LASIODIPLODIA SPECIES ASSOCIATED WITH DYING *EUPHORBIA INGENS* IN SOUTH AFRICA.

J.A. van der Linde[1], D.L. Six[2], M.J. Wingfield[1], and J. Roux[1]

[1]Department of Microbiology and Plant Pathology, DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, PBag X20, Hatfield, Pretoria, 0083, South Africa.

[2]College of Forestry and Conservation, Department of Ecosystem and Conservation Sciences, The University of Montana, Missoula, MT 59812, United States of America.

Corresponding author, e-mail: jolanda.roux@fabi.up.ac.za

Various species of *Euphorbia* occur in South Africa, including herbaceous, succulent and woody types. The largest of the succulent *Euphorbia* spp. in South Africa is *Euphorbia ingens*. These trees have been dying at an alarming rate in the Limpopo Province during the course of the last 15 years. Investigations into the possible causes of the death have included the possible role of fungal pathogens. Amongst the most common fungi isolated from diseased trees were species in the Botryosphaeriaceae. The aim of this study was to identify these fungi using morphology and DNA sequence data of two gene regions (TEF-1α & ITS). Results showed that *Lasiodiplodia theobromae* and *Lasiodiplodia mahajangana* were present. Pathogenicity studies showed that these *Lasiodiplodia* species can cause infections on healthy *E. ingens* trees, implicating them as contributors to the decline of *E. ingens.*

KEYWORDS

Botryosphaeriaceae, candelabra trees, tree diseases, insect infestations, climate change

INTRODUCTION

The genus *Euphorbia* includes more than 2100 species worldwide. *Euphorbia* species are known to vary dramatically in morphology and range from large woody trees to shrub like herbaceous plants and succulent cactus like plants (Palgrave 2002, PBI Euphorbia project, www.euphorbiaceae.org). There is a high diversity of woody to succulent euphorbias in Southern Africa, with the largest of these species being *Euphorbia ingens* E. Meyer: Boissier (Palgrave 2002, Gildenhuys 2006). *E. ingens* and similar species are characterised by woody main stems and fleshy succulent branches, giving the trees a candelabrum shape (Van Wyk and Van Wyk 1997, Palgrave 2002, Gildenhuys 2006). *E. ingens* is known only to occur in Africa with high densities in Southern Africa (Palgrave 2002, Gildenhuys 2006).

In the last 15 years, there have been alarming reports of large-scale decline and death of *E. ingens* trees in South Africa. Mortality of these trees has been particularly severe in the Limpopo Province. Symptoms associated with the death of trees include graying and spots on the succulent branches, infestation by branch and stem boring insects and brown to blue discolouration of the internal tissues of the branches and woody main stems (Roux *et al.* 2008, 2009). Preliminary investigations into the cause of this disease have yielded various fungi including species of Botryosphaeriaceae (Roux *et al.* 2008, 2009).

The Botryosphaeriaceae are known as opportunistic pathogens that cause cankers and death of numerous tree species, especially after periods of drought, frost, hail damage and other environmental conditions leading to stress (Punithalingam 1980, Slippers and Wingfield 2007). They are also known to be endophytes, infecting healthy trees and only causing disease after the onset of stress (Smith *et al*. 1996). In South Africa, fungi in the Botryosphaeriaceae are common, and known to be pathogens, especially on commercially grown plantation trees (Laughton 1937, Swart *et al.* 1985, Smith *et al.* 1996, Roux and Wingfield 1997), native tree

species such as *Pterocarpus angolensis* DC. (Mehl *et al*. 2011), *Syzygium* spp. (Pavlic *et al*. 2007) and *Acacia* spp. (Slippers *et al.* 2011). Various species are also known to occur on, and cause disease of fruit trees in the genera *Malus*, *Pyrus*, *Prunus*, *Populus*, *Syzygium* and *Vitis* (van Niekerk *et al.* 2004, Damm *et al*. 2007, Pavlic *et al.* 2007, Slippers *et al.* 2007). There are, however, no reports of Botryosphaeriaceae from succulent *Euphorbia* spp.

The aim of this study was to identify species of Botryosphaeriaceae collected during studies of dying *E. ingens* trees in the Limpopo Province of South Africa. We also tested the pathogenicity of the isolates on healthy *E. ingens* trees to consider their possible involvement in tree death.

MATERIALS AND METHODS

Collection of samples and isolations

Isolates were collected from diseased *E. ingens* (**Figure 1a**) at four sites in the Limpopo Province during 2009. Isolations were made from blue-black discoloured wood (**Figure 1b**) in the main woody stems of the trees, as well as from necrotic tissue and insect tunnels in the succulent branches. Isolations were also made from insects collected from rotting succulent branches and the woody main stems. Direct isolations were made from the plant material taken from the leading edges of lesions using a sterile scalpel. These tissue samples were plated on 2% Malt Extract Agar (MEA) (15g agar and 20g malt extract per 1000ml distilled water; Biolab, Merck, Midrand, South Africa) with streptomycin (0.4g/L; Sigma-Aldrich, St. Louis, USA). Isolations were made from insects by crushing them onto water agar (15g per 1000ml distilled water; Biolab) and incubating the plates for six weeks at 20˚C. Cultures from insects were purified by transferring mycelium to fresh 2% MEA. A second set of isolates was obtained from the culture collection (CMW) of the Forestry and Agricultural

