Plant Pathology & Quarantine 7(2): 160–163 (2017) ISSN 2229-2217
www.ppqjournal.org **Article**

Doi 10.5943/ppq/7/2/9

First confirmed report of *Cerotelium fici* causing leaf rust on *Ficus carica* in Mexico

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Solano-Báez AR, Jiménez-Jiménez B, Camacho-Tapia M, Leyva-Mir SG, Nieto-López EH, Tovar-Pedraza JM 2017 – First confirmed report of *Cerotelium fici* causing leaf rust on *Ficus carica* in Mexico. Plant Pathology & Quarantine 7(2), 160–163, Doi 10.5943/ppq/7/2/9

Abstract

During August to October 2015 and 2016, severe symptoms of rust were observed on fig leaves in orchards, nurseries, and backyard gardens located in Morelos, Puebla, State of Mexico, and Mexico City, Mexico. Based on morphology and analysis of nuclear large subunit rDNA, the fungus was identified as *Cerotelium fici.* This is the first confirmed report of *C. fici* causing fig leaf rust in Mexico.

Key words – *Cerotelium fici* – *Ficus carica* – morphology – sequence analysis

Introduction

Fig (*Ficus carica* L.), belongs to the family Moraceae, and it is an important crop worldwide for dry and fresh consumption and the commercial demand increases every year (Solomon et al. 2006). The major production is represented by a few cultivars within different regions as a result of selection through time among wild seedlings and volunteer open-pollinated seedlings of cultivated trees (Mawa et al. 2013).

Mexican fig production is considered as an incipient crop starting to increase in importance. During 2015, according to an official database, 1338 ha of figs were cultivated in Mexico, distributed mainly in the states of Baja California Sur, Morelos, and Puebla (SIAP 2016).

Many diseases, some of which can be quite destructive, affect the fig plant and fruit. Fungi cause most of these problems. The most important fungal diseases reported on fig plants are Alternaria internal rot, Alternaria leaf spot, brown leaf spot, Botrytis limb blight, canker, Cephalosporium leaf spot, Cercospora leaf spot, Macrophoma canker, fruit rot, soft rot, and rust (Michailides 2003).

Materials & Methods

Sample collection

Submitted 10 October 2017, Accepted 5 November 2017, Published 23 November 2017
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During August to October 2015 and 2016, a total of 100 fig leaves with symptoms and signs of rust were collected at orchards, nurseries, and backyard gardens located in Morelos, Puebla, State of Mexico, and Mexico City, Mexico. The symptoms on the mature leaves of the diseased plants consisted of angular necrotic lesions produced on the upper surface (Fig. 1A), while the lower surface was covered with reddish brown erumpent pustules (Fig. 1B). In all infected plants, premature defoliation was observed. A voucher specimen of five samples was deposited in the Herbarium of the Department of Agricultural Parasitology at the Chapingo Autonomous University (Texcoco, Estado de México, Mexico) as UACH H120–H124.

Morphology

For the morphological characterization of the fungus, glass slides with lactic acid were made with longitudinal sections of the fungal structures (uredinial stage) present on the lower surface of the leaves. Slides were examined using an Eclipse Ni-U compound microscope (Nikon®, Japan) and micrographs were taken using a Moticam 580 camera (Motic®, China). The morphological characteristics of 30 uredinia, 100 turgid urediniospores, and 30 uredinial paraphyses were recorded.

DNA extraction, PCR amplification and sequencing

To confirm the identity of the fungus, urediniospores were scraped from a sorus. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen®, USA) according to the manufacturer's protocol. The quality of the DNA was verified by electrophoresis in a 1% agarose gel. DNA concentrations were quantified using a NanoDrop Lite Spectrophotometer (Thermo Scientific®, USA) and the samples were diluted to 50 ng/µl for polymerase chain reaction (PCR). The nuclear large subunit (LSU) rDNA gene was amplified by a nested PCR using the primers Rust 2INV (Aime 2006) and LR7 (Vilgalys & Hester 1990). One microliter of the first reaction mixture was used for the second amplification with the primers LROR and LR6 (Vilgalys & Hester 1990) as described by Daly & Ono (2015). The amplified PCR product was sequenced by Macrogen (www. macrogen.com). The resulting 899 bp sequence was deposited in GenBank (Accession No. MF580676) and was compared with the unique LSU sequence available in the database.

Results

Microscopic observations of the fungus revealed hypophyllous uredinia (Fig. 1C), subepidermal, scattered, vesiculose, erumpent, 135–210 × 125–160 µm. Urediniospores were broadly ellipsoid or obovoid-globose, unicellular, echinulate, sessile, 23–27 × 16–19 µm (Fig. 1D). Uredinial paraphyses were cylindric to slightly claviform, thin-walled and hyaline. Germ pores were obscure and equatorial. Telia were not observed. The morphology of uredinia and urediniospores was the same for all samples.

BLASTn analysis of the LSU sequence showed a 94% similarity with that of *C. fici* from *Ficus* sp. from Australia (Accession No. KP753385).

Discussion

The results of morphological characterization coincided with the description of *Cerotelium fici* reported by McKenzie (1986, 2013) and Huseyin & Selcuk (2004). Currently, only one LSU sequence of *C. fici* is available in GenBank; our sequence (MF580676) is the second sequence of LSU rDNA of *C. fici* deposited in GenBank.

