Australasian Plant Pathol. (2012) 41:649–660
DOI 10.1007/s13313-012-0163-1

Lasiodiplodia species associated with dieback disease of mango (*Mangifera indica*) in Egypt

A. M. Ismail · G. Cirvilleri · G. Polizzi · P. W. Crous · J. Z. Groenewald · L. Lombard

Received: 27 February 2012 / Accepted: 2 August 2012 / Published online: 25 August 2012
© Australasian Plant Pathology Society Inc. 2012

Abstract *Lasiodiplodia theobromae* is a plurivorous pathogen of tropical and subtropical woody and fruit trees. In 2010, an investigation of mango plantations in Egypt resulted in the isolation of 26 *Lasiodiplodia* isolates that, based on previous reports from literature, were tentatively identified as *L. theobromae.* The aim of this study was to clarify the taxonomy of these isolates based on morphology and DNA sequence data (ITS and TEF1-α). In addition to *L. theobromae*, a new species, namely *L. egyptiacae,* was identified. Furthermore, *L. pseudotheobromae* is also newly recorded on mango in Egypt. Pathogenicity tests with all recognised species showed that they are able to cause dieback disease symptoms on mango seedlings.

A. M. Ismail
Plant Pathology Research Institute, Agriculture Research Centre,
12619 Giza, Egypt

G. Cirvilleri · G. Polizzi
Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali Sez. Patologia Vegetale,
95123 Catania, Italy

P. W. Crous · J. Z. Groenewald · L. Lombard (✉)
CBS-KNAW Fungal Biodiversity Centre,
Uppsalalaan 8,
3584 CT Utrecht, The Netherlands
e-mail: l.lombard@cbs.knaw.nl

P. W. Crous
Laboratory of Phytopathology,
Wageningen University and Research Centre (WUR),
Droevendaalsesteeg 1,
6708 PB Wageningen, The Netherlands

P. W. Crous
Microbiology, Department of Biology, Utrecht University,
Padualaan 8,
3584 CH Utrecht, The Netherlands

Keywords *Botryosphaeriaceae* · ITS · *Lasiodiplodia* · Mango · Morphology · TEF1-α

Introduction

Mango (*Mangifera indica*) is a popular fruit tree in Egypt, introduced from Bombay, India in 1825, and is cultivated along the Nile valley and some surrounding desert areas (El Tomi 1953; Abdalla et al. 2007). Most Egyptian mango cultivars, such as alphonso, balady, mabroka, pairi, succary and zebda, are polyembryonic, bearing fruit that are characterised by a sweet and spicy flavour, and low fibre content (Knight 1993; El-Soukkary et al. 2000). Among the wide range of destructive fungal pathogens that impact on mango fruit production are members of the *Botryosphaeriaceae* (Johnson 1992). *Botryosphaeriaceae* is a genus-rich family in the *Dothidiomycetes*, containing numerous species with a cosmopolitan distribution (Crous et al. 2006; Phillips et al. 2008). Some of the genera are important pathogens of fruit and woody trees causing symptoms such as leaf spot, dieback, stem-end rot, fruit rot and cankers that can result in tree mortality (Johnson et al. 1991, 1993; Ramos et al. 1991; Smith et al. 2001; Slippers et al. 2005; Damm et al. 2007). Most members of the *Botryosphaeriaceae* have a broad host range, and have been recognized as successful opportunistic pathogens that occasionally cause extensive disease symptoms when their plant hosts are subjected to unfavourable conditions (Johnson 1992; Slippers and Wingfield 2007; Sakalidis et al. 2011). Various factors play significant roles in the predisposition of mango trees to attack by members of *Botryosphaeriaceae* such as mechanical injuries, mineral deficiencies and environmental factors (Ramos et al. 1991; Ploetz et al. 1996a, b).

Lasiodiplodia theobromae, a member of *Botryosphaeriaceae*, is a cosmopolitan fungus occurring predominantly

throughout tropical and subtropical regions (Punithalingam 1980; Burgess et al. 2006). It has also been known as a human pathogen causing keratomycosis and phaeohyphomycosis (Punithalingam 1976; Summerbell et al. 2004), and as a plant pathogen associated with up to 500 plant hosts (Punithalingam 1980). The fungus has been reported as mango pathogen worldwide associated with several plant disease symptoms including decline, canker and dieback (Jacobs 2002; Khanzada et al. 2004a, b; Abdollahzadeh et al. 2010; de Oliveira Costa et al. 2010). In Egypt, the fungus is well established and has been considered as the main causal agent of fruit rot, stem-end rot, panicle brown rot and dieback of mango (Ragab et al. 1971; Abdalla et al. 2003). In addition to mango, it has also been reported to cause root rot of sugar beet (Abd-El Ghani and Fatouh 2005) and dieback, canker and soft rot of other hosts such as grapevine (El-Goorani and El Meleigi 1972), walnut (Haggag et al. 2007), maize (Diab et al. 1984), citrus (Abo-El-Dahab et al. 1992) and *Annona* spp. (Haggag and Nofal 2006) in Egypt. The taxonomic placement of *Botryosphaeria rhodina* (anamorph *L. theobromae*) has been complicated by several names associated with this fungus (Burgess et al. 2006). Punithalingam (1976) reduced several species (*L. nigra, L. triflorae*, and *L. tubericola*) to synonymy under *L. theobromae*. Subsequent to this treatment, several studies have led to the identification of cryptic species within the *L. theobromae* species complex (Pavlic et al. 2004, 2008; Burgess et al. 2006; Damm et al. 2007; Alves et al. 2008; Begoude et al. 2009; Abdollahzadeh et al. 2010). Presently, up to 13 cryptic species are recognised in the L. theobromae complex.

In recent years molecular DNA-based approaches have been widely employed in taxonomic studies of the *Botryosphaeriaceae* (Crous and Groenewald 2005). Several phylogenetic studies have relied on the sequence differences from the internal transcribed spacer (ITS) region of the rDNA operon to distinguish species within *Botryosphaeriaceae* (Denman et al. 2000, 2003; Alves et al. 2008). However, ITS sequence data alone can obscure cryptic species diversity and proved to be inadequate to separate closely related species (de Wet et al. 2003; Slippers et al. 2004a; Slippers and Wingfield 2007; Marincowitz et al. 2008). Interestingly, contemporary phylogenetic studies using multiple gene genealogies have increasingly revealed cryptic species in the *L. theobromae* complex (Pavlic et al. 2004, 2008; Burgess et al. 2006; Damm et al. 2007; Alves et al. 2008; Begoude et al. 2009; Abdollahzadeh et al. 2010).

Little is known of the aetiology of *Botryosphaeriaceae* diseases on mango in Egypt. By means of a morphological and DNA sequence data comparison, the present study represents the first attempt to characterise the variability within an Egyptian collection of isolates previously treated as *L. theobromae* or *Botryodiplodia theobromae.*

Materials and methods

Isolates

In February 2010, a routine survey was conducted in several areas in Egypt where mango is cultivated. Isolations were made from fresh symptomatic plant material showing twig and branch dieback and black lesions on leaves. Initially, samples were surface sterilised with a diluted potassium hydroxide solution (5 %) and EtOH (70 %). Approximately 3–5 mm diam pieces of plant material between the healthy and infected tissues were placed on 2 % Potato-Dextrose Agar (PDA) supplemented with Streptomycin sulphate (0.1 g/L^{-1}) and incubated at 25 °C in the dark. Pure cultures were obtained by hyphal tip excision from the colony margins on PDA, and subsequent incubation at 25 °C in the dark. All isolates obtained from mango were deposited in the collection of the Plant Pathology Research Institute, Egypt. Representative isolates used for morphological and molecular studies were also deposited in the collection of CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands (Table 1).

