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RAQUEL MARIA DA SILVA

SENSIBILIDADE À FUNGICIDAS DE ESPÉCIES DE *Lasiodiplodia* ASSOCIADAS À FRUTEIRAS NO NORDESTE BRASILEIRO E ADAPTABILIDADE DE ISOLADOS

Rio Largo-AL
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Tese de doutorado apresentada ao Programa de Pós-Graduação em Proteção de Plantas da Universidade Federal de Alagoas, como requisito para Defesa do doutordo em Proteção de Plantas.
Área de concentração: Fitopatologia

Orientadora: Prof. Dra. Kamila Câmara Correia
Coorientador: Prof. Dr. Sami Jorge Michereff

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(Universidade Federal do Cariri)

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Ó meu senhor, dá-me mais gratidão
Por tudo que Tu fizeste por mim
Por Tua graça no meu coração
Que me encheu de ventura sem fim!
Mais grato a Ti, mais grato a Ti
Mais consagrado, ó faz-me, Senhor!
Mais humildado e cheio de amor
Faz-me mais grato a Ti, mais grato a Ti!

Paulo Leivas Macalão

OFEREÇO.
A Deus, todo poderoso detentor de toda sabedoria

A minha querida irmã Rute Maria.
DEDICO

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RESUMO

A região Nordeste do Brasil se destaca como produtora e exportadora de frutas tropicais. As doenças causadas por espécies de *Lasiodiplodia* são importantes nessa região, principalmente em videira, mangueira, mamoeiro e serigueleira. Embora *L. theobromae* seja a espécie predominante, outras como *L. brasiliensis, L. euphorbiaceicola, L. hormozganensis, L. iraniensis* e *L. pseudotheobromae* também estão associadas a essas doenças. As informações são limitadas sobre a sensibilidade a fungicidas dessas espécies menos conhecidas de *Lasiodiplodia*. Este estudo teve como objetivo avaliar a sensibilidade e a adaptabilidade de isolados de cinco espécies de *Lasiodiplodia* de pomares de fruteiras no Nordeste do Brasil aos fungicidas imazalil, tebuconazol, tiabendazol e tiofanato-metílico. A sensibilidade de 153 isolados de *Lasiodiplodia* obtidos de uva, manga, mamão e seriguela aos fungicidas foi avaliada medindo a concentração efetiva (EC_{50}) necessária para inibir 50% do crescimento micelial. Posteriormente, cinco isolados com os valores de EC_{50} mais baixos (sensíveis-S) e mais altos (menos sensíveis-LS) foram examinados quanto à estabilidade da sensibilidade aos fungicidas, eficácia dos fungicidas no controle dos isolados em frutos e componentes de adaptabilidade, incluindo crescimento micelial, sensibilidade osmótica e virulência. A sensibilidade ao tebuconazol (EC_{50} média = 0,15 μg/mL) foi maior do que ao tiofanato-metílico (EC_{50} média = 0,34 μg/mL), tiabendazol (EC_{50} média = 1,15 μg/mL) e imazalil (EC_{50} média = 2,36 μg/mL). Foi detectada sensibilidade cruzada somente entre imazalil e tebuconazol. Isolados classificados como LS para imazalil exibiram um aumento na sensibilidade após subculturas sucessivas, enquanto os demais isolados mantiveram níveis de sensibilidade estáveis. A eficácia de controle de isolados LS para imazalil e tebuconazol em manga, mamão e seriguela foi significativamente menor do que a de isolados S. Custos de adaptabilidade foram observados apenas em isolados LS de tiabendazol, pois exibiram menor virulência em frutas de mamão em comparação com isolados S. Isolados classificados como LS para imazalil, tebuconazol e tiofanato metílico demonstraram maior capacidade de crescimento sob estresse salino em comparação aos isolados S. Os resultados deste estudo sugerem a presença potencial de isolados de *Lasiodiplodia* de pomares de uva, manga, mamão e seriguela no Nordeste do Brasil que são resistentes a imazalil, tebuconazol, tiabendazol e tiofanato- metílico.

Palavras-chave: Botryosphaeriaceae; doenças de fruteiras tropicais; controle químico; fungicidas DMI; fungicidas MBC; resistência a fungicidas; adaptabilidade.

ABSTRACT

The Northeast region of Brazil is an important producer and exporter of tropical fruits. In this region, diseases caused by *Lasiodiplodia* species significantly impact crops, particularly grapevine, mango, papaya, and red mombin. While *L. theobromae* is the dominant species, others such as *L. brasiliensis*, *L. euphorbiaceicola*, *L. hormozganensis*, *L. iraniensis*, and *L. pseudotheobromae* are also linked to these diseases. However, there is limited information on the fungicide sensitivity of these lesser-known *Lasiodiplodia* species. This study aimed to assess the sensitivity and fitness of isolates from five *Lasiodiplodia* species collected from fruit orchards in Northeast Brazil to the fungicides imazalil, tebuconazole, thiabendazole, and thiophanate-methyl. The sensitivity of 153 *Lasiodiplodia* isolates from grape, mango, papaya, and red mombin to the fungicides was assessed by measuring the effective concentration (EC_{50}) needed to inhibit 50% of mycelial growth. Subsequently, five isolates with the lowest (sensitive-S) and highest (less sensitive-LS) EC_{50} values for the fungicides were examined for sensitivity stability, fungicide effectiveness in controlling the isolates on detached fruits, and fitness components, including mycelial growth, osmotic sensitivity, and virulence. The sensitivity to tebuconazole (mean EC_{50} = 0.15 μg/mL) was greater than to thiophanate-methyl (mean EC_{50} = 0.34 μg/mL), thiabendazole (mean EC_{50} = 1.15 μg/mL), and imazalil (mean EC_{50} = 2.36 μg/mL). Cross-sensitivity was observed exclusively between imazalil and tebuconazole. Low-sensitive isolates to imazalil showed increased sensitivity after successive subcultures, whereas the other isolates maintained stable sensitivity levels. The control efficacy of LS isolates to imazalil and tebuconazole on mango, papaya and seriguela was significantly lower than that of S isolates. Fitness costs were observed only in LS isolates to thiabendazole, as they exhibited lower virulence on papaya fruits compared to S isolates. Isolates classified as LS to imazalil, tebuconazole, and thiophanate-methyl demonstrated a greater ability to grow under salt stress compared to S isolates. The findings of this study suggest the potential presence of *Lasiodiplodia* isolates from grape, mango, papaya, and red mombin orchards in Northeastern Brazil that are resistant to imazalil, tebuconazole, thiabendazole, and thiophanate-methyl.

Keywords: Botryosphaeriaceae; Tropical fruit diseases; Chemical control; DMI fungicide; MBC fungicide; Fungicide resistance; Fitness.

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INTRODUÇÃO GERAL E REVISÃO DE LITERATURA

1 INTRODUÇÃO GERAL

As fruteiras tropicais são acometidas por muitas doenças, mas no Nordeste brasileiro as causadas por espécies de Botryosphaeriaceae, principalmente *Lasiodiplodia*, têm grande destaque em mamoeiro, mangueira, serigueleira e videira. *Lasiodiplodia theobromae* é a espécie mais prevalente nessas fruteiras, mas várias outras foram identificadas causando doenças, incluindo *L. brasiliensis, L. euphorbiaceicola, L. hormozganensis, L. iraniensis* e *L. pseudotheobromae*.

