitted to test plants and young virus-free plants of *L. maculatum* using 0.01 M phosphate buffer (pH 7). The virus caused chlorotic local lesions on *Chenopodium quinoa*, while systemic mosaic was observed on *Capsicum annuum* 'Rotund,' *Nicotiana rustica*, *N. glutinosa*, *N. tabacum* 'White Burley,' and *Phaseolus vulgaris* 'Top Crop.' The virus was transmitted mechanically to *L. maculatum* and induced symptoms resembling those observed on the source plants. Inoculated plants were assayed by DAS-ELISA and all five inoculated plants of each species tested positive for CMV. The presence of CMV in *L. maculatum* as well as mechanically infected *N. glutinosa* plants was further confirmed by RT-PCR. Total RNA from symptomatic leaves was isolated using RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and RT-PCR was performed with the One-Step RT-PCR Kit (Qiagen) following the manufacturer's instructions. The primer pair, CMVAu1u/CMVAu2d, that amplifies the entire coat protein (CP) gene and part of 3'- and 5'-UTRs was used for both amplification and sequencing (4). Total RNA obtained from the Serbian CMV isolate from pumpkin (GenBank Accession No. HM065510) and a healthy *L. maculatum* plant were used as positive and negative controls, respectively. All naturally and mechanically infected plants as well as the positive control yielded an amplicon of the expected size (850 bp). No amplicon was observed in the healthy control. The amplified product derived from isolate 3-Lam was purified (QIAquick PCR Purification Kit, Qiagen), directly sequenced in both directions and deposited in GenBank (JX436358). Sequence analysis of the CP open reading frame (657 nt), conducted with MEGA5 software, revealed that the isolate 3-Lam showed the highest nucleotide identity of 99.4% (99.1% amino acid identity) with CMV isolates from Serbia, Australia, and the USA (GQ340670, U22821, and U20668, respectively). To our knowledge, this is the first report of the natural occurrence of CMV on *L. maculatum* worldwide and it adds a new host to over 1,241 species (101 plant families) infected by this virus (3). This is also an important discovery for the ornamental industry since *L. maculatum* is commonly grown together with other ornamental hosts of CMV in nurseries and the urban environment as well as in natural ecosystems.

**References:** (1) Y. K. Chen et al. *Arch. Virol.* 146:1631, 2001. (2) M. L. Daughtrey et al. *Plant Dis.* 81:1220, 1997. (3) M. Jacquemond. *Adv. Virus Res.* 84:439, 2012. (4) I. Stanković et al. *Acta Virol.* 55:337, 2011.

**First Report of *Grapevine leafroll-associated virus 7* in Two Native Grape Varieties in China.** M. D. Lyu, M. J. Li, J. Li, X. M. Li, and Y.-Q. Cheng, Department of Pomology/Laboratory of Stress Physiology and Molecular Biology for Tree Fruits, Key Laboratory of Beijing Municipality, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China. *Plant Dis.* 97:150, 2013; published online as <http://dx.doi.org/10.1094/PDIS-08-12-0760-PDN>. Accepted for publication 12 September 2012.

