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Tese de Doutorado

Dispersão temporal de conídios de Botryosphaeriaceae em parreirais no Vale do Siriji (Pernambuco) e sensibilidade de isolados do Nordeste brasileiro a fungicidas

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Recife – PE
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FÁBIO JÚNIOR ARAÚJO SILVA

DISPERSÃO TEMPORAL DE CONÍDIOS DE BOTRYOSPHAERIACEAE EM PARREIRAIS NO VALE DO SIRIJI (PERNAMBUCO) E SENSIBILIDADE DE ISOLADOS DO NORDESTE BRASILEIRO A FUNGICIDAS

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SUMÁRIO

RESUMO GERAL

A morte descendente da videira causada por espécies de Botryosphaeriaceae é uma importante doença que ocorre em vinhedos no mundo inteiro. Os patógenos infectam o tronco da videira, indo em direção às regiões centrais das plantas, promovendo a morte lenta do hospedeiro. Estudos recentes baseados em análises filogenéticas demonstraram uma ampla diversidade de espécies associadas aos sintomas característicos da morte descendente da videira em diferentes regiões produtoras de uva no Nordeste Brasileiro. No entanto, até o momento, são poucos os estudos envolvendo o conhecimento de fatores como a dispersão do patógeno em área de cultivo e a disponibilidade de métodos de manejo. Neste sentido, este estudo teve como objetivos: (a) Analisar a dispersão de conídios das espécies de Botryosphaeriaceae em vinhedos na região Nordeste do Brasil; e (b) Avaliar a sensibilidade de isolados de *Lasiodiplodia theobromae* obtidos em diferentes áreas produtoras de uva no Nordeste Brasileiro a diferentes grupos de fungicidas. O estudo da dispersão foi conduzido em quatro áreas produtoras de uva de mesa, localizadas no Vale do Sirijí, em Pernambuco/ Brasil. A avaliação da dispersão de conídios foi realizada a partir de plantas sintomáticas e restos de cultura após a poda. Os dados da dispersão de conídios apresentaram bom ajuste ao modelo matemático logístico indicando que o mesmo pode ser empregado para futuros estudos de previsão de doenças. No estudo de sensibilidade foi avaliada a sensibilidade *in vitro* e os componentes de adaptabilidade de 62 isolados de *L. theobromae*, obtidos de videiras sintomáticas cultivadas na região Nordeste do Brasil, a tebuconazol, tiofanato-metílico, azoxistrobina e iprodiona. A sensibilidade *in vitro* revelou que as populações de *L. theobromae* do Nordeste brasileiro apresentam diferentes graus de sensibilidade aos fungicidas testados com diferentes niveis de sensibilidade a tebuconazol, tiofanato-metílico, iprodiona e azoxystrobina, com EC_{50} variando de 0.044 a 3 μg mL^{-1} para a maioria dos isolados. O entendimento da dispersão de conídios de Botryosphaeriaceae nos vinhedos, bem como, o estado atual da sensibilidade das populações de *L. theobromae* a diferentes fungicidas podem fornecer parâmetros para estabelecer estratégias de manejo da morte descendente da videira.

Palavras-chave: Morte descendente, tebuconazol, videira, dispersão, iprodiona.

GENERAL ABSTRACT

The *Botryosphaeriaceae* dieback is an important disease that occurs in vineyards around the world. Pathogens infect the trunk of the vine by moving toward the central regions such as xylem and wood, promoting slow plant death. In Brazil, studies related to the *Botryosphaeriaceae* dieback in grapevine are scarce, the first reports of *Botryosphaeriaceae dieback* occurring in grapevine, dates back to 1992. Recent studies based on phylogenetic analyzes have demonstrated a wide variation of species associated with symptoms characteristic of this disease in different regions in the Northeast Region. For the proper management of phytopathogens, it is important to know a series of factors including the pathogen dispersal in the area of crop and the availability of methods for the management of the disease. In this sense, this thesis had as objectives: (a) To analyze the conidia dispersal of *Botryosphaeriaceae* species in vineyards in the Northeast region of Brazil; (b) To evaluate the sensitivity of isolates of *Lasiodiplodia theobromae* obtained in different grape producing areas in the Northeast of Brazil to different groups of fungicides. The dispersal study was conducted in four table grape producing areas, located in tropical region (Sirijí Valley) in the state of Pernambuco (Brazil). The conidia dispersal in symptomatic plants and pruning debris throughout the year was evaluated. The conidia dispersal data had a Goodness-of-fit to the logistic mathematical model, indicating that it could be used for future disease prediction studies. In the sensitivity study of *L. theobromae* the in vitro susceptibility and adaptability components of 62 symptomatic grapevine isolates originating in the Northeast of Brazil were evaluated for tebuconazole, thiophanate-methyl, azoxystrobin and iprodione. *In vitro* susceptibility to azoxystrobin revealed that the populations of *L. theobromae* in the Northeast of Brazil show different degrees of sensitivity to the fungicides tested with different values for sensitivity to tebuconazole, thiophanato-methyl, iprodione and azoxystrobin, with EC_{50} ranging from 0.044 to 3 $\mu g.ml^{-1}$ for most isolates. The understanding of the dispersal of *Botryosphaeriaceae* conidia in the vineyards as well as the current state of the sensitivity of *L. theobromae* populations to different fungicides can provide parameters to establish the best management strategies for *Botryosphaeriaceae* dieback.

Key-words: *Botryosphaeriaceae* dieback, tebuconazole, grapevine, dispersal, iprodione

Capítulo I

Introdução Geral

Dispersão temporal de conídios de Botryosphaeriaceae em parreirais no Vale do Siriji (Pernambuco) e sensibilidade de isolados do Nordeste brasileiro a fungicidas

INTRODUÇÃO GERAL

1. A cultura da videira

A videira (*Vitis* spp.) pertence à família Vitaceae, constitui-se como importante frutífera para a humanidade. Cultivada durante séculos, a videira dentro do setor agrícola é uma atividade de extrema importância, pela diversidade de produtos originados (sucos, vinhos, vinagre, consumo *in natura*, cosméticos etc.) que por serem apreciados em todo o mundo, tornam o comércio vitícola sempre aquecido economicamente, gerando montantes para os países produtores e contribuindo diretamente com o desenvolvimento socioeconômico local.

Segundo a FAO (2016), os principais produtores de uva em 2016 foram a China (14.763.000 t), Itália (8.201.914 t), Estados Unidos da América (7.097.723 t) e França (6.247.034 t). Esses países vêm ao longo do tempo apresentando um elevado crescimento na área de cultivo, especialmente os países asiáticos como a China, que aumentou a área plantada de 2000 a 2012 em 90% (KARLSON, 2013).

Na América do Sul, destacam-se como grandes produtores de uva e derivados o Chile e Argentina, somando ambos uma produção de 4.232.006 milhões de toneladas de uvas produzidas em 2016 (FAO, 2016). O Brasil é o terceiro no ranking de produção com 984.481 mil toneladas no ano de 2016 e crescimento estável ao longo dos anos 2013/14/15 (FAO, 2016). Essa estabilidade se deve às condições favoráveis que possibilitam o seu cultivo nas regiões sul e Nordeste, com aproximadamente 80 mil hectares plantados no ano de 2014 (IBGE, 2015) e comercialização de vinhos em torno de 226.836.639 milhões de litros (UVIBRA, 2015).

A produção no Brasil é regionalmente distinta, enquanto a região Sul apresenta-se como principal produtor de uvas, com cultivos destinados a produção de vinhos, a

região Nordeste aparece como outro importante polo de produção de uvas de mesa (VITAL, 2009).

Com um mercado em constante crescimento, novos polos de cultivos vêm sendo introduzidos, a exemplo de áreas que já se encontram com pomares instalados no estado do Rio Grande do Norte (Vale do Assú), Ceará (Tabuleiros e Cariri) e alguns municípios no estado de Goiás (Santa Helena de Goiás, Paraúna, Hidrolândia, Itaberaí e Aragoiânia) que juntos somaram 133 hectares plantados em 2014 (IBGE, 2015) e já contam com uma série de fábricas e vinícolas instaladas (OLIVEIRA, 2009).

2. A viticultura no Nordeste Brasileiro

O Nordeste do Brasil conta com o cultivo da uva sobre clima tropical. Atualmente existem dois polos vitícolas bem consolidados nesta região, o Vale do São Francisco e o Vale do Siriji. O Vale do São Francisco, está localizado em terras semiáridas e abrange os estados de Pernambuco e Bahia, que juntos detém grande parte da produção de uva de mesa, respondendo por 90% da produção de uvas no Nordeste (IBGE 2015). Neste polo, os cultivos são caracterizados por um sistema de produção com alta tecnologia desde o início da implantação do vinhedo, grandes plantios em extensão e comércio voltado à exportação.

O Vale do Siriji, polo situado na Zona da Mata de Pernambuco, está localizado em área com clima tropical úmido. Caracteriza-se pela produção em pequenas áreas e baixo perfil tecnológico. O grande diferencial dos polos vitícolas do Nordeste está na possibilidade de 2 safras anuais, devido a alguns fatores específicos como a prática da viticultura irrigada e a presença de elevado índice de radiação solar ao longo do ano, com seus diferentes comprimentos de onda, levando ao incremento da microbiota do solo (CIA et al., 2009) e consequentemente melhor qualidade da uva colhida.

No Nordeste do Brasil, as cultivares empregadas na implantação dos vinhedos compreendem a Italia, Benitaka, Red Globe, Sugraone, Thompson Seedless, Crimson Seedless e Isabel. Essas variedades apresentam uma série de características que as tonam ideais para o cultivo na região Nordeste do Brasil, dentre as quais podem ser citadas a adaptação às diferentes condições climáticas e situações extrema de estresse em que são submetidas ao longo do seu desenvolvimento (SOUZA LEÂO, 2004).

Todos esses fatores aliados a práticas de manejo do solo, fertilização adequada, boas práticas pré e pós-colheita estabelecem um sistema propício a atingir elevados de níveis de produção final de uva.

3. Morte descendente da videira

A viticultura está exposta a uma série de patógenos que influenciam na longevidade das plantas e na produção final, quando mal manejados (GENTA et al., 2010, CHAVARRIA; SANTOS, 2013). Dentre os problemas fitossanitários associados à videira, destacam-se as doenças fúngicas, uma vez que nas diferentes classes de fungos são encontradas espécies capazes de infectar as mais variadas partes das plantas. Um importante grupo de fungos para a viticultura mundial são os pertencentes à família Botryosphaeriaceae, que engloba uma diversidade de gêneros e espécies associados com algumas cultivares de uva, provocando declínio e perdas nos vinhedos ao redor do mundo (MORALES et al., 2012). Ao longo dos anos, esse grupo de patógenos tem sido relacionado especialmente com a morte descendente da videira.

Estudos demonstram como principais doenças associadas ao tronco da videira (Grapevine Trunk Diseases - GTDs) a doença de Petri, causada por numerosas espécies do gênero *Phaeoacremonium* e *Phaeomoniella Chlamydospora* (W. Gams, Crous, M. J. Wingf. & Mugnai) Crous & W. Gams (GROENEWALD et al., 2001), pé negro da videira associada às espécies *Cylindrocarpon* spp. (*Cylindrocarpon destructans* (Zinnsm.) Scholten, *C. liriodendra* J. D. MacDon. & E. E. Butler, *C. macrodidymum* Schroers, Halleen & Crous, e *C. pauciseptatum* Schroers & Crous) e *Campylocarpon* spp. (*Campylocarpon fasciculare* Schroers, Halleen & Crous e *Campylocarpon pseudofasciculare* Halleen, Schroers & Crous) (HALLEEN et al., 2004), escoriose *Cadophora luteoolivacea* (J. F. H. Beyma) T. C. Harr. & McNew e *Pleurostomophora richardsiae* (Nannf.) L. Mostert, W. Gams & Crous (basionym: *Ca. richardsiae* Nannf.) (NAVARRETE et al., 2011), morte de eutypa associada principalmente à *Eutypa lata* ((Pers.) Tul. & C. Tul. (1863) e *Eutypella vitis* ((Schwein.) Ellis & Everh. 1892) (CATAL et al., 2017) e a morte descendente da videira relacionada à espécies da família Botryosphaeriaceae (LINALDEDDU et al., 2015).

Alguns desses fitopatógenos apresentam um estilo de vida com características saprofíticas e endofíticas, além do desenvolvimento de uma fase de latência (SLIPPERS; WINGFIELD, 2007, PRANCHER et al., 2012), possibilitando sua

sobrevivência em uma diversidade de tecidos como flores, gemas dormentes e frutos em diferentes estágios fenológicos (WUNDERLICH et al., 2011). Ademais, alguns fatores podem contribuir para que ocorra um desenvolvimento parasitário, incluindo características ambientais, a suscetibilidade do hospedeiro, deficiência nutricional, solos compactados e mal drenados além da presença de ferimentos (FOURIER; HALLEEN, 2001, GARRIDO; SONEGO; GOMES, 2004).

Botryosphaeriaceae como grupo causador da morte descendente em videira inclue espécies pertencentes principalmente aos gêneros *Diplodia* Fr., *Dothiorella* Sacc., *Fusicoccum* Corda, *Neofusicoccum* Crous, Slippers & A. J. L.Phillips (2006) e *Lasiodiplodia* Ellis e Everh que ocorrem conforme a região. Mohammadi et al. (2013) caracterizaram, por meio de análises morfológicas e moleculares, as espécies *N. parvum* e *D. seriata* incidentes em vinhedos no Irã. Na Argélia a morte descendente e cancros em videiras têm sido associados às espécies *D. seriata*, *Botryosphaeria dothidea* e *N. parvum* (AMMAD et al., 2014). Em algumas regiões a diversidade de espécies é elevada. Como acontece na Nova Zelândia, onde foi identificado a presença de *N. parvum*, *N. luteum*, *N. australe*, *N. ribis*, *D. mutila*, *D. seriata*, *B. dothidea*, *Do. Iberica* e *Do. sarmentorum* (BASKARATHEVAN, et al., 2012).

Um dos fatores na qual a sintomatologia referente à morte descendente da videira está relacionada é a espécie do patógeno. Contudo, em alguns casos, são encontrados diferentes agentes causando sintomas semelhantes, como ocorre em sintomas externos causados por agentes associados ao pé-negro e doença de Petri (REGO et al., 2000), ou em outros casos, no qual mesmos agentes causam diferentes sintomas (ÚRBEZ-TORRES et al., 2006). Na maioria das vezes, os principais sintomas presentes nos vinhedos são constituídos por cancros nos caules, redução de crescimento da planta, coloração difusa, atrofiamento, podridão de frutos, redução de produção e morte da planta (LARIGNON; DUBOS, 2001, NIEKERK et al., 2006).