Dentre as medidas de manejo das doenças causadas por *Lasiodiplodia*, o controle químico é uma das opções. No entanto, os produtores de frutas tropicais no Brasil têm encontrado dificuldades para o manejo eficiente das doenças causadas por espécies de Botryosphaeriaceae devido ao reduzido número de fungicidas registrados para as culturas e à pouca eficácia demonstrada por alguns produtos ao longo dos anos.

Vários fatores podem levar a resultados desfavoráveis quando as doenças são manejadas com fungicidas, mas um dos principais fatores é a perda de eficácia devido à resistência das populações dos patógenos aos fungicidas. O surgimento de populações de fungos resistentes a fungicidas é resultante do uso intensivo de fungicidas sistêmicos com mecanismos específicos de ação, que causam um aumento na pressão de seleção nas populações de fungos não-sensíveis.

Os estudos envolvendo resistência aos fungicidas em populações de Botryosphaeriaceae de fruteiras tropicais têm sido restritos, em sua maioria, a isolados de *L. theobromae*. Diante disso, os objetivos dessa tese foram:(i) avaliar a sensibilidade de isolados de *L. brasiliensis, L. euphorbiaceicola, L. hormozganensis, L. iraniensis* e *L. pseudotheobromae*, coletados em pomares de mamoeiro, mangueira, serigueleira e videira no Nordeste brasileiro, aos fungicidas imazalil, tebuconazol, tiabendazol e tiofanato metílico; (ii) verificar a ocorrência de sensibilidade cruzada entre os fungicidas; (iii) avaliar a estabilidade da sensibilidade aos fungicidas em isolados sensíveis e menos sensíveis; (iv) avaliar a eficácia dos fungicidas no controle de isolados sensíveis e menos sensíveis em frutos destacados; e (v) verificar a relação entre a sensibilidade a esses fungicidas e as variáveis relacionadas à adaptabilidade.

2 REVISÃO BIBLIOGRÁFICA

2.1 Importância das fruteiras tropicais

As fruteiras tropicais desempenham um importante papel na alimentação, na geração de emprego e de renda e na promoção do desenvolvimento nacional, baseando-se em três pilares da sustentabilidade (econômico, social e ambiental) (FONSECA, 2022). No Brasil há cerca de 2,5 milhões de hectares plantados com frutíferas e a cada hectare cultivado, estima-se que ocorra o emprego de duas pessoas, ou seja, são gerados 5 milhões de empregos nesse setor agrícola (REINHARDT *et al*., 2021). O ramo da fruticultura é segmento de destaque na economia nacional, pois impulsiona o Brasil para uma integração econômica e global, sendo um país emergente na expansão comercial e um dos maiores produtores agrícolas do mundo (CARVALHO *et al*., 2024).

O Brasil possui solos com elevada aptidão agrícola apresentando grande diversidade física, química e biológica, além das condições climáticas que possibilitam a produção diversificada ao longo das quatro estações anuais, essas características destacam o país como um importante produtor e exportador de culturas agrícolas (GRACIA *et al*., 2023; PASCHOALINO; PARRÉ, 2023). Outro destaque no cultivo nacional é a adaptação aos biomas existentes, permitindo assim escalonamento produtivo em períodos não convencionais, no qual é realizado em muitas propriedades e em diversas regiões, diferenciando-se dos demais produtores mundiais (FERREIRA; GOMES; KNOX, 2023; SILVA *et al*., 2023). Em contrapartida, o clima predominantemente tropical proporciona o rápido crescimento de plantas invasoras e a proliferação de pragas e doenças, assim constituindo um dos principais entraves para os produtores (ANWAR *et al*., 2021; CÔTE *et al*., 2021; MERLE; HIPÓLITO; REQUIER, 2022).

A fruticultura brasileira apresenta uma relevante representatividade no âmbito da exportação, pela grande diversidade de frutas e elevada na qualidade dos produtos ofertados (BARROS *et al*., 2023), com destaque, dentre outras, para mamão (*Carica papaya* L.), manga (*Mangifera indica* L.), melancia (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), melão (*Cucumis melo* L.) e uva (*Vitis vinifera* L.) (ABRAFRUTAS, 2025b).

Em 2023, o Brasil foi o terceiro maior produtor mundial de frutas, superado apenas por China e Índia (FAO, 2025). Nesse ano, a produção brasileira de mamão foi de 1,13 milhões de toneladas, principalmente nos estados de Bahia (31,1%) e Espirito Santo (30,9%). A produção de manga foi de 1,76 milhões de toneladas, principalmente nos estados de Bahia (40,1%) e

Pernambuco (34,2%) e São Paulo (12,1%). A produção de uva foi de 1,76 milhões de toneladas, principalmente nos estados de Rio Grande do Sul (40,1%) e Pernambuco (28,2%) (ABRAFRUTAS, 2025a). Em relação às exportações de frutas frescas, a região Nordeste é responsável por mais de 90% das exportações de manga e uva (ABRAFRUTAS, 2025b).

A produção de algumas fruteiras tropicais, como a seriguela (*Spondias purpurea* L.), não aparecem nas bases estatísticas de produção, mas apresentam importância socioeconômica para pequenos agricultores da região Nordeste (FILGUEIRAS, 2001; FONSECA *et al*., 2017; SILVA JÚNIOR *et al*., 2008).

A preocupação com a sustentabilidade e o meio ambiente é um tema relevante no agronegócio brasileiro, pois é um desafio harmonizar o desenvolvimento econômico agrícola com a preservação ambiental, com intuito de minimizar os impactos negativos oriundos das práticas agrícolas (QUINTAM; ASSUNÇÃO, 2023).

Questões fitossanitárias e o manejo dos resíduos de fungicidas são as principais questões da fruticultura no país e estão diretamente ligadas à segurança alimentar, sustentabilidade ambiental e à saúde dos trabalhadores (BARROS; MOREIRA, 2023). A segurança alimentar é ameaçada e as perdas econômicas são ocasionadas, principalmente, devido a agentes fitopatogênicos (HENZ, 2024), com destaque para os de etiologia fúngica, responsáveis por mais de 50% das doenças descritas (DOEHLEMANN *et al*., 2017; OLIVER, 2024b).

As fruteiras tropicais são acometidas por muitas doenças (DRENTH, GUEST, 2016; PLOETZ, 2003; PLOETZ *et al*., 1994), mas no Nordeste brasileiro as causadas por espécies de Botryosphaeriaceae, principalmente *Lasiodiplodia* Ellis & Everh., têm grande destaque em cajueiro (NETTO *et al*., 2017), coqueiro (COELHO *et al*., 2022), mamoeiro (NETTO *et al*., 2014), mangueira (COSTA *et al*., 2010; MARQUES *et al*., 2013), serigueleira (COUTINHO *et al*., 2017; SOUZA, 2022) e videira (CORREIA *et al*., 2016; RÊGO *et al*., 2019).