Biotechnology Institute (FABI), University of Pretoria which were collected previously (Roux *et al.* 2009, 2010) from diseased *E. ingens* trees in the Limpopo Province. Purified isolates from plant tissue and insects were deposited in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Culture and Morphological Characteristics

Isolates were plated onto 1.5% Water Agar (WA; 15g per 1000ml distilled water; Biolab) containing sterilized pine needles to induce the formation of fruiting bodies. Cultures were incubated at 20°C under near-ultra violet (UV) light. Characteristic fungal structures (conidia, conidiogenous cells, paraphyses, conidiomata) were viewed using a Zeiss light microscope fitted with an Axiocam digital camera with Axiovision 3.1 software (Carl Zeiss Ltd., Germany). The fungal structures were placed on glass microscope slides and mounted in 75% lactic acid. Colors of resultant cultures were determined using the color notations of Rayner (1970).

DNA extraction and Polymerase Chain Reaction (PCR) amplification

Isolates, representing different collection sites and culture morphology, were grown for six days on 2% MEA, prior to DNA extraction. DNA extraction followed the protocol of Möller *et al.* (1992), after mycelium was scraped from the surfaces of the cultures and freeze dried for 24 hours in 2ml Eppendorf tubes. DNA concentration was determined using a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA).

The Polymerase Chain Reaction (PCR) was used to amplify the internal transcribed spacer regions and the 5.8S gene using the primers ITS 1 and ITS 4 (White *et al*. 1990) and the translation elongation Factor 1-α (TEF 1-α) gene region using the primers EF1-F and EF1-R (Jacobs *et al*. 2004). PCRs were done using an Applied Biosystems Veriti thermocycler

(Applied Biosystems, Foster City, USA) following the protocols described by Mohali *et al.* (2007).

PCR products were viewed using an agarose gel (2%; Whitehead Scientific, Cape Town, South Africa), loaded with GelRed (Anatech, USA), and visualised under UV illumination. The size of the PCR products was estimated using a 100bp DNA molecular marker (O'RangeRuler™ 100bp DNA ladder, Fermentas Life Sciences). Sephadex G-50 columns (1g in 15ml distilled water Water; SIGMA, Steinheim, Germany) were used to purify the amplified products in preparation for sequencing.

DNA sequencing

Confirmed PCR products were sequenced with an ABI3700 DNA analyzer (Applied Biosystems, Foster City, USA) using a Big Dye Cycle Sequencing kit Version 1.1 (Applied Biosystems). Sequences were edited based on forward and reverse sequences using Mega Version 4.0 (Tamura *et al*. 2007). To confirm gene identity and obtain related sequences, the correctly edited sequences were placed in the nucleotide database *blastx* (National Centre for Biotechnology Information, www.ncbi.nih.nlm.gov). Mafft version 5.851 (Katoh *et al.* 2002) was used to align the sequences from this study and those of closely related species obtained from the blast results. Phylogenetic analysis of each data set was done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002) and phylogenetic trees were constructed using random stepwise addition and tree bisection and reconstruction as branch swapping algorithms, based on heuristic searches. Bootstrap and maximum parsimony analyses were run using a 1000 replicates (Felsenstein 1985). A partition homogeneity test was used to determine whether the ITS and TEF1-α sequence data sets could be combined (Farris *et al*. 1995, Huelsenbeck *et al*. 1996). Two separate phylogenetic analyses, one including all the recently described species of *Lasiodiplodia* and another

considering only the clades including the isolates from this study, were conducted. Prior to the partition homogeneity test, data sets of individual gene regions were analysed separately. The data sets were rooted with the GenBank sequences of *Botryosphaeria sarmentorum* A.J.L. Phillips, Alves & Luque (CBS 12041) and *Lasiodiplodia crassispora* Burgess & Barber (UCD27Co) (**Table 1**).

Bayesian analysis was used to determine the posterior probabilities of each dataset (ITS, TEF1-α) based on the Monte Carlo Markov Chain (MCMC) method. A jModelTest 0.1.1 (Posada 2008) was used to determine the most appropriate nucleotide substitution model. The best-fitting models for the ITS and TEF1-α datasets, based on Akaike Information Criterion (AICc), were determined for the complete analysis (TPM1: ITS, K80+G: TEF1-α) and the specific analysis (TIM2+G: ITS, K80+G: TEF1-α). The Bayesian analysis was run on MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) and trees were recorded every 100 generations based on four chains producing 5 000 000 generations. The likelihood data were used in graphical analysis to estimate the burn-in values for each dataset. Mega Version 4.0 (Tamura *et al.* 2007) was used to produce consensus trees from the two analysed datasets from which the posterior probabilities were determined.

