Species of *Lasiodiplodia* responsible for table grape dieback in the main Brazilian exporting region

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Abstract This study aims to identify and characterize species of *Lasiodiplodia* responsible for table grape dieback in São Francisco Valley, the main Brazilian exporting region. Fungal identifications were made using a combination of morphology together with a phylogenetic analysis based on partial translation elongation factor 1-α sequence (EF1-α) and internal transcribed spacers (ITS). Eight species of *Lasiodiplodia* were identified: *L. brasiliense*, *L. crassispora*, *L. egyptiacae*, *L. euphorbicola*, *L. hormozganensis*, *L. jatrophicola*, *L. pseudotheobromae* and *L. theobromae*. Only three these species had previously been reported in grapevine, while all the other species are reported for the first time in association with this host in Brazil and worldwide. *Lasiodiplodia theobromae* was the prevalent species. All species of *Lasiodiplodia* were pathogenic on detached green shoots of grapevine, with *L. brasiliense* being the most virulent.

Keywords Botryosphaeriaceae, Phylogeny, Trunk disease, Virulence, *Vitis vinifera*

Introduction

Table grape (*Vitis* spp.) is an important fresh fruit exported by Brazil. In 2011, 59,400 t of table grapes were exported and accounting for US$ 136 million (FAO 2014). The Northeastern region is responsible for 99% of Brazilian exports of table grapes, where 9,600 ha are cultivated. The São Francisco Valley, located in the semi-arid region of Bahia and Pernambuco States, is the main table grape growing area in the region, accounting for 98% of the production (Lazzarotto and Fioravanço 2013).

Northeastern Brazil is a tropical region, thus the management systems for grapevine production are adapted to the specific environmental conditions of a tropical viticulture. In the dry tropic, the growth and cropping cycle of the vine can be manipulated to extend from 5 to 12 months by a combination of pruning, modifying vine water status and the use of chemical

regulators. Thus, it is possible to achieve two and a half to three vegetative cycles per year (Camargo et al. 2008; Possingham 2008).

Table grapes are affected by various pests and pathogens. Among the wide range of diseases that impact on table grape production in Brazil, dieback has become increasingly important (Garrido et al. 2011). The first report of grapevine dieback in Brazil was in 1992 (Ribeiro et al. 1992) and since then the intensity of the disease has increased leading, in some cases, to drastic reductions in longevity and productivity of the plants, greatly increasing production costs (Garrido et al. 2011).

Grapevine dieback is caused by a complex of fungi, but members of the Botryosphaeriaceae are considered to be the most important (Úrbez-Torres 2011; Larignon 2012; Úrbez-Torres et al. 2012). *Lasiodiplodia* Ellis & Everh. is a member of the Botryosphaeriaceae, a genus-rich family in the Dothideomycetes, containing numerous species with a cosmopolitan distribution that occur on a large variety of plant hosts, on which they are found as saprophytes, parasites, and endophytes (Slippers and Wingfield 2007; Liu et al. 2012; Phillips et al. 2013; Wikee et al. 2013). *Lasiodiplodia* species are common, especially in tropical and subtropical regions (Punithalingam 1980; Burgess et al. 2006), where they cause a variety of diseases in up to 650 plant hosts (Farr and Rossman 2014). The fungus has been reported as a grapevine pathogen worldwide associated with several disease symptoms including stunted growth, dieback of shoots, spurs and arms, canker of trunk or arms, wedge-shape cankers in the vascular tissue and mortality (Larignon et al. 2001; van Niekerk et al. 2004; Amponsah et al. 2011; Úrbez-Torres 2011; Úrbez-Torres et al. 2012).

Besides grapevine, several other crops of economic importance are affected by *Lasiodiplodia* in Brazil, especially avocado (*Persea americana* Mill.), banana (*Musa* spp.), barbados cherry (*Malpighia glabra* L.), cacao (*Theobromae cacao* L.), cashew (*Anacardium occidentale* L.), castor bean (*Ricinus communis* L.), citrus (*Citrus* spp.), coconut palm (*Cocos nucifera* L.), custard apple (*Annona squamosa* L.), guarana (*Paullinia cupana* Ducke), guava

(*Psidium guajava* L.), mango (*Mangifera indica* L.), muskmelon (*Cucumis melo* L.), papaya (*Carica papaya* L.), passion fruit (*Passiflora edulis* Sims), physic nut (*Jatropha curcas* L.), soursop (*Annona muricata* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) (Tavares 2002; Freire et al. 2003; Costa et al. 2010; Muniz et al. 2012; Marques et al. 2013; Machado et al. 2014; Netto et al. 2014).

The main features that distinguish *Lasiodiplodia* from other closely related genera are the presence of pycnidial paraphyses and longitudinal striations on mature conidia (Sutton 1980; Phillips et al. 2008). The taxonomic history of *Lasiodiplodia* is confused, but after 150 years this trend ended with the monograph of Punithalingam (1976), which reduced most species to synonymy with *L. theobromae* (Pat.) Griff. & Maubl. However, in recent years, the use of molecular tools has been offering meaningful advances at the species identification of *Lasiodiplodia* and 24 new species have been reported since 2004 (Phillips et al. 2013; Machado et al. 2014 ; Netto et al. 2014).

Four species of *Lasiodiplodia* have been found associated with grapevine dieback worldwide: *L. crassispora* T.I. Burgess & Barber, *L. missouriana* J.R. Úrbez-Torres, F. Peduto & W.D. Gubler, *L. theobromae L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous, L. *parva* A.J.L. Phillips, A. Alves & Crous, and *L. viticola* J.R. Úrbez-Torres, F. Peduto & W.D. Gubler (Phillips 2002; Taylor et al. 2005; Qiu et al. 2011; Úrbez-Torres 2011; Larignon 2012; Úrbez-Torres et al. 2012; Abreo et al. 2013; Bertsch et al. 2013; Billones-Baaijens et al. 2013; Correia et al. 2013; Pitt et al. 2013; Yan et al. 2013). Among these, *L. theobromae* has been reported to be the most prevalent and virulent in grapevine worldwide (van Niekerk et al. 2006; Úrbez-Torres et al. 2008; Qiu et al. 2011; Úrbez-Torres 2011; Pitt et al. 2013; Yan et al. 2013).

In Brazil, thirteen species of *Lasiodiplodia* were reported (Costa et al. 2010; Correia et al. 2013; Marques et al. 2013; Machado et al. 2014; Netto et al. 2014), and only *L. theobromae, L. crassispora, L. pseudotheobromae* and *L. parva* has been reported on

grapevine (Garrido et al. 2011; Correia et al. 2013). However, in some works, identifications of *L. theobromae* from grapevine were based primarily on morphological and cultural data, which is now considered to be unreliable for species discrimination since the morphological characteristics overlap with other species of *Lasiodiplodia* (Costa et al. 2010).

The increasing economic importance of dieback caused by *Lasiodiplodia* in grapevine and the recent discovery of several new species of fungus associated with tropical plants led us to speculate that more than one species of *Lasiodiplodia* may be associated with table grape dieback in São Francisco Valley, Northeastern Brazil. The disease etiology is crucial for epidemiological studies and for a better understanding of the distribution and importance of individual species, as well as finding effective management strategies to each pathogen. Therefore, the objective of this study were (a) to identify species of *Lasiodiplodia* associated with dieback of table grapes in São Francisco Valley, (b) to investigate the prevalence and distribution of the species in the region and (c) to evaluate their pathogenicity and virulence in excised green shoots of table grape.