2.2 Espécies de *Lasiodiplodia* em fruteiras tropicais

O gênero *Lasiodiplodia* pertence ao reino Fungi, classe Dothideomycetes, filo Ascomycota e família Botryosphaeriaceae (MYCOBANK, 2025). Distingue-se morfologicamente dos outros gêneros da família por possuir conídios pigmentados, porém podem permanecer hialinos por um tempo e, posteriormente, tornarem-se marrons, septado com estrias longitudinais e paráfises picnidiais (PHILLIPS *et al*., 2013; RATHNAYAKA *et al*., 2023). Filogeneticamente, está intimamente relacionado com *Diplodia* e *Neodeightonia*, entretanto, morfologicamente, os conídios estriados de *Lasiodiplodia* diferenciam-se de

Diplodia, enquanto a presença de paráfises o diferencia de *Neodeightonia*, que também possui conídios estriados (JAYAWARDENA *et al*., 2019; PHILLIPS *et al*., 2013).

A taxonomia dos gêneros e espécies de Botryosphaeriaceae foi confusa durante muito tempo devido à identificação baseada apenas em características morfológicas (PHILLIPS *et al*., 2013). No entanto, análises multilocus usando sequências de DNA da região do espaço interno transcrito (ITS) e dos genes fator de alongamento da tradução 1-α (EF1-α), β-tubulina (TUB2) e segunda maior subunidade da RNA polimerase II (RPB2) têm sido empregadas com sucesso para discriminar espécies crípticas e elucidar as relações filogenéticas em Botryosphaeriaceae (PHILLIPS *et al*., 2013; SLIPPERS *et al*., 2017; ZHANG *et al*., 2021). A combinação do uso de sequências ITS e EF1-α fornece resolução suficiente na identificação em nível de espécie em muitos gêneros de Botryosphaeriaceae (YANG *et al*., 2017). Porém é insuficiente para uma identificação filogenética robusta de *Diplodia*, *Neofusicoccum* e *Lasiodiplodia*, motivo pelo qual os loci adicionais RPB2 e TUB2 devem ser considerados. É aconselhável usar os quatro loci para determinar a singularidade de cada espécie, para evitar a identificação questionável ou não confiável de espécies intimamente relacionadas no gênero *Lasiodiplodia* (KO *et al*., 2023; RATHNAYAKA *et al*., 2023).

Membros da família Botryosphaeriaceae são endófitos, patógenos ou saprófitos, considerados cosmopolitas e com ampla gama de hospedeiros, que causam doenças de difícil controle em muitos cultivos economicamente importantes (PHILLIPS *et al*., 2013; RATHNAYAKA *et al*., 2023; SILVA-VALDERRAMA; ÚRBEZ-TORRES; DAVIES, 2024; SLIPPERS, B.; WINGFIELD, 2007). Além disso, são relacionados a induzirem doenças em plantas expostas a estresses bióticos (insetos pragas e outros agentes patogénicos) ou abióticos (estresse hídrico, geada, granizo e ferimentos mecânicos) (BELAIR *et al*., 2023). Também já foram relatados como patógenos oportunistas de humanos, causando infecções subcutâneas, oculares e de órgãos e internos (GÓMEZ; MOREIRA; LUIZ, 2021; KANAUJIA *et al*., 2022; PICOS-MUÑOZ *et al*., 2015)

A distribuição geográfica de algumas espécies de Botryosphaeriaceae está diretamente associada ao clima, devido ao seu estilo de vida e à sua adaptação a amplitudes de temperaturas, especialmente no atual contexto de alterações climáticas (LEAL *et al.*, 2024). O gênero *Lasiodiplodia* apresenta uma distribuição cosmopolita em regiões tropicais e subtropicais, não sendo encontrado em regiões polares (PHILLIPS *et al*., 2013; RATHNAYAKA *et al*., 2023). Dentre suas espécies, *L. theobromae* (Pat.) Griffon & Maubl. é considerada a espécie-tipo (PHILLIPS *et al*., 2013) e a mais prevalente em fruteiras tropicais no Nordeste brasileiro (COELHO *et al*., 2022; CORREIA *et al*., 2016; COSTA *et al*., 2010; MARQUES *et al*., 2013;

NETTO *et al*., 2014, 2017; RÊGO *et al*., 2019). Além dessa espécie, várias outras foram identificadas causando doenças em fruteiras no Nordeste brasileiro (Tabela 1).

Tabela 1 - Primeiro relato baseado em filogenia molecular de espécies de *Lasiodiplodia* causadoras de doenças em mamoeiro, mangueira, serigueleira e videira no Nordeste brasileiro.

Fruteira	**Espécie de *Lasiodiplodia***	**Referência Bibliográfica**
Mamoeiro	*L. brasiliensis* M.S.B. Netto, M.W. Marques & A.J.L. Phillips	NETTO *et al*., 2014
	L. euphorbiaceicola A.R. Machado & O. L. Pereira (sin. *L. marypalmiae* Netto, M.W. Marques, A.J.L. Phillips & M.P.S. Câmara)	NETTO *et al*., 2014
	L. hormozganensis Abdollahz., Zare & A.J.L. Phillips	NETTO *et al*., 2014
	L. pseudotheobromae A.J.L. Phillips, A. Alves & Crous	NETTO *et al*., 2014
	L. theobromae	NETTO *et al*., 2014
Mangueira	*L. brasiliensis*	MARQUES *et al*., 2013
	L. crassispora T.I. Burgess & Barber	MARQUES *et al*., 2013
	L. hormozganensis	MARQUES *et al*., 2013
	L. iraniensis Abdollahzadeh, Zare & A.J.L. Phillips	MARQUES *et al*., 2013
	*L. laeliocattleyae (*Sibilia) A. Alves (sin. *L. egyptiaca* A.M. Ismail, L. Lombard & Crous)	MARQUES *et al*., 2013
	L. pseudotheobromae	MARQUES *et al*., 2013
	L. theobromae	MARQUES *et al*., 2013
Serigueleira	*L. brasiliensis*	COUTINHO *et al*., 2017
	L. caatingaensis I.B.L. Cout., F.C.O Freire, C.S. Lima & J.E. Cardoso	COUTINHO *et al*., 2017
	L.euphorbiaceicola	SOUZA, 2022
	L. iraniensis	SOUZA, 2022
	L. jatrophicola	SOUZA, 2022
	L. mahajangana	SOUZA, 2022
	L. pontei F.C. Freire, I.BL. Cout., C.S. Lima & J.E. Cardoso	COUTINHO *et al*., 2017
	L. pseudotheobromae	SOUZA, 2022
	L. theobromae	SOUZA, 2022
Videira	*L. brasiliensis*	CORREIA *et al*., 2016
	L. crassispora	CORREIA *et al*., 2016
	L. euphorbiaceicola	CORREIA *et al*., 2016
	L. hormozganensis	RÊGO, 2018
	L. iraniensis	RÊGO, 2018
	L. jatrophicola A.R. Machado & O.L. Pereira	CORREIA *et al*., 2016
	L. laeliocattleyae	CORREIA *et al*., 2016
	L. mahajangana Begoude, Jol. Roux & Slippers (sin. *L. macroconidia* Y. Zhang ter & S. Lin)	NASCIMENTO, 2023
	L. newvalleyensis A.M. Ismail, S.M. El-Ganainy & E.S. Elshewy	NASCIMENTO, 2023
	L. pseudotheobromae	CORREIA *et al*., 2016
	L. theobromae	CORREIA *et al*., 2016
	L. viticola Úrbez-Torres, Peduto & Gubler	NASCIMENTO (2023

