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TAXONOMIA E EPIDEMIOLOGIA COMPARATIVA DE BOTRYOSPHAERIACEAE ASSOCIADA À GOMOSE DO CAJUEIRO

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Taxonomia e epidemiologia comparativa de Botryosphaeriaceae associada à gomose do cajueiro

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SUMÁRIO

RESUNO GERAL

A gomose destaca-se como uma das principais doenças relatadas em todas as regiões produtoras de caju no Brasil. No presente estudo identificamos, caracterizamos e avaliamos a epidemiologia comparativa das espécies de Botryosphaeriaceae associadas a gomose em *Anacardium* no Brasil. Um total de 138 isolados foram amostrados e identificados usando uma combinação de analise morfológica e filogenética baseados na sequencia parcial do translation elongation factor 1-α sequence (EF-1α), internal transcribed spacers (ITS) e sequencia do β-tubulin. dez espécies de Botryosphaeriaceae foram identificadas: *Lasiodiplodia brasiliense*, *L. euphorbicola*, *L. gonubiensis*, *L. iraniensis*, *L. jatrophicola*, *L. gravistriata* sp. nov., *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum batangarum* e *Pseudofusicoccum stromaticum*. Somente *L. theobromae* foi previamente descrito em cajueiro, enquanto que todas outras espécies são reportadas pela primeira vez em associação com cajueiro no Brasil e no mundo. *Lasiodiplodia theobromae* foi a espécie prevalente. Todas espécies de Botryosphaeriaceae foram patogênicas em ramos destacados de cajueiro. Houve diferença significativa entre as espécies, com *N. batangarum*, *L. iraniensis*, *L. jatrophicola* e *L. gravistriata* sendo as espécies mais agressivas, enquanto *L. euphorbicola*, *L. pseudotheobromae* foram as menos agressivas. Todas as espécies de Botryosphaeriaceae causaram sintomas nos hospedeiros alternativos testados exceto, *P. stromaticum*. *L. brasiliense*e *L. iraniensis* apresentaram as maiores lesões em abacate, banana, goiaba, mamão, manga e maracujá. *L. jatrophicola* mostrou os menores valores de agressividade nos hospedeiros, já *N. batangarum* não foi patogênico em maracujá e somente *L. gravistriata* causou sintomas em melão. Nosso resultado sugere que esses hospedeiros alternativos servem com uma fonte de inóculo potencial. *L. gravistriata* apresentou crescimento nas temperaturas de 5°C e 10°C. As espécies de Botryosphaeriaceae demostraram redução no crescimento micelial na presença dos fungicidas Tiofanato-metilico, difenoconazole e azoxistrobin, apresentando diferentes níveis de sensibilidade a cada um dos princípios ativos utilizados.

GENERAL ABSTRACT

The gummosis out as a major disease reported in all producing regions of cashew in Brazil. In this study we identify, characterize and evaluate the comparative epidemiology of species Botryosphaeriaceae associated with gummosis in *Anacardium* in Brazil. A total of 138 isolates were sampled and identified using a combination of morphological analysis and phylogenetic based on the partial sequence of the translation elongation factor 1-α sequence (EF-1α), internal transcribed spacers (ITS) and sequence of the β-tubulin. ten species were identified Botryosphaeriaceae: *Lasiodiplodia brasiliense*, *L. euphorbicola*, *L. gonubiensis*, *L.* iraniensis, *L. jatrophicola*, *L. gravistriata* sp. nov., *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum batangarum* and *Pseudofusicoccum stromaticum*. Only *L. theobromae* previously described in cashew tree, while all other species are reported for the first time in association with cashew trees in Brazil and worldwide. *Lasiodiplodia theobromae* was the prevalent species. All species of pathogenic Botryosphaeriaceae were highlighted in branches of cashew. There were significant differences between species with *N. batangarum*, *L. iraniensis*, *L. jatrophicola* and *L. gravistriata* being the most aggressive species, while *L. euphorbicola*, *L. pseudotheobromae* were less aggressive. All species of Botryosphaeriaceae caused symptoms in alternative hosts tested except *P. stromaticum*, *L. brasiliense* and *L. iraniensis* had the highest injury in avocado, banana, guava, papaya, mango and passion fruit. *L. jatrophicola* showed lower aggressive in the hosts, as *N. batangarum* was not pathogenic in passion fruit and only *L. gravistriata* caused symptoms in melon. Our results suggest that these alternate hosts serve as a potential source of inoculum. Species Botryosphaeriaceae demonstrated reduction in mycelial growth in the presence of Thiophanate-methyl fungicides, difenoconazole and azoxystrobin. The response sensitivity present variation according to the species of the fungicides and Botryosphaeriaceae.

Capítulo I

Introdução Geral

INTRODUÇÂO GERAL

1. A Cultura do Caju

O cajueiro (*Anacardium occidentale* L.), planta originária do Brasil, é cultivado mundialmente em diversas partes dos trópicos. Do processamento da castanha em casca (fruto verdadeiro), resulta a Amêndoa de Castanha-de-Caju (ACC) e o Líquido da Castanha-de-Caju (LCC), ambos de grande valor comercial. Do pedúnculo, são produzidas as bebidas (notadamente o suco e a cajuína) e outros produtos (principalmente doces e ração animal) Souza Filho et al. (2010). O regime pluviométrico mais adequado para a exploração racional do cajueiro está compreendido na faixa entre 800 e 1.600 mm anuais, distribuídos de cinco a sete meses, embora a planta tolere uma faixa mais ampla de período chuvoso. A umidade relativa do ar mais apropriada para a cultura situa-se na faixa entre 70% a 80%. Como planta de origem tropical, exige para seu desenvolvimento e produção, regime de altas temperaturas, sendo a média anual de 27º C a mais apropriada para o seu cultivo. O cajueiro deve ser cultivado, preferencialmente, em solos profundos, de textura média, em relevo plano ou suavemente ondulado, não sujeitos a encharcamento e com profundidade efetiva nunca inferior a 1,50 m.

Em 2009, a produção nos principais países envolvidos nessa atividade foi estimada em 2.804.266 toneladas, sendo o Brasil responsável por 8% desse valor (FAOSTAT, 2012). Assim, ocupando o 5º lugar, ficando atrás da Costa do Marfim, Nigéria, Índia e Vietnam (FAOSTAT, 2012). A Costa do Marfim pulou de 9% do total da produção dos cinco maiores países produtores, para 12% em 2009, evidenciando, assim, o crescimento da cajucultura nesse país. O crescimento da indústria processadora na África tem se estabelecido como uma ameaça para a indústria brasileira e indiana não devido ao seu possível crescimento no mercado internacional de ACC (SOUZA FILHO et al., 2010). Com relação aos países importadores, os Estados Unidos notadamente é o país que mais importa com um crescimento substancial passando de 10 mil toneladas no ano de 2012. Países Baixos, Canadá, Líbano e Reino Unido são também países que se destacam no mercado internacional em relação à importação de ACC.

Encontrado disperso em larga faixa do mundo tropical, o cajueiro constitui uma cultura de elevada importância econômica e social no nordeste brasileiro. No Brasil, a

cajucultura tem sido desenvolvida tanto em pequenas como em médias e grandes explorações rurais, apresentando grande variabilidade em termos de tecnificação (SOUZA FILHO et al., 2010).

A cajucultura comercial foi implantada no Nordeste na década de 1970 com apoio da Superintendência do Desenvolvimento do Nordeste (SUDENE). Nessa região, ocupa 670 mil hectares, que representa 99% da área com cajueiro no Brasil. A produção do cajueiro ocorre no período seco, portanto, na entressafra das demais espécies cultivadas na região, o que confere uma relevância estratégica na redução da flutuação na ocupação de mão-de-obra, principalmente, no campo. (SOUZA FILHO et al., 2010). Um parque industrial constituído por minifábricas e empresas de pequeno, médio e grande porte, instaladas principalmente nos Estados do Ceará, Rio Grande do Norte e Piauí beneficiaram, no ano de 2010, 104.342 toneladas de castanhas de caju, oriundas das regiões Norte, Nordeste e Centro-Oeste (IBGE, 2013). Dentro do segmento de fruticultura da pauta de exportação brasileira, a amêndoa da castanha de caju constitui-se o item de maior relevância, gerando, nos primeiros meses de 2013, divisas da ordem de US$ 22 milhões, valor que tem oscilado muito, em decorrência de flutuações nas produtividades (SINDICAJU, 2013).

No mercado interno, além da demanda pela amêndoa, o caju é ainda vendido como fruto de mesa e produtos oriundos do beneficiamento do pedúnculo do caju, fabricados em escala industrial: suco integral, suco concentrado, suco adoçado, cajuína, néctar, refrigerante, polpa congelada e doces. Entretanto, estima-se que mais de 90% do pedúnculo é desperdiçado, ou seja, é um subproduto pouco aproveitado na produção de ACC e LCC. (SOUZA FILHO et al., 2010).

No campo, a produtividade dos pomares é maior, quando comparada com há de 20 anos atrás, fato possível, devido que as pesquisas avançaram bastante no campo da propagação do cajueiro, identificando clones para finalidades específicas (amêndoa, fruta de mesa, fruta para indústria de sucos) como os clones do cajueiro anão precoce e técnicas de manejo, que, indiscutivelmente, propiciaram um aumento considerável no rendimento da castanha de caju, levando à obtenção de materiais de elevada qualidade de pedúnculo e de castanha. (LEITE; PESSOA, 2004). Na região semi-árida, por exemplo, apenas o clone CCP-76 responde por mais de 90% dos pomares implantados nas duas últimas décadas (PAIVA et al., 2002). No entanto, a uniformidade genética pode aumentar a vulnerabilidade das plantas ao ataque de patógenos. Em diversas áreas do Nordeste brasileiro, o cajueiro mantém uma grande diversidade genética, mas, também, grande conjunto de fitopatógenos e de insetos vetores ou predadores da cultura, requerendo, assim, permanente trabalho de melhoramento,

de monitoramento e de controle de pragas e doenças, tendo como premissa fundamental, a tecnificação contínua da cajucultura em direção à fruticultura moderna e competitiva. (LEITE; PESSOA, 2004).

Dentre as principais doenças que afetam o cajueiro no semi-árido nordestino, a resinose, causada pelo fungo *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., configura-se como séria ameaça à cultura, pela severidade dos seus sintomas, que incluem deficiência nutricional, murcha, queda de folhas, podridão seca dos ramos e formação de cancros nos ramos lenhosos e no tronco, geralmente acompanhada de exsudação de goma e escurecimento dos tecidos (FREIRE et al., 2002)

2. Resinose do cajueiro

As doenças do cajueiro destacam-se economicamente, pois sua presença acarreta severas perdas na produção, comercialização e exportação da amêndoa de Castanha-de-Caju. O cajueiro é infectado por patógenos foliares, bem como por causadores de podridões de pós-colheita. Entre as doenças foliares destacam-se aquelas causadas por fungos como: antracnose (*Colletotrichum gloeosporioides* (Penz) Pez. & Sacc.), Oídio (*Oidium anacardii* F. Noack), Mancha de Pestalotia (*Pestalotiopsis guepinii* (Desm.) Steyaert). O cajueiro está sujeito a outras doenças, ainda pouco estudadas, mas cuja incidência vem aumentando nas áreas de cultivos, entre elas a resinose causadas por fungos da família Botryosphaeriaceae (MENEZES, 2005). Na região do semi-áridado nordeste do país, recentemente, epidemias severas de resinose têm ocorrido sob deficiência hídrica, condição favorável ao desenvolvimento da doença, embora, ocasionalmente, ocorram sob condições de cultivo irrigado no Vale do São Francisco.

Atualmente esta doença já assume a posição de principal doença em algumas regiões do semi-árido nordestino. Apesar de a doença aparecer já no primeiro ano de cultivo, é apenas após o segundo ano que danos severos se manifestam (FREIRE et al., 2002).

Os sintomas da resinose são caracterizados por uma coloração escura dos tecidos da casca e do câmbio vascular, com exsudação de goma em casos mais avançados da doença. Na região Nordeste estes sintomas estão frequentemente associados com a poda da planta para substituição e uniformização da copa (MENEZES, 2005).

As perdas devido à resinose são decorrentes da redução da produtividade da planta, pelo bloqueio do movimento da seiva nos primeiros estágios de infecção e da produção do pomar, redução no transporte de água e nutrientes, redução na taxa fotossintética, destruição

dos ramos e morte da planta em virtude do avanço dos sintomas (MENEZES, 2005). A expansão do cultivo do clone CCP 76, altamente suscetível, tem propiciado grandes epidemias em algumas regiões do Nordeste do Brasil. (FREIRE et al., 2002, CARDOSO et al., 2009). Consequentemente, a modernização da cajucultura na região semi-árida requer, fundamentalmente, a adoção de medidas eficientes de controle da resinose. O caráter destrutivo, a disseminação através de propágulos vegetativos assintomáticos e a falta de estudos sobre a detecção precoce, mecanismos de infecção, defesa e controle eficiente transformam esta doença em um fator limitante do cultivo do cajueiro nas condições citadas (CARDOSO et al., 2009).

3. Aspectos taxonômicos de Botryosphaeriaceae

Na família Botryosphaeriaceae, o gênero *Botryosphaeria* Ces & De Not. foi inicialmente descrito por Cesati e De Notaris em 1863 e revisado por Saccardo em 1877 (PHILLIPS et al., 2005) e, mais recentemente, por Phillips et al. (2013) e Slippers et al. (2013). Trata-se de um Dothideomycetes com ascos bitunicados, produzidos num tecido estromático denominado ascotroma. Os ascósporos são hialinos, unicelulares e variam de fusoide, elipsoide a ovoide, tornando-se, em algumas espécies, marrom e com um a dois septos com o amadurecimento (PHILLIPS, 2013). Vários gêneros têm sido relatados como anamorfos de Botryosphaeriaceae: *Diplodia* Fr. in Mont., *Dothiorella* Sacc., *Fusicoccum* Corda in Sturm., *Lasiodiplodia* Ellis & Everh., *Phyllosticta* Pers. e *Sphaeropsis* Sacc. (JACOBS; REHNER, 1998; SLIPPERS et al., 2004a). Espécies de *Diplodia*, *Dothiorella* e *Lasiodiplodia* são claramente separadas daquelas de *Fusicoccum* por conídios de parede espessa e de menor razão comprimento e largura (LUQUE; MARTOS; PHILLIPS, 2005; PHILLIPS et al., 2005). Quando maduros, os conídios da maioria das espécies de *Diplodia*, *Dothiorella* e *Lasiodiplodia* são escuros e septados (ZHOU; STANOSZ, 2001). Apesar da semelhança das características dos conídios entre esses gêneros, no geral, os de *Lasiodiplodia* são asseptados e hialinos quando jovem e tornam-se escuros, septados, mais largos e ovoides e possuem parede estriada na maturidade (BURGESS et al., 2006). Conídios de *Dothiorella* tornam-se marrons e septados antes mesmo de serem liberados da célula conidiogênica, enquanto conídios em *Diplodia* são hialinos e tornam-se escuros e septados após a liberação da célula conidiogênica (PHILLIPS et al., 2005). Como na maioria dos outros fungos por mais de um século, o conceito de espécie morfológica tem predominado durante a descrição de novas espécies de Botryosphaeriaceae. Embora ainda útil em algumas situações, este

conceito de espécie tende a subestimar a verdadeira diversidade (TAYLOR et al. 2000). A atual identificação das espécies de Botryosphaeriaceae é difícil, uma vez que as formas sexuadas são raramente encontradas na natureza (JACOBS; REHNER, 1998; ZHOU; STANOSZ, 2001; VAN NIEKERK et al., 2004; SLIPPERS et al., 2004a; SLIPPERS et al., 2007). Além disso, a sua diversidade morfológica é limitada para permitir uma clara diferenciação, enquanto os anamorfos possuem uma larga diversidade morfológica (JACOBS; REHNER, 1998; LUQUE; MARTOS; PHILLIPS, 2005; PHILLIPS et al., 2005). A taxonomia de Botryosphaeriaceae é, portanto, frequentemente baseada nas características das formas assexuadas (JACOBS; REHNER, 1998; SLIPPERS et al., 2004b; BARBER et al., 2005; PHILLIPS et al., 2005; MOHALI; SLIPPERS; WINGFIELD, 2006; SLIPPERS et al., 2007;). Caracteres morfológicos de anamorfos dessa família, considerados úteis para sua delimitação taxonômica, incluem morfologia conidial como forma, tamanho, cor, septação, espessura e textura da parede, ornamentação, presença de microconídios e tipo de conidiogênese (JACOBS; REHNER, 1998; ZHOU; STANOSZ, 2001; SLIPPERS et al., 2004b). No entanto, esses caracteres exigem cuidadosa interpretação, uma vez que alguns deles se sobrepõem entre as espécies (MOHALI; SLIPPERS; WINGFIELD, 2006).

Os gêneros anamórficos de Botryosphaeriaceae não foram claramente delimitados e alguns têm sido reduzidos à sinonímia (PHILLIPS et al., 2005). Uma das razões é que várias espécies foram descritas baseadas na associação com hospedeiro ou distribuição geográfica (GURE; SLIPPERS; STENLID, 2005). Segundo Zhou, Smith e Stanosz (2001), problemas podem ser encontrados ao definir limites, especialmente entre espécies muito próximas dentro do mesmo gênero, devido à falta de variação morfológica distinta, a interdependência dos caracteres morfológicos e as influências ambientais na morfologia. Exemplos das dificuldades em diferenciar espécies muito próximas são aparentes dentro de Botryosphaeriaceae (JACOBS; REHNER, 1998).

4. Aspectos filogenéticos de Botryosphaeriaceae

Marcadores moleculares são usados como ferramentas para caracterização de diversidade genética de fitopatógenos, onde características morfológicas são ausentes ou incapazes de diferenciação de isolados (SHARMA; SINGH; PRACHI, 1999; SHARMA; GUPTA; SHARMA, 2005). Estudos recentes sobre a taxonomia de Botryosphaeriaceae têm empregado métodos moleculares para revelar relações filogenéticas entre as espécies (PHILLIPS et al., 2013; SLIPPERS et al., 2013; WIKEE et al., 2013) e para ajudar a resolver

complexo de espécies (SMITH et al. 2001; PHILLIPS et al. 2002; DENMAN, et al. 2003; ALVES et al. 2004; SLIPPERS et al. 2004a; BEGOUDE et al. 2010; SAKALIDIS et al. 2011). No gênero *Botryosphaeria* mais de 20 anamorfos já foram associados a esse gênero, sendo os mais comuns: *Diplodia, Dothiorella, Fusicoccum, Lasiodiplodia, Neoscytalidium* e *Sphaeropsis* (DENMAN et al. 2000; PHILLIPS et al., 2013).

