Disease Note

FIRST REPORT OF TWO DISTINCT STRAINS OF *PEPINO MOSAIC VIRUS* INFECTING TOMATOES IN GREENHOUSES IN LITHUANIA

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During 2010 and 2011, one hundred thirty one symptomatic samples of tomato leaves and fruits were collected from commercial greenhouses in Lithuania and analyzed for the presence of *Pepino mosaic virus* (PepMV). Two isolates of this virus (NV and KK) were found in two different greenhouses on a tomato plant exhibiting mild yellow leaf spotting and on tomato fruits with marbling symptoms, respectively. The presence of the virus was confirmed by RT-PCR and DAS-ELISA, using commercial antibodies (DSMZ, Germany). Filamentous virus particles were observed by electron microscopy. The coat protein (CP) gene of the two viral isolates was amplified using a two-step RT-PCR with the PepTGB-F/PepUTR-R primer pair (Mumford and Metcalfe, 2001). A specific 845 bp PCR product was obtained, purified and sequenced. The nucleotide sequence of the CP gene of PepMV-NV and -KK was submitted to GenBank under the accession numbers JQ979169 and JQ979170, respectively. Nucleotide sequence analysis showed that the two Lithuanian PepMV isolates differ from each other and belong to two distinct PepMV genotypes. PepMV-NV belongs to the EU genotype while PepMV-KK was assigned to the CH2 genotype. These results are consistent with an expansion of the distribution of PepMV in many European countries, including Poland (Pospieszny *et al*., 2008). They also show that both PepMV EU and CH2 genotypes are becoming common in Europe.

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Disease Note

FIRST REPORT OF *GIBBERELLA AVENACEA* CAUSING WET APPLE CORE ROT IN ITALY

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Gibberella avenacea R.J. Cook, [syn. *Fusarium avenaceum* (Fr.) Sacc.] is a widely distributed plant pathogen and the causal agent of wet apple core rot (wACR), a disease that develops inside the fruits and remains undetected until they are eaten (Sørensen *et al*., 2009). In 2012, wACR symptoms were observed in apples cv. Golden Delicious from a southern Italian orchard. Diseased carpel tissues were colonized by a white mycelium, whereas the surrounding mesocarp tissues showed a light-brown wet rot. Single spore cultures were grown on PDA slants and carnation leaf agar (CLA) plates. On PDA slants, after 14 days at alternating day/night temperatures of 25/20°C and a 12 h photoperiod, the fungus formed abundant floccose white mycelium with pale orange sporodochia and released a greyish-rose pigment in the agar. Macroconidia formed on CLA were 40-80×3.5-4 µm, slightly falcate, thin-walled, usually 5 septate, with a tapering apical cell. Microconidia and chlamydospores were absent. Based on these morphological characters the fungus was identified as *G. avenacea.* For molecular confirmation, DNA was extracted from the fungal mycelium (Sanzani *et al*., 2012), its internal transcribed spacer regions ITS1 and ITS2, including the 5.8S gene, were amplified using the universal primers ITS5/ITS4 (White *et al*., 1990) and sequenced (GenBank KC342826). BLAST analysis of the 424 bp amplicon showed 100% identity with other *G. avenacea/F. avenaceum* ITS sequences from database. This is the first report of *G. avenacea* as causal agent of wACR in Italy. *G. avenacea* infections constitute an economical problem for growers and a safety issue due to the potential production of mycotoxins such as moniliformin and enniatins (Sørensen *et al*., 2009).

Sanzani S.M., Schena L., De Cicco V., Ippolito A., 2012. Detection and quantification of Botrytis cinerea in symptomless table grape stamens and berries. *Postharvest Biology and Technology* **68**: 64-71.

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Disease Note

FIRST REPORT OF *GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS* IN TUNISIAN GRAPEVINES

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Grapevine rupestris stem pitting-associated virus (GRSPaV), a member of the genus *Foveavirus*, is associated with Rupestris stem pitting, a disease that, along with Kober stem grooving (KSG), Corky bark (CB) and LN33 stem grooving, constitute the rugose wood disease complex of grapevine. *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB), which are associated with the KSG and CB, respectively (Martelli, 1993), are common in Tunisian grapevines (Mahfoudhi *et al*., 1998), whereas no information is available on the presence of GRSPaV. Therefore, 140 samples of 10 table grape varieties (Italia, Superior Seedless, Down Seedless, Red Globe, Victoria, Early Sugar, Black Pearl, King's Ruby, Rich Baba and Sultanine) from different Tunisian grapevine-growing areas were collected and indexed for the presence of this virus by RT-PCR using as template total nucleic acid preparations extracted from phloem tissue (Foissac *et al*., 2001) and primers RSP-48 (5'-AGCTGGGATTATAAGGGAGGT-3') and RSP-49 (5'-CCAGCCGTTCCACCACTAAT-3') (Lima *et al*., 2006). A 330 bp product corresponding to a fragment of the GRSPaV coat protein gene was amplified from 112 samples, accounting for an infection rate of 80%. The virus was present in all the surveyed areas and grape cultivars tested. More specifically, it was detected in all samples of cv. Early Sugar, King's Ruby and Sultanine, whereas in the other cultivars the infection rate ranged from 17% (cv. Down Seedless) to 97% (cv. Italia). To our knowledge, this is the first report of GRSPaV in grapevines in Tunisia.

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Disease Note

FIRST REPORT OF *HOP STUNT VIROID* IN LEBANESE FIG TREES

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In the course of a survey carried out in 2010 in the main fig-growing areas of Lebanon (Bekaa and Mount Lebanon), a total of 100 samples representative of 15 different cultivars were collected and tested by RT-PCR for the presence of *Hop stunt viroid* (HSVd) using viroid-specific primers (Sano *et al.,* 2001). Distinct PCR products of the expected size (303 bp) were amplified from 12 fig trees of cvs Houmairi (6), Bayadi (5) and Sweidi (1), all originating from the Bekaa valley. This result was further confirmed by dot blot molecular hybridization using a HSVd-specific riboprobe. Only seven infected plants showed symptoms resembling those typical of Fig mosaic disease, the remaining being symptomless. Five of these HSVd isolates were sequenced (accession Nos HE662802-HE662806) and compared with the other HSVd isolates from GenBank. In Blast analysis, all the sequenced HSVd isolates showed 99-100% nucleotide identity among them and 94-95% identity with the HSVd type member (X0009). The only exception was the isolate D1, which confirmed the 94% identity with HSVd type but showed a lower identity level (94-95%) with the other HSVd Lebanese fig isolates. In a phylogenetic tree constructed with whole viroidal sequences the Lebanese fig isolates, except for D1, clustered in a separate group together with fig isolates from Syria and mulberry isolates from Lebanon (Elbeaino *et al*., 2012a, 2012b). To our knowledge this is the first report of HSVd in *Ficus carica* in Lebanon.

Elbeaino T., Abou Kubaa R., Ismaeil F., Mando J., Digiaro M., 2012a. Viruses and viroids of fig trees in Syria. *Journal of Plant Pathology* **94**: 687-691

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