2.3 Ciclo das doenças causadas por *Lasiodiplodia*

As principais fontes de inóculo de Botryospaheriacea incluem material de propagação infectado, corpos de frutificação do fungo dispersos nos órgãos da planta, ferramentas de poda contaminadas e restos culturais infectados. Além disso, culturas que sejam hospedeiras do patógeno e cultivadas próximo aos pomares, também são importantes fontes de inóculo (BATISTA *et al*., 2017; GARRIDO; GAVA; CAROLLO, 2017; VENTURA; COSTA; TATAGIBA, 2004).

A disseminação dos conídios (picnidiósporos) de Botryosphaeriaceae está intimamente relacionada com a elevada umidade relativa do ar e episódios de precipitação pluviométrica (SILVA *et al*., 2018; VALÊNCIA *et al*., 2015). A extrusão de cirros dos picnídios requer água livre, mas umidade relativa próxima a 100% pode fornecer umidade suficiente para a extrusão. Além disso, altas umidades relativas podem contribuir para manter a matriz gelatinosa dos cirros por longos períodos, favorecendo a viabilidade dos conídios liberados (SILVA *et al*., 2018). A disseminação dos conídios de *L. theobromae* em um plantio de coqueiro foi favorecida por precipitações pluviométricas entre 25 mm e 80 mm, mas prejudicadas em níveis superiores a esse limite (CORREIA; COSTA, 2005). Em curta distância, a disseminação ocorre pela água da irrigação e pelas gotículas das chuvas (PICOS-MUÑOZ *et al*., 2015). Outro mecanismo de disseminação de esporos é através dos insetos, como constatado em *L. pseudotheobromae* (BRAGARD *et al*., 2023). Na pós-colheita, a disseminação pode ocorrer através do contato físico de um fruto sadio com um doente (VENTURA; COSTA; TATAGIBA, 2004).

As principais vias de penetração de Botryosphaeriaceae nos hospedeiros são através de feridas produzidas por tratos culturais, como as podas mecânicas (BATISTA *et al*., 2017), ou ferimentos oriundos do ataque de insetos, durante a colheita, transporte, armazenamento de frutos e por causas naturais, como condições climáticas adversas (geadas, granizo) (BRAGARD *et al*., 2023; PICOS-MUÑOZ *et al*., 2015). A penetração direta nos tecidos pode ocorrer com ou sem a formação de apressório, e pelas aberturas naturais, como estômatos e lenticelas (NAVARRO; MOLINA; NOGUEIRA JÚNIOR, 2022; TERAO *et al*., 2019).

Os processos de colonização por Botryosphaeriaceae e suas interações com seus hospedeiros têm sido pouco estudados, apesar da sua importância agrícola (NAVARRO; MOLINA; NOGUEIRA JÚNIOR, 2022). É observado no sistema vascular dos hospedeiros a ocorrência de uma mudança de coloração pela presença do patógeno, os feixes vasculares são ocluídos progredindo para lesões necróticas (RANGEL-MONTOYA; ROLSHAUSEN; MARTINEZ-HERNANDEZ, 2021).

As espécies de Botryosphaeriaceae causam vários sintomas da doença, dependendo do hospedeiro e do tecido afetado, incluindo morte descendente de galhos, cancro do caule, gomose, declínio, podridão das raízes e podridão peduncular em pós-colheita (CORREIA *et al*., 2016; COUTINHO *et al*., 2017; MARQUES *et al*., 2013; NAVARRO; MOLINA; NOGUEIRA JÚNIOR, 2022; NETTO *et al*., 2014).

A incidência de *L. theobromae* é influenciada pela temperatura (maior que 30°C), pelo estresse hídrico e baixos níveis de nutrição da planta (PICOS-MUÑOZ *et al*., 2015; SLIPPERS *et al*., 2017).

2.4 Manejo das doenças causadas por *Lasiodiplodia*

Na perspectiva de minimizar as perdas ocasionadas por *Lasiodiplodia*, existe uma série de medidas que devem ser seguidas no manejo integrado, incluindo ações durante a fase de produção no campo como: utilização de materiais de propagação sadios; vistorias periódicas do pomar para verificar os primeiros sintomas da doença; realização de poda de limpeza para retirada de ramos com morte dos ponteiros; retirada de material infectado como caules e frutos, bem como plantas mortas ou que apresentem a doença em estágio avançado; utilização de ferramentas de poda frequentemente desinfestadas em solução de água sanitária (hipoclorito de sódio 2,0%); aplicação de pasta cúprica nos locais podados; pulverização das plantas com fungicidas registrados, nos períodos críticos da cultura; controle adequado de insetos que possam causar ferimentos que sirvam de porta de entrada para o fungo (BATISTA *et al*., 2016; 2017; LEAL *et al*., 2024; VENTURA; COSTA; TATAGIBA, 2004).

Para o controle das podridões na pós-colheita, as medidas de manejo devem atentar para cuidados durante a colheita e pós-colheita: realizar a colheita do fruto no estádio de maturação ideal, manusear cuidadosamente durante a colheita e pós-colheita para evitar danos físicos, manter o pedúnculo (1-2 cm), armazenar e transportar os frutos em ambiente refrigerado (BATISTA *et al*., 2017; DANTAS; OLIVEIRA, 2006; TERAO; BATISTA; BARBOSA, 2013; VENTURA; COSTA; TATAGIBA, 2004). O tratamento hidrotérmico dos frutos (água a 52 ºC durante 5 minutos) não é considerado eficaz no controle das podridões-pós colheita devido as espécies Botryosphaeriaceae serem mais termorresistentes que *Colletrotrichum*, patógeno principal para qual essa medida é utilizada (BATISTA *et al*., 2016; TERAO; BATISTA; BARBOSA, 2013).

