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Short communication

Characteristics of *Lasiodiplodia theobromae* from *Rosa rugosa* in South China

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ABSTRACT

Rosa rugosa Thunb., one of the most important cut flower crops in China, is mainly planted in the southern regions of this country. *Lasiodiplodia* species include pathogens of a wide variety of plant and cover a wide geographic distribution. Recently, canker disease caused by species of *Lasiodiplodia* was observed on *R. rugosa* branches in GuangDong Province in South China. Based on sequence comparisons for the ITS rDNA and translation elongation factor 1 alpha (TEF-1α) gene region, and combined morphological characteristics, the *Lasiodiplodia* isolates from diseased *R. rugosa* branches were identified as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. Pathogenicity tests showed that *L. theobromae* was virulent to *R. rugosa*. To our knowledge, this is the first report of *L. theobromae* infecting *Rosa* in China.

1. Introduction

Rose (*Rosa rugosa*) is one of the top ten cut flower crops in China; approx. 10,000 ha of rose have been planted in this country, mainly in the YunNan and GuangDong Provinces in South China (Liu et al., 2012). Studies of *R. rugosa* diseases in China are relatively limited; the reported pathogens of *R. rugosa* in China include *Actinonema rosae* (Lib.) Fr. (Chen, 2002), *Botrytis cinerea* Pers. (Zhang, 2006), *Cladosporium* sp. (Zhang, 2003), *Phragmidium* spp. (Chen, 2002), and *Sphaerotheca* spp. (Chen, 2002). These pathogens produce various disease symptoms but are particularly associated with spots on the branch and leaf (Chen, 2002; Zhang, 2003, 2006).

Lasiodiplodia (*Botryosphaeriaceae*, Botryosphaeriales, Ascomycetes) is a genus of fungi that has a wide geographic distribution and occurs on a wide range of monocotyledonous, dicotyledonous and gymnospermous hosts (Barr, 1987; Slippers and Wingfield, 2007). Fungi of *Lasiodiplodia* can cause various disease symptoms such as stem cankers, stem and branch gummosis, shoot blight and fruit rot (Slippers and Wingfield, 2007). Recently, diseased branches of *R. rugosa* with symptoms typical of those caused by species of *Lasiodiplodia* were observed in South China. Currently, in China, there has been no report of *Lasiodiplodia* spp. on *Rosa* spp. The objectives of this study were as follows: (1) to identify the species of *Lasiodiplodia* associated with diseased branches of *R. rugosa* using phylogenetic analyses and morphological characteristics and (2) to determine the virulence of the identified *Lasiodiplodia* species by pathogenicity tests.

2. Materials and methods

2.1. Sample collection and isolation

In Oct. 2014, diseased branches of *R. rugosa* were observed and collected in the ZhanJiang Region of GuangDong Province in South China (21°13′39″N, 110°23′33″E). Black cankers and abundant mature black conidial masses produced by the pycnidia, were observed on diseased branches and covered the diseased tissues of *R. rugosa* branches (Fig. 1a). Isolates were obtained by transferring mature conidia directly from diseased branches under a stereomicroscope (Carl Zeiss Co., Ltd., Oberkochen, Germany) onto 2% malt extract agar (MEA) medium (20 g malt extract powder and 20 g agar per L water). Cultures were incubated at 25 °C until colonies were observed. Fungi purified from the cultures of the original isolates were re-cultured for 1–2 days. Then single hyphal tips were obtained from these cultures. These single hyphal-tip cultures were deposited in the culture collection of the China Eucalypt Research

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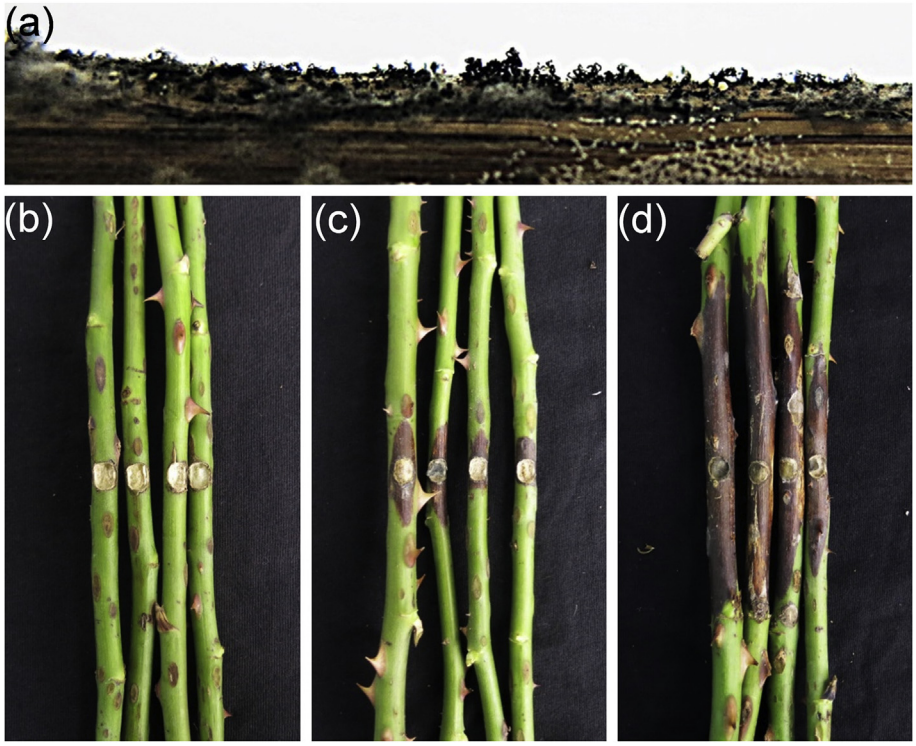