Materials and methods

Sampling and fungal isolation

During 2012 and 2013, isolates of *Lasiodiplodia* were obtained from 14 vineyards located in São Francisco Valley, Northeastern Brazil. These isolates represented three table grape populations (Casa Nova, Juazeiro and Petrolina) according to their geographical origin (Fig. 1). In each vineyard, 10 grapevines exhibiting dieback symptoms were sampled for tissue isolation. Symptomatic wood fragments taken from the margin of dead and healthy tissue, and from internal necroses were washed under running tap water, surface-disinfected for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile distilled water. Small

pieces (4-5 mm) of tissue were taken from the margin between necrotic and apparently healthy tissue to be plated onto potato dextrose agar (PDA) (Acumedia, Lansing, USA) amended with 0.5 g l^{-1} streptomycin sulfate (PDAS). Plates were incubated at 25°C in the dark for 3 to 4 days. Fungal colonies emerging from plant tissue pieces that were morphologically similar to species of Botryosphaeriaceae (Sutton 1980; Phillips 2006) were transferred to PDA plates and incubated at 25 °C in the dark, with observation after 3, 7 and 15 days. To obtain single-spore isolates, pycnidia were induced on 2 % water agar (WA) with autoclaved pine needles as a substrate after 3-week incubation at 25 °C under a 12 h daily photoperiod with near-ultraviolet light (Slippers et al. 2004). A single conidium was cut from each isolate under a stereo microscope (Zeiss Stemi DV4; Carl Zeiss, Berlin, Germany) and placed in 250 µl of sterile water to produce a conidial suspension. A 20 µl aliquot was spread on PDAS and incubated at 25 °C in the dark for 24 h. A single-conidia isolate was recovered for an individual sample and transferred to a fresh PDA plate. One-hundred and twelve isolates were morphologically identified as *Lasiodiplodia* based on morphological characteristics typical of the genus, namely conidiomatal paraphyses, conidia that were initially hyaline and aseptate, but in time developed a single median septum, the wall became dark brown and melanin granules deposited longitudinally on the inner surface of the wall giving the conidia a striate appearance (Sutton 1980; Alves et al. 2008). Stock cultures were stored in PDA slants at 5 °C in the dark.

DNA isolation, PCR amplification and sequencing

Using a sterile 10 µl pipette tip, a small amount of aerial mycelium was scraped from the surface of a 7 day old culture on PDA at 25 °C and genomic DNA was extracted using the AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Scientific Inc., Union City, USA) following the manufacturer's instructions. A portion of the translation elongation factor

1α (EF1-α) gene was sequenced for all the *Lasiodiplodia* isolates collected from vineyards. The internal transcribed spacer (ITS) region of rDNA was sequenced to confirm the identity of 32 representative isolates within EF1-α identified species. The ITS region was amplified using the primers ITS1 and ITS4 (White et al. 1990) as described by Slippers et al (2004) and EF1-α gene was amplified using the primers EF1-688F and EF1-1251R (Alves et al. 2008) as described by Phillips et al. (2005). Each 50 µl polymerase chain reaction (PCR) mixture included 21 µl of PCR-grade water, 1 µl of DNA template, 1.5 µM of each primer, and 1 µl of PCR Master Mix (2X) (0.05 u μl^{-1} de *Taq* DNA polimerase, reaction buffer, 4 mM MgCl2, 0.4 mM of each dNTP; Thermo Scientific,Waltham, USA). PCR reactions were carried out in a thermal cycler (Biocycler MJ 96; Applied Biosystems, Foster City, USA). The PCR amplification products were separated by electrophoresis in 1.5 % agarose gels in 1.0× Tris-acetate acid EDTA (TAE) buffer and were photographed under UV light after staining with with ethidium bromide (0.5 µg ml^{-1}) for 1 min. The PCR amplification products were separated by electrophoresis in 1.5% agarose gels in 1.0× Tris-acetate acid EDTA (TAE) buffer and were photographed under UV light after staining with ethidium bromide (0.5 µg ml^{-1}) for 1 min. PCR products were purified using the AxyPrep™ PCR Cleanup Kit (Axygen) following the manufacturer's instructions. ITS and EF1-α regions were sequenced in both directions using an ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems) at the Sequencing Platform LABCEN/CCB in the Universidade Federal de Pernambuco (Recife, Brazil).

Phylogenetic analyses

Sequences were aligned with ClustalX v. 1.83 (Thompson et al. 1997), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight =

0.5, delay divergent sequences = 25 %). Alignments were checked and manual adjustments were made where necessary. Phylogenetic information contained in indels (gaps) was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young and Healy 2003). Sequences of *Lasiodiplodia* type strains obtained from GenBank were included in the analyses (Table 1). *Diplodia seriata* De Not. (CBS 112555) and *D. mutila* Fr. (CBS 112553) were used as outgroup.

Phylogenetic analyses were performed using PAUP v. 4.0b10 (Swofford 2003) for maximum-parsimony and MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003) for Bayesian analyses. Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1000 bootstrap replications (Hillis and Bull 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI). In the Bayesian analysis, the full data set were run twice for 10^6 generations. Trees were sampled every 1000th generation for a total of 10 000 trees. The first 1 000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 9 000 trees. This analysis was repeated four times starting from different random trees to ensure trees from the same tree space were sampled during each analysis

Phylogenetic trees were viewed with Treeview (Page 1996). Sequences generated in this study were deposited in GenBank (Table 1) and the alignment in TreeBase (S14682). Representative isolates of different *Lasiodiplodia* species obtained in this study were deposited in the Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Brazil).

Morphology and cultural characteristics

The 32 *Lasiodiplodia* isolates that were identified in the phylogenetic analysis using the combined data set were used to study colony morphology and conidial characteristics. The color and aerial hyphal growth from isolates were recorded during 15 days of growth on 2 % malt extract agar (MEA) (Acumedia) at 25 ºC in the dark. Colony colors were recorded as per Rayner (1970). Characteristics of conidial morphology were observed after placing cultures on 2 % WA containing autoclaved pine needles and incubation under near-ultraviolet light, as previously described. Conidia and other structures were mounted in 100 % lactic acid and digital images recorded with a Leica DFC320 camera on a Leica DMR HC microscope fitted with Nomarski differential interference contrast optics (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The length and width of 50 conidia per isolate were measured with the Leica IM500 measurement module. Mean and standard errors of the conidial measurements, including mean length to width ratio (L/W) of the conidial measurements were calculated. Conidial color, shape, and presence or absence of septa was also recorded.

Isolates were also used to determine the effect of temperature on colony growth of different species. A 3-mm-diameter mycelial plug from the growing margin of a 3-day-old colony was placed in the center of a 90-mm-diameter 2 % MEA plate, and four replicates of each isolate were incubated at temperatures ranging from 10 °C to 40 °C in 5 °C intervals in the dark. After a 2-days incubation period, the colony diameter (mm) was measured in two perpendicular directions. The experiment was done twice. Colony diameters were plotted against temperature and a curve was fitted by a cubic polynomial regression ($y=a+bx+cx^2+dx^3$). Optimal temperature was estimated from the regression equation and numeric summary with TableCurve™ 2D v. 5.01 (SYSTAT Software Inc., Chicago, USA).