Dentre as medidas de manejo das doenças causadas por *Lasiodiplodia*, o controle químico é uma das opções. No sistema de registro de produtos fitossanitários autorizados para

uso no Brasil (AGROFIT), a única espécie de Botryosphaeriaceae com fungicidas registrados para uso em fruteiras tropicais é *L. theobromae*. Somente os princípios ativos difenoconazol (triazol) e hidróxido de cobre (inorgânico) + oxicloreto de cobre (inorgânico) possuem registros para controle de *L. theobromae* em mangueira. Em mamoeiro, os princípios ativos registrados para o controle de *L. theobromae* são flutriafol (triazol) e tiabendazol (benzimidazol), sendo o último exclusivamente para aplicação na pós-colheita. Não existem fungicidas registrados para o controle de *L. theobromae* em videira e serigueleira (MAPA, 2025). No entanto, diversos outros fungicidas pertencentes aos grupos metil benzimidazol carbamato (MBC) (tiofanato metílico) e inibidores da desmetilação de esteróis (DMI) (difeconazol, flutriafol, imazalil, tebuconazol e tetraconazol) são registrados e aplicados em pomares de mamoeiro, mangueira e videira no Nordeste brasileiro (CAVALCANTE *et al*., 2014; MAPA, 2025; PEREIRA *et al*., 2012; SANTOS *et al*., 2019; SILVA; LEITE; CAPUCHO, 2022).

Os produtores de frutas tropicais no Brasil têm encontrado dificuldades para o manejo eficiente das doenças causadas por espécies de Botryosphaeriaceae devido ao reduzido número de fungicidas registrados para as culturas e à pouca eficácia demonstrada por alguns produtos ao longo dos anos (BATISTA *et al*., 2016; CAVALCANTE *et al*., 2014; PEREIRA *et al*., 2012; SANTANA *et al*., 2007; SANTOS *et al*., 2019).

2.5 Controle químico de doenças fúngicas

O controle químico de doenças de plantas se baseia na utilização de moléculas orgânicas ou inorgânicas, obtidas naturalmente ou sintetizadas, denominadas genericamente como fungicidas químicos, para a proteção das plantas contra os patógenos (SILVA JÚNIOR; BEHLAU, 2018). Os fungicidas químicos são cruciais para garantir o abastecimento e a segurança alimentar global (STEINBERG; GURR, 2020), bem como o potencial produtivo em regiões de clima tropical e subtropical (BOLZAN *et al*., 2024).

Os fungicidas químicos são utilizados para evitar ou interromper os processos de sobrevivência, disseminação, penetração, colonização e reprodução dos fungos e oomicetos causadores de doenças em plantas. Os fungicidas podem apresentar maior ou menor especificidade às diferentes classes taxonômicas de fungos e oomicetos, bem como às diferentes fases da relação patógeno-hospedeiro (OLIVER; BECKERMAN, 2022; SILVA JÚNIOR; BEHLAU, 2018).

No início do século XIX foi descoberto o primeiro fungicida para uso na agricultura, a calda sulfocálcica, introduzida por William Forsyth e recomendado para o controle de oídio

(*Oidium* spp.) em fruteiras. O próximo marco foi a introdução por Millardet, em 1885, da calda bordalesa, uma preparação à base de cobre usada para combater o míldio da videira (*Plasmopara vitícola* (Berk. & M.A. Curtis) Berl. & De Toni). No início do século XX foram introduzidos os primeiros fungicidas orgânicos - compostos organomercuriais - para o tratamento de sementes de cereais. Desde a década de 1930, compostos orgânicos como os ditiocarbamatos e as ftalimidas tornaram-se ferramentas importantes para o controle de doenças em plantas. O modo de ação destes fungicidas é descrito como multisítio, uma vez que inibem simultaneamente uma gama de enzimas e estruturas celulares e proporcionam uma proteção preventiva de plantas contra várias doenças de maneira não sistêmica, na superfície da planta (HERMANN; STENZEL, 2019).

Os primeiros fungicidas com mecanismos específicos de ação - os benzimidazóis, carboxamidas e inibidores de biossíntese de esterol (SBIs) - foram descobertos na década de 1960 e início de 1970. No final dos anos 1970 e início dos anos 1980, dicarboximidas, fenilamidas, e os primeiros triazóis entraram no mercado. Fungicidas específicos controlam patógenos de plantas de forma mais eficaz e a uma concentração muito menor em comparação com fungicidas multisítio. A maioria, mas não todos os fungicidas específicos, têm propriedades sistêmicas e, portanto, são capazes de penetrar no tecido da planta e se distribuírem através dos vasos do xilema (apoplasto) em partes de plantas que não foram atingidas diretamente durante a aplicação. No geral, os fungicidas específicos com propriedades sistêmicas foram considerados um verdadeiro progresso na proteção de plantas, pois têm menor probabilidade de serem removidos pela chuva e também muitas vezes são redistribuídos na planta. Como resultado, eles permitiram uma redução considerável, não só na quantidade aplicada, mas também no número de aplicações por estação de cultivo. O modo de ação específico, no entanto, tornou-se a origem de um novo fenômeno - a seleção de indivíduos resistentes em populações fúngicas, e o desenvolvimento da resistência de campo (HERMANN; STENZEL, 2019).

Ao longo dos anos, os programas de pesquisa das indústrias têm investido no desenvolvimento de compostos químicos para proteção de culturas de alto valor agregado. As características desejáveis desses produtos incluem: especificidade, ações sistêmica, curativa e erradicante, e alta atividade mesmo a baixas doses (AVENOT; MICHAILIDES, 2010; OLIVER; BECKERMAN, 2022).

Em 2022, o Brasil foi o maior usuário de agrotóxicos químicos em nível mundial, com a aplicação de 800,6 mil toneladas de produtos, sendo 26,5% superior ao utilizado nos Estados Unidos da América (EUA), o segundo maior usuário. A quantidade média de agrotóxicos utilizada por área de cultivo no Brasil foi de 12,6 kg/ha (FAO, 2025b).

Os fungicidas correspondem a 22% do total de agrotóxicos químicos comercializados no Brasil, sendo superados pelos herbicidas (48%) (SINDIVEG, 2025). Os 10 agrotóxicos mais comercializados no Brasil, em 2023, atingiram 532,48 mil toneladas de produtos, com destaque para o herbicida glifosato e seus sais (48%), seguido do fungicida mancozebe (10%). O fungicida clorotalonil é outro integrante dessa lista e ocupou a quarta posição (9%) (IBAMA, 2025).

Os principais fungicidas químicos utilizados no controle de doenças de plantas pertencem aos grupos metil benzimidazol carbamatos (MBC), inibidores da desmetilação de esteróis (DMI), inibidores da respiração - quinona interna (QoI), inibidores da enzima succinato desidrogenase (SDHI), ditiocarbamatos e produtos inorgânicos (HERMANN; STENZEL, 2019; OLIVER; BECKERMAN, 2022; OLIVER, 2024; SILVA JÚNIOR; BEHLAU, 2018).

Os fungicidas dos grupos MBC e DMI se destacam pelo pioneirismo na descoberta da ação sistêmica, pelo tempo que estão no mercado de insumos agrícolas e pela efetividade no controle de uma ampla gama de fungos (OLIVER; BECKERMAN, 2022; YOUNG, 2015; ZIOGAS; MALANDRAKIS, 2015).

