Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium clavum* Causing Leaf Spot and Fruit Rot on Tomato in Italy

G. Gilardi,¹ S. Matic,¹ V. Guarnaccia,^{1,2} A. Garibaldi,¹ and M. L. Gullino^{1,†}

¹Centre of Competence for Innovation in the Agro-Environmental Sector (AGROINNOVA), University of Torino, 10095 Grugliasco, Italy

²DISAFA, University of Torino, 10095 Grugliasco, Italy

Funding: Funding was provided by Fondazione Cassa Risparmio Cuneo, Agroalimentare 4.0, SFIDA Project 'Low environmental impact management strategies for the horticultural sector'. Plant Dis. 105:2250, 2021; published online as <https://doi.org/10.1094/PDIS-05-20-1096-PDN>. Accepted for publication 22 February 2021.

During July to October 2019, an unknown leaf spot and fruit rot disease was observed on 90- to 120-day-old *Solanum lycopersicum* L. plants in plastic tunnels (0.5 ha) in Piedmont, Italy. Small circular, brown leaves spots (1 to 5 mm in diameter), with a well-defined border, surrounded by a yellow halo, were observed on 20 to 35% plants. Lesions expanded up to 30 mm in diameter, becoming necrotic and broken in the center, and eventually dried out. In September, 1 to 5% of fruit showed dry rot (up to 40 mm in diameter) and fruit mummification. Approximately 1-mm² tissue pieces of 50 symptomatic leaves and 20 fruits were dipped in 1% sodium hypochlorite for 1 min, rinsed in sterilized water, and placed on potato dextrose agar (PDA) with 25 mg/liter of streptomycin sulfate. *Fusarium* was isolated at 80% frequency from both tissues incubated at 22°C for 5 days. Single-spore cultures of two selected isolates, isolates 8-19.1 and 8-19.3, respectively, from the leaves and fruits, were subcultured on carnation leaf agar (CLA) (Leslie and Summerell 2006). Microconidia, although rare, were single-celled, hyaline, nonseptate, ovoid, and 10.1 to 14.5 × 3.2 to 3.9 µm. Macroconidia were hyaline one to five septate, with dorsiventral curvature, and measured 19.9 to 41.6 × 2.3 to 5.6 (mean 30.4 × 4.0, *N* = 40) µm. Chlamydospores developed in a 21-day-old culture grown on CLA, either terminally or intercalary, of 6.7 to 10.9 (mean 9.0, *N* = 40) µm. The DNA extraction was performed (E.Z.N.A. Fungal DNA Mini Kit, Omega Bio-Tek, Darmstadt, Germany). Subsequently, the partial calmodulin gene

(*cmdA*; Carbone and Kohn 1999; Groenewald et al. 2013) and partial RNA polymerase II beta subunit (*rpb2*; Xia et al. 2019) were amplified and sequenced, producing 579 to 580 bp (*cmdA*) and 874 to 876 bp (*rpb2*) (GenBank nos. MT182816 and MT182814 for isolate 8-19.1, and MT182817 and MT182815 for isolate 8-19.3). BLASTn analysis exhibited 100% identity with the reference strains of *Fusarium clavum* known as FIESC 5 (Villani et al. 2016; Xia et al. 2019) in *rpb2* portion (GenBank nos. MN170395 and LN901605) and 99% identity in *cmdA* portion (GenBank no. MN170328). Both isolates clustered in the same clade as *F. clavum* following the maximum parsimony algorithm on the concatenated alignment of *cmdA* and *rpb2* sequences (supplementary material). The suspension (1 × 10⁵ conidia/ml) from 15-day-old PDA cultures of both isolates was sprayed onto five 30-day-old plants (cv. Rugantino) covered with plastic bags for 7 days in a greenhouse at 20 to 26°C. Fruits were surface disinfected, wounded with a 5-mm borer (four fruits, five wounds/fruit), and inoculated (10⁴ conidia/ml of both isolates). Negative control leaves and fruits were treated with sterilized distilled water. Inoculated and negative control fruits were kept in a chamber at 22°C for 7 days. Both tests were repeated. Symptoms were observed 8 to 10 days after inoculation of leaves and 5 days after inoculation of fruit and progressed as observed in the field. All water-inoculated controls remained asymptomatic. *Fusarium* colonies were recovered from the inoculated leaves and wounded fruits. *F. equiseti* was found to cause tomato crown and root rot (Yezli et al. 2019) and fruit rot (Khokhar et al. 2019). This is the first report of *F. clavum*, a member of the *Fusarium incarnatum-equiseti* species complex (Xia et al. 2019), causing fruit rot and leaf spot on tomato in Italy. The new disease poses a threat to tomato cultivation in Italy and needs to be properly monitored and managed to prevent severe yield loss.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Groenewald, J. Z., et al. 2013. Stud. Mycol. 75:115.
- Khokhar, I., et al. 2019. Plant Dis. 103:1782.
- Leslie, J. F., and Summerell, B. A., eds. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Ames, IA.
- Villani, A., et al. 2016. Int. J. Food Microbiol. 234:24.
- Xia, J. W., et al. 2019. Persoonia 43:186.
- Yezli, K., et al. 2019. Plant Pathol. J. 101:1249.

The author(s) declare no conflict of interest.

Keywords: fungi, vegetables, epidemiology

[†]Indicates the corresponding author.
M. L. Gullino; marialodovica.gullino@unito.it