Fig. 1. Disease symptoms of *Rosa rugosa* branches naturally infected by *Lasiodiplodia theobromae* in the environment. Symptoms that appeared after *R. rugosa* branches were inoculated with *L. theobromae in vitro* are also shown. (a) Fruiting structures with abundant mature dark conidia on one *Rosa* branch; (b) *R. rugosa* branches inoculated with the negative control, showing a wound and the absence of lesion development; (c) lesions caused by *L. theobromae* isolate CERC3824; (d) lesions caused by isolate CERC3820.

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2.2. Identification

Fungal isolates from diseased *R. rugosa* branches were identified by phylogenetic analyses and morphological characteristics. Multigene phylogenetic analyses were conducted based on DNA sequence comparisons of the internal transcribed spacer (ITS) rDNA region and the translation elongation factor 1-alpha (TEF-1α) gene region. The primers ITS1 and ITS4 were used to amplify the ITS region (White et al., 1990), and the primers EF1-728F and EF1-986R (Carbone et al., 1999) were used to amplify part of the TEF-1α locus. Before conducting phylogenetic analyses, DNA extraction, DNA amplification and DNA sequencing for the selected isolates were performed using the method described by Li et al. (2015). Sequences of the ITS and TEF-1α gene regions were deposited in GenBank (http://www.ncbi.nlm.nih.gov). Sequences of the type specimen strains that were related to the fungi isolated in this study were obtained from GenBank using the method described by Li et al. (2015). All sequences were aligned using MAFFT (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013), with the iterative refinement method FFT–NS–i option. Alignments were checked and edited manually with MEGA 6.0 (Tamura et al., 2013) software where necessary.

The ITS, TEF-1α and combined ITS and TEF-1α datasets were analyzed using the Maximum Likelihood (ML) method using PHYML v. 3.0 (Guindon and Gascuel, 2003), and the best model for each dataset was determined with MODELTEST v. 3.7 (Posada and Crandall, 1998). Analyses were conducted using the method described by Li et al. (2015). *Diplodia mutila* (Fr.) Mont. (CBS 112553) and *D. seriata* De Not. (CBS 112555) were used as the outgroup taxa (Li et al., 2015).

The representative isolates selected in the phylogenetic analyses were also used to examine culture and conidia characteristics. The morphology of these isolates was studied using the method of Li et al. (2015).

2.3. Pathogenicity test

Isolates selected for pathogenicity tests were grown on 2% MEA at 25 °C for seven days, and 7.0-mm diameter MEA plugs covered with mycelium were made from the actively growing colonies for inoculations. The pathogenicity tests were carried out *in vitro* using healthy *R. rugosa* branches of 20 cm in length and 8–10 mm in diameter; ten branches were inoculated for each isolate. The branch cuts were inoculated and placed in sealed plastic boxes by the inoculation method for *Bougainvillea* sp. described in Li et al. (2015). Ten branches were inoculated with sterile MEA plugs as negative controls. Branches were inoculated on March 4, 2015, and the results were evaluated one week later by measuring the lesion lengths in the cambium. The inoculation experiment was repeated seven days after the first inoculation on ten *R. rugosa*, using the same fungal isolates. Re-isolations were performed by cutting small pieces of wood from the lesion edges and placing these on 2% MEA at 25 °C. Re-isolations were done from all branches inoculated as controls and from four randomly selected branches per isolate for each experiment. In order to confirm the identities of the re-isolated fungi, ITS sequences were generated for the re-isolates, and these were compared with those of the inoculated fungi. The results were analyzed in EXCEL (2010). Single factor analysis of variance (ANOVA) was used to define the effects of a fungal isolate on lesion length. To test the significance among means, F-values with $P < 0.05$ were considered significantly different. The standard