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Impact Factor: 3.02

ISSN: 0191-2917

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# plant disease

Editor-in-Chief: Alison E. Robertson  
Published by The American Phytopathological Society

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Posted online on March 18, 2016.  
<http://dx.doi.org/10.1094/PDIS-11-15-1298-PDN>

## DISEASE NOTES

## First Report of Cladode Brown Spot in Cactus Prickly Pear Caused by *Neofusicoccum batangarum* in Brazil

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## ABSTRACT

Cactus prickly pear (*Nopalea cochenilifera*) cladodes showing brown spot symptoms were collected of 18 fields of the State of Pernambuco, northeastern Brazil, from March to June 2014. The symptoms were prevalent in 100% of fields surveyed. Small pieces (4 to 5 mm) of necrotic tissues were surface sterilized for 1 min in 1.5% NaOCl, washed twice with sterile distilled water, and plated onto potato dextrose agar (PDA) amended with 0.5 g/liter streptomycin sulfate. Colonies morphologically similar to species of Botryosphaeriaceae were transferred to malt extract agar (MEA); five isolates (CMM 1424, CMM 1425, CMM 1426, CMM 1427, and CMM 1428) presented colonies forming concentric rings, and white mycelium becoming gray to gray-olivaceous after 5 days. Conidial characters were observed after growth on 2% water agar bearing sterilized pine needles for 3 weeks at 25°C under near-UV light. Conidiogenous cells holoblastic, hyaline, smooth, and cylindrical. Conidia were nonseptate, hyaline, smooth, fusoid to ovoid, thin-walled,  $15.3 \pm 1.4 \times 5.4 \pm 0.6 \mu\text{m}$  ( $n = 50$ ), L/W ratio = 2.8, which are morphological and cultural characteristics typical of *Neofusicoccum* spp. (Phillips et al. 2013). DNA sequencing of part of the elongation factor 1-alpha (EF1- $\alpha$ ) gene and the internal transcribed spacer (ITS1-5.8S-ITS2 rDNA) region were conducted to identify the species as described by (Marques et al. 2013). Sequences of the isolates were 99% similar to those of *N. batangarum* for EF1- $\alpha$  (GenBank Accession Nos. FJ900653 and FJ900654) and ITS (FJ900607 and FJ900608). A phylogenetic analysis using Bayesian inference and including published EF1- $\alpha$  and ITS data for *Neofusicoccum* spp. was carried out, showing that the isolated fungi belong to the *N. batangarum* clade. Sequences of the isolates were deposited in GenBank (ITS, KU052217 to KU052221; EF1- $\alpha$ , KT873814 and KT878644 to KT878647). Cultures were deposited in the Culture Collection of Phytopathogenic Fungi of the Universidade Federal Rural de Pernambuco, Recife, Brazil. Pathogenicity tests were performed with all five strains of *N. batangarum* on detached cladodes of cactus prickly pear ('Miuda'). Detached cladodes were wounded (~4 mm long, 2 mm deep) in two points (spaced 15 cm) using a sterilized scalpel. Mycelial plugs (0.5 mm diameter) from the margin of actively growing colonies (PDA) of each isolate were placed in the wounds. Non-colonized PDA plugs were used as control. Inoculated and control cladodes (10 each/isolate with two replications) were maintained in a moist chamber for 72 h at 25°C in the dark. After 11

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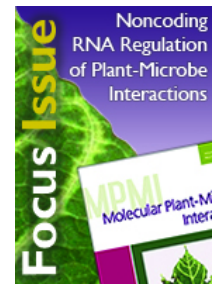
## Article History

Ahead of Print: 18 Mar 2016

First Look: 12 Jan 2016

Accepted: 5 Jan 2016

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days, all inoculated cladodes showed brown spots, with an average length of 1.5 cm ( $\pm 0.3$  cm) and depth of 1.0 cm ( $\pm 0.5$  cm). No symptoms were observed in the control cladodes. The pathogen was reisolated from all inoculated shoots and identified by conidia morphology to fulfill Koch's postulates. *Neofusicoccum batangarum* was identified as the causal agent of dieback on *Terminalia catappa* in Cameroon (Begoude et al. 2010), and on healthy seeds of *Schinus terebinthifolius* in the United States (Shetty et al. 2011). This is the first report of *N. batangarum* causing cladode brown spot in cactus prickly pear in Brazil and worldwide.

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