A filogenia molecular tem desempenhado um papel importante para distinguir as espécies de *B. dothidea* era parafilético. Mais tarde, para este grupo parafilético foi mostrado como representantes as espécies *B. dothidea e B ribis*. (ZHOU; STANOSZ, 2001; SMITH e STANOSZ, 2001; SMITH et al., 2001). Denman et al. (2000) revisou os gêneros anamorfos associados a *Botryosphaeria* e na base da sua filogenia reconheceu que as espécies estudadas separavam-se em dois clados principais, que correspondiam aos gêneros anamorfos *Fusicoccum* e *Diplodia*. Segundo esses autores a separação foi relacionada a características morfológicas de seus conídios. Assim espécies de *Fusicoccum* apresentavam conídios hialinos com paredes finas, enquanto espécies de *Diplodia* apresentavam conídios pigmentados com paredes grossas. Phillips et al (2002) também apoiaram a manutenção de apenas esses dois gêneros como anamorfos de espécies de *Botryosphaeria*. Isso mais tarde também foi apoiado por Zhou e Stanosz (2001), que dividiram o gênero *Botryosphaeria* dentro de seções Hyala (espécies com conídios hialinos e estreitos do tipo *Fusicoccum*) e seção Brunnea (espécies com conídios largos e marrons do tipo *Diplodia*). Zhou e Stanosz (2001) utilizaram a subunidade ribossomal mitocondrial (mtSSUrDNA) para tratar de questões filogenéticas em *Botryosphaeria*, e os resultados dessas análises não estavam de acordo com as filogenias da região ITS. Assim, a separação entre *Diplodia* e *Fusicoccum* não foi apoiada por filogenias mtSSUrDNA e algumas espécies de *Botryosphaeria* com anamorfos *Fusicoccum*, ou seja, *B. dothidea*e *B. corticis*, agruparam no clado *Diplodia*. No entanto, filogenias de mtSSUrDNA separaram claramente *Botryosphaeria* do gênero *Guignardia* intimamente relacionado (ZHOU; STANOSZ, 2001). Corroborando com Silva-Hanlin e Hanlin (1999), os quais através do seqüenciamento da região codificante da subunidade 18S do rDNA também, verificaram que os gêneros *Guignardia*e *Botryosphaeria* estão intimamente relacionados.

Alguns trabalhos consideram que *Lasiodiplodia* Ellis & Everh. e *Sphaeropsis* Sacc. deveriam ser sinônimos de *Diplodia*. (ZHOU; STANOSZ, 2001; ALVES et al., 2004). A sinonímia de *Sphaeropsis* sob *Diplodia* tem sido geralmente aceita pela maioria dos autores (DE WET et al., 2003; BURGESS et al., 2004; PAVLIC et al., 2004). No entanto, Xiao e Rogers (2004) descreveram recentemente uma nova espécie de

Sphaeropsis (Pyriputrescens S. Xiao & J. D. Rogers), preferindo, portanto, manter *Sphaeropsis* como um gênero separado. Por causa das características morfológicos (paráfises e conídios estriados) e análises filogenéticas (ITS e EF1-a), alguns autores (PAVLIC et al., 2004; BURGESS et al., 2006) também mantiveram *Lasiodiplodia* como um gênero separado de *Diplodia*.

Em um amplo estudo filogenético, Crous et al., (2006) avaliaram dados de seqüência da região 28S do rDNA de 113 isolados, representando a maioria das variações morfológicas atualmente reconhecidos em Botryosphaeriaceae. As análises revelaram 10 linhagens em Botryosphaeriaceae e neste estudo novos gêneros foram descritos. O gênero *Botryosphaeria*, atualmente, apresenta-se restrito a *B. dothidea* e *B. corticis* (Demaree & Wilcox) Arx & E. Müll. Conseqüentemente, o nome *Botryosphaeria* deixou de ser aceito para a maioria das espécies com anamorfos em *Fusicoccum* e *Diplodia*. No entanto, o gênero *Neofusicoccum* Crous, Slippers & A.J.L. Phillips foi descrito para acomodar Botryosphaeriaceae com anamorfos em *Fusicoccum*. Mas recentemente Phillips et al., (2013) aceitaram 17 gêneros na família Botryosphaeriaceae. Esses gêneros foram caracterizados com base em 17 linhagens em uma filogenia multi-locus. Embora a região ITS sozinha seja suficiente para separar as espécies dentro de cada gênero de Botryosphaeriaceae, a inclusão do EF1-α resultou em uma separação mais robusta, e foi considerada essencial para alguns gêneros, como *Diplodia*, *Lasiodiplodia* e *Neofusicoccum*. Recomendamos, assim, pelo menos, estes dois loci para a separação de espécies dentro do Botryosphaeriaceae.

Várias espécies de *Botryosphaeria* foram identificadas como importantes patógenos em videiras no mundo. Até então, somente a espécie *B. rhodina* tinha sido associada à doença do cancro em *Vitis vinifera*, na Califórnia (ÚRBEZ-TORRES et al., 2006). Os autores utilizaram dados morfológicos e o sequenciamento das regiões ITS1-5,8S-ITS2 do rDNA e parte do gene da β-tubulina para investigar a presença de *Botryosphaeria* spp. em 1735 amostras de 166 vinhedos do Estado da Califórnia. Estas análises revelarampelo menos sete espécies de *Botryosphaeria* colonizando as videiras, sugerindo que a relação destes fungos com o hospedeiro precisa ser considerada.

Apesar de 39 espécies de *Lasiodiplodia* serem conhecidas atualmente (*L. abnormis* Traverso & Spessa, *L. brasiliense* M. S. B. Netto, M. W. Marques & A. J. L. Phillips, *L. caatinguensis* I. B. L. Coutinho, F. C. O. Freire, C. S. Lima & J. E. Cardoso, *L. citri* Av. – Saccá, *L. citricola* Abdollahzadeh, Javadi & A. J. L. Phillips, *L. crassispora* T. I. Burgess & Barber, *L. egyptiacae* A.M. Ismail, L. Lombard & Crous, *L. euphorbicola* A. R. Machado &

O. L. Pereira, *L. exígua* Linaldeddu, Deidda & A. J. L. Phillips, *L. fiorii* Bacc., *L. frezaliana* Faurel & Schotter, *L. gilanensis* Abdollahzadeh, Javadi & A. J. L. Phillips, *L. gonubiensis* Pavlic, Slippers & M. J. Wingf., *L. hormozganensis* Abdollahzadeh, Zare & A. J. L. Phillips, *L. iraniensis* Abdollahzadeh, Zare & A. J. L. Phillips, *L.jatrophicola* A. R. Machado & O. L. Pereira, *L. macrospora* A. R. Machado & O. L. Pereira, *L. mahajangana* Begoude, Jol. Roux & Slippers, *L. margaritacea* Pavlic, T. I. Burgess & M. J. Wingf., *L. mediterranea* Linaldeddu, Deidda & Berraf-Tebbal, *L. missouriana* Úrbez-Torres, Peduto & Gubler, *L. nigra* K. R. Appel & Laubert, *L. paraphysaria* (Sacc.) Keissl., *L. parva* A. J. L. Phillps, A. Alves & Crous, *L. plurivora* Damm & Crous, *L. pontae* F. C. O. Freire, I. B. L. Coutinho, C .S. Lima & J. E. Cardoso, *L. pseudotheobromae* A. J. L. Phillips, A. Alves & Crous, *L. pyriformis* F. J. J. Van der Walt, Slippers & G. J. Marais, *L. ricini* Sacc., *L. rubropurpurea* T. I. Burgess, Barber & Pegg, *L. subglobosa* A. R. Machado & O. L. Pereira, *L. thailandica* T. Trakunyingcharoen, L. Lombard & Crous, *L. theobromae* (Pat.) Griffon & Maubl., *L. thomasiana* Sacc., *L. triflorea* B. B. Higgins, *L. tubericola* Ellis & Everh., *L. undulata* (Berk. & M. A. Curtis) Abbas, B. Sutton, Ghaffar & Abbas, *L. venezuelensis* T. I. Burgess, Barber & Mohali, *L. viticola* Úrbez-Torres, Peduto & Gubler), apenas 28 estão disponíveis (*L. brasiliense, L. caatinguensis*, *L. citricola*, *L. crassispora, L. egyptiacae, L. euphorbicola, L. exigua, L. gilanensis*, *L. gonubiensis*, *L. hormozganensis*, *L. iraniensis, L. jatrophicola, L. macrospora, L. mahajangana, L. margaritacea, L. mediterranea, L. missouriana, L. pontae*, *L. parva*, *L. plurivora, L. pseudotheobromae, L. pyriformis, L. rubropurpurea*, *L. subglobosa*, *L. thailandica*, *L. theobromae, L. venezuelensis* e *L. viticola* para análises filogéneticas e todas elas são espécies recém descritas, desde 2004 e possuem sequências de DNA depositadas em banco de dados, como por exemplo, o GenBank, por esta razão, torna-se possível incluir apenas essas espécies recentemente descritas nas análises filogenéticas. O recente aumento no número de espécies é reconhecido em grande parte devido à utilização de dados moleculares, mas é também devido à amostragem, em regiões até então pouco exploradas, incluindo Venezuela (BURGESS et al. 2006), Austrália (PAVLIC et al. 2008), Irã (ABDOLLAHZADEH et al. 2010), Egito (ISMAIL et al. 2012), Brasil (MARQUES et al. 2012, MARQUES et al. 2013, MACHADO et al. 2014, CORREIA et al. 2015, COUTINHO et al. 2016) e Tailândia (TRAKUNYINGCHAROEN et al. 2015). As espécies descritas mais recentemente foram separadas, não só na morfologia, mas também com base em dados da sequência ITS ("Internal Transcribed Spacer") e EF-1α ("Translation Elongation Factor").

Estudando o gênero *Lasiodiplodia* com dados de sequência de ITS distinguiram *L. gonubiensis* de *L. theobromae* (PAVLIC et al. 2004). Burgess et al. (2006) descreveram mais

três espécies novas de *Lasiodiplodia* claramente separada de *L. theobromae* baseados em sequências de ITS. Inclusão de sequências EF-1α na análise filogenética deu suporte para estas espécies (BURGESS et al. 2006).

Em um estudo de Botryosphaeriaceae em espécies de *Prunus* na África do Sul, Damm; Crous; Fourie (2007) descreveram *L. plurivora* como uma nova espécie. Esta espécie está intimamente relacionada com *L. theobromae*e as duas espécies não poderiam ser distinguidas unicamente na base de dados da sequência ITS mas foram claramente separadas quando os dados EF-1α foram incluídos. Alves et al. (2008) usaram ITS e EF-1α, juntamente com dados morfológicos para caracterizar uma coleção de isolados inicialmente identificados como *L. theobromae*. Dessa forma, os pesquisadores mostraram que *L. theobromae* é um complexo de espécies crípticas e descreveram como novas espécies *L. pseudotheobromae* e *L. parva*. As espécies atualmente reconhecidas não podem ser distinguida apenas em suas sequências da região ITS e a separação filogenética é efetivamente com base em uma região do gene único, ou seja, EF-1α. Pavlic et al. (2004) descreveram uma nova espécie *L. gonubiensis*, com base na morfologia e dimensões de conídios e sequência de dados. Essa foi a primeira espécie deste gênero a ser encontrada em árvores nativas na África do Sul.

Seis espécies de *Lasiodiplodia* foram associadas com uma variedade de sintomas em uma gama de hospedeiros lenhosos no Irã e descreveram quatro espécies novas (*L. citricola*, *L. gilanensis*, *L. hormozganensis*e *L. iraniensis*). Todas as quatro espécies podem ser distinguidas morfológica e filogeneticamente umas das outras e das espécies previamente descritas. Todas as espécies utilizadas foram isoladas de galhos mortos, mas não sabe-se se eles eram patógenos primários ou saprófitos que se desenvolveram no tecido doente. Mesmo sabendo-se que *L. citricola* foi isolado apenas de *Citrus* sp., não foi possível determinar qualquer grau de especificidade. Na verdade, as outras três novas espécies foram isoladas a partir de vários hospedeiros diferentes, sugerindo uma natureza polífaga (ABDOLLAHZADEH et al. 2010).

No Egito três espécies de *Lasiodiplodia* foram associadas causando morte descendente e podridão peduncular em mangueira, *L. pseudotheobromae*, *L. theobromae* e uma espécie recém descrita *L. egyptiacae*, dessa forma, *L. theobromae* é um complexo de espécies crípticas que atualmente há mais de 13 espécies crípticas reconhecidas neste complexo. (ISMAIL et al. 2012)

No Nordeste brasileiro, estudos recentes demonstraram uma grande diversidade de espécies Botryosphaeriaceae associadas à podridão peduncular em mangueira e descreveram sete espécies de *Lasiodiplodia* (*L. crassispora*, *L. egyptiacae*, *L. hormozganensis*, *L.*

iraniensis, *L. pseudotheobromae*, *L. theobromae* e uma possível nova espécie *Lasiodiplodia* sp.) e sete espécies de Botryosphaeriaceae (*Botryosphaeria dothidea*, *B. mamane*, *Fusicoccum fabicercianum*, *Neofusicoccum parvum*, *N. brasiliense* sp. nov, *Neoscytalidium dimidiatum* e *Pseudofusicoccum stromaticum*), sendo os primeiros relatos de *L. crassispora* e *Botryosphaeria mamane* associados à mangueira no mundo (MARQUES et al. 2012, MARQUES et al. 2013).

Apesar de *L. theobromae* ter sido relatado em mais de 500 hospedeiros, a gama de hospedeiros das espécies descritas nos últimos anos têm sido supostamente restrita (PAVLIC et al., 2004; BURGESS et al., 2006; DAMM; CROUS; FOURIE, 2007). No entanto, não está claro se a gama de hospedeiros restrita das espécies mais recentemente descritas é um reflexo de amostragem em vez de uma representação real da gama de hospedeiros. Assim, é possível que haja uma variação na amplitude de gama de hospedeiros entre espécies como visto de outros gêneros de Botryosphaeriaceae. Por exemplo, *Diplodia seriata* De Not. tem uma gama muito ampla de hospedeiro, enquanto *D. pinea* (Desm.) J. J. Kickx é restrita aos pinheiros e *D. corticola* A. J. L. Phillips, A. Alves & J. Luque está restrita às espécies do gênero *Quercus*. No Brasil não há estudos sobre a diversidade genética de Botryosphaeriaceae associadas à resinose do cajueiro que possivelmente pode incluir várias espécies recentemente descritas e/ou desconhecidas, identificadas como sinônimos de *L. theobromae*, que não podem ser separadas somente por caracteres morfológicos, como se tem visto em estudos envolvendo este patógeno em diversas partes do mundo, principalmente na África, Brasil, Egito, Irã e Venezuela (PAVLIC et al., 2004; BURGESS et al. 2006; DAMM; CROUS; FOURIE, 2007; ALVES et al., 2008; ABDOLLAHZADEH et al., 2010; ISMAIL et al., 2012; MARQUES et al., 2013). Portanto o objetivo desse trabalho é caracterizar e identificar espécies de Botryosphaeriaceae associadas à gonose do cajueiro no Nordeste brasileiro a fim de buscar um melhor entendimento da interação hospedeiro- patógeno, a relação com outros hospedeiros alternativos, a sensibilidade a alguns fungicidas utilizados no manejo de outras anacardiáceas, além de fornecer subsídios para uma melhor estratégia de controle.

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Capítulo II

Analysis of phylogeny, distribution and pathogenicity of Botryosphaeriaceae species associated with gummosis of *Anacardium* in Brazil, with a new species of *Lasiodiplodia*

Fungal Biology (Aceito)

Analysis of phylogeny, distribution and pathogenicity of Botryosphaeriaceae species associated with gummosis of *Anacardium* in Brazil, with a new species of *Lasiodiplodia*

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Resuno

Netto, M. S. B., Lima, W. G., Correia, K. C., da Silva, C. F. B., Thon, M., Martins, R. B., Miller, R. N. G., Michereff, S. J. and Câmara, M. P. S. 2016. Analysis of phylogeny, distribution and pathogenicity of Botryosphaeriaceae species associated with gummosis of *Anacardium* in Brazil, with a new species of *Lasiodiplodia*

Foram identificadas e caracterizadas as espécies Botryosphaeriaceae associados à gomose em *Anacardium* no Brasil. Um total de 138 isolados foram amostrados e identificados com base na morfologia e análise filogenética, por meio de sequências parciais do fator de alongação1-α (EF-1α), do espaçador transcrito interno do DNA ribossômico (ITS) e do gene β-tubulina (TUB). Foram identificados dez espécies, *Lasiodiplodia brasiliense*, *L. euphorbicola*, *L. gonubiensis*, *L. iraniensis*, *L. jatrophicola*, *L. gravistriata* sp. nov., *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum batangarum* e *Pseudofusicoccum stromaticum*. *L. theobromae* foi previamente relatado em caju e é a espécie mais prevalente. Todas as outras espécies são relatados aqui pela primeira vez neste hospedeiro. Todas as espécies de Botryosphaeriaceae foram patogênicas em ramos destacados de caju. Houve diferença significativa na agressividade entre as espécies, *N. batangarum*, *L. iraniensis*, *L. jatrophicola* e *L. gravistriata* foram caracterizadas como as espécies mais agressivas, enquanto que *L. euphorbicola* e *L. pseudotheobromae* foram identificadas como menos agressivas.

Palavras-chave

Agressividade, Caju, EF1-α, ITS, Patogenicidade, abordagem polifásica

Abstract

Netto, M. S. B., Lima, W. G., Correia, K. C., da Silva, C. F. B., Thon, M., Martins, R. B.,Miller, R. N. G., Michereff, S. J. and Câmara, M. P. S. 2016. Analysis of phylogeny, distribution and pathogenicity of Botryosphaeriaceae species associated with gummosis of *Anacardium* in Brazil, with a new species of *Lasiodiplodia*

We identified and characterized Botryosphaeriaceae species associated with gummosis on *Anacardium* in Brazil. A total of 138 isolates were sampled and identified on the basis morphology and phylogeny, through analysis of a partial translation elongation factor 1-α sequence (*EF-1α*), ribosomal DNA internal transcribed spacers (ITS) and β-tubulin (*TUB*) gene sequence data. Ten taxa were identified, namely, *Lasiodiplodia brasiliense*, *L. euphorbicola*, *L. gonubiensis*, *L. iraniensis*, *L. jatrophicola*, *L. gravistriata* sp. nov., *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum batangarum* and *Pseudofusicoccum stromaticum. Lasiodiplodia theobromae* has been previously reported in cashew and is the most prevalent species observed. All the other species are reported here for the first time on this host. All species of Botryosphaeriaceae were pathogenic on detached green cashew shoots. Significant differences in aggressiveness were observed among the species, with *Neofusicoccum batangarum*, *L. iraniensis*, *L. jatrophicola* and *L. gravistriata* characterized as the most aggressive species, whilst *L. euphorbicola* and *L. pseudotheobromae* were identified as the least aggressive.

Keywords

Aggressiveness, Cashew, EF1-α, ITS, Pathogenicity, Polyphasic approach

1 Introduction

Cashew (*Anacardium occidentale*) is a tropical evergreen crop cultivated worldwide with a centre of origin in the Amazonian forest of Brazil. In contrast to the other seven species within the genus *Anacardium*, only cashew (*A. occidentale*) is an economically important nut crop, with both an edible hypo carp (apple) and nutritious kernel arising from a drupe (Aliyu 2012). It is important as an export commodity, with considerable consumption in Europe and the USA. Brazilian production in 2013 reached 259,900 t, from a production area of 708,430 ha. In 2016, 12,165 t of cashew nuts were exported generating about US$ 79 M. The north-eastern region of Brazil is responsible for 99% of the country's production (Agrianual 2015), with the cashew industry in rural areas recognized to be of considerable socio-economic importance (Moreira et al. 2013).

Of the numerous diseases that compromise cashew production, cashew gummosis, which is caused by *L. theobromae*, is considered one of the most important diseases for the cashew industry (Cysne et al. 2010).This fungal species was first reported on cashew in 1990 (Freire 1991), and was soon recognized as one of the most important diseases of the crop in north-eastern Brazil (Freire et al. 2002; Moreira et al. 2013). The main symptoms of this disease comprise the appearance of cankers along the trunk or branches, which develop over time and release a characteristic resin-like gum. Gummosis subsequently results in reduced water and nutrient transport, branch dieback, inflorescence blight, reduction in photosynthesis and eventual plant death (Freire et al. 2002; Moreira et al. 2013).

To date only *L. theobromae* has been found associated with cashew gummosis (Freire et al. 2002; Cardoso et al. 2004; Muniz et al. 2012; Moreira et al. 2013). However, identification of

causal agents was based on analysis of fungal morphology and cultural characteristics, which are today considered insufficient for species identification in the genus *Lasiodiplodia* (Phillips et al. 2013).