Os fungicidas MBCs foram introduzidos na década de 1960 como fungicidas sistêmicos altamente efetivos e controlam uma ampla grama de espécies de fungos, mas são ineficazes contra oomicetos. Esses fungicidas atuam sobre a tubulina, proteína fúngica formada pelas subunidades α e β. A tubulina é o principal componente de filamentos de microtúbulos, os quais desempenham um papel central na divisão nuclear em todas as células eucarióticas. A função dos microtúbulos na divisão nuclear exige a montagem reversível da tubulina nos polímeros dos microtúbulos (YOUNG, 2015). Ao se ligarem à β-tubulina, os MBCs levam ao bloqueio da divisão nuclear, impedindo que ocorra a polimerização dos microtúbulos formadores do fuso mitótico e interrompendo a mitose na fase de metáfase (DELEN; TOSUN, 2004; YOUNG, 2015).

Os fungicidas MBCs possuem seis princípios ativos, pertencentes aos grupos químicos benzimidazol (benomil, carbendazim, fuberidazol e tiabendazol) e tiofanato (tiofanato e tiofanato metílico) (FRAC, 2024). Benomil foi retirado do mercado brasileiro em 1992 (MAY-DE MIO; LUO; MICHAILIDES, 2011), o que levou à grande utilização de tiofanato metílico e tiabendazol em diversas culturas. Em 2022, carbendazim foi proibido no mercado brasileiro (ANVISA, 2022), restringindo ainda mais as opções de produtos do grupo de fungicida.

Os fungicidas DMIs são classificados como inibidores da biossíntese do esterol (SBIs). Esteróis são constituintes essenciais na membrana celular de fungos, regulando sua estabilidade e permeabilidade, portanto a biossíntese de esterol é um alvo importante na inibição do

crescimento fúngico. Os DMIs inibem a desmetilação do esterol C14 durante o processo de formação de esterol em fungos. A desmetilação do lanosterol C14 é mediada por uma função mista de citocromo oxigenase P450, que é uma espécie de hemoproteína. DMIs ligam-se ao ferro do citocromo P450 por um átomo de nitrogênio e inibem a ligação de O_2 e sua transferência para o grupo metílico lanosterol C14, que é a principal fase no processo de desmetilação do lanosterol C14. A inibição da desmetilação resulta no desequilíbrio entre as membranas lipídicas, com a inibição da acumulação de fosfolípidos e ácidos graxos livres que atingem níveis tóxicos, destruindo a integridade e fluidez da membrana plasmática da célula fúngica (STENZEL; VORS, J-P, 2019; ZIOGAS; MALANDRAKIS, 2015).

Os fungicidas DMIs pertencem à cinco grupos químicos: piperazinas (triforina), piridinas (pirifenox e pirisoxazol), pirimidinas (fenarimol e nuarimol), imidazóis (imazalil, oxpoconazol, pefurazoato, procloraz e triflumizol) e triazóis (azaconazol, bitertanol, bromuconazol, ciproconazol, difenoconazol, diniconazol, epoxiconazol, etaconazol, fenbuconazol, fluquinconazol, flusilazol, flutriafol, hexaconazol, imibeconazol, ipconazol, mefentrifluconazol, metconazol, miclobutanil, penconazol, propiconazol, protioconazol, simeconazol, tebuconazol, tetraconazol, triadimefon, triadimenol e triticonazol) (FRAC, 2024).

Fungicidas pertencentes às piperazinas, pirimidinas e imidazóis foram os primeiros DMIs a entrar no mercado agrícola. No entanto, os triazóis dominam não apenas pela sua participação no mercado, mas também pelo número de compostos que atingiram o nível de mercado. Os triazóis são fungicidas orgânicos com ação acropetal, possuem alta fungitoxicidade, rápida penetração e translocação nos tecidos da planta. Os princípios ativos mais importantes do grupo dos triazóis incluem ciproconazol, difenoconazol, flutriafol, propiconazol, tebuconazol, triadimefon e triadimenol, enquanto do grupo dos imidazóis se destaca imazalil (STENZEL; VORS, J-P, 2019; ZIOGAS; MALANDRAKIS, 2015).

Mesmo com o uso intensivo de fungicidas, a produção agrícola ainda sofre perdas devido a patógenos fúngicos (FONES *et al*., 2020). Nas últimas décadas houve avanços e esforços realizados para minimizar os problemas relacionados ao uso intensivo de fungicidas. Esse tipo de uso representa riscos de poluição ambiental, intoxicação ao homem e redução das populações de microrganismos benéficos (PIMENTÃO *et al*., 2024; QUEIROZ *et al*., 2023). Além disso, a aplicação recorrente de fungicidas pode resultar no desenvolvimento de resistência aos compostos mais frequentemente utilizados (PIMENTÃO *et al*., 2024).

2.6 Resistência de fungos a fungicidas

Vários fatores podem levar a resultados desfavoráveis quando as doenças são manejadas com fungicidas, mas um dos principais fatores é a perda de eficácia devido à resistência das populações dos patógenos aos fungicidas (CERESINI *et al*., 2024; CORKLEY; FRAAIJE; HAWKINS, 2022; HOLLOMON, 2015; OLIVER; BECKERMAN, 2022; YIN *et al*., 2023).

A resistência a fungicida é definida como uma redução estável e hereditária na sensibilidade de um fungo a um fungicida (DELP; DEKKER, 1985). Constitui um problema originado no campo, que pode ser detectado por um declínio no desempenho do fungicida. Geralmente, nas populações de patógenos ocorre uma baixa frequência de indivíduos resistentes, não interferindo no controle da doença em campo, porém a situação torna-se crítica quando os indivíduos resistentes são predominantes (HOLLOMON, 2015).

O surgimento de populações de fungos resistentes a fungicidas é resultante do uso intensivo de fungicidas sistêmicos com mecanismos específicos de ação, que causam um aumento na pressão de seleção nas populações de fungos não-sensíveis (CERESINI *et al*., 2024; CORKLEY; FRAAIJE; HAWKINS, 2022; HOLLOMON, 2015; MILGROOM, 2015; OLIVER; BECKERMAN, 2022; THIND, 2022; YIN *et al*., 2023).

Já se passaram mais de 50 anos desde que a resistência aos fungicidas se tornou um grande problema no controle de doenças nas culturas (CERESINI *et al*., 2024; CORKLEY; FRAAIJE; HAWKINS, 2022; ISHII *et al*., 2024). A resistência a fungicidas acarreta sérias consequências a todos os segmentos da cadeia produtiva, desde as empresas fabricantes que perdem a confiabilidade de seus clientes, passando pelos produtores agrícolas, que ao perceberem que o fungicida não controla a doença, tendem aumentar as aplicações e as dosagens, chegando aos consumidores que recebem um produto com resíduos de fungicidas e preços elevados (GHINI; KIMATI, 2000).

Os sistemas de produção agrícola sob manejo convencional de doenças são determinantes para o surgimento e disseminação da resistência aos fungicidas e cinco fatores principais contribuem para essa situação: a) agricultura de monocultura; b) dependência de um número limitado de fungicidas com diferentes modos de ação; c) aplicação inadequada de fungicidas; d) falta de diversidade nas estratégias de manejo de doenças; e) pesquisa e desenvolvimento de novos fungicidas limitados (CERESINI *et al*., 2024).