Grapevine leafroll disease (GLD) is one of the most economically important diseases of cultivated grapevines (*Vitis vinifera*), causing decrease in yield, as well as decreasing the sugar levels and increasing the acidity of the berries (1). There are currently at least 10 serologically distinct viruses, referred to as grapevine leafroll-associated viruses (GLRAVs), from the family *Closteroviridae* that are associated with leafroll disease (4). China is one of the world's leading grape producers, and nearly 75% of the vineyards in China are located in Xinjiang Uygur Autonomous Region, and Hebei, Shandong, Gansu, Ningxia, and Yunnan provinces. *Grapevine leafroll-associated virus 7* (GLRAV-7) isolates have been reported so far in Liaoning (GQ849392, GQ849393, and JF927943) and Henan (EF093187) provinces in China (3). The four Chinese isolates were isolated respectively from grape varieties, Cabernet Sauvignon (GQ849392, GQ849393), Centennial Seedless (JF927943), and Semillon (EF093187), and these grape varieties are introduced from abroad. Cow's Nipple and Dragon's Eye are old grape varieties native to China. Cow's nipple is extensively cultivated in Xinjiang Uygur Autonomous Region, while Dragon's Eye is widely planted in Hebei Province. To determine if GLRAV-7 was present in these two varieties, six samples (three per variety) were collected from six individual grapevines showing GLD-like symptoms in two vineyards in Xinjiang Uygur Autonomous Region and Hebei Province, respectively, in September 2011. Total RNA extracts obtained from phloem scrapings of samples using the RNeasy plant mini kit (QIAGEN) were tested by reverse transcription (RT)-PCR with primers F1 (5'-TATATCCCAACGGAGATG GC-3') and R1 (5'-ATGTTCTCCACCAAAATCG-3') (2) specific to the heat shock protein 70 homologue (*HSP-70* gene) of GLRAV-7. All samples produced a single band of the expected size of 502 bp. One GLRAV-7-specific amplicon per variety was cloned into pMD 18-T simple vector (TaKaRa). Plasmid DNA was purified using Column Plasmid DNA<sub>OUT</sub> (TIANDZ, Beijing, China) from three individual clones and sequenced from both directions. The sequence of the two isolates (GenBank Accession Nos. JX494722 and JX494723) shared 97.81% identity at the nucleotide level and 100% identity at the amino acid level. A pairwise comparison of *HSP-70* sequences of the two isolates from this report with nine corresponding sequences of GLRAV-7 isolates (including four previously reported Chinese isolates) showed nucleotide sequence identities ranging from 91.24% (EF093187) to 98.80% (GQ849392). These samples were further analyzed by double antibody sandwich (DAS)-ELISA using antibody specific to GLRAV-7 (NEOGEN Europe, Ayr, Scotland) according to the manufacturer's instructions, and the results confirmed the presence of the virus in these samples that were positive by RT-PCR. To our knowledge, this is the first report of GLRAV-7 occurring in native grape varieties in China. These results could be helpful in developing sound diagnostic systems for implementing efficient disease management strategies.

**References:** (1) B. Akbaş et al. *Hort. Sci.* 36:97, 2009. (2) E. Engel et al. *Plant Dis.* 92:1252, 2008. (3) X. Fan et al. *Acta Hort. Sinica* 39:949, 2012. (4) G. P. Martelli. *Extended Abstr. 16th Meet. International Council for the Study of Virus and Virus-like Diseases of Grapevines (ICVG).* 15-23, 2009.

## e-Xtra\*

**First Report of *Tomato spotted wilt virus* on *Chrysanthemum* in Serbia.** I. Stanković, A. Bulajić, A. Vučurović, D. Ristić, K. Milojević, D. Nikolić, and B. Krstić, Institute of Plant Protection, Department of Phytopathology, University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia. This research was supported by grant III-43001 of the Ministry of Education and Science, Republic of Serbia. *Plant Dis.* 97:150, 2013; published online as <http://dx.doi.org/10.1094/PDIS-08-12-0778-PDN>. Accepted for publication 4 September 2012.

In July 2011, greenhouse-grown chrysanthemum hybrid plants (*Chrysanthemum × morifolium*) with symptoms resembling those associated with tospoviruses were observed in the Kupusina locality (West Bačka District, Serbia). Disease incidence was estimated at 40%. Symptomatic plants with chlorotic ring spots and line patterns were sampled and tested by double antibody sandwich (DAS)-ELISA using polyclonal antisera (Bioreba AG, Reinach, Switzerland) against the two of the most devastating tospoviruses in the greenhouse floriculture industry: *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV) (2). Commercial positive and negative controls and extracts from healthy chrysan-

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