Lasiodiplodia is a member genus of the Botryosphaeriaceae, a family in the Dothideomycetes. This family contains numerous fungal species which occur as saprophytes, parasites or endophytes on a diverse range of plant hosts (Slippers and Wingfield 2007; Phillips et al. 2013). In addition to cashew in Brazil, genera of Botryosphaeriaceae such as *Botryosphaeria*, *Fusicoccum*, *Macrophomina*, *Neofusicoccum*, *Neoscytalidium* and *Pseudofusicoccum* (Marques et al. 2013b; Machado et al. 2014) have been reported to cause disease in several other economically important crops including avocado (*Persea americana*), banana (*Musa* spp.), barbados cherry (*Malpighia glabra*), cacao (*Theobromae cacao*), castor bean (*Ricinus communis*), citrus (*Citrus* spp.), coconut palm (*Cocos nucifera*), custard apple (*Annona squamosa*), grapevine and table grape (*Vitis* spp.), guaraná (*Paullinia cupana*), guava (*Psidium guajava*), mango (*Mangifera indica*), muskmelon (*Cucumis melo*), papaya (*Carica papaya*), passion fruit (*Passiflora edulis*), physic nut (*Jatropha curcas*), sour sop (*Annona muricata*) and watermelon (*Citrullus lanatus*) (Costa et al. 2010; Marques et al. 2013a; Machado et al. 2014; Netto et al. 2014; Correia et al. 2016).

Although the taxonomy of the Botryosphaeriaceae has until recently been based upon morphology of asexual morphs, more recent phylogenetic inference based upon analysis of sequence data for target DNA loci has had considerable impact on the systematics of the Botryosphaeriaceae, with increased resolution enabling discrimination of species with overlapping morphological characteristics (de Wet et al. 2008; Phillips et al. 2013).

Despite the pathogenic importance attributed to Botryosphaeriaceae on diverse host plants, there have been no phylogenetic analyses of this family on cashew. Given the increasing

economic importance of cashew gummosis and the recent reports of new species of Botryosphaeriaceae occurring on tropical plants, it is possible that a number of species of this family may be associated with cashew gummosis in Brazil. For effective disease management, a clear understanding of disease aetiology is essential for determination of the distribution of individual species and their disease epidemiology. In this context, the objectives of this study were (i) to identify species of Botryosphaeriaceae associated with cashew gummosis in Brazil, (ii) to determine the prevalence and distribution of each species and (iii) to characterize isolates in terms pathogenicity and virulence using excised cashew green shoots.

2 Materials and methods

2.1 Isolation of fungal material

During 2013 and 2014, samples were obtained from 30 *Anacardium* orchards, located in six states of Brazil (Alagoas, Ceará, Minas Gerais, Pernambuco, Piauí and Rio Grande do Norte). In each orchard, a total of 15 *Anacardium* trees exhibiting gummosis symptoms were selected for isolation of fungal material. Symptomatic shoot material at the interface between necrotic and apparently uninfected tissue was surface sterilized using 70 % ethanol for 30 s followed by 1 % NaOCl for 1 min. Following rinsing once with sterile distilled water for 30 s, material was then dried and4–5 mm fragments plated onto potato dextrose agar (PDA) (Acumedia, Lansing, USA) containing 0.5 g l^{-1} streptomycin sulphate (PDAS). Following incubation at 25 °C in the dark for a period of 3 to 4 days, all colonies showing morphological characteristic typical of the Botryosphaeriaceae (Sutton 1980; Phillips 2006) were plated onto PDA and incubated at 25 °C in the dark and observed over a period 15 days. Pycnidial production was induced following growth

on 2 % water agar (WA) and autoclaved pine needles (PNA) as carbon source. After a 3-week incubation period at 25 °C under a 12 h daily photoperiod with near-ultraviolet light (Slippers et al. 2004a),individual pycnidia were from each isolate were examined under a stereo-microscope (Zeiss Stemi DV4; Carl Zeiss, Berlin, Germany), and transferred in 250 µl of sterile distilled water. A 20 µl aliquot of the resultant conidial suspension was spread onto PDAS and incubated at 28 °C in the dark for 24 h. Single spore isolates were prepared for each sample through transfer onto fresh PDA plates. Based upon morphological characteristics typical of the genus, a total of 138 isolates were identified as members of the Botryosphaeriaceae. All isolates were preserved on PDA slants at 5 °C in the dark.

2.2 Molecular-based amplification

For identification of the Botryosphaeriaceae, a region of the translation elongation factor 1α (*EF1*-α) gene was amplified and sequenced for all isolates collected from the cashew orchards. The rDNA internal transcribed spacer (ITS) regions was employed to support species identity based on EF1-α gene sequence data, with a portion of the β-tubulin gene for the fusicoccum-like representative isolates. Total DNA was extracted from aerial mycelium from 7-day-old cultures grown on PDA at 25 °C using an AxyPrepTM Multisource Genomic DNA Miniprep Kit (Axygen Scientific Inc., Union City, USA) according to the manufacturer's instructions.

The target region of the *EF1-α* gene was amplified using primer pairs EF-688F and EF-1251R (Alves et al. 2008), as described by Phillips et al. (2005). The rDNA ITS region was amplified using universal primers ITS1 and ITS4 (White et al. 1990) according to Slippers et al. (2004b). A portion of the β-tubulin (*TUB*) gene was amplified using the primers BT2a and BT2b (Glass and Donaldson 1995). Each PCR reaction contained 1 µl of total DNA, 1.5µM of each

primer, 25 µl of 2X PCR Master Mix (Thermo Scientific, Waltham, USA), containing 0.05 U of *Taq* DNA polymerase, 2 X reaction buffer, 4 mM $MgCl_2$ and 0.4 mM dNTPs. Reaction volumes were completed to 50 µl volumes using PCR-grade water. Temperature cycling was conducted with a thermo cycler (Biocycler MJ 96; Applied Biosystems, Foster City, USA). PCR products were photo documented under UV light after staining 1.5% agarose gels with ethidium bromide (0.5 µg ml^{-1}) for 1 min. Purification of PCR products was performed with the AxyPrepTM PCR Cleanup Kit (Axygen), according to the manufacturer's instructions. The rDNA ITS, EF1-α and β-tubulin regions were forward and reverse sequenced with an ABI 3730 XL DNA Analyzer (Applied Biosystems).

2.3 Phylogenetic analyses

Alignment of sequence data was conducted using ClustalX v. 1.83 (Thompson et al. 1997), with the following parameters: pair wise alignment (gap opening = 10, gap extension = 0.1); multiple alignment (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25 %). Sequences of two isolates of each species of Botryosphaeriaceae including the type strains available in GenBank were also included in the analyses and outgroup (Table 1). Only the type species of *Lasiodiplodia pontae* was included in the analysis because the two other strain available [isolate IBL 14 (GenBank accession number: ITS-KT151795; EF-1α-KT151792) and isolate IBL 18 (GenBank accession number: ITS-151796; EF-1α-KT151793)] did not cluster in the type strain clade (Coutinho et al. 2016).Simple indel coding, as implemented by GapCoder (Young and Healy 2003), was employed for incorporation of phylogenetic information present in indels (gaps) into the phylogenetic analyses. Tree robustness was evaluated following 1000 bootstrap replications (Hillis and Bull 1993).Sequence alignments were deposited in TreeBASE

(http://www.treebase.org/) under accession number S19242 for *Lasiodiplodia*, S19243 for *Neofusicoccum* and S19241 for *Pseudofusicoccum*. Phylogenetic analyses were conducted using the programme GENEIOUS v. 7.1.8 (Kearse et al. 2012). Maximum likelihood estimation was conducted using a plugin for PhyML (Guindon et al. 2010) and Bayesian analyses using MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003). Bayesian analysis was performed by four independent runs with the Markov Chain Monte Carlo (MCMC) algorithms (Larget and Simon1999). Data were partitioned according to locus, with nucleotide substitution model parameters for each partition set as described below. Four parallel MCMC chains were run, with a heating scheme set at 0.3, under a general time-reversible (GTR) substitution model with rate variation of gamma-distribution (G), and proportion of invariable site (I) (Rodríguez et al. 1990). Trees were sampled every 1,000th generation from a total of 10,000 trees, with the first 2,500 trees discarded as representing the burn-in phase of each analysis. The remaining 7,500 trees (stationary distribution) were employed for determination of posterior probabilities (Rannala and Yang 1996) and mapping onto the majority-rule consensus tree. FigTree v.1.4.2 (Rambaut 2009) was employed for tree visualization. Representative cultures of the species identified in this study were deposited in the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Universidade Federal Rural de Pernambuco, Brazil.

2.4 Morphology and cultural characteristics

Colony morphology and conidial characteristics were examined for a total of 33 representative isolates among the Botryosphaeriacea species identified following phylogenetic analysis.Colony colour and aerial hyphae were recorded after 15 days of growth on 2 % malt extract agar (MEA) (Acumedia) at 25 °C in the dark. Colony colours were examined according to Rayner (1970).

Conidial morphology characteristics were examined after growth under near-ultraviolet light on PNA, as previously described. Digital images for conidia and other structures mounted in 100% lactic acid were recorded using a Leica DFC320 camera fitted to a Leica DMR HC microscope with Nomarski differential interference contrast optics (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The Leica IM500 measurement module was employed to determine the length and width of 50 conidia per isolate, with mean values and standard errors calculated for all measurements. Conidial shape, colour, and septation were also recorded.

The effect of temperature on colony growth was examined across the different species identified. Four replicates were included per isolate, with experiments performed in duplicate. Mycelial plugs isolated from the growing margin of 3-day-old colonized plates were transferred onto 2 % MEA plates and incubation the dark at temperatures ranging from 5 °C to 35 °C, with 5 °C intervals. After a 2-day period, colony diameters (mm) were measured in two perpendicular directions. Mycelial growth rate (mm/day) was estimated based on colony diameters following growth at 30°C.In order to estimate the optimal growth temperature, recorded colony diameters were plotted against temperature and a curve fitted through cubic polynomial regression ($y = a + bx + cx^2 + dx^3$) using the programme TableCurveTM 2D v. 5.01 (SYSTAT Software Inc., Chicago, USA). One-way analyses of variance (ANOVA) were conducted on optimum temperature and mycelia growth rate data, with means for each species compared with Fisher's least significant difference (LSD) test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

2.5 Pathogenicity and aggressiveness assays

Pathogenicity and aggressiveness of all Botryosphaeriaceae isolates characterized morphologically was examined using detached green shoots of *A. occidentale* (cv. BRS 274) (Amponsahet *et al*. 2011; Correia *et al*. 2016). Healthy 30 cm sections of soft green shoots were obtained from cashew trees (cv. BRS 274) from a non-commercial orchard at the Universidade Federal Rural de Pernambuco where Botryosphaeriaceae species were considered absent, based upon extensive sampling. The cut ends were firstly dipped in wax then cut in the centre of each shoot using a sterilized scalpel. Each superficial wound (~4-mm length, 2-mm deep)was inoculated with a 4 mm diameter mycelial plug taken from the growing margin of a 5-day-oldPDA culture of each isolate. As negative control checks, non-colonized PDA plugs were used for inoculation of shoots. In order to prevent drying, all inoculated areas were covered with Parafilm (Pechiney Co., Chicago, USA) Shoots were then incubated in a growth chamber for a 10 day period at 25°C and 12-h photoperiod. Following incubation, Parafilm was removed and shoots were sliced lengthwise to enable visual observation of internal lesions. The presence of lesions advancing beyond the original 4-mm diameter inoculation point was considered indicative of pathogenicity. Isolate virulence was evaluated through accurate digital calliper-based (Mitutoyo Co., Kanagawa, Japan) measurement of lesions dimensions. The entire experiment was arranged in a completely randomized design, with four replicates employed per treatment (isolate) and one shoot per replicate. The entire experiment was conducted in duplicate. Differences in virulence were determined by analysis of data with a one-way ANOVA, with means compared by LSD test at the 5% significance level using the program STATISTIX.

3 Results

3.1 Phylogenetic analyses

Sampling from *Anacardium* spp. from numerous growing regions in Brazil (Fig. 1) resulted in isolation of 138 isolates of Botryosphaeriaceae. Phylogenetic analysis of the EF1-α gene was employed for identification of all isolates, with rDNA ITS sequences analysed for 17 isolates that represented *EF1-α* haplotypes, and partial *TUB* gene sequences for six fusicoccum-like isolates. The GenBank accession numbers are listed in Table 1.Analysed *EF1-α* and *TUB* sequences were approximately 450 bp in size, while rDNA ITS sequences were approximately 580 bp in size. The *EF1-α* and rDNA ITS sequences were combined in separate data sets, which corresponded to *Lasiodiplodia* species and *Pseudofusicoccum* species. The ITS, *EF1-α*and *TUB* sequences were combined in a third data set corresponding to *Neofusicoccum* species. Data sets were analyzed separately, resulting in three phylogenetic trees, one for each genus (Figs 2–4). The isolates obtained in this study grouped into 10 distinct clades. The combined *EF1-α* and rDNA ITS sequences for *Lasiodiplodia* contained data for 78 isolates, including two outgroup taxa. Out of a total of 1393 characters, 1136 were constant, 231 were variable and parsimony uninformative and 163 were parsimony informative. The Maximum Likelihood (ML) and Bayesian methods (BM) for phylogenetic analyses produced trees with nearly identical topologies (Bayesian tree not shown). The majority (76 isolates) grouped together in a large clade containing *L. theobromae* (CBS 16496). Nine isolates grouped with *L. iraniensis* (CBS 124710). Four isolates grouped with *L. brasiliense* (CMM 4015) and *L. jatrophicola* (CMM 3610). Three isolates grouped with *L. pseudotheobromae* (CBS 116459). Two isolates grouped with *L. euphorbicola* (CMM 3609) and *L. gonubiensis* (CBS 115812), respectively. Ten isolates did not cluster with any known *Lasiodiplodia* species (Fig 2). The *Neofusicoccum* combined ITS, *EF1-α* and β-tubulin dataset (which comprised two isolates from this study and 18 sequences originating from GenBank) comprised 1830 characters, with 1370 constant, 427 variables and parsimony uninformative and

366 parsimony informative. The two isolates clustered with *Neofusicoccum batangarum*(CBS 124924). The dataset of combined rDNA ITS and EF1-α sequence data for *Pseudofusicoccum* species comprised 17 isolates including the outgroup, with comprised total of 1411 characters, of which 1187 were constant, 162 were variable and parsimony uninformative and 137 parsimony informative. All 16 isolates clustered with *Pseudofusicoccum stromaticum*(CMW 13434).

3.2 Morphology and cultural characteristics

All the isolates that were identified based on the phylogenetic analyses using the combined data, comprising 23 *Lasiodiplodia* isolates [*L. brasiliense* (2), *L. euphorbicola* (1), *L. gonubiensis* (1), *L.iraniensis* (5), *L. jatrophicola* (2), *L. gravistriata* (5), *L. pseudotheobromae* (2), and *L. theobromae* (5)] and the 10 fusicoccum-like isolates [*Neofusicoccum batangarum* (5) and *Pseudofusicoccum stromaticum* (5)] were characterized on the basis of colony morphology and conidial characteristics. Growth was rapid for all isolates on PDA, with mycelia covering the entire surface of the solid media. Aerial mycelium was initially white, then turning dark greenish-grey or greyish after 4–5 daysincubation at 25°C in the dark. For all isolates, structures of the asexual morph appeared within 2–4 weeks colonization ofPNA. Sexual structures were absent throughout the growth period. Whilst all species showed morphological features typical of the genus (Punithalingam 1976, 1980). The new species of *Lasiodiplodia* described here showed differences in conidial size to previously described species. The conidial dimensions of *L. gravistriata* were also outside the range previously documented in the literature for this species (Table 2).

Only *L. gravistriata* and *L. pseudotheobromae* grew at 5 °C and 10 °C. The optimum temperature for mycelia growth and mycelia growth rate differed significantly ($P \leq 0.05$) among

the Botryosphaeriaceae species (Table 3). The optimum growth temperature for *N. batangarum* (27.9 °C) was significantly lower than observed for *P. stromaticum* (32.3 °C), *L. brasiliense* (31.2 °C), *L. jatrophicola* (31.0 °C) and five additional species (*L. gravistriata*, *L. gonubiensis, L. theobromae*, *L. pseudotheobromae* and *L. iraniensis*) where temperatures varied from 30.1 °C to 30.7 °C (Table 3). The mycelia growth rates of *L. gravistriata* (69.6 mm/day) and *L. iraniensis* (64.0 mm/day) were significantly higher than the other seven species, which varied from 24.8 mm/day (*L. pseudotheobromae*) to 53.7 mm/day (*L. theobromae*).

3.3 Taxonomy

Lasiodiplodia gravistriata M.S.B. Netto & M.P.S. Câmara, sp. nov.(Fig 5)

MycoBank MB816925

Etymology: In reference to the pronounced longitudinal striations compared to most species.

Mycelium immersed or superficial, branched, septate, dark brown. Aerial mycelia becoming olivaceous grey to violaceous black at the surface and dark mouse grey to olivaceous black. *Colonies* reaching 60 mm on MEA after 2 days in the dark at 25 °C. Optimum temperature formycelia growth at 31.2 °C. *Ascomata* not seen. *Conidiomata* stromatic, pycnidial, produced on pine needles on WA within 2–4 weeks, immersed or superficial, dark brown to black, covered with mycelium, mostly uniloculate, solitary, globose, thick-walled, non-papillate with a central ostiole. *Paraphyses* hyaline, cylindrical, aseptate, rounded at apex. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, not proliferating,discrete, hyaline, smooth, thin-walled, cylindrical, 9–14 × 3–5 µm. *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, with granular content, rounded at apex, base mostly truncate, wall <2µm, becoming pigmented,

verruculose, ellipsoid to ovoid, 1-septate with longitudinal striations, 24.5–28.5 × 10.5–16µm (Δ=26.2 × 13.8, n=50).

Habitat: On *Anacardium humile*.

Known distribution: Minas Gerais state, Brazil.

Holotype: Brazil, Minas Gerais, Coração de Jesus, on *Anacardium humile* stems, 2013, coll. M. S. B. Netto, holotype URM 89942 dry culture and dry pycnidium produced on pine needles, ex-type culture URM 7360 = CMM 4564.

Notes —*Lasiodiplodia gravistriata* is closely related to *L. subglobosa*, although conidia of *L. gravistriata*, are longer and narrower than those of *L. subglobosa* (Table 2). *Lasiodiplodia gravistriata* differs from its closest phylogenetic neighbour, *L. subglobosa*, by unique fixed alleles in two genomic DNA loci, based on alignments of the separate loci deposited in TreeBASE as study S19242: rDNA ITS position 23(T); EF-α positions 50(T), 56(A), 167(GAP), 187(T) and 227 (C). *L. gravistriata* is also distinguished from *L. subglobosa* on the basis of conidial size and the prominently longitudinal striations in *L. gravistriata*.