DNA isolation and amplification

Total genomic DNA was extracted from 8 to 10-day old cultures using the Ultraclean® Microbial DNA Isolation Kit (MO–BIO Laboratories, Inc, Carlsbad, USA) according to the manufacturer's protocol. The ITS region of the rDNA operon was amplified using the primers V9G (de Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990). Partial sequence of the translation elongation factor 1-alpha (TEF-1α) gene region was amplified using primers EF1-728F (Carbone and Kohn 1999) and EF2 (O'Donnell et al. 1998). For some isolates, the TEF-1α gene region was amplified using primers EF1-688F and EF1-1251R (Alves et al. 2008). Each PCR reaction contained a final concentration of 0.5 U/μL of *Taq* polymerase, 1X buffer 2–2.5 mM $MgCl_2$ (BIOLINE, San Diego, USA), 0.4–0.6 mM of each dNTP and 0.12–0.2 μm of each primer made up to a final volume of 12.5 μL with sterile deionized water. PCR conditions included the following steps: an initial step of denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, with a final elongation step at 72 °C for 7 min.

Phylogeny

The amplified fragments of the ITS gene region were sequenced in both directions using internal primers ITS4 and ITS5 (White et al. 1990), whereas the TEF-1α gene region was sequenced in both directions using the same primer pairs for amplification. Sequencing reactions were

Table 1 *Botryosphaeriaceae* isolates used in the phylogenetic analysis

Species	Isolate no.	Location	Host	Collector	GenBank accession no.[b]	
					ITS	TEF-1α
Diplodia corticola	CBS 112549[d]	Portugal	*Quercus suber*	A. Alves	AY259100	AY573227
	CBS 112545	Spain	*Q. suber*	M.E. Sánchez & A. Trapero	AY259089	AY573226
	CBS 112546	Spain	*Q. ilex*	M.E. Sánchez & A. Trapero	AY259090	EU673310
D. mutila	CBS 112553[d]	Portugal	*V. vinifera*	A.J.L. Phillips	AY259093	AY573219
	CBS 230.30	USA	*Phoenix dactylifera*	L.L. Huillier	DQ458886	DQ458869
Lasiodiplodia egyptiacae	**BOT-10 = CBS 130992**[d]	Egypt	*M. indica*	A.M. Ismail	*JN814397*	*JN814424*
	BOT-29	Egypt	*M. indica*	A.M. Ismail	*JN814401*	*JN814428*
L. citricola	IRAN1521C	Iran	*Citrus* sp.	A. Shekari	GU945353	GU945339
	IRAN1522C	Iran	*Citrus* sp.	J. Abdollahzadeh & A. Javadi	GU945354	GU945340
L. crassispora	CMW13488[d]	Venezuela	*Eucalyptus urophylla*	S. Mohali	DQ103552	DQ103559
	WAC12533	Australia	*Syzygium album*	T.I. Burgess & B. Dell	DQ103550	DQ103557
L. gilanensis	IRAN1501C	Iran	Unknown	J. Abdollahzadeh & A. Javadi	GU945352	GU945341
	IRAN1523C	Iran	Unknown	J. Abdollahzadeh & A. Javadi	GU945351	GU945342
L. gonubiensis	CBS 115812[d]	South Africa	*S. cordatum*	D. Pavlic	DQ458892	DQ458877
	CMW14078	South Africa	*S. cordatum*	D. Pavlic	AY639594	DQ103567
L. hormozganensis	IRAN1498C	Iran	*Mangifera indica*	J. Abdollahzadeh & A. Javadi	GU945356	GU945344
	IRAN1500C	Iran	*Olea* sp.	J. Abdollahzadeh & A. Javadi	GU945355	GU945343
L. iraniensis	IRAN921C	Iran	*M. indica*	N. Khezrinejad	GU945346	GU945334
	IRAN1517C	Iran	*Citrus* sp.	J. Abdollahzadeh & A. Javadi	GU945349	GU945337
L.mahajangana	CMW27820	Madagascar	*Terminalia catappa*	Unknown	FJ900597	FJ900643
	CMW27818	Madagascar	*T. catappa*	Unknown	FJ900596	FJ900642
L. margaritaceae	CBS 122519	Australia	*Adansonia gibbosa*	Unknown	EU144050	EU144065
	CBS 122065	Australia	*A. gibbosa*	Unknown	EU144051	EU144066
L. parva	CBS 356.59	Sri Lanka	*Theobromae cacao*	A. Riggenbach	EF622082	EF622062
	CBS 494.78	Colombia	Cassava-field soil	O. Rangel	EF622084	E 622064
L. plurivora	CPC4583[d]	South Africa	*V. vinifera*	F. Halleen	AY343482	EF445396
	CPC5803	South Africa	*Pinus salicina*	U. Damm	EF445362	EF445395
L. pseudotheobromae	CBS 116459[d]	Costa Rica	*Gmelina arborea*	J. Carranza-Velásquez	EF622077	EF622057
	CMW24700	China	*Eucalyptus* sp.	M. J. Wingfield & X. D. Zhou	HQ332192	HQ332208
	CMW24699	China	*Eucalyptus* sp.	M. J. Wingfield & X. D. Zhou	HQ332191	HQ332207
	CMW22667	South Africa	*Pterocarpus angolensis*	J. Mehl& J. Roux	FJ888471	FJ888449
	CBS 121773	Namibia	*Acacia mellifera*	F.J.J. van der Walt	EU101311	EU101356
	IRAN1518C	Iran	*Citrus* sp.	J. Abdollahzadeh & A. Javadi	GU973874	GU973866
	BOT-1	Egypt	*M. indica*	A. M. Ismail	*JN814375*	*JN814402*
	BOT-13[c]	Egypt	*M. indica*	A. M. Ismail	*JN814377*	*JN814404*
	BOT-14	Egypt	*M. indica*	A. M. Ismail	*JN814378*	*JN814405*
	BOT-16	Egypt	*M. indica*	A. M. Ismail	*JN814379*	*JN814406*
	BOT-28	Egypt	*M. indica*	A. M. Ismail	*JN814380*	*JN814407*
	BOT-18	Egypt	*M. indica*	A. M. Ismail	*JN814381*	*JN814408*
	BOT-2	Egypt	*M. indica*	A. M. Ismail	*JN814382*	*JN814409*
	BOT-11 = CBS 130990	Egypt	*M. indica*	A. M. Ismail	*JN814383*	*JN814410*
	BOT-3	Egypt	*M. indica*	A. M. Ismail	*JN814384*	*JN814411*

Table 1 (continued)