Optimum temperature was defined as the temperature that produced the maximum mycelial growth. The colony diameter data at 30 °C were used to calculate the mycelial growth rate (mm/day). One-way analyses of variance (ANOVA) were conducted with data obtained from optimum temperature and mycelial growth rate experiments, and means were compared by Fisher's least significant difference (LSD) test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

Distribution and diversity of *Lasiodiplodia* species

Based on the number of isolates of each *Lasiodiplodia* species recorded, it was calculated the relative frequency of each species in relation to overall number of isolates and to the total number of isolates within each table grape population (Zak and Willig 2004). The diversity of *Lasiodiplodia* species was estimated in terms of species richness (number of species in the sample) and evenness (dominance of species in the sample) by the Shannon-Wiener's index $H' = \Sigma_j (p_j \ln p_j)$, j = 1…… N_p, where N_p is the number of species identified among these isolates, and p_j is the proportion of individuals in the j^{th} species. The H' values increases with the number of species in a sample or reduces as one or a few species domain in the sample (Shannon and Weaver 1949). To quantify the degree of overlap between the *Lasiodiplodia* species in the table grape populations, a measure of the similarity between pairs of samples was calculated by the Jaccard's index $JI = a/(a+b+c)$, where *a* represents the number of species occurring in both samples, *b* represents the number of species restricted to sample 1, and *c* represents the number of species restricted to sample 2. The *JI* values ranges from 0 (no species shared) 1 (all species shared) (Kumar and Hyde 2004).

Pathogenicity and virulence on detached green shoots

Detached green shoots of cultivar Isabel were used to investigate the pathogenicity and virulence of the eight *Lasiodiplodia* species found on grapevines in San Francisco Valley. The isolates were the same used in the morphological characterization. The soft green shoots from unsprayed plants were cut in a commercial vineyard in São Vicente Férrer (Pernambuco), from which Botryosphaeriaceae species where not detected following repeated sampling and isolation. The shoots were immediately placed into large plastic containers filled with sterile water, with the shoots placed over a plastic grid. The plastic containers were partially sealed with plastic bags and transported to Universidade Federal Rural de Pernambuco. The cut ends were dipped in wax and in the centre of each shoot (30 cm long) a superficial wound (~4-mm length, 2-mm deep) was made using a sterilized scalpel. It was inoculated with a mycelial plug (4 mm in diameter) removed from the margin of a 5-day-old PDA culture of each isolate. Non-colonized PDA agar plugs were used as negative controls. The inoculated area was wrapped with Parafilm (Pechiney Co., Chicago, USA) to prevent rapid dehydration. Inoculated shoots were placed in large plastic containers, as described above, and incubated at 25 °C and 12-h photoperiod in a growth chamber. After 10 days, the Parafilm was removed, the shoots were sliced through lengthwise and the internal lesions visually observed. The isolates were considered pathogenic when the lesioned area advanced beyond the 4-mm diameter inoculated area. The virulence of the isolates was evaluated by measurement of the lesion lengths with a digital calliper (Mitutoyo Co., Kanagawa, Japan). The experiment was arranged in a completely randomized design with ten replicates per treatment (isolate) and one shoot per replicate. The experiment was conducted twice. Differences in virulence caused by *Lasiodiplodia* species were determined by one-way ANOVA and means were compared by LSD test at the 5 % significance level using STATISTIX.

Results

DNA sequencing and phylogenetic analyses

A total of 112 isolates of *Lasiodiplodia* spp. were obtained from table grape plants. All the isolates were identified based on phylogenetic analysis of the partial translation elongation factor 1α (EF1-α) gene. To confirm the identity of the isolates, the internal transcribed spacer (ITS) sequence was obtained for 32 isolates representing each putative species. The combined ITS and EF1-α data set consists of 83 taxa, including two outgroup. The alignment contained 746 characters, of which 579 were constant while 33 were variable and parsimony uninformative. A heuristic search of the remaining 134 parsimony-informative characters generated 8 equally parsimonious trees with (Tl=284; CI=0.739; RI=0.900; HI=0.261). Maximum-parsimony and Bayesian inference produced nearly identical topologies (Bayesian tree not shown). Sequences of ex-type isolates of *Lasiodiplodia* species from GenBank were included in the analysis together with isolates obtained in this study (Table 1). The combined dataset resulted in 25 well supported clades of which 23 clades corresponded to previously described *Lasiodiplodia* species. Six isolates clustered with clade containing *L. jatrophicola* (CMM 3610). The second group with five isolates clustered with L. *theobromae*. Four isolates clustered with *L. euphorbicola* (CMM 3651, CMM 3609 and CMM 3652) and *L. marypalme* (CMM 2173, CMM 2275 and CMM 2272). In clades *L. crassispora* and *L. brasiliense* (CMM 4011, CMM 4015) four isolates were clustered in each. Three isolates clustered with L. *egyptiacea* and other three isolates clustered with L. *pseudotheobromae*. In clade *L. hormozganensis* three isolates clustered (Fig. 2).

Morphology and cultural characteristics

The 32 *Lasiodiplodia* isolates [*L. brasiliense* (4), *L. crassispora* (4), *L. egyptiacae* (3), *L. euphorbicola* (4), *L. hormozganensis* (3), *L. jatrophicola* (6), *L. pseudotheobromae* (3) and *L.*

theobromae (5)] that were identified based in the phylogenetic analysis using the combined data were further characterized by colony morphology and conidial characteristics. All isolates produced anamorph structures on the pine needles on WA within 2–4 wk. No teleomorph structures were observed during this study. All species showed morphological features typical of the genus, namely slowly maturing conidia with thick walls and longitudinal striations (Punithalingam 1976, 1980). All isolates grew rapidly on PDA, covering the entire surface of the Petri dishes within 3 days. The aerial mycelium was initially white, turning dark greenish-grey or greyish after 4–5 days at 25 °C in the dark. The species of *Lasiodiplodia* found in this study show differences in conidial size. The conidial dimensions found in *L. euphorbicola* and *L. jatrophicola* are outside of the range previously described for these species in the literature (Table 2). All species of *Lasiodiplodia* used in this study grew at 10 °C. There were significant differences ($P \leq 0.05$) in growth rate among the *Lasiodiplodia* species and differences in the optimum temperature for mycelial growth. The optimum temperature for growth of *L. egyptiacae* (29.9 °C) was significantly lower than that of *L. euphorbicola* (32.6 °C). The other species (*L. brasiliense*, *L. crassispora*, *L. hormozganensis*, *L. jatrophicola, L. pseudotheobromae* and *L. theobromae*) presented intermediate values of optimum temperature for growth, without differing of observed extremes. The mycelial growth rate of *L. jatrophicola* (43.5 mm/day) was significantly higher than *L. brasiliense, L. euphorbicola* and *L. hormozganensis*, which varied from 36.9 to 39.8 mm/day. The other species (*L. crassispora*, *L. egyptiacae*, *L. pseudotheobromae* and *L. theobromae*) presented intermediate values of mycelial growth rate, without differing of *L. jatrophicola, L. euphorbicola* and *L. hormozganensis* (Table 3).

Taxonomy

Lasiodiplodia euphorbicola A.R. Machado & O.L. Pereira. Fungal Divers (DOI 10.1007/s13225-013-0274-1)

= *Lasiodiplodia marypalme* M.S.B. Netto, M.W. Marques, A.J.L. Phillips & M.P.S. Câmara. Fungal Divers (DOI 10.1007/s13225-014-0279-4)

Placed as synonymy with *L. euphorbicola*, the species *L. marypalme* was described by Netto et al. (2014) causing stem-end rot of papaya in Northeastern Brazil. The authors described this species by multi-locus phylogeny using the EF1-α and ITS genes. Machado et al. (2014) early described the species *L. euphorbicola* using sequence data of EF1-α, βT and ITS genes. Since the molecular data of *L. euphorbicola* were not available in public database, Netto et al. (2014) described *L. marypalme* as a new species. We sequenced the EF1-α and ITS genes for some isolates first described as *L. marypalme* (including the ex-type) and included them in our analysis. Morphological characteristics are similar for both species, differing only in the size of conidia (Table 1). The phylogenetic tree generated showed that this isolate nested with the ex-type of *L. euphorbicola* in a well supported clade.