3.4 Distribution of Botryosphaeriaceae species

Lasiodiplodia theobromae was the predominant species observed from on*Anacardium* spp. (66.7%), followed by *L. gravistriata* and *N. batangarum* (7.2%), *P. stromaticum* and *L. iraniensis* (6.5%), *L. brasiliense*, *L. jathrophicola* and *L. pseudotheobromae* (1.4%), *L. euphorbicola* and *L. gonubiensis* (0.7%). The overall distribution of these *Botryosphaeria* species differed among the Brazilian states sampled. *Lasiodiplodia theobromae* was found in five Brazilian states (Alagoas, Ceará, Minas Gerais, Pernambuco and Rio Grande do Norte). *Neofusicoccum batangarum* was found in four Brazilian states (Alagoas, Ceará, Pernambuco and Rio Grande do Norte). The new

356 species *L. gravistriata*, together with *L. euphorbicola* and *L. gonubiensis*, were found only in the
357 state of Minas Gerais (Fig. 1).
358
359 3.5Pathogenicity and virulence on detached green shoots
360
361 All isolates of Botryosphaeriaceae were found to be pathogenic on *A. occidentale* (cv. BRS 274),
362 with inoculated detached green shoots showing visible lesions 10 days after inoculation. Dark
363 brown necrotic lesions were observed both on the tissue surface and internally, advancing
364 upwards and downwards from the point of inoculation. Significant differences ($P \leq 0.05$) in
365 internal lesion lengths were apparent between the examined isolates for the different
366 Botryosphaeriaceae species.
367 The longest lesions were produced by *N. batangarum* (27.0 mm) and *L. iraniensis* (26.2
368 mm), which were thus considered to be the most aggressive species in this study. By contrast, the
369 shortest lesions were observed for the least aggressive species, *L. euphorbicola* and *L.*
370 *pseudotheobromae* (<12 mm), with lesion size differing significantly from *N. batangarum* and *L.*
371 *iraniensis*. The other species (*L. brasiliense*, *L. gonubiensis*, *L. jathrophicola*, *L. gravistriata*, *L.*
372 *theobromae*, and *P. stromaticum*) displayed intermediate aggressiveness, with lesions varying in
373 length from 15.5 mm to 22.2 mm(Fig 6).
374
375 **4 Discussion**
376
377 In this study, we describe the species of Botryosphaeriaceae which are associated with gummosis
378 of *Anacardium* in Brazil. Data was based on morphological, molecular and pathogenicity testing
379 for a large set of isolates from different growing regions across the country. Ten species of

Botryosphaeriaceae were identified associated with gummosis on *Anacardium* spp.: *L. brasiliense*, *L. euphorbicola*, *L. gonubiensis*, *L. iraniensis*, *L. jatrophicola*, *L. gravistriata*, *L. pseudotheobromae*, *L. theobromae*, *N. batangarum* and *Pseudofusicoccum stromaticum*. With the exception of *L. theobromae*, all the other species described represent first reports on *Anacardium*.

Following identification, *L. theobromae* was concluded to be both the most frequent species associated with gummosis of *Anacardium*, as well as was the most widespread of all the Botryosphaeriaceae species (Fig. 1). Similar findings were observed for this species when associated with dieback and stem-end rot of mango (Marques et al. 2013a), stem-end rot of papaya (Netto et al. 2014) and grapevine dieback (Correia et al. 2016) across the semi-arid region of north-eastern Brazil. Such data supports previous descriptions of this species as a pantropical pathogen occurring on a diverse range of hosts plants (Punithalingam 1980; Burgess et al. 2006). In recent years, a number of species have been described in the *L. theobromae* complex globally, which likely reflects the increased employment of DNA sequence data, as well as sampling of relatively unexplored areas, including Australia (Pavlic et al. 2008), Brazil (Marques et al. 2013a; Machado et al. 2014;Nettoet al. 2014;Correia et al. 2016; Coutinho et al. 2016), Egypt (Ismail et al. 2012), Iran (Abdollahzadeh et al. 2010), Italy, Alergia and Tunisia (Linaldeddu et al. 2015), Oman and The United Arab Emirates (Al-Sadiet al. 2013), Thailand (Trakunyingcharoen et al. 2015) and Venezuela (Burgess et al. 2006).

Lasiodiplodia gravistriata is recognized as a new species in the genus *Lasiodiplodia*, which is phylogenetically closely related to *L. subglobosa*. However, five nucleotides in the *EF1-α* gene distinguish *L. gravistriata* from *L. subglobosa*. The cashew derived isolates of *L. gravistriata* formed a clade strongly supported in both the Bayesian (1.00) and in the ML (98%) analyses. *Lasiodiplodia gravistriata* can also be distinguished from *L. subglobosa* on the basis of both conidial size, which are longer and narrower than those typical of *L. subglobosa* (Machado

et al. 2014), and the prominent longitudinal striations in which occur in the conidia of this species. This new species was also one of the most frequently occurring as pathogen of *A.humile* in Brazil (Fig. 1), and did not differ in virulence from *L. brasiliense*, *L. iraniensis*, *L. gonubiensis*, *L. jatrophicola*, *L. theobromae* and *N. batangarum*.

Lasiodiplodia iraniensis was described from Iran on the susceptible hosts *Mangifera indica* and *Juglans* sp. (Abdollahzadeh et al. 2010), then subsequently reported in Brazil associated with mango (Marques et al. 2013a). This current work represents the first report of this species as causing gummosis in *Anacardium* spp. Although *L. iraniensis* was only moderately prevalent, it wasone of the most aggressive species observed following inoculation of detached green cashew shoots and therefore *L. iraniensis* should be regarded as a potential threat to this crop. These findings contrast those reported by Marques et al. (2013a), where *L. iraniensis* isolates produced smaller lesions on mango fruits than other species.

Lasiodiplodia brasiliense was first described in Brazil in 2014 causing stem-end rot of papaya (Netto et al. 2014).Its identification in the present study represents the first report of this species causing gummosis on *Anacardium*. Although most closely related to *L. viticola* based on phylogenetic analyses, conidia in *L. brasiliense* are longer and wider than those typical of *L. viticola.* Genomic DNA for this species also differed from *L. viticola*, with specific alleles at ITS nucleotide positions: 2(C), 12(G), 42(T), 46(A), 50(C), 56(GAP), 62(GAP), 75(GAP), 123(T), and 370(A). *L. brasiliense* was pathogenic on detached green cashew shoots and one of the least prevalent species associated with *Anacardium*. This contrasts to reports for this species as being a prevalent species associated with stem-end rot of papaya (Netto et al. 2014) and grapevine dieback (Correia et al. 2016) in the Brazilian São Francisco Valley region.

Prior to this study, *L. jatrophicola* and *L. euphorbicola* were described on physic nut in Brazil (Machado et al. 2014). *L. jatrophicola* is phylogenetically closely related, yet clearly

distinct from *L. iraniensis*, with larger conidia and shorter paraphyses typical of this species. *L. euphorbicola* is phylogenetically closely related to *L. parva*. These two taxa share morphological characteristics, although paraphyses are smaller in *L. euphorbicola* (Machado et al. 2014). In this study, *L. jatrophicola* was the one of least prevalent species (0.7%), and only moderately aggressiveness on *A. occidentale*. Similary *L. euphorbicola* was rarely encountered and showed only low levels of aggressiveness. A similar result was found by Correia et al. (2016), where *L. jatrophicola* and *L. euphorbicola* isolates displayed moderate and low levels of aggressiveness, respectively, on grapevines. This study provides both the first report of these species causing gummosis in *Anacardium* anywhere in the world, and identification of a third host of these species in Brazil.

Lasiodiplodia pseudotheobromae was also identified on *A. occidentale* in Brazil (Coutinho et al. 2016). Globally, this species, like *L. theobromae*, has a wide distribution and a wide host range, having been reported on hosts that include *Acacia, Citrus, Coffea*, *Gmelina* and *Rosa* species (Alves et al. 2008; Phillips et al. 2008; Abdollahzadeh et al. 2010; Perez et al. 2010; Sakalidis et al. 2011; Ismail et al. 2012; Slippers et al. 2014; Trakunyingcharoen et al. 2015). In Brazil, *L. pseudotheobromae* has so far been reported on mango (Marques et al. 2013a), physic nut (Machado et al. 2014), papaya (Netto et al. 2014), and grapevine (Correia et al. 2016). Morphologically, this species differs from *L. theobromae* in terms of conidial dimension and form, with conidia generally being larger, more ellipsoid and with less pronounced tapering towards the base (Alves et al. 2008). In terms of pathogenicity, *L. pseudotheobromae* was the most aggressive species on mango in Australia (Sakalidis et al. 2011), and Egypt (Ismail et al. 2012) as well as on *Terminalia catappa* (Combretaceae) in Cameroon (Begoude et al. 2010). Here, by contrast, *L. pseudotheobromae* was only moderately aggressiveness on cashew shoots,

451 and was reported on mango (Marques et al. 2013a), papaya (Netto et al. 2014), grape (Correia et
452 al. 2016) and cashew (Coutinho et al. 2016) in Brazil.

453 *Lasiodiplodia gonubiensis* was the first species for the genus to be reported on native trees
454 in South Africa, where it was encountered as an endophytic fungus of *Syzygium cordatum* (Pavlic
455 et al. 2004). The present study represents the first report of *L. gonubiensis* in Brazil and causing
456 gummosis on *Anacardium*. Here, this species was isolated infrequently on *A. occidentale*, with
457 aggressiveness on this host similar to levels observed for *L. brasiliense*, *L. jatrophicola*, *L.*
458 *theobromae* and *P. stromaticum*.

459 The application of molecular tools has facilitated the recognition of species in the
460 Botryosphaeriaceae, with numerous species recently described on native vegetation, and
461 economically important crops (Liu et al. 2012; Phillips et al. 2013). In this work, two additional
462 genera were identified as associated with gummosis on *Anacardium*: *Neofusicoccum batangarum*
463 and *Pseudofusicoccum stromaticum*. Information on *N. batangarum* is scarce given it was only
464 recently described (Begoude et al. 2010). This fungus was reported as an endophytic fungus on *T.*
465 *catappa* in Cameroon (Shetty et al. 2011). As this fungus can cause pathogenic reactions on *T.*
466 *catappa* under greenhouse conditions (Begoude et al. 2010), however, the fungus may therefore
467 switch from an endophytic life style in plant organs to aggressive pathogen, when environmental
468 conditions become unfavorable for the tree host.In this study, this species was frequently isolated
469 from *A. accidentale* and produced the largest lesions on detached cashew green shoots.

470 *P. stromaticum* was also an abundant species, indicating this genus to be more widely
471 distributed than earlier considered. Previously, this species has been found on *Eucalyptus* and
472 *Acacia* spp. in Venezuela (Mohali et al. 2006; 2007) and on mango in Brazil (Marques et al.
473 2012). Pavlic et al. (2008) also reported *Pseudofusicoccum* spp. on native hosts plants in
474 undisturbed areas in Australia, providing evidence for these species to be native to the country.

Our study contradicts this suggestion, with *P. stromaticum* found in Brazil on native cashew (*Anacardium othonianum*).

Optimum growth temperatures of Botryosphaeriaceae species varied from 27.9 °C to 32.3 °C. *L. gravistriata* and *L. pseudotheobromae* also grew at as low as 5 °C and 10 °C. Such growth of *L. pseudotheobromae* at low temperatures contradicts a number of previous studies (Abdollahzadehet al. 2010; Marques et al. 2013a;Netto et al. 2014), although other studies (Alves et al. 2008; Ismail et al. 2012) clearly provide data showing that these *Lasiodiplodia* species have a much wider temperature range than was previously assumed.

In summary, this paper reports 10 species of Botryosphaeriaceae associated with *Anacardium* in Brazil. *L. theobromae*, although the species most frequently observed on this host, is neither the exclusive etiologic agent nor the most aggressive species. All species showed potential to cause cashew gummosis, with the species *N. batangarum* and *L. iraniensis* identified as the most aggressive species. Continued investigation of the epidemiology and impact of gummosis on cashew production is necessary, together with improved understanding of the ecology, distribution, host range and fungicide sensitivity of all Botryosphaeriaceae species reported on *Anacardium*. Such information will be crucial for the development of novel gummosis control strategies and genetic improvement of cashew for resistance to biotic stress.

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500

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Table 1 –Isolates of Botryosphaeriaceae species used in this study.

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

Table 3 –Optimum temperature for mycelial growth and mycelial growth rate at 30 °C of *Lasiodiplodia* species associated with gummosis of *Anacardium* in Brazil.

Fig 1 –Collection sites of Botryosphaeriaceae isolates associated with gummosis of *Anacardium* in seven different states of Brazil. Circles represent association frequency of each species with plants exhibiting symptoms of gummosis in each orchard sampled, *n* is the number of isolates analyzed in each orchard.

Fig 2 –Maximum likelihood tree resulting from the combined analysis of ITS and EF1-α sequence data. ML Bootstrap support values and Bayesian posterior probability scores are given at the nodes. The tree was rooted to *Diplodia mutila* and *Diplodia seriata*. Ex-type isolates are in bold. The scale bar represents the number of substitutions per site.

Fig 3 –Maximum likelihood tree resulting from the combined analysis of ITS, EF1-α and β-tubulin sequence data. ML Bootstrap support values and Bayesian posterior probability scores are given at the nodes. The tree was rooted to *Neofusicoccum macroclavatum*. Ex-type isolates are in bold. The scale bar represents the number of substitutions per site.

Fig 4 – Maximum likelihood tree resulting from the combined analysis of ITS and EF1-α sequence data. ML Bootstrap support values and Bayesian posterior probability scores are given at the nodes. The tree was rooted to *Botryosphaeria dothidea*. Ex-type isolates are in bold. The scale bar represents the number of substitutions per site.

Fig 5 –*Lasiodiplodia gravistriata* (CMM4564) a–b. Conidiogenous cells giving rise to conidia; c. mature conidia in two different focal planes to show the longitudinal striations; d. brown, 1-septate conidia. — Scale bars: a-d= 10 µm.

Fig 6 –Mean internal lesion lengths (mm) caused by 10 Botryosphaeriaceae species associated with cashew gummosis in Brazil, 10 days after inoculation with mycelium colonized agar plugs onto detached green shoots of *Anacardium occidentale* (cv. BRS 274). 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≤0.05).

Table 1 – Isolates of Botryosphaeriaceae species used in this study.

Taxon	Isolate code [a]	Host	Location	Collector	GenBank Accession No. [b]		
					ITS	EF1-α	β-tubulin
Botryosphaeria dothidea	CMW 8000	*Prunus* sp.	Switzeland	B. Slippers	AY236949	AY236898	
B. dothidea	CBS 110302*	*Vitis vinifera*	Portugal	A. J. L. Phillips	AY259092	AY573218	
Diplodia mutila	CBS 136015	*Populus alba*	Portugal	A. Alves	KJ361838	KJ361830	
D. seriata	CBS 112555*	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259094	AY573220	
Lasiodiplodia brasiliense	CMM 4011	*Mangifera indica*	Brazil	M.W. Marques	JX464074	JX464037	
L. brasiliense	CMM 4015*	*Mangifera indica*	Brazil	M.W. Marques	JX464063	JX464049	
L. brasiliense	CMM 4469	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325574**	**KT325580**	
L. brasiliense	CMM 4470	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325575**	**KT325579**	
L. citricola	CBS 124707*	*Citrus* sp.	Iran	J. Abdollahzadeh & A. Javadi	GU945354	GU945340	
L. citricola	CBS 124706	*Citrus* sp.	Iran	J. Abdollahzadeh & A. Javadi	GU945353	GU945339	
L. crassispora	CMW 13448	*Eucalyptus urophylla*	Venezuela	S. Mohali	DQ103552	DQ103559	
L. crassispora	WAC 12533*	*Santalum album*	Australia	T.I. Burgess/B. Dell	DQ103550	DQ103557	
L. egyptiacae	CBS 130992*	*Mangifera indica*	Egypt	A.M. Ismail	JN814397	JN814424	
L. egyptiacae	BOT-29	*Mangifera indica*	Egypt	A.M. Ismail	JN814401	JN814428	

L. euphorbicola	CMM 3651	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234553	KF226711
L. euphorbicola	CMM 3609*	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234543	KF226689
L. euphorbicola	CMM 3652	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234554	KF226715
L. euphorbicola	CMM 4473	*Anacardium humile*	Brazil	M.S.B. Netto	**KT325568**	**KT325581**
L. exigua	CBS 137785*	*Retama raetam*	Tunisia	B.T. Linaldeddu	KJ638317	KJ638336
L. exigua	BL 184	*Retama raetam*	Tunisia	B.T. Linaldeddu	KJ638318	KJ638337
L. gilaniensis	CBS 124704*	Unknown	Iran	J. Abdollahzadeh & A. Javadi	GU945351	GU945342
L. gilaniensis	CBS 124705	Unknown	Iran	J. Abdollahzadeh & A. Javadi	GU945352	GU945341
L. gonubiensis	CBS 115812*	*Syzigium cordatum*	South Africa	D. Pavlic	AY639595	DQ103566
L. gonubiensis	CBS 116355	*Syzigium cordatum*	South Africa	D. Pavlic	AY639594	DQ103567
L. gonubiensis	CMM 4468	*Anacardium humile*	Brazil	M.S.B. Netto	**KT325571**	**KT325584**
L. gravistriata	CMM 4564*	*Anacardium humile*	Brazil	M.S.B. Netto	**KT250949**	**KT250950**
L. gravistriata	CMM 4565	*Anacardium humile*	Brazil	M.S.B. Netto	**KT250947**	**KT266812**
L. gravistriata	CMM 4566	*Anacardium humile*	Brazil	M.S.B. Netto	**KT250946**	**KT266813**

L. gravistriata	CMM 4570	*Anacardium humile*	Brazil	M.S.B. Netto	**KT250948**	**KT266814**
L. hormozganensis	CBS 124709*	*Olea* sp.	Iran	J. Abdollahzadeh & A. Javadi	GU945355	GU945343
L. hormozganensis	CBS 124708	*Mangifera indica*	Iran	J. Abdollahzadeh & A. Javadi	GU945356	GU945344
L. iraniensis	CBS 124710*	*Mangifera indica*	Iran	N. Khezrinejad	GU945346	GU945334
L. iraniensis	CBS 124711	*Juglans* sp.	Iran	A. Javadi	GU943447	GU945335
L. iraniensis	CMM 4557	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325573**	**KT325586**
L. iraniensis	CMM 4559	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325572**	**KT325585**
L. jatrophicola	CMM 3610*	*Jatropha 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 4471	*Anacardium occidentale*	Brazil	M.S.B.Netto	**KT325569**	**KT325582**
L. jatrophicola	CMM 4472	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325570**	**KT325583**
L. macrospora	CMM 3833*	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234557	KF226718
L. mahajangana	CBS 124927*	*Terminalia catappa*	Madagascar	J. Roux	FJ900597	FJ900643
L. mahajangana	CBS 124925	*Terminalia catappa*	Madagascar	J. Roux	FJ900595	FJ900641
L. margaritaceae	CBS 122519*	*Adansonia gibbosa*	Australia	T.I. Burgess	EU144050	EU144065
L. margaritaceae	CBS 122065	*Adansonia gibbosa*	Australia	T.I. Burgess	EU144051	EU144066
L. mediterranea	CBS 137783*	*Quercus ilex*	Italy	B.T. Linaldeddu	KJ638312	KJ638331

L. mediterranea	CBS 137784	*Vitis vinifera*	Italy	S. Serra	KJ638311	KJ638330
L. mediterranea	ALG 36	*Citrus siensis*	Algeria	A. Berraf-Tebbal	KJ638314	KJ638333
L. missouriana	CBS 128311*	*Vitis* sp.	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288225	HQ288267
L. missouriana	CBS 128312	*Vitis* sp.	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288226	HQ288268
L. parva	CBS 45678*	Cassava-field soil	Colombia	O. Rangel	EF622083	EF622063
L. parva	CBS 49578	Cassava-field soil	Colombia	O. Rangel	EF622084	EF622064
L. plurivora	CBS 120832*	*Prunus salicina*	South Africa	U. Damm	EF445362	EF445395
L. pseudotheobromae	CBS 116459*	*Gmelina arborea*	Costa Rica	J. Carranza-Velásquez	EF622077	EF622057
L. pseudotheobromae	IRAN1518C	*Citrus* sp.	Iran	J. Abdollahzadeh & A. Javadi	GU973874	GU973866
L. pseudotheobromae	CMM 4474	*Anacardium humile*	Brazil	M.S.B. Netto	**KT728914**	**KT882611**
L. pseudotheobromae	CMM 4475	*Anacardium humile*	Brazil	M.S.B. Netto	**KT728915**	**KT882612**
L. pyriformis	CMW 25414*	*Acacia mellifera*	Namibia	F.J.J. van der Walt & J. Roux	EU101307	EU101352
L. pyriformis	CMW 25415	*Acacia mellifera*	Namibia	F.J.J. van der Walt & J. Roux	EU101308	EU101353
L. rubropurpurea	CBS 118740*	*Eucalyptus grandis*	Australia	T.I. Burgess/G. Pegg	DQ103553	EU673304
L. rubropurpurea	WAC 12536	*Eucalyptus grandis*	Australia	T.I. Burgess/G. Pegg	DQ103554	DQ103572

