Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Cladosporium tenuissimum* Causing Leaf Spots on Carnation in China

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Carnation (*Dianthus caryophyllus* L., Caryophyllaceae) is a popular and important market flower in China (Kayamori et al. 2012). Leaf spots of *Cladosporium tenuissimum* Cooke were first observed in early October 2016 on carnation grown at the academy of agricultural sciences as well as in parks in Xi'an city (108°54'18.6696" E, 34°15'12.9" N), with nearly 80% of leaves on individual plants infected. Carnation plants in five nearby parks were surveyed and showed 60 to 100% disease incidence. Initially, the symptoms appeared as small, dark, elliptical, necrotic lesions surrounded by a dark-brown halo. Some leaf spots in severe cases covered the entire leaf surface. Necrotic tissue was treated with lactophenol and used for microscopic examination. Sporulation was seen on the necrotic tissue. To identify the pathogen, 18 leaf pieces (3 to 5 mm) with both infected and healthy portions were taken at the edge of lesions and surface disinfected by placing them in 75% ethanol for 5 s, then transferred to a 0.1% aqueous mercuric chloride solution for 30 s and rinsed with sterilized water three times. Six sections were placed on each potato dextrose agar (PDA) at 25°C in the dark. Ten pure fungal cultures were obtained from single spores. Colonies on PDA were greyish to dark brown and showed a velvety texture. Subcylindrical to subclavate conidiophores were solitary or in loose groups, on leaves and stems erumpent through the cuticle or emerging through stomata, and measured 49 to 513 by 3 to 7 µm ($n = 50$). Ramoconidia were subcylindrical, 15 to 31 by 4 to 5 µm, aseptate, with basal hilum 2 to 3.5 µm in diameter. Morphological

characteristics of the pathogen were similar to *C. tenuissimum* Cooke (Bensch et al. 2010). For molecular identification, pure cultures of 10 single-spore isolates were extracted from mycelium using the Plant Genomic DNA Kit (Tiangen, China). Three different genomic DNA regions—ribosomal DNA internal transcribed spacer (ITS) region, partial translation elongation factor 1 α (EF), and actin (ACT)—were amplified using the primers ITS1/ITS4 (White et al. 1990), ACT-512F/ACT-783R (Carbone and Kohn 1999), and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. Phylogenetic analysis of multiple genes was conducted with the neighbor-joining method using MEGA 7 (Bakhshi et al. 2018). The sequences of our isolate (CTK) and seven published sequences of *C. tenuissimum* were clustered into one clade with a 100% bootstrap supporting level. The sequences of CTK have been deposited in GenBank with accessions MZ351731 for ITS, MZ351730 for ACT, and MZ351732 for TEF1. Isolate pathogenicity was tested on surface-disinfested leaves of 2-month-old carnation plants by spraying a 2×10^6 conidia/ml at an incubation temperature of 25°C. Another set of plants was sprayed with sterile water as a noninoculated control. Three replicates of every isolate were conducted, and each replicate included five carnation plants. After 12 days, only the inoculated leaves showed leaf blight symptoms resembling those observed on naturally infected carnation leaves. The pathogen was consistently reisolated from the infected leaves with the aim of completing Koch's postulates. To our knowledge, this is the first report of *C. tenuissimum* causing carnation leaf spots in China and worldwide. Thus, the identification of *C. tenuissimum* for this disease is important for future prospects for the advancement of effective prevention and control approaches.

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