Species	Isolate no.	Location	Host	Collector	GenBank accession no.[b]	
					ITS	TEF-1α
	BOT-17	Egypt	*M. indica*	A. M. Ismail	*JN814385*	*JN814412*
	BOT-12	Egypt	*M. indica*	A. M. Ismail	*JN814386*	*JN814413*
	BOT-24	Egypt	*M. indica*	A. M. Ismail	*JN814387*	*JN814414*
	BOT-26	Egypt	*M. indica*	A. M. Ismail	*JN814388*	*JN814415*
	BOT-27	Egypt	*M. indica*	A. M. Ismail	*JN814389*	*JN814416*
	BOT-22	Egypt	*M. indica*	A. M. Ismail	*JN814390*	*JN814417*
	BOT-15	Egypt	*M. indica*	A. M. Ismail	*JN814391*	*JN814418*
	BOT-25	Egypt	*M. indica*	A. M. Ismail	*JN814393*	JN814420
	BOT-21	Egypt	*M. indica*	A. M. Ismail	*JN814394*	JN814421
L. rubropurpurea	WAC12536[d]	Australia	*E. grandis*	T. I. Burgess & G. Pegg	DQ103554	DQ103572
	WAC12537	Australia	*E. grandis*	T. I. Burgess & G. Pegg	DQ103555	DQ103573
L. theobromae	CBS 112874	South Africa	*V. vinifera*	F. Halleen	EF622075	EF622055
	CBS 559.70	Unknown	*Zea mays*	H. A. van der Aa	EF622073	EF622053
	CBS 111530	Unknown	Unknown	Unknown	EF622074	EF622054
	CMW24702	China	*Eucalyptus* sp.	M. J. Wingfield & X.D. Zhou	HQ332194	HQ332210
	CMW24701	China	*Eucalyptus* sp.	M. J. Wingfield & X.D. Zhou	HQ332193	HQ332209
	MUCC709	Australia	*Lysiphyllum cunninghamii*	M. L. Sakalidis	GU199367	GU199393
	BOT-5	Egypt	*M. indica*	A.M. Ismail	*JN814376*	*JN814403*
	BOT-9	Egypt	*M. indica*	A.M. Ismail	*JN814392*	*JN814419*
	BOT-4 = CBS 130989	Egypt	*M. indica*	A.M. Ismail	*JN814395*	*JN814422*
	BOT-7	Egypt	*M. indica*	A.M. Ismail	*JN814396*	*JN814423*
	BOT-6	Egypt	*M. indica*	A.M. Ismail	*JN814399*	*JN814426*
	BOT-23	Egypt	*M. indica*	A.M. Ismail	*JN814400*	*JN814427*
L. venezuelensis	CMW13513[d]	Venezuela	*A. mangium*	S. Mohali	DQ103549	DQ103570
	WAC12540	Venezuela	*A. mangium*	S. Mohali	DQ103548	DQ103569
Phyllosticta capitalensis	CBS 115051	Brazil	*Spondias mombin*	K.F. Rodriquez	FJ538325	FJ538383
P. citricarpa	CBS 102374	Brazil	*C. aurantium*	Unknown	FJ538313	FJ538371

[a] CMW = culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; CBS = CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; CAA = A. Alves, Universidade de Aveiro, Portugal; CAP = A.J.L. Phillips, Lisbon, Portugal. WAC = Department of Agriculture Western Australia, Plant Pathogen Collection; BOT = A. M. Ismail, Plant Pathology Research Institute, Egypt; CPC = P.W. Crous working collection, maintained at CBS

[b] GenBank accession numbers in italics were generated in this study

[c] Isolate numbers in bold were selected for pathogenicity test

[d] Ex-type cultures

performed using Big Dye terminator sequencing kit v. 3.1 (Perkin-Elmer Applied Bio Systems, Foster City, CA, USA) following the manufacturer's instructions and run using an ABI PRISM™ 3730 DNA automated sequencer (Perkin-Elmer Applied BioSystems, Foster City, CA, USA).

The generated sequences were aligned together with other sequences obtained from GenBank using MAFFT v. 6.0 (Katoh and Toh 2010). The ambiguous sequences of both 5′ and 3′ ends were excluded from the final alignment and the aligned sequences were manually checked and corrected where necessary. Nucleotide substitution models were determined for each gene region using MrModel Test v.2.2 (Nylander 2004). The model HKY+G was selected for both ITS and TEF sequence datasets. Sequences for each gene region were individually analysed for conflict using 70 % reciprocal NJ (Neighbour-Joining) bootstrap analysis and the topology of the resulting trees were compared visually for inconsistency (Mason-Gamer and Kellogg 1996; Gueidan et al. 2007).

Bayesian analyses were performed with MrBayes v. 3.1.1 (Ronquist and Huelsenbeck 2003) using the Markov Chain Monte Carlo (MCMC) (Larget and Simon 1999) algorithm to generate trees with Bayesian probability values. Four chains were run simultaneously from a random tree topology and ended at 1,000,000 generations, and trees were saved every 100th generation. The burn-in value was graphically estimated from the likelihood scores and therefore, the first 1,000 trees were discarded from the analysis as the burn-in phase and the consensus tree was constructed from the remaining trees. Trees were rooted using *Phyllosticta capitalensis* (CBS 115051) and *P. citricarpa* (CBS 102374) (Glienke et al. 2011).

All the sequence datasets were also analysed to determine possible phylogenetic relationship among taxa using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2001). Maximum parsimony (MP) tests were conducted using the heuristic search option with random stepwise addition using 1,000 replicates, tree bisection and reconnection (TBR) as branch swapping algorithms, and random taxon addition sequences for the construction of maximum parsimony trees. Maxtrees was set to 10,000 branches of zero length were collapsed, and all multiple equally parsimonious trees were saved. In the analysis, all characters were unordered and had equal weight; gaps were treated as missing data. Values calculated for parsimony included tree length (TL), consistency index (CI), rescaled consistency index (RC) and the retention index (RI). Bootstrap support values were evaluated using 1,000 bootstrap replicates (Hillis and Bull 1993). All sequences generated in this study were deposited in GenBank (Table 1). The aligned sequences were deposited in TreeBASE (S12897).

Morphological characterisation

Sporulation was induced by plating representative isolates onto 2 % (w/v) water agar with sterilised pine needles (WAP) and incubated at 25±2 °C under near-ultraviolet (UV) light for 2 weeks. Plates were observed every 2 days for the formation of pycnidia. Gross morphological characteristics were determined by mounting fungal structures in clear lactic acid. Measurements of 50 conidia and at least 30 other fungal structures for each representative isolate were determined at ×1,000 magnification. Sections were made through pycnidia using a Leica CM1100 cryostat at −20 °C and the 10 µm sections were mounted in lactic acid. Gross morphological characteristics were observed as above. For the conidia, the 95 % confidence levels were calculated of 30 observations, with extremes given in parentheses. Only the extremes are indicated for the other fungal structures. Colony characteristics were determined after 7 days on PDA in the dark at 25 °C, using the colour charts of Rayner (1970). Optimal growth temperatures were determined for each selected isolate on PDA at 10–35 °C in 5 °C intervals in the dark, with three plates per isolate at each temperature. Descriptions, nomenclature and illustrations were deposited in MycoBank (Crous et al. 2004).

