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First Report of *Citrus tristeza virus* in the State Union of Serbia and

Montenegro. T. Papić, Ministry of International Economic Relations, Mihajla Pupina 2, 11000 Belgrade, Serbia and Montenegro; and C. Santos and G. Nolasco, CDCTPV, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. Plant Dis. 89:434, 2005; published on-line as DOI: 10.1094/PD-89-0434B. Accepted for publication 18 January 2005.

Citrus production in the State Union of Serbia and Montenegro has a strategic importance to the agricultural sector. Approximately 400,000 trees are now grown in the major citrus producing region, which is the Montenegrin Coastal Region. Satsuma mandarins and lemons grafted on *Poncirus trifoliata* are the most cultivated varieties. In December 2003, eight samples taken from the coastal region close to the towns of Bar and Ulcinj were analyzed using enzyme-linked immunosorbent assay (ELISA) with SP7 antibodies produced at Universidade do Algarve, Portugal (3). Further analysis was done using immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) targeting the entire coat protein (*CP*) gene (forward primer CTV1: 5(prime)-ATGGACGACGAAACAAAGAA-3(prime) and reverse primer CTV10: 5(prime)-ATCAACGTGTGTTGAATTTCC-3(prime)). Using both techniques, seven of eight samples analyzed were found to be infected by *Citrus tristeza virus* (CTV), including samples from five trees that exhibited chlorosis, gummosis, and fruit deformation, and two trees that were symptomless. When analyzed using agarose gel electrophoresis, PCR products from the positive samples consisted of a single amplicon of the expected size for the *CP* gene compared with a positive control. The PCR products of two samples were TA cloned (pGEM-T Easy Vector; Promega, Madison, WI) in *E. coli* cells and the *CP* inserts were analyzed using single-strand conformation polymorphism (SSCP) and DNA sequencing. In both cases, the SSCP analysis of several clones showed a variety of different patterns, suggesting the occurrence of infections with a mixture of genomic variants. Sequence analysis of different variants showed a *CP* gene with 669 nucleotides having greater than 90% nucleotide identity to most CTV *CP* gene sequences available in GenBank. A genomic variant (GenBank Accession No. AY764154) was closely related (98.5% nucleotide identity) to the T30 mild strain from Florida (GenBank Accession No. AF260651). However, other sequences obtained showed only 93% nucleotide identity with this variant and were closely related to other *CP* gene sequences obtained from Croatian isolates. A previous report (1) refers to the existence of CTV-infected Satsuma plants illegally introduced in Italy from the former Yugoslavia. The presence of CTV in the former Yugoslavia was

later confirmed (2) but in a region that became part of the Croatian Republic. To our knowledge, this is the first report of CTV in the State Union of Serbia and Montenegro, although a relationship with Croatian isolates cannot be excluded. Although a very small number of samples were analyzed in this study, CTV appears to be very common in the Satsuma orchards. This could be due to the traditional use of the trifoliolate rootstock that prevents the appearance of tristeza decline, thus enabling the unnoticed propagation of infected material. Because the kind of symptoms observed in five trees are not typical of CTV and two infected trees were symptomless, the virus is probably not responsible for the symptoms observed in the field.

References:(1) M. Davino et al. Pages 8-13 in: Proc. Conf. Int. Organ Citrus Virol. IOCV, Riverside, 1988. (2) A. Šarić and I. Dulić. Agric. Conspectus Sci. 55:171, 1990. (3) Z. Sequeira and G. Nolasco, Phytopathol. Mediterr. 41:552, 2002.

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