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Phylogeny and pathogenicity of *Lasiodiplodia* species associated with dieback of mango in Peru

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ABSTRACT

Mango, which is an important tropical fruit crop in the region of Piura (Peru), is known to be prone to a range of diseases caused by *Lasiodiplodia* spp. The aim of this study was to evaluate the incidence and prevalence of mango dieback in the region of Piura, and to identify the species of *Lasiodiplodia* associated with the disease and evaluate their pathogenicity towards mango. Mango dieback was present in all orchards surveyed but incidence varied with location. Identification of fungal isolates was based on morphological and cultural characteristics as well as sequence data of the rDNA internal transcribed spacer region (ITS) and translation elongation factor 1-alpha gene (*tef1-α*). The following *Lasiodiplodia* species were identified: *Lasiodiplodia brasiliense*, *Lasiodiplodia egyptiacae* (for which the new combination *Lasiodiplodia laeliocattleyae* is introduced), *Lasiodiplodia iraniensis*, *Lasiodiplodia pseudotheobromae*, *Lasiodiplodia theobromae*, and a *Lasiodiplodia* sp. Individual and combined gene genealogies suggest that this *Lasiodiplodia* sp. is possibly a hybrid of *Lasiodiplodia citricola* and *Lasiodiplodia parva*. Apart from *Lasiodiplodia theobromae*, which was the most prevalent species, all other species are newly reported from Peru. Moreover, *L. iraniensis* is reported for the first time on mango. Inoculation trials of mango plants confirmed Koch's postulates, and revealed differences in aggressiveness among species and isolates.

Introduction

Mango (*Mangifera indica*) is an economically relevant fruit crop grown worldwide in tropical climates. In the region of Piura, located in the northwest coast of Peru, it represents one of the main export products. In this region, which has a desert to semi-desert climate without marked seasons, mango fruit (mostly Kent variety) production occupies 17 000 ha, corresponding to 75 % of the national production of mango for export (Webb & Fernández 2013).

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One of the main diseases affecting mango production is dieback, which in recent years has drawn the attention of producers due to an alarming increase of affected plants in Peru. The symptoms occur in plants of all ages and are characterised by shoot and branch necrosis, defoliation and panicle necrosis. The most typical symptoms of the disease are seen at the foliar level with groups of necrotic leaves dispersed throughout the canopy, covering large sections or even the entire canopy. Sections of affected branches and stems show necrotic wood tissue areas that can spread downwards, towards the root region. With time the disease can progress to cause plant death. The pathogen enters the plant chiefly via pruning wounds and colonizes tissues basipetally, causing dieback.

The impact of mango dieback in Piura was firstly studied in 1998 in an area covering 5118 ha. A prevalence of 100 % was reported but with a low incidence ranging from 0.09 % of affected plants in Alto Piura, to 0.89 % in San Lorenzo (Rodríguez-Gálvez *et al.* 1999). At the time, based on morphological characters, the causal agent of mango dieback was identified as *Lasiodiplodia theobromae* (Rodríguez-Gálvez *et al.* 1999). This species, which is a member of the family *Botryosphaeriaceae*, is a well-known and widespread plant pathogen occurring mostly in tropical and sub-tropical regions, and has been reported on more than 500 host plants (Punithalingam 1980).

Using morphological and phylogenetic data, Alves *et al.* (2008) revealed the existence of cryptic species within what was formerly regarded as *L. theobromae*. Since then a large number of species have been described, and the genus currently comprises 30 species known from culture (Marques *et al.* 2013; Phillips *et al.* 2013; Netto *et al.* 2014; Prasher & Singh 2014; Slippers *et al.* 2014; Chen *et al.* 2015; Trakunyingcharoen *et al.* 2015). Of these 30 species, at least seven including *Lasiodiplodia crassispora*, *Lasiodiplodia egyptiacae*, *Lasiodiplodia hormozganensis*, *Lasiodiplodia iraniensis*, *Lasiodiplodia pseudotheobromae*, *Lasiodiplodia thailandica*, and *Lasiodiplodia theobromae* have been reported from mango (Abdollahzadeh *et al.* 2010; Costa *et al.* 2010; Sakalidis *et al.* 2011; Ismail *et al.* 2012; Marques *et al.* 2013; Phillips *et al.* 2013; Trakunyingcharoen *et al.* 2015). *Lasiodiplodia* species have been associated with several disease symptoms on mango plants including fruit rot, stem-end rot, panicle brown rot, decline, canker and dieback (Costa *et al.* 2010; Sakalidis *et al.* 2011; Ismail *et al.* 2012; Marques *et al.* 2013) but are also known from asymptomatic plants, where they occur as latent endophytes (Trakunyingcharoen *et al.* 2015).

