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DIVERSIDADE E SENSIBILIDADE A FUNGICIDAS DE ESPÉCIES DE FUNGOS ASSOCIADAS À MORTE DO ABACATE NO BRASIL

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DIVERSIDADE E SENSIBILIDADE A FUNGICIDAS DE ESPÉCIES DE FUNGOS ASSOCIADAS À MORTE DO ABACATE NO BRASIL

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DIVERSIDADE E SENSIBILIDADE A FUNGICIDAS DE ESPÉCIES DE FUNGOS ASSOCIADAS À MORTE DO ABACATE NO BRASIL

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RESUMO

O Brasil está entre os maiores produtores mundiais de abacate (*Persea americana*), com a maior parte da produção destinada ao mercado interno. No entanto, houve um crescimento significativo das exportações nos últimos anos, tanto para a indústria farmacêutica quanto para o mercado de frutas frescas. Dada a expansão da cultura, desafios fitossanitários comprometem a produtividade, incluindo a morte descendente, uma doença fúngica caracterizada pela colonização dos vasos do xilema e consequente morte dos tecidos vegetais. Estudos taxonômicos sobre a etiologia da doença em outros países revelam vários fungos associados à doença e a ocorrência de espécies de acordo com a região geográfica. No entanto, espécies da família Botryosphaeriaceae são predominantes nessa condição sintomática. A identificação precisa dos agentes causadores da podridão do abacateiro no Brasil ainda não foi alcançada. Além disso, é necessário elucidar a sensibilidade desses fungos aos fungicidas comumente utilizados nas culturas. Diante desse cenário, o presente estudo teve como objetivo identificar os fungos associados à morte descendente do abacateiro no Brasil e avaliar a sensibilidade dos isolados a dois fungicidas. Foram realizadas coletas de plantas sintomáticas nas principais regiões produtoras do país – São Paulo, Minas Gerais, Distrito Federal e Santa Catarina – para posterior isolamento dos fungos. A identificação das espécies foi baseada em sequenciamento de DNA e análises filogenéticas. Testes de patogenicidade foram realizados em plantas e frutos. Para avaliar a sensibilidade aos fungicidas, foram determinados os EC50 para tiofanato-metil e azoxistrobina. Os gêneros *Lasiodiplodia*, *Neofusicoccum*, *Pseudofusicoccum*, *Neopestalotiopsis*, *Neocosmospora*, *Nectria* e *Cytospora* foram identificados como causadores de doenças em abacate, sendo os dois primeiros os mais frequentes. As espécies *L. macrospora*, *N. kwambonambiense*, *N. ribis*, *Neopestalotiopsis arecacearum*, *Neocosmospora bostrycoides*, *Nectria pseudotrichia* e *Cytospora viridistroma* foram relatadas pela primeira vez como causadoras de doenças em abacate. Testes de patogenicidade confirmaram que todas as espécies isoladas induziram doenças em plantas e frutos de abacate, exceto *L. laeliocattleyae* que não apresentou sintomas nas plantas. Os ensaios de fungicida indicaram a presença de isolados de *L. pseudotheobromae* resistentes ao tiofanato-metil. Para azoxistrobina, foi constatado que todas as espécies de Botryosphaeriaceae testadas apresentaram isolados resistentes. A aplicação de fungicidas em frutos de abacate reduziu significativamente a severidade da doença quando os isolados foram sensíveis ao tratamento. Esses resultados destacam a diversidade de fungos associados à morte descendente de abacateiros no Brasil e reforçam a necessidade de estratégias de manejo eficazes, incluindo o monitoramento da resistência fúngica a fungicidas.

Palavras-chave: Botryosphaeriaceae, Complexo de doenças de plantas, Metil benzimidazol carbamatos, Inibidores da quinona externa, *Persea americana*

ABSTRACT

Brazil is among the world's largest avocado (*Persea americana*) producers, with the majority of production destined for the domestic market However, there has been significant export growth in recent years, both for the pharmaceutical industry and the fresh fruit market. Given the expansion of the crop, phytosanitary challenges compromise productivity, including dieback, a fungal disease characterized by the colonization of xylem vessels, and subsequent death of plant tissues. Taxonomic studies on the etiology of the disease in other countries reveal several fungi associated with the disease and the occurrence of species according to the geographic region. However, species of the Botryosphaeriaceae are predominant in this symptomatic condition. Accurate identification of the causative agents of avocado rot in Brazil has not yet been achieved. Furthermore, it is necessary to elucidate the sensitivity of these fungi to fungicides commonly used in crops. Given this scenario, the present study aimed to identify the fungi associated with the dieback of avocado trees in Brazil and the sensitivity of the isolates to two fungicides. Collections were made from symptomatic plants in the main producing regions of the country – São Paulo, Minas Gerais, Distrito Federal, and Santa Catarina – for subsequent isolation of the fungi. Species identification was based on DNA sequencing and phylogenetic analyses. Pathogenicity tests were performed on plants and fruit. To assess fungicide sensitivity, the EC50 for thiophanate-methyl and azoxystrobin were determined. The genera *Lasiodiplodia*, *Neofusicoccum*, *Pseudofusicoccum*, *Neopestalotiopsis*, *Neocosmospora*, *Nectria* and *Cytospora* were identified as causing disease in avocado, with the first two being the most frequent. The species *L. laeliocattleyae*, *L. macrospora*, *N. kwambonambiense*, *N. ribis*, *Neopestalotiopsis arecacearum*, *Neocosmospora bostrycoides*, *Nectria pseudotrichia*, and *Cytospora viridistroma* were reported for the first time causing disease in avocado. Pathogenicity tests confirmed that all isolated species induced disease in avocado plants and fruits, except *L. laeliocattleyae* which showed no symptoms on plants. Fungicide assays indicated the presence of *L. pseudotheobromae* isolates resistant to thiophanate-methyl. For azoxystrobin, it was found that all Botryosphaeriaceae species tested presented resistant isolates. The application of fungicides to avocado fruits significantly reduced the severity of the disease when the isolates were sensitive to the treatment. These results highlight the diversity of fungi associated with the dieback of avocado trees in Brazil and reinforce the need for effective management strategies, including monitoring of fungal resistance to fungicides.

Palavras-chave: *Botryosphaeriaceae*, Plant disease complex, Methyl benzimidazole carbamates, Quinone outside inhibitors, *Persea americana*

INTRODUÇÃO

O abacateiro (*Persea americana*) é uma planta perene da família Lauraceae, nativa do continente americano (Koller, 1984). Seu cultivo é restrito às regiões tropicais e subtropicais, onde temperatura, disponibilidade de recursos e altitude são essenciais para seu desenvolvimento, concentrando-se principalmente na América Central e do Sul (Schaffer et al., 2012).

A produção mundial totaliza 8.059.359 toneladas em uma área de 807,47 mil hectares, com a maioria concentrada nas Américas (71,7%), seguida pela África (12,6%) e Europa (12,1%). O México é o maior produtor mundial, enquanto o Brasil ocupa o sexto lugar (FAOSTAT, 2020). No Brasil, os principais estados produtores são São Paulo, Minas Gerais, Paraná, Rio Grande do Sul e Distrito Federal (IBGE, 2021). Os híbridos utilizados comercialmente no Brasil incluem Geada, Margarida, Breda, Fortuna, Quintal e Hass (Yamanishi, 2011). Esses híbridos apresentam diferentes adaptações climáticas e a escolha da variedade pode determinar o sucesso da produção de frutos (Duarte, 1998).

O cultivo do abacateiro é economicamente rentável e apresenta baixo risco comercial devido às suas propriedades nutricionais e ao crescente consumo global (Araújo et al., 2018). Em vários países, a demanda supera a oferta, impulsionada pela crescente divulgação dos benefícios à saúde da fruta, seu uso na culinária e na produção de cosméticos e fármacos (Araújo et al., 2018; Sampaio & Whately, 2022). Sua importância se deve principalmente ao seu alto valor nutricional em comparação a outras frutas, sendo uma rica fonte de proteínas, vitaminas C, E, B1 e B2 e antioxidantes (Duarte et al., 2016). Além disso, a polpa da fruta contém aproximadamente 60% de óleo, o que tem despertado crescente interesse das indústrias farmacêuticas, cosméticas e alimentícias (Flores et al., 2019). Entre 2018 e 2022, o consumo global aumentou 40% e no Brasil, o consumo médio per capita passou de 600 gramas em 2016 para 900 gramas em 2018 (ABPA, 2022).

Para fortalecer os pequenos agricultores e aumentar a lucratividade do cultivo de abacate, o manejo eficiente de doenças é essencial. A cultura pode ser afetada por várias doenças tanto no campo quanto na pós-colheita, levando a perdas significativas na produção e comercialização de frutas (Piccinin & Pascholati, 2018). Entre as principais doenças, a antracnose, causada por patógenos do gênero *Colletotrichum*, é uma das principais doenças pós-colheita, frequentemente caracterizada pelo aparecimento de

lesões escuras e necróticas no fruto (Tozze Júnior et al., 2016; Sharma et al., 2017; Ramírez-Gil & Peterson, 2019). A sarna, causada por *Sphaceloma perseae*, leva ao declínio na aparência e a queda prematura dos frutos (Bautista-Baños et al., 2020). Patógenos do solo, como espécies de *Phytophthora*, afetam diretamente o sistema radicular, causando produção excessiva de frutos de baixo peso e inadequados para comercialização (Salgadoe et al., 2018; Ramírez-Gil et al., 2017). Outra doença que tem se destacado é a morte descendente caracterizada pelo murchamento e necrose dos galhos, que eventualmente leva a morte da planta (Sampaio & Whately, 2022).

A morte descendente é observada no tronco e nos galhos, aparecendo inicialmente como uma área escurecida que, à medida que a doença progride, leva à depressão e ressecamento do tecido (Pérez-Jiménez, 2008). Quando os sintomas afetam os galhos ou o caule devido à colonização fúngica, o xilema morre, causando murcha, seguido pelo colapso e morte de galhos ou árvores inteiras (Twizeyimana et al., 2013). Em alguns casos, pequenas manchas podem aparecer no fruto, escurecendo progressivamente e afetando a polpa, o que reduz sua vida útil ou os torna inadequados para comercialização (Dann et al., 2013; Piccinin & Pascholati, 2018). Pode ser causado por fatores bióticos e abióticos, mas principalmente pela combinação de ambos (Camarero et al., 2015). O estresse abiótico, como deficiências nutricionais, estresse por seca, temperaturas elevadas, geada e irrigação excessiva, pode impactar as atividades fisiológicas celulares e o processo de sinalização celular, reduzindo a eficácia da defesa da planta contra os patógenos causadores de morte descendente (Jeandet et al., 2022; Rolland et al., 2006; Valencia et al., 2019).

Estudos taxonômicos utilizando ferramentas moleculares em vários países revelaram *Bionectria*, *Colletotrichum*, *Diaporthe*, *Diplodia*, *Fusarium*, *Lasiodiplodia*, *Neocosmospora*, *Neofusicoccum*, *Neopestalotiopsis*, *Phytophthora*, *Pestalotiopsis*, *Pseudofusicoccum*, *Raffaelea* e *Rosellinia* associadas a esta doença em abacate (Burbano-Figueroa et al., 2020; Fiorenza et al., 2023; Guarnaccia et al., 2018; Hernández et al., 2023; Lin et al., 2018; Piccin, 2018; Ploetz et al., 2017; Ramírez-Gil and Peterson, 2019; Trakunyingcharoen et al., 2015; Valencia et al., 2022). No Brasil, *Colletotrichum*, *Nectria*, *Neofusicoccum*, *Phytophthora*, *Rosellinia* e *Verticillium* foram identificados com base em características morfológicas (Piccinin, 2018), enquanto a identificação molecular foi realizada apenas para *Colletotrichum*. Fungos do gênero *Phytophthora* têm sido os principais associados aos sintomas de morte descendente em abacate, causado pela

podridão radicular, que compromete o sistema radicular e desencadeia diversos sintomas secundários como murchamento das folhas e redução no crescimento vegetativo, levando a morte de membros inteiros (Fischer & Firmino, 2023; Piccinin & Pascholati, 2018). Observa-se, entretanto, que espécies da família Botryosphaeriaceae (Dothideomycetes, Botryosphaeriales) estão frequentemente associadas a esses sintomas em plantas. Em diversos estudos, a maior parte dos isolados identificados pertence a essa família (Arjona-Girona et al., 2019; Fiorenza et al., 2023; Guarnaccia et al., 2016; Hernández et al., 2023, Mcdonald & Eskalen, 2009; Mendoza-Churape et al., 2024; Möller et al., 2025; Rodríguez-Gálvez et al., 2021; Rodríguez-Gálvez et al., 2021; Twizeyimana et al., 2013; Valencia et al., 2019).

Os fungos da família Botryosphaeriaceae pertencem aos Dothideomycetes e incluem espécies fitopatogênicas, saprofíticas, endofíticas e epífitas. Esses fungos geram dois tipos distintos de corpos de frutificação: os peritécios, que contêm ascas bitunicadas com ascósporos, os quais podem ser hialinos ou pigmentados, representando o estágio sexual, e os picnídios, responsáveis pela liberação de conídios, também hialinos ou pigmentados, que correspondem ao estágio assexual (Hyde et al., 2013; Schoch et al., 2006). Este último é o mais comumente observado (Möller et al., 2025; Phillips et al., 2013). Eles são geralmente associados a plantas lenhosas, incluindo gimnospermas e angiospermas (Slippers & Wingfield, 2007). São considerados fitopatógenos oportunistas, que em condições normais vivem endofiticamente, mas podem se tornar patogênicos quando a planta hospedeira sofre estresse ou mudanças ambientais (Slippers & Wingfield, 2007; Chethana et al., 2016).

A identificação de espécies dentro da família Botryosphaeriaceae é realizada principalmente por meio da integração de características morfológicas e análises filogenéticas (Dissanayake et al., 2016). Em geral, aspectos morfológicos, como forma, cor e tamanho dos conídios, além da estrutura dos conidióforos e picnídios, são mais úteis para distinguir gêneros do que para diferenciar espécies dentro do mesmo gênero, devido à grande similaridade morfológica (Osorio et al., 2017). Por esse motivo, a abordagem filogenética multigênica tornou-se essencial para a identificação precisa das espécies (Slippers et al., 2017). As regiões gênicas mais utilizadas para inferir relações filogenéticas dentro da família Botryosphaeriaceae incluem o espaçador transcrito interno (ITS), o fator de alongamento da tradução 1-alfa (TEF1-α), a β-tubulina (TUB) e a subunidade da RNA polimerase II (RPB2). Essas regiões foram eficazes para a

identificação específica de diversas espécies em diferentes estudos (Feng et al., 2023; Díaz et al., 2022; Bautista-Cruz et al., 2019; Marques et al., 2012; Li et al., 2023). Porém, a maioria das identificações em campo são feitas por sintomatologia enquanto as caracterizações morfológica e filogenética são negligenciadas.

O manejo da morte descendente no abacateiro é baseado em práticas que seguem os princípios de proteção e exclusão, além de estratégias para minimizar os danos causados pelo patógeno após seu estabelecimento na planta (Tavares et al., 2005; Saeed et al., 2017). Em geral, recomenda-se remover e queimar partes infectadas, eliminar resíduos da cultura, realizar podas sanitárias, desinfetar ferramentas e controlar insetos que podem causar ferimentos e facilitar a infecção. Uma vez estabelecida a doença, o manejo deve incluir o uso de métodos químicos e biológicos para conter seu avanço (Tavares et al., 2005; Syed et al., 2014; Kamil et al., 2018).

Os produtos registrados para o controle de doenças na cultura do abacate no Brasil incluem inibidores de desmetilação (DMI), carbamatos de metil benzimidazol (MBC), inibidores externos de quinona (QoI), anilino pirimidinas (AP), inibidores de succinato desidrogenase (SDHI) e outros com ações multissítio como cloronitrilas, ditiocarbamatos e compostos inorgânicos (MAPA, 2025). Porém não há nenhum produto registrado para o controle de fungos da família Botryosphaeriaceae no abacate.

Os fungicidas MBC (benzimidazóis) são um grupo sistêmico, de amplo espectro e foi o primeiro fungicida sítio único, produzindo atividade antifúngica baseado no seu anel benzimidazol (Bai et al., 2024). Apresentam baixa toxicidade e são altamente eficientes no controle de uma ampla gama de microrganismos (Hawkins & Fraaije, 2016). Eles atuam inibindo a polimerização da tubulina, interferindo na formação do fuso mitótico, comprometendo a divisão celular e desenvolvimento fúngico (Bai et al., 2024). Para a cultura do abacate os ingredientes ativos registrados são tiofanato metílico e tiabendazol para controle de antracnose (*Colletotrichum* sp.) (MAPA, 2025).

Os QoIs, também conhecidos como estrobilurinas, são fungicidas de sítio único e são ativos contra uma ampla gama de patógenos de plantas e amplamente registrados em várias culturas no Brasil, incluindo árvores frutíferas (Fernández-Ortuño et al., 2010; MAPA, 2025). Eles funcionam bloqueando a respiração mitocondrial fúngica, impedindo a transferência de elétrons no complexo III da cadeia de transporte de elétrons, especificamente no sítio Qo do citocromo bc1 oxidorredutase, resultando na cessação da

produção de ATP e morte do patógeno (Fernández-Ortuño et al., 2008). Em abacates produtos a base de azoxistrobina são registrados para controle de *Colletotrichum gloeosporioides* e *Pseudocercospora purpurea* (MAPA, 2025).

Embora os benzimidazóis e estrubirulinas sejam eficazes no controle de uma ampla gama de patógenos, seu uso contínuo e sem a devida gestão pode levar a seleção de isolados menos sensíveis, reduzindo a eficácia e a vida útil dos fungicidas (Hollomon, 2015; Mikaberidze et al., 2017). Os MBCs e os QoIs são bastante utilizados na cultura do abacate para o controle de antracnose (Fischer & Firmino, 2023). Isolados de botriosfaeráceos resistentes a ingredientes ativos desses grupos já foram identificados em outras frutíferas pelo uso indevido dos produtos químicos (Cavalcante et al., 2014; Chen et al., 2020; Dong et al., 2022). Todos os fungicidas estão sujeitos ao desenvolvimento de resistência por patógenos, e o risco dessa adaptação depende de fatores como o ciclo do patógeno (monocíclico ou policíclico), a intensidade do uso do fungicida, a especificidade do hospedeiro e os sistemas de produção adotados (Grimmer et al., 2015; Brent & Hollomon, 2007; Corkley et al., 2022).

Fungicidas de sítio específico, como MBCs e QoIs, têm um risco significativamente maior de desenvolvimento de resistência em comparação com fungicidas multissítio (Mikaberidze et al., 2017). As respostas fúngicas a esses compostos são complexas e envolvem várias modificações moleculares que conferem maior capacidade de sobrevivência (Yin et al., 2023). Alterações na proteína alvo, superexpressão do sítio alvo, bombas de efluxo e evasões metabólicas são exemplos de mecanismos de resistência conhecidos em fungos resistentes (Hawkins & Fraaije, 2022).

A resistência a benzimidazois são conhecidas em diversos fungos e geralmente estão associadas a mutações no gene da β-tubulina, sendo as mais conhecidas E198A/G/K, F200Y (FRAC, 2025; Yin et al., 2023). No caso de *Lasiodiplodia theobromae*, a redução da sensibilidade a tiofanato metil foi associada a mutação E198K da β-tubulina (Chen et al., 2020). Quanto às estrobilurinas a resistência é frequentemente causada por mutações no local alvo do gene *Cytb* (G143A, F129L) (FRAC, 2025; Lucas et al., 2015). No entanto, até o momento, não foram relatadas mutações ou superexpressão do gene *Cytb* em isolados de *Lasiodiplodia* e *Neofusicoccum* resistentes aos fungicidas azoxistrobina e piraclostrobina, que são os ingredientes ativos utilizados nas pesquisas (Chen et al., 2020; He et al., 2021; Tennakoon et al., 2018; Xu et al., 2015). Por outro

lado, mutações no gene da oxidase alternativa (AOX), uma enzima envolvida na cadeia respiratória mitocondrial, foram observadas em isolados altamente resistentes (Dong et al., 2022).

No Brasil, casos de resistência de isolados de Botryosphaeriaceae aos princípios ativos azoxistrobina, piraclostrobina e tiofanato metilico já foram relatados em manga e mamão (Cavalcante et al., 2014; da Silva Pereira et al., 2012; dos Santos et al., 2019) e, embora não existam produtos registrados para morte descendente em abacate, o uso de fungicidas para outras doenças pode influenciar a dinâmica das populações fúngicas e contribuir para o desenvolvimento de resistência (Brent & Hollomon, 2007).

Neste contexto, este estudo teve como objetivo: (a) esclarecer a etiologia da morte descendente em abacateiro nas principais regiões produtoras; (b) avaliar a patogenicidade e agressividade de fungos associados à morte descendente de abacateiros. (c) caracterizar a sensibilidade de isolados de *Lasiodiplodia* e *Neofusicoccum* à azoxistrobina e ao tiofanato-metil in vitro e in vivo; (d) investigar as bases moleculares associadas à mutações de resistência; (e) comparar isolados sensíveis e resistentes quanto a adaptabilidade.

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CHAPTER 1

Unraveling the Diversity of Fungi Causing Avocado Dieback in Brazil

Abstract

Brazilian avocado production has been severely impacted by canker and dieback. Producers have identified *Phytophthora* as the primary causal agent of this disease. Despite the diversity of fungal species associated with these symptoms, botryosphaeriaceous fungi remain the most prevalent worldwide. As accurate identification is essential for controlling this disease, this study aimed to characterize the isolates through morpho-molecular analysis and determine the pathogenicity and virulence of different fungal species. All 158 isolates obtained from four states were classified into eleven distinct species within Botryosphaeriales. Although *Lasiodiplodia brasiliensis*, *L. euphorbiaceicola*, *L. laeliocattleyae*, *L. macrospora*, *L. parva*, *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum kwambonambiense*, *N. parvum*, *N. ribis*, and *Pseudofusicoccum stromaticum* were identified, *N. parvum* (39.7%) and *L. pseudotheobromae* (22%) together accounted for more than half of the isolates. Additionally, *Neofusicoccum* isolates were more prevalent in regions with a tropical wet and dry climate, while *Lasiodiplodia* isolates were more common in areas with a humid subtropical climate. In this study, *Neofusicoccum caryigenum* was synonymized with *N. ribis*, while *N. hyperici*, *N. sichuanense*, *N. vaccinii*, and *N. yunnanense* were synonymized with *Neofusicoccum parvum* based on multi-locus phylogenetic analysis and GCPRS criteria. Pathogenicity tests on fruits and seedlings confirmed that all species are pathogenic. To our knowledge, this is the first report of *L. macrospora*, *N. kwambonambiense*, and *N. ribis* causing dieback in avocado worldwide. This study demonstrates that regional climatic conditions largely determine the prevalence of botryosphaeriaceous fungal species.

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Key words: Botryosphaeriaceae, Identification, Pathogenicity, *Persea americana*.

1.Introduction

The avocado tree (*Persea americana*) is a low-risk crop that has seen a 40% increase in global consumption over the past four years (ABPA, 2022; Araújo et al., 2018). This growing demand is driven by the fruit's health benefits, its culinary versatility, and its applications in the cosmetics and pharmaceutical industries (Araújo et al., 2018). Brazil is among the main producers, with the majority of its production destined for domestic consumption (ABRAFRUTAS, 2022; Andrade, 2022; IBGE, 2021). The Hass variety is the most widely cultivated globally due to its firmer texture and higher content of essential oils (Dreher & Davenport, 2013). In Brazil, varieties that produce larger fruits with smooth green skin, a fibrous texture, and higher water content are more common (Barbieri et al., 2023).

Mitigating the factors that limit production is essential to maintaining production and quality levels (Dann et al., 2013). Climate change, defined by rising temperatures and prolonged drought periods, combined with nutritional deficiencies, frost, and overirrigation, imposes physiological stress on plants, making them more vulnerable to fungal diseases (Nnadi & Carter, 2021; Raza & Bebber, 2022; Singh et al., 2023; Valencia et al., 2022). These conditions along with the intensification of cropping systems have intensified dieback epidemics in various fruit trees (Avenot et al., 2023; Camarero & Gazol, 2022; Kenfaoui et al., 2022; Marchin et al., 2022; Martino et al., 2024; Rodríguez-Gálvez et al., 2017; Sharma et al., 2024). In Brazil, the monitoring of diseases in avocado orchards has revealed plant death due to the occurrence of cankers and dieback (Silva et al., 2025).

The canker is recognized by fissures and darkening of the trunk's bark, while dieback begins with yellowing of the leaves and darkening of the branches, which gradually wither and dry out. In both cases, fungi colonize the xylem disrupting nutrient and water transport and leading to the death of entire branches and trees (Pérez-Jiménez, 2008; Twizeyimana et al., 2013; Ramírez-Gil et al., 2017; 2021). Despite the diversity of fungal species associated with these symptoms (Arjona-Girona et al., 2019; Silva et al., 2025), brazilian producers believe that these symptoms, including the formation of a brown-red exudate on the cankers, are the result of *Phytophthora* infection (personal communication).

However, in major avocado-producing countries, fungi from the Botryosphaeriaceae family are the main causal agents of canker and dieback (Eskalen et al., 2012; Trakunyingcharoen et al., 2015; Çalış et al., 2024; Guarnaccia et al., 2020; Hernandez et al., 2023; Möller et al., 2025). In addition to reduced fruit yield and plant death caused by botryosphaeriaceous fungi, the greatest losses are observed in post-harvest due to stem-end rot of the fruit (Chen et al., 2024; Firmino et al., 2016; Guarnaccia et al., 2016; Xu et al., 2024; Valencia Bernal et al., 2025).

Although the prevalence of botryosphaeriaceous fungi varies according to regional climatic conditions (Hernandez et al., 2023; Moller et al., 2025; Valentina-Bernal et al., 2025), species of *Lasiodiplodia*, *Neofusicoccum*, *Diplodia*, *Fusicoccum*, and *Dothiorella* are commonly reported in avocado (Auger et al., 2013; Fiorenza et al., 2022; McDonald & Eskalen, 2011; Rodríguez-Gálvez et al., 2021; Trakunyingcharoen et al., 2015; Valencia et al., 2019).