L. subglobosa	CMM 3872*	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234558	KF226721
L. subglobosa	CMM 4046	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234560	KF226723
Lasiodiplodia sp.	CPC 22800	*Mangifera indica*	Thailand	T. Trakunyingcharoen	KJ193643	KJ193687
L. thailandica	CPC 22755	*Phyllanthus acidus*	Thailand	T. Trakunyingcharoen	KM00633	KM006464
L. thailandica	CPC 22795	*Mangifera indica*	Thailand	T. Trakunyingcharoen	KJ19367	KJ193681
L. theobromae	CBS 16496*	Fruit along coral reef coast	New Guinea	A. Aptroot	AY64025	AY640258
L. theobromae	CMM 0310	*Vitis vinifera*	Brazil	M. A. Silva	KJ41790	KJ417880
L. theobromae	CMM 0384	*Vitis vinifera*	Brazil	M. A. Silva	KJ417904	KJ417876
L. theobromae	CMM 2269	*Carica papaya*	Brazil	J.H.A. Monteiro	KC484821	KC481585
L. theobromae	CMM 4499	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325578**	**KT325587**
L. theobromae	CMM 4508	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325576**	**KT325588**
L. theobromae	CMM 4513	*Anacardium occidentale*	Brazil	M.S.B. Netto	**KT325577**	**KT325589**
L. venezuelensis	CBS 118739*	*Acacia mangium*	Venezuela	S. Mohali	DQ103547	DQ103568
L. venezuelensis	WAC 12540	*Acacia mangium*	Venezuela	S. Mohali	DQ103548	DQ103569

L. viticola	CBS 128313	*Vitis vinifera*	USA	R.D. Cartwright & W.D. Gubler	HQ288227	HQ288269	
L. viticola	UCD 2604MO	*Vitis vinifera*	USA	K. Striegler & W.D. Gubler	HQ288228	HQ288270	
Neofusicoccum batangarum	CBS 124924*	*Terminalia catappa*	Africa	D. Begoude/J. Roux	FJ900607	FJ900653	FJ900615
N. batangarum	CBS 124923	*Terminalia catappa*	Africa	D. Begoude/J. Roux	FJ900608	FJ800654	FJ900616
N. batangarum	CMM 4547	*Anacardium othonianum*	Brazil	M.S.B. Netto	**KT728916**	**KT728920**	**KT728912**
N. batangarum	CMM 4553	*Anacardium othonianum*	Brazil	M.S.B. Netto	**KT728917**	**KT728921**	**KT728913**
N. brasiliense	CMM 1269*	*Mangifera indica*	Brazil	M.W. Marques	JX513629	JX513609	KC794932
N. brasiliense	CMM 1285	*Mangifera indica*	Brazil	M. W. Marques	JX513628	JX513608	KC794030
N. cordaticola	CBS 123634*	*Syzigium cordatum*	South Africa	D. Pavilic	EU821898	EU821868	EU821838
N. cordaticola	CBS 123635	*Syzigium cordatum*	South Africa	D. Pavilic	EU821903	EU821843	EU821843
N. kwambonambiense	CBS 123639*	*Eucalyptus grandis*	South Africa	D. Pavilic	EU821900	EU821870	EU821840
N. kwambonambiense	CBS 123641	*Eucalyptus grandis*	South Africa	D. Pavilic	EU821949	EU821889	EU821859
N. macroclavatum	WAC 12445*	*Eucalyptus globulus*	Australia	T.I. Burguess	DQ093197	DQ093218	DQ093207
N. macroclavatum	WAC 12446	*Eucalyptus globulus*	Australia	T.I. Burguess	DQ093219	DQ093219	DQ093208
N. occulatum	CBS 128008*	*Eucalyptus grandis* hybrid	Australia	T.I. Burgess	EU730103	EU339509	EU339472
N. occulatum	MUCC 296	*Eucalytus pellita*	Australia	T.I. Burgess	EU301034	EU339512	EU339475

N. parvum	PD 106	*Prunus dulcis*	USA	T.J. Michailides	GU251139	GU251271	KC794029
N. parvum	ATCC 58189*	*Malus sylvestris*	New Zealand	G.J. Samuels	AF243395	AY236883	AY236912
N. ribis	CBS 12126*	*Ribis rubrum*	USA	B. Slippers	AF241177	AY236879	AY236908
N. ribis	CMW 7772	*Ribis* sp.	USA	B. Slippers & G. Hudler	AY236925	AY236877	AY236906
N. umdonicola	CMW 14058*	*Syzigium cordatum*	South Africa	D. Pavilic	EU821934	EU821874	EU821844
N. umdonicola	CMW 14060	*Syzigium cordatum*	South Africa	D. Pavilic	EU821935	EU821875	EU821845
Pseudofusicoccum adansoniae	CBS 122054*	*Eucalytus* sp.	Australia	D. Pavilic	EF585532	EF585570	
P. adansoniae	WAC 13299	*Mangifera indica*	Australia	J. Ray	GU172404	GU172436	
P. ardesiacum	CBS 122062*	*Adansonia gibbosa*	Australia	D. Pavilic	EU144060	EU144075	
P. ardesiacum	WAC 13294	*Mangifera indica*	Australia	J. Ray	GU172405	GU172437	
P. artocarpi	CPC 22796	*Artocarpus heterophyllus*	Thailand	T. Trakunyingcharoen	KM006452	KM006483	
P. kimberleyense	CBS 122061*	*Ficus oppsita*	Australia	D. Pavilic	EU144059	EU144074	
P. kimberleyense	WAC 13293	*Mangifera indica*	Australia	J. Ray	GU172406	GU172438	
P. olivaceum	CBS 124939*	*Pterocarpus angolensis*	Africa	J. Roux	FJ888459	FJ888437	
P. olivaceum	CBS 124940	*Pterocarpus angolensis*	Africa	J. Mehl & J Roux	FJ888462	FJ888438	
P. stromaticum	CMW 13435	*Eucalyptus hybrid*	Venezuela	S. Mohali	DQ436935	DQ436936	
P. stromaticum	CMW 13434*	*Eucalyptus hybrid*	Venezuela	S. Mohali	AY693974	AY693975	
P. stromaticum	CMM 4541	*Anacardium othonianum*	Brazil	M.S.B. Netto	**KT728918**	**KT728922**	

P. stromaticum	CMM 4544	*Anacardium othonianum*	Brazil	M.S.B. Netto	**KT728919**	**KT728923**
P. violaceum	CBS 124936*	*Pterocarpus angolensis*	Africa	J. Mehl & J Roux	FJ888474	FJ888442
P. violaceum	CBS 124937	*Pterocarpus angolensis*	Africa	J. Roux	FJ888458	FJ888440

a *ALG* = A. Berraf-Tebbal, Université Saad Dahleb, Blida, Algeria; *ATCC* = American Type Culture Collection, Manassas, USA; *BL* = B.T. Linaldeddu, Università degli Studi di Sassari, Sassari, Italy; *BOT* = A. M. Ismail, Plant Pathology Research Institute, Giza, Egypt; *CBS* = Centraalbureau voor Schimmelcultures Utrecht, Netherlands; *CMM* = Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes", Universidade Federal Rural de Pernambuco, Recife, Brazil; *CMW* = Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; *CPC* = Culture Collection of P.W. Crous, housed at CBS; *MUCC* = Murdoch University Culture Collection, Perth, Australia; *IRAN* = Culture Collection of the Iranian Research Institute of Plant Protection, Tehran, Iran*; PD* = Culture Collection, University of California, Davis, USA*; STE-U* = Culture Collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; *UCD* = Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California, Davis, USA; *WAC* = Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia. * ex-type or ex-epitype. b Sequences derived in this study are emphasized in bold

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

Species	Conidia (µm)	L/W ratio	Reference
Lasidodiplodia brasiliense	22.7 – 29.2 × 11.7 – 17.0	1.8	Netto et al. 2014
L. citricola	(20-)24.5(-31) × (10.9-)15.4(-19)	1.6	Abdollahzadeh et al. 2010
L. crassispora	(27-)28.8(-33) × (14-)16(-17)	1.8	Burgess et al. 2006
L. egyptiacae	(17-)22(-27) × (11-)12(-13)	2	Ismail et al. 2012
L. euphorbiicola	15 - 23 × 9 -12	-	Machado et al. 2014
L. exigua	(19.6-)21.8(-24.3) × (10.8-)12.3(-13.3)	1.8	Linaldeddu et al. 2015
L. gilanensis	(25.2-)31(-38.8) × (14.4-)16.6(-19)	1.9	Abdollahzadeh et al. 2010
L. gonubiensis	(28-)33.8(-39) × (14-)17.3(-21)	1.9	Pavlic et al. 2004
L. gravistriata	(24.7-)26.2(-28.7) × (10.6-)13.8(-16.1)	1.9	Present study
L. hormozganensis	(15.3-)21.5(-25.2) × (11-)12.5(14)	1.7	Abdollahzadeh et al. 2010
L. iraniensis	(15.3-)20.7(-29.7) × (11-)13(-14)	1.6	Abdollahzadeh et al. 2010

L. jatrophicola	22 - 26 × 14 -17	-	Machado et al. 2014
L. lignicola	(15-)16(-17.5) × (8-)8.5 – 10.5(-11)	1.7	Phillips et al. 2013
L. macrospora	28 - 35 × 15 -17	-	Machado et al. 2014
L. mahajangana	(13.5-)17.5(-21.5) × (10-)11.5(-14)	1.4	Begoude et al. 2010
L. margaritacea	(12-)15.3(-19) × (10.-)11.4(-12.5)	1.3	Pavlic et al. 2008
L. mediterranea	(26.3-)30.6(-37) × (13.5-)16.1(-18)	1.9	Linaldeddu et al. 2015
L. missouriana	(16.1-)18.5(-21) × (8.1-)9.8(-11.8)	1.9	Úrbez-Torres et al. 2012
L. parva	(15.5-)20.2(-24.5) × (10-)11.5(-14.5)	1.8	Alves et al. 2008
L. plurivora	(22-)29.6(-35) × (13-)15.6(-18.5)	1.9	Damm et al. 2007

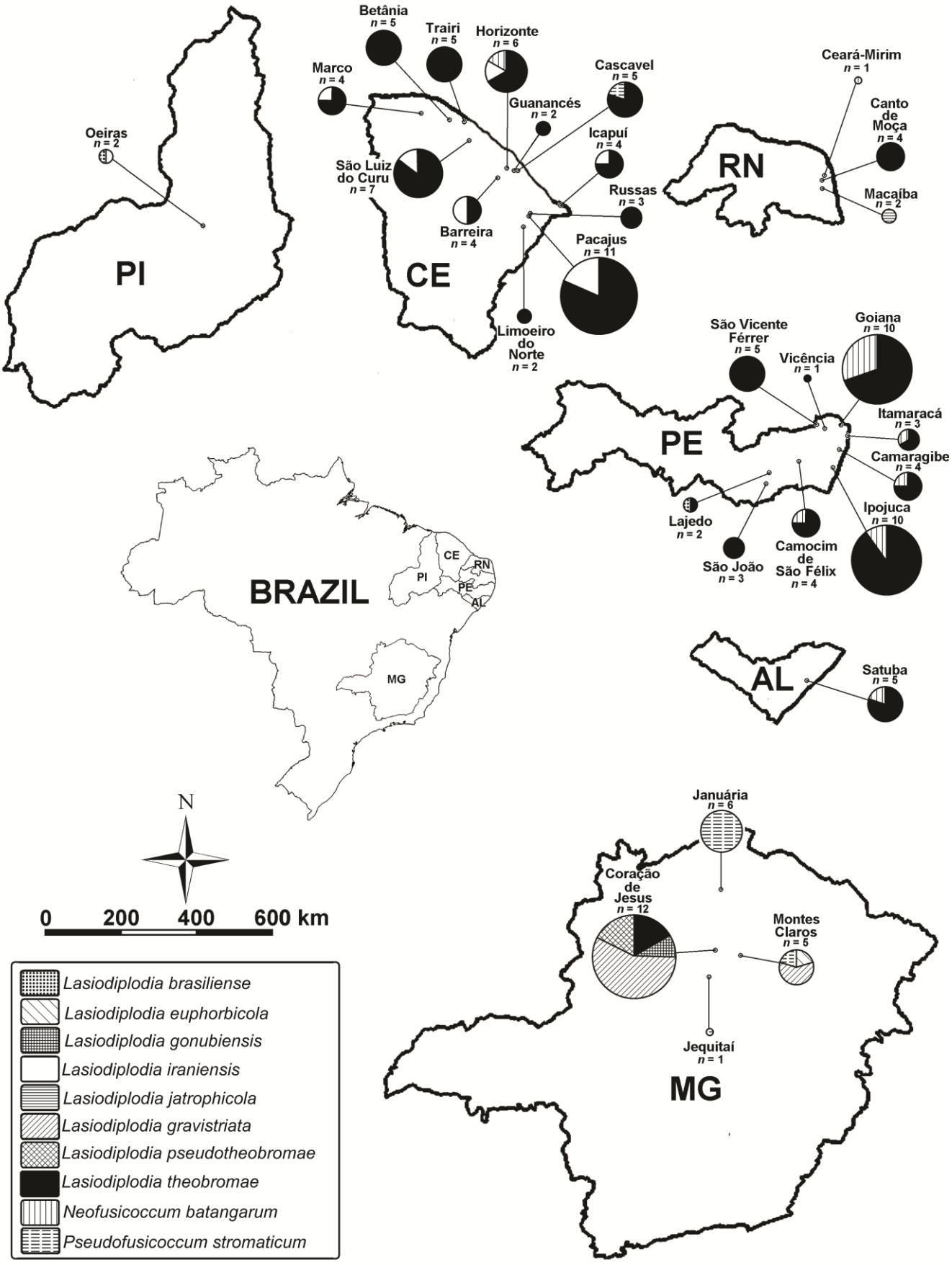
Table 2 (Continued)

Species	Conidia (µm)	L/W ratio	Reference
L. pseudotheobromae	(22.5-)28(-33) × (13.5-)16(-20)	1.7	Alves et al. 2008
L. pyriformis	(19-)21.5-25(28-) × (13.5)-15.5-19.5 (-21.5)	1.3	Slippers et al. 2014
L. rubropurpurea	(24-)28.2(-33) × (13-)14.6(-17)	1.9	Burgess et al. 2006
L. subglobosa	16 - 23 × 11 – 17	-	Machado et al. 2014
L. thailandica	(20-)22-25(-26) × (12-)13-15(-16)	-	Trakunyingcharoen et al. 2015
L. theobromae	(19-)26.2(-32.5) × (12-)14.2(-18.5)	1.9	Phillips et al. 2013
L. venezuelensis	(26-)28.4(-33) × (12-)13.5(-15)	2.1	Burgess et al. 2006
L. viticola	(16.8-)19.5(-22.9) × (7.9-)9.5(-10.7)	2.1	Úrbez-Torres et al. 2012

Table 3 – Optimum temperature for mycelial growth and mycelial growth rate at 30 °C of *Lasiodiplodia* species associated with gummosis of *Anacardium* in Brazil.

Species	n	Optimum temperature (°C) ± SE	Mycelial growth rate (mm/day) ± SE
Lasiodiplodia brasilense	2	31.2 ± 0.64 ab	42.0±3.11 bc
L. euphorbicola	1	30.6± 0.42 b	56.8±4.20 ab
L. gonubiensis	1	28.4 ± 0.81 cd	44.1±5.18 bc
L. gravistriata	5	30.7 ± 0.40 b	69.6±3.22 a
L. iraniensis	5	30.1 ± 0.41 bc	64.0±3.22a
L. jatrophicola	2	31.0 ± 0.60 ab	31.2±5.57 cd
L. pseudotheobromae	2	30.1 ± 0.59bc	24.8±3.15d
L. theobromae	5	30.5± 0.36 b	53.7±3.19b
Neofusicoccum batangarum	5	27.9± 0.52 d	32.0± 3.12cd
Pseudofusicoccum stromaticum	5	32.3± 0.34 a	33.1±2.76cd