A incidência da resistência tem sido restrita a fungicidas sistêmicos que agem em alvos bioquímicos de inibição em um único sítio, o que inclui a maior parte dos grupos de fungicidas. A ocorrência de isolados resistentes pode ocorrer depois de poucos anos após esses compostos serem introduzidos no mercado devido a seu uso intensivo (HOLLOMON, 2015; OLIVER; BECKERMAN, 2022; THIND, 2022; YIN *et al*., 2023). A capacidade de determinar esse risco

de resistência é de grande ajuda na seleção dos produtos químicos a serem utilizados e no estabelecimento de estratégias para aumentar sua durabilidade (CERESINI *et al*., 2024; HOLLOMON, 2015).

O desenvolvimento da resistência é um processo evolutivo populacional baseado na seleção natural. A evolução ocorre quando uma população variável encontra uma pressão seletiva, neste caso uma pressão exercida por um ou mais fungicidas. Os isolados que são resistentes à pressão de seleção são mais aptos isto é, produzem esporos viáveis mais rapidamente - e, portanto, são selecionadas (CERESINI *et al*., 2024; MILGROOM, 2015; OLIVER; BECKERMAN, 2022). Os fungicidas funcionam matando ou retardando o crescimento e a reprodução de fungos e, portanto, representam pressões de seleção potentes. Geralmente a evolução ocorre ao longo de milhões ou bilhões de anos, mas as populações de patógenos geralmente podem evoluir muito rapidamente, em questão de semanas ou meses. Primeiro, eles têm enormes tamanhos populacionais, um campo infectado típico pode produzir da ordem de 10^{10} esporos/ha. Segundo eles têm genomas altamente variáveis devido a propriedades mutagênicas convencionais e específicas de fungos. Terceiro, eles geralmente têm ciclos de vida curtos e podem se reproduzir em uma semana ou menos em alguns casos. Quarto, muitas espécies de fungos podem recombinar geneticamente genes de resistência a fungicidas por meio da reprodução sexual. Por fim, eles podem se mover por longas distâncias em produtos agrícolas, por meio de respingos de chuva ou como esporos transportados pelo ar (OLIVER; BECKERMAN, 2022).

O monitoramento e a detecção da resistência a fungicidas podem ser realizados utilizando as técnicas *in vivo* (patógenos obrigatórios) ou *in vitro.* As técnicas *in vivo* podem ser realizadas com utilização de plantas em ambientes controlados submetidas aos fungicidas em diferentes concentrações. As *in vitro* se baseiam na análise do crescimento micelial exposto ao fungicida, que podem ocorrer simultaneamente com a análise de germinação de esporos, a depender da morfologia do fungo. Essas técnicas são essenciais para avaliar e acompanhar a sensibilidade aos fungicidas, independente dos mecanismos envolvidos. No entanto, os diagnósticos moleculares estão desempenhando um papel importante em muitos aspectos da pesquisa de resistência a fungicidas (HOLLOMON; ISHII, 2015).

A resistência a fungicidas pode acontecer por diversos mecanismos, mas os quatro principais são alteração do sítio alvo para que a sensibilidade ao fungicida seja reduzida, desintoxicação ou metabolismo do fungicida, superexpressão do alvo e exclusão ou expulsão do fungicida do local alvo (CAPOTE *et al*., 2012; DORIGAN *et al*., 2023; ISLAM *et al*., 2024; MA; MICHAILIDES, 2005; SÁNCHEZ-TORRES, 2021; YIN *et al*., 2023). O conhecimento

dos mecanismos moleculares de resistência permite o monitoramento das populações não-sensíveis dos fungos de maneira rápida e eficiente, permitindo direcionar a utilização dos princípios ativos e grupos de fungicidas de maneira eficiente (ISLAM *et al*., 2024; MA; MICHAILIDES, 2005; OLIVER; BECKERMAN, 2022).

Logo após a introdução dos fungicidas MBCs houve o desenvolvimento de resistência em um alto nível, despertando intenso interesse no modo de ação desses fungicidas e no mecanismo de resistência (YOUNG, 2015). Esses fungicidas são classificados como de alto risco para resistência a fungicidas e mais de 150 espécies fúngicas já apresentaram resistência a esse grupo (FRAC, 2024). A velocidade com que a resistência se desenvolveu para diferentes patógenos foi maior para fungos com muitos ciclos de vida por estação de cultivo (policíclicos) (YOUNG, 2015).

A resistência a MBCs tem sido relacionada, na maioria dos casos, a mutações pontuais no gene β-tubulina, resultando em alterações na sequência de aminoácidos nos códons 6, 50, 134, 165, 167, 198, 200 e 240. No entanto, as mutações E198A/G/K/Q e F200Y são as mais prevalentes (DORIGAN *et al*., 2023; FRAC, 2024; MA; MICHAILIDES, 2005; SÁNCHEZ-TORRES, 2021; YOUNG, 2015). Há resistência cruzada positiva entre os membros do grupo MBC e resistência cruzada negativa aos N-fenil carbamatos (FRAC, 2024). Em muitos casos, foi demonstrado que fungos mutantes resistentes persistem na população por muitos anos, mesmo após o uso de MBCs ter sido interrompido (YOUNG, 2015).

Independentemente do seu modo de ação sítio-específico, o desenvolvimento de resistência em DMIs evoluiu de uma maneira típica gradual, levando a uma eficácia prolongada por mais de quatro décadas e tornando esses fungicidas um paradigma único (ZIOGAS; MALANDRAKIS, 2015). O risco de resistência aos DMIs é considerado baixo ou moderado, pois a velocidade das mudanças causadas pela resistência é determinada pela epidemiologia do patógeno e pela frequência ou duração da pressão de seleção aplicada (PEREIRA *et al*., 2012).

Vários mecanismos de resistência agindo individualmente ou em combinação foram identificados ao longo dos anos como associados à diminuição da sensibilidade a esses compostos em certas populações de patógenos no campo, incluindo modificação do sítio-alvo no gene desmetilase C14 (CYP51), superexpressão do CYP51 durante a formação do ergosterol, aumento do efluxo e múltiplos parálogos do gene-alvo (DORIGAN *et al*., 2023; SÁNCHEZ-TORRES, 2021; ZIOGAS; MALANDRAKIS, 2015). Várias mutações na região codificadora do gene CYP51geralmente conferem diferentes níveis ou nenhuma resistência a diferentes membros de DMIs, ao contrário daqueles que levam à superexpressão do gene-alvo, o que diminui a sensibilidade dos isolados a todos os membros do grupo DMI. O aumento do

efluxo mediado por transportadores de fungicidas pertencentes às famílias de transportadores ABC ou MFS resulta em resistência a todos os membros DMI (ZIOGAS; MALANDRAKIS, 2015). Uma combinação desses mecanismos pode contribuir para a diminuição da eficácia desse grupo de fungicidas no campo (DORIGAN *et al*., 2023; SÁNCHEZ-TORRES, 2021 ZIOGAS; MALANDRAKIS, 2015). As mutações Y134F, Y136F e Y137F no gene CYP51 são as mais prevalentes associadas à resistência em DMIs (DORIGAN *et al*., 2023). Geralmente é aceito que a resistência cruzada esteja presente entre fungicidas DMI ativos contra o mesmo fungo. Os fungicidas DMI pertencem à classe I dos SBIs, mas não apresentam resistência cruzada com fungicidas de outras classes (II, III e IV) de SBIs (FRAC, 2024).