In Brazil, species of *Lasiodiplodia* and *Neofusicoccum* have been identified on avocado through morphological comparisons of a limited number of isolates (Fischer & Firmino, 2023; Piccinin & Pascholati, 2018; Firmino et al., 2016). As morpho-molecular characterization combined with extensive sampling is crucial to enhancing the understanding of these pathogens and developing effective management strategies (Martino et al., 2024; Silva et al, 2025; Moller et al., 2025), our objective is to clarify the etiology and pathogenicity of botryosphaeriaceous fungi causing dieback and canker in the main avocado-producing regions of Brazil.

2.Material and Methods

Sampling and isolation

The collections were carried out in avocado-producing areas in Minas Gerais, São Paulo, Santa Catarina, and the Distrito Federal between 2019 and 2023 (**Table S1**). Symptomatic branches and trunk were collected from at least three plants sampled per orchard (**Figure 1**). The samples were placed in paper bags and transported to the laboratory. Small pieces were removed from the margins between the healthy and symptomatic tissue and were disinfected by immersion in 70% ethanol for 1 minute and then in 1% hypochlorite for 2 minutes and rinsed with water three consecutive times. The disinfested samples were deposited on potato dextrose agar (PDA) medium and incubated at 25°C in the dark for 7 to 14 days. The mycelium was transferred to water agar to remove

hyphal tips and obtain pure cultures. The isolates were stored in the Coleção Micológica da Universidade de Brasília (CCUB) by the Castellani method (de Capriles et al., 1989) and -80°C in 10% glycerol.

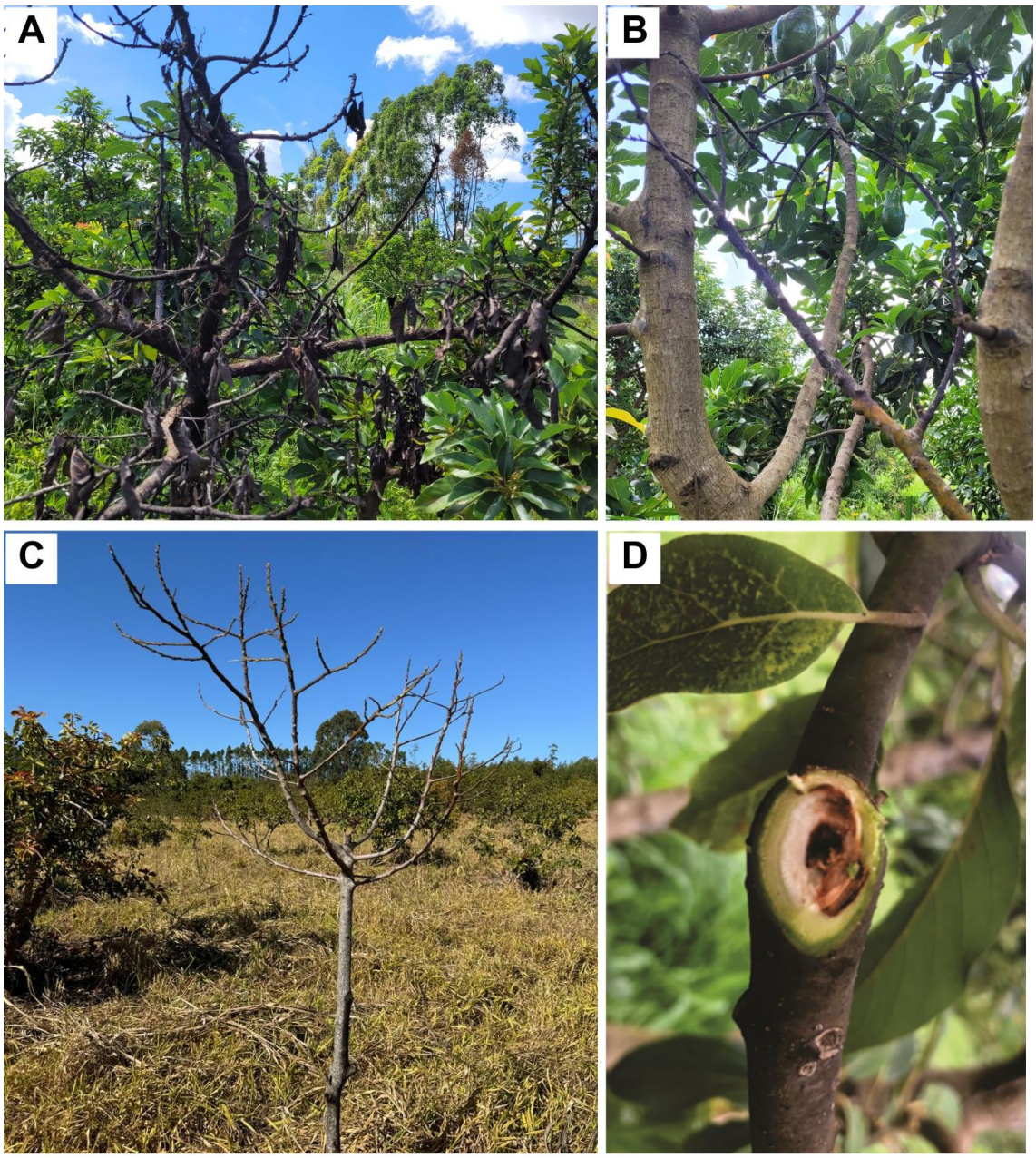

Figure 1. (A-B) Avocado branches with symptoms of drought. (C) tree with symptoms of dieback. (D) darkening of the vascular system of branches with symptoms.

DNA extraction and sequencing

The fungi were grown in a Petri dish containing PDA covered with cellophane and incubated at 25ºC for 3 days in the dark. Approximately 40 mg of mycelium were

removed and placed in 1.5 mL tubes containing 40 µL of Tris-EDTA (TE) buffer. Genomic DNA extraction was performed using the Wizard Genomic DNA Purification Kit (Promega Corporation, WI, U.S.A.) according to Pinho et al. (2013). The polymerase chain reaction (PCR) was initially performed for the translation elongation factor 1-alpha (tef1-α) region using primers EF1F and EF2R (Jacobs et al., 2004) to estimate species diversity. Subsequently, two representative isolates of each species were selected for accurate identification using primers recommended for Botryosphaeriaceae (**Table 1**). The PCR products were analyzed by electrophoresis in 1% agarose gel stained with fluorescent dye GelRedTM (Biotium, Hayward, California, USA) and visualized under ultraviolet light to verify the size and purity. The PCR products were purified and sequenced by the Sanger method.

Table 1. List of primers that were used in this study.

Locus	Primer name	sequence (5'–3')	Orientation	(Anealing/ Extension)	Reference
Elongation factor 1-α (*tef1-α*)	EF1F1	TGC GGT GGT ATC GAC AAG CGT	Forward	54°-45''/72°-45''	(Jacobs et al., 2004)
	EF2R1	AGC ATG TTG TCG CCG TTG AAG	Reverse	54°-45''/72°-45''	(Jacobs et al., 2004)
RNA polymerase II second largest subunit (*rpb2*)	5F2	GGG GWG AYC AGA AGA AGG	Forward	54°-45''/72°-45''	(Sung et al., 2007)
	7Cr	CCC ATR GCT TGY TTR CCC AT	Reverse	54°-45''/72°-45''	(Liu et al., 1999)
β-tubulin (*tub2*)	Bt2-a	AAC ATG CGT GAG ATT GTA AGT	Forward	55°-45''/72°-60''	(Glass and Donaldson, 1995)
	Tub_R1	TTG GGG TCG AAC ATC TGC TG	Reverse	55°-45''/72°-60''	(Chen et al., 2020)
28S nrRNA (*LSU*) e Internal transcribed spacer (*ITS*)	V9G	TTA CGT CCC TGC CCT TTG TA	Forward	53°-45''/72°-45''	(de Hoog and van den Ende, 1998)
	LR5	TCC TGA GGG AAA CTT CG	Reverse	53°-45''/72°-45''	(Vilgalys and Hester, 1990)

Phylogenetic analyses

The electropherograms were aligned to assemble the consensus sequence using DNA Dragon (https://www.dna-dragon.com). All sequences were deposited in GenBank (**Table S2**). The sequences of the type and reference isolates of all known species for each genus were downloaded from GenBank and combined with the newly generated sequences by MEGA 7 (Kumar et al., 2016) (Table 2) and aligned with the online version of MAFFT version 7 with the iterative refinement method G-INS-I (Katoh & Standley, 2013).

Maximum likelihood (ML) and Bayesian inference (BI) analyses were used for the phylogeny of each locus and all concatenate data. Sequences were concatenated using

SequenceMatrix 1.8 (Vaidya et al., 2011). ML analyses were performed by IQ-TREE v. 2.1.2 (Nguyen et al., 2015) and model parameters were considered separately for each partition using ModelFinder (Kalyaanamoorthy et al., 2017; Minh et al., 2020) with bootstrap support SH-alrt ≥80% with 1000 sampling (Guindon et al., 2010). BI analyses were performed using MrBayes 3.2.6 1 (Ronquist et al., 2012) implemented in the CIPRES Science Gateway. The best model was selected according to the Akaike Information Criteria (AIC) performed by MrModeltest 2.3 (Nylander, 2004). The matrices were subjected to Bayesian Inference analysis and the posterior probabilities were determined by Markov Chain Monte Carlo (MCMC) sampling under analysis of 10 million generations with sampling every 1,000, with subsequent discarding of 20% of the first trees. The consensus tree was visualized in FigTree v.1.4 (Ronquist et al., 2012).

The Genealogical Concordance Phylogenetic Species Recognition (GCPSR) approach was used to identify the phylogenetic lineages for which a clade was recognized as an independent evolutionary lineage if at least one of the GCPSR criteria was met for genealogical concordance or genealogical non-discordance (Dettman et al., 2003; Taylor et al., 2000).

Species prevalence

Species prevalence by sampling geography (state) was recorded as a percentage of the total number of isolates by sampling stratum. The prevalence of each species by state was calculated as the total number of isolates per species in a state divided by the total number of isolates in that state and expressed as a percentage.

Pathogenicity test

Representative isolates of each species were used for pathogenicity and aggressiveness tests on avocado seedlings and immature fruits of the 'Margarida' cultivar. Cuts of 6 mm were made at 25 cm from the tips of the seedlings and in the center of the fruits. Mycelium plugs of 5 mm of fungi grown on PDA for 5 days at 25 ± 2 ºC were deposited on the wounds. The seedlings were kept in humidity chambers for 7 days and then kept in a greenhouse at 25 ± 5 °C for 40 days. The fruits were kept in a humid chamber for 5 days at 25 ± 2 ºC. In control, PDA plugs were deposited on the wounds. Aggressiveness was assessed by measuring the lesions vertically. The experiments followed a completely randomized design with three replicates, each consisting of one avocado seedling or fruit. Data underwent normality testing using Fisher's LSD test, and

means were compared using analysis of variance (ANOVA) followed by the Scott-Knott test ($p > 0.05$) in R software.

3.Results

Fungal isolates

Avocado canker and dieback were observed across all monitored orchads. A total of 158 fungal isolates were obtained from symptomatic avocado tissues of nine cultivars namely 'Margarida', 'Hass', 'Breda', 'Campinas', 'Fortuna', 'Geada', 'Ouro Verde' and 'Quintal', collected from in the states of São Paulo (n =71), Santa Catarina (n=5), Minas Gerais (n=22) and Distrito Federal (n=60).

Phylogenetic analysis

The identification using the comparison of TEF1-α sequences in GenBank with the BLASTn tool confirmed eleven fungal genera—*Colletotrichum*, *Cytospora*, *Calonectria*, *Fusarium*, *Lasiodiplodia*, *Nectria*, *Neocosmospora*, *Neofusicoccum*, *Neopestalotiopsis*, and *Pseudofusicoccum*—associated with avocado canker and dieback. As 158 out of the 141 isolates represent botryosphaeriaceous fungi, we proceeded with the accurate identification of this fungal group.

The concatenated sequences of *Lasiodiplodia* consisted of 91 Operational Taxonomic Units (OTUs) and an aligned matrix of 1,564 characters. The loci boundaries in the alignment were: *tub2* 1-357, *ITS* 358-815, *rpb2* 816-1348, and *tef1*-α 1348-1564. The selected models for BI were GTR+G, K80+I, SYM+G, and K80+G, respectively.

The *Lasiodiplodia* were classified into six species based on multilocus analyses utilizing ML and IB. The identification was confirmed using GCPSR criteria. *Lasiodiplodia pseudotheobromae* was the most frequent species (n=31) followed by *L. theobromae* (n=13), *L. euphorbiaceicola* (n=9), *L. parva* (n=5), *L. macrospora* (n=4), *L. brasiliensis* (n=3) and *L. laeliocattleyae* (n=1) (**Figure 2**).

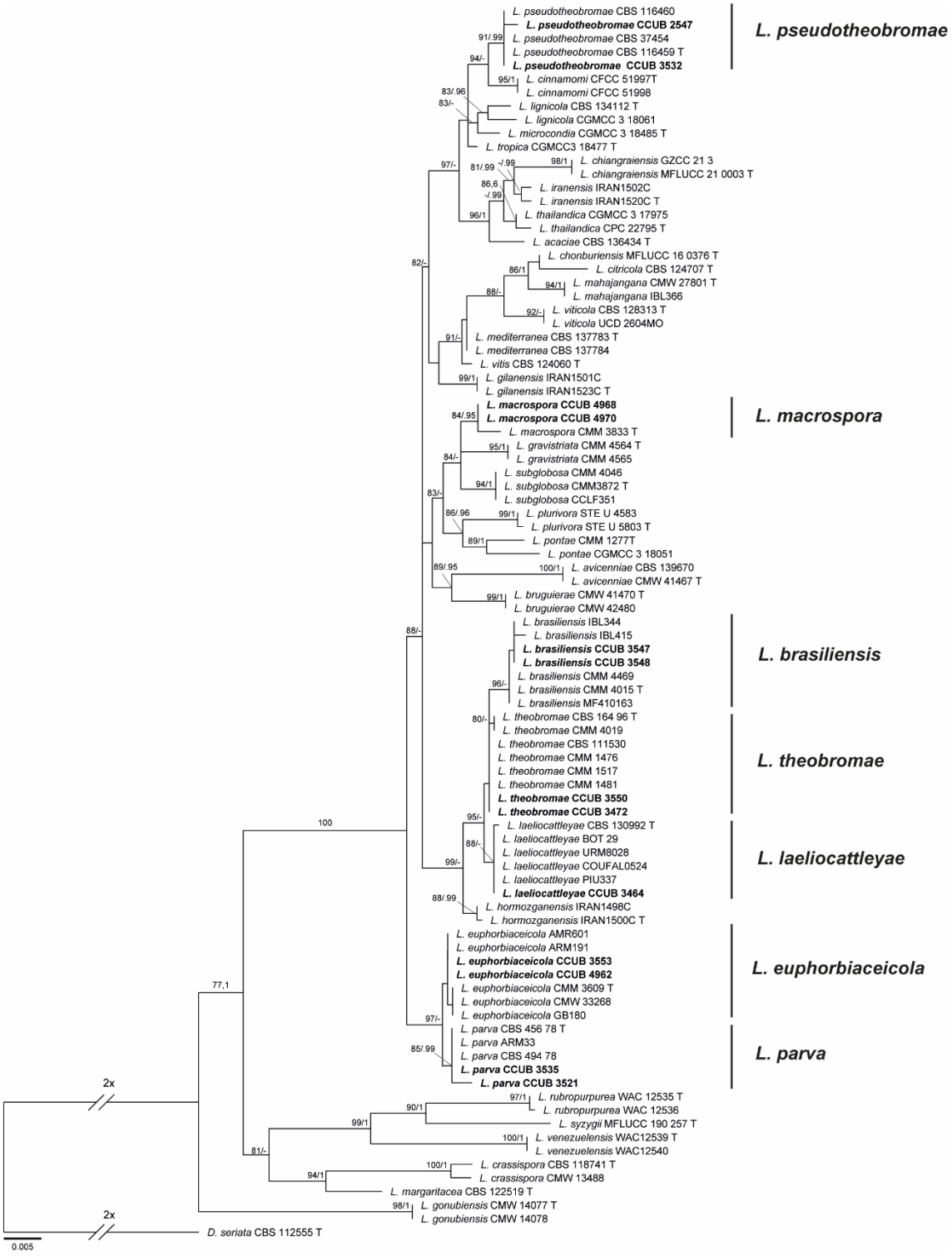

Figure 2: Maximum likelihood tree of the *Lasiodiplodia* from a concatenated alignment of *ITS*, *tef1*-α, *rpb2* and *tub2* sequences. Significant SH-aLRT bootstrap supports (≥ 80) and posterior Bayesian probability (≥ 95) are shown above the nodes. The tree was rooted with *Diplodia seriata*. Ex-type isolates are indicated with "T" and isolates obtained in

this study are highlighted in bold. The scale bar indicates the average number of substitutions per site.

The concatenated sequences of the *Neofusicoccum* resulted in an aligned matrix consisting of 1,738 characters with 64 OTUs. The loci boundaries in the alignment were: *ITS* 1-505, *rpb2* 506-1,070 *rpb2* 1,071-1,342, and *tef1*-α 1343- 1,738 and the selected models for BI were HKY+I, GTR+I, HKY+G, and GTR+I, respectively. The isolates were identified as *N. parvum* (n=56 isolates), *N. ribis* (n=8), and *N. kwambonambiense* (n=4) based on multilocus phylogenetic analysis.

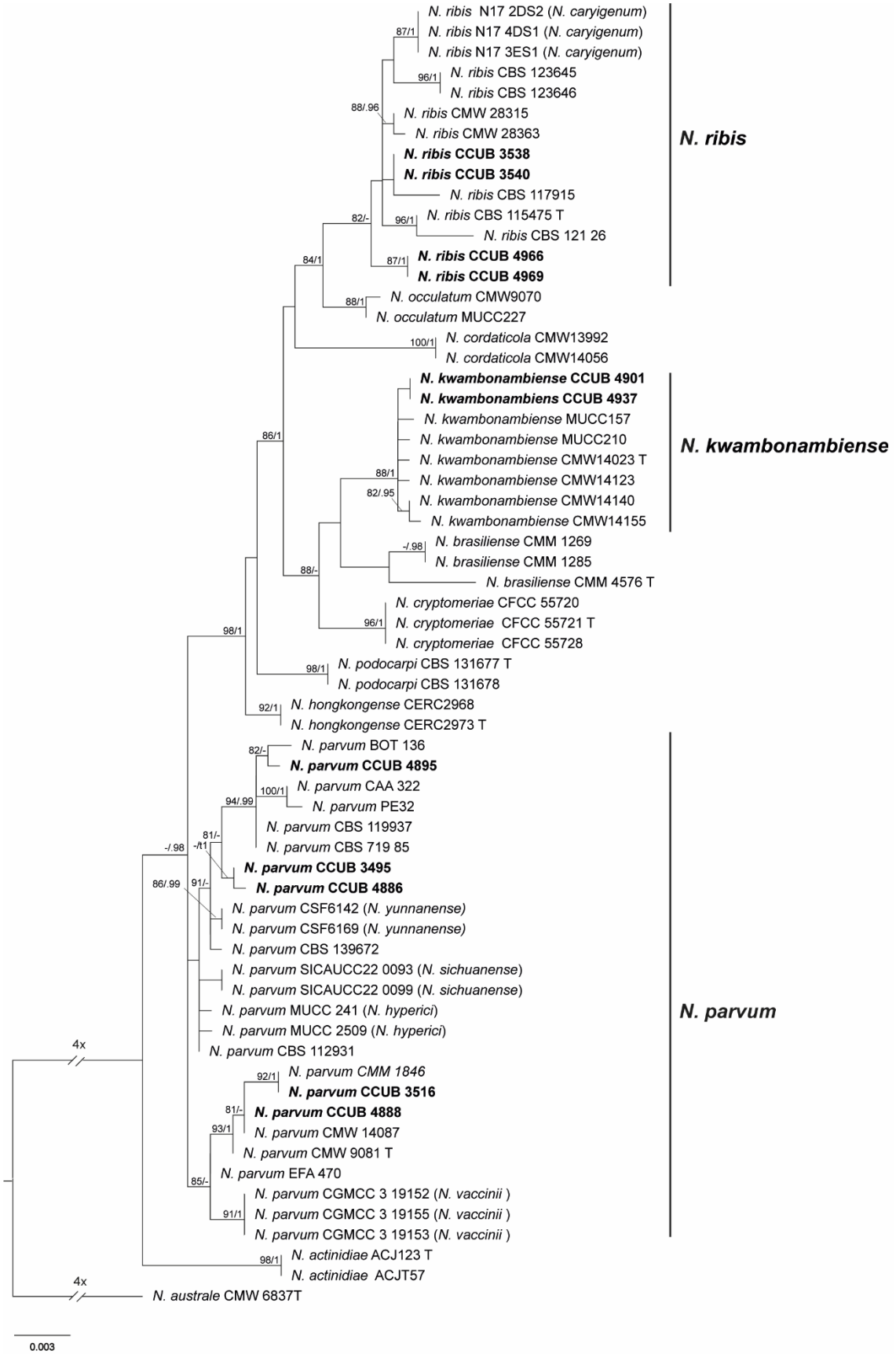

Figure 3: Maximum likelihood tree of the *Neofusicoccum* from a concatenated alignment of *ITS*, *tef1*-α, *rpb2* and *tub2* sequences. Significant SH-aLRT bootstrap supports (≥ 80)

and posterior Bayesian probability (≥ 95) are shown above the nodes. The tree was rooted with *N. australe* (CMW 6837). Ex-type isolates are indicated with "T" and isolates obtained in this study are highlighted in bold. The scale bar indicates the average number of substitutions per site.

The concatenated sequences of *Pseudofusicoccum* consisted of 24 OTUs and an aligned matrix of 890 characters. The loci boundaries in the alignment were: *ITS* 1-526 and *tef1*-α 527-890. The models selected for BI were K80+I and HKY+G respectively. The *Pseudofusicoccum* were classified into one species based on multilocus analyses utilizing ML and IB. All isolates were identified as *P. stromaticum* considering the GCPSR criteria (**Figure 4**).

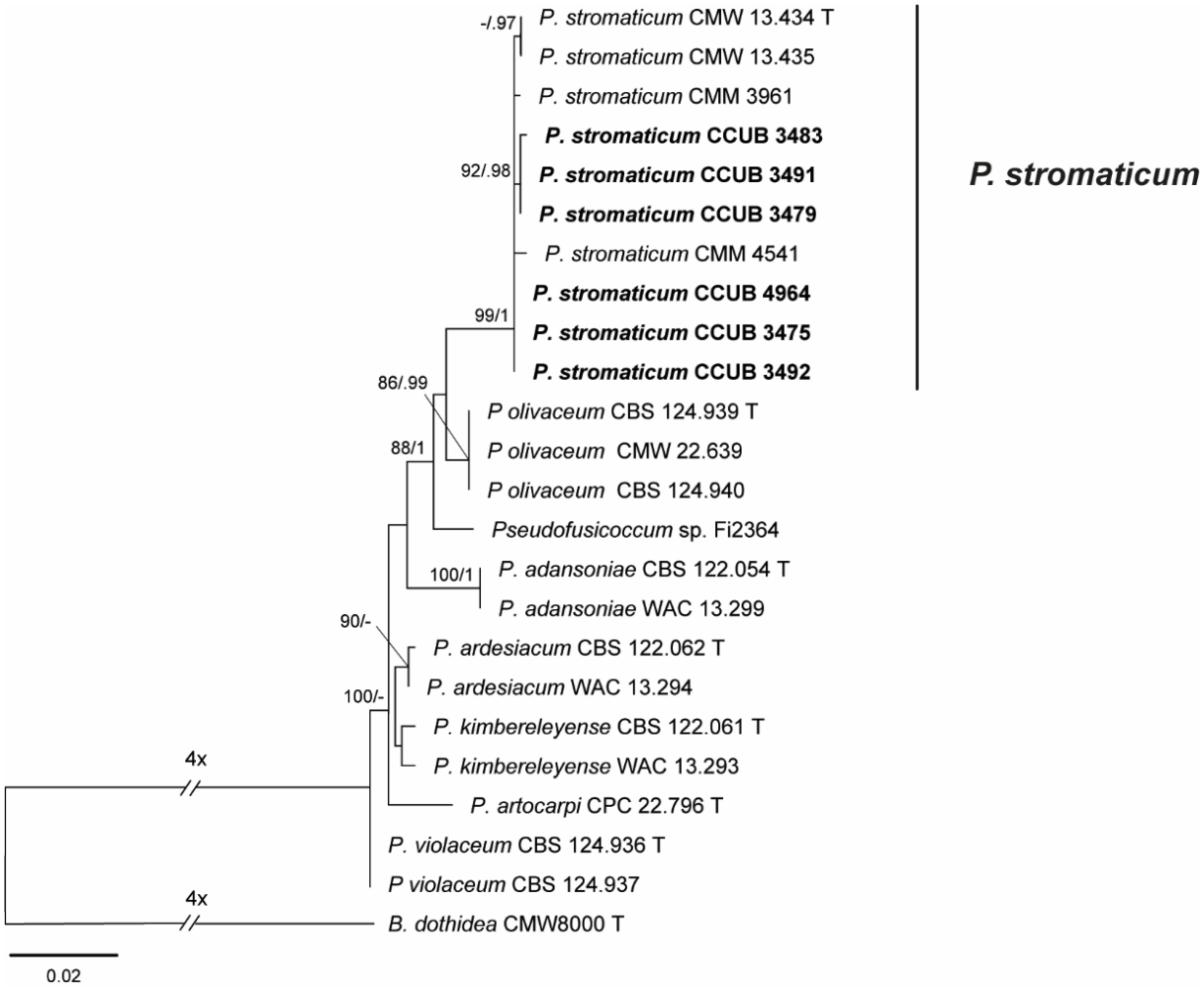

Figure 4: Maximum likelihood tree of *Pseudofusicoccum* from a concatenated alignment of *ITS* and *tef1*-α sequences. Significant SH-aLRT bootstrap supports (≥ 80) and posterior Bayesian probability (≥ 95) are shown above the nodes. The tree was rooted with *Botryosphaeria dothidea* (CMW 8000). Ex-type isolates are indicated with "T" and

isolates obtained in this study are highlighted in bold. The scale bar indicates the average number of substitutions per site.

