

First Report of Hop stunt viroid Infecting Citrus Trees in Morocco

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DISEASE NOTES

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Citation

 Open Access.

Citrus fruits are an important contributor to the Moroccan economy; the industry employs thousands of people and it also serves as a significant source of hard currency. During July 2014, a field survey for the presence of *Hop stunt viroid* (HSVd) on citrus was conducted in the Gharb region (northwestern Morocco). HSVd is a member of the *Pospiviroidae* family and the *Hostuviroid* genus. Its best known host is hop, but the viroid has a wide host range, including several woody and herbaceous crops. Forty samples without symptoms of viroid infection were randomly collected from four citrus orchards of different varieties: Common and Sidi Aissa Clementines (*Citrus clementina* hort. ex Tanaka), and Valencia Late and Washington Navel sweet oranges (*C. sinensis* (L.) Osbeck). Total RNA extraction was performed on the samples using RNeasy Plant Mini Kit (Qiagen, Germany) and was then used for reverse transcription (RT)-PCR assays with primers specific to HSVd (Kofalvi et al. 1997). The RT-PCR results showed that three samples tested positive to HSVd, yielding the expected

size of 302 bp in agarose gel. Two positive samples were detected from two Common Clementine trees and the third was from a Valencia Late orange tree. RT-PCR products from the positive samples from Clementine were cloned to the pUC18 plasmid vector (Agilent Technologies, USA), then one clone from each isolate was sequenced and deposited in GenBank under accession numbers KU640953 and KU640954. BLAST analysis of these sequences indicated 95 to 96% nucleotide identity with HSV-cit isolate from Etrog citron (Accession No. X06719). The positive results obtained by RT-PCR were confirmed by biological indexing for citrus cachexia using graft inoculation on Parson's Special mandarin on rough lemon rootstock. After 16 months of incubation under greenhouse conditions (28 to 32°C), indicator plants graft-inoculated with bark tissue from HSVd-positive trees presented, at the bud-union, stem pitting and gumming affecting the Parson's Special mandarin scion but not the rough lemon rootstock (Roistacher et al. 1973). To our knowledge, this is the first report of HSVd in citrus trees in Morocco, though this viroid was previously reported on apricot by Amari et al. (2000). Cachexia is an economically important disease of citrus, and is included in the national health certification program for citrus. Researchers are currently studying the biological properties of these newly identified HSVd isolates, in addition to the field surveys for other viroids being undertaken in Morocco's main citrus growing areas.

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