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


PI
Oeiras
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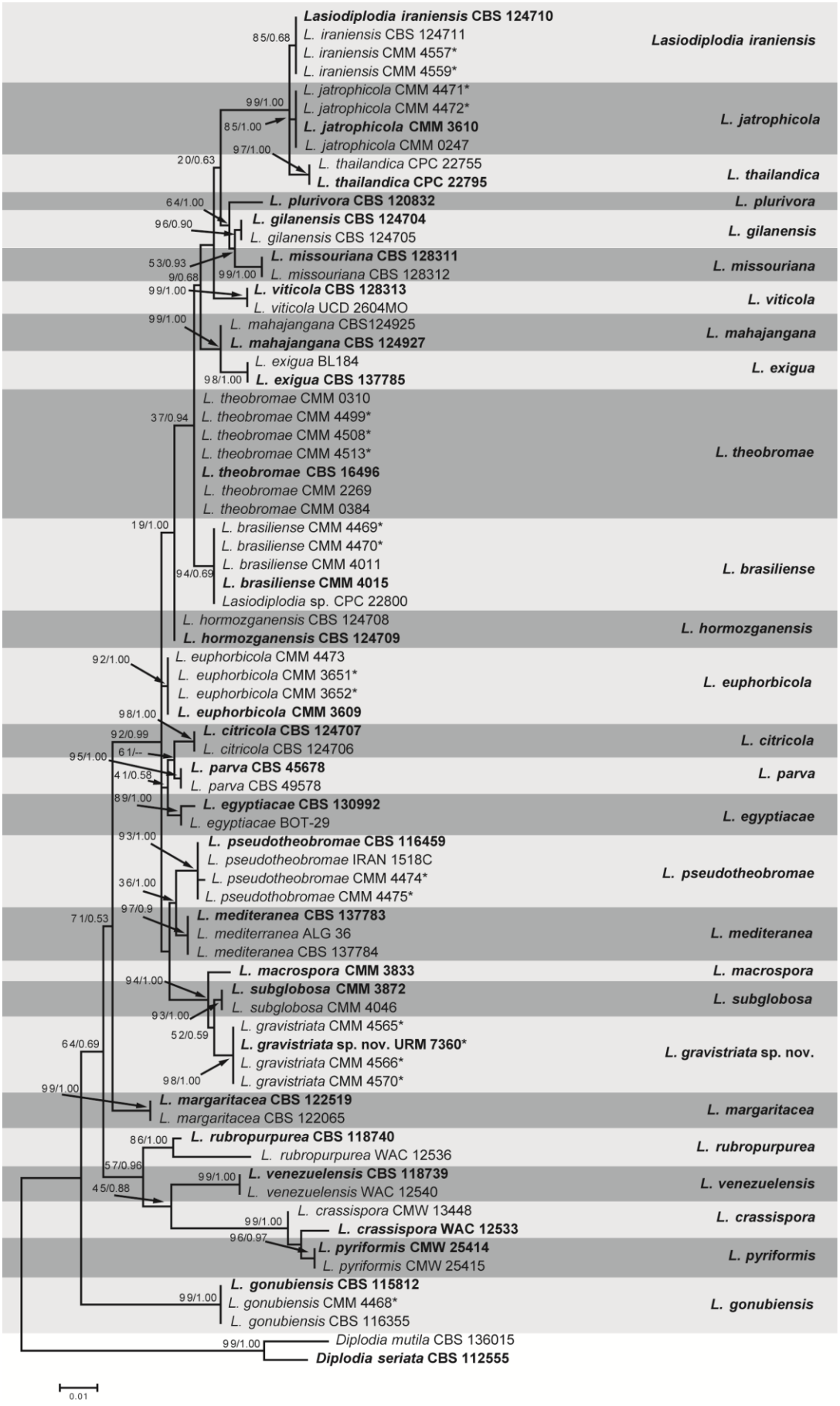
CE
Betânia
n = 5
Trairi
n = 5
Horizonte
n = 6
Marco
n = 4
Cascavel
n = 5
Guanancés
n = 2
Icapuí
n = 4
São Luiz
do Curu
n = 7
Russas
n = 3
Barreira
n = 4
Pacajus
n = 11
Limoeiro
do
Norte
n = 2
RN
Ceará-Mirim
n = 1
Canto
de
Moça
n = 4
Macaíba
n = 2
PE
São Vicente
Férrer
n = 5
Vicência
n = 1
Goiana
n = 10
Itamaracá
n = 3
Camaragibe
n = 4
Ipojuca
n = 10
Lajedo
n = 2
São João
n = 3
Camocim
de
São Félix
n = 4
AL
Satuba
n = 5
BRAZIL
CE
RN
PI
PE
AL
MG
N
0
200
400
600 km
MG
Januária
n = 6
Coração
de
Jesus
n = 12
Montes
Claros
n = 5
Jequitaí
n = 1
Lasiodiplodia brasiliense
Lasiodiplodia euphorbicola
Lasiodiplodia gonubiensis
Lasiodiplodia iraniensis
Lasiodiplodia jatrophicola
Lasiodiplodia gravistriata
Lasiodiplodia pseudotheobromae
Lasiodiplodia theobromae
Neofusicoccum batangarum
Pseudofusicoccum stromaticum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L. thailandica CPC 22755
L. thailandica CPC 22795
L. plurivora CBS 120832
L. gilanensis CBS 124704
L. gilanensis CBS 124705
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L. missouriana CBS 128312
L. viticola CBS 128313
L. viticola UCD 2604MO
L. mahajangana CBS124925
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L. exigua BL184
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L. theobromae CMM 0310
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L. theobromae CMM 0384
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L. brasiliense CMM 4015
Lasiodiplodia sp. CPC 22800
L. hormozganensis CBS 124708
L. hormozganensis CBS 124709
L. euphorbicola CMM 4473
L. euphorbicola CMM 3651*
L. euphorbicola CMM 3652*
L. euphorbicola CMM 3609
L. citricola CBS 124707
L. citricola CBS 124706
L. parva CBS 45678
L. parva CBS 49578
L. egyptiacae CBS 130992
L. egyptiacae BOT-29
L. pseudotheobromae CBS 116459
L. pseudotheobromae IRAN 1518C
L. pseudotheobromae CMM 4474*
L. pseudothobromae CMM 4475*
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L. mediterranea ALG 36
L. mediteranea CBS 137784
L. macrospora CMM 3833
L. subglobosa CMM 3872
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L. gravistriata CMM 4565*
L. gravistriata sp. nov. URM 7360*
L. gravistriata CMM 4566*
L. gravistriata CMM 4570*
L. margaritacea CBS 122519
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L. rubropurpurea CBS 118740
L. rubropurpurea WAC 12536
L. venezuelensis CBS 118739
L. venezuelensis WAC 12540
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L. gonubiensis CBS 116355
Diplodia mutila CBS 136015
Diplodia seriata CBS 112555
Lasiodiplodia iraniensis
L. jatrophicola
L. thailandica
L. plurivora
L. gilanensis
L. missouriana
L. viticola
L. mahajangana
L. exigua
L. theobromae
L. brasiliense
L. hormozganensis
L. euphorbicola
L. citricola
L. parva
L. egyptiacae
L. pseudotheobromae
L. mediteranea
L. macrospora
L. subglobosa
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L. gonubiensis
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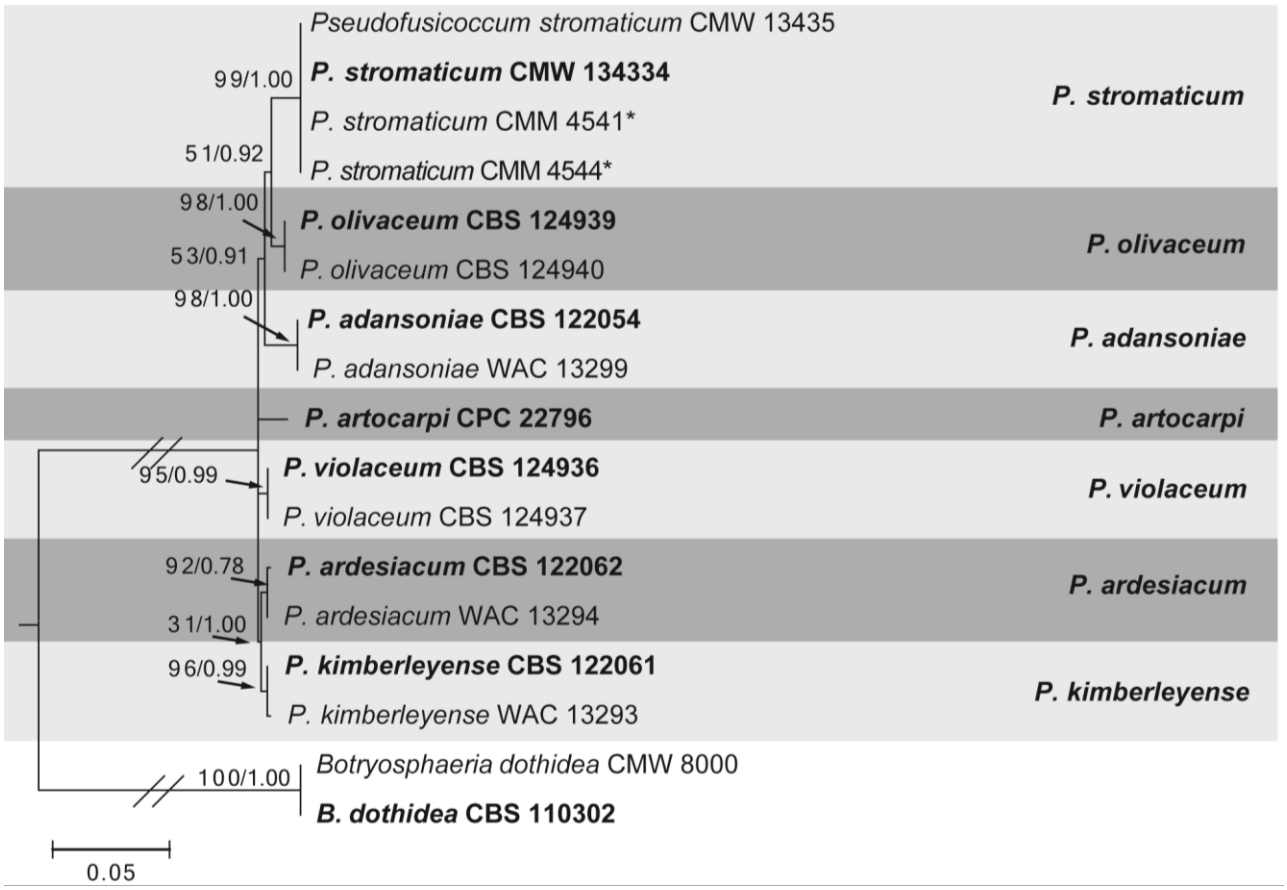
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P. artocarpi
95/0.99
P. violaceum CBS 124936
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P. violaceum CBS 124937
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P. ardesiacum WAC 13294
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96/0.99
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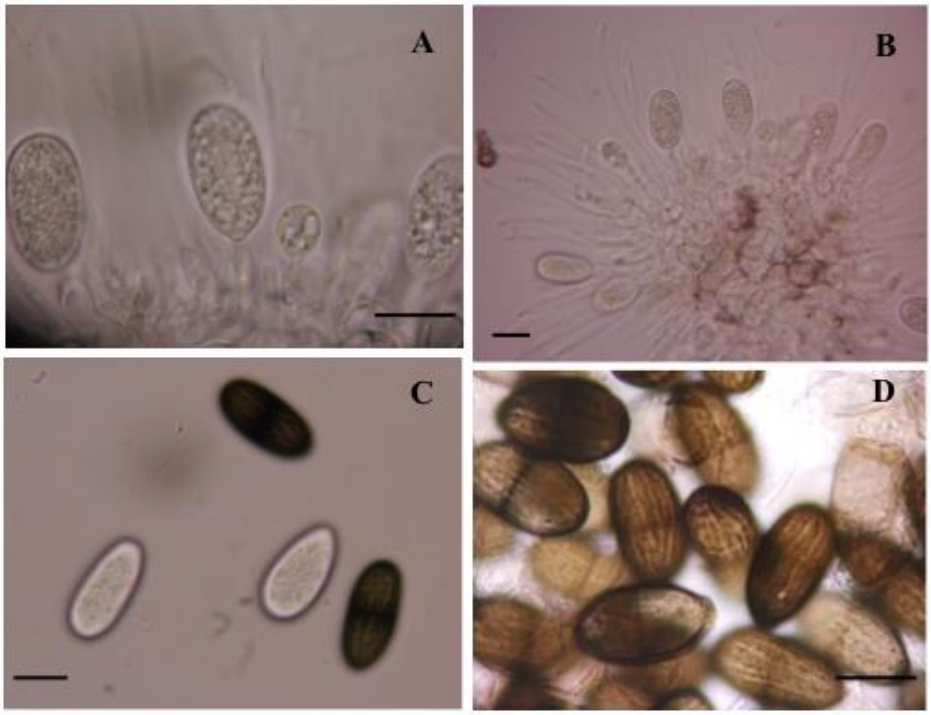
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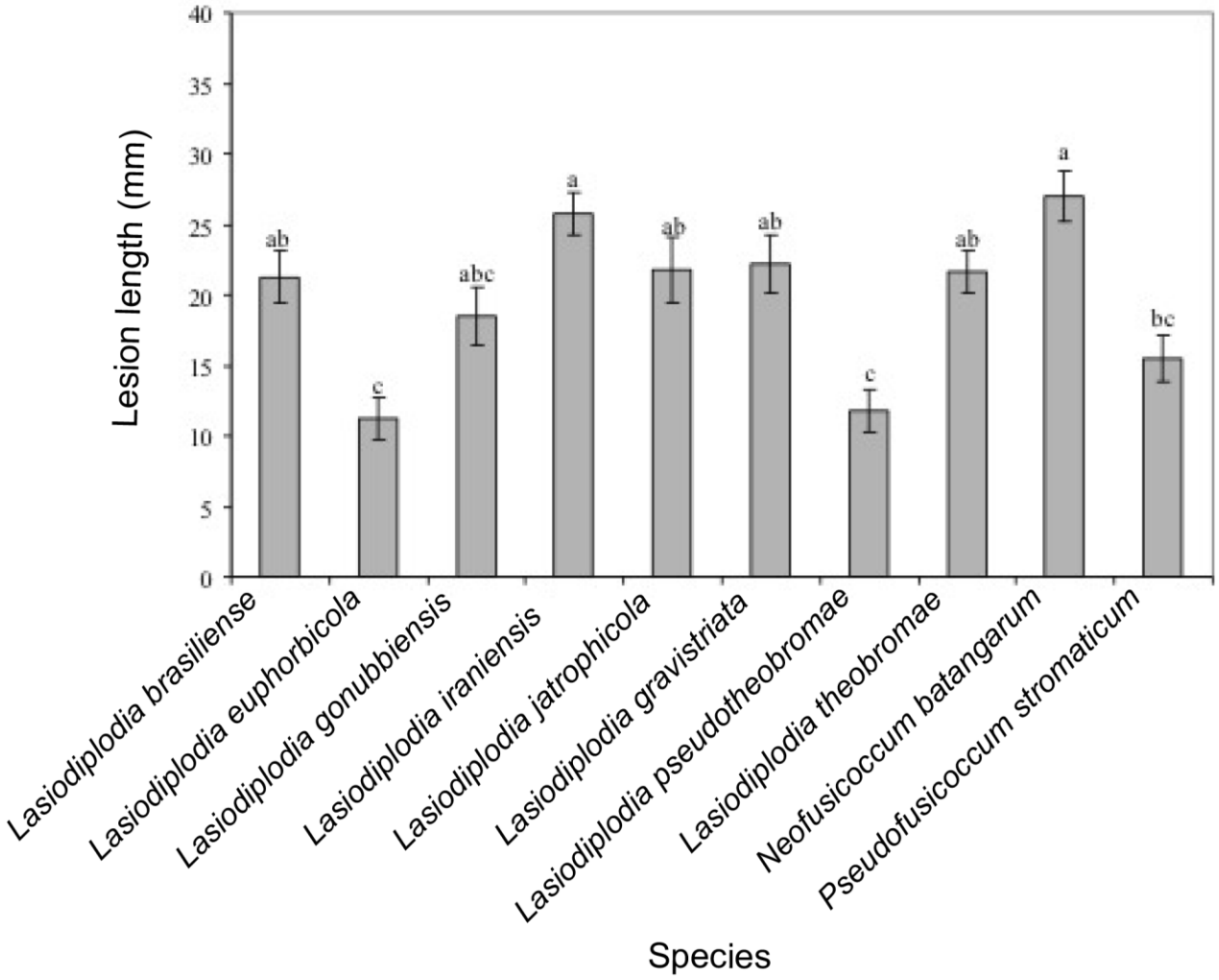
Lesion length (mm)
40
35
30
25
20
15
10
5
0
ab
c
abc
a
ab
ab
c
ab
a
bc
Lasiodiplodia brasiliense
Lasiodiplodia euphorbicola
Lasiodiplodia gonubbiensis
Lasiodiplodia iraniensis
Lasiodiplodia jatrophicola
Lasiodiplodia gravistriata
Lasiodiplodia pseudotheobromae
Lasiodiplodia theobromae
Neofusicoccum batangarum
Pseudofusicoccum stromaticum
Species

Capítulo III

Comparative epidemiology of Botryosphaeriaceae species from *Anacardium* in Brazil

Plant Pathology (Submetido)

Comparative epidemiology of Botryosphaeriaceae species from *Anacardium* in Brazil

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Resumo

Dentre as principais doenças que afetam o cajueiro no Brasil, a gomose, causada por fungos da família Botryosphaeriaceae, configura-se como séria ameaça à cultura, pela severidade dos seus sintomas e logo se tornou uma das doenças mais importantes para *Anacardium* spp. No presente trabalho, foram investigadas a virulência de sete espécies de Botryosphaeriaceae predominantes no Brasil (*Lasiodiplodia brasiliense*, *L. iraniensis*, *L. jatrophicola*, *L. gravistriada*, *L. theobromae*, *Neofusicoccum batangarum* e *Pseudofusicoccum stromaticum*) em relação a sete hospedeiros alternativos e o efeito da temperatura sobre o crescimento da colônia. Determinou-se também a influência de fungicidas no crescimento micelial dessas espécies. Em geral, os hospedeiros alternativos foram suscetíveis as sete espécies de Botryosphaeriaceae (abacate, banana, goiaba, manga, melão, mamão e maracujá) indicando que os patógenos não demonstram especificidade do hospedeiro, exceto *P. stromaticum* que só causou lesão em ramos de cajueiro. Esses resultados sugerem que esses hospedeiros alternativos podem servir como uma fonte potencial de inóculo. *L. gravistriata* apresentou crescimento nas temperaturas de 5°C e 10°C. As espécies Botryosphaeriaceae demonstraram uma

redução do crescimento micelial na presença dos fungicidas tiofanato-metilo, difenoconazole e azoxistrobina, independentemente do ingrediente ativo. A resposta de sensibilidade variou de acordo com o fungicida e as espécies de Botryosphaeriaceae.

Palavras-chave

Gama de hospedeiro, Fungicidas, Patogenicidade, *Anacardium occidentale*, Sensibilidade

Abstract

Gummosis caused by Botryosphaeriaceae species is considered one of the most important diseases for the cashew industry and soon became one of the most important diseases of *Anacardium* spp. This disease limits production, which directly affects the export of the nuts. In this study, we investigated the virulence of seven species of Botryosphaeriaceae prevalent in the orchards in Brazil (*Lasiodiplodia brasiliense*, *L. iraniensis, L. jatrophicola, L. gravistriada, L. theobromae, Neofusicoccum batangarum* and *Pseudofusicoccum stromaticum*) with respect to effect of temperature on colony growth and seven alternative hosts. We also determined the influence of fungicides on the mycelial growth of the fungi. In general, the alternative hosts were susceptible to the seven species of Botryosphaeriaceae (avocado, banana, guava, mango, melon, papaya and passion fruit) indicated that the pathogens do not demonstrate host specificity except *P. stromaticum*. Our results suggest that these alternative hosts may serve as a potential inoculum source. Only *L. gravistriata* grew at 5 °C and 10 °C. The Botryosphaeriaceae species demonstrated reduced mycelial growth in the presence of thiophanate-methyl, difenoconazole and azoxystrobin fungicides, regardless of the active ingredient. The sensitivity response varied according to the fungicide and the

48 species of Botryosphaeriaceae.

49 **Keywords**

50 Host range, Fungicides, Pathogenicity, *Anacardium occidentale*, sensitivity

Introduction

Brazil is the fifth-largest producer of cashew (*Anacardium occidentale* L.) worldwide, surpassed by Vietnam, Nigeria, India and Ivory Cost respectively. Annual production represents approximately 8 % of total world production, which in 2009 was equivalent to 2.804.266 million tons (FAOSTAT, 2012). Cashew is cultivated in northeastern Brazil, where the fruits are produced primarily for export. Among the wide range of diseases that affect cashew production in Brazil, gummosis have become increasingly important. The disease is caused by the fungus belonging to the Botryosphaeriaceae family (Netto et al., 2016). The gummosis appears as a serious threat to the culture. Disease symptoms include nutritional deficiency, wilting, leaf fall, rot dry branches and cankers in woody branches and trunk, usually accompanied by gum exudation and darkening of tissues (Freire et al., 2002).

Botryosphaeriaceous especies are known to wide distribution and more than 20 different species have been associated with dieback, stem-end rot, decline, gummosis, canker and occurs mainly in tropical and sub-tropical regions. They include a host range, mainly on woody hosts including fruits and forest trees (Phillips et al., 2013) but litle is known about the host specificity and relative importance of majority of species that have been described to date. Different species of Botryosphaeriaceae can be isolated from diseased and healthy tissues of the same host (Mohali; Slippers; Wingfield, 2006; Pavlic et al. 2007; Slippers et al. 2007).

Endophytic isolates obtained from healthy material have been shown to cause disease symptoms in greenhouse trials (Pavlic et al. 2007). Pathogenic activity of the Botryosphaeriaceae, as latent opportunistic pathogens, is expected to increase due to climate changes (Desprez-Loustau et al. 2006; Slippers et al., 2007). However, cultural characters (colony morphology, chromogenicity and temperature effects on mycelial

growth) have been used to distinguish closely related species within "*Botryosphaeria*" anamorphs (Alves et al. 2008).