Taxonomy

Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, Studies in Mycology 55: 248. 2006.

Basionym. Fusicoccum parvum Pennycook & Samuels, Mycotaxon 24:455. 1985.

New synonyms. Neofusicoccum hyperici Y. Hattori & C. Nakash., Mycoscience 62:252. 2021.

Neofusicoccum sichuanense X.L. Xu & C.L. Yang, Frontiers Microbiol. 13:1016548. 2022.

Neofusicoccum vaccinii Y. Zhang ter & L. Zhao., Plant Dis. 106:9. 2022.

Neofusicoccum yunnanense G.Q. Li & S.F. Chen, IMA Fungus 22:39. 2020.

Notes — The *Neofusicoccum parvum* species complex comprises *N. hyperici*, *N. sichuanense*, *N. vaccinii*, and *N. yunnanense*. In the past, *N. algeriense*, *N. italicum*, and *N. pandanicola* were synonymized with *N. parvum* due to minimal base pair differences in the *ITS*, *tef1*-α, and *tub2* regions, while the rpb2 sequences were identical (Zhang et al., 2021). *N. hyperici*, *N. sichuanense, N. vaccinii,* and *N. yunnanense* differ in few base pairs, these differences are less in the *rpb2* and *tub2* genes. Furthermore, the lack of concordance between individual gene trees and low branch support justify the synonymization. Although the clade containing *N. yunnanense*, *N. sichuanense*, *N. hyperici* and the isolates of this study (CCUB 4895, CCUB 3495, CCUB 4886) is positioned in a distinct clade from the Ex-type of *N. parvum*, it is observed that the genetic similarities between these isolates vary between 96% and 100% for all genes analyzed, indicating a high genetic similarity.

Neofusicoccum ribis (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55:249. 2006.

Basionym. Fusicoccum ribis Slippers et al., Mycologia 96:96. 2004.

New synonyms. Neofusicoccum caryigenum M.T. Brewer & C.J. Cameron, Mycologia 113 (3):593. 2021.

Notes — *Neofusicoccum caryigenum* are reduced to synonymy with *Neofusicoccum ribis* (**Figure 3**). The ex-type culture of *N. ribis* has the following nucleotide similarities with the sequences of the ex-type of *N. caryigenum*. On *ITS*, *tef1*-α, *tub2* and *rpb2*: 492/494(99%), 213/214(99%), 409/410(99%), 540/541(99%) respectively. The species concordance is observed only in the *ITS* tree, however, in the individual trees of the other genes, discordance is identified, which justifies the synonymization.

Prevalence of Botryosphaeriales species according to geographic region

The most common species were *N. parvum* (39.7%) and *L. pseudotheobromae* (22%), followed by *L. theobromae* (9.2%), *L. euphorbiaceicola* (6.4%), *N. ribis* (5.7%), *P. stromaticum* (5%), *L. parva* (3,5%), *L. macrospora* (2.8%), *N. kwambonambiense* (2.8%), *L. brasiliensis* (2.1%) and *L. laeliocatteyae* (0.7%). In the Federal District, the highest frequency of isolates was of the species *N. pavum*, associated with tropical humid and dry climates (Aw). In Minas Gerais, where the climate is humid subtropical (Cfa), the prevalence of isolates was dominated by *L. pseudotheobromae*, followed by *N. parvum*. In São Paulo and Santa Catarina, with a subtropical highland climate (Cwb), the prevalence was mainly of fungi of the genus *Lasiodiplodia*, especially *L. pseudotheobromae* and *L. theobromae* (**Figure 5; Table S1**).

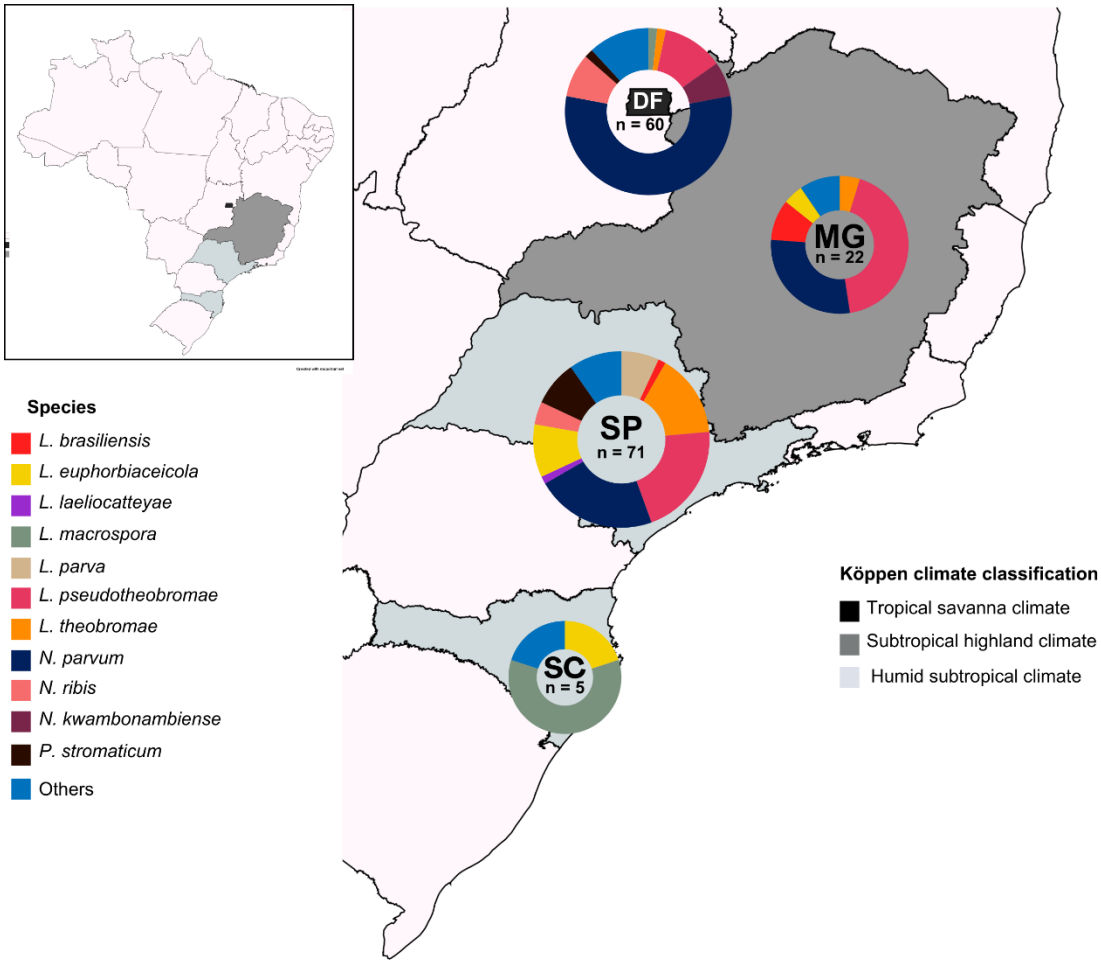

Figure 5: Species prevalence of 158 fungal isolates collected from avocado in the Federal District (DF), Minas Gerais (MG), Santa Catarina (SC) and São Paulo (SP).

Pathogenicity test

The inoculations confirmed that all species of botryosphaeriaceous fungi causing lesions in avocado fruits. The symptoms observed included darkening of the skin, followed by rotting of the pulp and, in some cases, the development of mycelium on the fruit's surface. The species *L. brasiliensis*, *L. parva*, *L. euphorbiaceicola*, *L. pseudotheobromae*, and *N. parvum* were the most aggressive to the fruits. In the seedlings, *N. kwambonambiense* was the most aggressive, followed by *L. parva*, *N. parvum* and *N. ribis* (Figure 6; Table 3). Of all the species, only *L. laeliocattleyae* did not cause lesions in the vascular system of the seedlings.

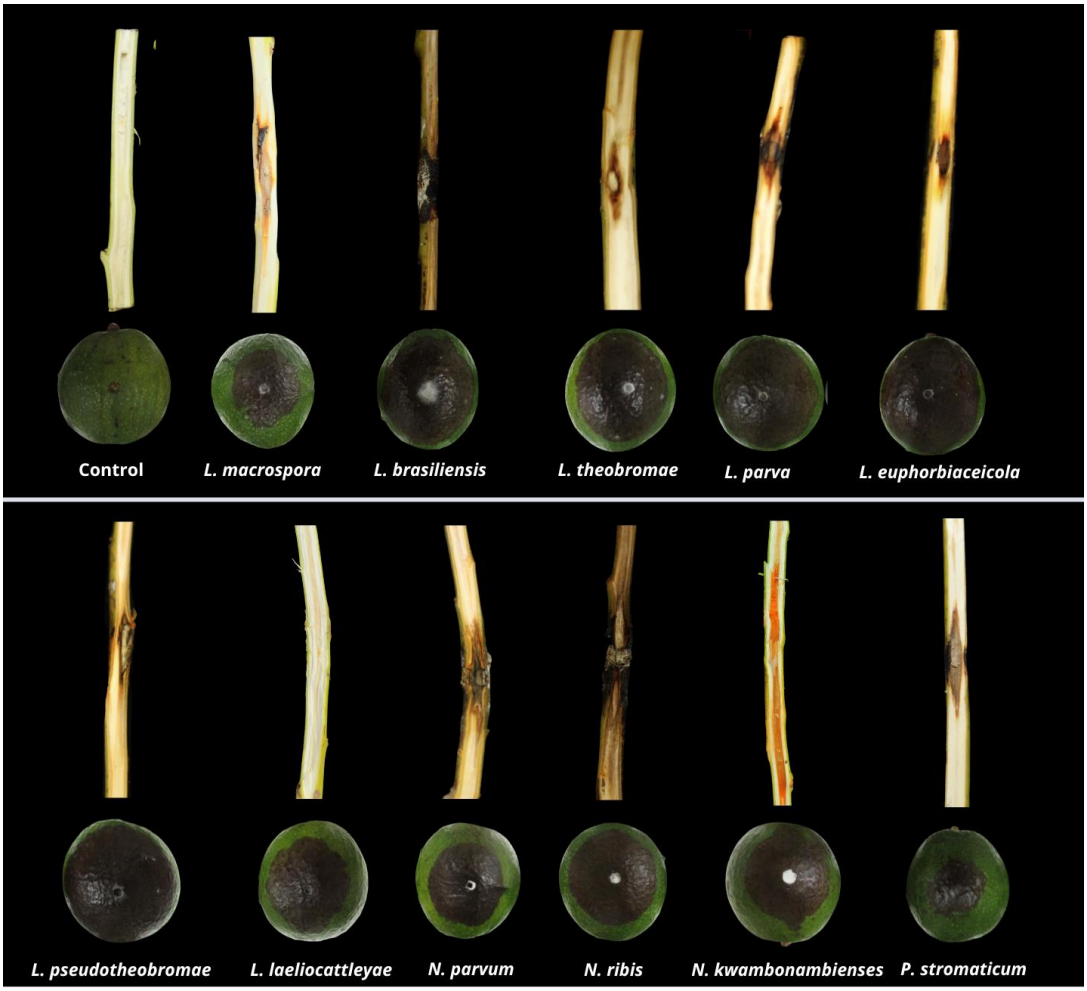

Figure 6: Symptoms in fruits and seedlings of avocado cultivar Margarida inoculated with representative isolates of *Lasiodiplodia*, *Neofusicoccum* and *Pseudofusicoccum* species. The fruits and seedlings were evaluated after 5 and 40 days of inoculation, respectively.

Table 3: Mean lesion length (mm) caused by *Lasiodiplodia* and *Neofusicoccum* species on fruit and seedlings of avocado.

Species	Fruit (mm)**	Seedling* (mm)**
L. laeliocatteyae	78.7 ± 1.3 a	0 ± 0 a
L. macrospora	95.7 ± 3.9 a	56.7 ± 7.6 a
L. theobromae	104.2 ± 17.4 a	79.6 ± 5.4 a
L. brasiliensis	110.0 ± 6.4 b	76.7 ± 25.2 a
L. euphorbiaceicola	120.5 ± 13.3 b	40.5 ± 8.4 a
L. parva	118.5 ± 12.6 b	155 ± 34.2 b
L. pseudotheobromae	132.8 ± 12 b	123.1 ±30.5 a
N. ribis	99.7 ± 7.8 a	142.8 ± 52.5 b
N. kwambonambiense	98.0 ± 2.7 a	213 ± 36.9 c
N. parvum	110.5 ± 22.4 b	135.8 ± 78.2 b
P. stromaticum	71.0 ± 13.2 a	23.6 ± a

cv(%)	10.9	35.3

* Internal length of the lesion on the stems of avocado seedlings.
** Mean lesion length followed by different letters in each column are significantly different according to Scott Knott test (p = 0.05).

4.Discussion

This study represents the first survey of species causing dieback in avocado trees in the main producing regions of Brazil with a significant number of isolates, integrating molecular identification, prevalence, virulence, and aggressiveness. In this study, we found that the main species associated with symptoms belonged to fungi of the order Botryosphaeriales, namely *L. brasiliensis*, *L. euphorbiaceicola*, *L. laeliocattleyae*, *L. macrospora*, *L. parva*, *L. pseudotheobromae*, *L. theobromae*, *N. kwambonambiense*, *N. parvum*, *N. ribis*, and *P. stromaticum*, with the most prevalent species being *N. parvum* followed by *L. pseudotheobromae*.

The species *N. parvum* was the prevalent species and is known to cause twig dieback and fruit rot, affecting the quality and production of fruit crops (Feng et al., 2023; Hattori et al., 2021). In avocado, for example, it is associated with twig dieback and fruit rot (Becerra Morales et al., 2019; Berraf-Tebbal et al., 2014; Haenzi et al., 2021; McDonald et al., 2009; Molina-Gayosso et al., 2012; Serret-López et al., 2017; Thomidis et al., 2011). In Brazil, *N. parvum* has been reported to cause diseases in strawberries and mangoes, two commercially important fruits for the country (Lopes et al., 2014; Marques et al., 2013). The species *N. kwambonambiense* and *N. ribis* have not been reported to cause diseases in avocados but are known to cause dieback in fruit trees (Maduke et al., 2024; Nogueira Junior et al. 2016; Ilyukhin & Ellouze, 2024; Tennakoon et al., 2019). The three species are part of the *N.parvum–N.ribis* complex, presenting similar morphological characteristics requiring molecular methods for identification (Marques et al., 2013; Sakalidis et a., 2013).

In several locations and climates, fungi of the *N. parvum–N. ribis* complex were isolated, *N. parvum* was observed to be the most dominant and abundant. This pattern of dominance and distribution of *N. parvum* has been associated with disturbed habitats or those close to human activities, where generalist species tend to be more efficient in invading human-modified environments (Pavlic-Zupanc et al., 2015). In addition, it was noted that *Neofusicoccum* species prevailed in areas with a tropical savanna climate. It is known that species of this genus have a wide distribution in different climatic zones and

can grow in various temperature ranges. Still, the relative proportion decreased in hot and humid environments, as pointed out by Burgess et al. (2019).

Molecular evidence has synonymized the species *N. hyperici*, *N. sichuanense*, *N. vaccinii*, and *N. yunnanense* under *N. parvum* and *N. caryigenum* as *N. ribis* in the present study. Recently, a study by Zhang et al. (2021) revisited the species within the order Botryosphaeriales, synonymizing important species of the *N. parvum*/*N. ribis* complex. However, subsequent research did not adopt this synonymization and proposed new species within the same complex (Brewer et al., 2021; Hattori et al., 2021; Zhao et al., 2022). Accurate identification of pathogenic fungal species is crucial for preventing and controlling plant diseases. GCPSR is currently most widely implemented in the field of fungal systematics for the identification of phylogenetic lineages (Laurence et al., 2014; Liu et al., 2016; Manawasinghe et al., 2021; Qiao et al., 2022; Taylor et al., 2000) and through its application it was possible to synonymize species.

Lasiodiplodia pseudotheobromae was the second species most associated with symptoms, it is a cryptic species within the *L. theobromae* complex, which has a wide geographic distribution, affecting a range of woody hosts, some of which are of economic importance such as eucalyptus, grapes, mangoes, citrus fruits, coffee, grapes, among others, the last two already reported in Brazil (Bragard et al., 2023; Correia et al., 2013; Freitas-Lopes et al., 2020; Lu et al., 2024; Saucedo-Picazo et al., 2022). The other species have already been reported causing dieback in avocados, except for *L. macrospora* (Hernández et al., 2023; Rodríguez-Gálvez et al., 2021; Xu et al., 2024; Rossell, 2023). Species of the genus *Lasiodiplodia* are frequently recognized as aggressive pathogens in a wide range of fruit trees. Identification has been mainly using molecular techniques using the four regions as in our study, since differentiation by morphology is particularly difficult (Huda-Shakirah et al., 2022; Rodríguez-Gálvez et al., 2021, Wang et al., 2024).

Species of the genus *Lasiodiplodia* were found in all sampled states, evidencing their ability to adapt to different regions and climates. However, a higher prevalence was observed in areas with a subtropical highland climate (Cwb) and humid subtropical climate (Cfa). The adaptation of these species to different climatic conditions and temperature variations is already known, however, their best adaptation seems to be associated with hot and humid environments (Rodríguez-Gálvez et al., 2021; Xu et al., 2015).

Pseudofusicoccum stromaticum belongs to the Phyllostictaceae family, which includes fungi known for their role as endophytes, saprophytes, and pathogens of woody plants, associated with stems, branches, and twigs (Phillips et al., 2019; Silveira et al., 2018; Sobreira et al., 2018). In Brazil, this species has been identified both as an endophyte in Cerrado plants and as a pathogen, especially in mango crops, associated with plant death (wilting of the tips) (Marques et al. 2012). A study by Marques et al. (2013), which investigated the species related to death and stem end rot in mango, revealed that *P. stromaticum* was the second most abundant species among the samples.

Pathogenicity tests showed that all species evaluated were capable of causing diseases in seedlings and fruits of the avocado cultivar Margarida under the experimental conditions used. Among these species, *L. parva*, *N. ribis*, and *N. parvum* were identified as the most aggressive to seedlings. Notably, N. parvum has previously been reported as a highly aggressive and harmful species to avocado (Valencia et al., 2019). However, findings in the literature vary, as different studies indicate that the most aggressive species within the Botryosphaeriaceae family may differ (Rodríguez-Gálvez et al., 2021; Qiu et al., 2020; Arjona-Girona et al., 2019; Fiorenza et al., 2023; Bernal et al., 2025). For instance, in our study, *L. laeliocattleya* did not induce symptoms in seedlings, while another research suggests this species is pathogenic (Rodríguez-Gálvez et 2021). These discrepancies may be due to genetic variations of the isolates found in diverse environments and populations, factors related to the genetic variability of the host plant, and differing experimental conditions. Despite these differences, it is crucial to emphasize the capability of the tested fungi to cause symptoms in avocado crops, highlighting their phytopathogenic potential.

These fungi have part of their life cycle as endophytes and can develop without causing symptoms (Chethana et al., 2016). Thus, healthy plants can harbor the pathogen without it being detected, facilitating its spread through human activities, especially by transporting infected seedlings over long distances. Short-distance spread can occur through contaminated tools during pruning and the use of machinery, as these fungi penetrate plants mainly through wounds (Moral et al., 2019). Consequently, the management of dieback in avocado is based on protection and exclusion practices, such as the removal of infected parts, elimination of crop residues, sanitary pruning, disinfection of tools, insect control, and correct irrigation practices (Tavares et al., 2005; Moral et al., 2019). Additionally, strategies to minimize the damage caused by the

pathogen after its establishment in the plant include the use of chemical and biological products (Kamil et al., 2018; Syed et al., 2014).

The combination of increased transport of plant material, the introduction of new species and crops, and changes in global climates have contributed to the expansion of the geographic distributions of many practices (Bebber, 2015; Bebber et al., 2014; Silva-Valderrama et al., 2024). In this context, a series of pathogens belonging to the order Botryosphaeriales, responsible for dieback and canker symptoms, which were previously unknown in Brazil or attributed to other etiological agents, were presented. Based on these findings, we suggest that new studies be carried out, focusing on fruit trees of economic importance to Brazil, preferably covering a wide geographic area to evaluate the distribution and severity of the disease in new hosts.

5.Conclusion

This study confirms that botryosphaeriaceous fungi are the main causal agents of canker and dieback in avocado in Brazil. The identification of eleven species using a multigene approach highlights the importance of comprehensive studies in understanding species diversity and their geographical distribution. These combined findings revealed that regional climatic conditions largely determine the prevalence of botryosphaeriaceous fungal species. As stem-end rot of the fruits causes severe post-harvest losses, pathogenicity tests highlight the need for frequent orchard monitoring and use of elite mother trees for high-quality seedling production. This information aids in understanding the diseases caused by botryosphaeriaceous fungi and is essential for recommending effective management strategies that promote avocado cultivation

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Supplementary material

Table S1. Botryosphaeriales species isolated in avocado: Geographical data and Köppen climate classification.

Isolate	Species	City	State[1]	Coordinates	Köppen climate classification[2]
CCUB 3469	*L parva*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3473	*L parva*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3480	*L parva*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3521	*L parva*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3535	*L parva*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3466	*L. brasiliensis*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3547	*L. brasiliensis*	Prata	MG	19°17′56″S 48°54′51″O	Aw
CCUB 3548	*L. brasiliensis*	Prata	MG	19°17′56″S 48°54′51″O	Aw
CCUB 3478	*L. euphorbiaceicola*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3498	*L. euphorbiaceicola*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3499	*L. euphorbiaceicola*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3500	*L. euphorbiaceicola*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3501	*L. euphorbiaceicola*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3507	*L. euphorbiaceicola*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3520	*L. euphorbiaceicola*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3553	*L. euphorbiaceicola*	Prata	MG	19°17′56″S 48°54′51″O	Aw
CCUB 4962	*L. euphorbiaceicola*	Tijucas	SC	27°14′26″S 48°38′4″O	Cfa
CCUB 3464	*L. laeliocatteyae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 4960	*L. macrospora*	Tijucas	SC	27°14′26″S 48°38′4″O	Cfa
CCUB 4968	*L. macrospora*	Tijucas	SC	27°14′26″S 48°38′4″O	Cfa
CCUB 4970	*L. macrospora*	Tijucas	SC	27°14′26″S 48°38′4″O	Cfa
CCUB 4972	*L. macrospora*	Gama	DF	16°01′10″S 48°04′01″O	Aw
CCUB 3467	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3470	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3472	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa

CCUB 3477	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3486	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3487	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3489	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3493	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3494	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3518	*L. theobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3531	*L. theobromae*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3550	*L. theobromae*	Prata	MG	19°17′56″S 48°54′51″O	Cwb
CCUB 4933	*L. theobromae*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 2541	*L.pseudotheobromae*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 2547	*L.pseudotheobromae*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 2549	*L.pseudotheobromae*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 2550	*L.pseudotheobromae*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 2551	*L.pseudotheobromae*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 2552	*L.pseudotheobromae*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 2554	*L.pseudotheobromae*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 3504	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3508	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3509	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3510	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3511	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3514	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3522	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3523	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3524	*L.pseudotheobromae*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3526	*L.pseudotheobromae*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3529	*L.pseudotheobromae*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa

CCUB 3532	*L.pseudotheobromae*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3533	*L.pseudotheobromae*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3536	*L.pseudotheobromae*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3539	*L.pseudotheobromae*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3551	*L.pseudotheobromae*	Prata	MG	19°17′56″S 48°54′51″O	Aw
CCUB 3555	*L.pseudotheobromae*	Prata	MG	19°17′56″S 48°54′51″O	Aw
CCUB 4880	*L.pseudotheobromae*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4882	*L.pseudotheobromae*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4890	*L.pseudotheobromae*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4891	*L.pseudotheobromae*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4971	*L.pseudotheobromae*	Gama	DF	16°01′10″S 48°04′01″O	Aw
CCUB 4978	*L.pseudotheobromae*	Gama	DF	16°01′10″S 48°04′01″O	Aw
CCUB 4979	*L.pseudotheobromae*	Gama	DF	16°01′10″S 48°04′01″O	Aw
CCUB 4892	*N. kwambonambiense*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4901	*N. kwambonambiense*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4902	*N. kwambonambiense*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4937	*N. kwambonambiense*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 2546	*N. parvum*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 3474	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3495	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3496	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3497	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3502	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3503	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3505	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3527	*N. parvum*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3541	*N. parvum*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3544	*N. parvum*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa

CCUB 3546	*N. parvum*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3549	*N. parvum*	Prata	MG	19°17′56″S 48°54′51″O	Aw
CCUB 4886	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4895	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4896	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4897	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4899	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4900	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4903	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4905	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4906	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4907	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4908	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4921	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4932	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4963	*N. parvum*	São Gotardo	MG	19°18′39″S 46°02′56″O	Cwb
CCUB 4965	*N. parvum*	Gama	DF	16°01′10″S 48°04′01″O	Aw
CCUB 4973	*N. parvum*	Paracatu	MG	17°13′21″S 46°52′31″O	Cwb
CCUB 5307	*N. parvum*	Fazenda Ágra Limpa	DF	15°56' 25"S 47°56'26"W	Aw
CCUB 2553	*N. parvum*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 2556	*N. parvum*	São thomé das letras	MG	21°43′25″S 44°58′53″O	Cwb
CCUB 3465	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3468	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3482	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3506	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3516	*N. parvum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 4879	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4884	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw

CCUB 4885	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4887	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4888	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4893	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4911	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4912	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4913	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4916	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4917	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4918	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4919	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4920	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4923	*N. parvum*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4936	*N. parvum*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4961	*N. parvum*	Tijucas	SC	27°14′26″S 48°38′4″O	Cfa
CCUB 4977	*N. parvum*	Asa norte	DF	15°45′47″S 47°53′01″O	Aw
CCUB 5312	*N. parvum*	Fazenda Ágra Limpa	DF	15°56' 25"S 47°56'26"W	Aw
CCUB 3525	*N. ribis*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3538	*N. ribis*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 3540	*N. ribis*	Mogi Morim	SP	22°25′55″S 46°57′30″O	Cfa
CCUB 4878	*N. ribis*	Sobradinho	DF	15°39′11″S 47°47′29″O	Aw
CCUB 4966	*N. ribis*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 4969	*N. ribis*	Brazlandia	DF	15°40′30″S 48°12′03″O	Aw
CCUB 5301	*N. ribis*	Asa norte	DF	15°45′47″S 47°53′01″O	Aw
CCUB 5304	*N. ribis*	Fazenda Ágra Limpa	DF	15°56' 25"S 47°56'26"W	Aw
CCUB 3475	*P. stromaticum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3476	*P. stromaticum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3479	*P. stromaticum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa

CCUB 3483	*P. stromaticum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3491	*P. stromaticum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 3492	*P. stromaticum*	Santo antonio de posse	SP	22°36′24″S 46°55′9″O	Cfa
CCUB 4964	*P. stromaticum*	Gama	DF	16°01′10″S 48°04′01″O	Aw

[1] Distrito Federal (DF); Minas Gerais (MG); Santa Catarina (SC); São Paulo (SP).
[2] Humid subtropical climate (Cfa); Tropical savanna climate (Aw); Subtropical highland climate (Cwb).

