***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 Heibei 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′-ATGTTCCTCCACCAAAATCG-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 $DNA_{OUT}$ (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 Hortic. 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-

themum tissue were included in each ELISA. TSWV was detected serologically in 16 of 20 chrysanthemum samples and all tested samples were negative for INSV. The virus was mechanically transmitted from ELISA-positive chrysanthemum samples to five plants each of both *Petunia* × *hybrida* and *Nicotiana tabacum* 'Samsun' using chilled 0.01 M phosphate buffer (pH 7) containing 0.1% sodium sulfite. Inoculated plants produced local necrotic spots and systemic chlorotic/necrotic concentric rings, consistent with symptoms caused by TSWV (1). The presence of TSWV in ELISA-positive chrysanthemum plants and *N. tabacum* 'Samsun' was further confirmed by conventional reverse transcription (RT)-PCR. Total RNAs were extracted with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RT-PCR was performed with the One-Step RT-PCR Kit (Qiagen) using primers TSWVCP-f/TSWVCP-r specific to the nucleocapsid protein (N) gene (4). A Serbian isolate of TSWV from tobacco (GenBank Accession No. GQ373173) and RNA extracted from a healthy chrysanthemum plant were used as positive and negative controls, respectively. An amplicon of the correct predicted size (738-bp) was obtained from each of the plants assayed, and that derived from chrysanthemum isolate 529-11 was purified (QIAqick PCR Purification Kit, Qiagen) and sequenced (JQ692106). Sequence analysis of the partial N gene, conducted with MEGA5 software, revealed the highest nucleotide identity of 99.6% (99% amino acid identity) with 12 TSWV isolates deposited in GenBank originating from different hosts from Italy (HQ830186-87, DQ431237-38, DQ398945), Montenegro (GU355939-40, GU339506, GU339508), France (FR693055-56), and the Czech Republic (AJ296599). The consensus maximum parsimony tree obtained on a 705-bp partial N gene sequence of TSWV isolates available in GenBank revealed that Serbian TSWV isolate 529-11 from chrysanthemum was clustered in the European subpopulation 2, while the Serbian isolates from tomato (GU369723) and tobacco (GQ373172-73 and GQ355467) were clustered in the European subpopulation 1 denoted previously (3). The distribution of TSWV in commercial chrysanthemum crops is wide (2). To our knowledge, this is the first report of TSWV infecting chrysanthemum in Serbia. Since chrysanthemum popularity and returns have been rising rapidly, the presence of TSWV may significantly reduce quality of crops in Serbia.

*References*: (1) Anonymous. OEPP/EPPO Bull. 34:271, 2004. (2) Daughtrey et al. Plant Dis. 81:1220, 1997. (3) I. Stanković et al. Acta Virol. 55:337, 2011. (4) A. Vučurović et al. Eur. J. Plant Pathol. 133:935, 2012.

* The *e*-Xtra logo stands for "electronic extra" and indicates this Disease Note online contains supplemental material not included in the print edition.

## Others

**First Report of Laurel Oak as a Host for the Pecan Root-Knot Nematode, *Meloidogyne partityla*, in Florida.** J. A. Brito, Division of Plant Industry, Gainesville, FL 32614-7100; H. Han, Division of Forest Insect Pests and Diseases, Korea Forest Research Institute, Seoul, Republic of Korea, 130-712; J. D. Stanley, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL 32614-7100; M. Hao, Division of Plant Industry, Gainesville, FL 32614-7100; and D. W. Dickson, Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620. Plant Dis. 97:151, 2013; published online as http://dx.doi.org/10.1094/PDIS-02-12-0201-PDN. Accepted for publication 20 June 2012.