Pathogenicity trials

Two isolates (CMW36766, CMW36765) obtained in this study were used to inoculate healthy *E. ingens* trees in the North West Province. Cultures were first grown on 2% MEA for five days and then used to inoculate wooden toothpicks first soaked in Malt Extract (5g malt extract per 250ml distilled water; Biolab) and then placed on the surface of MEA in Petri dishes. Mycelium-colonised toothpicks, and sterile toothpicks for the controls, were inserted into the succulent branches (five branches for each isolate) to a depth of 3mm. After six weeks the results were determined by measuring the surface lesions, cambium lesions and

internal lesions after cutting branches in half at the point of inoculation. Isolations were made on MEA from inoculated tissue to comply with Koch's postulates. To determine significance between means, a student's t test was done with $P < 0.05$ as being significant. Since there was no variance in the controls, the data for each isolate were Bonferroni – corrected for multiple comparisons ($\alpha = 0.05$). All tests were conducted using JMP version 9.0.2 (SAS institute 2011).

RESULTS

Collection of samples and isolations

Isolates from this study were obtained from lesions on a total of 23 trees. Isolates resembling the Botryosphaeriaceae were obtained from only six trees. A total number of six isolates were obtained from Mokopane, one from Capricorn and one from the Louis Trichardt area. Of the eight isolates collected, six were from diseased plant material and the remaining two were isolated from insects infesting diseased tissue.

Culture and morphological Characteristics

Fungal structures showed typical features of *Lasiodiplodia* spp., with aseptate, hyaline conidia becoming dark brown and septate with striations as they matured. Cultures were white with abundant, fluffy aerial mycelium which became an olivaceous grey (23'''''b) with time (after 10 days). Pycnidia were produced after five days on the WA with sterilized pine needles and were black in colour, unilocular, solitary, immersed in the media and were formed on the top surfaces of the pine needles (**Figure 2**).

DNA sequence analyses

The ITS and TEF-1α datasets were combined based on a value of P = 0.350 (complete *Lasiodiplodia* species group, 43 taxa) and P = 0.140 (specific *Lasiodiplodia* species clade, 29 taxa) obtained from the partition homogeneity test done in PAUP (**Figure 3 and 4**). MP analyses of the individual gene region data sets did not give a good resolution in terms of species identity and with P > 0.05, trees were combined for this study. The MP analysis for the combined datasets for the complete *Lasiodiplodia* group (characters = 655, 9% of characters parsimony informative) and the specific *Lasiodiplodia* species clade (649 characters, 2% of characters parsimony informative) generated 7 (TL = 182, CI = 0.780, RI = 0.832, RC = 0.649) and 100 trees respectively (TL = 53, CI = 0.849, RI = 0.855, RC = 0.0.726), with similar topology for both groups. Both of the combined datasets had strong bootstrap and Bayesian support with statistically significant values. However bootstrap analysis produced trees with limited resolution not resolving the final identity of the species. Bayesian analysis produced trees with high resolution and was used as the final model to identify the species. Burn-in values were obtained for all analyses (burn-in values: complete analysis; 52 and specific analysis; 122).

In the final phylogenetic analyses, the data set including all described *Lasiodiplodia* spp. gave rise to seven clades while three clades emerged for the data set containing only selected *Lasiodiplodia* spp. The complete *Lasiodiplodia* data set did not show a good resolution but indicated that the isolates from this study resided in clade one. Analyses of the reduced *Lasiodiplodia* data set showed that isolates represented *Lasiodiplodia mahajangana* Begoude, Jol. Roux, Slippers (CMW36765) and *Lasiodiplodia theobromae* (Patouillard) Griffon & Maubl (CMW26225, CMW26592, CMW26593, CMW26594, CMW26595, CMW36766, CMWxxxx88) with strong Bayesian support (**Figure 3 and 4**). *L. mahajangana* was isolated from blue stain in the wood from one tree (**Figure 1b**) near the Capricorn Toll Plaza (S23 21.910 E29 44.621), while *L. theobromae* was isolated from diseased plant

material (CMW26225, CMW26592, CMW26593, CMW26594, CMW26595) form Mokopane (S24 10.291 E29 01.131) as well as the insects *Cyrtogenius africus* Wood (CMW36766) and *Cossonus* Claireville (CMWxxxx88) from Mokopane and Last Post Private Nature Reserve (S23 17.738 E29 55.467) (Louis Trichardt site), respectively.

Pathogenicity trials

L. mahajangana (CMW36765) and *L. theobromae* (CMW36766) produced lesions on the exterior, cambium and internal core of healthy *E. ingens* branches (**Figure 1c**). The most severe damage caused by the fungi was in the internal core of the succulent branches which had rotten. Lesions on the exterior were conspicuous at the point of inoculation with necrotic tissue and a black discharge. *L. mahajangana* and *L. theobromae* lesions were brown and circular at the points of inoculation with necrotic tissue in the internal core. The control also had small brown circular lesions at the point of inoculation but had no signs of discoloration in the cambium or internal core (**Figure 1d**) of the succulent branches. Statistical analysis did not show significant differences in pathogenicity between species except for the cambium lesion length with P values of 0.025 (DF = 12.51), 0.1303 (DF = 18.00) and 0.4261 (DF = 14.06) for the cambium lesion, internal lesion depth and internal lesion width data, respectively (**Figure 5**). Isolations from the sites of inoculation yielded *L. theobromae* and *L. mahajangana* identified based on characteristic morphological features.