Distribution and diversity of *Lasiodiplodia* species

Lasiodiplodia theobromae was the predominant species isolated from table grape plants (46.4 %) followed by *L. brasiliense* (15.2 %), *L. jatrophicola* (11.6 %), *L. hormozganensis* (7.1 %), *L. crassispora* (7.1 %), *L. egyptiacae* (5.4 %), *L. euphorbicola* and *L. pseudotheobromae* (3.6 %). The distribution of *Lasiodiplodia* species differed between the three table grape populations of São Francisco Valley. Only in the population of Casa Nova all *Lasiodiplodia* species were found. In Petrolina population the only species that was not present was *L. crassispora*. *Lasiodiplodia jatrophicola*, *L. hormozganensis* and *L. theobromae* were found in all populations. *Lasiodiplodia theobromae* was the predominant species in all populations.

Lasiodiplodia brasiliense, L. egyptiacae, *L. euphorbicola* and *L. pseudotheobromae* were found in populations of Casa Nova and Petrolina. *Lasiodiplodia crassispora* was found only in population of Casa Nova, where it was the second most prevalent species (Fig. 1).

A comparison of the Shannon-Wiener's diversity index (*H´*) showed that populations of Casa Nova and Petrolina are similar in diversity of *Lasiodiplodia* species causing dieback in table grapes (*H´* = 1.55 and *H´* = 1.56, respectively), while the population of Juazeiro had the lowest diversity (*H´* = 0.90) (Fig. 1).

The comparison between the *Lasiodiplodia* species recovered from different table grape populations was computed using a Jaccard's index for possible pairs of populations. The highest overlap (*JI* = 0.89) was observed for the *Lasiodiplodia* species from populations of Casa Nova and Petrolina, followed by populations of Juazeiro and Petrolina (*JI* = 0.43). The lowest value of similarity (*JI* = 0.38) was observed between populations of Casa Nova and Juazeiro.

Pathogenicity and virulence in detached green shoots

All isolates of *Lasiodiplodia* were pathogenic to detached green shoots of table grape, resulting in visible lesions 10 days after inoculation. The symptoms observed both on the surface and internally were dark brown necrotic lesions which extended upward and downward from the point of inoculation. There were significant differences ($P \leq 0.05$) in internal lesion lengths produced by the different *Lasiodiplodia* species. The longest lesions were produced by *L. brasiliense* (253.0 mm), wherein was most virulent, followed by *L. theobromae* (197.6 mm). The small lesions were produced by *L. egyptiacae* and *L. euphorbicola* (<20 mm), than did not differ significantly from each other and were considered the less virulent species. The others species *(L. crassispora*, *L. hormozganensis*, *L.*

jatrophicola and *L. pseudotheobromae*) presented intermediate virulence, with lesions varying from 109.3 mm to 148.1 mm (Fig. 3).

Discussion

This study represents the first survey of species of *Lasiodiplodia* associated with table grape dieback in the main Brazilian exporting region with an extensive collection of isolates, and integrating morphology, pathology and molecular data. Eight species of *Lasiodiplodi*a were identified as causing table grape dieback: *L. brasiliense, L. crassispora, L. egyptiacae, L. euphorbicola* (*L. marypalme*), *L. hormozganensis, L. jatrophicola, L. pseudotheobromae*, and *L. theobromae*. Except for *L. crassispora, L. pseudotheobromae*, and *L. theobromae*, all the other species are reported for the first time on grapevine worldwide.

In this work, *L. theobromae* was the most frequently isolated species associated with table grape dieback (46.4 %), and also the most widespread species in vineyards of São Francisco Valley, Northeastern Brazil. Similar results were obtained when the frequency of *Lasiodiplodia* species associated with dieback and stem-end rot of mango (Marques et al. 2013) and stem-end rot of papaya (Netto et al. 2014) was investigated in the semi-arid region of Northeastern Brazil. This species is considered a pantropical pathogen occurring in a wide range of hosts (Punithalingam 1980; Burgess et al. 2006; Farr and Rossman 2014). In Brazil, *L. theobromae, L. crassispora, L. parva* and *L. pseudotheobromae* had been reported in grapevine (Gava et al. 2010; Correia et al. 2013). In this work, five more species were found in this host.

Worldwide, several species have been described in the *L. theobromae* complex, mostly due to the increase in the application of DNA sequence data, but also because of the increased sampling of relatively unexplored areas, including Venezuela (Burgess et al. 2006), Australia (Pavlic et al. 2008), Iran (Abdollahzadeh et al. 2010), Egypt (Ismail et al. 2012), Brazil

(Marques et al. 2013; Machado et al. 2014; Netto et al. 2014), Oman and United Arab Emirates (Al-Sadi et al. 2013).

Lasiodiplodia hormozganensis was described in Iran associated with mango and *Olea* sp. (Abdollahzadeh et al. 2010), in Australia associated with *Adansonia digitata* L. (Sakalidis et al. 2011), in Brazil associated with mango (Marques et al. 2013) and papaya (Netto et al. 2014) and in Oman associated with *Citrus*, date palm (*Phoenix dactylifera* L.) and mango (Al-Sadi et al. 2013). This work represents the first report of this species causing table grape dieback worldwide. In this study, *L. hormozganensis* presented intermediate virulence in detached green shoots of table grape. This result differs from that found by Sakalidis et al. (2011), Marques et al. (2013) and Netto et al. (2014) where *L. hormozganensis* isolates produced the largest lesions in mango branches, mango fruits and papaya fruits, respectively.

Another species associated with table grape dieback in Brazil was *L. pseudotheobromae*. This species was described from *Acacia*, *Citrus*, *Coffea*, *Gmelina* and *Rosa* species, and differs from *L. theobromae* in its bigger conidia that are more ellipsoid and do not taper as strongly towards the base (Alves et al. 2008). Worldwide, *L. pseudotheobromae* has been reported on numerous hosts (Alves et al. 2008, Phillips et al. 2008, Begoude et al. 2010, Perez et al. 2010, Wright and Harmon 2010**,** Zhao et al. 2010, Abdollahzadeh et al., 2010, Sakalidis et al. 2011, Ismail et al. 2012), but in Brazil it has been reported only on grapevine (Correia et al. 2013), mango (Marques et al. 2013), physic nut (Machado et al. 2014) and papaya (Netto et al. 2014). This shows an increase in the spread of the fungus, suggesting that *L. pseudotheobromae*, like *L. theobromae,* has a worldwide distribution and a wide host range. Regarding the pathogenicity, *L. pseudotheobromae* was the most virulent species in mango fruits in Australia (Sakalidis et al. 2011), in mango seedlings in Egypt (Ismail et al. 2012) and in young trees of *Terminalia catappa* L. in Cameroon (Begoude et al. 2011). However, in the present work, *L. pseudotheobromae* had relatively low level of virulence in detached green shoots of table grape, similar to that

observed when this species was inoculated in mango fruits (Marques et al. 2013) and papaya fruits (Netto et al. 2014) in Brazil. The divergent results indicate that there is a great variability in virulence within this species and that the Brazilian isolates might represent a population with low virulence.