Table S2. Isolates used in the phylogenetic analyses of *Lasiodiplodia* with their corresponding GenBank accession numbers. Strains reported here have been highlighted in bold. (* = type species).

Species	**Isolate**	**Host**	**Origin**	**ITS**	**TEF**	**TUB**	**RPB2**
Diplodia seriata	CBS:112555	*Vitis vinifera*	Portugal	AY259094	AY573220	DQ458856	KX463962
Lasiodiplodia acaciae	CBS:136434*	*Acacia* sp.	Indonesia	MT587421	MT592133	MT592613	MT592307
L. avicenniae	CMW41467	*Avicennia marina*	South Africa	KP860835	KP860680	KP860758	KU587878
L. avicenniae	CBS:139670	*Avicennia marina*	South Africa	KU587957	KU587947	KU587868	KU587880
L. brasiliensis	CMM4015*	*Mangifera indica*	Brazil	JX464063	JX464049	-	-
L. brasiliensis	CMM4469	*Anacardium occidentale*	Brazil	KT325574	KT325580	-	-
L. brasiliensis	IBL344	fruit trees	Brazil	KT151808	KT151802	KT151805	-
L. brasiliensis	IBL415	fruit trees	Brazil	KT151806	KT151800	KT151803	-
L. brasiliensis	CERC 2284	*Eucalyptus* sp.	China	KX278010	KX278115	KX278219	MF410163
L. brasiliensis	**CCUB 3548**	***Persea Americana***	**Brazil**	**CCUB_3548**	**CCUB_3548**	**CCUB_3548**	**CCUB_3548**
L. brasiliensis	**CCUB 3547**	***Persea Americana***	**Brazil**	**CCUB_3547**	**CCUB_3547**	**CCUB_3547**	**CCUB_3547**
L. bruguierae	CMW41470*	*Bruguiera gymnorrhiza*	South Africa	KP860832	KP860677	KP860755	KU587875
L. bruguierae	CMW42480	*Bruguiera gymnorrhiza*	South Africa	KP860834	KP860679	KP860757	KU587876
L. chiangraiensis	MFLU:21-0003	Unknown host	Thailand	MW760854	MW815630	MW815628	-
L. chiangraiensis	GZAAS 21-0014	Unknown host	Thailand	MW760853	MW815629	MW815627	-
L. chonburiensis	MFLUCC_16_0376*	*Pandanus* sp.	Thailand	MH275066	MH412773	MH412742	-
L. cinnamomi	CFCC 51997*	*Cinnamomum camphora*	China	MG866028	MH236799	MH236797	MH236801

L. cinnamomi	CFCC 51998	*Cinnamomum camphora*	China	MG866029	MH236800	MH236798	MH236802
L. citricola	CBS:124707*	*Citrus* sp.	Iran	GU945354	GU945340	KU887505	KU696351
L. crassispora	CBS:118741*	*Santalum album*	Australia	DQ103550	DQ103557	KU887506	KU696353
L. crassispora	CMW13488	*Eucalyptus urophylla*	Venezuela	DQ103552	DQ103559	KU887507	KU696352
L. euphorbiaceicola	CMM3609T	*Jatropha curcas*	Brazil	KF234543	KF226689	KF254926	-
L. euphorbiaceicola	CMW33268	*Adansonia* sp.	Senegal	KU887131	KU887008	KU887430	KU887367
L. euphorbiaceicola	AMR601	*Manihot esculenta*	Brazil	OP832620	OP866772	-	OP866778
L. euphorbiaceicola	GB180	*Anacardium occidentale*	Boloma	MT981878	MW315827	MW315866	-
L. euphorbiaceicola	ARM191	*Manihot esculenta*	Brazil	MK480484	MK500928	-	MN935465
L. euphorbiaceicola	**CCUB 3553**	***Persea Americana***	**Brazil**	**CCUB_3553**	**CCUB_3553**	**CCUB_3553**	**CCUB_3553**
L. euphorbiaceicola	**CCUB 4962**	***Persea Americana***	**Brazil**	**CCUB_4962**	**CCUB_4962**	**CCUB_4962**	**CCUB_4962**
L. gilanensis	IRAN1523C*	*Citrus* sp.	Iran	GU945351	GU945342	KU887511	KP872462
L. gilanensis	IRAN1501C	*Citrus* sp.	Iran	GU945352	GU945341	KU887510	KP872463
L. gonubiensis	CMW14077*	*Syzygium cordatum*	South Africa	AY639595	DQ103566	DQ458860	-
L. gonubiensis	CMW14078	*Syzygium cordatum*	South Africa	AY639594	DQ103567	EU673126	-
L. gravistriata	CMM4564*	*Anacardium humile*	Brazil	KT250949	KT250950	-	-
L. gravistriata	CMM4565	*Anacardium humile*	Brazil	KT250947	KT266812	-	-
L. hormozganensis	IRAN1500C*	*Olea* sp.	Iran	GU945355	GU945343	KU887515	KP872466
L. hormozganensis	IRAN1498C	*Mangifera indica*	Iran	GU945356	GU945344	KU887514	KP872467
L. iranensis	IRAN1520C*	*Salvadora persica*	Iran	GU945348	GU945336	KU887516	KP872468
L. iranensis	IRAN1502C	*Juglans* sp.	Iran	GU945347	GU945335	KU887517	KP872469
L. laeliocattleyae	CBS:130992*	*Mangifera indica*	Egypt	NR_120002	KU507454	KU887508	KU696354
L. laeliocattleyae	BOT 29	*Mangifera indica*	Egypt	JN814401	JN814428	-	-
L. laeliocattleyae	COUFAL0524	*Musa* sp.	Brazil	PP465987	PP460494	PP460496	-
L. laeliocattleyae	PIU337	*Persea americana*	Peru	OP747498	OP839084	OP839101	-
L. laeliocattleyae	URM8028	*Manihot esculenta*	Brazil	MK480480	MK495378	-	MN886573
L. laeliocattleyae	**CCUB 3464**	***Persea Americana***	**Brazil**	**CCUB_3464**	**CCUB_3464**	**CCUB_3464**	**CCUB_3464**
L. lignicola	CBS:134112*	Dead wood	Thailand	JX646797	KU887003	KT852958	KU696364

L. lignicola	CGMCC:3-18061*	Woody branch	China	NR_152983	KX499927	KX500002	KX499965
L. macrospora	CMM3833*	*Jatropha curcas*	Brazil	NR_147349	KF226718	KF254941	-
L. macrospora	**CCUB 4968**	***Persea Americana***	**Brazil**	**CCUB_4968**	**CCUB_4968**	**CCUB_4968**	**CCUB_4968**
L. macrospora	**CCUB 4970**	***Persea Americana***	**Brazil**	**CCUB_4970**	**CCUB_4970**	**CCUB_4970**	**CCUB_4970**
L. mahajangana	CMW27801*	*Terminalia catappa*	Madagascar	NR_147325	FJ900641	FJ900630	-
L. mahajangana	IBL366	*Terminalia catappa*	Madagascar	FJ900596	FJ900642	FJ900631	-
L. margaritacea	CBS:122519*	*Australia*	EU144065	KT852959	EU144065	KU887520	KU696367
L. mediterranea	CBS:137783*	*Quercus ilex*	Italy	KJ638312	KJ638331	KU887521	KU696368
L. mediterranea	CBS:137784	*Vitis vinifera*	Italy	KJ638311	KJ638330	KU887522	KU696369
L. microcondia	CGMCC:3-18485*	*Aquilaria crassna*	Laos	KY783441	KY848614	-	KY848561
L. parva	CBS:456.78*	*Cassava*-field soil	Colombia	EF622083	EF622063	KU887523	KP872477
L. parva	CBS:494.78	*Cassava*-field soil	Colombia	EF622084	EF622064	EU673114	KU696373
L. parva	ARM33	*Manihot esculenta*	Brazil	MK480477	MK495375	-	MN935460
L. parva	**CCUB 3535**	***Persea Americana***	**Brazil**	**CCUB_3535**	**CCUB_3535**	**CCUB_3535**	**CCUB_3535**
L. parva	**CCUB 3521**	***Persea Americana***	**Brazil**	**CCUB_3521**	**CCUB_3521**	**CCUB_3521**	**CCUB_3521**
L. plurivora	STE-U 5803*	*Prunus salicina*	South Africa	EF445362	EF445395	KP872421	KP872479
L. plurivora	STE-U 4583	*Vitis vinifera*	South Africa	AY343482	EF445396	KU887525	KU696375
L. pontae	CMM1277*	*Spondias purpurea*	Brazil	KT151794	KT151791	KT151797	-
L. pontae	CGMCC:3-18051	-	China	MK510560	MK510671	MK510634	MK510597
L. pseudotheobromae	CBS:116459*	*Gmelina arborea*	Costa Rica	EF622077	EF622057	EU673111	KU696376
L. pseudotheobromae	CBS:116460	*Acacia mangium*	Costa Rica	EF622078	EF622058	KU198428	-
L. pseudotheobromae	CBS:37454	*Coffea sp.*	Zaire	KX464139	KX464633	KX464906	-
L. pseudotheobromae	**CCUB 3532**	***Persea Americana***	**Brazil**	**CCUB_3532**	**CCUB_3532**	**CCUB_3532**	**CCUB_3532**
L. pseudotheobromae	**CCUB 2547**	***Persea Americana***	**Brazil**	**CCUB_2547**	**CCUB_2547**	**CCUB_2547**	**CCUB_2547**
L. rubropurpurea	WAC12535*	*Eucalyptus grandis*	Australia	DQ103553	DQ103571	EU673136	KP872485
L. rubropurpurea	WAC12536	*Eucalyptus grandis*	Australia	DQ103554	DQ103572	KU887530	KP872486
L. subglobosa	CMM3872*	*Jatropha curcas*	Brazil	KF234558	KF226721	KF254942	-
L. subglobosa	CMM4046	*Jatropha curcas*	Brazil	KF234560	KF226723	KF254944	-

L. subglobosa	CCLF351	*Persian Lime*	Mexico	PP783656	PP792921	-	-
L. syzygii	MFLUCC 190-257*	*Syzygium samarangense*	Thailand	MT990531	MW016943	MW014331	-
L. thailandica	CPC 22795*	*Mangifera indica*	Thailand	KJ193637	KJ193681	-	-
L. thailandica	CGMCC:3-17975	*Acacia confusa*	China	NR_152982	KX499917	KX499992	KX499955
L. theobromae	CBS 164.6*	*Fruit along coral reef coast*	New Guinea	NR_111174	AY640258	KU887532	KU696383
L. theobromae	CBS111530	*Leucospermum sp.*	USA	EF622074	EF622054	KU887531	KU696382
L. theobromae	CMM1476	*Mangifera indica*	Brazil	JX464083	JX464057	-	-
L. theobromae	CMM1481	*Mangifera indica*	Brazil	JX464095	JX464021	-	-
L. theobromae	CMM1517	*Mangifera indica*	Brazil	JX464060	JX464054	-	-
L. theobromae	CMM4019	*Mangifera indica*	Brazil	JX464096	JX464026	-	-
L. theobromae	**CCUB 3550**	***Persea Americana***	**Brazil**	**CCUB_3550**	**CCUB_3550**	**CCUB_3550**	**CCUB_3550**
L. theobromae	**CCUB 3472**	***Persea Americana***	**Brazil**	**CCUB_3472**	**CCUB_3472**	**CCUB_3472**	**CCUB_3472**
L. tropica	CGMCC3 18477*	*Aquilaria crassna*	Laos	KY783454	KY848616	KY848540	KY848574
L. venezuelensis	WAC12539*	*Acacia mangium*	Venezuela	DQ103547	DQ103568	KU887533	KP872490
L. venezuelensis	WAC12540	*Acacia mangium*	Venezuela	DQ103548	DQ103569	KU887534	KP872491
L. viticola	CBS 128313*	*Vitis vinifera*	USA	HQ288227	HQ288269	HQ288306	KU696385
L. viticola	UCD2604MO	*Vitis vinifera*	USA	HQ288228	HQ288270	HQ288307	KP872493
L. vitis	CBS:124060*	*Vitis vinifera*	Italy	KX464148	KX464642	KX464917	KX463994

Table S3. Isolates used in the phylogenetic analyses of *Neofusicoccum* with their corresponding GenBank accession numbers. Strains reported here have been highlighted in bold. (* = type species).

Species	**Isolate**	**Host**	**Origin**	**ITS**	**TEF**	**TUB**	**RPB2**
Neofusicoccum actinidiae	ACJ123*	*Actinidia chinensis*	Hubei	MW540517	MW553093	MW553095	MW553097
N. actinidiae	ACJT57	*Actinidia chinensis*	Hubei	MW540518	MW553094	MW553096	MW553098
N. australe	CMW:6837*	*Persea Americana*	Brazil	AY339262	AY339270	AY339254	EU339573
N. brasiliense	CMM1269	*Mangifera indica*	Brazil	JX513629	JX513609	KC794032	-

N. brasiliense	CMM1285	*Mangifera indica*	Brazil	JX513628	JX513608	KC794030	-
N. brasiliense	CMM4576*	*Mangifera indica*	Brazil	KT247455	KT247457	-	-
N. caryigenum	N17-2DS2*	Pecan	Georgia	MW405114	MW393657	MW393679	MW393668
N. caryigenum	N17-3ES1	Pecan	Texas	MW405115	MW393658	MW393680	MW393669
N. caryigenum	N17-4DS1	Pecan	Texas	MW405116	MW393659	MW393681	MW393670
N. cordaticola	CMW13992	*Syzygium cordatum*	South Africa	EU821898	EU821868	EU821838	EU821928
N. cordaticola	CMW14056	*Syzygium cordatum*	South Africa	EU821903	EU821873	EU821843	EU821933
N. cryptomeriae	CFCC 55721*	*Cryptomeria japonica*	China	ON209700	OP056461	OP056458	OP056455
N. cryptomeriae	CFCC 55720	*Cryptomeria japonica*	China	ON209698	OP056459	OP056456	OP056453
N. cryptomeriae	CFCC 55728	*Cryptomeria japonica*	China	ON209699	OP056460	OP056457	OP056454
N. hongkongense	CERC2968	*Araucaria cunninghamii*	China	KX278051	KX278156	KX278260	KX278282
N. hongkongense	CERC2973*	*Araucaria cunninghamii*	China	KX278052	KX278157	KX278261	KX278283
N. hyperici	MUCC 241*	*Hypericum patulum*	Japan	LC589125	LC589137	LC589147	LC589160
N. hyperici	MUCC 2509	*Hypericum patulum*	Japan	LC589126	LC589138	LC589148	LC589161
N. kwambonambiense	MUCC157	*Eucalyptus dunnii*	E Australia	EU339522	EU339516	EU339479	-
N. kwambonambiense	MUCC210	*Corymbia torelliana*	NE Australia	EU301016	EU339515	EU339478	EU339564
N. kwambonambiense	CMW14023	*Syzygium cordatum*	South Africa	EU821900	EU821870	EU821840	EU821930
N. kwambonambiense	CMW14123	*Syzygium cordatum*	South Africa	EU821924	EU821894	EU821864	EU821954
N. kwambonambiense	CMW14140	*Syzygium cordatum*	South Africa	EU821919	EU821889	EU821859	EU821949
N. kwambonambiense	CMW14155	*Syzygium cordatum*	South Africa	EU821923	EU821893	EU821863	EU821953
N. kwambonambiense	**CCUB 4937**	***Persea Americana***	**Brazil**	**CCUB_4937**	**CCUB_4937**	**CCUB_4937**	**CCUB_4937**
N. kwambonambiense	**CCUB 4901**	***Persea Americana***	**Brazil**	**CCUB_4901**	**CCUB_4901**	**CCUB_4901**	**CCUB_4901**
N. occulatum	MUCC227*	*Eucalyptus grandis hybrid*	NE Australia	EU301030	EU339509	EU339472	EU339558
N. occulatum	CMW9070	*Wollemia nobilis*	E Australia	AY615164	AY615156	AY615148	EU339556
N. parvum	PE32	*Eucalyptus globulus*	Portugal	KT440952	KT441012	KX871765	-
N. parvum	CAA 322	*Malus domestica*	Portugal	KX505906	KX505894	KX505916	-
N. parvum	CBS:719-85	*Malus domestica*	New zealand	KX464151	KX464646	KX464921	KX464000
N. parvum	BOT 136	*Cedrus atlantica*	Montenegro	KF729053	KF729383	KF729343	KF729296

N. parvum	CBS 119937	*Quercus suber*	Italy	MT587444	-	MT592642	MT592338
N. parvum	CMW 14087	*Syzygium cordatum*	South Africa	EU821909	EU821879	EU821849	EU821939
N. parvum	CMM 1846	*Fragaria vesca*	Brazil	KC507812	KC507809	KC507806	KC507803
N. parvum	CMW_9081	*Populus nigra*	New Zealand	AY236943	AY236888	AY236917	EU821963
N. parvum	EFA 470	*Vitis vinifera*	Spain	MG547971	MH118930	MH118939	MH118948
N. parvum	CBS 139672	*Bruguiera gymnorrhiza*	South Africa	MT587446	MT592156	MT592646	MT592342
N. parvum	CBS 112931	*Vitis vinifera*	South Africa	AY343466	AY343358	MT592644	MT592340
N. parvum	**CCUB 3516**	***Persea Americana***	**Brazil**	**CCUB_3516**	**CCUB_3516**	**CCUB_3516**	**CCUB_3516**
N. parvum	**CCUB 4888**	***Persea Americana***	**Brazil**	**CCUB_4888**	**CCUB_4888**	**CCUB_4888**	**CCUB_4888**
N. parvum	**CCUB 3495**	***Persea Americana***	**Brazil**	**CCUB_3495**	**CCUB_3495**	**CCUB_3495**	**CCUB_3495**
N. parvum	**CCUB 4886**	***Persea Americana***	**Brazil**	**CCUB_4886**	**CCUB_4886**	**CCUB_4886**	**CCUB_4886**
N. parvum	**CCUB 4895**	***Persea Americana***	**Brazil**	**CCUB_4895**	**CCUB_4895**	**CCUB_4895**	**CCUB_4895**
N. podocarpi	CBS:131677*	*Podocarpus henkelii*	South Africa	MT587508	MT592223	MT592715	MT592412
N. podocarpi	CBS:131678	*Podocarpus henkelii*	South Africa	MT587509	MT592224	MT592716	MT592413
N. ribis	CMW 28315	*Terminalia catappa*	Cameroon	FJ900606	FJ900652	FJ900633	FJ900614
N. ribis	CMW 28363	*Terminalia catappa*	Cameroon	FJ900607	FJ900653	FJ900634	FJ900615
N. ribis	CBS 123645	*Syzigium cordatum*	South Africa	EU821904	EU821874	EU821844	EU821934
N. ribis	CBS 117915	*Eucalyptus urophylla*	Venezuela	MT587476	MT592189	MT592681	MT592379
N. ribis	CBS_121.26	*Ribes rubrum*	USA	AF241177	AY236879	AY236908	EU821960
N. ribis	CBS_115475*	*Ribes rubrum*	USA	AY236935	AY236877	AY236906	EU821958
N. ribis	CBS_123646	*Syzigium cordatum*	South Africa	EU821905	EU821875	EU821845	EU821935
N. ribis	**CCUB 3538**	***Persea Americana***	**Brazil**	**CCUB_3538**	**CCUB_3538**	**CCUB_3538**	**CCUB_3538**
N. ribis	**CCUB 3540**	***Persea Americana***	**Brazil**	**CCUB_3540**	**CCUB_3540**	**CCUB_3540**	**CCUB_3540**
N. ribis	**CCUB 4969**	***Persea Americana***	**Brazil**	**CCUB_4969**	**CCUB_4969**	**CCUB_4969**	**CCUB_4969**
N. ribis	**CCUB 4966**	***Persea Americana***	**Brazil**	**CCUB_4966**	**CCUB_4966**	**CCUB_4966**	**CCUB_4966**
N. sichuanense	SICAUCC22-0099*	*Juglans regia*	China	OP058990	OP066336	OP066363	OP066355
N. sichuanense	SICAUCC22-0093	*Juglans regia*	China	OP058984	OP066333	OP066357	OP066349

N. vaccinii	CGMCC 3.19152	Blueberries	China	MN444625	MN444638	MN444651	-
N. vaccinii	CGMCC 3.19155*	Blueberries	China	MN444628	MN444641	MN444654	-
N. vaccinii	CGMCC 3.19154	Blueberries	China	MN444627	MN444640	MN444653	-
N. yunnanense	CSF6142*	*Eucalyptus globulus*	China	MT028667	MT028833	MT028999	MT029112
N. yunnanense	CSF6169	*Eucalyptus globulus*	E. globulus	MT028665	MT028831	MT028997	MT029110

Table S4. Isolates used in the phylogenetic analyses of *Pseudofusicoccum* with their corresponding GenBank accession numbers. Strains reported here have been highlighted in bold. (* = type species).

Species	**Isolate**	**Origin**	**ITS**	**TEF**
Botryosphaeria dothidea	CMW8000	Switzerland	AY236949.1	AY236898.1
Pseudofusicoccum adansoniae	CBS 122.054*	Australia	EF585532	EF585570
P. adansoniae	WAC 13.299	Australia	GU172404	GU172436
P. ardesiacum	CBS 122.062*	Australia	EU144060	EU144075
P. ardesiacum	WAC 13.294	Australia	GU172405	GU172437
P. artocarpi	CPC 22.796*	Thailand	KM006452	KM006483
P. kimbereleyense	CBS 122.061*	Australia	EU144059	EU144074
P. kimbereleyense	WAC 13.293	Australia	GU172406	GU172438
P. olivaceum	CBS 124.939*	South Africa	FJ888459	FJ888437
P. olivaceum	CMW 22.639	South Africa	FJ888463	FJ888439
P. olivaceum	CBS 124.940	South Africa	FJ888462	FJ888438
P. stromaticum	CMW 13.434*	Venezuela	AY693974	AY693975
P. stromaticum	CMW 13.435	Venezuela	DQ436935	DQ436936
P. stromaticum	CMM 3961	Brazil	JX464103	JX464110
P. stromaticum	CMM 4541	Brazil	KT728918	KT728922
P. stromaticum	Fi2364	Uruguay	KT191029	MW110607
P. stromaticum	**CCUB 3475**	**Brazil**	**CCUB_3475**	**CCUB_3475**

P. stromaticum	**CCUB 3479**	**Brazil**	**CCUB_3479**	**CCUB_3479**
P. stromaticum	**CCUB 3483**	**Brazil**	**CCUB_3483**	**CCUB_3483**
P. stromaticum	**CCUB 3491**	**Brazil**	**CCUB_3491**	**CCUB_3491**
P. stromaticum	**CCUB 4964**	**Brazil**	**CCUB_4964**	**CCUB_4964**
P. stromaticum	**CCUB 3492**	**Brazil**	**CCUB_3492**	**CCUB_3492**
P. violaceum	CBS 124.936*	South Africa	FJ888474	FJ888442
P. violaceum	CBS 124.937	South Africa	FJ888458	FJ888440

CHAPTER 2

Four New Fungal Pathogens Causing Avocado Dieback in Brazil

Abstract

Dieback is a disease with a complex etiology that results in the death of branches or entire plants in avocado producing regions. Losses associated with this disease have already been reported in the main producing regions of Brazil. The etiological agents of this disease are different and an association between fungi is often observed. This study aimed to identify fungi associated with avocado dieback in Brazil. Fungal isolates were collected from symptomatic avocado stems from orchards in São Paulo state. Identification was based on phenotypic characteristics and multilocus phylogenetic analysis of the translation elongation factor 1-alpha, RNA polymerase II, β-tubulin, actin, the internal transcribed spacer (ITS) region of rDNA, and the large subunit ribosomal RNA regions, according to the previously identified genus. The pathogenicity test confirmed that all species were pathogenic to avocado seedlings and fruits. This is the first report of *Neopestalotiopsis arecacearum*, *Neocosmospora bostrycoides*, *Nectria pseudotrichia* and *Cytospora viridistroma* causing avocado dieback in Brazil and worldwide. Identifying emerging pathogens that cause losses is crucial for avocado-producing countries with different climatic conditions, as it helps understand the geographic distribution of these phytopathogens and the conditions that favor their development.

Keywords: Canker, Diagnosis, *Persea americana***,** Plant Disease Complex, Phylogeny

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1.Introduction

The avocado is an important fruit tree distributed mainly in the Midwest, Southeast, and South of Brazil. Most fruit production is commercialized for domestic consumption, while only a small portion is exported (Instituto Brasileiro de Geografia e Estatística, 2024; Sommaruga and Eldridge, 2021). Its nutritional value and unique characteristics make it highly attractive to the pharmaceutical industry, positioning it as the fastest-growing commodity among major tropical fruits (FAO, 2021; Flores et al., 2019).