4. Morte descendente da videira no Brasil

A morte descendente da videira é uma doença comumente presente em vinhedos em todo o mundo, sendo a primeira descrição em 1974 na Hungria (LEHOCZKY, 1974). A importância dessa doença para a produção vitícola só tem sido notada nos últimos anos, possivelmente devido a capacidade dos patógenos envolvidos infectarem plantas jovens e levarem a um declínio constante até a morte (MUGNAI et al., 1990).

No Brasil, estudos envolvendo patógenos causadores da morte descendente são escassos. Os primeiros relatos na literatura dessa doença datam de 1992 com a descrição de uma nova doença causada por *Lasiodiplodia theobromae* (RIBEIRO et al., 1992). Com a condução de pesquisas, outras espécies envolvidas com a morte descendente vêm sendo identificadas constantemente em todo o país (PARADELA FILHO et al.,1993; SÔNEGO; INÁCIO; DIANESE, 1999; GARRIDO et al., 2011; DOS SANTOS et al., 2014).

Os principais sintomas associados à morte descendente da videira encontrados no Brasil incluem podridão seca, crescimento atrofiado, morte de brotos, esporas e ramos laterais, cancro do tronco ou nos ramos laterais, cancros em forma de cunha no tecido vascular, redução no crescimento inicial, mostrando amarelecimento das folhas, murcha, listras longitudinais marrons e pretas que apareceram como pontos negros necróticos (CORREIA et al., 2013; CORREIA et al., 2016; PEIXINHO; RIBEIRO; AMORIM, 2017). Sintomas decorrentes da morte descendente geralmente levam um tempo para serem expressas, com altas severidades geralmente observadas com o aumento da idade dos vinhedos (GUBLER et al., 2005).

A morte descendente da videira corresponde a uma importante doença para as áreas brasileiras produtoras de uva, ocorrendo em vinhedos situados em regiões com diferentes condições climáticas. Nos estados que compõe a região Sul, principal polo brasileiro de produção de uvas destinadas à produção de vinho tem sido registrado a presença das espécies: *N. luteum*, *Fusicoccum aesculi* e *Lasiodiplodia theobromae* (GAVA et al., 2010; SÔNEGO; GARRIDO; GRIGOLETTI JÚNIOR, 2005; GARRIDO et al., 2011).

No Vale do São Francisco, onde são cultivadas uvas de mesa na região Nordeste, foi detectado espécies de *Lasiodiplodia* e *Neofusicoccum*. No entanto, a maior frequência observada em videiras corresponde a espécies de *Lasiodiplodia* (CORREIA et al., 2013). Em trabalho recente, foi identificado oito espécies de *Lasiodiplodia* associadas a morte descendente da videira em áreas distintas nessa região, das quais cinco compreenderam espécies ainda não descritas em videira no Brasil (CORREIA et al., 2016). Na região Nordeste, o Vale do Sirijí também se destaca pela produção de uvas de mesa, similarmente ao que ocorre no Vale do São Francisco, e isolados de patógenos associados à GTDs têm sido relatados em uvas nesse polo de produção (CIMMINO, 2017).

Além da ocorrência da morte descendente em uvas na região Nordeste, o cajueiro, a mangueira e o mamoeiro apresentam-se como potenciais hospedeiros, principalmente de espécies pertencentes ao gênero *Lasiodiplodia* (NETTO et al., 2014; NETTO et al., 2017; MARQUES et al., 2013, CAVALCANTE et al., 2014). Esse fato é relevante para o Brasil, uma vez que o país destaca-se por estar entre os principais produtores desses cultivos (FAO, 2016). Além disso, as áreas destinadas ao cultivo de videira muitas vezes podem estar implantadas nas áreas vizinhas destinadas a diferentes cultivos, possibilitando a troca constante de inóculo entre ambos.

Mesmo com a falta de dados que relacione morte descendente com a diminuição nos rendimentos das culturas, a incidência dessa doença vem se tornando cada vez mais importante, principalmente para a cultura da videira, pois sua infecção poderá levar a uma redução drástica na longevidade dos parreirais (GRAMAJE; ARMENGOL, 2011). Neste sentido, o conhecimento a cerca dos patógenos causadores da morte descendente nos vinhedos torna-se de grande importância para o agronegócio da viticultura no Nordeste Brasileiro.

5. Epidemiologia da morte descendente da videira

A aplicação de métodos e medidas de controle nos patossistemas passa primariamente pelo estudo e desenvolvimento de conhecimento a cerca dos fatores que estão envolvidos com as epidemias.

A morte descendente da videira é uma doença mundialmente conhecida, relacionada a uma diversidade de patógenos da família Botryosphaeriaceae (CORREIA et al., 2013, CORREIA et al., 2016), os quais tem se demonstrado serem cosmopolitas (CROUS et al., 2016). São capazes de infectar todas as partes da planta, podendo causar cancros, morte dos braços e lesões descoloridas (AMPONSAH et al., 2011, WUNDERLICH et al., 2011) que levarão a morte lenta do hospedeiro. Uma das características dessa classe de patógenos é a alta variação nos componentes epidemiológicos, que seguirão padrões conforme a espécie de patógeno envolvido, do hospedeiro, das condições climáticas e de outros elementos relacionados ao manejo empregado (ÚRBEZ-TORRES et al., 2010a).

Segundo Morales et al. (2012), vinhedos a partir de oito anos de estabelecimento tendem a apresentar maiores níveis de suscetibilidade a ocorrência da morte descendente. Isso se deve não necessariamente a idade dos tecidos vegetais, mas,

principalmente a algumas práticas de condução dos vinhedos que se iniciam no preparo das mudas e seguem ao longo dos ciclos da cultura, como a presença de ferimentos em porta enxertos sem realização de um tratamento adequado, utilização de ferramentas sem desinfestação, transporte inadequado das estacas, irrigação e sistema de condução do vinhedo (GRAMAJE; DI MARCO, 2015).

Ao longo do tempo de condução, as condições relacionadas ao ambiente com as variações sazonais de temperatura, umidade e velocidade do vento, poderão desencadear epidemias. A dispersão de conídios, por exemplo, é um importante fator para o processo de novas infecções, estando relacionado diretamente com a precipitação (ÚRBEZ-TORRES et al., 2010a; VALÊNCIA et al., 2015). Kuntzmann e Villaume (2009) observaram em vinhedos situados na França, picos de liberação de conídios da espécie *D. seriata* após episódios de chuvas, assim também como Úrbez-Torres et al. (2010b), em que os maiores picos da liberação dos conídios das espécies de *B. dothidea*, *D. seriata*, *D. mutila*, *L. theobromae*, e *N. parvum* foram observados logo após eventos de chuvas. Um fato já bem conhecido é a necessidade de umidade para liberação de conídios, confirmada em algumas regiões de clima seco como no semiárido. Nesse tipo de clima ocorre baixa precipitação e, nesse curto intervalo favorável, ocorrere a dispersão dos conídios (VALÊNCIA et al., 2015).

Apesar da atuação da chuva no favorecimento da liberação dos conídios, Van Niekerk et al. (2010), ressaltam ainda, a cerca da presença da temperatura, umidade relativa e velocidade do vento governando os fenômenos de liberação e dispersão de conídios. Esses autores caracterizaram, em ordem de importância, os fatores ambientais ligados aos eventos de incidência de conídios do grupo Botryosphaeriaceae em vinhedos na África do Sul: precipitação, máxima umidade relativa, temperatura mínima e temperatura máxima. Alguns relatos demonstram diferenças na sensibilidade de *D. seriata* e *L. theobromae* a diferentes temperaturas *in vitro*, no qual apresentaram níveis elevados na germinação de conídios a 40 °C, o que não ocorreu para outras espécies como *B. dothidea* e *Dothiorella iberica* (ÚRBEZ-TORRES et al., 2010a).

A facilidade da ocorrência de epidemias da morte descendente em vinhedos, também está relacionada à capacidade de sobrevivência do patógeno em restos culturais ao serem deixados nas proximidades dos vinhedos, possibilitando futuras infecções conforme as condições sejam favoráveis. Estudos focados na sobrevivência e disponibilidade de corpos de frutificação com conídios viáveis demonstram à capacidade de algumas espécies de Botryosphaeriaceae produzirem picnídios em

bastões de videiras, apesar dos conídios perderem a viabilidade de infecção com o tempo. Esse fato indica a presença de detritos de podas como fontes de inóculo por um tempo considerável (ELENA; LUQUE, 2016).

Além dos fatores ambientais envolvidos com epidemias, um agravante são as técnicas e práticas empregadas ao longo do processo de produção de mudas. Um conjunto de estudos tem demonstrado que os processos de infecção por Botryosphaeriaceae poderão iniciar nos viveiros, durante todas as fases que compõem as etapas de preparo de mudas até o armazenamento de porta-enxertos (GRAMAJE; DI MARCO, 2015). Um conjunto de práticas adotadas que incluem, por exemplo, a hidratação de porta-enxertos, ausência de sanitização nos vinhedos, armazenamento e a própria irrigação, contribuirão com infecções por diferentes espécies, que se desenvolverão no viveiro e serão levadas para os campos de produção (FOURIER; HALLEN, 2006, ÚBEZ-TOREES et al., 2010b, WAITE et al., 2013).

Esses conjuntos de fatores podem contribuir com o surgimento de epidemias, tornando-se necessário o conhecimento das diferentes condições ambientais e de manejo que os influenciam para a tomada de decisões adequadas.

6. Sensibilidade de fitopatógenos a fungicidas

Manejo de doenças de plantas compreende um conjunto de métodos e técnicas empregados na proteção das plantas contra doenças, com objetivo de reduzir o crescimento dos patógenos, avanços de epidemias e consequentemente minimização de perdas e custos (VAN LOON, 1992). Dentro do conjunto de técnicas empregadas na proteção de plantas, o manejo químico vem sendo utilizado de maneira eficiente ao longo dos anos em diferentes patossistemas (BOWEN; HAGAN; WEEKS, 1997; LEGARD et al.; 2001; LEHNER et al., 2017).

Assim como nos demais patossistemas, o manejo químico da morte descendente da videira vem sendo estudado, inicialmente com aplicações de arsenito de sódio, que teve grande importância na proteção contra fungos causadores de doenças do tronco. Arsenito de sódio compreende uma derivação da molécula de arsênico (As) encontrada naturalmente em todos os solos (CULLEN; REIMER, 1989; SMEDLEY; KINNIBURGH, 2002). Essa molécula foi empregada na tentativa de manejo de GTDs, principalmente na proteção contra esca da videira (LARIGNON; DUBOS, 1997). A importância desse composto para a proteção dos cultivos contra GTDs está na sua alta

toxicidade induzindo a produção de compostos antimicrobianos que migrarão das folhas para as partes baixas da planta (CARBONELL-BARRACHINA; BURLÓ, 1997; LARIGNON et al., 2008).

Outro importante aspecto do arsenito diz respeito à alta toxicidade a humanos e ao meio ambiente. A principal forma de absorção se dá via inalação ou oral (HONG; SONG; CHUNG, 2014). Os riscos associados a esta molécula levaram a proibição do seu uso em cultivos agrícolas (BERTSCH et al., 2013; DECOIN, 2001; LARIGNON et al., 2008). Consequentemente, o manejo de plantas sofreu um grande impacto no que se refere a proteção dos cultivos, levando a aumentos de incidência de GTDs em videira nas regiões produtoras em todo o mundo (SURICO; MUGNAI; MARCHI, 2006).

Após a proibição do arsenito de sódio, houve a necessidade do desenvolvimento de produtos e novas técnicas alternativas de manejo dessas doenças incluindo, produtos químicos, agentes de controle biológico, moléculas naturais e métodos de saneamento. As tentativas de obtenção de um método único de manejo para GTDs têm sido limitadas, uma vez que o processo de infecção pelos patógenos associados a essas doenças conduzem muitas vezes a limitações no que diz respeito à presença de sintomas, levando em alguns casos a presença de elevadas proporções de falso positivo (WHITEMAN et al., 2007).

Para um adequado manejo dos patógenos causadores de GTDs, grande atenção deve ser dada ao material vegetal do viveiro (planta matriz e porta-enxerto) uma vez que estes podem ser portadores de patógenos (AROCA et al., 2010; DUBROVSKY; FABRITIUS, 2007; FOURIE; HALLEEN, 2004). Outro aspecto importante corresponde aos danos ocasionados por patógenos associados à GTDs, como a morte descendente da videira, que requeren um maior período de tempo para expressarem os sintomas. Considerando esses fatos, os processos de proteção das plantas devem iniciar desde o momento de preparação das mudas (GRAMAJE; ÚRBEZ-TORREZ; SOSNOWSKI, 2018). Neste sentido, estudos envolvendo o manejo químico vêm sendo desenvolvidos ao longo dos anos desde a implantação das plantas nos viveiros durante as diversas fases no processo de propagação, como enraizamento de estacas, imersão das estacas e embebição das plantas enxertadas (REGO et al., 2009, FOURIE; HALLEEN, 2004; HALLEEN et al., 2007), reduzindo significativamente a incidência de patógenos (FOURIE; HALLEN, 2006). Outro método efetivo corresponde ao tratamento químico dos ferimentos resultantes de podas, uma vez que estudos realizados demonstram níveis saisfatorios na proteção de patógenos associados à GTDs (OLMO;

GRAMAJE; ARMENGOL, 2017). Com relação à morte descente da videira, ferimentos de poda podem representar a principal porta de entrada para futuras infecções, podendo elevar o tamanho dos cancros e consequentemente a morte das plantas (DOLL; MICHAILIDES; ROLSHAUSEN, 2013).

No entanto, apesar da eficiência do emprego de moléculas químicas na proteção dos cultivos agrícolas, cuidados devem ser tomados no que se refere principalmente ao emprego de dosagens inadequadas, onde uma vez negligenciado esse fato, níveis de resistência podem ser desencadeados nos patógenos alvos (MIKABERIDZE et al., 2017).

Na literatura, inúmeros estudos apontam para o surgimento de resistência a grupos químicos de fungicidas envolvendo diferentes patossistemas. Resistência a ditiocarbamatos, por exemplo, tem sido observada em isolados de *Botrytis cinerea* causador do morfo cinzento em frutos de amora (*Rubus plicatus*) (CHEN et al., 2011). De acordo com os autores, o tamanho de lesões ocasionadas por isolados resistentes foram maiores que nos isolados sensíveis. Em Pêssego (*Prunus persica*) e mirtilo (*Vaccinium myrtillus*), um dos principais métodos de manejo da antracnose ocasionada por *Colletotrichum siamensis* consiste em aplicações consecutivas de fungicidas, onde alguns relatos informam a presença de resistência de isolados expostos a thiophanato-metil e azoxistrobina (HU et al., 2015). Da mesma forma, em plantios de mangueira no Nordeste brasileiro, foram identificados isolados de Botryosphaeriaceae apresentando variações nos níveis de sensibilidades a thiophanato metil e tiabendazol (SANTOS et al., 2018).