Lasiodiplodia crassispora was described in Australia causing canker in *Santalum album* L. (Burgess et al. 2006). In terms of morphology, *L. crassispora* resembles *L. pseudotheobromae* and the only feature that distinguishes the two species is that in *L. crassispora* the pseudoparaphyses are mostly septate, while in *L. pseudotheobromae* they are mostly aseptate (Phillips et al. 2013). In 2010 it was described in *Eucalyptus urophylla* L. (Perez et al. 2010) and grapevine (Úrbez-Torres et al 2010; van Niekerk et al. 2010). In 2013, *L. crassispora* was described in Brazil associated with trunk disease in grapevine (Correia et al. 2013), and dieback and stem-end rot of mango (Marques et al. 2013). In this study, *L. crassispora* showed low prevalence and low virulence.

Other species found in this work associated with table grape dieback was *L. egyptiacae*. This species was described first on mango in Egypt (Ismail et al. 2012), and recently was reported in Brazil in the same host (Marques et al, 2013) and physic nut (Machado et al, 2014). This species is morphologically and phylogenetically closely related to other previously reported species previously associated with table grape dieback in Brazil, *L. pseudotheobromae* and *L. parva* (Correia et al. 2013), but it can be distinguished based on the dimensions of conidia and paraphyses. In this study, *L. egyptiacae* showed low levels of virulence in detached green shoots of table grape compared to other *Lasiodiplodia* species. Similar results were obtained in mango fruits (Ismail et al. 2012; Marques et al. 2013) and in physic nut plants (Machado et al., 2014). More sampling is necessary to understand the host range, distribution and variability of this species. This work represents the first report of this species causing dieback in table grape worldwide.

Lasiodiplodia jatrophicola and *L. euphorbicola* were recently described in Brasil associated with physic nut. *L. jatrophicola* is phylogenetically close but clearly separate from *L. iraniensis* and the new species has larger conidia and smaller paraphyses. *L. euphorbicola* is phylogenetically close but clearly separate from *L. parva*. The two taxa have several similar morphological characteristics, but the proposed new species has smaller paraphyses (Machado et al. 2014). In this work, *L. jatrophicola* was the third most prevalent species with moderate virulence, whereas *L. euphorbicola* showed low levels of virulence and low prevalence. Machado et al. (2014) found only one isolate of *L. jatrophicola* and three of *L. euphorbicola* associated with physic nut. This work is the first report of these species causing table grape dieback worldwide, identifying one second host of these species, which may indicate that these species are distributed in the country. The species were identified in regions distant from one another and under different climatic conditions.

Other species described in Brazil in 2014, but associated with stem-end rot of papaya, were *L. brasiliense* and *L. marypalme*. Phylogenetically *L. brasiliense* is closely related to *L. viticola*, but conidia of *L. brasiliense*, 22.7–29.2 × 11.7–17.0 µm, are longer and wider than those of *L. viticola* 18.2–20.5 × 8.8–10.1µm. *L. brasiliense* differs from its closest phylogenetic neighbor, *L. viticola*, by unique fixed alleles in one loci. In this work *L. brasiliense* was the most virulent species in detached green shoots from table grape and second species most prevalent in São Francisco Valley. Netto et al. (2014) it also showed that *L. brasiliense* was the second prevalent species associated with stem-end rot of papaya in São Francisco Valley, but the virulence of this species did not differ significantly from *L. marypalme, L. pseudotheobromae* and *L. theobromae*. This is the first world record of *L. brasiliense* causing dieback in grapevine.

Phylogenetically, *L. marypalme* is closely related to *L. pseudotheobromae* and *L. citricola*, but conidia of *L. citricola*, 22.5–26.6 × 13.6–17.2 µm, and *L. pseudotheobromae*, 23.5–32 × 14–18, are longer and wider than those of *L. marypalme*, 18.0–24.4 × 9.8–15.3 µm.

L. marypalme differs from its closest phylogenetic neighbor, *L. pseudotheobromae* and *L. citricola*, by unique fixed alleles in two loci (Netto et al. 2014). In this study, we observed that *L. marypalme* is synonymy of *L. euphorbicola*. The phylogenetic tree generated showed that this isolate nested within the former type of *L. euphorbicola* in a well-supported clade. Therefore, the 24 species reported *Lasiodiplodia* (Phillips et al.2013; Machado et al. 2014; Netto et al. 2014), 23 may be considered valid species.

Regarding cultural characteristics, the optimum temperature for mycelial growth for *Lasiodiplodia* species from table grape varied between 29.9 °C and 31.2 °C. In addition, all the species in this study grew at 10 °C. This growth at low temperature corroborates the work of Abdollahzadeh et al. (2010), Marques et al. (2013) and Netto et al (2014) and is in contrast to other studies that show only *L. pseudotheobromae* as capable of growing at this temperature (Alves et al. 2008; Ismail et al. 2012). As can be observed, cultural characteristics may vary widely among isolates of the same species and therefore are of limited value in the determination of species.

Regarding the distribution of species in the sampled populations, a greater diversity of *Lasiodiplodia* species was observed in populations of Petrolina and Casa Nova, while population of Juazeiro presented lower species diversity. *Lasiodiplodia theobrome* was the predominant species in all populations and it was the species that had the largest number of isolates (52). *Lasiodiplodia crassispora* was found only in population of Casa Nova, where was the second most prevalent species.

This paper reports eight species of the genus *Lasiodiplodia* associated with table grape dieback in São Francisco Valley, the main Brazilian exporting region. *L. theobromae*, despite being the most frequent species, is not the only etiologic agent and neither the most virulent. All the species found in in São Francisco Valley have potential to cause table grape dieback, but *L. brasiliense* was the most virulent species. Information about this species is scarce due to its recent description (Netto et al. 2014). Studies are needed on the epidemiology and

impact on table grape production together with information referring to ecology, distribution, host range and fungicide sensitivity of all species of *Lasiodiplodia* found in this study. The results of this study will certainly be crucial to a better formulation of dieback control strategies and genetic improvement programs for the table grape.

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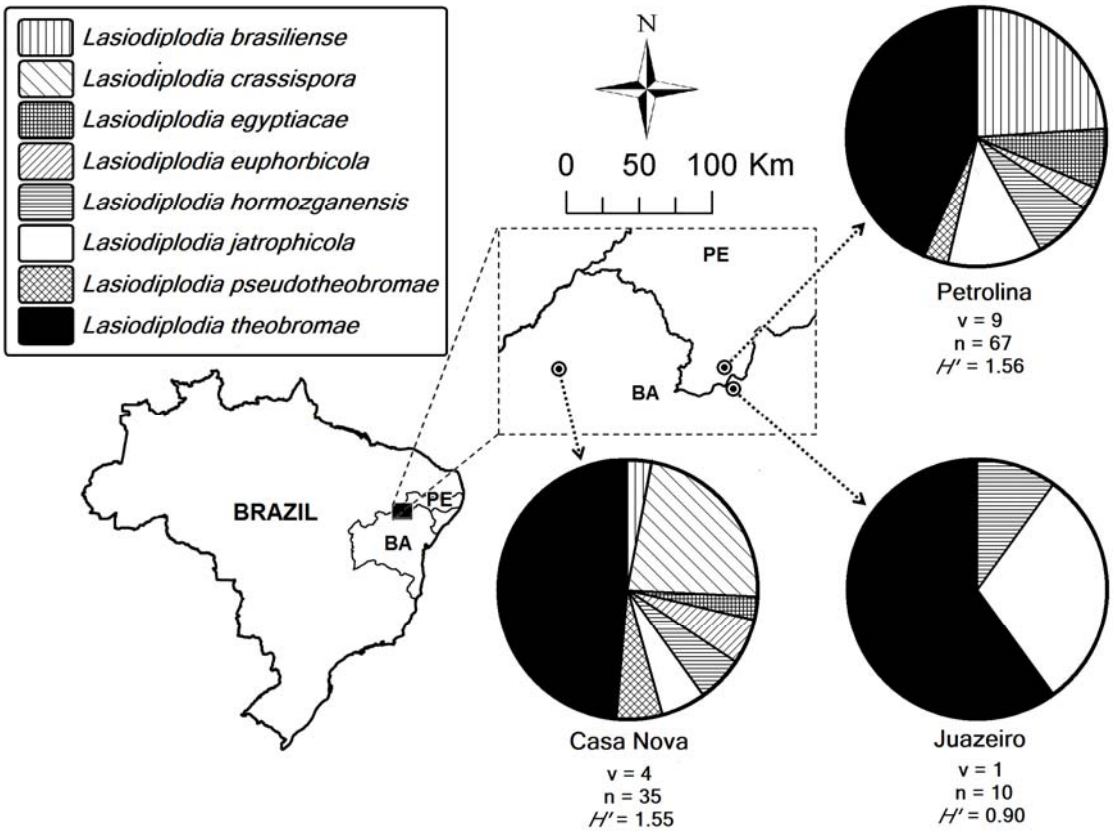