DISCUSSION

Results of this study showed that two species of *Lasiodiplodia*, *L. theobromae* and *L. mahajangana* are associated with die-back symptoms on *E. ingens*. These fungi were identified based on morphological characteristics and DNA sequence comparisons. They are

both well-known from trees in Southern Africa (Crous *et al.* 2000, Burgess *et al.* 2003, Pavlic *et al.* 2007, Begoude *et al.* 2010), but have not previously been found on *E. ingens*.

L. mahajangana is a recently described species from healthy branches of *T. catappa* in Madagascar (Begoude *et al.* 2010). The current study represents only the second report of this fungus and very limited information is, therefore, available regarding its possible origin or importance. In this study *L. mahajangana* was isolated from blue stain in the wood of the main stem of an *E. ingens* tree. Blue stain is a common symptom of wood infected by species in the Botryosphaeriaceae, resulting from the dark colour of the mycelium of these fungi (Slippers and Wingfield 2007). Blue stain is, however, a secondary symptom on dead or dying wood, suggesting that *L. mahajangana* obtained from *E. ingens* in this study, is not the primary cause of tree decline but rather causes secondary infections due to the trees being stressed.

L. theobromae was obtained from diseased plant material and insects collected from the internal parts of dying *E. ingens* trees. These insects, *Cyrtogenius africus* (Curculionidae: Scolytinae), and a *Cossonus* sp. (Curculionidae: Cossoninae) were not surface disinfected and inoculum of *L. theobromae* could have been on their surfaces or related to tissue that they had consumed and so occurring in their guts. Based on morphology, variation was found between previously identified *L. theobromae* and isolates from this study. Isolates from *E. ingens* have smaller conidia than previously described *L. theobromae* isolates (**Table 2**).

The Botryosphaeriaceae typically disperse via rain splash and are not adapted to insect dispersal. But species such as *L. theobromae* have previously been isolated from insects such as *Hypocryphalus mangiferae* Stebbing (Scolytinae) after surface sterilization, implying that it might be carried in the gut or mycangia (Masood *et al.* 2010). Both insect families have previously been associated with succulent *Euphorbia* (Wollaston) (Jordal 2006, 2009). Recently, previously described *L. theobromae* species were re-analysed to determine their

genetic variability and cryptic speciation based on morphology and the combined sequence analyses of ITS and TEF-1α (Alves *et al.* 2008). Three distinct morphological groups were discovered based on conidial size and phylogenetic analysis clearly showing *Lasiodiplodia parva* AJL Phillips, A Alves & Crous (small conidia) and *Lasiodiplodia pseudotheobromae* AJL Phillips, A Alves & Crous (larger conidia) being separate from *L. theobromae* as two new species (Alves *et al.* 2008). Phylogenetic analyses based on ITS and TEF-1α gene sequences, in this study, showed poor resolution in the *L. theobromae* clade (**Figure 3 and 4**) with limited differences between isolates of *L. theobromae* and *Lasiodiplodia hormozganensis* Abdollahzadeh, Zare & A.J.L. Phillips. Additional analyses will be needed to resolve this group more clearly, either by including other gene regions or using specific microsatellite markers to consider the problem at a population level.

Inoculation studies, using *L. theobromae* and *L. mahajangana*, showed that both these fungi have the potential to cause disease on *E. ingens*. Both produced extensive internal rot of the succulent branches of these trees, within six weeks. It was not surprising to find that *L. theobromae* and *L. mahajangana* were able to cause disease symptoms on *E. ingens*. Previously, *L. theobromae* was shown to be pathogenic to *Eucalyptus* clones (GC540) (Pavlic *et al.* 2007), grapevines (Úrbez-Torres *et al.* 2008, Úrbez-Torres and Gubler 2009) and *T. catappa* (Begoude *et al.* 2010). Similar to results in the study by Begoude *et al.* (2010), in our study, *L. mahajangana* produced smaller lesions in artificial inoculation studies than *L. theobromae*.

CONCLUSIONS

Since environmental and other stress factors play an important role in the epidemiology of diseases caused by fungi in the Botryosphaeriaceae, the symptoms observed on dying *E. ingens* trees in South Africa could, at least in part, be attributed to *L. theobromae* and L.

mahajangana. Reports by Van der Linde *et al.* (2011) of increased temperature and decreased rain in the study areas in the Limpopo Province over the last 40 years may be a possible stress factor for *E. ingens*. It does appear that a link to an environmental and/or an anthropogenic trigger initiated the the sudden and severe decline of these trees, in combination with various pathogens and insects. Further and more extensive surveys will be required to fully understand the diversity and distribution of Botryosphaeriaceae on native *Euphorbia* trees and to establish the possible triggers enabling these fungi to attack and thrive on these trees.