Avaliação da sensibilidade em populações de fitopatógenos representa uma importante técnica para avaliação dos estados de resistência a um determinado grupo de fungicidas (LEHNER et al., 2015). Levando em conta que redução na sensibilidade representa um dos fatores mais importantes na eficácia do controle químico, é de grande importância que estudos no monitoramento da resistência em populações de fungos expostos a fungicidas sejam conduzidos nas areas de cultivos.

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Capítulo II

Temporal conidia dispersal pattern of Botryosphaeriaceae species on table-grape vineyards in Northeastern Brazil

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Temporal conidia dispersal pattern of Botryosphaeriaceae species on table-grape vineyards in Northeastern Brazil

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Abstract

A field experiment was conducted between mid-August 2016 and mid-October 2017 in four table-grape vineyards in the Siriji Valley, Pernambuco State (Northeastern Brazil), to study the conidial dispersal dynamics of Botryosphaeriaceae fungi, causing grapevine trunk diseases (GTDs). Conidial dispersal was assessed by exposing microscope slides coated with petroleum gel close to symptomatic plants and pruning debris. The slides

were replaced every 2 weeks for a total of 30 sampling periods. Conidia of the genera *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were enumerated based on morphological characters. Conidia were collected from all four table-grape vineyards, confrming that these fungi are present as aerial inoculum and could be associated with GTDs in the region. Conidia of *Diplodia* and *Lasiodiplodia* were present in all the sampling periods, and those of *Lasiodiplodia* were the most abundant. Conidia of *Neofusicoccum* were found less frequently, and in less numbers than the other genera. Significant correlation between the number of conidia sampled and the amount of rain was observed for *Diplodia* only. Greater numbers of conidia were collected from pruning debris than from symptomatic plants. For *Diplodia* and *Lasiodiplodia*, the numbers of conidia gradually increased in September, increased sharply between March and June, and then decreased. These dynamics were described by a logistic equation, with hydro-thermal time (i.e., a combination of degree-days and relative humidity) as the independent variable ($R^2 > 0.998$).

Keywords: spore traps, *Vitis vinifera*, tropical climate, hydro-termal time.

Introduction

Viticulture is an important agricultural sector worldwide. In Brazil, the total grape production in 2016 was 1,499,353 t, distributed between table-grapes (47.88%) and wine-grapes (52.12%) (De Mello, 2016). The Siriji Valley located in the northeastern region of Brazil (Pernambuco state) is one of the main areas of table-grape production in this country. The Siriji Valley mainly produces table grape cv. Isabel, with areas of production characterized by family farms. The valley has a tropical climate (Aw in the Koppen-Geiger climate classification as indicated by Peel *et al.*

(2007)) with an average of 1075 mm rain per year and warm temperatures (yearly average of 26ºC) (Tavares and Lima, 2009).

Grapevine trunk diseases (GTDs), caused by several fungal species, are one of the main limiting factors for productivity and longevity of vines plants (Gramaje *et al*., 2018; Mondello *et al*., 2018). Botryosphaeria dieback, caused by Ascomycete fungi in the Botryosphaeriaceae (Philips *et al*., 2013; Yang *et al*., 2017), has emerged as one of the most damaging GTDs worldwide (Úrbez-Torres, 2011), because of: i) adaptability to diferent climatic conditions (Amponsah *et al*., 2009; Kuntzmann *et al*., 2009), ii) survival in pruning debris and dead plant material (Elena and Luque, 2016), and iii) prolifc inoculum production (Van Niekerk *et al*., 2010; Valencia *et al*., 2015).

Symptoms of Botryosphaeria dieback include foliar discoloration, fruit rot, dead canes and cordons, vascular necrosis and streaking, perennial cankers, and plant dieback (Amponsah *et al.,* 2011; Úrbez-Torres, 2011; Dissanayake *et al*., 2013; Gramaje *et al*., 2018). In Brazil, Botryospaheriaceae were firstly associated to grapevines affected by GTDs by Paradela *et al.* (1992) in the ‘90s. *Lasiodiplodia* and *Neofusicoccum* were the genera of Botryosphaeriaceae more frequently associated with GTDs in nurseries and vineyards (Batista *et al*., 2010; Correia *et al*., 2013; Correia *et al*., 2016; Garrido *et al*., 2017).

In the last decades, the taxonomy of the fungal taxa associated with GTDs and the etiology of these diseases have been studied in detail (van Niekerk *et al.*, 2004; Yang *et al.*, 2017). It has been suggested that these fungi overwinter as pycnidia and/or perithecia embedded in the vine bark or on the surface of dead grapevine wood. Therefore, the permanence of pruning debris above the vineyard soil can be an important source of inoculum for subsequent seasons (Elena and Luque, 2016). Conidia and/or ascospores are mainly released by these fruiting bodies during rain events or

under moist conditions, with temperatures above freezing (Úrbez-Torres, 2011; Gramaje *et al*., 2018). Infection of grapevine tissues can occur through natural openings, although it is mainly associated with the existence of pruning wounds or weak graft unions (Úrbez-Torres, 2011). Pruning shears can contribute to spread of GTD pathogens in afected vineyards (Agustí-Brisach *et al*., 2015).

The dynamics of Botryosphaeriaceae spores in vineyard environments have been studied recently because of their relevance for disease management (Amponsah *et al*., 2009; Kuntzmann *et al*., 2009; Úrbez-Torres *et al*., 2010; Van Niekerk *et al*., 2010; Baskarathevan *et al*., 2013; Valencia *et al*., 2015). The temporal dispersal patterns of these spores varied among studies, probably due to the geographical location, the fungal species, and the spore sampling methods (Gramaje *et al*., 2018). Since all these studies have been conducted in regions with temperate climates, no information exists about the dispersal of Botryosphaeriaceae spores under tropical conditions. Moreover, the dispersal pattern of conidia from pruning debris has not been considered in previous studies.

The objectives of the present research were: i) describe the temporal dynamics of conidial dispersal of Botryosphaeriaceae in four table-grape vineyards in the Siriji Valley, characterized by tropical climate, considering symptomatic plants and pruning debris as inoculum sources; ii) examine environmental variables associated with conidial dispersal; and iii) develop equations for predicting the temporal dispersal pattern of Botryosphaeriaceae spores under tropical climate conditions, which could be used to identify the periods with high risk of spore dispersal.

Material and methods

Vineyards

The study was conducted in four table-grape vineyards located in St Vicente Ferrer, in the tropical region (Siriji Valley) of Pernambuco state (Brazil). Vineyard one (7°34'53.4''S, 35°31'01.2''W), area 1.2 ha, was 14 years old; Vineyard two (7°35'27.2''S, 35°31'35.8''W), area 0.5 ha, was 18 years old; Vineyard three (7°35'21.3''S, 35°31'20.9''W), area 0.8 ha, was 9 years old; and Vineyard four (7°.34'.53.7'S, 35°30'05.3''W), area 1.4 ha, was 15 years old. All these vineyards were afected by GTDs at diferent levels of incidence (Vineyard 1, 22% of plants afected; Vineyard 2, 13%; Vineyard three, 23%; Vineyard 4, 98%). All four vineyards were planted with cv. Isabel on double cordon system, and cultural practices (irrigation, fertilizer plant protection applications) were performed following the usual practice. No fungicides were applied to control GTDs.

Data of temperature (T, °C), rainfall (R, mm), and relative humidity (RH, %) were obtained from the Agência Pernambucana de Água e Clima (APAC), responsible of the agrometeorological service in the region. Hourly records of T °C and RH were obtained from two weather stations situated in Carpina (7°50'57.3"S 35°14'19.5"W) and Goiana (7°38'24.1"S 34°57'21.4"W), located in opposite directions, respectively 42 km north-east and 62 km south-east from the vineyards. All three locations (the vineyards and the two weather stations) are in a fat area of the Siriji Valley. A preliminary analysis of the weather data recorded in Goiana (y) and Carpina (x) showed close linear relationship for T °C and RH, the regression equations being, for T, $y = 1.009x$ ($R^2 = 0.997$; $P < 0.001$) and for RH, $y = 1.030x$ ($R^2 = 0.994$; $P < 0.001$). Therefore, the weather data measured at both stations was considered representative of the vineyards under study. Thus, daily averages of T °C and RH were calculated using

126 the values from the two stations. Daily rainfall data were obtained from APAC for St
127 Vicente Ferrer, the location of the four vineyards.
128
129 **Conidial dispersal from symptomatic plants and pruning debris**
130 In each vineyard, conidial dispersal was evaluated by exposing spore traps
131 between 22 August 2016 and 17 October 2017, a period comprising two grape harvests.
132 Spore traps were microscope slides coated on one side with petroleum gel (Vaseline).
133 Spore traps were located, (i) in symptomatic plants or (ii) close to pruning debris. For
134 (i), ten plants with the typical Botryosphaeria dieback symptoms were selected, and one
135 microscope slide per plant was attached (oriented horizontally) to the cane with a clip,
136 the gel side upward. For (ii), pruning debris were collected at the beginning of the
137 experiment and arranged to form three piles (diam. 50 cm and height 30 cm), each pile
138 with canes from 50 symptomatic vines. Two spore trap devices were located at 10 cm
139 from the pruning debris and 30 cm above the soil (supported by a metal stake). Each
140 spore -trap device comprised two microscope slides inserted into slots on a polystyrene
141 slab; the slides were in parallel, with the adhesive gel side oriented towards the pile of
142 pruning debris. The slides were covered by a Petri dish to avoid washing by rain
143 (González-Domínguez *et al*., 2014).
144 Microscope slides were replaced every 2 weeks, commencing on 5 September
145 2016, for a total of 30 sampling periods. Microscope slides were brought to the
146 laboratory, and each prepared for microscope observation by adding lactophenol cotton
147 blue to the gel side and a cover slip. Conidia of *Diplodia*, *Lasiodiplodia* and
148 *Neofusicoccum* were identified based on the morphological characteristics described by
149 Philips *et al*. (2013), and were enumerated using a compound microscope (40×
150 magnifcation). All the cover slip area (400 mm^2) of each slide was considered. The

151 ascospores of these genera were not considered because their sexual stages have rarely
152 been found in grapevines (Phillips *et al*., 2013, Úrbez-Torres *et al*., 2013), so that
153 conidia are considered to be the principal sources of infection for these fungi (Úrbez-
154 Torres *et al*., 2013). The numbers of conidia were expressed as the cumulative numbers
155 collected over a 2 week period per cm^2 of trap surface. In aggregate, there were 1,200
156 microscope slides were on symptomatic plants and 1,440 were near pruning debris
157 piles.

158 At each sampling period, the growth stage of vines was assessed using the scale
159 described by Lorenz *et al*. (1995).

160

161 **Data analysis**

162 All data analyses were performed using the software R (v 3.4.0; R CoreTeam,
163 2014). In all the analyses, the four vineyards were considered as replicates.

164 The relationship between the amount of rain (mm) and the number of
165 Botryosphaeriaceae conidia found in the microscope slides (for each fungal genus and
166 inoculum source) was evaluated through the non parametric Spearman rank correlation
167 coefficient (by using the function cor.test from the 'stats' package).

168 The cumulative number of conidia at different times was expressed as a
169 proportion of the total seasonal conidia (PSC) for each vineyard, Botryosphaeriaceae
170 genus, and inoculum source. Then, the average and the standard error for the different
171 vineyards were calculated for the genera *Diplodia* and *Lasiodiplodia*, and for both
172 inoculum sources (symptomatic plants and pruning debris). *Neofussicoccum* was not
173 considered in this analysis because of the low number of conidia found during the study.

Average PSC values were regressed against time (t=1 is the first day in which the microscope slides were exposed) by using a logistic equation in the following form (Campbell and Madden, 1990):

$$y=1/(1+(a \times \exp(-b \times t))) \quad (1)$$

where: y is the PSC; a is the equation parameter accounting for the length of the lag phase of the S-shaped curve; b is the rate parameter; and t is the time. The equations were fitted to the data using the nls function of the 'stats' package.

To assess the effect of the environment in the dynamics of conidial dispersal, PSC values were regressed against the thermal or hydro-thermal time (Lovell *et al.*, 2004). PSC was calculated as the sum of conidia from symptomatic plants and pruning debris, and this value was averaged between the vineyards. Average PSC value of both genera, *Diplodia* and *Lasiodiplodia,* was also calculated. Time was expressed as a function of thermal time or hydro-thermal in three different forms: (i) the combination of degree-days and RH (DD-RH), (ii) temperature dependent mycelial growth rate (MGR) and (iii) the combination of MGR and RH (MGR-RH). For (i), the daily values of temperature and RH were accumulated during the experimental period in the form: $\sum T \times RH/1000$. For (ii) daily values of temperature were accumulated as a function of mycelial growth rate (MGR). The MGR was calculated using data from a laboratory experiment developed by Netto *et al.* (2017) with four isolates of *Lasiodiplodia theobromae* from the Pernambuco state (Brazil). Supplementary material S1 describe briefly the experiment developed by Netto *et al.* (2017) and the non-linear model build in this work. For (iii) daily values of temperature and RH were accumulated in the form: $\sum MGR \times RH/100$.