Fig. 1 Collection sites of *Lasiodiplodia* isolates associated with grapevine dieback in three different populations located in São Francisco Valley (Casa Nova, Juazeiro and Petrolina), Northeastern Brazil. Circles represent association frequency of each species with plants exhibiting symptoms of dieback in each population sampled, *v* is the number of vineyards sampled in each population, *n* is the number of isolates analyzed in each population, and *H'* is the Shannon-Wiener's diversity index

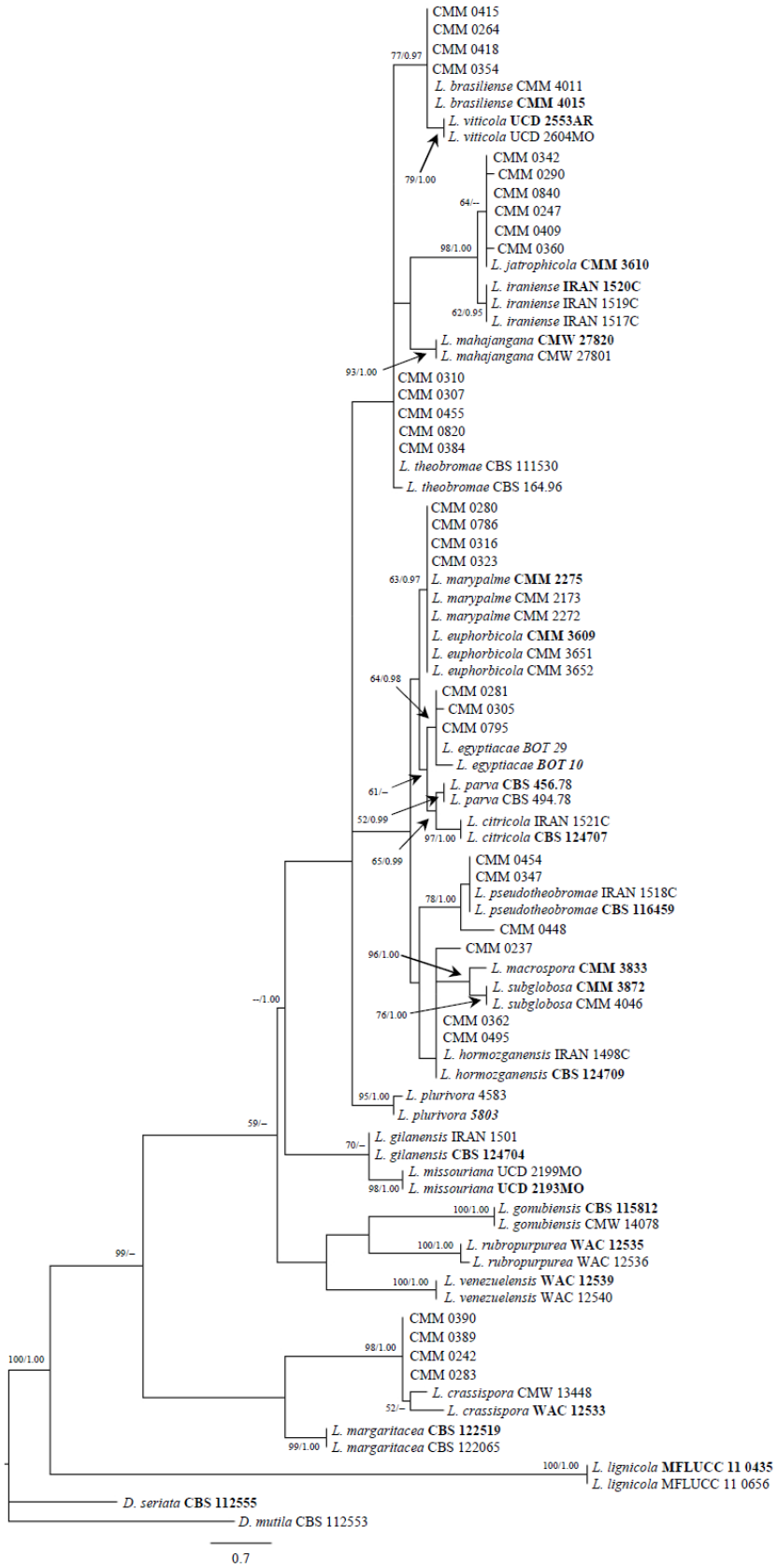

Fig. 2 One of 8 most parsimonious trees (Tl=284; CI=0.739; RI=0.900; HI=0.261) obtained from combined ITS and EF1-α sequence data. Maximum parsimony bootstrap support values from 1000 replications and Bayesian posterior probability scores are shown at the nodes. Ex-type isolates are in **bold**

