

DISEASE NOTE

**FIRST REPORT OF SPECIES IN THE
CLADOSPORIUM CLADOSPORIOIDES
COMPLEX CAUSING PECAN LEAF SPOT
IN BRAZIL**

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From January to April 2014, sixteen pecan (*Carya ilinoensis*) orchards in southern Brazil were surveyed to determine the causal agent of a small black circular leaf spot disease. Forty isolates obtained on potato dextrose agar (PDA) were identified on the basis of morphological features and sequence analysis of the translation elongation factor 1- α gene (Bensch *et al.*, 2012). Morphological features and sequence homology confirmed the isolates to be either *Cladosporium cladosporioides*, *C. pseudocladosporioides* or *C. subuliforme*, all species included in the *C. cladosporioides* complex. On PDA, *C. cladosporioides* (2/1PR, GenBank accession No. KT991541) and *C. pseudocladosporioides* (11RS, KT991560) both produced colonies of greenish-gray pigmentation with velvety and floccose aerial mycelium, whereas *C. subuliforme* (16/2RS, KT995114) produced colonies with similar pigmentation but more abundant aerial mycelium. Isolates 2/1PR, 11RS and 16/2RS produced on average colonies that were 72.2, 68.1 and 74.6 mm in diameter, respectively; conidia were $5.1 \times 2.6 \mu\text{m}$, $5.7 \times 2.5 \mu\text{m}$ and $5.3 \times 2.9 \mu\text{m}$ in length; ramoconidia were $23.7 \times 4.2 \mu\text{m}$, $22.5 \times 2.8 \mu\text{m}$ and $16.0 \times 4.3 \mu\text{m}$ in length. To confirm pathogenicity, young detached leaflets were inoculated with twenty-six isolates. One-tenth ml of conidial suspension (1×10^6 conidia ml⁻¹) divided into six individual drops was applied on each leaflet surface with eight replicates. Leaflets receiving only sterile distilled water served as a non-inoculated control. Black leaf spots were observed after 22 days on all inoculated leaflets. The original *Cladosporium* species was re-isolated from inoculated leaflets. To our knowledge, this is the first report of *C. cladosporioides*, *C. pseudocladosporioides* and *C. subuliforme* causing pecan leaf spot in Brazil as well as in the world.

Bensch K., Braun U, Groenewald J. Z., Crous P. W., 2012. The genus *Cladosporium*. *Studies in Mycology* 72: 1-401.

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**FIRST REPORT OF LEAF SPOT CAUSED
BY *COLLETOTRICHUM KAHAWAE* ON
AMERICAN SWEETGUM (*LIQUIDAMBAR
STYRACIFLUA*) IN ITALY**

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Liquidambar styraciflua (American sweetgum, Altingiaceae) is a deciduous tree native to warm temperate areas of eastern North America and tropical montane regions of Mexico and Central America. In Italy it is a popular ornamental tree. During summer-fall 2014, a new leaf spot was observed on 30-year-old plants of *L. styraciflua*, at temperatures ranging between 15 and 26°C and high relative humidity. All of the five plants in a private garden close to Biella (northern Italy) were affected. Seventy to eighty percent of leaves showed necrotic spots, 10 to 60 mm in diameter, with a purple margin, often interesting the margins of the leaves. *Colletotrichum* sp. was consistently recovered with a frequency of 70% from several isolations from infected leaf tissues carried out on potato dextrose agar (PDA) amended with 25 mg/l of streptomycin sulphate (Bailey and Jeger, 1992). Hyaline, cylindrical, aseptate and thin walled conidia (13.0 to 18.7×3.8 to 5.9 , average $16.2 \times 4.7 \mu\text{m}$ size) were abundantly produced in acervuli in a gray mycelium on PDA medium. DNA was extracted with E.Z.N.A. Plant DNA Kit (Omega Bio-Tek) and PCR reactions were performed using primers ITS1/ITS4, and primers T1 (O'Donnell and Cigelink, 1997) and $\beta\text{t}2\text{b}$ (Glass and Donaldson, 1995). The 499 bp and 725 bp products were sequenced (GenBank accession Nos. KT375326 and KT375325, respectively) and confirmed to correspond to *Colletotrichum kahawae*. Pathogenicity tests were performed under growth chamber at 24 to 26°C and 12 h photoperiod by spraying *L. styraciflua* leaves with 1×10^5 conidia/ml or sterile water covered with plastic bags for 5 days. About 7 to 10 days after inoculation, lesions developed only on inoculated leaves and *Colletotrichum* sp. was consistently reisolated. This is the first report of *Colletotrichum kahawae* on American sweetgum in Italy as well as worldwide. Due to the increased number of report of *C. kahawae* in Italy on species such as olive, mango and cultivated rocket, the adoption of preventative strategies to contain the spread of this pathogen is suggested.

Bailey J.A., Jeger M.J., 1992. *Colletotrichum*. Biology, Pathology and Control, CAB International, UK.

Glass N.L., Donaldson G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323-1330.

O'Donnell K., Cigelink E., 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7: 103-116.

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