In a preliminary analysis, logistic and Gompertz equations were fitted to the PSC by using the thermal or hydro-thermal time parameters as independent variables (i.e.,

199 DD-RH, MGR, and MGR-RH) (Madden *et al.*, 2007). Logistic equations were selected
200 because their better goodness of fit. Goodness-of-fit of the different equations was
201 assessed by using the adjusted R^2, the magnitude of the standard error of the parameters,
202 the coefficient of residual mass (CRM) and the concordance correlation coefficient
203 (CCC) (Nash and Sutcliffe 1970; Lin 1989; Madden *et al.*, 2007). CRM is a measure of
204 the tendency of the equation to overestimate or underestimate the observed values (a
205 negative CRM indicates a tendency of the model toward overestimation). The CCC is
206 the product of two terms: the Pearson correlation coefficient and the coefficient *Cb*,
207 which indicates the difference between the best fitting line and the perfect agreement
208 line (CCC = 1 indicates perfect agreement). CCC was calculated by using the *epi.ccc*
209 function of the 'epi.R' package.
210

211 # Results

212 **Meteorological conditions**

213 A summary of the environmental conditions registered during the experiment is
214 shown in Figure 1. The daily temperature showed low variability, with an average of
215 26.4ºC and fluctuations < 5°C (Figure 1A). Daily relative humidity was always > 70%
216 (Figure 1B). The precipitation accumulated during the 2-week sampling periods showed
217 high variability, with an average of 46.6 mm and a maximum of 167.5 mm rain. No
218 rainfall was recorded in 4 of the 30 sampling periods, and accumulated rainfall was
219 lower than 40 mm in 17 periods; in 10 and 4 periods, > 60 and > 100 mm rainfall
220 occurred (Figures 1C and D).
221

222 **Conidia from symptomatic plants and pruning debris**

223 Conidia of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were found on
224 microscope slides placed close to both inoculum sources, symptomatic plants and
225 pruning debris. No other Botryosphaeriaceae spores (i.e., ascospores or conidia of other
226 genera of the family) were found. A total of $3.72x10^6$ conidia/cm^2 were enumerated;
227 91.0% of the conidia were *Lasiodiplodia*, 6.5% were of *Diplodia* and 2.5% were of
228 *Neofusicoccum*. Conidia of *Diplodia* and *Lasiodiplodia* were observed in all the 30
229 sampling times, whereas *Neofusicoccum* was observed in 22 of the 30 sampling periods
230 from symptomatic plants, and 23 of the 30 sampling periods from pruning debris
231 (Figure 2). Of total conidia, 18.8% were found on microscope slides close to pruning
232 debris. These diferences were greater for *Lasiodiplodia* than for the other two genera,
233 with a total of 5.98 × 105 cm^{-2} trapping surface from symptomatic plants, and 8.69 ×
234 104 conidia cm^{-2} from pruning debris (Figure 2).
235
236 **Temporal dynamics of the conidia**
237 Peaks of *Diplodia* and *Lasiodiplodia* conidia were mainly found in 2-week
238 periods with high rainfall (Figure 3). On symptomatic plants, the maximum number of
239 *Diplodia* conidia (334 conidia/cm^2) was found on microscope slides collected on 28
240 December 2016, after a 2-week period with a total of 78.9 mm. Close to debris, the peak
241 of *Diplodia* conidia (413 conidia/cm^2) was observed on 17 May 2017, after a 2-week
242 period with a total of 129.4 mm rainfall (Figures 3A and 3B). A positive correlation was
243 found between the number of *Diplodia* conidia from either symptomatic plants (r=0.62,
244 $P<0.001$) or pruning debris (r=0.59, $P<0.001$) and the amount of rain.
245 Few conidia of *Lasiodiplodia* were found at the beginning of the season, most of
246 the conidia being sampled from February to mid-June (45.1% of the total conidia) from
247 symptomatic plants, and from January to July (95.0%) from pruning debris (Figure 3C).

248 Conidia of *Neofusicoccum* were sampled from symptomatic plants mainly from
249 mid-October to mid-December 2016. Near pruning debris, most of the conidia were
250 sampled on 23 August 2017, representing > 65% of the total conidia (Figure 3D). No
251 significant correlations were found between rainfall and numbers of *Lasiodiplodia* or
252 *Neofussicoccum* conidia.

253 When the conidia were expressed as the proportions of the seasonal conidia
254 (PSC), dynamics over time were similar and fitted to logistic regressions, with $R^2 > 0.95$
255 (Figure 4 and Table 1). The variability in PSC in the diferent vineyards (standard errors,
256 in Figure 4) was within the 95% confidence intervals of the logistic equations (dashed
257 lines in Figure 4). This confirmed that the logistic equations represent the diferent
258 vineyards well.

259 When time was expressed as thermal or hydrothermal time (i.e., DD-RH, MGR,
260 or MGR-RH), the goodness of fit of the logistic equation increased, with R^2>0.98 and
261 CCC>0.99 for both *Diplodia* and *Lasiodiplodia* (Table 2); CRM values were low,
262 indicating that the equation did not under- or overestimate the real data. The fit of the
263 logistic equation to the PSC values was also good when data from *Diplodia* and
264 *Lasiodiplodia* were pooled, indicating that the conidial dispersal pattern were similar for
265 the two genera. In general, based on AIC and CRM, the DD-RH better represents the
266 temporal dynamics and should be selected to express time. When DD-RH was selected
267 and both genera were considered together, the R^2 and CCC of the model were 0.998, the
268 lowest value of CRM was obtained (0.007) and the SE of the parameters was low (Table
269 2).

270

271

272

Discussion

To our knowledge, this is the first study addressing the dispersal of Botryosphaeriaceae conidia under these conditions.

In this research, the temporal dynamics of conidial dispersal of Botryosphaeriaceae fungi (specifically *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*) were investigated by using spore samplers. The samplers were located on symptomatic plants or near pruning debris, in four table-grape vineyards of Northeastern Brazil, under tropical climatic conditions. The tropical climate is characterized by precipitation distributed regularly through each year, and warm temperatures and high relative humidity (Peel *et al*., 2007).

The spore samplers used to study the conidial dispersal were slightly different for symptomatic plants microscope slides attached to plants were used, and these are suitable for trapping rain-splashed spores. In the latter case (pruning debris), vertical microscope slides were used, which are suitable for trapping airborne spores (Campbell and Madden, 1990). This difference may have affected the numbers of conidia trapped by the two spore sampler types. However, the inoculum from infected plants may be mainly splash-borne, and mainly air-borne (i.e., wind-driven, splashing droplets) when produced from pruning debris above the vineyard soil. Thus, the different types of spore samplers used probably reflect this difference.

Conidia of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were collected from all four of the table-grape vineyards, confirming that these fungi are associated with GTDs in Northeastern Brazil (Cimmino *et al*., 2017). However, conidia of *Neofusicoccum* were found less frequently, and in lower numbers, than the other two genera, both from symptomatic plants and pruning debris. In this region, the occurrence of *Neofusicoccum* may be less than *Lasiodiplodia* and *Diplodia*. Only

298 recently has the occurrence of *Neofusicoccum* spp. associated with GTDs been
299 reported in Brazil (Correia *et al*., 2013). Similar to the results of the present study,
300 fewer conidia of *Neofusicoccum* than *Diplodia* were trapped in a 2-year experiment
301 conducted in Chile (Valencia *et al*., 2015). Further studies are required to provide
302 understanding of the implication of *Neofusicoccum* spp. in the development of GTDs
303 in grapevines in Brazil, as well as in other countries.

304 *Lasiodiplodia* and *Diplodia* conidia were trapped in all the sampling periods,
305 and in high numbers in the case of *Lasiodiplodia*. The dispersal of both genera was
306 related to rainfall events, as rainfall occurred in 27 of the 30 sampling periods. This
307 result is similar to those from previous vineyard spore-trapping studies, in which
308 Botryosphaeriaceae conidia were captured during and/or following rainfall (Úrbez-
309 Torres *et al*., 2010; Van Niekerk *et al*., 2010; Valencia *et al*., 2015). Studies in South
310 Africa detected conidia of these fungi during or after as little as 0.25 mm rainfall (Van
311 Niekerk *et al*., 2010), and found significant correlations between numbers of conidia
312 and the amounts of rainfall; these correlations were also found by Úrbez-Torres *et al*.
313 (2010) in California. In the present study, statistically significant found for numbers of
314 *Diplodia* conidia, but not for those of *Lasiodiplodia* or *Neofusicoccum*.

315 Conidia of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* were also found in the
316 four periods when no rainfall was recorded. In other pycnidia-producing fungi,
317 moderate to warm temperatures and high relative humidity (> 80%) promote the
318 production of pycnidia, and increase the number of conidia produced per pycnidium
319 (Lalancette *et al*., 2003; Anco *et al*., 2013; Onesti *et al*., 2017). The extrusion of cirri
320 from pycnidia requires free water; but RHs close to 100% can provide sufficient
321 moisture for extrusion (Janex-Favre *et al*., 1993; Onesti *et al*., 2017). In addition, high
322 RHs may contribute to maintaining the gelatinous matrix of the cirri for long periods,

323 favoring the viability of released conidia (Moore and Ostry, 2015). All this information
324 collectively makes it plausible that in tropical climates, with warm temperatures and
325 high RH throughout the year, the dispersal of Botryosphaeriaceae conidia may occur in
326 the absence of rainfall.

327 To our knowledge, this is the first study that has evaluated dispersal of conidia
328 of Botryosphaeriaceae from pruning debris. A larger number of conidia were trapped
329 from pruning debris than from symptomatic plants, especially for *Lasiodiplodia*. Elena
330 and Luque (2016) found that conidia of *D. seriata* remained viable in debris up to 42
331 months after pruning, and warned that pruning debris left in vineyards were long-
332 lasting inoculum sources for this pathogen. The present study confirms this finding for
333 *Diplodia* and *Lasiodiplodia*. Therefore, the elimination of pruning debris (by removal
334 or burning) is a key disease management strategy, to reduce the amounts of pathogen
335 inoculum in vineyards (Gramaje *et al*., 2018). The incorporation of pruning debris into
336 the soil after composting is an alternative disease management practice. Lecomte *et al*.
337 (2006) showed that inoculum of *D. seriata*, *Phaeomoniella chlamydospora*,
338 *Phaeoacremonium minimum*, and *Eutypa lata* was eliminated from grapevine wood
339 tissues after composting for 6 months. Petruta *et al*. (2016) also demonstrated the
340 potential of composting vine pruning debris to control *D. seriata*. However, before
341 recommending this practice, further research is required to confirm these results, and
342 to consider other GTD pathogens.

343 Although the numbers of *Diplodia* and *Lasiodiplodia* conidia differed for the
344 two inoculum sources, the patterns of conidia dispersal were similar for both genera.
345 Dispersal of *Diplodia* and *Lasiodiplodia* conidia increased gradually at the beginning
346 of the experiment (in September), greatly increased in March and June, and then
347 increased at slow rates between August and mid-October. These trends of conidial

dispersal were described by logistic equations (as demonstrated by goodness-of-ft), in which the thermal-time was used as the driving variable. The logistic, Gompertz and monomolecular equations have been widely used in analyses of disease progress (Campbell and Madden, 1990), and thermal or hydro-thermal time have also been widely used (Lovell *et al*., 2004; Rossi *et al*., 2010b; Onesti *et al*., 2018). In the present study, the logistic equations did not over- or under-estimate the dispersal of conidia for both *Diplodia* and *Lasiodiplodia*. Over-estimation or under-estimation of the real data may limit the predictive capacity of conidia dispersal models, which are essential components of epidemiological models (Dewolf and Isard, 2007). Thus, the equations developed here can potentially be used to predict periods of high risk of conidial dispersal of *Diplodia* and *Lasiodiplodia* in vineyards in the tropical region of Northeastern Brazil. Before recommending their use, these equations need to be validated to assess their accuracy (i.e., closeness of predicted to observed values), with data from different years and locations (Rossi *et al*., 2010a). However, these equations are likely to ft well to new, independent data, because of the stability of tropical climates, where the environment is not undergoing major changes.

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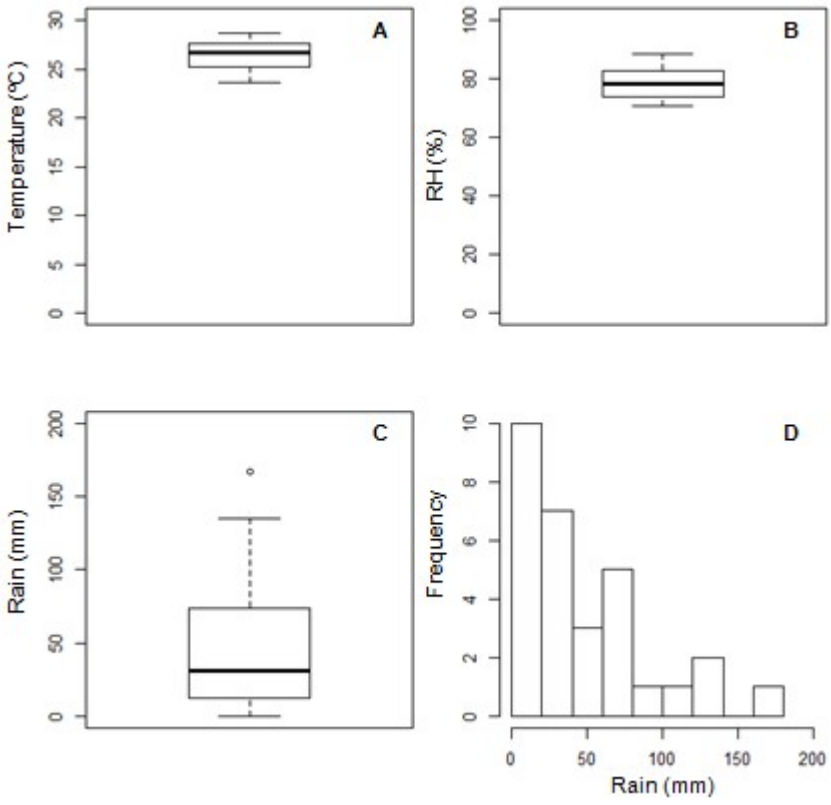
505