There are no recent data on dieback of mango in Peru, and previous identifications of *L. theobromae* were based solely on morphology. This study was undertaken with the aim of reevaluating the incidence, severity and prevalence of mango dieback in plantations in the region of Piura, as well as confirming the identification of the causal agents.

Materials and methods

Field survey and sampling

Field surveys were carried out between March and November 2012 in the following regions of Piura (Peru): Valle de San Lorenzo (Hualtaco, Malingas, Partidor, Somate, San Isidro and Valle de los Incas), Valle del Chira (Cieneguillo Norte, Cieneguillo Centro and Cieneguillo Sur) and Valle del Alto Piura (Campanas, La Matanza and Yapatera) (Fig 1). Several plantations covering a total area of 4076 ha were evaluated and in each, which was traversed in a zigzag movement, 10 % of the plants (Table 1) were visually inspected for dieback symptoms.

Disease incidence (I) was determined following the approach of Teng & James (2001) using the formula: $I\ (\%) = (ni/N) \times 100$ (ni: total number of affected plants, N: total number of evaluated plants). Disease prevalence in a given geographic area was also determined and expressed as a percentage (Teng & James 2001). Disease severity was determined according to the formula proposed by French and Hebert (1982): $\text{Severity} = n(L0) + n(L1) + n(L2) + n(L3) + n(L4) + n(L5)/\sum n$, where n = number of diseased plants in each corresponding damage level (L). For this purpose six damage levels were considered: L0: healthy plant; L1: plants with shoot necrosis; L2: plants with defoliation in 25 % of the canopy; L3: plants with defoliation in 50 % of the canopy; L4: plants with defoliation in 75 % of the canopy and L5: dead plant.

Fungal isolation and morphology

Samples collected from symptomatic plants (one per plant) were washed under tap water, dried and briefly flamed. Small pieces of wood were taken from the interface between healthy and diseased plant tissue, submerged in 2.5 % sodium hypochlorite for 2 min, and washed twice in sterile distilled water. Wood pieces were plated on potato dextrose agar medium (PDA, Merck, Darmstadt, Germany) and incubated at 28 °C for 5 d. Fungal colonies were transferred to fresh PDA plates and after sporulation single spore cultures were obtained. In order to identify botryosphaeriacous isolates, micromorphological characteristics (e.g. conidial size, shape, colour, striation, septation, conidiogenous cells, presence of paraphyses) of the isolates were observed with a Nikon 80i microscope and pictures captured with a Nikon DS-Ri1 camera.

Molecular identification of isolates

Genomic DNA was extracted from mycelium as described by Alves *et al.* (2004). PCR reactions were carried out with NZYTaq 2 × Green Master Mix (NZYTech, Lisboa, Portugal).

The internal transcribed spacer (ITS) region of the ribosomal DNA cluster and part of the translation elongation factor 1-alpha (*tef*1-α) were sequenced for the 32 selected isolates as described previously (Alves *et al.* 2004, 2008), and the sequences deposited in GenBank (Table 2). The amplified PCR fragments were purified with the DNA Clean and Concentrator™-5 kit (Zymo Research, California, USA). Both strands of the PCR products were sequenced at GATC Biotech (Germany). The nucleotide sequences were read and edited with FinchTV v. 1.4.0 (Geospiza Inc. http://www.geospiza.com/finchtv).

The ITS and *tef*1-α sequences of the isolates from mango were combined and aligned with sequences retrieved from GenBank, representing 28 species of the genus *Lasiodiplodia*.