



# First detection of the mosaic leafhopper, *Orientus ishidae*, in northern Italian vineyards infected by the flavescence dorée phytoplasma

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*Orientus ishidae* Matsumura 1902 [Hemiptera, Auchenorrhyncha, Cicadellidae], known as the mosaic leafhopper, was recently found by Lombardy Plant Health Service technicians (Servizio Fitosanitario Regione Lombardia) in northern Italian (Varese) vineyards affected with flavescence dorée (FD) disease. FD has the greatest economic impact among grapevine yellows diseases caused by phytoplasmas in Europe and is associated with significant production losses in European vineyards (Belli *et al.*, 1997). FD is caused by a phytoplasma (proposed as 'Candidatus Phytoplasma vitis' within group 16SrV, 'Elm yellows'). FD phytoplasma is a prokaryotic, cell wall-less, uncultivable bacteria-like organism that belongs to the class Mollicutes, and is transmitted by *Scaphoideus titanus* Ball, its known natural vector. *O. ishidae* is a polyphagous leafhopper which originates from Palearctic region. It was reported for the first time in Europe in Switzerland (Gunthart & Muhlethaler, 2002) and later in Slovenia (Seljak, 2004). In Italy, *O. ishidae* was previously found in ornamental plants in the Tuscany Region (Mazzoni, 2005).

In 2010, *O. ishidae* was found infected by FD phytoplasma in Slovenia (Mehle *et al.*, 2010), so surveys for *O. ishidae* were conducted in vineyards of Lombardy Region to search for potential FD phytoplasma vectors. A total of 75 yellow sticky traps were used to capture 251 *O. ishidae* specimens, which were stored in 96% ethanol until DNA extraction and PCR testing. Total DNA was extracted from 32/251 specimens and subjected to nested PCR with specific primers that amplify the 16S rRNA gene of the 16SrV phytoplasma group: P1/P7 for the first PCR reaction, and R16(V)F1/R1 (Lee *et al.*, 1994) for the nested PCR step that yielded PCR products of expected size (approximately 1800 bp for P1/P7; 1100 bp for R16(V)F1/R1). RFLP analyses with restriction endonucleases *TaqI* and *BfaI* were carried out for the nested PCR amplicons for preliminary characterisation of the phytoplasma group. Nested PCR and RFLP products were analysed in 1% and 3% agarose gels, respectively, stained with ethidium bromide and visualised under UV light (Figs. 1, 2). Six out of 32 *O. ishidae* specimens were found positive for the presence of 16SrV phytoplasma group products by nested PCR. RFLP analysis of the six nested PCR products yielded patterns identical to those of 16SrV-D subgroup isolated from grapevines.

Nested PCR amplicons from two representative samples were purified

(MiniElute®PCR Purification Kit-Qiagen®) and directly sequenced. The partial 16S rDNA sequence of the phytoplasma detected in *O. ishidae* showed 99% sequence identity with that of the FD phytoplasma (GenBank Accession No.JN641804). The high populations of *O. ishidae* in vineyards and its capability to acquire the FD phytoplasma compared with the low captures of *S. titanus* (only three specimens on the same 75 yellow sticky traps), may suggest a role of *O. ishidae* in FD epidemiology. Transmission trials to transmit the FD phytoplasma from infected to healthy plants are in progress to prove vector capacity of *O. ishidae* for FD.

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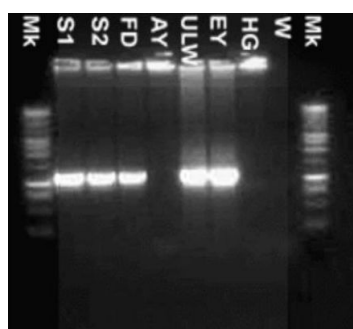


Figure 1

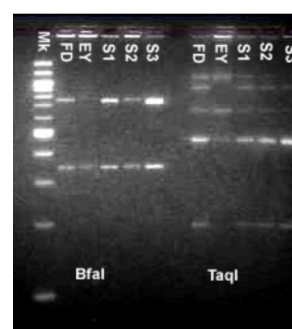


Figure 2

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