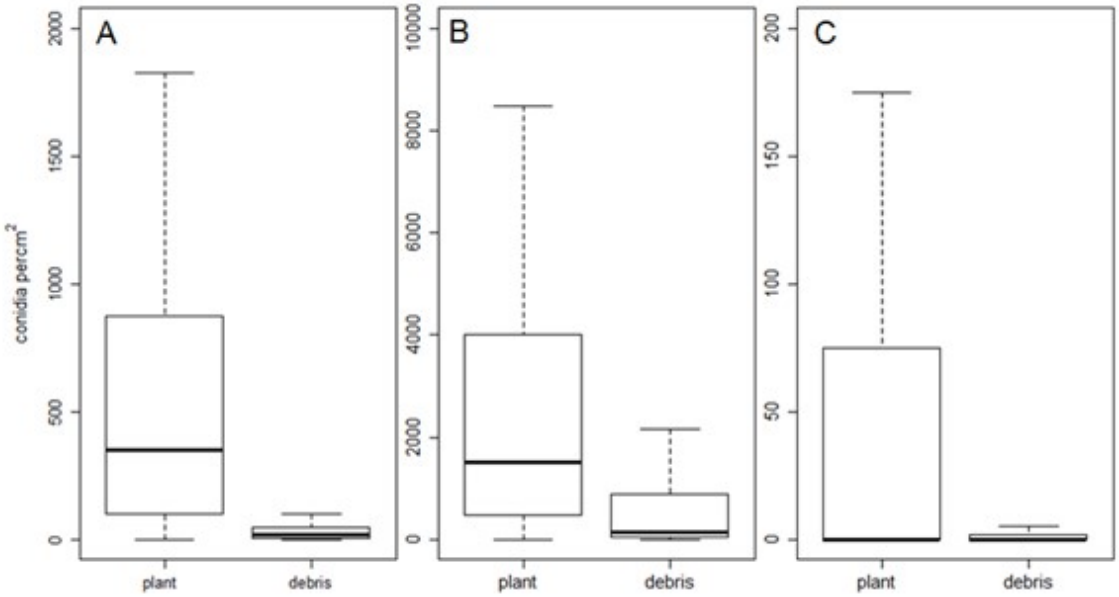
506 **Figure captions:**



507

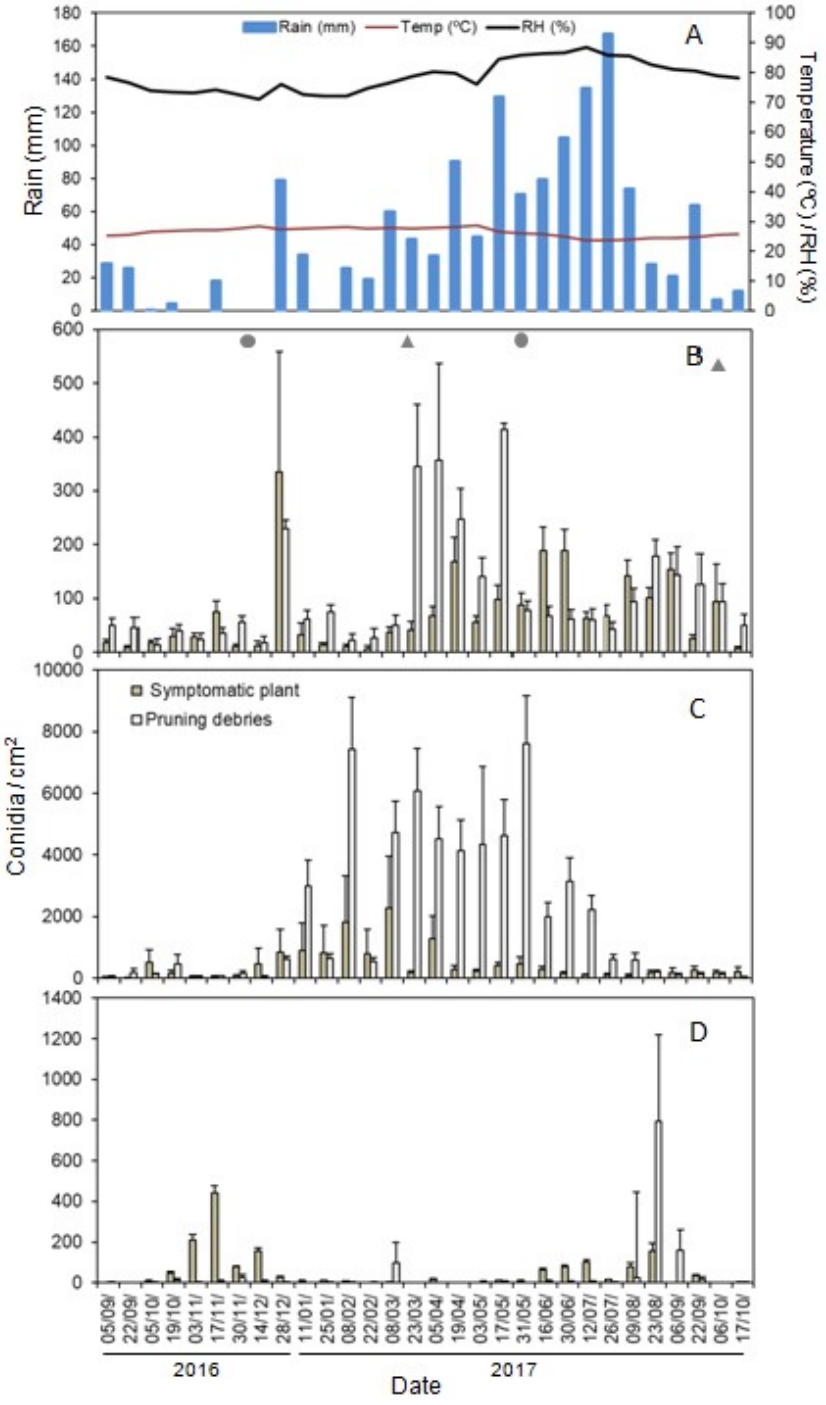
508 **Figure 1.** Boxplots of the distribution of temperature (T, ºC) (A), relative humidity
509 (RH, %) (B), accumulated rain (in mm) (C), and frequency distribution of accumulated
510 rain (in mm) (D) registered during the 30 2-week long periods in which the dispersal of

511 *Diplodia*, *Lasiodiplodia*, and *Neofusicoccum* conidia was studied in four Brazilian
512 vineyards. Boxes include the 2nd and 3rd quartile, the dotted line is the median, whiskers
513 extend to minimum and maximum values, and points are outliers.



514

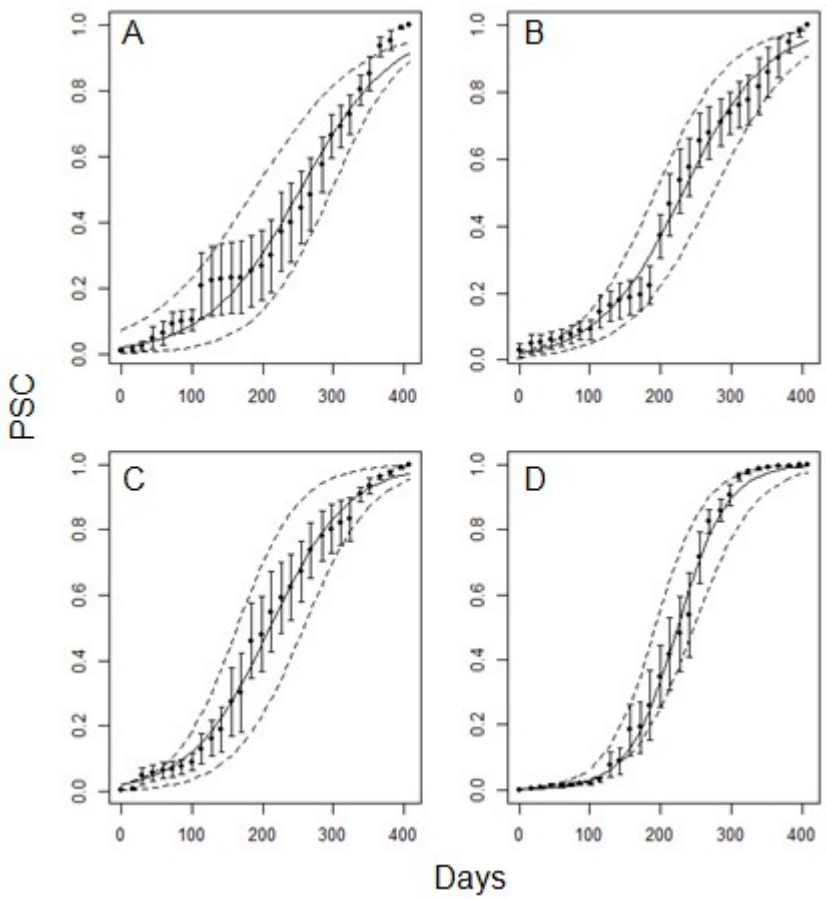
515 **Figure 2.** Boxplot of the distribution of the number of conidia sampled per cm^2 of spore
516 trap surface from symptomatic plants and pruning debris found during the 30 2-week
517 long sampling periods for *Diplodia* (A), *Lasiodiplodia* (B), and *Neofusicoccum* (C).
518 Boxes include the 2nd and 3rd quartile, the dotted line is the median, and whiskers extend
519 to minimum and maximum values. Outliers are not represented.



520

521 **Figure 3.** Numbers of conidia of *Diplodia* (B), *Lasiodiplodia* (C), and *Neofusicoccum*
522 (D) sampled per cm^2 of spore trap surface from symptomatic plants and pruning debris
523 found between August 2016 to October 2017 in four vineyards in St Vicente Ferrer
524 (Brazil); in A, the weather data are also presented. Spore traps were replaced every 2
525 weeks, commencing from 5 September 2016. Bars are averages for the four vineyards,
526 and whiskers represent the standard errors. In B, grey triangles show the full flowering

527 stage of plants (stage 65; Lorenz et al., 2005) and grey circles show the berries ripe
528 stage (stage 89).
529



530

531 **Figure 4.** Proportion of the seasonal *Diplodia* conidia (PSC), sampled from
532 symptomatic plants (A) or pruning debris (B), and for *Lasiodiplodia* from
533 symptomatic plants (C) or pruning debris (D), in St Vicente Ferrer (Brazil), between
534 August 2016 and October 2017. Points represent average values for four vineyards and
535 bars represent the standard errors. Lines show the logistic equation fitted to the data
536 (—), and its 95% confidence limits (---) (see Table 1 for parameters of the four
537 equations).

538 **Table 1.** Estimated parameters of logistic equations used to describe the proportions of
539 seasonal *Diplodia* and *Lasiodiplodia* conidia (PSC) found over time from symptomatic
540 plants or pruning debris in four vineyards in St Vicente Ferrer (Brazil), between August
541 2016 and October 2017.

		Estimated parameters[a]		R^2
Source	**Genera**	***a***	***b***	
Plant	*Diplodia*	7.78 (12.77-300)	0.008 (0.013-0.019)	0.976
	Lasiodiplodia	45.30 (46.59-196.40)	0.018 (0.023-0.002)	0.994
Debries	*Diplodia*	50.81 (41.39-105.60)	0.017 (0.019-0.017)	0.989
	Lasiodiplodia	609.10 (263.50-300.00)	0.028 (0.029-0.023)	0.997

542 [a] Regression equation is $y=1/(1+(a \times \exp(-b \times t)))$ where y is the proportion of seasonal conidia (PSC), a
543 and b are the equation parameters, and t is the time (t=1 is the first day in which the microscope slides
544 were exposed). Confidence intervals of the estimated parameters are shown in parentheses.

Table 2. Parameters and goodness-of-ft statistics of the equations used to describe the effects of different physiological units on the cumulative numbers of Botryosphaeriaceae conidia sampled from symptomatic grapevine plants or pruning debris, in four vineyards in St Vicente Ferrer (Brazil) between August 2016 and October 2017.

Fungi	Physiological units[a]	Estimated parameters[b]		Goodness-of-fit[c]			
		a	*b*	R^2	CRM	CCC	AIC
Diplodia	DD-RH	48.93 (6.69)	0.07 (0.002)	0.991	0.017	0.996	-117.03
	MGR	75.50 (16.25)	1.97 (0.093)	0.984	0.032	0.991	-98.49
	MGR-RH	58.10 (10.46)	2.45 (0.103)	0.987	0.026	0.993	-104.17
Lasiodiplodia	DD-RH	132.30 (13.64)	0.10 (0.002)	0.998	0.002	0.999	-154.46
	MGR	193.91 (29.94)	2.63 (0.072)	0.997	0.015	0.998	-134.99
	MGR-RH	143.13 (18.29)	3.30 (0.082)	0.997	0.009	0.998	-142.12
Both	DD-RH	71.01 (5.61)	0.08 (0.001)	0.998	0.007	0.998	-157.76
	MGR	108.27 (15.77)	2.24 (0.06)	0.999	0.021	0.997	-128.15
	MGR-RH	81.75 (9.37)	2.79 (0.07)	0.996	0.015	0.998	-137.79

[a] DD-RH (degree-days and RH) were calculated by accumulating the daily values of temperature and RH during the sampling period; MGR (temperature dependent mycelial growth rate) was calculated by regressing data from a *Lasiodiplodia* spp. laboratory experiment (Netto *et al.*, 2017) as indicated in the supplementary material S1. MGR-RH was calculated by accumulating daily values of MGR and RH.

[b]Regression equation is $y=1/(1+(a \times \exp(-b \times t)))$ where y is the proportion of seasonal conidia (PSC), a and b are the equation parameters, and t is the time expressed as physiological units. Standard errors of the estimated parameters are shown in parentheses.

[c]R^2, coefficient of determination; CRM, coefficient of residual mass; CCC, concordance correlation coefficient; AIC: Akaike's information criterion.

Capítulo III

Sensitivity and fitness of *Lasiodiplodia theobromae* from Brazilian Northeast grapevine vineyards to fungicides

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Sensitivity and fitness of *Lasiodiplodia theobromae* from Brazilian Northeast grapevine vineyards to fungicides

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Abstract

Botryosphaeriaceae dieback is an important trunk disease of grape in Brazil. Thiophanate-methyl, tebuconazole, azoxistrobin and iprodione are common fungicides used in crops all over the world, but there are no records for grapevine. However, it is important to know the state of the sensitivity of the *Lasiodiplodia theobromae*. The effective concentration that results in 50% of mycelial growth inhibition (EC_{50}) of 62 isolates, representing different populations of the pathogen was estimated in vitro. Ten isolates with lower (sensitive, S) and high (less-sensitive, LS) values of EC_{50} were evaluated in relation to stability of sensitivity, efficacy of the fungicides described above to disease control in grapevine and five components of fitness. The isolates of *L. theobromae* showed difference in the sensitivity to tebuconazole, thiophanate-methyl, iprodione and azoxystrobin fungicides, with EC_{50} values ranging from 0.044 to 3 μg ml^{-1} for the majority of isolates. Analysis of fitness components (mycelial growth, osmotic potential, pycnidia and conidia production) showed significant differences (P = 0.05) within each fungicide studied. This work represents one of the few studies that clarify the situation of isolates of *L. theobromae* from vineyards affected with Botryosphaeriaceae dieback and sensibility relationships with different groups of fungicides.

Keywords Grape · vineyard · Botryosphaeriaceae dieback · Fungicide resistance · Trunk disease

Introduction

Grapes (*Vitis* spp.) are one of the most commonly produced crops worldwide, which can adapt to different types of climates and a variety of soils. In Brazil, the cultivation of

grapes is distributed in two regions destined to the production of wine and in nature consumption, in the South and Northeast respectively. In 2016, the Brazilian area of planting corresponded to 127860 ha with a production of 984,481 t (FAO 2016). In the Northeast the principally region there are three production pole Jaguaribe, Sirijí and São Francisco Valley. The São Francisco Valley is the principal pole of production, characterized by a technical management where, fine varieties are used for in natura consumption with a production of 294,307 t in 2016 (De Melo 2017).

During grape cultivation the plants are attacked by diseases associated to wide range of pathogens which lead to a poor performance of vines and decreasing plant life (Gramaje and Armengol 2011). One of these corresponds to a trunk disease caused by different fungal groups such as species relative to the Brotryosphaeriaceae family as *Lasiodiplodia* (Úrbez-Torres 2011) and *Neofusicoccum* (Billones-Baaijens et al. 2015).

Lasiodiplodia is a fungi genus belonging to the Botryosphaeriaceae family that are associated with trunk disease in grapes principally dieback disease (Rodríguez-Gálvez; Maldonado; Alves, 2015). Recent studies have revealed the presence of large number of *Lasiodiplodia* species involved with these diseases in the Brazilian Northest (Correia et al. 2015). In this region, *L. theobromae* has been the most frequent species associated with Botryosphaeriaceae dieback in table grape (Correia et al. 2013, 2015).