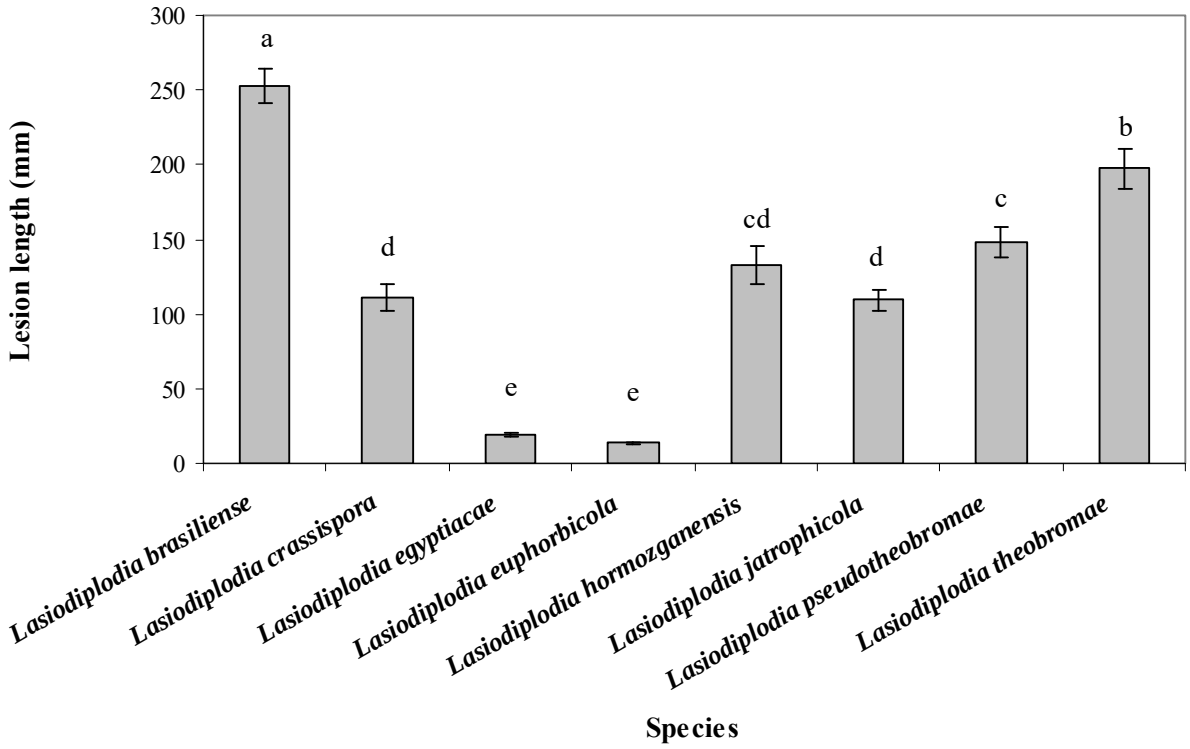

Fig. 3 Mean internal lesion lengths (mm) caused by eight *Lasiodiplodia* species associated with grapevine dieback in São Francisco Valley (Northeastern Brazil), 10 days after inoculation with mycelium colonized agar plugs onto wounded detached green shoots of Isabel cultivar. Bars above columns are the standard error of the mean. Columns with same letter do not differ significantly according to Fisher's LSD test ($P \leq 0.05$)

Table 1 Isolates of *Lasiodiplodia* species used in this study

Taxon	Culture Accession No.[a]	Host	Location	Collector	GenBank Accession No.[b]	
					ITS	EF1-α
Diplodia mutila	CBS 112553	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259093	AY573219
D. seriata	CBS 112555	*V. vinifera*	Portugal	A.J.L. Phillips	AY259093	AY573220
Lasiodiplodia brasiliense	CMM 4011	*Mangifera indica*	Brazil	M.W. Marques	JX464074	JX464037
L. brasiliense	CMM 0418	*V. vinifera*	Brazil	M.A. Silva	**KJ417850**	**KJ417846**
L. brasiliense	CMM 0354	*V. vinifera*	Brazil	M.A. Silva	**KJ417849**	**KJ417845**
L. brasiliense	CMM 0415	*V. vinifera*	Brazil	M.A. Silva	**KJ417852**	**KJ417848**
L. brasiliense	CMM 0264	*V. vinifera*	Brazil	M.A. Silva	**KJ417851**	**KJ417847**
L. brasiliense	CMM 4015	*M. indica*	Brazil	M.W. Marques	JX464063	JX464049
L. citricola	CBS 124707	*Citrus sp.*	Iran	J. Abdollahzadeh & A. Javadi	GU945354	GU945340
L. citricola	IRAN 1521C	*Citrus sp.*	Iran	A. Shekari	GU945353	GU945339
L. crassispora	CMW 13448	*Eucalyptus urophylla*	Venezuela	S. Mohali	DQ103552	DQ103559
L. crassispora	CMM 0390	*V. vinifera*	Brazil	M.A. Silva	**KJ417881**	**KJ417856**
L. crassispora	CMM 0389	*V. vinifera*	Brazil	M.A. Silva	**KJ417882**	**KJ417855**
L. crassispora	CMM 0242	*V. vinifera*	Brazil	M.A. Silva	**KJ417883**	**KJ417854**
L. crassispora	CMM 0283	*V. vinifera*	Brazil	M.A. Silva	**KJ417884**	**KJ417853**
L. crassispora	WAC 12533	*Santalum album*	Australia	T.I. Burgess & G. Pegg	DQ103550	DQ103557
L. egyptiacae	BOT 29	*M. indica*	Egypt	A.M. Ismail	JN814401	JN814428
L. egyptiacae	CMM 0305	*V. vinifera*	Brazil	M.A. Silva	**KJ417885**	**KJ417858**
L. egyptiacae	CMM 0281	*V. vinifera*	Brazil	M.A. Silva	**KJ417886**	**KJ417859**
L. egyptiacae	CMM 0795	*V. vinifera*	Brazil	M.A. Silva	**KJ417887**	**KJ417857**

L. egyptiacae	BOT 10	*M. indica*	Egypt	A.M. Ismail	JN814397	JN814424
L. euphorbicola	CMM 3651	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234553	KF226711
L. euphorbicola	CMM 3609	*J. curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234543	KF226689
L. euphorbicola	CMM 0323	*V. vinifera*	Brazil	M.A. Silva	**KJ417888**	**KJ417860**
L. euphorbicola	CMM 0280	*V. vinifera*	Brazil	M.A. Silva	**KJ417889**	**KJ417863**
L. euphorbicola	CMM 0316	*V. vinifera*	Brazil	M.A. Silva	**KJ417890**	**KJ417861**
L. euphorbicola	CMM 0786	*V. vinifera*	Brazil	M.A. Silva	**KJ417891**	**KJ417862**
L. euphorbicola	CMM 3652	*J. curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234554	KF226715
L. gilaniensis	CBS 124704	*Unknown*	Iran	J. Abdollahzadeh & A. Javadi	GU945351	GU945342
L. gilaniensis	IRAN 1501C	*Unknown*	Iran	J. Abdollahzadeh & A. Javadi	GU945352	GU945341
L. gonubiensis	CBS 115812	*Syzigium cordatum*	South Africa	D. Pavlic	DQ458892	DQ458860
L. gonubiensis	CMW 14078	*S. cordatum*	South Africa	D. Pavlic	AY639594	DQ103567
L. hormozganensis	CBS 124709	*Olea sp.*	Iran	J. Abdollahzadeh & A. Javadi	GU945355	GU945340
L. hormozganensis	CMM 0495	*V. vinifera*	Brazil	M.A. Silva	**KJ417892**	**KJ417865**
L. hormozganensis	CMM 0362	*V. vinifera*	Brazil	M.A. Silva	**KJ417893**	**KJ417864**
L. hormozganensis	CMM 0237	*V. vinifera*	Brazil	M.A. Silva	**KJ417894**	**KJ417866**
L. hormozganensis	IRAN 1498C	*M. indica*	Iran	J. Abdollahzadeh & A. Javadi	GU945356	GU945344
L. iraniensis	IRAN 1520C		Iran	A. Javadi	GU945346	GU945334
L. iraniensis	IRAN 1519C	*M. indica*	Iran	A. Javadi	GU945350	GU945338
L. iraniensis	IRAN 1517C	*Citrus* sp.	Iran	A. Javadi	GU945349	GU945337
L. jatrophicola	CMM 3610	*J. curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234544	KF226690
L. jatrophicola	CMM 0247	*V. vinifera*	Brazil	M.A. Silva	**KJ417895**	**KJ417870**
L. jatrophicola	CMM 0360	*V. vinifera*	Brazil	M.A. Silva	**KJ417896**	**KJ417867**
L. jatrophicola	CMM 0409	*V. vinifera*	Brazil	M.A. Silva	**KJ417897**	**KJ417868**
L. jatrophicola	CMM 0342	*V. vinifera*	Brazil	M.A. Silva	**KJ417898**	**KJ417872**