Avocado trees can be impacted by various diseases both in the field and post-harvest, resulting in significant losses in production and commercialization (Piccinin and Pascholati, 2018; Fischer and Firmino, 2023). Dieback is a disease with a complex etiology that causes leaf yellowing, wilting, and necrosis, followed by the gradual death of branches and stems. It begins with the drying of the tips and can lead to the death of entire branches and trees (Hultberg et al., 2020; Valor et al., 2020). It can be caused by both biotic and abiotic factors, but primarily by the combination of both (Camarero et al., 2015). The colonization of the xylem by fungi limits the accumulation of nutrient reserves, which can hinder fruit development or even result in the death of the plant (Auger et al., 2013). In addition, infected fruits may develop mycelial growth or necrotic lesions, which reduces their shelf life or makes them unsuitable for commercialization (Valencia et al., 2019).

Taxonomic studies using molecular tools in several countries revealed different etiological agents (Fiorenza et al., 2023; Guarnaccia et al., 2018; Hernández et al., 2023; Lin et al., 2018; Piccin, 2018; Ploetz et al., 2017; Ramírez-Gil and Peterson, 2019; Trakunyingcharoen et al., 2015; Valencia et al., 2022). Despite the high diversity of fungal species, traditionally the diagnosis of diseases is based only on the symptoms observed in the field. In Brazil, *Colletotrichum*, *Lasiodiplodia*, *Nectria*, *Neofusicoccum*, *Phytophthora*, *Rosellinia*, and *Verticillium* have been reported to cause dieback in avocado (Piccinin and Pascholati, 2018; Fischer and Firmino, 2023). However, only *Colletotrichum* has been identified through phylogenetic analyses (Oliveira et al., 2024; Soares et al., 2021). Accurate identification of phytopathogens enables effective disease management. The monitoring of avocado diseases revealed plants exhibiting symptoms

of dieback. Therefore, this study aimed to identify fungal species associated with avocado dieback in Brazil and to assess their pathogenicity in avocado seedlings and fruits.

2. Materials and Methods

Sampling and isolation

The collections were carried out on branches of seven avocado trees in producing areas of Mogi Mirim - SP in 2020 (22° 25′ 55″S, 46° 57′ 28″O). Briefly, pieces of approximately 1 cm of symptomatic tissue were superficially sterilized with 70% alcohol for 1 minute and then in 1% hypochlorite for 2 minutes, followed by consecutive washes in distilled water three times. The symptomatic tissues were then transferred to Petri dishes containing Potato-Dextrose-Agar (PDA) medium. The plates were incubated at 25 ± 2 °C in the dark. Emerging hyphal tips from the infected tissues were transferred to new PDA plates, and the pure cultures of seven fungal isolates were stored in the Coleção de Culturas da Universidade de Brasília (CCUB).

Cultural and morphological characterization

Morphological analyses were performed using a single isolate from each identified species. The fungal colonies were grown on PDA for color evaluation. Agar-agar (AA), synthetic nutrient-poor agar (SNA; 1,000 mL H_2O, KH_2PO_4 1 g, KNO_3 1 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, glucose 0.2 g, sucrose 0.2 g, and agar 20 g), and avocado branches were added when necessary to induce sporulation. The colonies were incubated at 25°C with 12 hours of light and 12 hours of darkness. Fungal structures were mounted in 10% lactic acid for microscopic observation. Images were obtained with a Leica DFC 490 digital camera attached to a Leica DM 2500 light microscope (Leica Microsystems, Nussloch, Germany). Image capture and measurement were performed using Leica Qwin-Plus.

DNA extraction and sequencing

DNA extractions from all seven fungal isolates were performed using the Wizard Genomic DNA Purification Kit (Promega Corporation, WI, U.S.A) according to Pinho et al. (2013). Firstly, the region of the translational elongation factor (TEF1-α) was amplified for all isolates for fungal genus identification. Subsequently, for the multilocus analysis, the regions translation elongation factor 1-alpha (TEF1-α), RNA Polymerase II

(RPB2), β-tubulin (BTUB), actin (ACT), internal transcribed spacer of rDNA (ITS), and large subunit ribosomal ribonucleic acid (LSU) were sequenced according to the genus previously identified. The PCR conditions for each genomic region are detailed in Table 1. PCR products were purified and bidirectionally Sanger-sequenced.

Phylogenetic analysis

Previous study sequences were obtained from GenBank (Supplementary Table 1) and aligned using multiple sequence alignments performed with MAFFT version 7 (Katoh and Standley, 2013). Alignments were concatenated in Sequence Matrix 1.8 (Vaidya et al., 2011). Phylogenetic relationships were inferred using maximum likelihood (ML) in RaxML-HPC2 (Stamatakis, 2014). Bayesian inference (BI) was conducted using the best-fit models of nucleotide substitution selected according to the corrected Akaike information criterion (AIC). The matrices were submitted by the Monte Carlo method, using Markov Chain (MCMC) carried out in MrBayers 3.1.2 (Ronquist and Huelsenbeck, 2003) within the CIPRES platform (Miller et al., 2010) under analysis of 10 million generations with sampling every 1,000, with subsequent discarding of 20% of the first trees under analysis. The trees of *Neocosmospora*, *Neopestalotiopsis*, *Cytospora*, and *Nectria* were rooted with *Fusarium cicatricum*, *Pestalotiopsis theae*, *Cytospora rostrata*, and *Cosmospora coccinea*, respectively.

Pathogenicity Test

The test was conducted on 6-month-old Margarida avocado seedlings and green Hass fruits. The fruits were washed with 70% alcohol for 1 minute and 2% hypochlorite for 2 minutes, then rinsed with water three times. Six-millimeter wounds were made in the pulp of the fruits and in the stem of the seedlings for inoculation. The isolates were grown on PDA for 7 days, and 5 mm mycelium discs taken from the colony's edge were placed in the wounds and secured with damp cotton and Parafilm®. The negative control used only PDA. The fruits were kept in boxes in a humid chamber for two days. The plants were maintained in a greenhouse at a temperature of 30/18°C day/night with a photoperiod of 16/8 hours day/night. Pathogenicity was checked 5 and 40 days after inoculation for the fruits and seedlings, respectively. Symptoms of wilting or darkening of the vascular system in the seedlings and darkening of the fruit were assessed. Subsequently, the fungi were reisolated. The tests were independently repeated twice.

3. Results

Sampling and fungal isolation

Two plantations were evaluated in the main producing region of São Paulo. Symptoms observed included wilting, severe leaf drop, and dieback in avocado trees, as well as necrotic internal tissues. The collected samples resulted in seven isolates with typical characteristics of the Nectriaceae, Pestalotiopsidaceae, and Cytosporaceae families (Table 2). All isolates were selected for phylogenetic analyses and pathogenicity tests.

Phylogenetic analysis and morphological characterization

Based on the first approximation of taxonomic identity by BLASTn, using the TEF1-α sequence, the isolates were assigned to four genera: *Neocosmospora* (CCUB 3537 and 3542), *Neopestalotiopsis* (CCUB 3534 and 3545), *Nectria* (CCUB 3530) and *Cytospora* (CCUB 3490 and 3528).

The TEF and RPB2 genes were utilized for the identification of *Neocosmospora* species, resulting in matrices containing 43 taxa. The concatenated alignment included 1,381 sites, of which 1,284 were conserved and 97 were informative for parsimony. The results confirmed that the isolates grouped with *N. bostrycoides* were well-supported in both ML and BI multilocus analyses (Figure 1). The pure cultures of isolates CCUB 3542 and 3537 showed typical morphology of *Neocosmospora* sp. with yellow coloration and white aerial mycelium in both cultures. In PDA, microconidia formation was seen from the 5th day of cultivation with a size of 3.5−7.0 × 2.0−4.0 µm (5.28 ± 0.91 × 2.91 ± 0.4 µm, n=30). The production of macroconidia was only seen in synthetic nutrient-poor agar after 15 days in the dark, with a size of 25.5−34.5 × 2.5−4.0 µm (31.01 ± 2.06 × 3.09 ± 0.26 µm, n=30) with five to six septa (Figure 5A-C). Chlamydospores were not observed.

The combined matrix of the genes TEF1-α, BTUB, and ITS was used for the genus *Neopestalotiopsis*, consisting of 31 taxa, totaling 2,221 characters, of which 77 were informative for parsimony and 2,144 were conserved. After the analysis, it was confirmed that the isolates CCUB 3534 and 3545 grouped with *N. aracacearum* (Figure 2). Isolates CCUB 3534 and 3545 showed colonies with radial growth, with a cotton white color observed on the upper side and light yellow on the reverse side, confirming the morphology of *Neopestalotiopsis*. Cultures were covered with dark cells after 14 days of

incubation. Conidia were fusiform to ellipsoid, with CCUB 3534 exhibiting a length and width of 19.5−28.5 × 5.5−8.0 (24.20 ± 2.26 × 6.75 ± 0.26 μm, n=30), with 5 versicolor cells, hyaline apical and basal cells and three brown median cells. They have between two and four apical and one basal appendage (Figure 5D-F).

The ML analyses combined with BI of the individual TEF1-α partitions for the *Nectria* isolate included 31 ingroup taxa. The alignment resulted in 798 bp, with 212 parsimony-informative sites. The posterior probabilities of BI were plotted on the ML tree, indicating that CCUB 3530 belongs to *N. pseudotrichia* with high branch support (Figure 3). The *Nectria* isolate grew rapidly on PDA, and after 11 days, the entire plate was completely covered with a white and brown colony against a reddish background. No reproductive structure was observed (Figure 5L).

The genes ITS, ACT, LSU, RPB2, TEF1-α, and BTUB were used for the phylogenetic analysis of the *Cytospora* isolates, including 40 taxa, resulting in a concatenated alignment of 3,741 characters, of which 701 were conserved and 2,688 were informative for parsimony. In our analysis of individual trees using ML and BI (Supplementary material), the isolates CCUB 3490 and 3528 were grouped into a single clade containing isolates of *C. viridostroma* (Figure 4). The Isolates CCUB 3490 and 3528 showed characteristic morphology of *Cytospora* with growth on PDA in 7-10 days with white, flat color, uniform texture, and regular growth. Sexual reproduction structures were not visualized. The asexual structure was visualized in the avocado stem in PDA after 40 days of cultivation under fluorescent light. Pycnidia were dark brown with a size of 180.0−310.5 × 126.5−182.0 μm (246.80 ± 57.69 × 159.61 ± 26,29 μm, n=20). Unbranched hyaline conidiophores, aseptate hyaline conidia with CCUB 3490 sizes of 2.5−4.0 × 1−1.5 (3.48 ± 0.27 × 1.40 ± 0.17, n=50) (Figure 5G-K).

Pathogenicity Test

All the isolates were pathogenic to the avocado fruit and seedlings. The symptoms observed on the surface of the fruits were dark brown necrotic lesions around the inoculation sites. In the seedlings, there was darkening of the vascular tissue, indicating pathogen colonization. In the control, seedlings and fruits remained asymptomatic (Figure 6).

4. Discussion

In Brazil, dieback is commonly observed in commercial avocado plantations. However, causal agents have not yet been accurately identified through phylogenetic analysis. Considering that the symptoms result from the combined effects of plant stress and the attack of fungi, it is assumed that different genera and species can cause dieback (Hrycan et al., 2020; Jurskis, 2005). In this study, isolates of *Neocosmospora*, *Neopestalotiopsis*, *Cytospora* and *Nectria* were recovered from *P. americana* showing symptoms of dieback between 2020 and 2022 in Brazil. Pathogenicity tests, combined with morphological characteristics and phylogenetic analyses, confirm that *Cytospora viridistroma, Nectria pseudotrichia, Neocosmospora bostrycoides,* and *Neopestalotiopsis aracacearum* cause dieback and fruit rot in avocados. All species are reported for the first time on avocado in Brazil.

In this study, *Neocosmospora bostrycoides* cause disease in the stem and fruit of avocado. In Brazil, this fungus was only reported on yellow passion fruit plants (Ninos et al., 2021) but has been reported in other hosts including coffee and *Ficus carica* in Puerto Rico and Italy, respectively (Gusella et al., 2024; Serrato-Diaz et al., 2024). Although *Neocosmospora euwallaceae* have been reported on avocados in South Africa and *Neocosmospora perseae* in Italy and Greece (Guarnaccia et al., 2022, 2018; van den Berg et al., 2019), this is the first report of *Neocosmospora bostrycoides* causing avocado dieback worldwide.

Neopestalotiopsis siciliana and *N. rosae* have previously been reported to cause dieback on avocado in Italy and Turkey, respectively (Çalış et al., 2024; Fiorenza et al., 2022). Although *Neopestalotiopsis arecacearum* has been previously reported in Brazil on *Caryota mitis*, *Dypsis lutescens*, *D. madagascariensis*, and *Ptychosperma elegans* (Guterres et al., 2023), this is the first global report of this species causing dieback in avocado.

Nectria pseudotrichia has been reported to cause stem-end rot in avocado fruits in Kenya (Wanjiku et al., 2020). In Brazil, this species has been reported to cause canker in Japanese pear (*Pyrus pyrifolia*) and as an endophyte in brazilwood (*Caesalpinia echinata*) (Becker, 2003; Cota et al., 2018). In this study, we observed *N. pseudotrichia* causing branch dieback in avocados for the first time.

Cytospora is a genus that causes diseases in the branches and trunks of tree species, with symptoms appearing as sunken, discolored areas on the bark of the stem and subsequent plant death (Fan et al., 2020; Lawrence et al., 2018; Pan et al., 2021; Rumbos, 1988). *Cytospora viridistroma* causes disease in eucalyptus (Adams et al., 2004; Jiang et al., 2020), but this is the first report of it causing dieback in avocado worldwide.

All isolates showed symptoms on the stems of seedlings, indicating colonization of vascular tissues, a common characteristic of the genera studied in this work. These genera are widely known to cause canker and dieback (Jiang et al., 2018; Lawrence et al., 2018; Pan et al., 2020; Yang et al., 2018; Crespo et al., 2019). In fruits inoculated with *Cytospora* and *Neopestalotiopsis*, lesions remain restricted to the inoculation site. Although lesions on fruits caused by these fungi are uncommon, some reports confirm these findings (Ayoubi and Soleimani, 2015; Baggio et al., 2021; Mincuzzi et al., 2017; Venter et al., 2017).

Dieback is a complex symptom that can be associated with various causal agents, exhibiting similar symptomatology (Arjona-Girona et al., 2019; Hilário et al., 2020; Ismail et al., 2012; McDonald et al., 2009; Rodríguez-Gálvez et al., 2017). This makes the diagnosis and effective control of the disease particularly challenging. Accurate identification of the causal agents of avocado dieback in Brazil is essential for monitoring and providing recommendations for effective control methods for avocado growers (Fischer and Firmino, 2023).

The management of dieback is based on practices that prioritize protection and exclusion, aiming to delay damage caused by disease progression (Saeed et al., 2017; Tavares et al., 2005). Generally, it is recommended to remove and dispose of infected plant parts, eliminate crop residues, perform pruning, disinfect tools, and control damaged insects. Once established, the disease can be reduced through applications of triazole, strobilurin, phenylpyridinyloamine, and phenylpyrrole fungicides, as well as the biological control agent *Bacillus velezensis* (Bernal et al., 2025; Li et al., 2024; Tavares et al., 2005; Twizeyimana et al., 2025).

Identifying emerging pathogens that may cause losses is crucial for avocado-producing countries with different climatic conditions, as it helps understand the

geographic distribution of these phytopathogens and the conditions that favor their development.

5. Conclusion

To our knowledge, this is the first report of *Neopestalotiopsis arecacearum*, *Neocosmospora bostrycoides*, *Nectria pseudotrichia* and *Cytospora viridistroma* causing avocado dieback in Brazil and worldwide. Since dieback is exacerbated by nutritional deficiencies, drought stress, elevated temperatures, frost, and over-irrigation, these conditions should be avoided or mitigated. This should be combined with branch protection using fungicides and proper sanitation of pruning tools to prevent the proliferation of these fungal species.

CRediT authorship contribution statement

Thais França Silva: Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. João Lucas Pimentel Duarte: Writing – review & editing, Visualization, Investigation. Jefferson Bertin Vélez Olmedo: Writing – review & editing, Visualization, Investigation. Willie Anderson dos Santos Vieira: Writing – review & editing, Visualization, Software. Luiz Eduardo Bassay Blum: review & editing, Visualization, Investigation, Conceptualization. Danilo Batista Pinho: Writing – review & editing, Visualization, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Data availability

The datasets generated in this study are available in GenBank, and the results are included within the contents of this report.

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Table 1. List of primers that were used in this study.

Locus	Primer name	sequence (5'–3')	Orientation	(Anealing/ Extension)	Reference
Elongation factor 1-α (EF1-α)	EF1F1	TGCGGTGGTATCGACAAGCGT	Forward	56°-45''/72°-45''	(Jacobs et al., 2004)
	EF2R1	AGCATGTTGTCGCCGTTGAAG	Reverse	56°-45''/72°-45''	(Jacobs et al., 2004)
RNA polymerase II second largest subunit (RPB2)	5F2	GGGGWGAYCAGAAGAAGG	Forward	54°-45''/72°-45''	(Sung et al., 2007)
	7Cr	CCCATRGCTTGYTTRCCCAT	Reverse	54°-45''/72°-45''	(Liu et al., 1999)
β-tubulin (BTUB)	T1	AACATGCGTGAGATTGTAAGT	Forward	55°-45''/72°-45''	(O'Donnell and Cigelnik, 1997)
	Bt2-b	ACCCTCAGTGTAGTGACCCTTGGC	Reverse	55°-45''/72°-45''	(Glass and Donaldson, 1995)
28S nrRNA (LSU) e Internal transcribed spacer (ITS)	V9G	TTACGTCCCTGCCCTTTGTA	Forward	53°-45''/72°-45''	(Vilgalys and Hester, 1990)
	LR5	TCCTGAGGGAAACTTCG	Reverse	53°-45''/72°-45''	(De Hoog and Gerrits Van Den Ende, 1998)
Actin (ACT)	ACT-512F	ATGTGCAAGGCCGGTTTCGC	Forward	58°-45''/72°-25''	(Carbone and Kohn, 1999)
	ACT-783R	TACGAGTCCTTCTGGCCCAT	Reverse	58°-45''/72°-25''	(Carbone and Kohn, 1999)

Table 2. List of families of Brazilian avocado isolates included in this study

Family	Isolate code*	Local
Nectriaceae	CCUB 3537	Mogi Morim (SP) 22° 25′ 55″ S, 46° 57′ 30″ W
	CCUB 3542	Mogi Morim (SP) 22° 25′ 55″ S, 46° 57′ 30″ W
Pestalotiopsidaceae	CCUB 3530	Mogi Morim (SP) 22° 25′ 55″ S, 46° 57′ 30″ W
	CCUB 3534	Mogi Morim (SP) 22° 25′ 55″ S, 46° 57′ 30″ W
	CCUB 3545	Mogi Morim (SP) 22° 25′ 55″ S, 46° 57′ 30″ W
Cytosporaceae	CCUB 3528	Mogi Morim (SP) 22° 25′ 55″ S, 46° 57′ 30″ W
	CCUB 3490	Santo Antonio de Posse (SP) 22° 36′ 24″ S, 46° 55′ 9″ W

*Isolate code from the Coleção de Cultura da Universade de Brasília (CCUB) at the Universidade de Brasília (UnB), Brazil

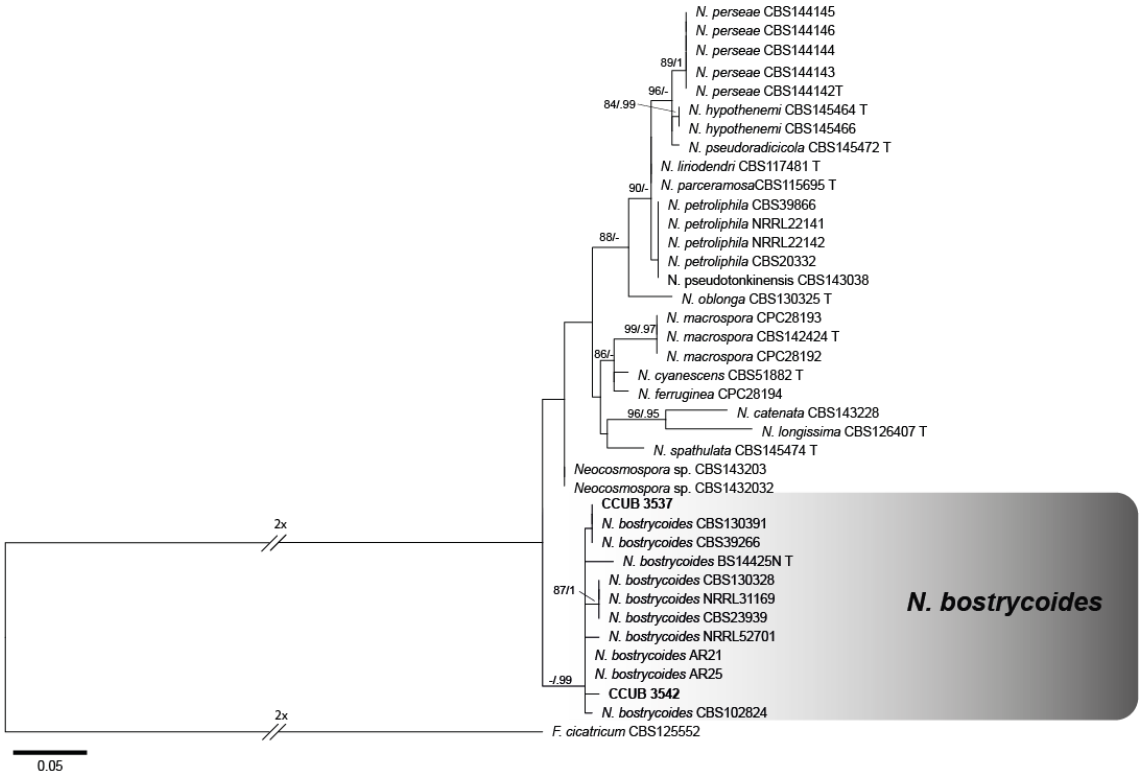

Figure 1. The Bayesian inference tree of the *Neocosmospora* from a concatenated alignment of TEF1-α and RPB2. Significant SH-aLRT bootstrap supports (≥ 80) and posterior Bayesian probability (≥ 95) are shown above the nodes. The tree was rooted with *F. cicatricum*. Ex-type isolates are indicated with "T" at the end of taxon labels. Isolates from the present study are highlighted in bold font. The scale bar indicates the average number of substitutions per site.

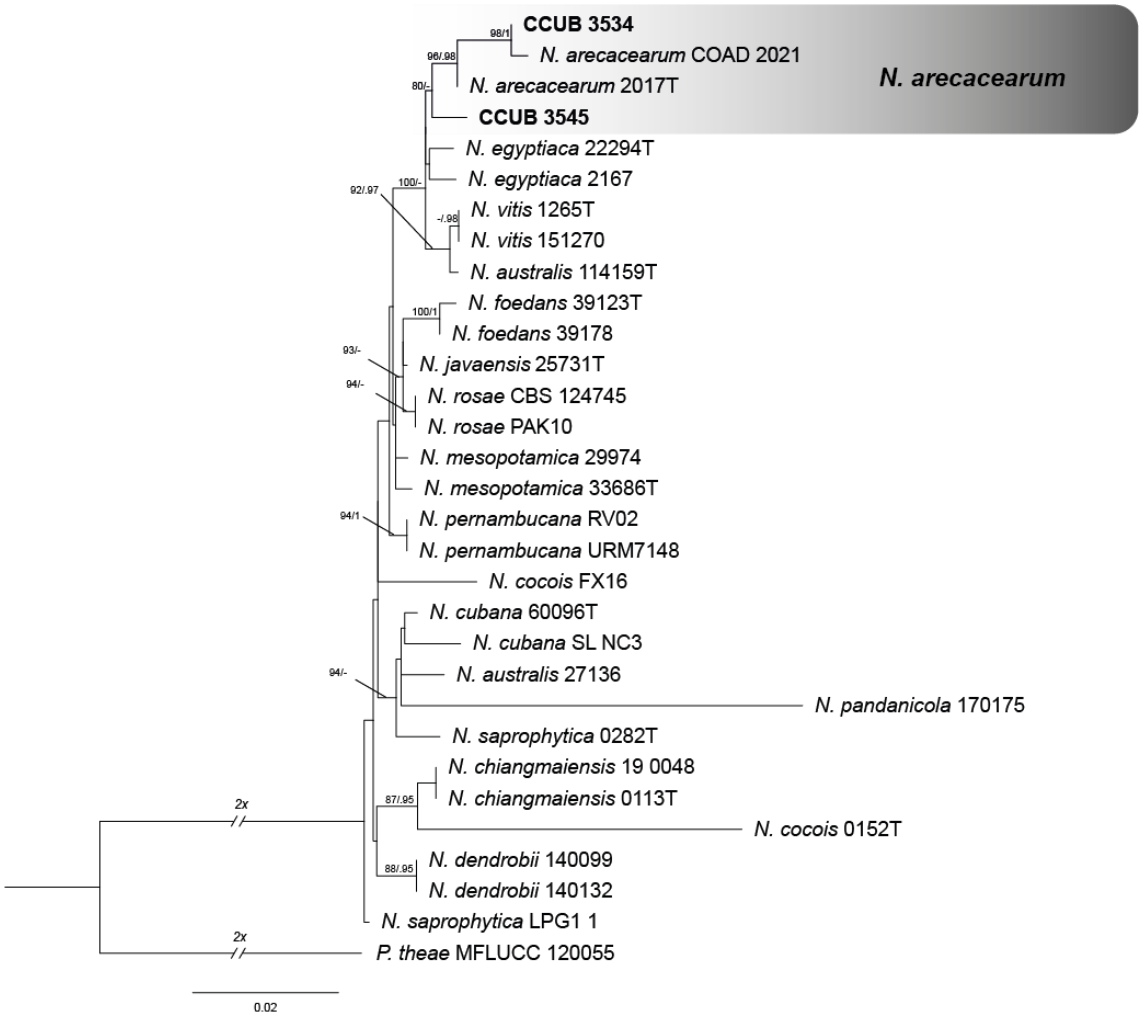

Figure 2. The Bayesian inference tree of the *Neopestalotiopsis* from a concatenated alignment of TEF1-α, BTUB and ITS. Significant SH-aLRT bootstrap supports (≥ 80) and posterior Bayesian probability (≥ 95) are shown above the nodes. The tree was rooted with *Pestalotiopsis theae*. Ex-type isolates are indicated with "T" at the end of taxon labels. Isolates from the present study are highlighted in bold font. The scale bar indicates the average number of substitutions per site.