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Table 1: Isolates of Botryosphaeriaceae used in this study and obtained from *E. ingens* and GenBank

Species	Culture Number	Host	Origin	GenBank Accession Number	
				ITS	TEF-1α
Botryosphaeriasarmentorum	CBS120.41	*Pyrus communis*	Norway	AY573207	AY573224
Lasiodiplodiacitricola	IRAN1521C	*Citrus* sp.	Iran	GU945353	GU945339
L. citricola	IRAN1522C	*Citrus* sp.	Iran	GU945354	GU945340
L. crassispora	CMW13488	*Eucalyptus urophylla*	Venezuela	DQ103552	DQ103559
L. crassispora	WAC12533	*Santalum album*	Australia	DQ103550	DQ103557
L. crassispora	UCD27Co	Grapevines	USA	GU799457	GU799488
L. gilanensis	IRAN1501C	Unknown	Iran	GU945352	GU945341
L. gilanensis	IRAN1523C	Unknown	Iran	GU945351	GU945342
L. gonubiensis	CBS115812	*Syzygium cordatum*	South Africa	DQ458892	DQ458877
L. gonubiensis	CMW14078	*S. cordatum*	South Africa	AY639594	DQ103567
L. hormozganensis	IRAN1498C	*Mangifera indica*	Iran	GU945356	GU945344
L. hormozganensis	IRAN1500C	*Oleo* sp.	Iran	GU945355	GU945343
L. iraniensis	IRAN921C	*Mangifera indica*	Iran	GU945346	GU945334

L. iraniensis	IRAN1502C	*Juglans* sp.	Iran	GU945347	GU945335
L. mahajangana	CMW36765	*Euphorbia ingens*	South Africa	JN098457	JN098464
L. mahajangana	CMW27801	*Terminalia catappa*	Madagascar	FJ900595	FJ900641
L. mahajangana	CMW27818	*T. catappa*	Madagascar	FJ900596	FJ900642
L. mahajangana	CMW27820	*T. catappa*	Madagascar	FJ900597	FJ900643
L. margaritacea	CBS122519	*Adansonia gibbosa*	Australia	EU144050	EU144065
L. margaritacea	CBS122065	*Adansonia gibbosa*	Australia	EU144051	EU144066
L. parva	CBS494.78	Cassava-field soil	Colombia	EF622084	EF622064
L. parva	CBS456.78	Cassava-field soil	Colombia	EF622083	EF622063
L. parva	CBS356.59	*Theobroma cacao*	Sri Lanka	EF622082	EF622062
L. plurivora	STEU-5803	*Prunuss alicina*	South Africa	EF445362	EF445395
L. plurivora	STEU-4583	*Vitis vinifera*	South Africa	AY343482	EF445395
L. pseudotheobromae	CBS116459	*Gmelinea arborea*	Costa Rica	EF622077	EF622057
L. pseudotheobromae	CBS374.54	*Coffea* sp.	Zaire	EF622080	EF622059
L. pseudotheobromae	CBS447.62	*Citrus aurantium*	Suriname	EF622081	EF622060
L. rubropurpurea	CBS118740	*Eucalyptus grandis*	Australia	DQ103553	DQ103571

L. rubropurpurea	CMW15207	*E. grandis*	Australia	DQ103554	DQ103572
L. theobromae	CMW36766	*Euphorbia ingens*	South Africa	JN098457	JN098465
L. theobromae	CMW88	*E. ingens*	South Africa	JN098458	JN098466
L. theobromae	CMW26225	*E. ingens*	South Africa	JN098459	JN098467
L. theobromae	CMW26592	*E. ingens*	South Africa	JN098460	JN098468
L. theobromae	CMW26593	*E. ingens*	South Africa	JN098461	JN098469
L. theobromae	CMW26594	*E. ingens*	South Africa	JN098462	JN098470
L. theobromae	CMW26595	*E. ingens*	South Africa	JN098463	JN098471
L. theobromae	CBS111530	Unknown	Unknown	EF622074	EF622054
L. theobromae	CMW30105	*Syzygium cordatum*	Zambia	FJ747642	FJ871116
L. theobromae	CMW30104	*S. cordatum*	Zambia	FJ747641	FJ871115
L. theobromae	CMW28317	*Terminalia catappa*	Cameroon	FJ900602	FJ900648
L. theobromae	CMW28319	*T. catappa*	Cameroon	FJ900603	FJ900649
L. theobromae	IRAN1233C	*Mangifera indica*	Iran	GU973868	GU973860
L. theobromae	IRAN1496C	*M. indica*	Iran	GU973869	GU973861
L. venezuelensis	WAC12539	*Acacia mangium*	Venezuela	DQ103547	DQ103568

L. venezuelensis	WAC12540	*A.mangium*	Venezuela	DQ103548	DQ103569

Table 2: Conidial measurements comparing *L. theobromae* isolates from *E. ingens* and previous studies.

Species	Previous studies	Reference
L. theobromae (Pat.) Griffon & Maubl.	23.6 - 28.8 x 13 - 15.4	Alves *et al*. 2008
L. theobromae	22.5 - 26 x 12.5 - 15	Begoude *et al.* 2010
L. theobromae	22.4 - 24.2 x 12.9 - 14.3	Abdollahzadeh *et al.* 2010
L. theobromae - *E. ingens*	18.1 - 21.3 x 11.6 - 13.3	This study