Pathogenicity test

Ten isolates representing three species of *Lasiodiplodia* (Table 1) were used for pathogenicity trials on mango seedlings cv. "Kensington Pride". The plants were 3–4-month old, 40–60 cm tall, and maintained in a greenhouse under artificial light (10/14 h light-and-dark cycles) at 25±2 °C and 70–80 % relative humidity (RH). Four plants for each isolate and the controls were used and arranged in a randomised design. The epidermis of the stem was disinfected with 70 % ethanol, washed with sterile distilled water and left to dry. A 5-mm cut was made into the epidermis, between two nodes and below the apex of the stem. A 5-mm diam mycelial PDA plug was removed from the edge of actively growing cultures, and placed onto the stem wounds, with the mycelium facing the cambium. The inoculated wounds were wrapped with Parafilm®, (Laboratory Film, Chicago, IL, USA) to prevent desiccation and contamination. Control plants were inoculated with sterile PDA plugs. Six weeks after inoculation the bark lesion lengths as well as the length of cambium discolouration were measured to assess the pathogenicity of the tested isolates. Re-isolation of the tested isolates was done from the margins of the necrotic lesions on PDA to prove Koch's Postulates.

Results

Phylogeny

Amplicons of approx. 570 bp were generated for ITS using primer pairs ITS5 and ITS4 and approx. 500 bp for TEF-1α were obtained using the EF1-728F and EF2 primers pairs. Amplicons of approx. 700 bp was obtained using primers EF1-688 and EF1-1252. The 70 % reciprocal NJ bootstrap analysis indicated congruence in the tree topology of both ITS and TEF-1α trees. The combined data set consisted of 69 taxa including the outgroup taxa composed of 920 characters including gaps, of which 589 were constant, 91 were variable and parsimony uninformative and 240 were parsimony informative. Maximum parsimony analysis resulted in one most parsimonious tree (TL=1774 steps, CI=0.581, RI=0.756, RC=0.894) presented in Fig. 1. In this tree, the *Lasiodiplodia*-like isolates obtained in this study fell into four distinct clades. The majority of isolates (BOT-1, BOT-2, BOT-3, BOT-11, BOT-12, BOT-13, BOT-14, BOT-15,

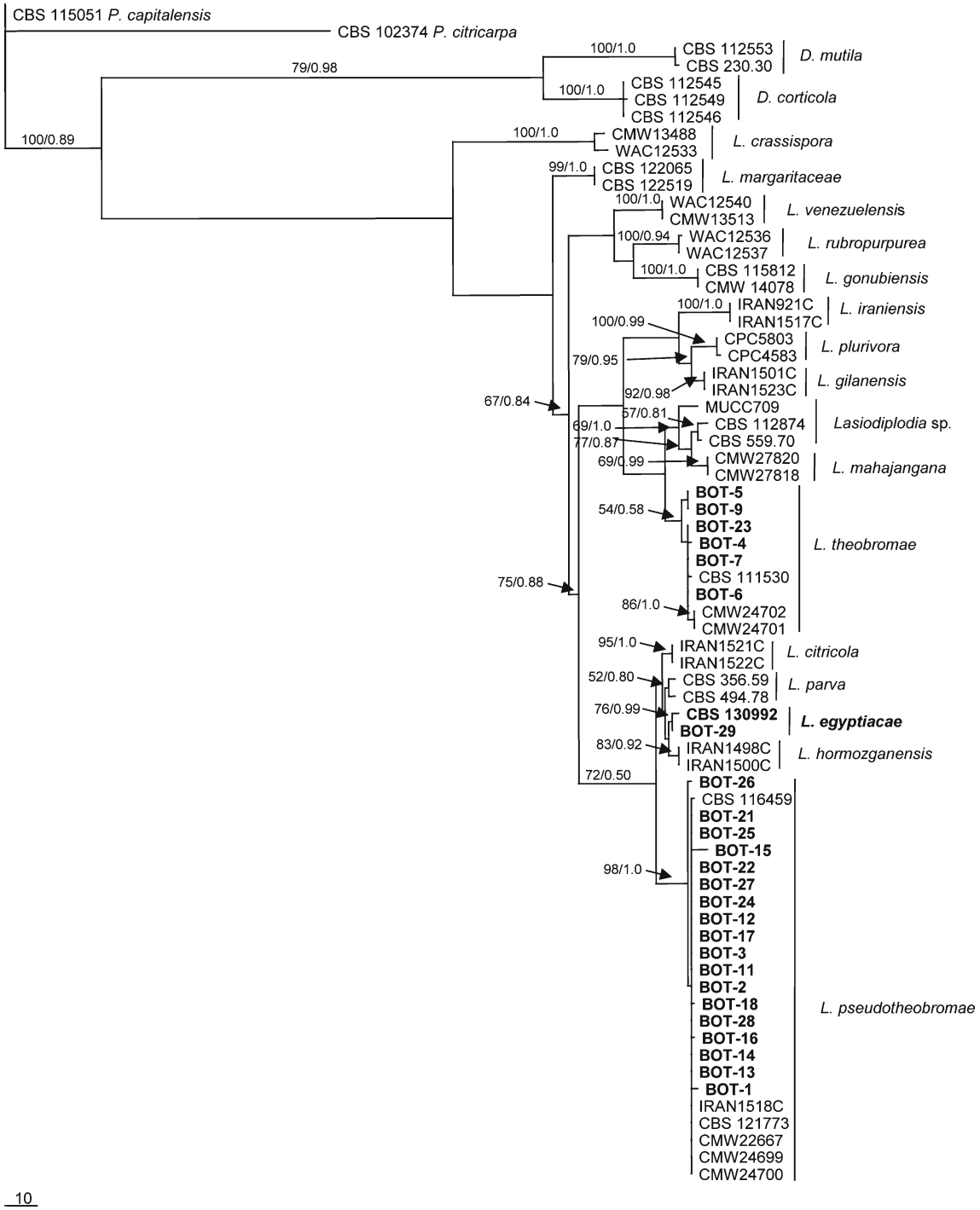

Fig. 1 The most parsimonious trees obtained from the maximum parsimony analysis using heuristic search with 1,000 random additions of the combined ITS and TEF-1α sequence alignments. *Scale bar* shows ten changes and bootstrap support and Bayesian posterior probability values are indicated at the nodes. The tree was rooted to *P. capitalensis* CBS 115051 and *P. citricarpa* CBS 102374

BOT-16, BOT-17, BOT-18, BOT-21, BOT-22, BOT-24, BOT-25, BOT-26, BOT-27 and BOT-28) clustered together in a large clade containing *L. pseudotheobromae* (CBS 116459, culture ex-type) supported by a bootstrap (BP) value of 98 and a Bayesian posterior probability (BPP) value of 1.0. A second well-supported clade (BS/BPP: 76/0.99) accommodated two *Lasiodiplodia*-like isolates (CBS 130992 and BOT-29), possibly representing a novel phylogenetic species. A further six isolates (BOT-4, BOT-5, BOT-6, BOT-7, BOT-9 and BOT-23) clustered together with *L. theobromae* (CMW 24701, CMW 24702, CBS 111530; Chen et al. 2011), with low support (BS/BPP: 54/0.58).