The genus *Lasiodiplodia* currently comprises 40 species known (Phillips et al. 2013; Netto et al. 2016), of which eight have been reported from cashews: *L. brasiliense*, *L. euphorbicola*, *L. gonubiensis*, *L. gravistriata*, *L. iraniensis*, *L. jatrophicola*, *L. pseudotheobromae*, *L. theobromae* and two new genus *Neofusicoccum* and *Pseudofusicoccum* able to induce gummosis in the main area for cashews production (Netto et al. 2016). Until recently, *Lasiodiplodia theobromae* was attributed exclusively to gummosis occurs mainly in tropical and subtropical regions, causing severe damage in almost 500 host plants (Burgess et al. 2006). Little is known about the epidemiological aspects of the disease. Reports on the subject of gummosis are scarce and have contributed little to elucidating the problems related to the incidence and severity of the disease. Fungicides have become the most important means of controlling fungal pathogens (Pedraza et al. 2013). Several in vitro tests have determined the sensitivity of Botryosphaeriaceae to fungicides, however, there are no reports of in vitro tests for the control of Botryosphaeriaceae isolates obtained from cashew. Information on fitness components of sensitive fungi to fungicides is useful for preventing the development of resistance, and also to determine certain disease management strategies. With the recent identification of several new species of Botryosphaeriaceae, associated with this disease the objective of this study was: 1- to establish if these species are host-specific; 2- if they have wide range of hosts; 3- if they show differences in aggressive; 4- effect of temperature on mycelial growth of Botryosphaeriaceae species and 5- establish the effectiveness of the current fungicides used to control the disease. All these aspects have important implications in any strategy used for disease control and quarantine measures.

Materials and methods

Fungal isolates

Twenty-nine isolates of seven Botryosphaeriaceae species (*Lasiodiplodia brasiliense*, *L. iraniensis*, *L. jatrophicola*, *L. gravistriada*, *L. theobromae*, *Neofusicoccum batangarum* and *Pseudofusicoccum stromaticum*) obtained from *Anacardium* orchards in Brazil (Table 1) were used in the experiments. The isolates were identified through phylogenetic inference based on the complete sequence of the internal transcribed spacer (ITS) and partial sequences of elongation factor 1α (EF1-α) as described previously (Netto et al. 2016). The isolates were deposited in the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Pernambuco, Brazil). The stock cultures were maintained on potato dextrose agar (PDA) media slants (Acumedia, Lansing, MI, USA) at 5 °C in the dark.

2.5 Pathogenicity and aggressiveness of Botryosphaeriaceae species in cashew and a range of hosts

The Pathogenicity and aggressiveness of the seven Botryosphaeriaceae species was evaluated in detached green shoots of *A. occidentale* (cv. BRS 274) (Amponsahet *et al*. 2011; Correia *et al*. 2016) and seven potential alternative hosts, including papaya (cv. Golden), banana (cv. Pacovan) and guava (cv. Paluma) at stages 3, 5 and 5 of maturation (Ministério da Integração Nacional 2000), respectively, avocado (cv. Fortuna), mango (cv. Tommy Atkins), melon (cv. Amarelo), pasion fruit (cv. Azedo) at the time of harvest. These hosts were selected based on proximity of the crops to *Anacardium* orchards in Brazil. The fruits were washed using detergent and running water, disinfested by dipping in a solution of 1 % sodium hypochlorite (NaOCl) for 3

125 min and washed with sterile distilled water. After drying, the epidermis of each fruit
126 was perforated at a point in the middle to a depth of 3 mm, using a sterilized pin. For
127 each isolate, an agar plug (5 mm in diameter) containing mycelia was removed from the
128 margin of a colony (grown on PDA for 7 days) and transferred to each puncture point
129 on the epidermis. Fruits with PDA plugs that were not colonized by the fungus were
130 used as control. The fruits were placed in plastic trays lined with paper towels
131 moistened with sterile distilled water and covered with a plastic bag to maintain a
132 higher relative humidity. The trays were incubated in the dark at 25 °C. The plastic bags
133 and paper towels were removed after 48 h, and the fruits were maintained at 25 °C. The
134 hosts were inoculated separately, using a completely randomized experimental design
135 for each host, with four replicates per treatment (isolate) and two fruits per replicate.
136 The virulence of the isolates was evaluated 7 days after inoculation by measuring the
137 diameter of the lesions (cm) in two perpendicular directions and calculating the mean
138 lesion diameter. Differences in aggressiveness were determined by analysis of data with
139 a one-way ANOVA, with means compared by LSD test at the 5% significance level
140 using the program STATISTIX

141 **Effect of fungicides on the mycelial growth of the Botryosphaeriaceae species**

142 The sensitivity of the seven Botryosphaeriaceae species to fungicides was determined in
143 culture media supplemented with fungicide, and the mycelial growth was evaluated. For
144 each of the seven Botryosphaeriaceae species a pre-test was performed to identify the
145 required concentration of each fungicide. Commercial formulations of thiophanate-
146 methyl (Cercobin 700 WP, 700 g kg^{-1} of active ingredient (a.i.), Iharabras, São Paulo,
147 SP, Brazil), difenoconazole (Score EC, 250 g l^{-1} a.i., Syngenta, São Paulo, SP, Brazil)
148 and azoxystrobin (Amistar 500 WG, 500 g kg^{-1} a.i., Syngenta, São Paulo, SP, Brazil)

fungicides were used. The thiophanate-methyl, difenoconazole and azoxystrobin fungicides were dissolved in dimethyl sulphoxide (DMSO) and added to molten PDA media (45 °C) to obtain final concentration of 1 μg a.i. ml^{-1}. For each fungicide sample (including the control), the final concentration of DMSO in the culture media was 0.1 % (v/v). Agar plugs (5 mm in diameter) containing mycelia from each isolate were removed from the margin of the colony (grown on PDA for 7 days) and were transferred to the center of the Petri dishes containing PDA supplemented with the fungicides. Petri dishes containing PDA without fungicide were used as the control. In total, four plates (replicates) were used per isolate/fungicide combination. After 7 days incubation in the dark at 25 °C, the radial growth (diameter) of each colony was measured in two perpendicular directions, and the mean diameter of the colony was obtained. The percentage of mycelial growth inhibition (MGI) was calculated using the formula MGI = [(C – T)/C] × 100, where C is the colony diameter of the control (without fungicide) and T is the diameter of the colony growing in PDA treated with fungicide. The experiment was performed twice, for each fungicides experiment were subjected to ANOVA and the means were compared using the Fisher's least significant difference (LSD) test (P= 0.05). The ANOVA and comparisons of means were performed with Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA.).

Effect of temperature on mycelial growth and optimal temperature

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. Four replicates of each isolate were incubated in the dark at temperatures ranging from 5 °C to 35 °C at 5 °C intervals. After a 2-day 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 cubic polynomial regression (y = a + bx + cx 2 + dx 3). The optimal temperature was estimated from the regression equation and numeric summary with TableCurveTM 2D v. 5.01 (SYSTAT Software Inc., Chicago, USA) as the temperature that produced 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 on optimum temperature and mycelia growth rate data, with means for each species compared with Fisher's least significant difference (LSD) test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

Results

Pathogenicity and aggressiveness of Botryosphaeriaceae species in cashew and a range of hosts

All Botryosphaeriaceae isolates but *Pseudofusicoccum stromaticum* caused symptoms in inoculated hosts (Table 2). The Botryosphaeriaceae species showed significant differences in aggressiveness when inoculated in avocado, banana, guava, mango, melon, papaya and passion fruit. In avocado, larger lesions were induced by *L. brasiliense*, *L. iraniensis* and *L. theobromae*. These lesions differed significantly in size from those induced by *L. gravistriata*, *L. jatrophicola* and *N. batangarum*. In banana and guava the lesions induced by *L. brasiliense* and *L. iraniensis* were significantly difference from those induced by *L. gravistriata*, *L. theobromae* and *N. batangarum*. *L. jatrophicola* showed less aggressiveness in comparison with the other species Botryosphaeriaceae in banana, mango and papaya. In melon only *L. gravistriata* induced symptoms. *N. batangarum* did not show symptoms in passionfruit. When inoculated in detached green shoots, all Botryosphaeriaceae species where pathogenic but no difference was observed in virulence and *P. stromaticum* showed symptoms in cashew detached green shoots (Table 2).

Effect of fungicides on the mycelial growth of Botryosphaeriaceae species

The mycelial growth inhibition of Botryosphaeriacea species varied according to the fungicide tested (Fig.1). The azoxistrobin induced a mycelial growth inhibition between 47.6 and 18.7%. For this fungicide, *Neofusicoccum batangarum* was the most sensitive species showing the highest rate of inhibition while *L. brasiliense*, *L. jatrophicola* and *L. theobromae* together were the least sensitive. The inhibition by difenoconazole ranged from 52.7 to 100%, and *L. gravistriata* was the most tolerant species. Finally,

209 the thiophanate-methyl inhibited the mycelial growth from 79.6 to 51.5%, showed
210 significant difference amongst the species.

211 **Effect of temperature on mycelial growth and optimal temperature for mycelial**
212 **growth**

213 Amongst all species, only *L. gravistriata* grew at 5 °C and 10 °C. There were no
214 significant differences (*P*=0.08) in the optimum temperature for mycelial growth among
215 all seven Botryosphaeriaceous species evaluated. The optimum temperature for
216 mycelial growth range from 27.9 °C (*L. jatrophicola*) to 32.9 °C (*P. stromaticum*)
217 (Table 3). The mycelial growth rates of *L. gravistriata* (69.5mm/day) and *L. iraniensis*
218 (64.9mm/day) were significantly higher than the other seven species, which varied from
219 42.5mm/day (*L. brasiliense*) to 53.8mm/day (*L. theobromae*) (Figure 2).
220

221

Discussion

This study represents the comparative epidemiological investigation of the Botryosphaeriaceae species prevalent in cashew orchards in Brazil. The aggressiveness of Botryosphaeriaceae species was investigated in fruits from different hosts, effect of temperature on mycelial growth and the effect of fungicides on mycelial growth were investigated.

In this study all species tested proved to pathogenic to all alternative hosts tested. The species *L. brasiliense* was described in Brazil in 2014 associated with stem-end rot of papaya (Netto et al. 2014), was aggressiveness species in alternative hosts. *Lasiodiplodia brasiliense* was the second most prevalent species associated with stem-end rot of papaya (Netto et al. 2014) and grapevine dieback (Correia et al. 2015) in the São Francisco Valley. *L. iraniensis* was described in Iran associated with mango and Juglans sp. (Abdollahzadeh et al. 2010), in Brazil associated with mango (Marques et al. 2013a; b). In this study, *L. iraniensis* was one of the most aggressiveness species except in melon, proving to be a potential threat. This result differs from that found by Marques et al. (2013a) where *L. iraniensis* isolates produced the smaller lesions in mango fruits. *Lasiodiplodia jatrophicola* was described in Brazil associated with physic nut (Machado et al. 2014). Correia et al. (2015) reported on grape branches with low levels of aggressiveness. In their study, *L. jatrophicola* showed less aggressiveness in comparison with the other Botryosphaeriaceae species.

In this work, *L. gravistriata* is recognized as a new species in the genus *Lasiodiplodia*. This species was pathogenic to all altenative host and not differing in aggressiveness from *L. jatrophicola, L. theobromae, Neofusicoccum batangarum* and *Pseudofusicoccum stramaticum*. *Lasiodiplodia theobromae* was aggressiveness species

247 in alternative hosts except in melon. This species is considered a pantropical pathogen
248 occurring in a wide range of hosts (Burgess et al. 2006). Worldwide, several species
249 have been described in the *L. theobromae* complex, mostly due to the increase in the
250 application of DNA sequence data, but also because of the increased sampling of
251 relatively unexplored areas, including Venezuela (Burgess et al. 2006), Australia (Pavlic
252 et al. 2008), Iran (Abdollahzadeh et al. 2010), Egypt (Ismail et al. 2012), Brazil
253 (Marques et al. 2013a; Machado et al. 2014; Netto et al. 2014; Correia et al. 2015),
254 Oman and United Arab Emirates (Al-Sadi et al. 2013), Italy, Alergia and Tunisia
255 (Linaldeddu et al. 2015).
256 The Botryosphaeriaceae species *Pseudofusicoccum stromaticum* only showed
257 symptoms in cashew stem and the non-pathogenicity when inoculated in avocado,
258 banana, guava, mango, melon, papaya and passion fruit. Recently, *P. stromaticum* was
259 reported for the first time in mango orchards in Brazil (Marques et al. 2012), even
260 though this species was described originally symptomatic and non-symptomatic plants
261 of *Eucalyptus* and *Acacia* spp. with and without symptoms in Venezuela (Mohali et al.
262 2006; 2007). However, in the present study, *P. stromaticum* had a relatively low level of
263 aggressiveness, similar result was observed when this species was inoculated in mango
264 fruits (Marques et al. 2013b) and grape branches (Correia et al. 2015) in Brazil.
265 Pathogenicity tests with *P. stromaticum* should be conducted to determine its relative
266 importance as plant pathogen.This confirmed this specie is more widely distributed than
267 it believed earlier. The genus *Neofusicoccum* occurrence on native and non-native hosts,
268 *Neofusicoccum batangarum* was described and reported in *Terminalia catappa* in
269 Cameroon (Begoude et al. 2010). This species was recently reported in Cactus prickly
270 pear in Brazil causing brown spot symptoms (Conforto et al. 2016). In this study *N.*
271 *batangarum* produced symptoms in avocado, banana, guava, mango, papaya except in

melon and passion fruit.

The pathogenicity of the six Botryosphaeriaceae species on the alternative hosts (avocado, banana, guava, mango, melon, papaya and passion fruit) indicated that the pathogens do not demonstrate host specificity. The wide host range for the Botryosphaeriaceae species associated with cashews in Brazil is a serious problem for the gummosis disease management.

Various studies suggest that global climate change will cause a shift in potential areas and activity of pathogens and in host susceptibility (Piškur et al. 2011). Botryosphaeriaceae are known to be latent pathogens, and their pathogenic impact is predicted to increase under stress-related conditions, such as those of drought (Slippers et al. 2007). Culture morphology has rarely been used as a character for species separation in Botryosphaeriaceae. Thus, cultural characters can vary widely between isolates of any given species, and thus are of limited value in species determination. In this paper, the Botryosphaeriaceae species grew from 15ºC to 35ºC in contrast to the report of Abdollahzadeh et al. (2010), except *L. gravistriata* grew in 5ºC and 10ºC also. This showed that the species can grow in a wide range of temperature or in warm climates.

This is the first report on the effect of thiophanate-methyl, difenoconazole and azoxystrobin fungicides on Botryosphaeriaceae species associated with cashew gummosis worldwise. The in vitro sensitivity test revealed that most of the Botryosphaeriaceae species are thiophanate-methyl, difenoconazole and azoxystrobin sensitive.

Although gummosis causes several damages on cashew trees, there are no fungicides registered for the disease control (MAPA 2016). According to our fungicide sensitivity in vitro assay, the fungicides tested were capable to inhibit the growth of

Botryophaeriaceae species. Azoxystrobin was the fungicide less effective in reduce the micelial growth on all species tested. This result indicates that this fungicide mightnot be effective to control the disease in field. However, thiophanate-methyl was the fungicide most efficient in reducing mycelial growth of the Botryosphaeriaceae species. Thus, this fungicide could efficiently control the gummosis, independently of the species associated with the disease. Moreover, the use of difenoconazole together with thiophanate-methyl could possibly be the best way to control the disease since difenoconazole showed inhibition of mycelial growth in vitro.

This fungicides are not registered for gummosis control. This comparative epidemiological study of Botryosphaeriaceae species prevalent in Brazil provides important information for an effective strategy to control of gummosis on cashew orchards. Information about these species is scarce because of its recent descriptions. Studies are needed on the epidemiology and impact on cashew production together with information referring to ecological role, distribution, diversity and host range of all species of Botryosphaeriaceae found in this study.

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Table 1– *Lasiodiplodia, Neofusicoccum* and *Pseudofusicoccum* isolates from *Anacardium* spp. from Brazil.

Table 2– Virulence (diameter of lesion) of the seven Botryosphaeriaceae species associated with gummosis in Brazil in avocado (Fortuna), banana (cv. Pacovan), cashews stem (cv. BRS 274), guava (cv. Paluma), mango (Tommy Atkins), melon (Amarelo), papaya (Golden) and passion fruit (Amarelo).

Table 3– Optimum temperature for mycelial growth and mycelial growth rate at 30 °C of Botryosphaeriaceae species associated with gummosis of *Anacardium* in Brazil

Fig.1– Sensitivity of the seven Botryosphaeriaceae species associated with cashew gummosis in Brazil to thiophanate-methyl (1µga.i.ml−1), difenoconazole (1µga.i.ml−1) and azoxystrobin (1.0µga.i.ml−1) fungicides. The bars represent the standard error, and columns with the same letter do not differ significantly from each other according to the Fisher's LSD test (P=0.05)

Fig.2 – Influence of temperature on mycelial growth of the seven Botryosphaeriaceae species associated with gummosis in Brazil.

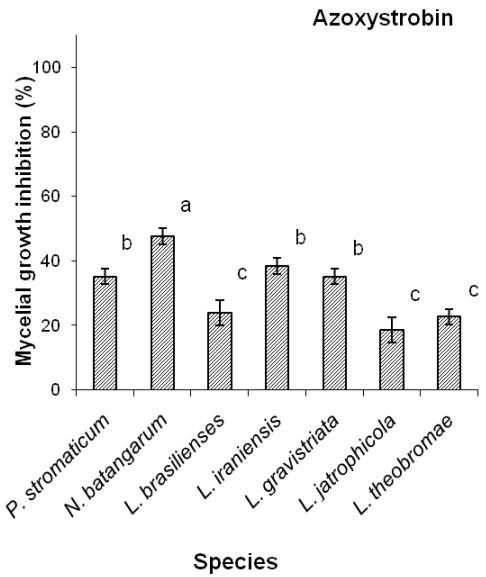
Isolate	Species	Municipality (State)
CMM 4469	*Lasiodiplodia brasiliense*	Lajedo (PE)
CMM 4470	*L. brasiliense*	Oeiras (PI)
CMM 4556	*L. iraniensis*	Icapuí (CE)
CMM 4557	*L. iraniensis*	Horizonte (CE)
CMM 4548	*L. iraniensis*	São Luiz do Curu (CE)
CMM 4559	*L. iraniensis*	Pacajus (CE)
CMM 4562	*L. iraniensis*	Barreira (CE)
CMM 4564	*L. gravistriata*	Coração de Jesus (MG)
CMM 4565	*L. gravistriata*	Coração de Jesus (MG)
CMM 4566	*L. gravistriata*	Coração de Jesus (MG)
CMM 4570	*L. gravistriata*	Montes Claros (MG)
CMM 4572	*L. gravistriata*	Montes Claros (MG)
CMM 4471	*L. jatrophicola*	Macaíba (RN)
CMM 4472	*L. jatrophicola*	Macaíba (RN)
CMM 4485	*L. theobromae*	Limoeiro do Norte (CE)
CMM 4499	*L. theobromae*	Porto de Galinhas (PE)
CMM 4509	*L. theobromae*	São Vicente (PE)
CMM 4508	*L. theobromae*	Goiana (PE)
CMM 4513	*L. theobromae*	São Vicente (PE)
CMM 4536	*Pseudofusicoccum stromaticum*	Montes Claros (MG)
CMM 4544	*P. Stromaticum*	Januária (MG)
CMM 4538	*P. Stromaticum*	Jequitaí (MG)
CMM 4537	*P. Stromaticum*	Januaria (MG)
CMM 4540	*P. Stromaticum*	Cascavel (CE)
CMM 4546	*Neofusicoccum batangarum*	Goiana (PE)
CMM 4552	*N. batangarum*	Ceara-Mirim (RN)
CMM 4547	*N. batangarum*	Aldeia (PE)
CMM 4545	*N. batangarum*	Porto de Galinhas (PE)
CMM 4554	*N. batangarum*	Camocin de São Felix (PE)

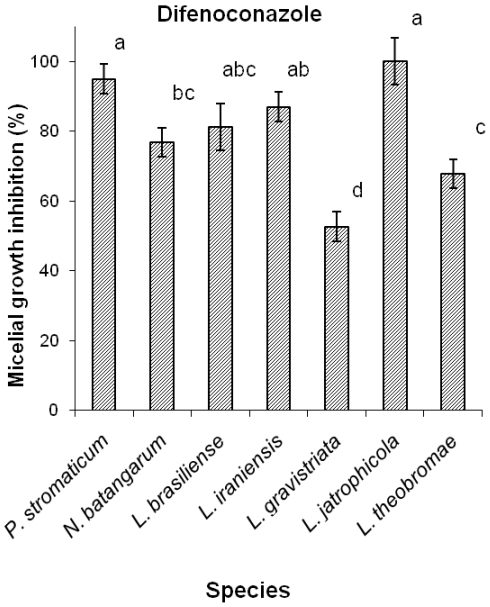
[a]CMM Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" at the Universidade Federal Rural de Pernambuco (Recife, Pernambuco, Brazil).