List of Figures

Figure 1

Figure 2

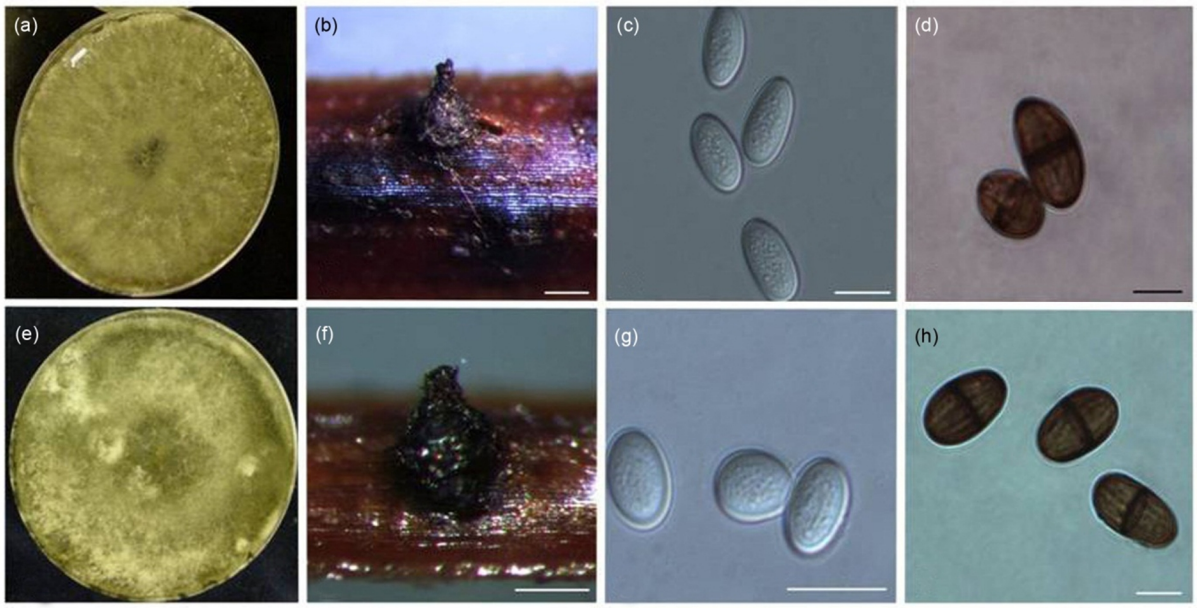
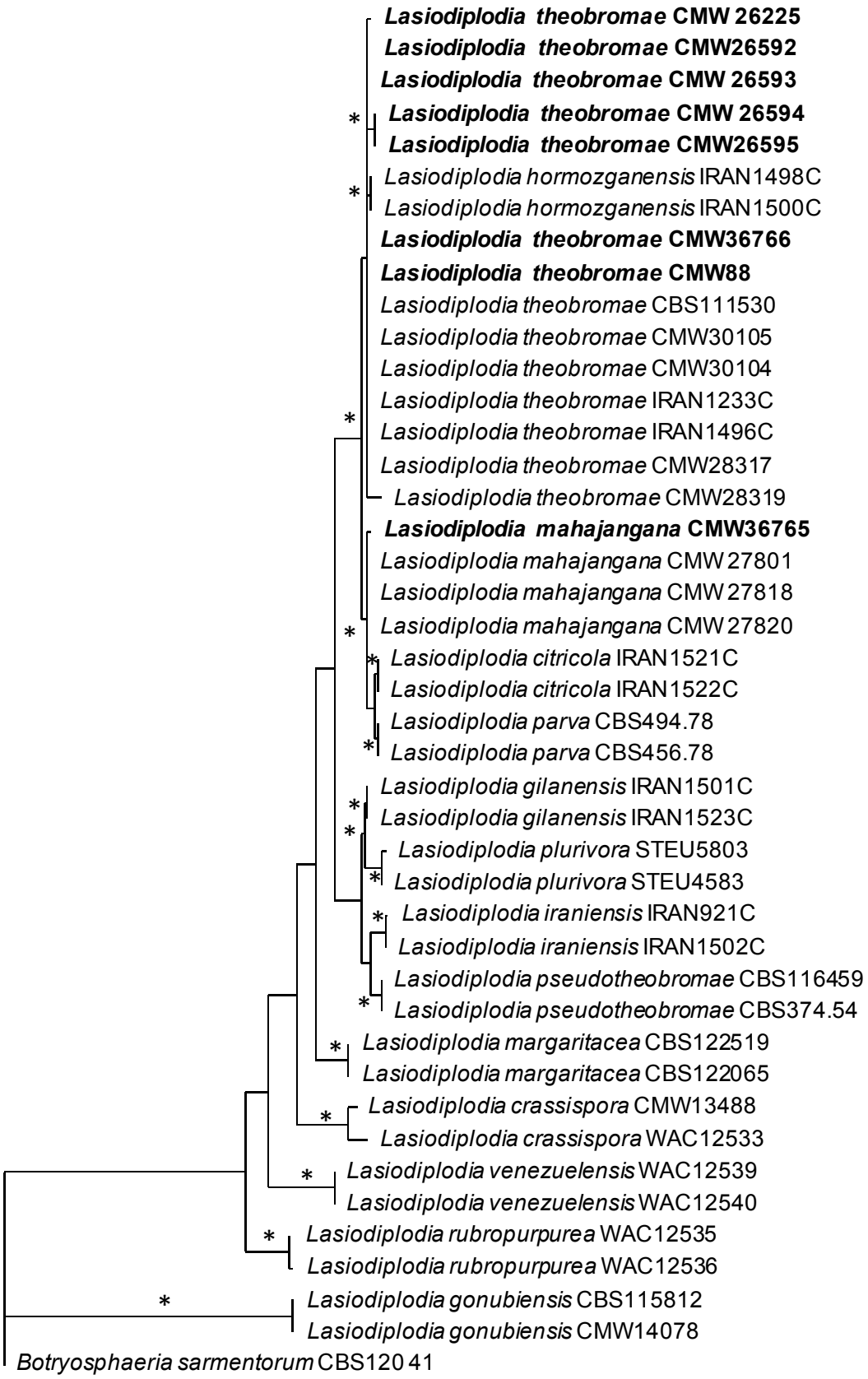