Morphological characterisation

In this study a total of 26 isolates representing species of *Botryosphaeriaceae* were obtained from mango trees. Of these, 12 isolates were obtained from branches, 11 from leaves and three from twigs. No teleomorph structures were observed in this study. Based on cultural and conidial characteristics isolates were considered to belong to *Lasiodiplodia*. All isolates were included in the phylogenetic analysis. Based on DNA sequence data and conidial morphology three species were identified which included *L. theobromae*, *L. pseudotheobromae* and a new species which is described here.

Lasiodiplodia egyptiacae A.M. Ismail, L. Lombard & Crous, **sp. nov.** MycoBank MB564516, Fig. 2

Etymology: The name refers to Egypt, the country where this fungus was collected.

Conidiomata stromatic, pycnidial, produced on WAP within 12 days, mostly solitary, or aggregated, dark-grey to black, globose to subglobose, covered with dense mycelium, papillate with centralostiole, conidiomata semi-immersed, becoming erumpent when mature, mostly multi-loculate to uni-loculate; wall of two regions: outer region composed of 5–7 layers of dark brown, thick-walled cells of *textura angularis*, followed by an inner region of hyaline, thin-walled cells of *textura angularis*. *Paraphyses* hyaline, subcylindrical, arising between the conidiogenous cells, aseptate, wider at base, rounded or slightly swollen at apex, up to 57 μm long, 2–3 μm wide. *Conidiogenous cells* holoblastic, hyaline, thin-walled, cylindrical, sometimes slightly swollen at the base, with rounded apex, proliferating percurrently to produce 1–2 min annellations, 5–11×3–5 μm. *Conidia* oozing from pycnidia in conidial cirri, initially hyaline, smooth, thick-walled, aseptate, obovoid to ellipsoid, granular, mostly somewhat tapered at apex, and rounded at base, becoming brown, 1-septate, with longitudinal striations on the inner surface of the conidia wall due to the melanin deposits, measuring (17–)20–24(−27) × (11–)11–12(−13) μm (av. ± SD=22±2 μm long, 12±1 μm wide, L/W ratio=2).

Culture characteristics: Colonies on PDA with moderately dense, raised mycelium mat, initially white to smoke-grey, turning greenish grey on the surface and greenish grey in reverse, becoming dark slate-blue with age. Cardinal temperature requirements for growth; minimum 15 °C, maximum 35 °C, optimum 25 °C.

Specimens examined: Egypt, Sharkia Province, El Menayar, isolated from *M. indica* leaf, 2 Feb. 2010, A.M. Ismail, holotype CBS H-20736, culture ex-type BOT-10 = CBS 130992; Sharkia Province, El Menayar, isolated from mango leaf, 2 Feb. 2010, A.M. Ismail, culture BOT-29.

Notes: *Lasiodiplodia egyptiacae* is phylogenetically closely related to *L. hormozganensis* (Abdollahzadeh et al. 2010), but it can be distinguished based on the morphology of its conidia and paraphyses (Table 2). The conidia of *L. egyptiacae* are ovoid to sub-ovoid, whereas those of *L. hormozganensis* are ellipsoid to cylindrical. In addition, paraphyses of *L. egyptiacae* are aseptate and shorter (up to 57 μm), whilst the paraphyses of *L. hormozganensis* are 1–7-septate and longer (up to 83 μm). Furthermore, *L. egyptiacae* is still distinct from *L. citricola* and *L. parva* in terms of paraphyses morphology. The paraphyses of *L. egyptiacae* are aseptate, shorter and narrower (57×2–3 μm) while those of *L. citricola* and *L. parva* are septate, longer and wider (125×3–4 μm), (105×3–4 μm), respectively (Table 2).

Pathogenicity test

Six weeks after inoculation, all isolates displayed levels of pathogenicity. Observed symptoms included brown, necrotic bark lesions around the inoculation sites extending upwards and downwards, leading to wilting and drying of the apical as well as the terminal leaves (Fig. 4). Cracking of the stem cortex was observed for most of the isolates, and

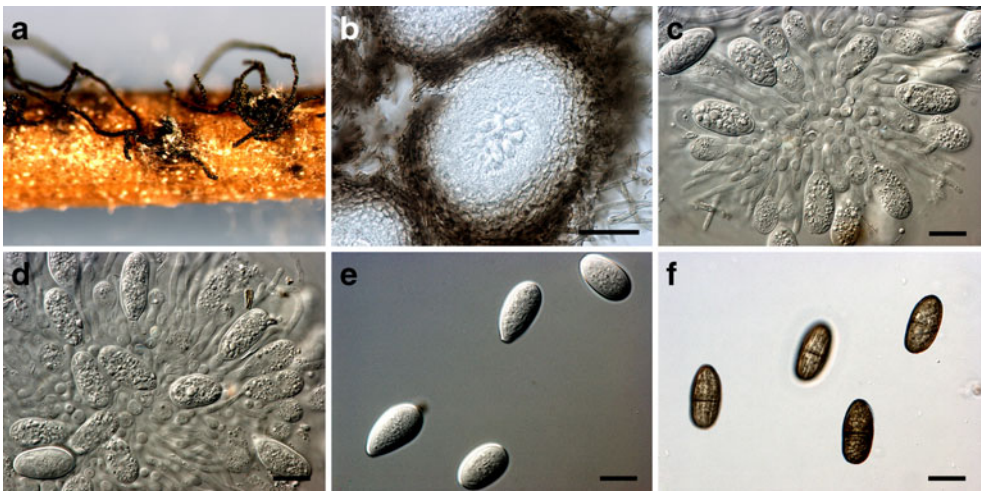

Fig. 2 *Lasiodiplodia egyptiacae* **a** pycnidia formed on WAP; **b** vertical section through pycnidia; **c** hyaline, aseptate paraphyses formed between conidiogenous cells; **d** conidiogenous cells; **e** hyaline immature thick-walled conidia; **f** dark mature conidia showing longitudinal striation. *Scale bars*: **b**=20 μm; **c**–**f**=10 μm

Table 2 Morphological comparison of conidia and paraphyses of *Lasiodiplodia* spp.

Identity	Conidial size (av. µm)	L/w ratio	Paraphyses (µm)			References
			Length	Width	Septation	
L. egyptiacae	22×12	1.8	57	2–3	Aseptate	This study
L. citricola	24.5×15.4	1.6	125	3–4	Septate	Abdollahzadeh et al. (2010)
L. crassispora	28.8×16	1.8	45.7	2.7	Septate	Burgess et al. (2006)
L. gilanensis	31×16.6	1.9	95	2–4	Septate	Abdollahzadeh et al. (2010)
L. gonubiensis	33.8×17.3	1.9	70	4	Aseptate	Pavlic et al. (2004)
L. hormozganensis	21.5×12.5	1.7	83	2–4	Septate	Abdollahzadeh et al. (2010)
L. iraniensis	20.7×13	1.6	127	2–4	Septate	Abdollahzadeh et al. (2010)
L.mahajangana	17.5×11.5	1.4	43	3	Aseptate	Begoude et al. (2009)
L. margaritaceae	15.3×11.4	1.3	37.1	2.2	Septate	Pavlic et al. (2008)
L. parva	20.2×11.5	1.8	105	3–4	Septate	Alves et al. (2008)
L. plurivora	29.6×15.6	1.9	130	2–5	Septate	Damm et al. (2007)
L. pseudotheobromae	28×16	1.7	58	3–4	Aseptate	Alves et al. (2008)
	26.7×12.3	2.1	52	2–3	Aseptate	This study
L. rubropurpurea	28.2×14.6	1.9	42.4	2.6	Aseptate	Burgess et al. (2006)
L. theobromae	26.2×14.2	1.9	55	3–4	Septate	Alves et al. (2008)
	23.7×13.3	1.7	44	2–3	Septate	This study
L. venezuelensis	28.4×13.5	2.1	28.3	3.5	Septate	Burgess et al. (2006)