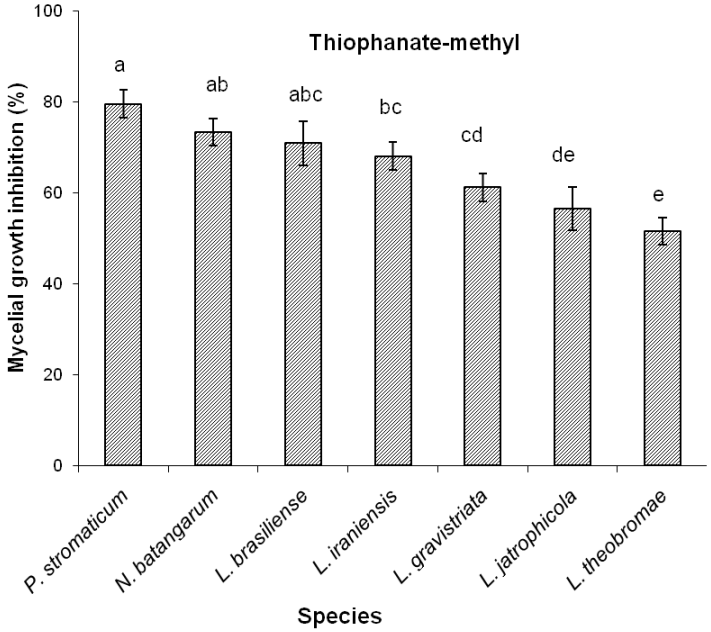
[b]CE Ceara, MG Minas Gerais, PE Pernambuco, PI Piauí, RN Rio Grande do Norte.

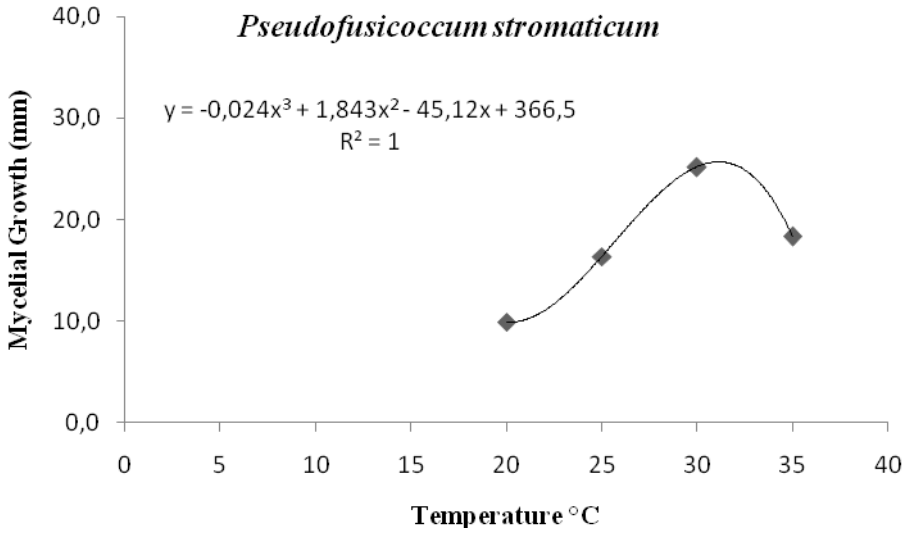
Species	Avocado	Banana	Cashwes stem	Guava	Mango	Melon	Papaya	Passion fruit
Lasiodiplodia brasiliense	63,2 ± 1.8 a	25,8 ± 1.4 a	21,4 ± 1.3 ab	50 ± 1.7 a	64.3 ± 1.8 a	0,0 b	28,7 ± 1.4 a	13 ± 1.8 a
L. iraniensis	60,7 ± 1.7 a	25 ± 1.3 a	24,0 ± 1.3 a	46,4 ± 1.6 a	60,5 ± 1.7 a	0,0 b	31,5 ± 1.4 a	13,1 ± 1.8 a
L. gravistriata	39,6 ± 1.5 b	17,4 ± 1.2 b	22,3 ± 1.3 a	30,8 ± 1.4 b	45,1 ± 1.46 ab	11,5 ± 0,9 a	27,2 ± 1.4 a	13,1 ± 1.8 a
L. jatrophicola	32,5 ± 1.5 b	8,9 ± 0.9 c	22,5 ± 1.3 ab	25,1 ± 1.4b	26,8 ± 1.2 c	0,0 b	13,4 ± 1.1 b	13,3 ± 1.8 a
L. theobromae	52,6 ± 1.7 a	14,5 ± 1.1 b	21,7 ± 1.3 ab	30,1 ± 1.4 b	50,8 ± 1.6 ab	0,0 b	32,8 ± 1.4 a	11,2 ± 1.8 a
Neofusicoccum batangarum	35,8 ± 1.5 b	13,9 ± 1.1 b	27,0 ± 1.4 a	33,3 ± 1.5 b	39,2 ± 1.5 b	0,0 b	29,2 ± 1.4 a	0,0 b
Pseudofusicoccum stromaticum	0,0 c	0,0 d	17,5 ± 1.2 b	0,0 c	0,0 d	0,0 b	0,0 c	0,0 b

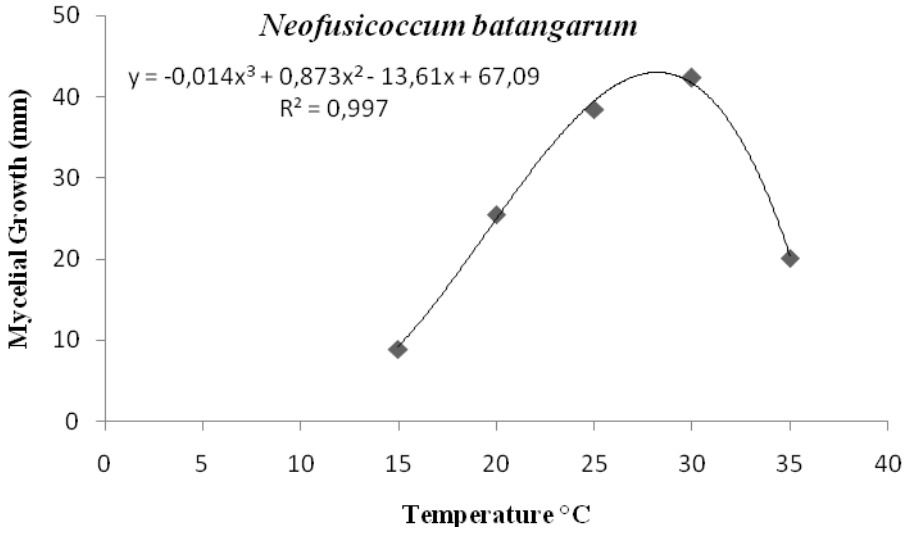
Species	n	Optimum temperature (ºC)	Mycelial growth rate (mm/day) ± SE
Lasiodiplodiabrasilense	10	30.8	42.5±0.69c
L. gravistriata	25	32.5	69.5±0.99a
L. iraniensis	25	30.0	64.9±0.94a
L. jatrophicola	10	27.9	31.6±0.59c
L. theobromae	25	30.4	53.8±0.82b
Neofusicoccumbatangarum	25	28.0	32.2±0.60c
Pseudofusicoccumstromaticum	25	32.9	33.0±0.61c

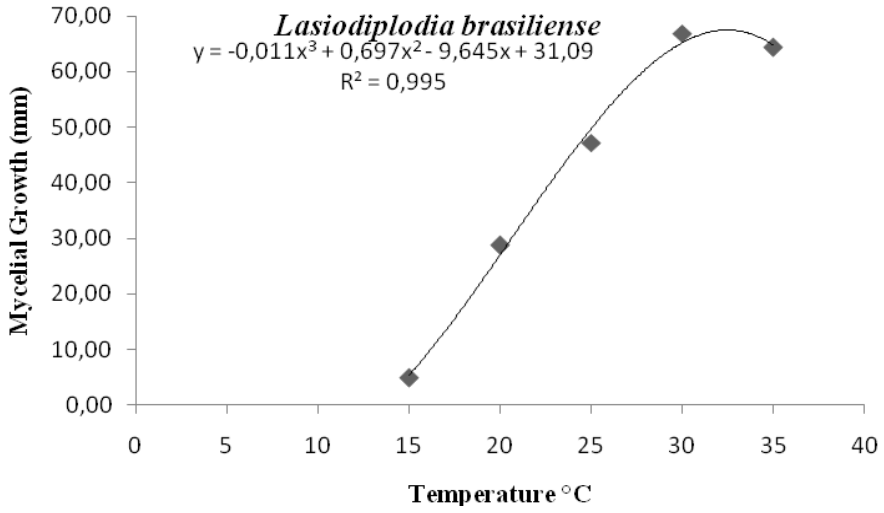

Azoxystrobin
Mycelial growth inhibition (%)
0
20
40
60
80
100
b
a
c
b
b
c
c
P. stromaticum
N. batangarum
L. brasiliense
L. iraniensis
L. gravistriata
L. jatrophicola
L. theobromae
Species

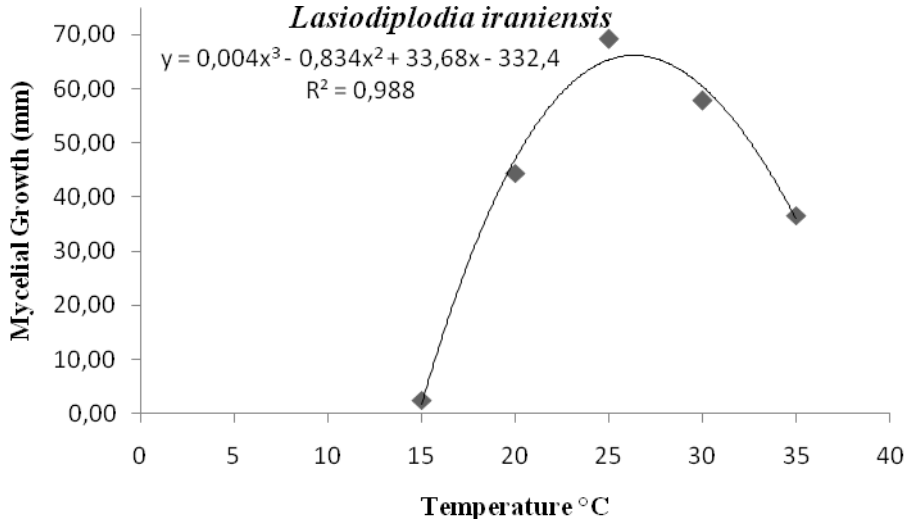

Difenoconazole
Micelial growth inhibition (%)
0
20
40
60
80
100
a
bc
abc
ab
d
a
c
P. stromaticum
N. batangarum
L. brasiliense
L. iraniensis
L. gravistriata
L. jatrophicola
L. theobromae
Species

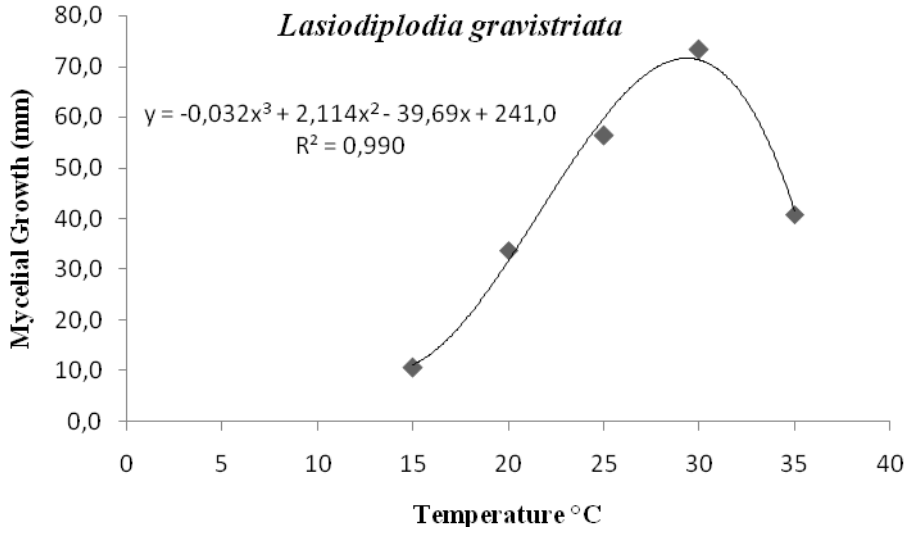

Thiophanate-methyl
Mycelial growth inhibition (%)
0
20
40
60
80
100
a
ab
abc
bc
cd
de
e
P. stromaticum
N. batangarum
L. brasiliense
L. iraniensis
L. gravistriata
L. jatrophicola
L. theobromae
Species

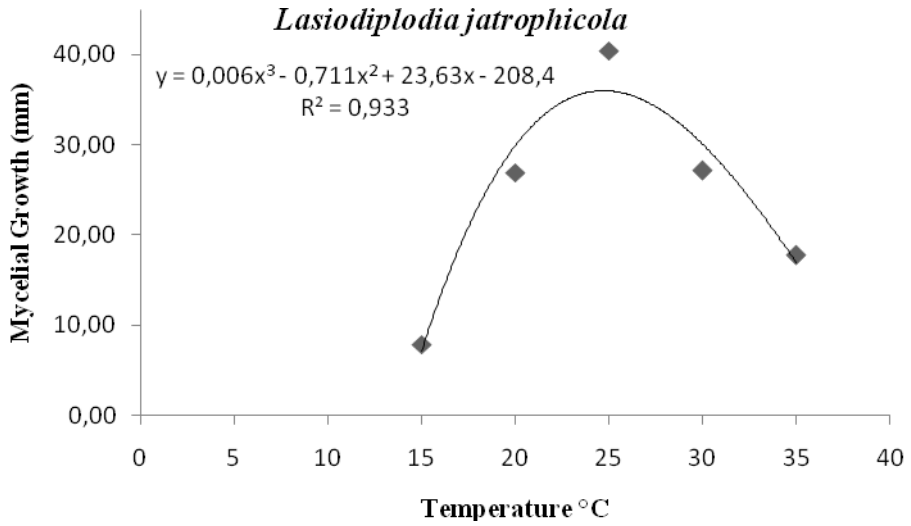

Pseudofusicoccum stromaticum
y = -0,024x³ + 1,843x² - 45,12x + 366,5
R² = 1
Mycelial Growth (mm)
Temperature °C
40,0
30,0
20,0
10,0
0,0
0
5
10
15
20
25
30
35
40

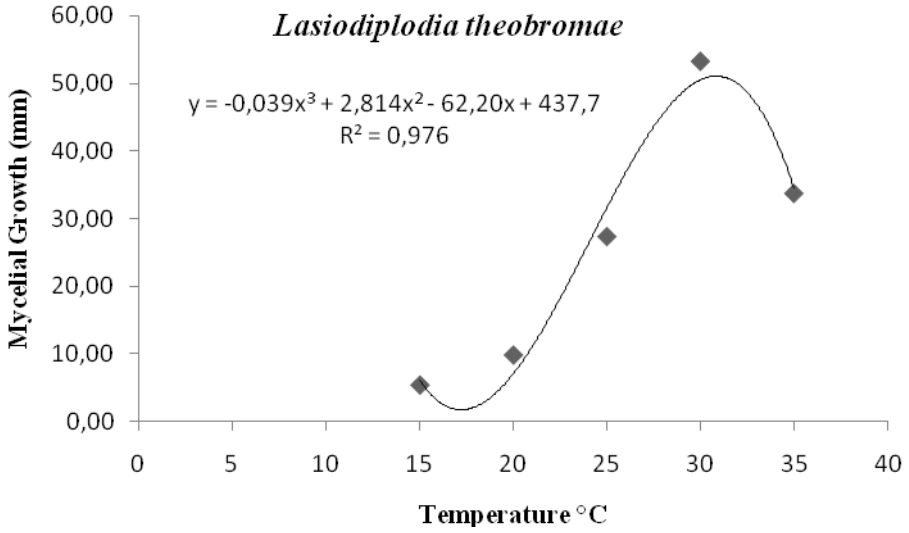

Neofusicoccum batangarum
y = -0,014x³ + 0,873x² - 13,61x + 67,09
R² = 0,997
Mycelial Growth (mm)
Temperature °C
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40


Lasiodiplodia brasiliense
y = -0,011x³ + 0,697x² - 9,645x + 31,09
R² = 0,995
Mycelial Growth (mm)
Temperature °C
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
0
5
10
15
20
25
30
35
40


Lasiodiplodia iraniensis
y = 0,004x³ - 0,834x² + 33,68x - 332,4
R² = 0,988
Mycelial Growth (mm)
Temperature °C
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
0
5
10
15
20
25
30
35
40


Lasiodiplodia gravistriata
y = -0,032x³ + 2,114x² - 39,69x + 241,0
R² = 0,990
Mycelial Growth (mm)
Temperature °C
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
0
5
10
15
20
25
30
35
40


Lasiodiplodia jatrophicola
y = 0,006x³ - 0,711x² + 23,63x - 208,4
R² = 0,933
Mycelial Growth (mm)
Temperature °C
40,00
30,00
20,00
10,00
0,00
0
5
10
15
20
25
30
35
40


Lasiodiplodia theobromae
y = -0,039x³ + 2,814x² - 62,20x + 437,7
R² = 0,976
Mycelial Growth (mm)
60,00
50,00
40,00
30,00
20,00
10,00
0,00
0
5
10
15
20
25
30
35
40
Temperature °C

Capítulo IV

Conclusões Gerais

CONCLUSOES GERAIS

- A gomose do cajueiro no Brasil é causada por dez espécies de Botryosphaeriaceae: *Lasiodiplodia brasiliense*, *L. euphorbicola*, *L. gonubiensis*, *L. iraniensis*, *L. jatrophicola*, *L. gravistriata* sp. nov., *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum batangarum* e *Pseudofusicoccum stromaticum* sendo *L. theobromae* a mais frequente;
- As espécies de Botryosphaeriaceae causadoras de gomose diferem quanto a agressividade, sendo que *L. iraniensis* e *N. batangarum* são as mais agressivas e as espécies *L. euphorbicola* e *L. pseudotheobromae* as menos agressivas;
- Os fungicidas utilizados Tiofanato-metilico, difenoconazole e azoxistrobin são capazes de inibir o crescimento micelial das diferentes espécies de Botryosphaeriaceae *in vitro*, sendo difenoconazole o mais eficiente;
- Com exceção de *L. gravistriata*, nenhuma das espécies avaliadas crescem em temperaturas de 5°C e 10°C. E a temperatura ótima varia de 27,9°C e 32,9°C;