Disease Note

FIRST DETECTION OF RASPBERRY LEAF BLOTCH VIRUS IN RED RASPBERRY IN BULGARIA

I. Mavrič Pleško[1], M. Viršček Marn[1], S. Lazarova[2], V. Peneva[2], S. Širca[1] and G. Urek[1]

[1] Agricultural Institute of Slovenia, Hacquetova 17, SI-1000 Ljubljana, Slovenia
[2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2, Gagarin Street, 1113 Sofia, Bulgaria

Severe chlorotic mottling, yellow chlorotic spots and patches were observed on a red raspberry plantation near Smolyan (south Bulgaria). Many patches of dead plants suggested that a nematode-transmitted virus could be associated with the disease. Soil samples were taken for nematode search and leaves for virus detection. *Xiphinema diversicaudatum* (34 nematodes per 100 g of soil) was found in two of the four soil samples analyzed but no common nematode-transmitted viruses were apparently present in the leaf samples analyzed by DAS-ELISA. Therefore, the presence of Raspberry leaf blotch virus (RLBV), which is associated with raspberry leaf blotch disorder in Scotland and England (McGavin *et al*., 2012) and is also found in Serbia and Finland (Bi *et al*., 2012), was investigated because of the symptoms observed on the Bulgarian plants. Total RNA was extracted from samples using the RNeasy plant mini kit (Qiagen, USA) and tested for RLBV by RT-PCR using primers 1571/1286 (McGavin *et al*., 2012). DNA fragments of the expected size (376 bp) from two RLBV isolates were cloned and sequenced. Two sequence variants (GenBank accession Nos HG738848 and HG738849) were obtained with one nucleotide difference in the non-coding region of RNA-5. They shared over 93% nucleotide and 96.1% amino acid identity with RLBV RNA-5 from the UK (FR823303). Both variants were equally present in one of the two samples tested while the second variant (HG738849) was predominant in the other sample. To our knowledge, this is the first report of RLBV in red raspberry in Bulgaria.

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Corresponding author: I. Mavrič Pleško
Fax: +386.1.2805255
E-mail: irena.mavric@kis.si

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Disease Note

FIRST REPORT OF FUSARIUM WILT OF LICORICE BY *FUSARIUM OXYSPORUM* IN IRAN

M. Abedi-Tizaki and D.M. Zafari

Department of Plant Protection, College of Agriculture, Buali Sina University, Hamedan, Iran

In September 2011, licorice (*Glycyrrhiza glabra*) plants were observed in a field in the Iranian province of Yazd that showed symptoms consisting of wilting, leaf necrosis, vascular discoloration, and death of the plant. Fragments of vascular tissues plated on potato dextrose agar amended with 0.5 g/l streptomycin sulfate yielded white to pale-violet fungal colonies that produced macro- and microconidia. In carnation leaf agar medium, the macroconidia were three-septate and sickle-shaped whereas microconidia were elliptical, and formed abundantly in false heads. The 5.8S subunit and flanking internal transcribed spacer of the rDNA gene were amplified using the ITS1/ITS4 primers (White *et al*., 1990) and sequenced. The ITS sequence was 99% similar to that of *F. oxysporum* (GenBank accession No. JQ045558.1), hence confirming the observed morphological traits typical of this species (Nelson *et al*., 1983). Wounded roots of 4-week-old plants of *G. glabra* were dipped for 10 min in a conidial suspension (1×10^5 spores/ml), while control plants were dipped in sterile tap water. Seedlings were transplanted into pots (13×15 cm) and maintained in a growth chamber at 25°C. Typical symptoms on the leaves developed after five weeks and *F. oxysporum* was successfully re-isolated from artificially infected plants. Control plants remained symptomless. Previously, *F. oxysporum* had been reported as a root rot agent of Russian licorice (*Glycyrrhiza uralensis*) in China (Cao *et al*., 2013). To our knowledge, this is the first report of *F. oxysporum* causing wilt of *G. glabra* in Iran.

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Corresponding author: D.M. Zafari
Fax: +98.8114424190
E-mail: Zafari_d@yahoo.com

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