



First report of *Stemphylium vesicarium* on chilli pepper in Italy

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In 2014 during a survey of fields of chilli pepper, grey leaf spot (GLS) symptoms were observed on leaves in two different Italian areas, Rieti (central Italy) and Cosenza (southern Italy). Diseased leaves were characterised by numerous tiny round spots (0.5 to 2.5 mm diameter, average 1.3 mm) that were white to grey with a sunken centre and brown edge, resulting in premature defoliation (Fig. 1). No other part of the plant was affected. The varieties 'Fatalii White' (*Capsicum chinense*) and 'Diavolicchio Calabrese' (*C. annuum*) were the most susceptible in central and southern Italy, respectively. In both locations disease incidence on the two chilli pepper varieties was >40%.

Tissue from leaves with typical GLS symptoms was plated on potato dextrose agar amended with streptomycin and ampicillin (100 ppm each) and incubated at 22 ±0.5°C with a 12 hour photoperiod. Single spore isolations were performed to obtain pure cultures (Fig. 2) and the morphological characteristics were consistent with the type description of *Stemphylium vesicarium* (Wallroth) Simmons (Simmons, 1969). To confirm morphological identification, a representative isolate from each of the two Italian areas (ISPaVe2162 and ISPaVe2165) were subjected to molecular analysis. The internal transcribed spacer (ITS) and glyceraldehyde 3-phosphate dehydrogenase (*gpd*) gene were amplified using universal primers ITS5 and ITS4 (White *et al.*, 1990) and *gpd*1 and *gpd*2 (Berbee *et al.*, 1999), respectively and sequences were deposited in the European Nucleotide Archive (Accession Nos. LN896692 and LN896693 for the ITS, and LN896694 and LN896695 for the *gpd* gene, respectively). A BLAST search in GenBank showed 100% identity with *S. vesicarium* in both the ITS region (JX424810) and *gpd* gene (DQ000654). To further support identification, phylogenetic analyses using the Maximum Composite Likelihood method (Kimura2/parameter model) was performed (MEGA 5.2). The isolates ISPaVe2162 and ISPaVe2165 clustered together with *S. vesicarium* isolates (Fig. 3).

Pathogenicity tests of the two isolates were performed by artificial inoculations on detached leaves of the chilli pepper varieties 'Diavolicchio Calabrese' and 'Fatalii White'. A 10 µl drop of a 1 × 10⁵ conidia/ml suspension was placed on the abaxial leaf surface. A 10 µl drop of sterile

distilled water was used as negative control. The inoculated leaves were incubated in sterile Petri plates, 20 mm in diameter, containing water-saturated sterile blotting paper at room temperature (24 ±2°C). Brown lesions started to be visible on detached leaves three days after inoculation. Subsequently, lesions enlarged in size and typical GLS were evident at all inoculation points on both chilli pepper varieties (Fig. 4). No symptoms developed on the leaf inoculated with sterile distilled water.

In Italy, *S. vesicarium* has been reported as the causal agent of leaf spots on tomato (Porta-Puglia *et al.*, 2001) and wilting and root rot on radish sprouts (Belisario *et al.*, 2008). To our knowledge this is the first report of *S. vesicarium* on chilli pepper in Italy. The presence of *S. vesicarium* in two distinct geographic areas in Italy suggests an association with seed contamination and *Stemphylium* spp. are generally seed-borne. Consequently more strict control of seed health for chilli pepper production would be advisable.

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Figure 1

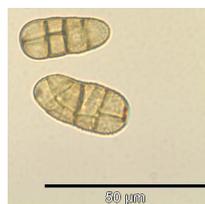


Figure 2

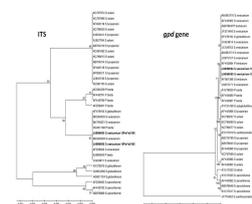


Figure 3



Figure 4

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