fungal structures (stromatic pycnidia and mycelium) developed on the necrotic lesions around the inoculation sites. Under the outer cortex, necrotic xylem vessels and brown discolouration extended along the length of the stems (Fig. 4). Symptoms observed on the control plants could be due to wound reaction as no *Lasiodiplodia* was isolated. There was a significant difference ($p<0.05$) in the lesions produced by *Lasiodiplodia* isolates compared to control lesions. Isolates BOT-11 and BOT-28 (*L. pseudotheobromae*) developed the longest bark (av. 63.3 mm and 62.6 mm, respectively) and cambium (av. 64.1 mm and 63.6 mm, respectively) lesions, followed by isolate BOT-4 (*L. theobromae*), which produced a bark lesion of av. 56.5 mm and cambium lesion of av. 60.7 mm in length (Fig. 3). These three isolates were the only to induce dieback symptoms similar to those observed during the survey (Fig. 4). Isolates CBS 130992 and BOT-29 (*L. egyptiacae*) produced smaller lesions (av. 38.8 mm and 35.1 mm, respectively), however, still longer than the controls (av. 25.8 mm).

Discussion

Three species of *Lasiodiplodia* associated with dieback and leaf lesions of mango trees were identified in the present study. These were *L. theobromae*, *L. pseudotheobromae* and the newly described *L. egyptiacae*. The latter new species is distinguished from other species of *Lasiodiplodia* based on morphological characters and phylogenetic inference.

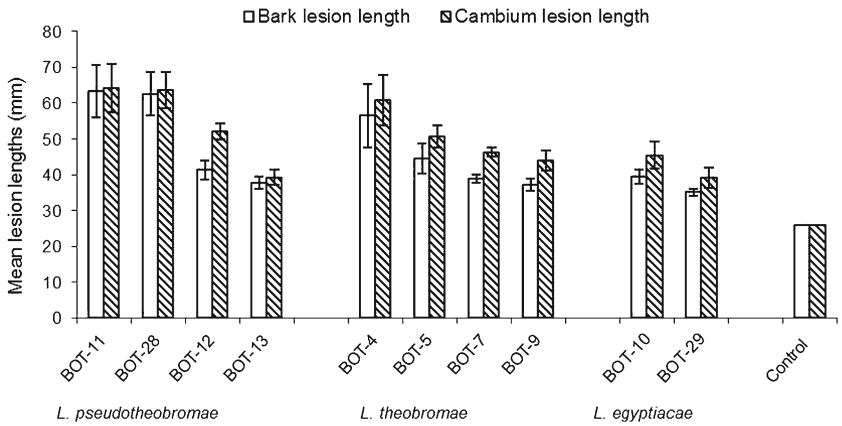

Fig. 3 Mean lengths (mm) of log-transformed bark and cambium lesions 6 weeks after inoculation on mango plants cv. Kensington Pride with four species of *Lasiodiplodia*. *Bars above columns* are the standard error of the mean of bark and cambium lesions lengths

Fig. 4 Results of the pathogenicity trial. **a** Black necrosis and cracks developing around the inoculation sites; **b** necrosis and brown discolouration of the cambium tissues extended up and down from the inoculation point; **c** typical dieback symptoms of mango seedling 4-weeks after stem inoculation; **d** mycelial growth on the necrotic tissues of a dead twig after complete defoliation of the apical leaves

Morphological characteristics combined with ITS and TEF1-α sequence data enabled us to separate *L. egyptiacae* from the other *Lasiodiplodia* species. Several authors have in the past relied on DNA sequence data (ITS and TEF1-α) and morphological characteristics to separate species in this genus, namely conidia (shape, dimensions and septation), paraphyses (size and septation) culture morphology, and cardinal temperature requirements for growth (Pavlic et al. 2004; Burgess et al. 2006; Damm et al. 2007; Alves et al. 2008; Abdollahzadeh et al. 2010). Although morphological characters can overlap (Charles 1970; Pennycook and Samuels 1985; Slippers et al. 2004a; Kim et al. 2005; Abdollahzadeh et al. 2010), they are still useful complimentary features when combined with DNA phylogeny to distinguish new species in the *Botryosphaeriaceae*. Therefore, using morphological features as discriminatory criteria alone should be done with care. In the present study, the shape and length of paraphyses were used to differentiate *L. egyptiacae* from the phylogenetically closely related species such as *L. hormozganensis, L. parva and L. citricola*. Burgess et al. (2006) relied on the septation of paraphyses to discriminate between *Lasiodiplodia* spp. and indicated that *L. gonubiensis*, *L. venezuelensis* and *L. crassispora* have septate paraphyses, whereas in other species they were aseptate. Damm et al. (2007) were able to distinguish *L. plurivora* from *L. crassispora* and *L. venezuelensis* based on dimensions of their paraphyses. This was also followed by Abdollahzadeh et al. (2010) to distinguish *L. gilanensis* from *L. plurivora,* and *L. hormozganensis* from *L. parva* and *L. citricola*.

Culture characteristics have also played an important role in distinguishing *Lasiodiplodia* species. Alves et al. (2008) discriminated *L. parva* and *L. pseudotheobromae* from *L. theobromae* based on the production of a pink pigment in culture. In contrast, the findings of Abdollahzadeh et al. (2010) revealed that *L. theobromae* and other *Lasiodiplodia* species with the exception of *L. hormozganensis,* produced a pink pigment on PDA at 35 °C. In the present study, only *L. theobromae* produced a dark pink pigment in PDA after 2 days at 35 °C, with the colour becoming darker with age. Moreover, *L. pseudotheobromae* was the only species that could grow at 10 °C, which is in agreement with the observations made by Alves et al. (2008), and in contrast to the study of Abdollahzadeh et al. (2010) who reported that all *Lasiodiplodia* species could grow at this temperature.

The phylogenetic inferences based on multiple gene sequences have played an important role in delimiting species in the genus *Lasiodiplodia.* In this study, combined ITS and TEF-1α sequence data allowed us to better characterise a new cryptic species within the *L. theobromae* species complex, described here as *L. egyptiacae*. Based on the phylogeny, the new species was distinct from *L. hormozganensis* and morphological characters reinforced this conclusion. In a first attempt to discover new cryptic species of *Lasiodiplodia,* Pavlic et al. (2004) were able to distinguish *L. gonubiensis* from *L. theobromae* based on the original description of *L. theobromae* (Patouillard and De Lagerheim 1892; Clendinin 1896), along with ITS sequence data. Due to the absence of the herbarium specimens and cultures, the authors relied on the available data in the literature to discriminate between the two species. Several studies have confirmed that using a single gene region is insufficient to delimit cryptic species in *Botryosphaeriaceae* (de Wet et al. 2003; Slippers et al. 2004a, b) and therefore, to resolve species boundaries in the genus *Lasiodiplodia,* more than one gene region is required (Alves et al. 2008; Abdollahzadeh et al. 2010).