Roots of laurel oak (*Quercus laurifolia* Michx.), member of the family Fagaceae, were found to be heavily galled by the pecan root-knot nematode, *Meloidogyne partityla*, in two separate home gardens between 2010 and 2012, in Alachua Co., FL. Distinct round galls were observed on secondary and tertiary roots. Internally, root-knot nematode females were clearly visible when the roots were thinly sliced and egg masses were seen protruding from the root surfaces. The nematode species identification was performed using morphology of the male stylet, selected characters of the second-stage juveniles (J2), female perineal patterns, and esterase (EST) and malate dehydrogenase (Mdh) isozyme phenotypes. Morphology of perineal patterns of females, body, stylet, and tail length of the J2 and males all matched those of the original description of *M. partityla* (2). A swollen deeply grooved rectum was observed in the J2. The male stylet had a blunt tip with a prominent thickening at the junction between the cone and shaft. The stylet knobs of males and females were bipartite, each incised by a deep medium longitudinal groove (2). The isozyme phenotypes (EST = Mp3; Mdh = N1a) were consistent with those previously reported for *M. partityla* from Florida (1). Mitochondrial DNA (mtDNA) (3) and ribosomal internal transcriber spacer (ITS) DNA (4) of females were amplified to further confirm the nematode species identification. The mtDNA amplification using the C2F3/1108 primer set (3) and the ITS amplification using a recently available *M. partityla* specific primer set (4) produced fragments of approximately 530 bp and 550 bp, respectively. These were consistent with those already reported for this nematode species. This first report of a plant host for the pecan root-knot nematode outside of the family Juglandaceae indicates that the nematode may have migrated from *Quercus* species to pecan trees during the period when orchards were being established in Florida. To our knowledge, this is the first report of the pecan root-knot nematode infecting laurel oak.

*References*: (1) J. A. Brito et al. Nematology 10:757, 2008. (2) Kleynhans, K. P. N. Phytophylatica 18:103, 1986. (3) T. O. Powers et al., J. Nematol. 37:226, 2005. (4) R. A. Stamler. M. S. thesis, New Mexico State University, Las Cruces, 2009.

## *e*-Xtra*

**First Report of *Pratylenchus neglectus* on Winter Wheat in China.** H. Y. Wu, Agricultural College of Guangxi University, Nanning 530004, China; Z. Z. Jia, J. Liu and J. Luo, College of Plant Protection, Shandong Agricultural University, Taian 271018, Shandong, China; and D. L. Peng, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Chinese Academy of Agricultural Sciences, Beijing 100193, China. Plant Dis. 97:151, 2013; published online as http://dx.doi.org/10.1094/PDIS-04-12-0332-PDN. Accepted for publication 6 September 2012.

Root-lesion nematodes are major pathogens of wheat and have been reported in the United States, Mexico, India, Australia, Egypt, Canary Islands, South Africa, Iran, Japan, the Netherlands, Belgium, Italy, Germany, and Yugoslavia (1). They can also cause injury in a large number of crops, including grasses, cereal grains, and vegetables. In 2009 and 2010, a survey was conducted for nematodes in winter wheat fields near Taian city, Shandong, northern China. Root tissues were stained via the acid fuchsin tissue stain technique, and nematode numbers were recorded under a stereo microscope. Sixty-eight root samples were collected during the winter wheat growing season, and root lesion nematode was found in all samples. The highest average lesion nematode populations in fresh roots were 154.3 nematodes/g in 2009 and 236.7 nematodes/g in 2010. Nematodes were collected from infested wheat roots by a modified Baermann funnel method. Dimensions of the nematodes were: length, 0.42 to 0.54 mm; a, 18.8 to 24.2; b, 4.4 to 5.7; c, 19.8 to 25.4; V, 80.4 to 84.8; and spear, 17.1 to 18.9 µm. DNA was extracted from individual nematodes using liquid nitrogen. Amplification of rDNA-internal transcribed spacer region using the forward primers 5′-CGTAACAAGGTAGCTGTAG-3′ and the reverse primer 5′-TTTCACTCGCCGTTACTAAGG-3′ yielded a PCR fragment of approximately 900 bp. PCR products were purified using Universal Plant DNA Purification Kit (Tiangen, China) and ligated to the pMD18-T vector system (TaKaRa Bio, Japan) and transformed to *E. coli* strain DH5α. Plasmid DNA carrying the insert was extracted and used as the template for DNA sequencing. DNA sequencing was carried out in an ABI 3730, compared and aligned using MEGA 5.0. Sequences showed 96% sequence identity with those of *Pratylenchus neglectus* (GenBank Accession No. FR692291.1). The sequence was submitted to the GenBank database (JX228136). To our knowledge, this is the first report of *P. neglectus* infesting winter wheat in China. *P. neglectus* has been reported as causing economically significant damage to wheat production of up to 70% yield loss in the Pacific Northwest. Damage from lesion nematode may therefore be potentially significant to wheat production in Shandong Province, and further information should be obtained on its prevalence.

*References*: (1) P. A. A. Loof. The family *Pratylenchidae* Thorne, 1949. W. R. Nickle, ed., Manual of Agricultural Nematol. Marcel Dekker, Inc. New York, 1991. (2) R. W. Smiley et al. J. Nematol. 37:45, 2005.

* The *e*-Xtra logo stands for "electronic extra" and indicates this Disease Note online contains supplemental material not included in the print edition.

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