For Botryosphaeriaceae dieback, widely management is aimed at preventing infection and delaying symptom development by reducing inoculum at its source. This includes prevention, by breaking the disease cycle removing inoculum source, and reducing the risk of further infection, reducing the impact on the vineyard by bringing diseased grapevines back into full production pruning and removal of symptomatic branches (Sosnowski et al. 2010; Úrbez-Torres 2010).

The chemical management of diseases is one of the most commonly used

methods to control plant diseases, a fact confirmed by the growing demand for these products (fungicides, insecticides and herbicides), each year (Euro stat 2013). In Brazil, there are no fungicides registered to control of *Lasiodiplodia* spp. in grape, and in other fruit crops such as mango, only difenoconazole (demethylation inhibitor group-DMI) is registered (MAPA 2018). However, throughout the cultivation of the grape *Lasiodiplodia* species are frequently exposed to different chemical groups for instance: strobilurin and methyl benzimidazole carbamate (MBC) that are commonly applied in the management of other diseases in the orchards in all the country (Gomes et al. 2011; Zaffari and Borba 2016).

Over the years it has been reported the occurrence of sensitivity to different groups of fungicides by isolates of *Lasiodiplodia* species collected in mango (Santos et al. 2018) and papaya (Cavalcante et al. 2014) in the northeast of Brazil. This fact is important, since the use of chemical molecules represents the main method of management of diseases in grapevines. In addition, the fungicides groups MBC, DMI (Demethylation Inhibitor), azoxystrobin and dicarboximides are registered for the control of oidium and mildew in grape (MAPA 2018), broadly exposing the populations of *Lasiodiplodia* of the vineyards.

These fungicides represent different groups of active ingredients specifically as the site of action. The mechanism of MBC action is via binding to fungal tubulin (Davidse 1975). The DMI (Demethylation inhibitor) fungicide, have a broad spectrum of activity and acts in the inhibition of sterol synthesis (Zweytick et al. 2000). Dicarboximides fungicides interfere with the osmotic signal transduction pathway through histidine kinase and MAP kinase cascades (Fujimura 2003). Strobilurins (QoI) group, controllers of a wide variety of fungal diseases, display a single-site mode of action associated with inhibition of mitochondrial respiration by binding at the Qo site

of the cytochrome bc1 enzyme complex (complex III) (Fisher and Meunier 2008).

The constant exposure to fungicides can contribute to the appearance of resistance in fungi. In this sense, this study aimed to estimate the sensitivity of *L. theobromae* isolates to MBCs, DMI, strobilurins and diocarboximida fungicides, which are commonly used next to table grape areas in northeastern Brazil and the relationship between sensitivity to fitness-related parameters.

Materials and methods

Fungal isolates

A total of 62 isolates of *L. theobromae* (Table 1), were obtained from symptomatic plants showing symptoms of Botryosphaeriaceae dieback in commercial vineyard from Pernambuco (PE), Bahia (BA), and Ceará (CE) states, Brazil, during 2014 (Fig. 1).

In each field, a sample of trunk and braches was collected and placed in labeled plastic bags. Dead and healthy tissue were collected from the internal necroses, were washed under running tap water, surface-disinfected for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile distilled water. Small pieces (4–5 mm) of tissue were taken from the margin between necrotic and apparently healthy tissue and plated onto potato dextrose agar (PDA) (Acumedia) amended with 0.5 g L-1 streptomycin sulphate (PDAS). Plates were incubated at 25°C in the dark for 7 days. To obtain monosporic isolates, plugs of colony margin were plated onto AW (agar-water) and collected a single hypha under a stereomicroscope (Stemi DV4; Zeiss) and transferred to PDA. All isolates were stored on PDA slants at 5 °C in the dark.

The isolates were identified through phylogenetic inference based on the partial sequences of the elongation factor 1-α gene (EF1-α) and complete sequence of the internal transcript space (ITS) and were maintained at the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Federal Rural University of Pernambuco (Recife, Pernambuco, Brazil) and stock cultures were stored in PDA slants at 5 °C in the dark.

In vitro sensitivity of the *Lasiodiplodia theobromae* isolates to thiophanate-methyl, tebuconazole, azoxystrobin and iprodione

Commercial formulation of thiophanate-methyl (Cercobin 700 WP, 700 g/ kg^{-1} active ingredient (a.i.), Iharabras, Sorocaba-SP, Brazil), tebuconazole (Folicur 200 EC g/L^{-1} a.i., Bayer CropScience LP, São Paulo-SP, Brazil), azoxistrobin (Amistar 500 TOP g/kg a.i., Syngenta Limited, São Paulo-SP, Brazil) and iprodione (Rovral 500 SC g/kg^{-1} a.i., FMC do Brasil, Campinas-SP, Brazil) were evaluated by a mycelial growth assay. Fungicides were solubilized in sterile distilled water and added to molten (45 °C) potato dextrose agar (PDA) medium at final concentrations: 0.1, 0.5, 1.0, 2.0, 4.0 and 6.0 µg a.i. ml^{-1} for tebuconazole; 0.3, 0.5, 1.0, 3.0, 4.0 and 16.0 µg a.i. ml^{-1} for thiophanate-methyl; 0.1, 0.3, 0.5, 1.0, 3.0 and 6.0 µg a.i. ml^{-1} for azoxystrobin; and 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 µg a.i. ml^{-1} for iprodione. Mycelial plugs (5 mm in diameter) were removed from the margins of 4-day-old cultures of *L. theobromae* and transferred to the center of the PDA plates amended with the different fungicide concentrations. Petri plates containing medium without fungicide were used as controls. Four replicates of each isolate were used to test each fungicide concentration and control. After a 48-h incubation period at 30 °C in the dark conditions, the diameter of each colony was

146 measured in two perpendicular directions, and the original mycelial plug diameter (5
147 mm) was subtracted from this measurement. The percentage of mycelial growth
148 inhibition related to the control was calculated for all the fungicide concentrations. The
149 effective fungicide concentration (μg ml^{-1}) to inhibit 50% of mycelial growth (EC_{50})
150 was calculated for individual isolates. Frequency distributions of the isolates between
151 the intervals of EC_{50} values were established. The ten most-sensitive (S) and the ten
152 least-sensitive (LS) isolates were grouped and mean EC_{50} values were determined for
153 each sensitivity class (Table 2).
154
155 Analysis of fitness components
156
157 Fitness components were determined for S and LS isolates exposed previsously to
158 thiophanate-methyl, tebuconazole, azoxystrobin and iprodione: mycelial growth rate,
159 pycnidia and conidia production, and osmotic sensitivity.
160 To evaluate the mycelial growth rate (MGR), a mycelia plug (5 mm in diameter)
161 was removed from the margin of a 4-day-old culture of each selected isolate and
162 transferred to the center of a Petri plate containing fungicide-free PDA. The plates were
163 incubated in the dark at 30 °C. Five replicates per isolate were used. The colony
164 diameter was measured at 36 h in two perpendicular directions, and the average was
165 used to calculate the MGR (mm $hour^{-1}$).
166 For pycnidia and conidia production, 3 stereli fragments of wood (5 cm) (cv.
167 Isabel) were arranged triangularly in 2% water-agar (WA). Mycelial plugs (5 mm in
168 diameter) were removed from the margin of a 4-day-old culture of each isolate and
169 transferred to the center of Petri plates. The plates were incubated at 27 °C for a period
170 of 5 weeks under a photoperiod of 12 hours of ultraviolet light. The experimental design

was completely randomized, with four replicates (plates) per treatment (isolated). The pycnidia quantification was performed by counting in all fragments and pycnidia number was expressed as the total found in each replicate. Count of conidia, was performed by the mycelial scraping of the fragments and arranged in test tubes containing 30 ml of water and subsequent mechanical stirring (vortex) for 1 minute. The suspension obtained from each isolate was filtered on a double layer of gauze with sterilized water. Four drops of suspension were counted from each tube.

To determine the osmotic sensitivity, mycelial plugs (5 mm in diameter) were removed from the margins of 4-day-old cultures of each isolate and transferred to the center of the PDA Petri plates amended with 1, 2, 4, 6 or 8% (wt vol^{-1}) NaCl. Petri plates containing medium without NaCl were used as controls. Four replicate plates per isolate-NaCl concentration combination were used. After 36-h incubation period at 30 °C in the dark, the diameter of each colony was measured in two perpendicular directions, and the original mycelial plug diameter (5 mm) was subtracted from this measurement. The percentage of mycelial growth inhibition related to the control was calculated for all the concentrations of the NaCl. The effective NaCl concentration (%) to inhibit 50% of mycelial growth ($EC_{50}N$) was calculated for individual isolates.

Data analysis

The experiment about *in vitro* sensitivity, the EC_{50} was calculated by linear regressions of the mycelial growth inhibitions versus the $\log_{10}$ transformation of the fungicide concentrations. Then, the EC_{50} values of ten isolates most-sensitive (S) and least-sensitive (LS) were compared using Student's *t*-test (P=0.05). In the experiment of fitness components, the difference between EC_{50} values prior and after transfers for

196 least-sensitive and sensitive isolates was determined using a Student's *t*-test (P=0.05). In
197 the experiment of osmotic sensitivity, the $EC_{50}N$ was calculated for individual isolates
198 by linear regression of the mycelial growth inhibitions versus NaCl concentrations. In
199 all fitness experiments, for each variable (MGR, pycnidia and conidia production, and
200 $EC_{50}N$), differences between the sensitive (S) and least-sensitive (LS) isolates were
201 determined using a Student's *t*-test (*P*=0.05). All analyses were performed using the R
202 v. 3.1.5 software.
203

204 ## Results

205

206 In vitro sensitivity of *Lasiodiplodia theobromae* isolates to thiophanate-methyl,
207 tebuconazole, azoxystrobin and iprodione
208

209 The isolates of *L. theobromae* showed difference in the sensitivity to
210 tebuconazole, thiophanate-methyl, azoxystrobin and iprodione fungicides, with EC_{50}
211 values ranging from 0.044 to 3 μg ml^{-1} for the majority of isolates (Fig. 2). The EC_{50}
212 values were high exceeding 50.67, 29.03 and 32.65 μg ml^{-1} for thiophanate-methyl,
213 iprodione and azoxystrobin, respectively. For tebuconazole, the EC_{50} values were low,
214 and did not exceed 0.73 μg ml^{-1}. Overall, the sensitivity to thiophanate-methyl,
215 iprodione and azoxystrobin was higher (EC_{50} mean = 1.46 μg ml^{-1}), (EC_{50} mean = 2.12
216 μg ml^{-1}) and (EC_{50} mean = 2.95 μg ml^{-1}) respectively than to tebuconazole (EC_{50} mean =
217 0.28 μg ml^{-1}) (Fig. 2).

218 The estimated EC_{50} values for tebuconazole ranged from 0 to 5.42 μg ml^{-1}, and
219 were >0.1 μg ml^{-1} in 32.2581% of the isolates, from 0.11 to 0.5 μg ml^{-1} in 53.2258%,

220 from 0.51 to 1 μg ml^{-1} in 8.06452%, from 1.1 to 2 in 4.83871%, from 2.1 to 4 in
221 1.6129%, there were no isolates at concentrations 4.1 to 6 and >6 μg ml^{-1} (Fig. 3).
222 For thiophanate-methyl, the estimated EC_{50} values ranged from 0 to 50.68 μg
223 ml^{-1}, and were >0.3 μg ml^{-1} in 19.3548% of the isolates, from 0.31 to 0.5 μg ml^{-1} in
224 24.1935%, from 0.51 to 1 in 41.9355% of the isolates, from 1.1 to 2 in 9.67742%, from
225 2.1 to 4 in 0%, from 4.1 to 6 in 3.22581% and >16 in 1.6129% of the isolates (Fig. 3).
226 The estimated EC_{50} values in azoxystrobin, ranged from 0 to 32.65 μg ml^{-1}, and
227 were >0.1 μg ml^{-1} in 32.2581% of the isolates, from 0.11 to 0.3 μg ml^{-1} in 17.7419%,
228 from 0.31 to 0.5 μg ml^{-1} in 9.67742%, from 0.51 to 1 μg ml^{-1} in 9.67742%, from 1.1 to
229 3 μg ml^{-1} in 12.9032%, from3.1 to 6 in 4.83871 and >6 μg ml^{-1} in 12.9032% of the
230 isolates (Fig. 3).
231 For iprodione, the estimated EC_{50} values ranged from 0 to 57.58 μg ml^{-1}, and
232 were >0.5 μg ml^{-1} in 29.0323% of the isolates, from 0.51 to 1 μg ml^{-1} in 50%, from 1.1
233 to 2 μg ml^{-1} in 11.2903%, from 2.1 to 4 μg ml^{-1} in 0%, from 4.1 to 8 μg ml^{-1} in
234 6.45161% and >16 μg ml^{-1} in 3.22581% of the isolates (Fig. 3).
235 For tebuconazole, the EC_{50} values of the 10 most sensitive (S) isolates ranged
236 from 0.001 to 0.52 μg ml-1 (mean 0.14 μg ml^{-1}) and were significantly ($P \leq 0.05$) when
237 compared to the 10 LS isolates, whose EC_{50} values ranged from 0.19 to 5.41 μg ml^{-1}
238 (mean 1.26 μg ml^{-1}) (Tables 2 and 3). Thiophanate-methyl EC_{50} values of the 10 most
239 sensitive (S) isolates ranged from 0.16 to 0.52 μg ml^{-1} (mean 0.32 μg ml^{-1}) and were
240 significantly ($P \leq 0.05$) lower than the 10 LS isolates, whose EC_{50} values ranged from
241 0.28 to 3.09 μg ml^{-1} (mean 1.42 μg ml^{-1}) (Tables 2 and 3). Azoxystrobin EC_{50} values
242 of the 10 most sensitive (S) isolates ranged from 0.00029 to 5.15 μg ml^{-1} (mean 0.74087
243 μg ml^{-1}) and were significantly ($P \leq 0.05$) lower than the 10 LS isolates, whose EC_{50}
244 values ranged from 1.13 to 28.4319 to μg ml^{-1} (mean 11.73917 μg ml^{-1}) (Tables 2 and

245 3). As well as iprodione where EC_{50} values of the 10 most sensitive (S) isolates ranged
246 from 0.039 to 0.42 μg ml^{-1} (mean 0.28 μg ml^{-1}) and were significantly ($P \leq 0.05$) lower
247 than the 10 LS isolates, whose EC_{50} values ranged from 0.32 to 10.89 μg ml^{-1} (mean
248 4.28 μg ml^{-1}) (Tables 2 and 3).
249
250 Analysis of fitness components
251
252 Evaluation of the fitness components using the mycelial growth on fungicide-free
253 medium and pycnidia production were not significantly ($P > 0.05$) between S and LS
254 isolates for tebuconazole. However, for ability of growing under salt stress (osmotic
255 sensitivity) and conidia production were significantly ($P \leq 0.05$) (Table 4). For
256 thiophanate-methyl there was a significant difference ($P \leq 0.05$) between S and LS
257 isolates for all the fitness components with mean osmotic potential of S greater than LS.
258 (Table 4). For azoxystrobin, there was significant difference ($P \leq 0.05$) for mycelial
259 growth and pycnidia production (Table 4). Evaluation with iprodione only mycelial
260 growth showed a significant difference ($P \leq 0.05$) between S and LS isolates (Table 4).
261

262 ## Discussion

263
264 This is the first study of the sensitivity of *L. theobromae* isolates from vineyards
265 orchards in the Brazilian Northeast to the fungicides tebuconazole, thiophanate-methyl,
266 azoxystrobin and iprodione, and their influence on fitness components of isolates.
267 Previous studies have reported resistance to fungicides and sensitivity reduction in *L.*
268 *theobromae* populations from other fruit orchards in northeastern Brazil (Pereira et al.
269 2012; Cavalcante et al. 2014; Bandeira 2014; Tsuji 2016; Santos et al. 2018).