Figure 3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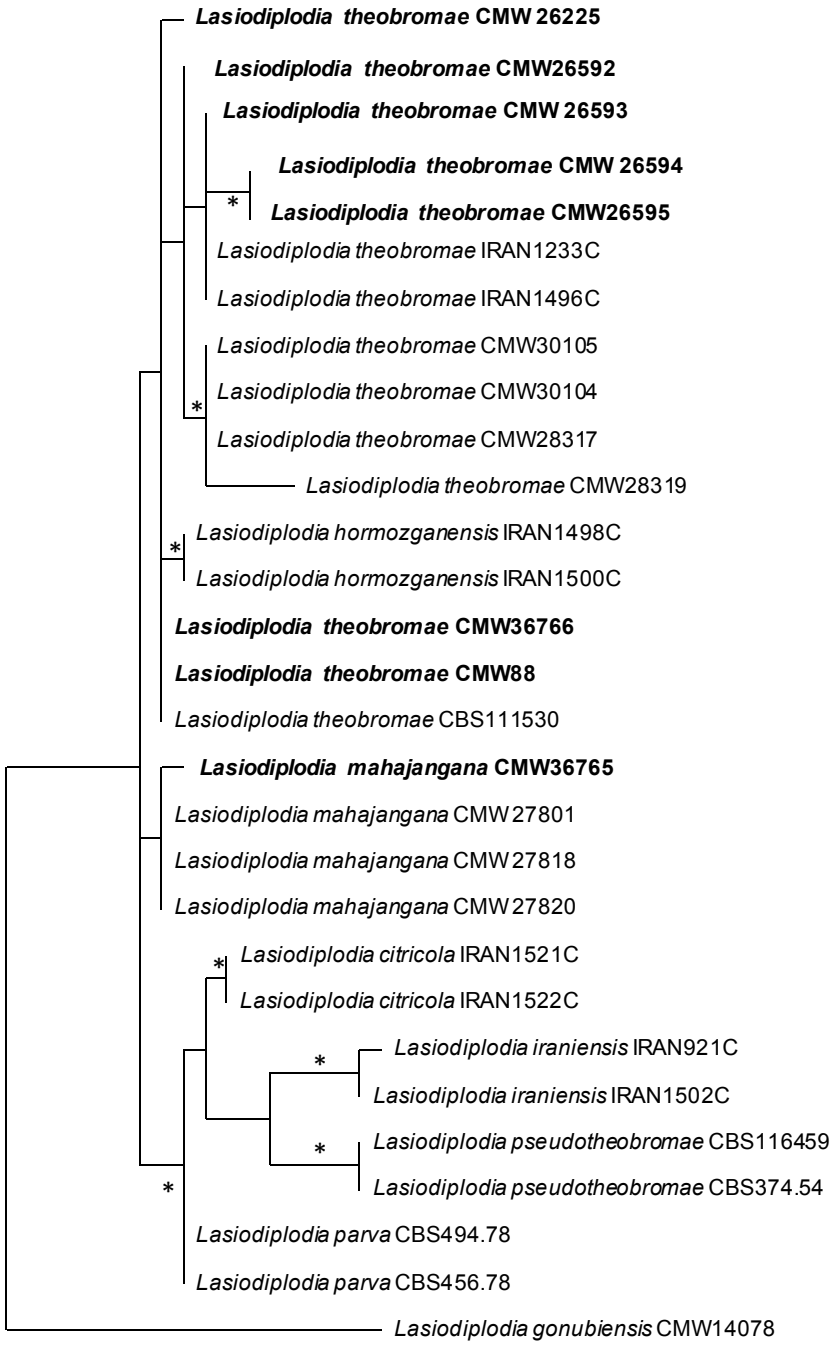
Lasiodiplodia theobromae CMW26592
Lasiodiplodia theobromae CMW 26593
Lasiodiplodia theobromae CMW 26594
Lasiodiplodia theobromae CMW26595
Lasiodiplodia hormozganensis IRAN1498C
Lasiodiplodia hormozganensis IRAN1500C
Lasiodiplodia theobromae CMW36766
Lasiodiplodia theobromae CMW88
Lasiodiplodia theobromae CBS111530
Lasiodiplodia theobromae CMW30105
Lasiodiplodia theobromae CMW30104
Lasiodiplodia theobromae IRAN1233C
Lasiodiplodia theobromae IRAN1496C
Lasiodiplodia theobromae CMW28317
Lasiodiplodia theobromae CMW28319
Lasiodiplodia mahajangana CMW36765
Lasiodiplodia mahajangana CMW 27801
Lasiodiplodia mahajangana CMW 27818
Lasiodiplodia mahajangana CMW 27820
Lasiodiplodia citricola IRAN1521C
Lasiodiplodia citricola IRAN1522C
Lasiodiplodia parva CBS494.78
Lasiodiplodia parva CBS456.78
Lasiodiplodia gilanensis IRAN1501C
Lasiodiplodia gilanensis IRAN1523C
Lasiodiplodia plurivora STEU5803
Lasiodiplodia plurivora STEU4583
Lasiodiplodia iraniensis IRAN921C
Lasiodiplodia iraniensis IRAN1502C
Lasiodiplodia pseudotheobromae CBS116459
Lasiodiplodia pseudotheobromae CBS374.54
Lasiodiplodia margaritacea CBS122519
Lasiodiplodia margaritacea CBS122065
Lasiodiplodia crassispora CMW13488
Lasiodiplodia crassispora WAC12533
Lasiodiplodia venezuelensis WAC12539
Lasiodiplodia venezuelensis WAC12540
Lasiodiplodia rubropurpurea WAC12535
Lasiodiplodia rubropurpurea WAC12536
Lasiodiplodia gonubiensis CBS115812
Lasiodiplodia gonubiensis CMW14078
Botryosphaeria sarmentorum CBS120 41