DNA sequence data and morphological comparisons were able to delimit *L. pseudotheobromae* from a collection of *Lasiodiplodia*-like isolates previously treated as *L. theobromae*. The distribution and host range of *L. pseudotheobromae* is poorly understood (Begoude et al. 2009). Alves et al. (2008) proposed that this fungus had a narrow host range, which included *Rosa* spp. in the Netherlands, *Gmelina arborea* and *Acacia mangium* in Costa Rica, *Coffea* sp. in Democratic Republic of Congo and *Citrus aurantium* in Suriname. However, recent studies have demonstrated that the host range of *L. pseudotheobromae* should be expanded to include *Terminalia catappa* in Cameron, South Africa and Madagascar (Begoude et al. 2009), and *M. indica* in Western Australia (Sakalidis et al. 2011) and *Citrus* sp. in

Iran (Abdollahzadeh et al. 2010). In addition, Zhao et al. (2010) recently reported *L. pseudotheobromae* on *Mangifera sylvatica* and on other tropical and subtropical trees in China. This study represents the first report of *L. pseudotheobromae* on mango in Egypt associated with severe twig and branch dieback, leading to tree mortality. In Egypt, *L. theobromae* was the second most dominant species isolated during the survey with mango trees showing symptoms of twig and branch dieback. This fungus has a cosmopolitan distribution occurring on a broad spectrum of woody plant hosts, especially in temperate climates (Punithalingam 1980; Burgess et al. 2006; Begoude et al. 2009). In addition to Egypt, this fungus is a well-known mango pathogen associated with gummosis, twig and branch dieback and decline around the world (Jacobs 2002; Al Adawi et al. 2003; Khanzada et al. 2004a, b; Abdollahzadeh et al. 2010; de Oliveira Costa et al. 2010).

Results of the pathogenicity trial revealed that of the three species tested, *L. pseudotheobromae* and strains representing *L. theobromae* were the most virulent on mango. Although previous pathogenic studies have been conducted using *L. theobromae* isolates (Ragab et al. 1971; Khanzada et al. 2004a; Sakalidis et al. 2011), little information is available on the virulence of *L. pseudotheobromae*. Pathogenicity results revealed that some isolates of *L. pseudotheobromae* were more virulent than *L. theobromae* on mango. The importance of *L. pseudotheobromae* has been overlooked in the past, as it was treated as *L. theobromae* (Begoude et al. 2009). Therefore, the expansion in host range of this fungus, and its importance as a pathogen of mango should be taken in consideration when establishing control strategies.

All isolates of *Lasiodiplodia* in this study were able to spread asymptomatically through the internal tissues above and below points of inoculation resulting in brown to black discolouration of vascular tissues. Previous studies (Ramos et al. 1991; Ploetz et al. 1996a; Khanzada et al. 2004a) support these findings, namely that inoculation of mango plants with *Lasiodiplodia* species can manifest various external and internal symptoms such as bark necrosis, vascular discolouration, defoliation, apical dieback and gummosis. However, no gummosis was observed in the present study. The upward and downward progress inside the apparently healthy tissues along the mango stem can reflect the well-known endophytic nature of these fungi (Ploetz 2004; Ploetz et al. 1996a; Slippers and Wingfield 2007). Hence, the external and internal symptoms that developed after inoculation reveal the capacity of recognised species to cause disease and to spread rapidly through the vascular tissues even if their hosts are not subjected to stress.

Lasiodiplodia egyptiacae has been isolated at low frequency from plant material showing brown to black leaf lesions and branch dieback. Limited information is available regarding its ecology and distribution in mango plantations in Egypt, and whether it possibly originates from alternative hosts in close proximity to the surveyed mango plantations. However, the ability of the newly described species to cause lesions on mango reveals that it could pose a potential threat to mango plantations elsewhere. Further surveys from different geographical areas and additional pathological studies are required to determine its potential threat to the Egyptian mango industry.

Acknowledgments This work was partially funded by the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands, University of Catania, Italy, and the Plant Pathology Research Institute, Giza, Egypt. The first author would like to thank all the staff at the CBS for their guidance and technical support.

References

Abdalla MA, Safie MH, El-Boghdady MM, Soltan HHM (2003) Fruit coating with certain plant oils for controlling post-harvest diseases of mangoes with special reference to stem end rot. Egypt J Appl Sci 18:116–136

Abdalla AEM, Darwish SM, Ayad EHE, El-Hamahmy RM (2007) Egyptian mango by-product 1. Compositional quality of mango seed kernel. Food Chem 103:1134–1140

Abd-El Ghani HS, Fatouh HM (2005) First record of sugar beet root rot disease caused by *Botryodiplodia theobromae* in Egypt. Egypt J Phytopathol 33(1):107–108

Abdollahzadeh J, Javadi A, Mohammadi Goltapeh E, Zare R, Phillips AJL (2010) Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. Persoonia 25:1–10

Abo-El-Dahab MK, El-Kazazz SA, Shoeib AA, El-Sheikh MA (1992) Biochemical changes in citrus fruits infected with *Botryodiplodia theobromae*. J Agric Sci Mansoura Univ 17:3525–3532

Al Adawi AO, Deadman ML, Al Rawahi AK, Khan AJ, Al Maqbali YM (2003) *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. Plant Pathol 52:419

Alves A, Crous PW, Correia A, Phillips AJL (2008) Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Divers 28:1–13

Begoude BAD, Slippers B, Wingfield MJ, Roux J (2009) Botryosphaeriaceae associated with *Terminalia catappa* in Cameron, South Africa and Madagascar. Mycol Prog 9:101–123

Burgess TI, Barber A, Mohali S, Pegg G, De Beer W, Wingfield MJ (2006) Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. Mycologia 98(2):423–435

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91 (3):553–556

Charles ML (1970) Effects of temperatures on conidium characteristics of *Ulocladium chartum* and *Stemphylium floridanum*. Mycologia 62(5):1071–1076

Chen SF, Pavlic D, Roux J, Slippers B, Xie YJ, Wingfield MJ, Zhou XD (2011) Characterization of Botryosphaeriaceae from plantation-grown *Eucalyptus* species in South China. Plant Pathol 60:739–751

Clendinin L (1896) *Lasiodiplodia* Ellis. & Everh. n. gen. Bot Gaz 21:92–93

Crous PW, Groenewald JZ (2005) Hosts, species and genotypes: opinions versus data. Australas Plant Pathol 34:463–470

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. Stud Mycol 50:19–22

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas FO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ (2006) Phylogenetic lineages in the Botryosphaeriaceae. Stud Mycol 55:235–253