L. jatrophicola	CMM 0290	*V. vinifera*	Brazil	M.A. Silva	**KJ417899**	**KJ417871**
L. jatrophicola	CMM 0840	*V. vinifera*	Brazil	M.A. Silva	**KJ417900**	**KJ417869**
L. lignicola	MFLUCC 11 0435	Dead wood of unknown host	Thailand	A.D. Ariyawansa	GU945346	GU945334
L. lignicola	MFLUCC 11 0656	Dead wood of unknown host	Thailand	A.D. Ariyawansa	JX646798	JX646863
L. macrospora	CMM 3833	*J. curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234557	KF226718
L. mahajangana	CMW 27820	*Terminalia catappa*	Madagascar	J. Roux	FJ900597	FJ900643
L. mahajangana	CMW 27801	*T. catappa*	Madagascar	J. Roux	FJ900595	FJ900641
L. margaritaceae	CBS 122065	*Adansonia gibbosa*	Western Australia	T.I. Burgess	EU144051	EU144066
L. margaritaceae	CBS 122519	*A. gibbosa*	Western Australia	T.I. Burgess	EU144050	EU144065
L. marypalme	CMM 2173	*C. papaya*	Brazil	J.H.A. Monteiro	KC484839	KC481563
L. marypalme	CMM 2272	*C. papaya*	Brazil	J.H.A. Monteiro	KC484842	KC481566
L. marypalme	CMM 2275	*C. papaya*	Brazil	J.H.A. Monteiro	KC484843	KC481567
L. missouriana	UCD 2193MO	*V. vinifera*	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288225	HQ288267
L. missouriana	UCD 2199MO	*V. vinifera*	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288226	HQ288268
L. parva	CBS 456.78	*Cassava-field soil*	Colombia	O. Rangel	EF622083	EF622063
L. parva	CBS 494.78	*Cassava-field soil*	Colombia	O. Rangel	EF622084	EF622064
L. plurivora	STE-U 5803	*Prunus salicina*	South Africa	U. Damm	EF445362	EF445395
L. plurivora	STE-U 4583	*V. vinifera*	South Africa	F. Halleen	AY343482	EF445396
L. pseudotheobromae	CBS 116459	*Gmelina arborea*	Costa Rica	J. Carranza-Velásquez	EF622077	EF622057
L. pseudotheobromae	CMM 0454	*V. vinifera*	Brazil	M.A. Silva	**KJ417901**	**KJ417875**
L. pseudotheobromae	CMM 0448	*V. vinifera*	Brazil	M.A. Silva	**KJ417902**	**KJ417873**
L. pseudotheobromae	CMM 0347	*V. vinifera*	Brazil	M.A. Silva	**KJ417903**	**KJ417874**
L. pseudotheobromae	IRAN 1518C	*Citrus sp.*	Iran	J. Abdollahzadeh/A. Javadi	GU973874	GU973866
L. rubropurpurea	WAC 12535	*E. grandis*	Queensland	T.I. Burgess & G. Pegg	DQ103553	DQ103571

L. rubropurpurea	WAC 12536	*E. grandis*	Queensland	T.I. Burgess & G. Pegg	DQ103554	DQ103572
L. subglobosa	CMM3872	*J. curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234558	KF226721
L. subglobosa	CMM 4046	*J. curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234560	KF226723
L. theobromae	CBS 111530	*Unknown*	Unknown	Unknown	AY622074	AY622054
L. theobromae	CMM 0384	*V. vinifera*	Brazil	M.A. Silva	**KJ417904**	**KJ417876**
L. theobromae	CMM 0820	*V. vinifera*	Brazil	M.A. Silva	**KJ417905**	**KJ417877**
L. theobromae	CMM 0307	*V. vinifera*	Brazil	M.A. Silva	**KJ417906**	**KJ417879**
L. theobromae	CMM 0310	*V. vinifera*	Brazil	M.A. Silva	**KJ417907**	**KJ417880**
L. theobromae	CMM 0455	*V. vinifera*	Brazil	M.A. Silva	**KJ417908**	**KJ4177878**
L. theobromae	CBS 164.96	*Fruit on coral reef coast*	New Guinea	A. Aptroot	AY640255	AY640258
L. venezuelensis	WAC 12539	*Acacia mangium*	Venezuela	S. Mohali	DQ103547	DQ103568
L. venezuelensis	WAC 12540	*A. mangium*	Venezuela	S. Mohali	DQ103548	DQ103569
L. vitícola	UCD 2553AR	*V. vinifera*	USA	K. Striegler & G.M. Leavitt	HQ288227	HQ288269
L. vitícola	UCD 2604MO	*V. vinifera*	USA	K. Striegler & G.M. Leavitt	HQ288228	HQ288270

[a] *CBS* Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; *CMW* Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; *WAC* Department of Agriculture Western Australia Plant Pathogen Collection, University of Western Australia, Perth, Australia; *CMM* Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes", Universidade Federal Rural de Pernambuco, Recife, Brazil; *STE-U* Culture Collection of the Department of Plant Pathology, University of Stellenbosch, Stellenbosch, South Africa; *UCD* Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California, Davis, USA; *BOT* A. M. Ismail, Plant Pathology Research Institute, Giza, Egypt; *IRAN* Culture Collection of the Iranian Research Institute of Plant Protection, Tehran, Iran

[b] Sequence numbers in *bold* were obtained in the present study

Table 2 Comparison of conidial size of *Lasiodiplodia* species examined in this study and previous studies

Species	Conidial size (μm)	L/W ratio	References
Lasiodiplodia brasiliense	22.3–28.7 × 11.9–16.7	1.8	This study
	22.7–29.2 × 11.7–17.0	1.8	Netto et al. 2014
L. crassispora	27.2–29.6 × 15.3–16.9	1.8	This study
	27–30 × 14–17	1.8	Burgess et al. 2006
L. egyptiacae	20.4–23.1 × 11.2–13.1	1.8	Present study
	20–24 × 11–13	1.8	Ismail et al. 2012
L. euphorbicola	18.0–24.4 × 9.8–15.3	1.8	This study
	15−23 × 9−12	1.7	Machado et al. 2014
L. hormozganensis	19.8–22.7 × 11.8–13.2	1.8	This study
	19.6–23.4 × 11.7–13.3	1.7	Abdollahzadeh et al. 2010
L. jatrophicola	23.6–28.5 × 11.0–14.8	1.9	This study
	22−26 × 14−17	1.6	Machado et al. 2014
L. pseudotheobromae	25.3–29.6 × 14.7–16.8	1.8	This study
	25.5–30.5 × 14.8–17.2	1.7	Alves et al. 2008
L. theobromae	24.5–28.2 × 13.3–15.1	1.8	This study
	23.6–28.8 × 13–15.4	1.9	Alves et al. 2008

Table 3 Optimum temperature for mycelial growth and mycelial growth rate at 30 °C of *Lasiodiplodia* species associated with dieback of table grape in São Francisco Valley, Northeastern Brazil

Species	n	Optimum temperature (ºC) ± SE	Mycelial growth rate (mm/day) ± SE
Lasiodiplodia brasiliense	4	31.2 ± 0.43 ab	36.9 ± 2.11 c
L. crassispora	4	30.3 ± 0.40 b	40.6 ± 1.10 ab
L. egyptiacae	3	29.9 ± 0.86 b	41.5 ± 3.18 ab
L. euphorbicola	4	32.6 ± 0.71 a	39.8 ± 1.93 bc
L. hormozganensis	3	30.9 ± 0.59 ab	39.9 ± 1.57 bc
L. jatrophicola	6	30.8 ± 0.60 ab	43.5 ± 2.16 a
L. pseudotheobromae	3	30.1 ± 0.55 b	40.3 ± 1.86 ab
L. theobromae	5	30.0 ± 0.33 b	41.1 ± 0.74 ab

Mean ± standard error. Values within columns followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leq 0.05$)