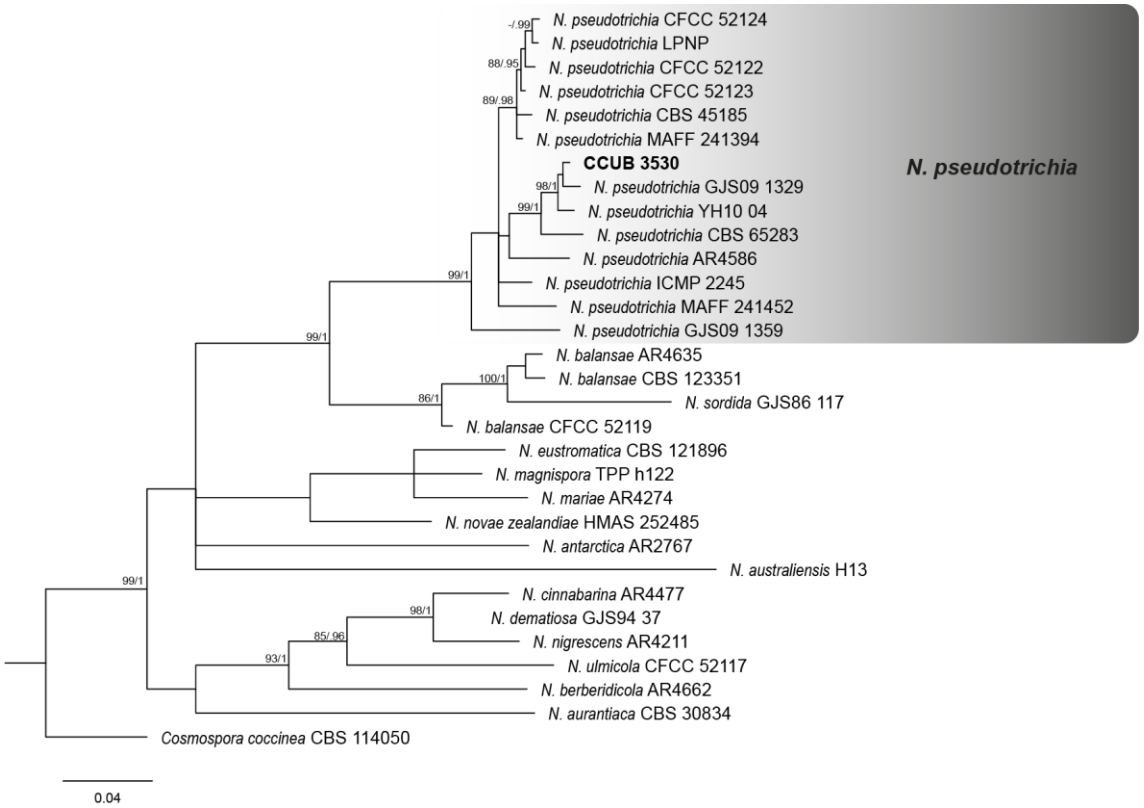

Figure 3. The Bayesian inference tree of the *Nectria* from a concatenated alignment of ITS and TEF1-α. Significant SH-aLRT bootstrap supports (≥ 80) and posterior Bayesian probability (≥ 95) are shown above the nodes. The tree was rooted with *Cosmospora coccinea.* Ex-type isolates are indicated with "T" at the end of taxon labels. Isolates from the present study are highlighted in bold font. The scale bar indicates the average number of substitutions per site.

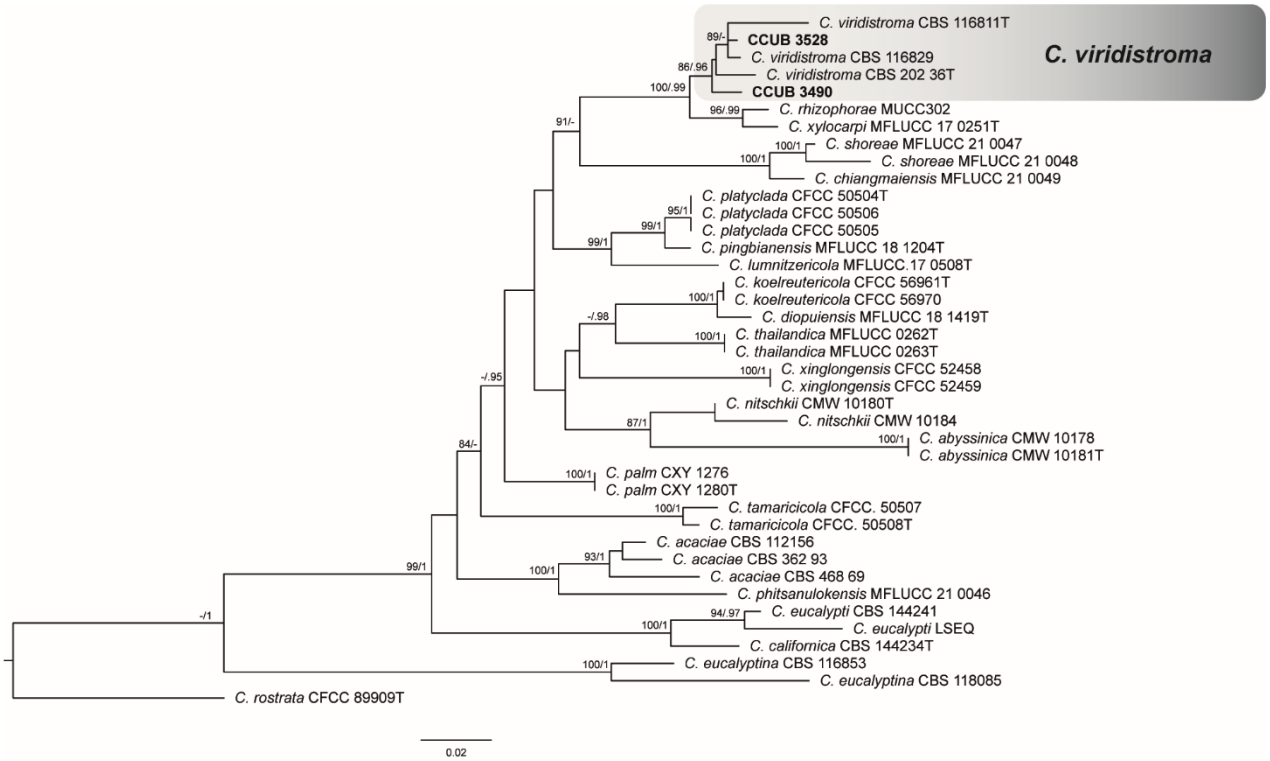

Figure 4. The Bayesian inference tree of the *Cytospora* from a concatenated alignment of ITS, ACT, LSU, RPB2, TEF1-α, and BTUB. Significant SH-aLRT bootstrap supports (≥ 80) and posterior Bayesian probability (≥ 95) are shown above the nodes. The tree was rooted with *C. rostrata*. Ex-type isolates are indicated with "T" at the end of taxon labels. Isolates from the present study are highlighted in bold font. The scale bar indicates the average number of substitutions per site.

Figure 5. Morphological characteristics of *Neocosmospora bostrycoides* (A-C), *Neopestalotiopsis aracacearum* (D-F), *Cytospora viridistroma*. (G-K) and *Nectria*

pseudotrichia (L). Front and back from culture of *N. bostrycoides* grown in PDA after 14 days at 25°C (A); macroconidium (B); microconidia (C); Front and back from culture of *N. aracacearum* grown in PDA after 12 days at 25°C (D); acervulus (E); conidia (F); Front from culture of *N. aracacearum* grown in PDA after 12 days at 25°C under near light (G); pycnidia (H); conidia (I); horizontal section of the stroma showing the conidioma (J); conidiogenic cells (K).

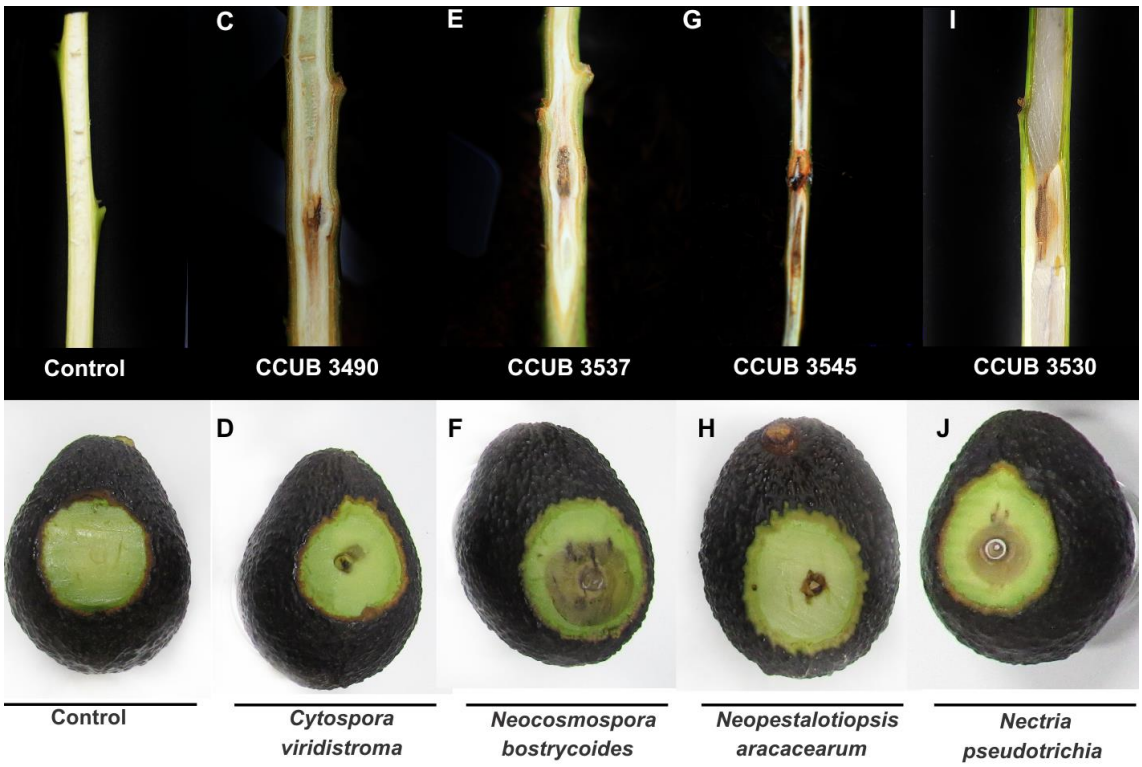

Figure 6. Stems of the "Margarida" variety and fruits of the "Hass" avocado inoculated with isolates of *Cytospora* (C and D), *Neocosmospora* (E and F), *Neopestalotiopsis* (G and H), *Nectria* (I and J) and control (A and B). Stems were inoculated using the mycelium disc method and evaluated after 40 days. Fruits were inoculated with mycelium disc directly into the pulp, with evaluation after 7 days.

Supplementary material

Table 1: Isolates used in the phylogenetic analyses of *Neocosmospora* with their corresponding GenBank accession numbers. Strains reported here have been highlighted in bold. (*T = type species).

Species	Strain	Host	Country	TEF1-α	RPB2
N. bostrycoides	**CCUB 3537**	***Persea americana***	**Brazil**	**PP187229**	**PP187218**
N. bostrycoides	**CCUB 3542**	***Persea americana***	**Brazil**	**PP187216**	**PP187217**
N. bostrycoides	CBS 239 39	*Atta sp. fungus garden*	Unknown	LR583595	LR583816
N. bostrycoides	CBS 102824	*Leaf litter*	Colombia	LR583596	LR583817
N. bostrycoides	BS 144 25N *T	Soil	Honduras	LR583597	LR583818
N. bostrycoides	CBS 392 66	*Bertholletia excelsa*	Unknown	LR583598	LR583819
N. bostrycoides	AR2.5	*Passiflora edulis*	Brazil	MW684268	MW684279
N. bostrycoides	AR2.1	*Passiflora edulis*	Brazil	MW684267	MW684278
N. bostrycoides	NRZ1427	*Homo sapiens*	Germany	MZ359482	NA
N. bostrycoides	NRRL 25101	*Homo sapiens*	Germany	JF740728	NA
N. catenata	CBS 143228	*Stegostoma fasciatum*	USA	KC808213	KC808354
N. cyanescens	CBS 518 82 *T	Human foot	Netherlands	LR583605	LR583826
N. ferruginea	CPC 28194	*Citrus sinensis*	Italy	LR583602	LT746341
N. ferruginea	CPC 28195	*Citrus sinensis*	Italy	LR583603	LT746342
N. hypothenemi	CBS 145464 *T	*Hypothenemus hampei*	Benin	JF740850	JF741176
N. hypothenemi	CBS 145466	*Hypothenemus hampei*	Uganda	JF740851	JF741177
N. liriodendri	CBS 117481 *T	*Liriodendron tulipifera*	USA	AF178340	EU329506
N. longissima	CBS 126407 *T	Tree bark	New Zealand	LR583621	LR583846
N. macrospora	CBS 142424 *T	*Citrus sinensis*	Italy	LT746218	LT746331
N. macrospora	CPC 28192	*Citrus sinensis*	Italy	LT746219	LT746332
N. macrospora	CPC 28193	*Citrus sinensis*	Italy	LT746220	LT746333
N. oblonga	CBS 130325 *T	Human eye	USA	LR583631	LR583853
N. parceramosa	CBS 115695 *T	Soil	South Africa	JX435149	JX435249
N. perseae	CBS 144142 *T	*Persea americana*	Italy	LT991902	LT991909
N. perseae	CBS 144143	*Persea americana*	Italy	LT991903	LT991910
N. perseae	CBS 144144	*Persea americana*	Italy	LT991904	LT991911
N. perseae	CBS 144145	*Persea americana*	Italy	LT991905	LT991912
N. perseae	CBS 144146	*Persea americana*	Italy	LT991906	LT991913
N. petroliphila	CBS 203 32	*Pelargonium sp.*	South Africa	DQ246835	LR583857
N. petroliphila	CBS 398 66	*Saccharum officinarum*	Brazil	LR583633	LR583859
N. petroliphila	NRRL 22141	*Cucurbita sp.*	New Zealand	AF178329	EU329491
N. petroliphila	NRRL 22142	*Cucurbita sp.*	USA	AF178347	FJ240379
N. pseudoradicicola	CBS 145472 *T	*Diseased cocoa pods*	New Guinea	JF740757	JF741084
N. pseudoradicicola	NRRL 25138	*Diseased cocoa pods*	New Guinea	JF740758	JF741085
N. pseudotonkinensis	CBS 143038	*Human cornea*	Netherlands	LR583640	LR583867
N. spathulata	CBS 145474 *T	Human synovial fluid	USA	DQ246882	EU329542
N. bostrycoides	CBS 130328	Human oral wound leukemia	USA	DQ246923	EU329564
N. bostrycoides	CBS 130391	*Homo sapiens*	EUA	HM347127	EU329665

N. bostrycoides	NRRL 52701	-	-	JF740784	JF741110
N. bostrycoides	NRRL 31169	Human oral wound leukemia	USA	DQ246923	EU329564
Neocosmospora	CBS 143203	Sea turtle	USA	DQ246937	EU329571
Neocosmospora	CBS 143203	Treefish	USA	DQ246945	EU329575
G. cicatricum	CBS 125552	Dead twig	Slovenia	HM626644	HQ728153

Table 2: Isolates used in the phylogenetic analyses of *Neopestalotiopsis* with their corresponding GenBank accession numbers. Strains reported here have been highlighted in bold. (*T = type species).

Species	**Isolate**	**Host**	**Country**	**ITS**	**TUB**	**TEF1-α**
N. arecacearum	COAD 2017 *T	*Dypsis madagascariensis*	Brazil	MH463406	MH460830	MH460838
N. arecacearum	COAD_2021	*Ptychosperma elegans*	Brazil	MH463409	MH460833	MH460841
N. arecacearum	**CCUB 3534**	***Persea americana***	**Brazil**	**PQ143172**	**PP187221**	**PP187219**
N. arecacearum	**CCUB 3545**	***Persea americana***	**Brazil**	**PQ143173**	**PP187222**	**PP187220**
N. australis	CPO 27.136	*Byrsonima crassifolia*	Mexico	NA	ON316846.1	ON316848.1
N. australis	CBS 114159 *T	*Telopea* sp.	Australia	KM199348	KM199432	KM199537
N. chiangmaiensis	MFLUCC 19–0048	*Magnolia liliifera*	China	MW248391	NA	MW259070
N. chiangmaiensis	MFLUCC 180113 *T	*Pandanus* sp.	Thailand	N/A	MH412725	MH388404
N. cocois	FX16	*Liquidambar*	China	NA	OK746277.1	OK746266.1
N. cocois	MFLUCC 150152 *T	*Cocos nucifera*	Thailand	KX789687	N/A	KX789689
N. cubana	SL-NC3	*Tomato*	China	NA	OK533395.1	OK533398.1
N. cubana	CBS 600.96 *T	*Leaf Litter*	Cuba	KM199347	KM199438	KM199521
N. dendrobii	MFLUCC 14-0099	*Dendrobium cariniferum*	Thailand	MK993570	MK975834	MK975828
N. dendrobii	MFLUCC 14–0132	*Dendrobium* sp.	Thailand	MK993572	NA	MK975830
N. egyptiaca	CBS H–22294 *T	*Mangifera indica*	Egypt	KP943747	KP943746	KP943748
N. egyptiaca	COAD 2167	*Psidium guajava*	Brazil	NA	MG692401.1	MG692403.1
N. foedans	CGMCC 3.9178	*Neodypsis decaryi*	China	JX398989	JX399024	JX399055
N. foedans	CGMCC 3.9123 *T	*Mangrove plant*	China	JX398987	JX399022	JX399053
N. javaensis	CBS 257.31 *T	*Cocos nucifera*	Java	KM199357	KM199437	KM199543
N. mesopotamica	CBS 299.74	*Eucalyptus* sp.	Turkey	KM199361	KM199435	KM199541
N. mesopotamica	CBS 336.86 *T	*Pinus brutia*	Iraq	KM199362	KM199441	KM199555
N. pandanicola	KUMCC 170175	*Pandanus* sp.	China	N/A	MH412720	MH388389
N. pernambucana	RV02	*Vismia guianensis*	Brazil	KJ792467	NA	KU306740
N. pernambucana	URM7148	*Vismia guianensis*	Brazil	KJ792466	N/A	KU306739
N. rosae	CBS_124745	*Paeonia suffruticosa*	USA	KM199360	KM199430	KM199524
N. rosae	PAK10	*Vaccinium corymbosum*	Peru	MK860755	MN000342	MN000339
N. saprophytica	MFLUCC 120282 *T	*Magnolia* sp.	China	JX398982	JX399017	JX399048
N. saprophytica	LPG1-1	Persimmon	China	NA	OM688188.1	OM688191.1
N. vitis	MFLUCC 15-1270	*Vitis vinifera*	China	KU140699	KU140690	KU140681
N. vitis	MFLUCC 151265 *T	*Vitis vinifera*	China	KU140694	KU140685	KU140676
P_theae	MFLUCC_120055	*Camellia sinensis*	Thailand	JQ683727	JQ683711	JQ683743

Table 3: Isolates used in the phylogenetic analyses of *Nectria* with their corresponding GenBank accession numbers. Strains reported here have been highlighted in bold. (*T = type species).

Species	Isolate	Host	Country	ITS	TEF1-α
N. antarctica	AR2767	*Mahonia aquifolium*	USA	HM484556	HM484516
N. aurantiaca	CBS 30834	*Ulmus* sp.	UK	JF832628	JF832519
N. australiensis	H13	-	China	GU075855	HM054076
N. balansae	CBS 123351	*Coronilla* sp.	France	HM484552	HM484525
N. balansae	CBS 129349	Twigs	China	JF832653	JF832522
N. balansae	CFCC 52119	*Hibiscus syriacus*	French Guiana	MG231956	MG232019
N. berberidicola	AR4662	*Berberis vulgaris*	France	JF832662	JF832538
N. cinnabarina	AR4477	*Aesculus* sp.	France	HM484548	HM484527
N. dematiosa	CBS 126570	Bark	USA	HM484557	HM484534
N. eustromatica	CBS 121896	*Hippocrepis emerus*	China	HM534896	HM534875
N. magnispora	CBS 129362	Twigs	Japan	JF832663	JF832539
N. mariae	AR4274	*Buxus sempervirens*	France	JF832629	JF832542
N. nigrescens	AR4211	Dicotyledonous tree	USA	HM484707	HM484672
N. novae-zealandiae	HMAS 252485	-	China	KM026503	KM026506
N. polythalama	CBS 128672	Twigs	New Zealand	JF832638	JF832523
N. pseudocinnabarina	CBS 129366	Dead wood	Venezuela	JF832642	JF832533
N. pseudotrichia	LPNP	*Camellia japonica*	China	OK562743	OL440127
N. pseudotrichia	CBS 551.84	-	Japan	HM484554	HM484532
N. pseudotrichia	MAFF 241394	Twigs	Japan	JF832639	JF832527
N. pseudotrichia	ICMP 2245	*Theobroma cacao*	Japan	JF832640	JF832532
N. pseudotrichia	MAFF 241452	Bark	New Guinea	JF832649	JF832531
N. pseudotrichia	CBS 65283	Bark	Japan	JF832648	JF832528
N. pseudotrichia	YH10 04	*Acer* sp.	Venezuela	JF832650	JF832529
N. pseudotrichia	GJS09 1329	-	USA	JF832647	JF832530
N. pseudotrichia	CFCC 52122	*Kadsura longipedunculata*	Venezuela	MG231953	MG232016
N. pseudotrichia	CFCC 52123	*Ulmus pumila*	China	MG231954	MG232017
N. pseudotrichia	CFCC 52124	*Juglans regia*	China	MG231955	MG232018
N. pseudotrichia	**CCUB 3530**	***Persea americana***	**Brasil**	**PQ142690**	**PP187230**
N. sordida	GJS86 117	Living woody vine	Croatia	HM484857	HM484848
N. ulmicola	CFCC 52117	*Ulmus davidiana*	China	MG231959	MG232022
Cosmospora coccinea	CBS 114050	-	China	FJ474072	AY489629

Table 4: Isolates used in the phylogenetic analyses of *Cytospora* with their corresponding GenBank accession numbers. Strains reported here have been highlighted in bold. (*T = type species).