The isolates of *L. theobromae* showed difference average EC_{50} values in the sensitivity to tebuconazole (0.36 μg ml^{-1}), thiophanate-methyl (1.46 μg ml^{-1}), azoxystrobin (2.95 μg ml^{-1}) and iprodione (2.12 μg ml^{-1}) fungicides. These values become more relevant considering that are not these fungicide registered to control of *L. theobromae* on grapevine orchards in Brazil. These fungicides are the group with the highest risk of resistance induction (FRAC 2015). This difference in sensitivity may be associated with a natural characteristic of the fungicide or in consequences that the *L. theobromae* populations had been more exposed to two fungicides used to control other pathogens, which may led to the selection of less sensitive isolates in the population.

The maximum EC_{50} value for tebuconazole observed here (5.41 μgml^{-1}) is high in comparison to the EC_{50} reported for tebuconazole in *L. theobromae* from grapevine (0.17 μgml^{-1}) (Bester et al. 2007) and papaya (4.05 μg ml^{-1}) (Pereira et al. 2012) and for species phylogenetically related *Botryosphaeria dothidea* (0.82 μg ml^{-1}) from Chinese hickory (Dai et al. 2017).

The average EC_{50} values to tebuconazole for *L. theobromae* sensitive isolates (0.14 μg ml^{-1}) and less-sensitive isolates (1.26 μg ml^{-1}) were similar, respectively, than those found by Pereira et al. (2012) for populations of *L. theobromae* sensitive (S) (0.25 μg ml^{-1}) and less-sensitive (LS) (1.18 μg ml^{-1}), and were lowest by Bandeira (2014) for *L. theobromae* sensitive isolates (0.10 μg ml^{-1}) and less-sensitive isolates (7.27 μg ml^{-1}) to difenoconazole.

The average EC_{50} value (0.36 μg ml^{-1}), is similar in comparison to the EC_{50} for *L. theobromae* from papaya for imazali (0.63 μg ml^{-1}), prochloraz (0.20μg ml^{-1}), tebuconazole (0.49 μg ml^{-1}), and for species phylogenetically related from grapevine, such as *Neofusicoccum austral* (0.002 and 0.098 μg ml^{-1}), *Neofusicoccum luteum* (0.003

294 and 0.076 μg ml^{-1}), *Diplodia mutila* 0.001 and 0.010 μg ml^{-1}) for flusilazole and
295 tebuconazole, respectively (Amponsah et al. 2012).

296 Tebuconazole is registered for the control of other commonly occurring
297 pathogens in grapevine, such as of *Colletotrichum gloeosporioides, Phakopsora euvitis*
298 and *Uncinula necator* (MAPA 2018). Thus, we may assume that low sensitivity to
299 tebuconazole may be the result of exposure of the *L. theobromae* population in orchards
300 used to control of these pathogens. This group of systemic fungicides target cell
301 membrane integrity by inhibiting C14 demethylation during sterol formation. A number
302 of resistance mechanisms acting individually or in combination can reduced sensitivity
303 have been identified to be associated with decreased sensitivity, including target-site
304 modification, target gene (cyp51) overexpression, increased efflux, and multiple
305 paralogues of the target gene (Ziogas and Malandrakis 2015).

306 The DMIs used in agriculture to control a wide range of pathogens, dominating
307 the agricultural fungicides market (Ziogas and Malandrakis 2015), intensive use of
308 DMIs in agriculture has led to a stepwise manner resistance development leading to
309 practical control problems have been reported in several phytopathogenic fungi (Ma and
310 Michailides 2005; Hollomon 2015).

311 The maxim EC_{50} value for thiophanate-methyl observed here 50,67 μg ml^{-1} is
312 high in comparison to the EC_{50} reported for isolates *L. theobromae* from mango showed
313 a maximum EC_{50} value of 2.60 μg ml^{-1} for thiophanate-methyl and 1.06 μg ml^{-1} for
314 thiabendazole (Santos et al. 2018) and for species phylogenetically related, such as *L.*
315 *pseudotheobromae* (2.69 μg ml^{-1} and 0.98 μg ml^{-1}), *L. viticola* (1.81 μg ml^{-1} and 1.15
316 μg ml^{-1}), *L. iraniensis* (0.89 μg ml^{-1} and 0.54 μg ml^{-1}) for thiophanate-methyl and
317 thiabendazole, respectively, from mango orchards of the Brazilian Northeast (Santos et

318 al. 2018). However, much lower than those shown by isolates of *L. theobromae* from
319 papaya orchards (482.90 μg ml^{-1}) (Cavalcante et al. 2014).

320 The average EC_{50} values for *L. theobromae* sensitive isolates (0.32 μg ml^{-1}) and
321 less-sensitive isolates (1.14 μg ml^{-1}) to thiophanate-methyl were similar than those
322 found by Santos et al. (2018) for populations of *L. theobromae* S (0.35 μg ml^{-1}) LS
323 (1.96 μg ml^{-1}). The average EC_{50} value (1.46 μg ml^{-1}), is similar to greater in
324 comparison to the EC_{50} for species phylogenetically related *Neofusicoccum austral*
325 (1.079 μg ml^{-1}), *Neofusicoccum luteum* (1.237 μg ml^{-1}), *Diplodia mutila* (0.485 μg ml^{-1})
326 (Amponsah et al. 2012), *Neofusicoccum parvum* (0.25 μg ml^{-1}) for benomyl (Latorre et
327 al. 2013).

328 The MBC group fungicides have been extensively used to control various plant
329 diseases caused by fungi, is presents a high risk of pathogens resistance, with cases in
330 150 species of phytopathogenic fungi (FRAC 2016). The isolates located in the city of
331 Petrolina in the state of Pernambuco, which showed the lower sensitivity to thiophanate-
332 methyl, may be a result of high exposure of this region which is intensely technified.
333 Therefore, it may be a result of exposure of *L. theobromae* populations to thiophanate-
334 methyl it is commonly used to control of *Botrytis cinerea*, *C. gloeosporioides* and
335 *Elsinoe ampelina*, *Plasmopara viticola, Pseudocercospora vitis* and *U. necator* (MAPA
336 2018). This fungicides act in the mitosis and cell division by inhibition of microtubule
337 assembly by binding to β-tubulin (FRAC 2016). Resistance to fungicides has usually
338 resulted from certain point mutations in the target β-tubulin gene, mutations at codons
339 198, 200, 240 have been associated with a moderate, high, and low to moderate level of
340 resistance, respectively. Mutations detected in previous study demonstrated that *L.*
341 *theobromae* isolates were resistant to thiophanate-methyl with a mutation in the codon
342 198 (E198K) of the β-tubulin gene from papaya in Brazilian Northeastern (Tsuji 2016).

343 The maxim EC_{50} value for azoxystrobin observed here (32.68µgml^{-1}) is high in
344 comparison to the EC_{50} reported for species phylogenetically *Neofusicoccum parvum*
345 (2.0 µg ml^{-1}) for pyraclostrobin (Latorre et al. 2013) and *Botryosphaeria dothidea*
346 (13.24 µg ml^{-1}) for Kresoxim-methyl (Dai et al. 2017).

347 The reduction of sensitivity it may be a result of exposure of *L. theobromae*
348 populations to azoxystrobin, it is registered for the control of *E. ampelina, P. viticola*
349 and *U. necator* in Brazil (MAPA 2018). The QoI fungicides have a very broad spectrum
350 and strong activity with a wide range of agronomical applications against many
351 pathogens from all taxonomic groups. It is well known that QoI fungicides are rated as
352 having a high risk for development of fungicide resistance (FRAC 2015), QoI
353 fungicides, inhibitors of mitochondrial respiration by inhibiting cytochrome bc1
354 complex (complex III), is usually conferred by point mutations G143A, F129L, and
355 G137R in cyt b gene in the mitochondrial cytb gene causing amino acid substitutions in
356 the target protein (Sierotzki 2015).

357 The maxim EC_{50} value for iprodione observed here (57.58 µgml^{-1}) is high in
358 comparison to the EC_{50} reported for *L. theobromae* from mamey (0.448 µgml^{-1}) (Tovar
359 Pedraza et al. 2013) and for species phylogenetically related, such as *Botryosphaeria*
360 *dothidea* from pistachio (2.726 µg ml^{-1}) (Ma et al. 2001). The average EC_{50} values (2.12
361 µg ml^{-1}) is high in comparison for species phylogenetically related for iprodione
362 *Neofusicoccum austral* (0.273 µg ml^{-1}), *Neofusicoccum luteum* (0.314 µg ml^{-1}),
363 *Diplodia mutila* (0.247 µg ml^{-1}) (Amponsah et al. 2012).

364 The low sensitivity to present it may be a result of exposure of *L. theobromae*
365 populations to iprodione it is registered for the control of *B. cinerea* (MAPA 2018). The
366 resistance mechanisms against HK inhibitors have been studied for various fungi
367 agricultural and economic importance. The fungicides over activate Hog-like mitogen

368 activated protein kinases in the osmotic signal transduction pathway and result in cell
369 death. The dicarboximides group fungicides present of the intrinsic risk medium to high
370 for resistance evolution, studies revealed that five types of point mutations in position of
371 resistant mutation in BcOS1 two specific amino acid (I365 and Q369) residues
372 conferred resistance. Dicarboximide fungicides have been used to protect various crops
373 and widely used to control diseases caused by the taxonomically related pathogens
374 (Fujimura 2015).

375 Among the factors that may influence the development of resistant individuals in
376 the population is the fitness (Mikaberidze and Mcdonald 2015), which is expressed as
377 the competitive efficiency of resistant individuals in relation to the susceptible. The
378 differential fitness between resistant and sensitive populations, if any, seems to be
379 closely related among the different characteristics that define a good adaptive potential,
380 can be experimentally measured as mycelial growth rate, sporulation capacity, spore
381 production, osmotic sensitivity, are just some examples of fitness components in
382 populations with the ability to compete with sensitive individuals in the absence of the
383 fungicide (Mikaberidze and Mcdonald 2015).

384 Most of the studies point out that the loss of adaptability of the isolates is low or
385 even non-existent of *L. theobromae* from vineyards in the Brazilian Northeast (Pereira
386 et al. 2012; Cavalcante et al. 2014; Bandeira 2014; Santos et al. 2018). The chances of
387 resistance are variable prevalent in a population are higher for those who have not
388 suffered decrease in their capacity (Ishii 2015). This study shows that tebuconazole to
389 the evaluation of fitness components did not show significant differences between S and
390 LS isolates, such as mycelial growth rate and pycnidia production increased capacity in
391 LS to osmotic sensitivity and conidia production. No difference was found (P>0.05)
392 between the difenoconazole-sensitive and less-sensitive isolates for mycelial growth on

393 fungicide-free agar medium, optimum temperature for mycelial growth, spore
394 production, osmotic sensitivity and virulence on papaya fruits (Bandeira 2014).
395 The thiophanate-methyl presented significant differences between S and LS for
396 all fitness components. This similarity in the fitness between S and LS isolates has also
397 been reported in *L. theobromae* from Brazilian papaya orchards to thiophanate-methyl
398 (Cavalcante et al. 2014). However, differential fitness between S and LS populations the
399 LS isolates showed for species phylogenetically related, such as *B*. *dothidea* and *N.*
400 *pavum*, better fitness, as demonstrated by their increased capacity to grow under salt
401 stress for thiophanate-methyl and thiabendazole from mango orchards of the Brazilian
402 Northeast (Santos et al. 2018). For instance, fitness penalty was observed in LS to
403 mycelial growth rate, pycnidia production and conidia production. Regarding, no
404 differences were found in mycelial growth on fungicide-free medium, the optimum
405 temperature for mycelial growth, pycnidia production, spore germination to
406 thiophanate-methyl, benomyl and thiabendazol (Pereira et al. 2012; Cavalcante et al.
407 2014; Santos et al. 2018).
408 The azoxystrobin to the evaluation of fitness components did not show
409 significant differences between S and LS isolates, such as osmotic sensitivity and
410 conidia production. In the present study, the LS isolates showed better fitness, as
411 demonstrated by their increased capacity to mycelial growth rate and pycnidia
412 production. In this sense, impact on efficacy can not be disseminated same the wind-
413 dispersed spores of the pathogen enable resistant strains to be widely dispersed.
414 This is the first report of this the fitness to iprodione and azoxystrobin in
415 populations of *Lasiodiplodia*. Iprodione did not show significant differences between S
416 and LS of fitness components, except mycelial growth rate. There was no decrease in
417 osmotic sensitivity is a well studied component for this group of fungicides, due

418 activated protein kinases in the osmotic signal transduction pathway. This behavior was
419 consistent with observations in mutations in the HK domain, which should never cause
420 unfavorable changes in stress response and osmotic and oxidative aptitude as compared
421 to the susceptible phenotype (Ma et al. 2007; Luo et al. 2013).

422 The present study showed the occurrence of *L. theobromae* isolates with low
423 sensitivity for the main fungicides used from important producing regions of grapevine
424 in Brazil. The reduction in sensitivity of tebuconazole, thiophanate-methyl,
425 azoxystrobin and iprodione there was fitness significant penalty. The main production
426 pole presented lower sensitivity was observed. Thus, the implementation of monitoring
427 programs is essential for assessing the risk of resistance and early detection of reduction
428 in sensitivity. This information will provide subsidies for a more effective management
429 of disease involving *L. theobromae* in grapevine orchards in the producing and
430 exporting regions in the Brazilian Northeast.

431

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435

436 **Compliance with Ethical Standards**

437 All principles of ethical and professional conduct have been followed during this
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439

440 **Conflict of interest** - The authors declare that they have no conflict of interest.