Figure 4


Lasiodiplodia theobromae **CMW 26225**
Lasiodiplodia theobromae **CMW26592**
Lasiodiplodia theobromae **CMW 26593**
Lasiodiplodia theobromae **CMW 26594**
Lasiodiplodia theobromae **CMW26595**
Lasiodiplodia theobromae IRAN1233C
Lasiodiplodia theobromae IRAN1496C
Lasiodiplodia theobromae CMW30105
Lasiodiplodia theobromae CMW30104
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Lasiodiplodia theobromae CMW28319
Lasiodiplodia hormozganensis IRAN1498C
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Lasiodiplodia theobromae **CMW36766**
Lasiodiplodia theobromae **CMW88**
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Lasiodiplodia mahajangana CMW27801
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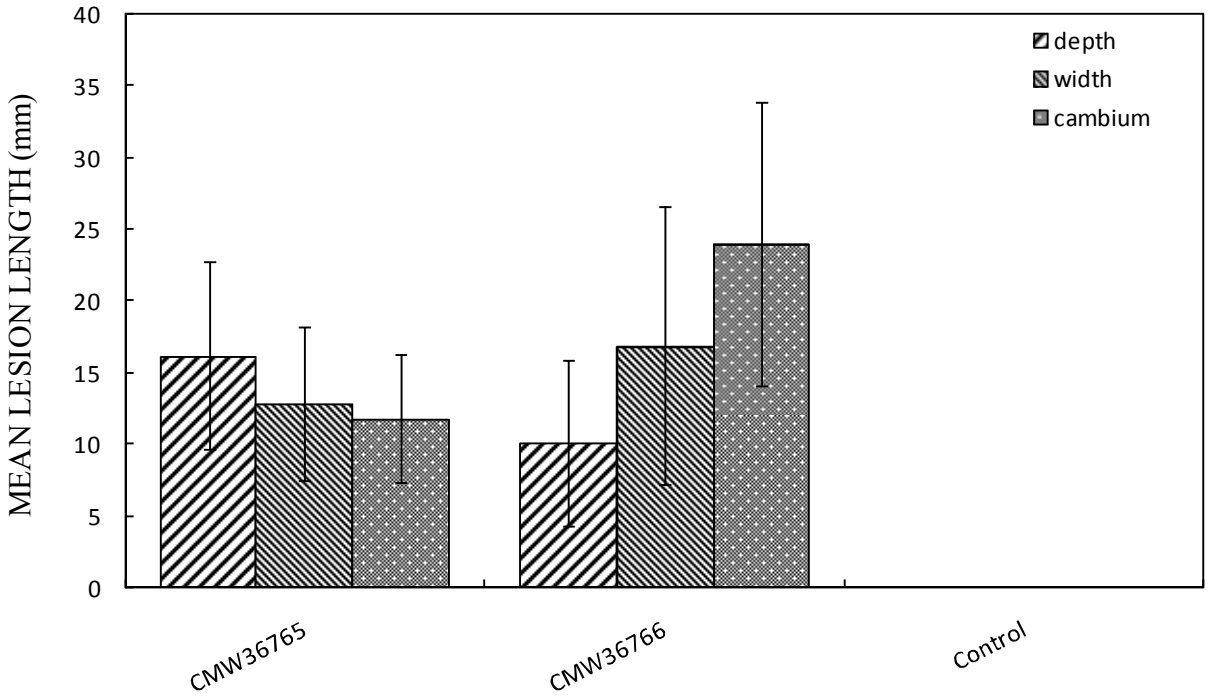
Lasiodiplodia pseudotheobromae CBS374.54
Lasiodiplodia parva CBS494.78
Lasiodiplodia parva CBS456.78
Lasiodiplodia gonubiensis CMW14078

Figure 5



LASIODIPLODIA ISOLATES USED IN PATHOGENICITY TRIALS