Species	Isolate	Host	Country	ITS	LSU	ACT	RPB2	TEF1-α	TUB
C. brevispora	CBS 116829	*Eucalyptus grandis*	Venezuela	KY051803	KX965267	KX964709	KX965477	KX965073	KX964909
C. brevispora	CBS 116811 *T	*Eucalyptus grandis* × *tereticornis*	Congo	AF192315	NA	NA	NA	NA	NA
C. viridistroma	CBS 202.36 *T	Cercis canadensis Castigl.	USA	MN172408	MN172388	NA	NA	MN271853	NA
C. lumnitzericola	MFLUCC 17-0508 *T	*Lumnitzera racernosa*	Tailand	MG975778	MH253453	MH253457	MH253461	NA	NA
C. pingbianensis	MFLUCC 18-1204 *T	Undefined wood	Yunnan, China	MK912135	MK571763	MN685817	MN685826	NA	NA
C. platyclada	CFCC 50504 *T	*Platycladus orientalis*	Yunnan, China	MH933645	MH933679	MH933552	MH933610	MH933516	MH933581
C. platyclada	CFCC 50505	*Platycladus orientalis*	Yunnan, China	MH933646	MH933680	MH933553	MH933611	MH933517	MH933582
C. platyclada	CFCC 50506	*Platycladus orientalis*	Yunnan, China	MH933647	MH933681	MH933554	MH933612	MH933518	MH933583
C. eucalyptina	CBS 118085	*Eucalyptus globulus*	Columbia	KY051848	KX965315	N/A	N/A	N/A	KX964935
C. eucalyptina	CBS 116853	*Eucalyptus globulus*	Columbia	KY051822	KX965288	KX964727	N/A	N/A	KX964918
C. rhizophorae	MUCC302	*Eucalyptus grandis*	Australia	EU301057	NA	NA	NA	NA	NA
C. xylocarpi	MFLUCC 17-0251 *T	*Xylocarpus granatum*	Thailand	MG975775	MH253454	MH253458	MH253462	NA	NA
C. diopuiensis	MFLUCC 18-1419 *T	Undefined wood	Thailand	MK912137	MK571765	MN685819	NA	NA	NA
C. koelreutericola	CFCC 56961 *T	*Koelreuteria paniculata*	Beijing, China	ON376918	NA	ON390905	ON390908	ON390914	ON390923
C. koelreutericola	CFCC 56970	*Koelreuteria paniculata*	Beijing, China	ON376917	NA	ON390904	ON390907	ON390913	ON390922
C. thailandica	MFLUCC 17-0262 *T	*Xylocarpus moluccensis*	Thailand	MG975776	MH253455	MH253459	MH253463	NA	NA
C. thailandica	MFLUCC 17-0263 *T	*Xylocarpus moluccensis*	Thailand	MG975777	MH253456	MH253460	MH253464	NA	NA
C xinglongensis	CFCC 52458	*Castanea mollissima*	China	MK432622	MK429892	MK442946	MK578082	NA	NA
C xinglongensis	CFCC 52459	*Castanea mollissima*	China	MK432623	MK429893	MK442947	MK578083	NA	NA
C. nitschkii	CMW 10180 *T	*Eucalyptus globulus*	Ethiopia	AY347356	NA	NA	NA	NA	NA
C. nitschkii	CMW 10184	*Eucalyptus globulus*	Ethiopia	AY347355	NA	NA	NA	NA	NA

C. palm	CXY 1276	*Cotinus coggygria*	Beijing, China	JN402990	NA	NA	NA	KJ781296	NA
C. palm	CXY 1280 *T	*Cotinus coggygria*	Beijing, China	JN411939	NA	NA	NA	KJ781297	NA
C. acaciae	CBS 362.93	*Ceratonia siliqua*	Spain	KY051929	KX965386	KX964811	KX965547	KX965173	KX964983
C. acaciae	CBS 112156	*Abies alba*	Switzerland	KY051776	KX965241	KX964688	N/A	N/A	KX964888
C. acaciae	CBS 468.69	*Ceratonia siliqua*	Spain	DQ243804	NA	NA	NA	NA	NA
C. phitsanulokensis	MFLUCC 21-0046	Decaying leaves	Thailand	MZ356517	MZ356521	MZ451160	MZ451168	MZ451164	MZ451172
C. californica	CBS 144234 *T	*Juglans regia*	California, USA	MG971935	NA	MG972083	NA	MG971645	NA
C. eucalypti	LSEQ	*Sequoia sempervirens*	USA	AY347340	NA	NA	NA	NA	NA
C. eucalypti	CBS 144241	*Eucalyptus globulus*	California, USA	MG971907	NA	MG972056	NA	MG971617	MG971772
C. chiangmaiensis	MFLUCC 21-0049	Decaying leaves	Thailand	MZ356514	MZ356518	MZ451157	MZ451165	MZ451161	MZ451169
C. shoreae	MFLUCC 21-0047	Decaying leaves	Thailand	MZ356515	MZ356519	MZ451158	MZ451166	MZ451162	MZ451170
C. shoreae	MFLUCC 21-0048	Decaying leaves	Thailand	MZ356516	MZ356520	MZ451159	MZ451167	MZ451163	MZ451171
C. tamaricicola	CFCC 50507*	*Rosa multifolora*	Yunnan, China	MH933651	MH933686	MH933559	MH933616	MH933525	MH933587
C. tamaricicola	CFCC 50508 *T	*Tamarix chinensis*	Yunnan, China	MH933652	MH933687	MH933560	MH933617	MH933523	MH933588
C. abyssinica	CMW 10181 *T	*Eucalyptus globulus*	Ethiopia	AY347353	NA	NA	NA	NA	NA
C. abyssinica	CMW 10178	*Eucalyptus globulus*	Ethiopia	AY347354	NA	NA	NA	NA	NA
Cytospora	**CCUB 3528**	**Persea americana**	**Brazil**	**PQ144547**	**CCUB_4629**	**PP187228**	**PP187226**	**PP187224**	**CCUB_4629**
Cytospora	**CCUB 3490**	**Persea americana**	**Brazil**	**PQ144548**	**CCUB_4599**	**PP187227**	**PP187225**	**PP187223**	**CCUB_4599**
C. rostrata	CFCC 89909 *T	Salix cupularis	Gansu, China	KR045643	KR045722	KU711009	KU710974	KU710932	KR045684

CHAPTER 3

Sensitivity of Botryosphaeriaceous Fungi Associated with Avocado Dieback in Brazil to Azoxystrobin and Thiophanate-Methyl

Abstract

Fungi of the Botryosphaeriaceae family are important pathogens in avocado crops, causing dieback symptoms and significantly impacting production in Brazil. To develop effective management strategies, it is essential to evaluate the sensitivity of these fungi to fungicides. In this study, 111 isolates of the *Lasiodiplodia* and *Neofusicoccum*, collected from symptomatic avocado orchards, were tested for sensitivity to thiophanate-methyl and azoxystrobin. *L. pseudotheobromae* and *N. parvum* were used for in vivo tests, molecular analyses, and fitness assessment. All *Neofusicoccum* isolates were sensitive to thiophanate-methyl and nine isolates of *L. pseudotheobromae*, from orchards in Minas Gerais and São Paulo, were resistant. Molecular analyses identified mutations in the codons E198A, E198K, and F200Y in the β-tubulin gene, never before described for *L. pseudotheobromae*. Furthermore, it is the first report of the E198A and F200Y mutations for the genus *Lasiodiplodia*. Most isolates, including all *Lasiodiplodia* isolates, *Neofusicoccum parvum*, and *N. ribs* isolates, were resistant to azoxystrobin. Analysis of the *Cytb* region revealed no differences between resistant and sensitive isolates. In vivo tests, thiophanate-methyl significantly reduced symptoms in fruits inoculated with susceptible isolates, while resistant isolates did not differ statistically from the control. Resistant and sensitive isolates had reduced lesion sizes when fruits were treated with azoxystrobin. Fitness tests indicated no significant differences in growth, osmotic sensitivity, or virulence between sensitive and resistant isolates to either fungicide. The discovery of *Lasiodiplodia* and *Neofusicoccum* isolates resistant to thiophanate-methyl and azoxystrobin in different producing regions and the fact that the resistant isolates have sufficient fitness to compete with sensitive pathogens in the field highlight significant obstacles to managing fungicide resistance in avocado crops.

Keywords: Fungicide resistance, MBC fungicide, Strobilurins, β-tubulin

1.Introduction

The growing demand for avocado has led to significant market growth and increased production due to their nutritional benefits (Daiuto et al., 2013). In the last 12 years, Brazil has seen a production increase of more than 160% (Daiuto Silva & Ledesma, 2014; IBGE, 2021). Biotic diseases are among the main factors that compromise avocado yield (Fischer & Firmino, 2023). Dieback disease, caused by Botryosphaeriaceous fungi (such as *Lasiodiplodia* spp. and *Neofusicoccum* spp.), is one of the most significant fungal diseases affecting the crop (Arjona-Girona et al., 2019; Trakunyingcharoen et al., 2015; McDonald & Eskalen, 2011).

Dieback is a disease caused by a fungal infection that primarily occurs through natural openings or wounds in the plant (Navarro et al. 2021). After infection, the fungus can remain quiescent for long periods, but the plant becomes more susceptible to colonization under stressful conditions (Chethana et al., 2016). Xylem colonization by the fungus disrupts water transport and results in drying and death of branches (Twizeyimana et al., 2013). In more severe cases, it can cause the complete death of the plant (Fischer & Firmino, 2023). Fruit infection can lead to premature fall or browning, making them unsuitable for sale (Dann et al., 2013; Piccinin & Pascholati, 2018).

Dieback management is mainly based on practices that follow the principles of protection and eradication (Saeed et al., 2017). Management recommendations generally include removal of affected plant parts, disposal of crop residues, pruning, disinfection of used equipment, control of harmful insects, biological control, and chemical control (Epstein et al., 2008; Kamil et al., 2018; Syed et al., 2014). No products specifically registered for dieback in avocado in Brazil (MAPA, 2025). However, fungicides such as thiophanate-methyl (a benzimidazole carbamate, MBC) and azoxystrobin (a quinone external inhibitor, QoIs) are commonly used for anthracnose (*Colletotrichum* sp.) and Cercospora spot (*Pseudocercospora* sp.). Continued use of these fungicides may contribute to developing resistance in other fungal populations, such as those causing dieback.

QoI fungicides inhibit mitochondrial respiration by binding to the ubiquinol oxidation center (Qo) of the cytochrome bc1 enzyme complex (Sierotzki, 2015). The first representatives of this class were strobilurins, which quickly conquered the market and became one of the most important and widely used agricultural fungicides (Fernández-Ortuño et al., 2010). However, the highly specific mode of action of these substances

resulted in a hereditary reduction in the sensitivity of fungi (Fernández-Ortuño et al., 2008). Reduced sensitivity in *Lasiodiplodia* has been frequently observed, including in Brazil, with effective concentrations (EC) higher than 100 ug/mL in papaya (Dong et al., 2022; He et al., 2021; Chen et al., 2020). Regarding *Neofusicoccum*, few studies have investigated this resistance, but the available results indicate ECs below 2 ug/mL (Antony et al., 2024; Latorre et al., 2013). The main mechanism of resistance to QoIs is associated with point mutations in the *Cytb* gene, which prevent the binding of fungicides to the target protein. The G143A and F129L mutations are the best-known and most frequently associated with this phenomenon (Dorigan et al., 2023; Sun et al., 2024).

Fungicides in the MBC group act to inhibit microtubule formation during cell division (Bai et al., 2024). This compound has been available on the market for many years and has been extensively researched in the literature for its effectiveness in controlling various plant pathogens (Bai et al., 2024; Quaranta, 2012). MBC is utilized in multiple crops, including fruits, vegetables, and grains (Bubici et al., 2019; Soliman et al., 2017; G. Zhang et al., 2021). The continuous use of these fungicides can lead to the rapid development of resistance in certain fungal populations in a short period (Chen et al., 2013; Thind, 2022). Resistance is often associated with mutations at several positions, with the most common being E198A/G/K and F200Y in the β-tubulin gene (FRAC, 2022; Ma & Michailides, 2005; Shao et al., 2021). In Brazil, sensitivity studies on *Lasiodiplodia* and *Neofusicoccum* isolates from mango and papaya have already identified resistant strains to MBC fungicide (Cavalcante et al., 2014; da Silva Pereira et al., 2012; dos Santos et al., 2019).

Considering the potential of avocado cultivation in Brazil and the limited information on the sensitivity of dieback fungi to fungicides, this study aimed to: characterize the sensitivity of *Lasiodiplodia* and *Neofusicoccum* isolates to thiophanate-methyl and azoxystrobin in vitro and in vivo, investigate the molecular basis associated with resistance mutations, and to compare the fitness of the sensitive and resistant isolates.

2.Materials and methods

Fungal isolates

A total of 111 isolates including the species *L. brasiliensis* (3), *L. euphorbiaceicola* (9), *L. macrospora* (4), *L. parva* (4), *L. pseudotheobromae* (26), *L. theobromae* (9), *N. kwambonambiense* (3), *N. parvum* (44) and *N. ribis* (9) were obtained from the Coleção de Cultura da Universidade de Brasília (CCUB) at the Universidade de Brasília (UnB), Brasília, Brazil (**Table S1**). The isolates originated from avocado fruits and stems collected in four Brazilian states, Distrito Federal, Minas Gerais, São Paulo and Santa Catarina, and were previously identified by phylogenetic inference.

In vitro azoxystrobin and thiophanate-methyl sensitivity assay

Sensitivity to azoxystrobin (Amistar, 500 g/kg, Syngenta Crop Protection, São Paulo, Brazil) was determined by growth rate inhibition assay on fungicide-amended medium. The fungicide was dissolved in DMSO and added to PDA medium at final concentrations of 0.01, 0.1, 1, 10 and 100 ug/mL. Less sensitive isolates were subjected to a second series of sensitivity tests with new concentrations of 10, 50, 150, 250, 350, and 500 ug/mL. SHAM was added to the medium at a final concentration of 100 ug/mL to suppress the alternative oxidase pathway. Similarly, thiophanate-methyl (Cercobin 850 g/kg, Iharabras, São Paulo, Brazil) was diluted in sterile distilled water and added to PDA medium at final concentrations of 0.25, 0.5, 0.75, 1.25, 1.75, 2.25, and 2.75 ug/mL for sensitivity assessment. The less sensitive isolates were tested with new concentrations of 10, 25, 50, and 100 ug/mL.

Mycelial plugs (5 mm diameter) were taken from the margins of 5-day-old colonies and transferred to the center of PDA plates amended with the fungicides. Petri dishes containing mediums without fungicides were used as negative control. Each isolate had three replicates, each plate being one replicate and incubated at 25°C in the dark. The experiment was repeated twice in time. Orthogonal diameters were measured of colonies grown in 24h for *Lasiodiplodia* isolates and 48h for *Neofusicoccum* isolates. The percentage of mycelial growth inhibition compared with the control was calculated for all fungicide concentrations and the fungicide concentration. The EC_{50} was calculated through linear regressions of mycelial growth inhibition versus the log10 transformation for each fungicide concentration.

Isolates were divided into three groups based on sensitivity levels: EC_{50} values <10 ug/mL were classified as sensitive (S), EC_{50} values between 10 and 100 ug/mL were classified as resistant (R), and EC_{50} values >100 ug/mL were classified as highly resistant (HR) (Yang et al., 2021;Yin et al., 2015).

In vivo thiophanate-methyl and azoxystrobin sensitivity assay

Eight isolates, including 2 azoxystrobin-sensitive (CCUB 5307 and CCUB 5312, Azx-S), 2 resistant (CCUB 3549 and CCUB 4906, Azx-R), 2 thiophanate-methyl-sensitive (CCUB 3522, CCUB 3532, Thm-S) and 2 resistant (CCUB 3514 and CCUB 3526, Thm-R) were used to evaluate the control efficacy in fruits treated with a commercial formulation of azoxystrobin (80 ug/mL) and thiophanate-methyl (600 ug/mL).

Commercially immature avocado fruits (cv. Breda) were washed in running water and surface disinfected in 1% sodium hypochlorite (NaOCl) for 3 minutes, then rinsed in sterile distilled water. Next, 6 mm wounds were made in the center of each fruit. The injured fruits were completely immersed in the fungicide solution for 10 seconds. The negative control was represented by fruits treated with distilled water. The fruits were inoculated with a 5 mm mycelium disk placed over the wound, then kept in a humid chamber inside boxes lined with moistened filter paper for 5 days at 25°C. Three replicates were conducted, with one fruit used for each replicate. Disease severity was assessed by measuring the mean radial diameter of the lesions.

Amplification and sequencing analysis of fungicide-target genes

To investigate the genetic variability associated with different levels of sensitivity to azoxystrobin, the Cytochrome B (Cytb) gene was amplified using the primers CytbF130 (ACAGGTGTAACATTAGCAATG) and CytbR810 (TCACTCAGGTACAATAGCAG) with an annealing temperature of 53ºC for 45s and extension of 72°C for 50s (Chen et al., 2020) to generate amplicons of approximately 700 pb length. The *Cytb* sequence of *L. theobromae* (GenBank accession number MH880818) was included in the analysis as a reference for the assignment of codon positions and detection of point mutations.

Partial sequences of the β-tubulin gene (TUB2) were analyzed to investigate the presence of genetic variability correlated with different levels of sensitivity to thiophanate-methyl. The primers used were Bt2-a (AACATGCGTGAGATTGTAAGT)

and Tub_R1 (TTGGGGTCGAACATCTGCTG) with an annealing temperature of 55°C for 45s and extension of 72°C for 60s to generate amplicons of approximately 750 pb length (Chen et al., 2020; Glass & Donaldson, 1995). The TUB2 sequence of a wild-type *L. pseudotheobromae* isolate (GenBank accession number EU673111) was included in the analysis.

PCR products were verified on a 1% agarose gel under UV light. The PCR products were subsequently purified using ExoSAP-IT™ reagent according to the manufacturer's instructions and sequenced by the sanger method. The sequences were assembled, aligned and analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (MEGA, Pennsylvania, USA). The sequences obtained in this study were deposited in GenBank (**Table S2**).

Analysis of fitness components

Three fitness components were determined: mycelial growth rate, osmotic sensitivity and virulence. The eight isolates tested in the *in vivo* test were used. For all assays 5 mm diameter mycelial disk from the edge of a 5-day cultured isolate to the center of a Petri dish containing potato dextrose agar (PDA) without fungicide was applied. Each isolate had three replicates, and the experiment was conducted twice.

The mycelial growth rate was evaluated at 5, 10, 15, 20, 25, 30, and 35 °C. The plates were then incubated in the dark for 24 hours. The colony diameter was measured in two perpendicular directions, and the mean mycelial growth rate (MRG) was calculated in mm/h.

The osmotic sensitivity test was conducted by examining mycelial growth in PDA supplemented with varying concentrations of NaCl: 0% (control), 1%, 2%, 4%, 6%, and 8% (w/v). Plates and incubated in the dark at 25°C and after 24 hours of incubation, perpendicular measurements were taken. The NaCl concentration that inhibited fungal development by 50% ($EC_{50}N$) was determined for each individual across all NaCl concentrations.

Virulence was assessed by evaluating the virulence of untreated shoots for each isolate using the in vivo thiophanate-methyl and azoxystrobin sensitivity assay. Untreated fruits and shoots inoculated with PDA without fungal growth were used as negative controls.

Data analysis

Analysis of variance (ANOVA) was used to evaluate the variance of EC50 values for thiophanate-methyl and azoxystrobin from two experiments. ANOVAs were performed individually for each fitness component. Data underwent normality testing using Shapiro-Wilk test (P=0.05). Differences between different groups of fungicidal reaction phenotypes were compared by Student's t-test (P = 0.05).

3.Results

In vitro azoxystrobin and thiophanate-methyl sensitivity assay.

The EC_{50} values for azoxystrobin of 111 isolates ranged from 0.02 to >500 ug/mL, with 60% of all isolates being classified as sensitive to azoxystrobin (Figure 1). *Neofusicoccum* isolates demonstrated EC_{50} values ranging from 0.03 to 130.1 ug/mL. Among the 56 *Neofusicoccum* isolates, 9 presented EC_{50} values greater than 10 ug/mL. All *Lasiodiplodia* isolates presented EC_{50} values greater than 10 ug/mL, ranging from 11.7 to >500 ug/mL.

All *Neofusicoccum* isolates demonstrated sensitivity to thiophanate-methyl, with EC_{50} ranging from 0.37 to 2.93 µg/mL. For *Lasiodiplodia*, the EC_{50} ranged from 0.44 to >100 µg/mL. The sensitive isolates presented EC_{50} values between 0.44 and 2.03 µg/mL, while the nine resistant isolates presented EC_{50} >100 µg/mL. All resistant isolates belonged to the species *L. pseudotheobromae* and were collected in the states of Minas Gerais and São Paulo.

In vivo and fitness tests were conducted with the prevalent species*, Lasiodiplodia pseudotheobromae* and *Neofusicoccum parvum*. *N. parvum* exhibited phenotypic variation only for azoxystrobin, with 37 sensitive isolates (Azx-S, EC_{50} < 10 ug/mL), 6 resistant isolates (Azx-R, 10 ug/mL < EC_{50} < 100 ug/mL) and 1 highly resistant isolate (Azx-HR, EC_{50} > 100 ug/mL). *L. pseudotheobromae* showed phenotypic variation only for thiophanate-methyl, with 17 sensitive isolates (Thm-S, EC_{50} < 10 ug/mL) and 9 highly resistant isolates (Thm-HR, EC_{50} > 100 ug/mL).

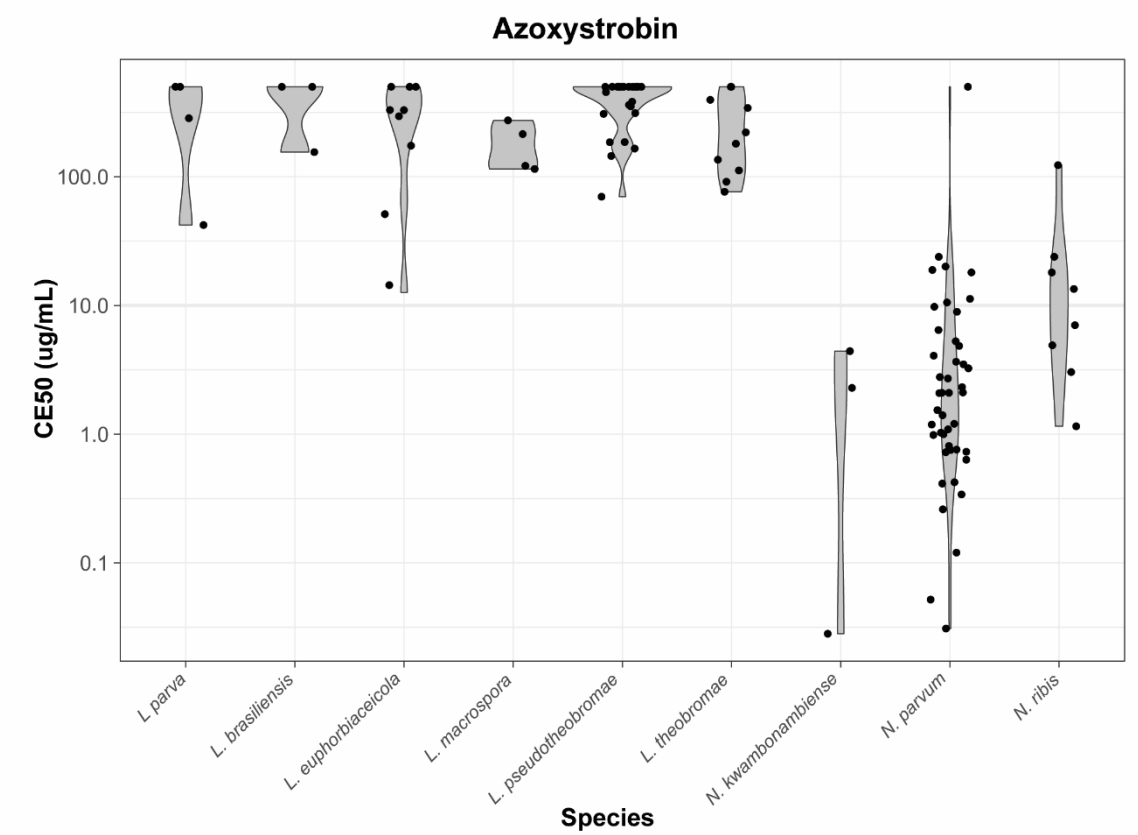

Figure 1: Distribution of effective fungicide con$_{ce}$ntrations to inhibit 50% of the mycelial growth (EC_{50}) of different *Neofusicoccum* and *Lasiodiplodia* isolates to azoxystrobin.

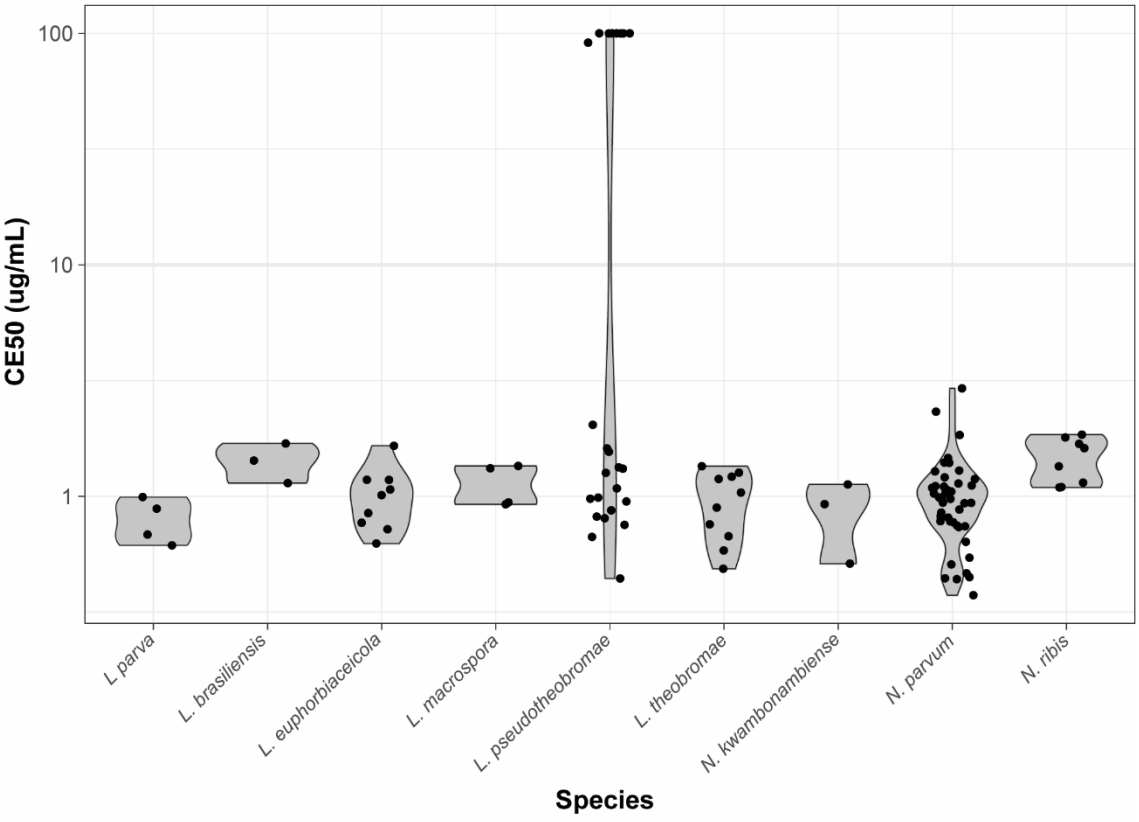

Figure 2. Distribution of effective fungicide concentrations to inhibit 50% of the mycelial growth (EC_{50}) of different *Neofusicoccum* and *Lasiodiplodia* isolates to thiophanate-methyl

In vivo azoxystrobin and thiophanate-methyl sensitivity assay

No symptoms were observed in treated fruit inoculated with azoxystrobin-sensitive isolates. Resistant isolates caused rot symptoms in treated fruit. Disease severity was significantly lower in treated fruit than in untreated fruit (P = 0.05) (**Figure 3**).

Thiophanate-methyl-sensitive *L. pseudotheobromae* isolates caused symptoms in treated and untreated fruits, however, fungicide application significantly reduced disease severity. Resistant isolates exhibited similar lesion diameters regardless of fungicide treatment (p = 0.05) (**Figure 3**).