Damm U, Crous PW, Fourie PH (2007) *Botryosphaeriaceae* as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99(5):664–680

de Hoog GS, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous basidiomycetes. Mycoses 41:183–189

de Oliveira Costa VS, Michereff SJ, Martins RB, Gava CAT, Mizubuti ESG, Câmara MPS (2010) Species of Botryosphaeriaceae associated on mango in Brazil. Eur J Plant Pathol 127:509–519

de Wet J, Burgess T, Slippers B, Preisig O, Wingfield BD, Wingfield MJ (2003) Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. Mycol Res 107:557–566

Denman S, Crous PW, Taylor JE, Ka JC, Pacose I, Wingfield MJ (2000) An overview of the taxonomic history of *Botryosphaeria* and re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Stud Mycol 45:129–140

Denman S, Crous PW, Groenewald JZ, Slippers B, Wingfield BD, Wingfield MJ (2003) Circumscription of *Botryosphaeria* species associated with Proteaceae based on morphology and DNA sequence data. Mycologia 95:294–307

Diab MM, Kahlil I, Dawood NA, El-Assiuty EM (1984) Ear and grain rot of maize caused by *Botryodiplodia theobromae* pathogens in Egypt. Minufiya J Agric Res 9:129–138

El Tomi AL (1953) Subtropical fruit industry in Egypt. Proc Fla State Hortic Soc 66:195–198

El-Goorani MA, El Meleigi MA (1972) Dieback of grapevine by *Botryodiplodia theobromae* Pat. in Egypt. Phytopathol Mediterr 11:210–211

El-Soukkary FAH, El-Sahn MA, Mohamed HMA (2000) Physico-chemical and nutritional evaluation of mango seed kernel and its utilization for pan bread supplementation. Zagazig J Agric Res 27:1319–1342

Glienke C, Pereira OL, Stringari D, Fabris J, Kava-Cordeiro V, Galli-Terasawa L, Cunnington J, Shivas RG, Groenewald JZ, Crous PW (2011) Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. Persoonia 26:47–56

Gueidan C, Roux C, Lutzoni F (2007) Using multigene phylogeny analysis to assess generic delineation and character evolution in Verrucariaceae (Verrucariales, Ascomycota). Mycol Res 111:1145–1168

Haggag WM, Nofal MA (2006) Improving the biological control of *Botryodiplodia* disease on some *Annona* cultivars using single or multi-bioagents in Egypt. Biol Control 38:341–349

Haggag WM, AbouRayya MSM, Kasim NE (2007) First report of a canker disease of walnut caused by *Botryodiplodia theobromae* in Egypt. Plant Dis 91:226

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42:182–192

Jacobs R (2002) Characterization of *Botryosphaeria* species from mango in South Africa. Dissertation, University of Pretoria

Johnson GI (1992) Biology and control of stem end rot pathogens of mango. Dissertation, University of Queensland

Johnson GI, Cooke AW, Mead AJ, Wells IA (1991) Stem end-rot of mango in Australia: causes and control. Acta Hortic 219:288–295

Johnson GI, Cooke T, Mead A (1993) Infection and quiescent of mango stem-end rot pathogens. Acta Hortic 341:329–336

Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 26:1899–1900

Khanzada MA, Lodhi A, Shahzad S (2004a) Mango dieback and gummosis in Sindh, Pakistan caused by *Lasiodiplodia theobromae*. Plant Health Prog. doi:10.1094/PHP-2004-0302-01-DG

Khanzada MA, Lodhi AM, Shahzad S (2004b) Pathogenicity of *Lasiodiplodia theobromae* and *Fusarium solani* on mango. Pak J Bot 36(1):181–189

Kim YK, Xiao CL, Rogers JD (2005) Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*. Mycologia 97(1):25–32

Knight RJ (1993) Evaluating important fruit characters in mango germplasm. Fruit Var J 47(1):25–30

Larget B, Simon D (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol Biol Evol 16:750–759

Marincowitz S, Groenewald JZ, Wingfield MJ, Crous PW (2008) Species of Botryosphaeriaceae occurring on Proteaceae. Persoonia 21:111–118

Mason-Gamer R, Kellogg E (1996) Testing for phylogenetic conflict among molecular datasets in the tribe Tiriceae (Graminae). Syst Biol 45:524–545

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci U S A 95:2044–2049

Patouillard N, De Lagerheim G (1892) Champignons de l'equateur (Pugillus II). Bull Soc Mycol Fr 8:113–140

Pavlic D, Slippers B, Coutinho TA, Gryzenhout M, Wingfield MJ (2004) *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. Stud Mycol 50:313–322

Pavlic D, Wingfield MJ, Barber P, Slippers B, Hardy GESJ, Burgess TI (2008) Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. Mycologia 100 (6):851–866

Pennycook SR, Samuels GJ (1985) *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. Mycotaxon 24:445–458

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21:29–55

Ploetz RC (2004) The major diseases of mango: strategies and potential for sustainable management. Proc Mango Acta Hort 645:137–150

Ploetz RC, Benscher D, Vázquez A, Colls A, Nagel J, Schaffer B (1996a) Mango decline: research in Florida on an apparently widespread disease complex. Proceeding of International Mango Symposium. Acta Hortic 455:547–553

Ploetz RC, Benscher D, Vázquez A, Colls A, Nagel J, Schaffer B (1996b) A re-examination of mango decline in Florida. Plant Dis 80:664–668

Punithalingam E (1976) *Botryodiplodia theobromae*. CMI descriptions of pathogenic fungi and bacteria, No.519. Commonwealth Mycological Institute, Key, Surrey, England

Punithalingam E (1980) Plant diseases attributed to *Botryodiplodia theobromae* Pat. J. Carmer. Vaduz

Ragab MM, Sabet KA, Dawood NA (1971) *Botryodiplodia theobromae* Pat. The cause of fruit rot and die back of mango in A.R.E. Agric Res Rev 49:81–97

Ramos LJ, Lara SP, McMillan RT, Narayanan KR (1991) Tip die back of mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. Plant Dis 75:315–318

Rayner RW (1970) A mycological colour chart. CMI and British Mycological Society, Kew, Surrey, UK

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Sakalidis ML, Ray JD, Lanoiselet V, Hardy GES, Burgess TI (2011) Pathogenic Botryosphaeriaceae associated with *Mangifera indica* in the Kimberley region of Western Australia. Eur J Plant Pathol 130:379–391

Slippers B, Wingfield MJ (2007) Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and their impact. Fungal Biol Rev 21:90–106

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ (2004a) Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96(1):83–101

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2004b) Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. Mycologia 96 (5):1030–1041

Slippers B, Johnson GI, Crous PW, Coutinho TA, Wingfield B, Wingfield MJ (2005) Phylogenetic and morphological re-evolution of the *Botryosphaeria* species causing diseases of *Mangifera indica*. Mycologia 97(1):99–110

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD (2001) *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. Mycologia 93(2):277–285

Summerbell RC, Krajden S, Levine R, Fuksa M (2004) Subcutaneous phaeohyphomycosis caused by *Lasiodiplodia theobromae* and successfully treated surgically. Med Mycol 42:543–547

Swofford DL (2001) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinaur Associates, Sunderland

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. San Diego, California, pp 315–322

Zhao JP, Lu Q, Liang J, Decock C, Zhang XY (2010) *Lasiodiplodia pseudotheobromae*, a new record of pathogenic fungus from some subtropical and tropical trees in southern China. Cryptogam Mycol 31:431–439