Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Sardiniella urbana* (Botryosphaeriaceae) Causing Decline of *Celtis australis* in Mallorca Island (Balearic Islands, Spain)

Ángel Pintos-Amengual,¹ Alberto M. Stchigel,^{2,†} and José F. Cano-Lira²

¹Interdisciplinary Ecology Group, Universitat de les Illes Balears, Palma de Mallorca, Spain

²Unitat de Micologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, 43201 Reus, Spain

Plant Dis. 105:3748, 2021; published online as <https://doi.org/10.1094/PDIS-01-21-0154-PDN>. Accepted for publication 19 May 2021.

European nettle (*Celtis australis* L.; Ulmaceae) is deciduous and a common ornamental tree in Palma de Mallorca (PdM) city (Balearic Islands, Spain). Little is known about fungal diseases of *C. australis* in Mallorca Island, with only a single report of foliar disease caused by *Helicoceras celtidis* (Biv.) Linder (Siquier et al. 2019). Surveys from 2018 onward on 30 trees in two streets in PdM and one street in Calvià revealed serious disease symptoms on European nettle. Symptoms included leaf chlorosis, defoliation, stem and branch dieback, and wedge-shaped wood necrosis (in cross section) of infected branches. Samples were collected from three specimens, two in PdM (AP25120B1, AP28120) and one in Calvià (AP81219). Six samples, two from each tree with branch canker symptoms, were collected. Small pieces (0.5 cm²) were cut from lesion margins and surface sterilized in 70% EtOH for 2 min. Samples were placed on PDA supplemented with 500 mg/liter of chloramphenicol (Cl) (PDA+Cl) in 90-mm-diameter Petri dishes and incubated for 7 days at 25°C. Pure cultures were obtained by transferring in parallel actively growing hyphae to Petri plates containing PDA+Cl and to tap water agar (TWA; 1.5% w/v agar to tap water) with 500 mg/liter Cl and containing several pieces (2 to 3 cm long) of sterilized pine needles to stimulate sporulation. Six morphologically identical isolates were obtained, one per sample; characteristics match *Sardiniella urbana* (Linaldeddu et al. 2016). Colonies growing on PDA at 25°C reached 80 mm after 7 days, have a velvety texture with irregular margins, are white but turn dark green with age, and the reverse is dark gray. Pycnidia were formed on TWA with pine needles after 3 weeks; they are unilocular, ostiolate, glabrous, pyriform, 210 to 320 × 140 to 200 µm, with an outer wall of textura angularis, 40 to 50 µm thick, five- to seven-layered, composed by flattened, polyhedral, brown to

subhyaline peridial cells. Conidiogenous cells percurrently proliferating, forming inconspicuous annellations at the top, hyaline, cylindrical, 4 to 10 × 3 to 9 µm. Conidia enteroblastic, solitary, initially hyaline and unicellular, becoming brown and bicelled, with a medial septum, rough and thick-walled, from ellipsoidal to ovoid, 19.2 to 26.3 × 9.8 to 14.5 µm. AP24420 and AP25120B1 (Ángel Pintos personal culture collection) were used for molecular identification. Total DNA was extracted from pure cultures grown on PDA+Cl for 4 days at 25°C using a DNeasy Plant Mini Kit (Qiagen). The complete ITS region and a fragment of the EF-1α were amplified by PCR using primers pairs ITS5/ITS4 and EF1-983F/EF1-2218R, respectively, for AP24420 and AP25120B1. PCR products were sequenced by Macrogen (Madrid, Spain). Nucleotide sequences of both isolates showed the same similarity values using a MegaBLAST search (Morgulis et al. 2008): 99.80% similar to the ITS sequence of the type strain of *S. urbana* CBS 141580 and 94.07% similar to the EF-1α sequence of *Sardiniella celtidis* MFLUCC_17_981. Sequences were deposited in GenBank: LR993312 (ITS) and LR993315 (EF-1α) for AP24420, and LR993316 (ITS) and LR993317 (EF-1α) for AP25120B1. Pathogenicity was tested by inoculating each *S. urbana* isolate to 10 *C. australis* plants (3 years old). Before inoculation, the bark was surface sterilized by a cotton pad with 70% v/v EtOH for 2 min. Then, 2 cm² of the bark was resected by sterilized scalpel. A PDA+Cl plug (2 cm²) with actively growing mycelium was inoculated in the wound, covered with sterilized aluminum foil, and sealed with tape. Negative control seedlings (10) used a noninoculated PDA+Cl plug. Inoculated seedlings were grown at 18 to 22°C for 40 days, with 12-h light/12-h dark. Plants inoculated with the isolates developed brownish necrotic wood lesions (25 to 50 mm diameter), which penetrated a few millimeters into the sapwood; control seedlings developed no symptoms. The pathogen was reisolated from 100% of the lesions from experimentally infected plants and the identity confirmed using the methodology above; no fungal isolates were recovered from control plants, fulfilling Koch's postulates. This is the first report of *S. urbana* causing decline of *C. australis* in Mallorca Island, demonstrating that it is an emerging disease in the Mediterranean area.

References:

- Linaldeddu, B. T., et al. 2016. Mycosphere 7:893.
- Morgulis, A., et al. 2008. Bioinformatics 24:1757.
- Siquier, J. L., et al. 2019. Rev. Cat. Micol. 40:3. <https://www.raco.cat/index.php/Micologia/article/view/378937>.

The author(s) declare no conflict of interest.

Keywords: *Celtis australis*, infection, Mallorca, *Sardiniella*, Spain

[†]Indicates the corresponding author.

A. M. Stchigel; albertomiguel.stchigel@urv.cat