Based on the combination of morphological characterization and sequence analysis of nuclear large subunit rDNA, the fungus was identified as *C. fici.* This plant pathogenic fungus has been reported on several species of *Ficus* around the world (Farr & Rossman 2017). Alvarez (1976) reported *C. fici* (as *Uredo fici*) as the causal agent of leaf rust on *F. carica* in Mexico. However, that report was not supported by morphological or molecular analysis, so that report should not be considered as valid due to the lack of scientific evidence. In addition, León-Gallegos & Cummins (1981) registered *Cerotelium fici* on *Ficus involuta* based on morphological characterization in the

states of Oaxaca and Campeche in Mexico. To our knowledge, this is the first morphological and molecular confirmation of the presence of *Cerotelium fici* causing leaf rust on *Ficus carica* in orchards, nurseries, and backyard gardens from Mexico.

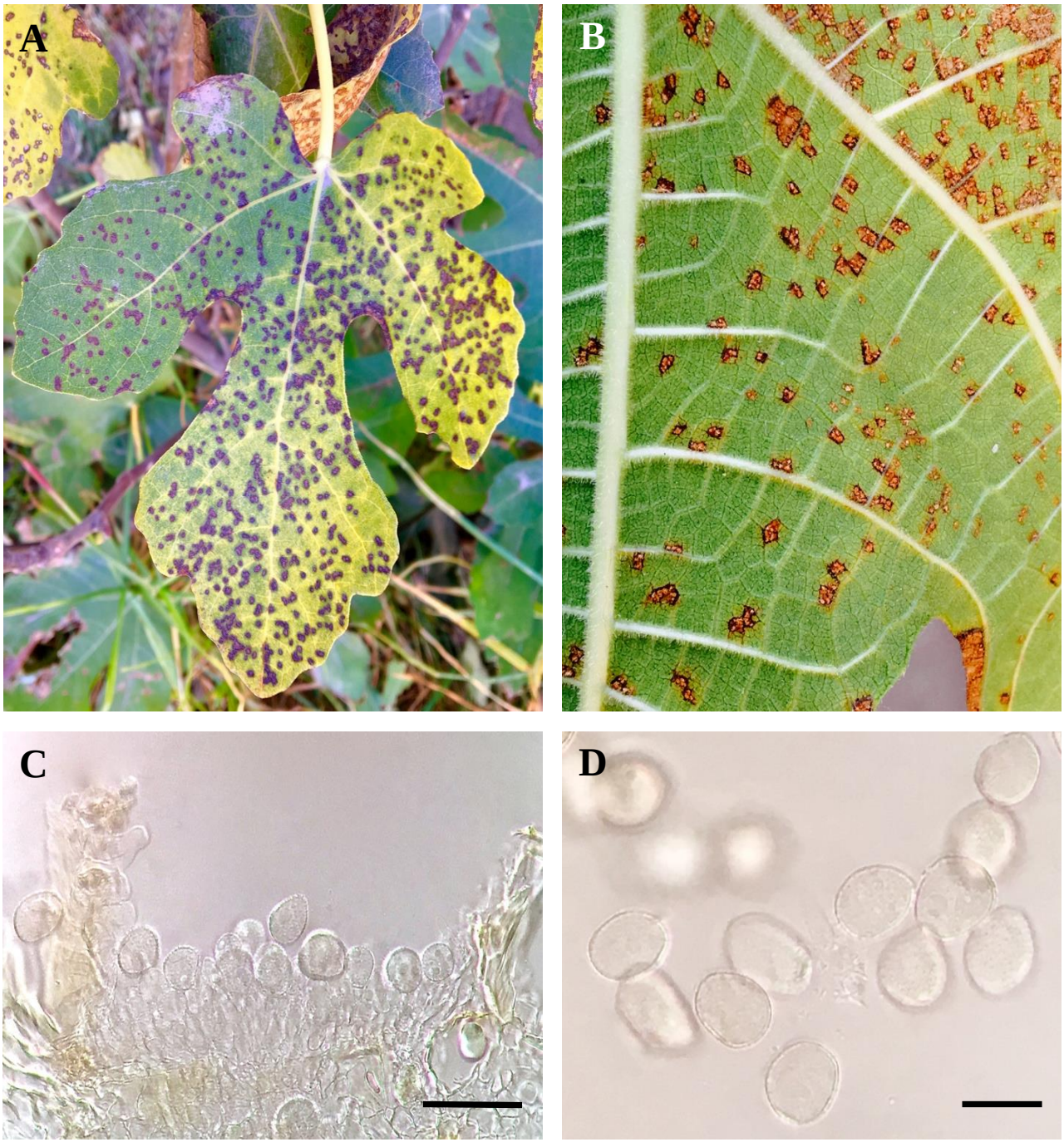

Fig 1 – *Cerotelium fici* on *Ficus carica*. A, Severe symptoms on upper surface of a fig leaf. B, Signs (uredinia) on lower surface of a fig leaf. C, Longitudinal section of an uredinium. D, Urediniospores. – Bars C = 50 µm, D = 20 µm.

Disease management of fig rust is achieved by chemical and cultural control. Fallen leaves should be removed from the orchard and destroyed. Fig rust can be controlled with preventive applications of fungicides such as copper sulfate, sulfur, zineb and maneb (McKenzie 1986, Michailides 2003). However, further studies are needed to evaluate strategies for the management of this disease in the fig producing areas in Mexico.

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