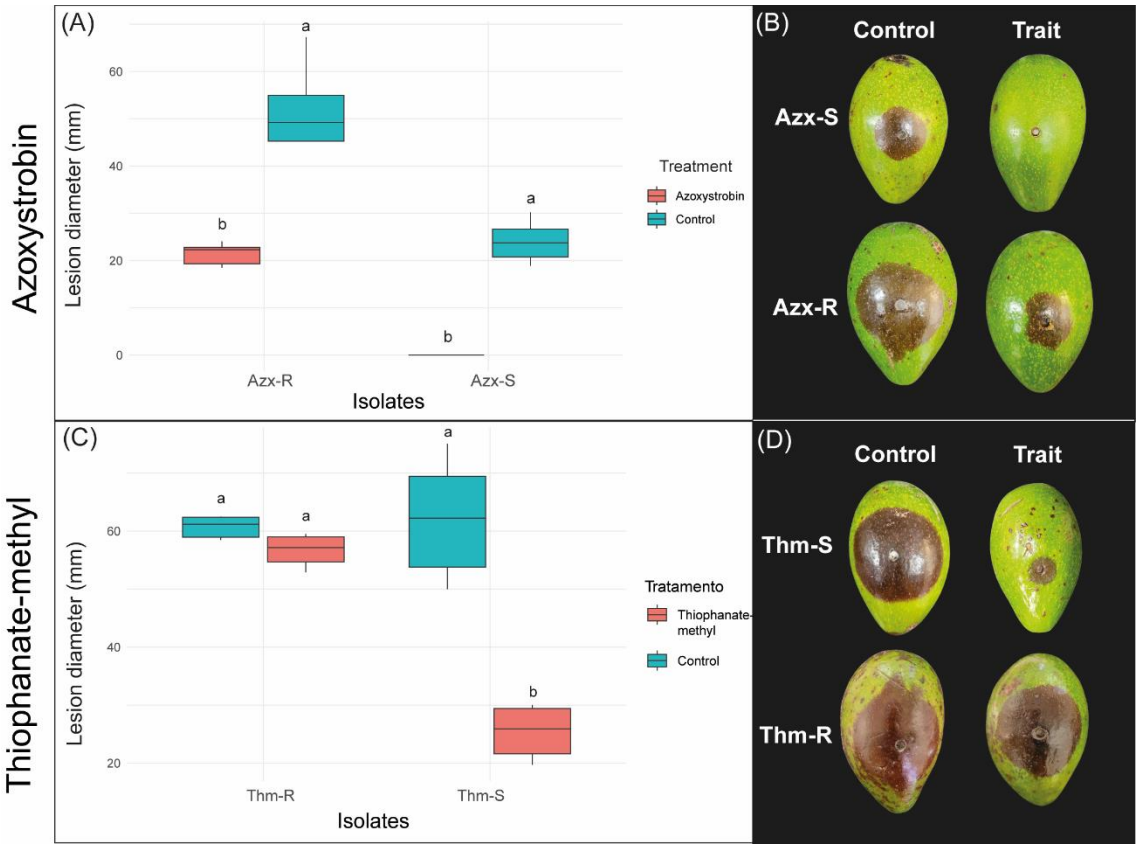

Figure 3: Efficiency of azoxystrobin and thiophanate-methyl in the control of *Lasiodiplodia pseudotheobromae* and *Neofusicoccum parvum*, respectively. Fruits treated with azoxystrobin and lesion size in fruits inoculated with resistant and sensitive isolates of *L. pseudotheobromae* (a-b). Fruits treated with thiophanate-methyl and lesion size in fruits inoculated with resistant and sensitive isolates of *N. parvum* (c-d). Different

letters within each isolate indicate statistically significant differences according to the student's t-test (p = 0.05) (b-d).

Nucleotide sequence analysis of the Cytb and β-tubulin gene

Gene-specific *Cytb* primer produced an amplificon with 561 bp in length, encoding 163 amino acids in *N. parvum*. The sequences of azoxystrobin-resistant and -sensitive isolates were identical to *L. theobromae*, with no mutation points correlated with the different phenotypes. (**Figure 4**).

Gene-specific TUB2 primer produced an amplificon with 658 bp in length, encoding 201 amino acids in *L. pseudotheobromae.* Nonsynonymous mutations in the TUB2 sequences of *L. pseudotheobromae* isolates were correlated with variation in thiophanate-methyl resistance. Sensitive isolates were identical in sequence in the TUB2 coding region to the wild-type *L. pseudotheobromae* sequence. Resistant isolates presented three-point mutations in our sequencing: a guanine (G) to adenine (A) transversion at position 409, adenine (A) to cytosine (c) transversion at position 410, and thymine (T) to adenine (A) transversion at position 416. These positions correspond to codons 198 and 200, resulting in amino acid changes from glutamic acid to lysine (E198K) or alanine (E198A) and from phenylalanine to tyrosine (F200Y), respectively. The resistant isolates presented only one of the three-point mutations mentioned (**Figure 5**).

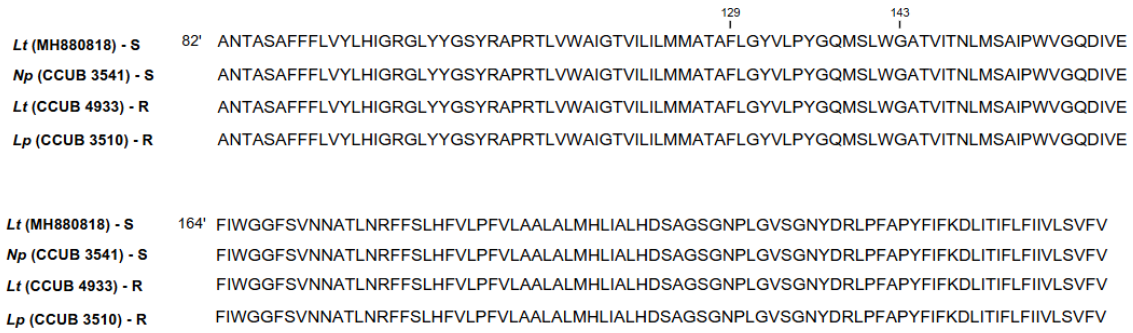

Figure 4. Amino acid alignment of the partial cytochrome b peptide sequence from *N. parvum* (*Np*), *L. theobromae* (*Lt*) and *L. pseudotheobromae* (*Lp*) with the characterized *L. theobromae* MH880818 sequence. The numbering in the upper left part of the alignment refers to the amino acid sequence.

Figure 5: Partial nucleotide and amino acid sequences of the β-tubulin gene (TUB2) from thiophanate-methyl sensitive and resistant isolates of *Lasiodiplodia pseudotheobromae* from Brazil. The intron sequence is depicted in a solid line box with an arrow showing the insertion site. The β-tubulin sequence of MBC-sensitive *L. pseudotheobromae* (accession number: EU673111) was used for comparison. The nucleotides and amino acids in red correspond to the mutations and corresponding amino acid changes.

Analysis of fitness components

No significant statistical differences were observed between the azoxystrobin- and thiophanate-methyl-sensitive and resistant isolates regarding osmotic sensitivity and virulence (**Tables 1-2**). None of the isolates grew at temperatures of 5ºC and 10ºC. The resistant isolates of *N. parvum* to azoxystrobin showed greater mycelial growth at temperatures of 15ºC and 35ºC (**Table 1**). The resistant isolates of *L. pseudotheobromae* to thiophanate-methyl exhibited greater mycelial growth at temperatures of 15ºC and 20ºC; however, at temperatures of 25ºC and 30ºC, the sensitive isolates showed greater growth (**Table 2**).

Table 1: Comparison of mycelial growth rate at different temperatures, osmotic sensitivity (EC50), and virulence on avocado fruit (VIR) between azoxystrobin sensitive and resistant isolates of *Neofusicoccum parvum*

	MGR (mm/h)						$EC_{50}N$	VIR (mm)
RT[1]	15ºC	20ºC	25ºC	30ºC	35ºC	40ºC		
Azx-R	0.14 a	0.36 a	0.85 a	1.01 a	0.73 a	0.0	1.52 a	49.1 a
Azx-S	0.07 b	0.24 a	0.65 a	0.66 a	0.10 b	0.0	1.56 a	44.1 a

P-value[2]	0.02	0.16	1.00	0.37	4E-04		0.40	0.56

*Reaction Type: azoxystrobin-resistant (Azx-R); azoxystrobin-sensitive (Azx-S)
** Values mean mycelial growth of isolates from two trials. Numbers in each column followed by the same letter are not statistically different according to Student's t-test (P=0.05)

Table 2: Comparison of mycelial growth rate (MGR) at different temperatures, osmotic sensitivity ($EC_{50}N$), and virulence on avocado fruit (VIR) between thiophanate-methyl sensitive and resistant isolates of *Lasiodiplodia pseudotheobromae*

	MGR (mm/h)						$EC_{50}N$	VIR (mm)
RT*	15ºC	20ºC	25ºC	30ºC	35ºC	40ºC		
Thm-R	0.15 a	0.43a	0.54 b	1.09 b	1.17 a	0.15 a	1.58 a	60.4 a
Thm-S	0.04 b	0.29 b	1.07 a	1.47 a	1.17 a	0.13 a	1.60 a	60.2 a
P-value**	0.002	0.005	0.03	0.04	0.86	0.93	0.7	0.94

*Reaction Type: thiophanate-methyl-resistant (Thm-R); thiophanate-methyl-sensitive (Thm-S)
** Values mean mycelial growth of isolates from two trials. Numbers in each column followed by the same letter are not statistically different according to Student's t-test (P=0.05)

4.Discussion

This study presents the first report on the sensitivity of fungi of the Botryosphaeriaceae family associated with avocado, collected in four producing regions of Brazil, to two chemical groups of fungicides: thiophanate-methyl (MBC) and azoxystrobin (QoIs). In general, thiophanate-methyl demonstrated greater in vitro efficacy, with only 8.1% of isolates resistant, while azoxystrobin showed resistance in almost 60% of the isolates tested. Resistance to MBC was observed exclusively in *Lasiodiplodia pseudotheobromae* isolates from the producing regions of São Paulo and Minas Gerais. In contrast, resistance to azoxystrobin was detected in isolates from all areas analyzed. Although fungicides from the MBC and QoIs groups are not registered for the control of these fungi in avocado crops (MAPA, 2025), they are recommended for other relevant diseases in the crop, and their frequent use may have exerted selective pressure, favoring the emergence of resistant isolates within the Botryosphaeriaceae family.

FRAC classifies fungicides in the QoI group as high risk for developing resistance. Our results confirm this trend since more than half of the tested isolates were resistant to azoxystrobin. This resistance has been broadly reported in the literature, both in species of the Botryosphaeriaceae family and in other fungal families (Chen et al., 2020; Dong et al., 2022; Primiano et al., 2017; Qin et al., 2016; Rosenzweig et al., 2008). Any mutation was found in the resistant isolates in the present study. Similar results were reported by Chen et al. (2020), who investigated the molecular mechanisms of resistance to azoxystrobin in *L. theobromae* isolates from papaya, without finding mutations in the *Cytb* gene. Similarly, Dong et al. (2022) analyzed *Lasiodiplodia* isolates from mango in Hainan province resistant to pyraclostrobin, without detecting mutations in the cytochrome b gene. These findings suggest that alternative mechanisms, such as

overexpression of the alternative respiration (AOX), efflux transport, overexpression in the mitochondrial *cytochrome b* gene, and heteroplasmic state of *Cytb* gene alleles may be involved in resistance to QoIs in these isolates (Fernández-Ortuño et al., 2008; Pereira et al., 2017; Sun et al., 2024; Yin et al., 2023).

Another important aspect to consider is the intrinsic resistance of isolates to the active ingredient. Reduced sensitivity of *Lasiodiplodia* isolates to QoI fungicides is frequently reported, including in natural populations, suggesting that this phenomenon may occur naturally (Chen et al., 2020; Dong et al., 2022; Valencia Bernal et al., 2025; Xu et al., 2024). Intrinsic resistance refers to the natural insensitivity of certain fungi to specific chemical groups (Lucas et al., 2015). Fungi that produce benzimidazole compounds as secondary metabolites are typically resistant to QoI fungicides, a phenomenon observed mainly in basidiomycetes (Iqbal et al., 2018; Lucas et al., 2015; Nofiani et al., 2018). Furthermore, other mechanisms of intrinsic resistance have been documented, including the presence of paralogs, overexpression of the target gene, or even mutations in isolates that have never been exposed to the fungicide, have also been documented (Liu et al., 2011; Mao et al., 2023; Nakaune & Nakano, 2007). Given that resistance to QoI fungicides may arise from multiple mechanisms, further studies are required to determine which factors predominantly contribute to resistance in the isolates analyzed in this research.

The mutations identified in *L. pseudotheobromae* in this study were E198A, E198K, and F200Y confer resistance to thiophanate-methyl, and are among the most frequently reported in different species under field conditions (Hawkins & Fraaije, 2016). Although mutations in *L. pseudotheobromae* have not been previously described, resistance mechanisms within the genus had already been reported by Chen et al. (2020), who identified the orthologous mutation E198K in the β-tubulin gene in resistant isolates of *L. theobromae*. The other mutations found in this study (E198A and F200Y) had not recorded in the genus *Lasiodiplodia*. However, these mutations have already been described in other important agricultural pathogens, including *Penicillium*, *Colletotrichum*, *Cercospora*, *Botrytis*, *Mycosphaerella*, *Fusarium* and *Sclerotinia*, among others, and are responsible for resistance to fungicides from the benzimidazole group. (Baraldi et al., 2003; Cañas-Gutiérrez et al., 2006; Suga et al., 2011; Trkulja et al., 2013; Wong et al., 2008; Yin et al., 2010; Zhang et al., 2010). Our results indicate that the use of thiophanate-methyl is favoring the selection of resistant isolates in the *L.*

pseudotheobromae population. Although the frequency of resistance is low, producers must take precautions when using MBCs for disease control in avocado.

The in vivo results show high efficacy of azoxystrobin in controlling sensitive isolates, but for resistant isolates there was only a reduction in disease severity. Since most isolates are resistant, this result may have practical implications for disease control. In contrast, the application of thiophanate-methyl did not prevent the development of symptoms in either sensitive or resistant isolates, with sensitive isolates showing a reduction in severity, while resistant isolates did not differ from the control. This limited effect of thiophanate-methyl raises concerns about the persistence of symptoms, generating significant economic losses. Thus, the application of thiophanate-methyl, even for sensitive isolates, may represent an obstacle for producers.

Our results revealed no significant differences in osmotic sensitivity and virulence between azoxystrobin-resistant and -sensitive isolates of *N. parvum*. When evaluating growth at different temperatures, the resistant phenotypes exhibited greater development at both high and low temperatures, while no differences were observed in the other thermal ranges. This improved growth under extreme conditions may favor the survival of the pathogen, as suggested by Hawkins and Fraaije (2018). The absence of fitness costs indicates that these resistant isolates have the potential to compete effectively with other members of the population, regardless of the presence of the fungicide.

Similarly, isolates of *L. pseudotheobromae* that were resistant and sensitive to thiophanate-methyl did not show significant differences in osmotic sensitivity and virulence. However, the resistant isolates demonstrated lower growth rates at 25°C and 30°C, which are typical temperatures observed in avocado-producing regions. This suggests that, under field conditions, these resistant isolates may be less competitive in terms of growth at room temperature.

The low sensitivity to azoxystrobin observed in most isolates in this study is relevant, especially because this fungicide is not yet registered for the control of Botryosphaeriaceae associated with dieback in avocado. In addition, we identified isolates of *L. pseudotheobromae* resistant to thiophanate-methyl, highlighting the need for effective strategies for resistance management. This result reinforces the importance of implementing management strategies such as the rotation of fungicides with different mechanisms of action, the use of multisite fungicides, and the implementation of non-chemical control strategies. In addition, monitoring the sensitivity of each species

involved in the symptomatic conditions is crucial for more efficient management. This information will contribute to the development of more sustainable and effective strategies for controlling dieback in avocado, especially in producing regions of Brazil.

Conclusion

The research revealed the occurrence of resistance to the fungicides thiophanate-methyl (MBC) and azoxystrobin (QoI) in isolates of *Lasiodiplodia* and *Neofusicoccum*, two major pathogens of avocado. Novel mutations were identified in the codons E198A, E198K, and F200Y in *L. pseudotheobromae*, with E198A and F200Y not reported in the genus *Lasiodiplodia*. Azoxystrobin resistance was widespread, covering all species tested and distributed across all regions sampled. Furthermore, analysis of the *Cytb* region showed no mutations associated with resistance, suggesting an alternative mechanism of resistance. In vivo tests, thiophanate-methyl was effective only against sensitive isolates. Azoxystrobin reduced symptoms in all cases, but only sensitive isolates did not develop symptoms on treated fruits. There were no significant differences in growth, osmotic sensitivity, or virulence between sensitive and resistant isolates. The detection of resistant isolates in different regions highlights the need for resistance management programs, promoting more effective strategies for controlling dieback in avocado trees.

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Supplementary material

Table S1. Isolate code, species, collected states and effective fungicide concentrations to inhibit 50% of mycelial growth (EC_{50}) of different *Neofusicoccum* and *Lasiodiplodia* isolates for azoxystrobin and thiophanate-methyl.

			EC_{50}	
Isolate	**Species**	**State**	**Azoxystrobin**	**Thiophanate-methyl**
CCUB 4972	*L. macrospora*	Distrito Federal	121,63	0,94
CCUB 4933	*L. theobromae*	Distrito Federal	112,03	1,22
CCUB 4891	*L.pseudotheobromae*	Distrito Federal	145,09	0,99
CCUB 4971	*L.pseudotheobromae*	Distrito Federal	307,77	0,98
CCUB 4882	*L.pseudotheobromae*	Distrito Federal	>500	0,95
CCUB 4880	*L.pseudotheobromae*	Distrito Federal	>500	1,56
CCUB 4890	*L.pseudotheobromae*	Distrito Federal	>500	1,61
CCUB 4892	*N. kwambonambiense*	Distrito Federal	2,29	0,51
CCUB 4901	*N. kwambonambiense*	Distrito Federal	0,03	0,93
CCUB 4937	*N. kwambonambiense*	Distrito Federal	4,42	1,13
CCUB 4884	*N. parvum*	Distrito Federal	0,73	0,54
CCUB 4885	*N. parvum*	Distrito Federal	2,32	0,74
CCUB 4886	*N. parvum*	Distrito Federal	4,85	2,93
CCUB 4887	*N. parvum*	Distrito Federal	2,11	0,64
CCUB 4888	*N. parvum*	Distrito Federal	2,71	0,78
CCUB 4893	*N. parvum*	Distrito Federal	0,03	0,81
CCUB 4896	*N. parvum*	Distrito Federal	1,03	0,94
CCUB 4897	*N. parvum*	Distrito Federal	0,51	0,98
CCUB 4899	*N. parvum*	Distrito Federal	1,40	1,21
CCUB 4903	*N. parvum*	Distrito Federal	0,12	1,29
CCUB 4906	*N. parvum*	Distrito Federal	27,90	1,06
CCUB 4907	*N. parvum*	Distrito Federal	2,08	0,85
CCUB 4908	*N. parvum*	Distrito Federal	2,10	0,51
CCUB 4911	*N. parvum*	Distrito Federal	3,49	0,47
CCUB 4912	*N. parvum*	Distrito Federal	0,42	0,75
CCUB 4913	*N. parvum*	Distrito Federal	0,76	0,88
CCUB 4916	*N. parvum*	Distrito Federal	1,00	1,04
CCUB 4917	*N. parvum*	Distrito Federal	2,78	0,99
CCUB 4918	*N. parvum*	Distrito Federal	18,04	1,19
CCUB 4919	*N. parvum*	Distrito Federal	5,27	1,14
CCUB 4920	*N. parvum*	Distrito Federal	1,54	0,99
CCUB 4921	*N. parvum*	Distrito Federal	0,34	0,93
CCUB 4923	*N. parvum*	Distrito Federal	4,07	1,11
CCUB 4932	*N. parvum*	Distrito Federal	1,19	1,08
CCUB 5306	*N. parvum*	Distrito Federal	3,25	1,11
CCUB 5307	*N. parvum*	Distrito Federal	0,26	0,44
CCUB 5312	*N. parvum*	Distrito Federal	1,60	0,44
CCUB 4878	*N. ribis*	Distrito Federal	123,00	1,80
CCUB 4966	*N. ribis*	Distrito Federal	23,88	1,10

CCUB 4969	*N. ribis*	Distrito Federal	3,04	1,69
CCUB 5301	*N. ribis*	Distrito Federal	1,15	1,62
CCUB 5304	*N. ribis*	Distrito Federal	7,02	1,15
CCUB 3547	*L. brasiliensis*	Minas Gerais	>500	1,69
CCUB 3548	*L. brasiliensis*	Minas Gerais	155,42	1,14
CCUB 3553	*L. euphorbiaceicola*	Minas Gerais	>500	1,65
CCUB 2541	*L. pseudotheobromae*	Minas Gerais	>500	>100
CCUB 2547	*L.pseudotheobromae*	Minas Gerais	185,69	0,82
CCUB 2549	*L.pseudotheobromae*	Minas Gerais	362,01	1,08
CCUB 2550	*L.pseudotheobromae*	Minas Gerais	454,49	2,04
CCUB 2552	*L.pseudotheobromae*	Minas Gerais	>500	>100
CCUB 2554	*L.pseudotheobromae*	Minas Gerais	>500	0,80
CCUB 3555	*L.pseudotheobromae*	Minas Gerais	352,66	1,34
CCUB 3550	*L. theobromae*	Minas Gerais	221,20	0,63
CCUB 2546	*N. parvum*	Minas Gerais	11,26	0,37
CCUB 2553	*N. parvum*	Minas Gerais	0,99	1,28
CCUB 2556	*N. parvum*	Minas Gerais	23,88	0,82
CCUB 3549	*N. parvum*	Minas Gerais	130,10	0,94
CCUB 4963	*N. parvum*	Minas Gerais	0,63	0,45
CCUB 4973	*N. parvum*	Minas Gerais	0,41	1,40
CCUB 4962	*L. euphorbiaceicola*	Santa Catarina	329,60	1,01
CCUB 4960	*L. macropora*	Santa Catarina	214,51	0,92
CCUB 4968	*L. macrospora*	Santa Catarina	115,05	1,35
CCUB 4970	*L. macrospora*	Santa Catarina	274,63	1,32
CCUB 4961	*N. parvum*	Santa Catarina	0,81	1,05
CCUB 3466	*L. brasiliensis*	São Paulo	>500	1,43
CCUB 3478	*L. euphorbiaceicola*	São Paulo	174,53	1,18
CCUB 3498	*L. euphorbiaceicola*	São Paulo	>500	0,72
CCUB 3499	*L. euphorbiaceicola*	São Paulo	51,24	0,77
CCUB 3500	*L. euphorbiaceicola*	São Paulo	>500	0,85
CCUB 3501	*L. euphorbiaceicola*	São Paulo	329,98	1,18
CCUB 3520	*L. euphorbiaceicola*	São Paulo	295,74	0,63
CCUB 3473	*L parva*	São Paulo	>500	0,99
CCUB 3480	*L parva*	São Paulo	>500	0,68
CCUB 3521	*L parva*	São Paulo	285,03	0,89
CCUB 3535	*L parva*	São Paulo	42,17	0,61
CCUB 3504	*L.pseudotheobromae*	São Paulo	312,45	>100
CCUB 3508	*L.pseudotheobromae*	São Paulo	>500	0,67
CCUB 3509	*L.pseudotheobromae*	São Paulo	383,15	0,44
CCUB 3510	*L.pseudotheobromae*	São Paulo	>500	1,26
CCUB 3511	*L.pseudotheobromae*	São Paulo	>500	0,75
CCUB 3514	*L.pseudotheobromae*	São Paulo	39,50	>100
CCUB 3522	*L.pseudotheobromae*	São Paulo	165,68	1,32
CCUB 3523	*L.pseudotheobromae*	São Paulo	185,96	>100
CCUB 3524	*L.pseudotheobromae*	São Paulo	>500	0,87
CCUB 3526	*L.pseudotheobromae*	São Paulo	>500	>100

CCUB 3532	*L.pseudotheobromae*	São Paulo	>500	1,07
CCUB 3533	*L.pseudotheobromae*	São Paulo	69,97	>100
CCUB 3536	*L.pseudotheobromae*	São Paulo	>500	>100
CCUB 3539	*L.pseudotheobromae*	São Paulo	>500	>100
CCUB 3467	*L. theobromae*	São Paulo	396,01	1,35
CCUB 3470	*L. theobromae*	São Paulo	91,55	1,19
CCUB 3472	*L. theobromae*	São Paulo	342,25	1,04
CCUB 3477	*L. theobromae*	São Paulo	76,44	0,89
CCUB 3487	*L. theobromae*	São Paulo	>500	0,49
CCUB 3493	*L. theobromae*	São Paulo	180,58	0,67
CCUB 3494	*L. theobromae*	São Paulo	>500	0,58
CCUB 3518	*L. theobromae*	São Paulo	135,71	0,76
CCUB 3468	*N. parvum*	São Paulo	9,77	2,32
CCUB 3482	*N. parvum*	São Paulo	6,45	0,78
CCUB 3495	*N. parvum*	São Paulo	10,55	1,40
CCUB 3497	*N. parvum*	São Paulo	18,88	1,03
CCUB 3502	*N. parvum*	São Paulo	2,10	1,10
CCUB 3503	*N. parvum*	São Paulo	0,75	0,77
CCUB 3506	*N. parvum*	São Paulo	0,05	1,09
CCUB 3516	*N. parvum*	São Paulo	3,65	0,74
CCUB 3527	*N. parvum*	São Paulo	8,93	1,84
CCUB 3541	*N. parvum*	São Paulo	0,72	1,46
CCUB 3525	*N. ribis*	São Paulo	13,44	1,85
CCUB 3538	*N. ribis*	São Paulo	4,90	1,09
CCUB 3540	*N. ribis*	São Paulo	18,04	1,35

*CCUB – Coleção de Cultura da Universidade de Brasília

Table S2. Sequences of resistant and sensitive isolates from this study deposited in Genbank.

Isolado	**Specie**	**Gene***	**Reaction****	**Genbank*****
CCUB 3541	*N. parvum*	Cytb	Azx-S	-
CCUB 4933	*L. theobromae*	Cytb	Azx-R	-
CCUB 3535	*L parva*	Cytb	Azx-R	-
CCUB 3510	*L.pseudotheobromae*	Cytb	Azx-R	-
CCUB 3507	*L. euphorbiaceicola*	Cytb	Azx-R	-
CCUB 3532	*L.pseudotheobromae*	TUB2	Thm-S	-
CCUB 2541	*L.pseudotheobromae*	TUB2	Thm-R	-
CCUB 3514	*L.pseudotheobromae*	TUB2	Thm-R	-
CCUB 3523	*L.pseudotheobromae*	TUB2	Thm-R	-
CCUB 3526	*L.pseudotheobromae*	TUB2	Thm-R	-
CCUB 3533	*L.pseudotheobromae*	TUB2	Thm-R	-
CCUB 3536	*L.pseudotheobromae*	TUB2	Thm-R	-
CCUB 3539	*L.pseudotheobromae*	TUB2	Thm-R	-

* Cytochrome b (Cytb); β-tubulin (TUB2)
**Reaction Type: azoxystrobin-resistant (Azx-R); azoxystrobin-sensitive (Azx-S); thiophanate-methyl-resistant (Thm-R); thiophanate-methyl-sensitive (Thm-S).
*** sequences not deposited