441 **Research involving Human Participants and/or Animals** - Not applicable.

442 **Informed consent** - Not applicable.

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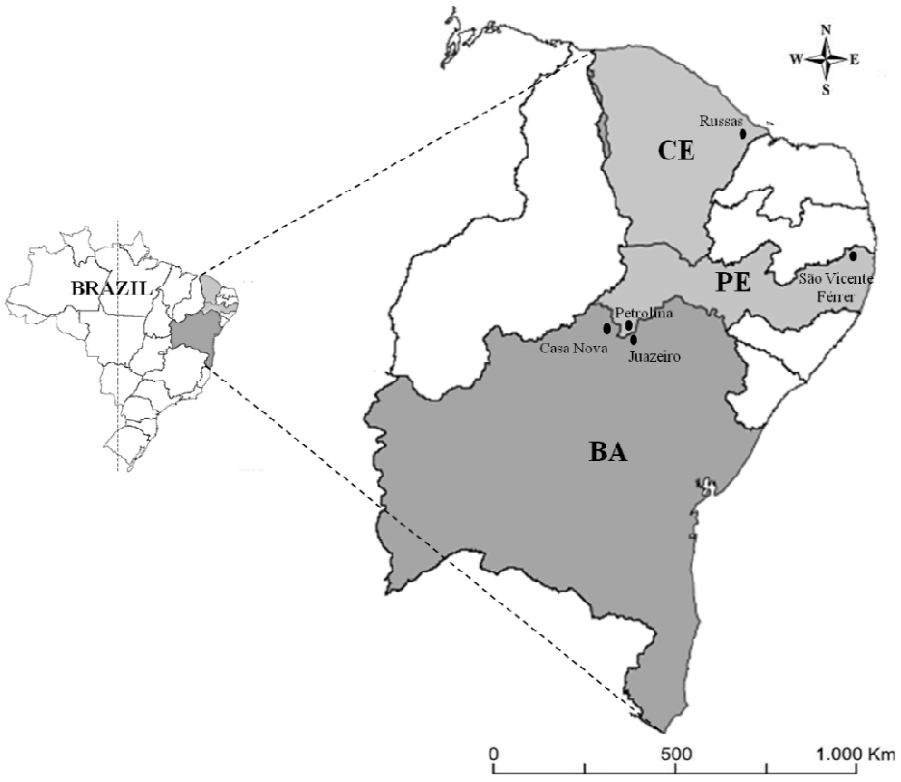
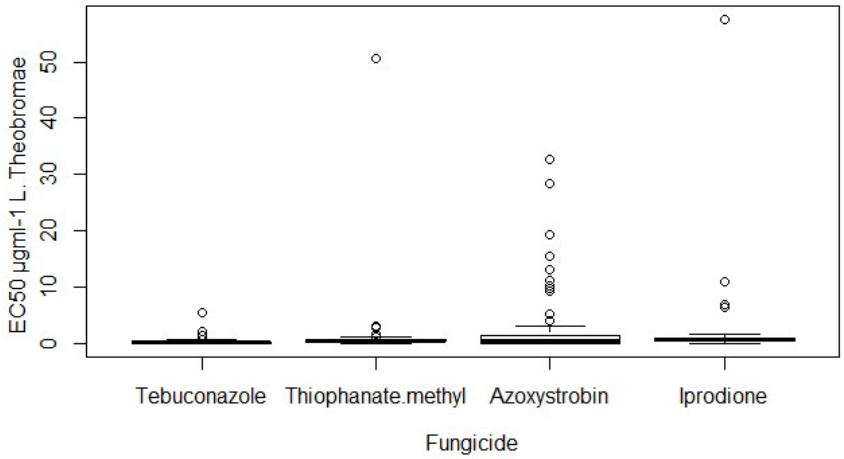


573

574 **Fig. 1** Collection sites of *Lasiodiplodia theobromae* isolates from Brazilian vineyards,
575 located in the states of Bahia (BA), Ceará (CE) and Pernambuco (PE).

576

577



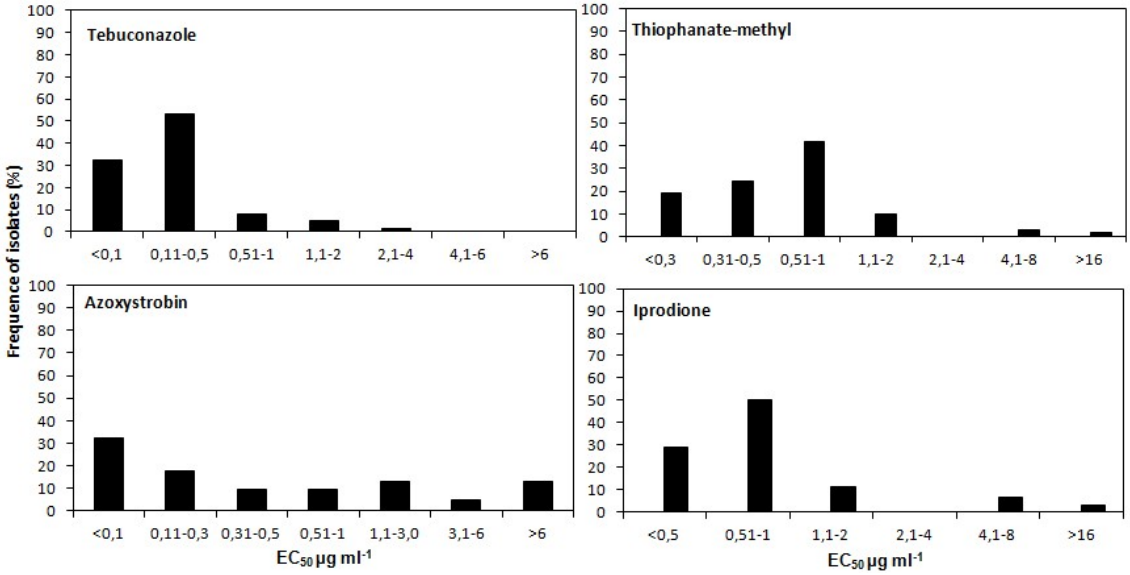
578

579 **Fig. 2** Box plot of (EC_{50}) values from *Lasiodiplodia theobromae*

580 to tebuconazole, thiophanate, azoxystrobin and iprodione

581 fungicides.

582



583

584 **Fig. 3** Frequency and distribution of effective fungicide concentrations required to

585 inhibit 50% of the mycelial growth (EC_{50}) of 62 isolates of *Lasiodiplodia theobromae*

586 collected from Brazilian vineyards.

Table 1 *Lasiodiplodia* species obtained of table grape vineyard with symptoms of Botryosphaeriaceae dieback collected in northeast region of Brazil.

Collection point (city, state)	Isolate (CMM)*	State	Number
Petrolina	0178, 0227, 0230, 0232, 0236, 0246, 0249, 0252, 0278, 0291, 0307, 0310, 0329, 0332, 0333, 0366, 0384, 0406, 0413, 0421, 0455, 0456, 0474, 0490, 0497, 0597, 0600, 0685, 0820, 4127.	Pernambuco	30
Juazeiro	0225, 0244, 0296, 0297, 0361, 0446.	Bahia	6
Casa Nova	0239, 0287, 0289, 0295, 0298, 0320, 0324, 0330, 0344, 0367, 0369, 0370, 0377, 0405, 0424, 0567, 0688.	Bahia	17
São Vicente Ferrer	0855, 0920, 0922, 0954, 0957.	Pernambuco	5
Russas	1024, 1032, 1043, 1044.	Ceara	4

*CMM = Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Pernambuco, Brasil).

Table 2 Sensitivity to tebuconazole, thiophanate methyl, iprodione and azoxystrobin of sensitive and less sensitive isolates of Botryosphaeriaceae from Brazilian vineyards.

Isolate class[a]	**EC_{50} (µg i.a. ml^{-1})**[b]			
	Tebuconazole	**Thiophanate- methyl**	**Azoxystrobin**	**Iprodione**
Sensitive	0.14 (0.03) b	0.32 (0.03) b	0.74 (0.42) b	0.28 (0.04) b
Less sensitive	1.26 (0.28) a	1.42 (0.20) a	11.73 (2.67) a	4.28 (0.68) a

[a] Each class is composed of 10 isolates, selected by the lowest and highest EC_{50} values for tebuconazole, thiophanate-methyl, iprodione and azoxystrobin.

[b] Values (µg i.a. ml^{-1}) are the means of three repetition. Averages followed by the same letter in the column do not differ significantly according to Student's t-test (P = 0.05). Values in the parentheses represent the standard error.

612 **Table 3** List of *L. theobromae* species from Brazilian vineyards selected in this study
613 showing the lowest (sensitive -S) and highest (less sensitive - LS) EC_{50} values for
614 tebuconazole, thiophanate-methyl, azoxystrobin and iprodione.

Code isolate *	EC_{50} (µg i.a. ml^{-1})[b]							
	Tebuconazole		Thiophanate methyl		Iprodione		Azoxystrobin	
	S	LS	S	LS	S	LS	S	LS
CMM-225	0.044	-	-	0.67	-	-	-	-
CMM-230	-	1.31	-	3.09	-	-	-	6.44
CMM-236	-	0.44	-	-	-	15.62	-	7.01
CMM-249	-	0.74	-	1.01	0.25	-	0.36	-
CMM-252	-	0.19	0.17	-	-	-	-	-
CMM-278	-	5.41	-	-	-	28.43	-	0.77
CMM-287	0.06	-	0.25	-	0.079	-	0.37	-
CMM-295	-	-	-	-	-	4.00	-	-
CMM-296	-	0.22	-	-	-	-	-	6.79
CMM-307	-	-	0.28	-	-	11.28	-	0.82
CMM-320	-	1.38	-	-	-	7.52	-	-
CMM-324	-	-	-	-	-	-	-	-
CMM-330	-	-	0.41	-	0.001	-	0.20	-
CMM-332	0.01	-	-	-	0.01	-	-	-
CMM-333	-	-	-	-	-	-	-	-
CMM-344	0.004	-	-	2.82	-	-	0.38	-
CMM-366	0.005	-	0.43	-	0.022	-	0.39	-
CMM-369	-	-	0.46	-	0.01	-	0.43	-
CMM-384	-	0.72	0.34	-	-	10.24	-	-
CMM-413	0.014	-	0.16	-	-	5.15	0.42	-
CMM-421	0.038	-	-	-	0.11	-	0.09	-
CMM-424	-	0.13	-	1.22	-	13.00	-	0.96
CMM-455	-	-	-	1.64	-	1.13	0.15	-
CMM-597	-	2.03	-	1.67	0.92	-	-	073
CMM-685	0.525	-	-	0.95	0.015	-	-	1.39
CMM-688	0.220	-	0.25	0.52	-	19.25	0.40	-
CMM-920	-	-	-	-	-	-	-	-
CMM-954	-	-	0.16	-	0.004	-	-	7.74
CMM-1024	0.094	-	-	1.26	-	-	-	6.40

615

616 * Isolate code from the Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes (CMM) at the
617 Universidade Federal Rural de Pernambuco (Recife, Pernambuco, Brazil).

Table 4 Fitness components between isolates of Botryosphaeriaceae sensitive and less sensitive to tebuconazole, thiophanate-methyl, iprodione and azoxystrobin fungicides.

Isolate class[a]	**Tebuconazole**			
	MGR (mm h^{-1})[b]	$EC_{50}N$ (%NaCl)[b]	Pycnidia (N^a)	Conidia (x10^4 ml^{-1})
Sensitive	23.75 (2.08) a[c]	0.54 (0.07) b	68.17 (10.17) a	13.39 (3.85) b
Less sensitive	24.01 (1.60) a	0.91 (0.13) a	111.87 (17.05) a	30.66 (6.48) a
	Thiophanate-methyl			
	MGR (mm h^{-1})	$EC_{50}N$ (%NaCl)	Pycnidia (N^a)	Conidia (x10^4 ml^{-1})
Sensitive	28.05 (1.96) a	0.82 (0.10) b	133.3 (11.50) a	26.53 (5.60) a
Less sensitive	17.43 (1.16) b	0.89 (0.13) a	46.25 (8.39) b	4.23 (1.10) b
Isolate class[a]	**Azoxystrobin**			
	MGR (mm h^{-1})	$EC_{50}N$ (%NaCl)	Pycnidia (N^a)	Conidia (x10^4 ml^{-1})
Sensitive	22.14 (1.93) b	0.87 (0.13) a	38.64 (7.67) b	16.48 (4.23) a
Less sensitive	29.19 (1.71) a	0.73 (0.10) a	99.92 (16.64) a	22.09 (5.54) a
	Iprodione			
	MGR (mm h^{-1})	$EC_{50}N$ (%NaCl)	Pycnidia (N^a)	Conidia (x10^4 ml^{-1})
Sensitive	23.31 (1.92) a	0.65 (0.09) a	77.60 (11.60) a	12.57 (3.76) a
Less sensitive	20.52 (1.96) b	0.89 (0.13) a	80.17 (13.95) a	19.84 (4.97) a

[a]Each class is composed of ten isolates, selected by the lowest and highest EC_{50} values for thiabendazole and thiophanate-methyl.

[b]MGR: Mycelial growth rate in fungicide-free PDA medium; $EC_{50}N$: osmotic sensitivity.

[c] Values are the means of two independent experiments because no heterogeneity was detected between them according to Levene's test ($P > 0.05$). Averages followed by the same letter in the column in the same fungicide do not differ significantly according to Student's t-test ($P=0.05$). Values (±) in the parentheses represent the standard error.

Conclusões Gerais

CONCLUSÕES GERAIS

1. Os fatores meteorológicos no vale do Sirijí apresentaram-se estáveis no período que compreendeu o monitoramento da dispersão de conídios de Botryosphaeriaceae de plantas sintomáticas e restos de poda de videira;

2. O modelo logístico ajustou-se adequadamente aos dados da dispersão de conídios de *Diplodia*, *Lasiodiplodia* e *Neofusicoccum* na região Nordeste do Brasil;

3. Elevadas quantidades de conídios dos gêneros *Diplodia*, *Lasiodiplodia* e *Neofusicoccum* são liberados em plantas sintomáticas e restos de poda em vinhedos situados na região Nordeste do Brasil;

4. Isolados de *L. theobromae* apresentam baixa sensibilidade aos principais fungicidas utilizados no manejo de importantes doenças de plantas no Brasil;

5. Não houve penalidade significativa na adaptabilidade dos isolados de *L. theobromae* com a redução na sensibilidade a iprodiona, azoxistrobina, tiofanato-metil e tebuconazol;

6. Isolados originários do principal polo de produção de videira no Nordeste Brasileiro apresentaram baixa sensibilidade a fungicidas.