2.7 Adaptabilidade e estabilidade da resistência de fungos a fungicidas

A adaptabilidade, a estabilidade da resistência e a capacidade competitiva de isolados resistentes, são fatores importantes no que se refere ao risco de desenvolvimento de resistência a fungicidas (HAWKINS; FRAAIJE, 2018; ISHII, 2015; MIKABERIDZE; MCDONALD, 2015; THIND, 2022). A adaptabilidade é a habilidade relativa dos organismos para continuarem em um ambiente por um longo período de tempo (NELSON, 1979). O custo de adaptabilidade pode ser definido como a redução da capacidade de um isolado de se desenvolver, reproduzir, sobreviver e causar doenças. Isolados resistentes deixarão mais descendentes em comparação a isolados sensíveis na presença do fungicida. Porém, a persistência de genótipos resistentes está determinada pela utilização do fungicida, uma vez que eles sejam selecionados. Em muitos casos, isolados resistentes podem ter adaptabilidade menor do que isolados sensíveis, não podendo sobreviver na ausência da pressão de seleção de fungicidas (CERESINI *et al*., 2024; HAWKINS; FRAAIJE, 2018; MA; MICHAILIDES 2005; MIKABERIDZE; MCDONALD, 2015; THIND, 2022). A adaptabilidade de isolados resistentes é frequentemente examinada em experimentos de laboratório e/ou estufa medindo, por exemplo, o crescimento micelial, a produtividade esporos, a capacidade de germinação, a competitividade e a agressividade (BROWN, 2006; MIKABERIDZE; MCDONALD, 2015; MILGROOM, 2015).

A estabilidade da resistência a fungicidas é definida como a capacidade do patógeno de manter o mesmo nível de insensibilidade a fungicidas após gerações sucessivas de exposição ou nenhuma exposição ao fungicida-alvo. A adaptabilidade diferencial entre populações resistentes e sensíveis, se houver, parece estar intimamente relacionada com a estabilidade da resistência no campo. Se as populações resistentes tiverem uma séria penalidade de adaptabilidade em comparação com as sensíveis, elas declinariam mais cedo ou mais tarde na

ausência de pressão de seleção de fungicidas, e então o fungicida problemático poderia ser reutilizado. Os casos mais conhecidos de estabilidade da resistência são relacionados a fungicidas MBCs, pois em várias situações isolados resistentes representaram apresentaram elevados níveis populacionais aos 5 anos após a última aplicação do fungicida. Em contraste com a durabilidade encontrada na resistência aos fungicidas MBCs, a estabilidade da resistência aos DMIs é mais variável (ISHII, 2015).

2.8 Manejo da resistência a fungicidas

A resistência não é inevitável, mas depende do impacto das propriedades dos patógenos e dos fungicidas nas populações. Alguns fatores podem ser manipulados para minimizar o risco de resistência e realizar estratégias anti-resistência (HOLLOMON, 2015).

Preocupações públicas sobre a perda de eficácia de fungicidas químicos, devido à resistência, são crescentes. Devido a essa conjuntura, surgiu a necessidade de desenvolver diretrizes consistentes para o manejo da resistência, o que levou à formação do Comitê de Ação para Resistência (FRAC) (CORKLEY *et al*., 2021).

Alguns princípios fundamentais no manejo da resistência a fungicidas, como o uso de diferentes modos de ação e a limitação de aplicações repetidas de produto com um modo de ação único, estão consolidados há muito tempo, pois é de conhecimento que que esses fungicidas têm maior de resistência em comparação aos fungicidas multissítios. No entanto, outros aspectos têm sido debatidos, tais como restringir o número de tratamentos aplicados por temporada, a utilização de taxas de dosagem mais altas ou mais baixas, misturas ou alternâncias, e se deve pulverizar de forma protetora ou apenas se for atingido um limiar de doença, implementar estratégias de manejo integrado das doenças (CERESINI *et al*., 2024; CORKLEY *et al*., 2021).

2.9 Resistência aos fungicidas MBC e DMI em espécies de Botryosphaeriaceae de fruteiras tropicais

Os estudos envolvendo resistência aos fungicidas MBC e DMI em populações de Botryosphaeriaceae de fruteiras tropicais têm sido restritos a isolados de *L. theobromae* coletados em pomares de mamoeiro (BANDEIRA, 2016; CAVALCANTE *et al*., 2014; CHEN *et al*., 2020; LI *et al*., 2020; PEREIRA *et al*., 2012) no Nordeste brasileiro, bem como de mangueira na China (WANG *et al*., 2021, 2023; YAN *et al*., 2021). Somente um estudo de resistência a fungicidas MBC foi realizado com isolados de outras espécies de *Lasiodiplodia*,

obtidos em pomares do Nordeste brasileiro (SANTOS *et al*., 2019).

A análise de 120 isolados de *L. theobromae* coletados de mamoeiro no Nordeste brasileiro mostrou que 8,4% apresentaram baixa sensibilidade aos MBCs (concentração efetiva necessária para inibir 50% do crescimento micelial - CE_{50} > 100 µg/mL), enquanto a maioria da população (91,6%) se mostrou sensível a benomil (CE_{50} média = 0,08 µg/mL) e tiabendazol (CE_{50} média = 0,76 µg/mL). Esses mesmos isolados foram sensíveis aos DMIs, imazalil (CE_{50} média = 0,63 µg/mL), procloraz (CE_{50} média = 0,20 µg/mL) e tebuconazole (CE_{50} média = 0,49 µg/mL). Não houve evidência de resistência múltipla, mas foi constatada resistência cruzada. Não foram constatados custos de adaptabilidade em isolados com baixa sensibilidade a MBCs em relação a crescimento micelial e virulência em frutos de mamão (PEREIRA *et al*., 2012).

Em outro estudo, foram analisados 109 isolados *de L. theobromae* de mamoeiro quanto à resistência ao fungicida tiofanato metílico, sendo constatado que 20,2% dos isolados foram resistentes ao fungicida, com CE_{50} > 300 µg/mL, enquanto 79,8% foram sensíveis (CE_{50} média = 1,87 µg/mL). Isolados resistentes mostraram capacidade de esporulação significativamente menor que os isolados sensíveis, indicando um custo de adaptabilidade (CAVALCANTE *et al*., 2014). Utilizando isolados de *L. theobromae* previamente caracterizados quanto a resistência a tiofanato metílico, foi constatado que isolados resistentes (>300 µg/mL) apresentavam mutação no códon 198, com a substituição de ácido glutâmico por lisina (CHEN *et al*., 2020).

Em relação a difenoconazol, foram avaliados 107 isolados de *L. theobromae* de mamoeiro, dos quais 2,8% tiveram CE_{50} > 10 µg/mL, enquanto 64,5% dos isolados apresentaram CE_{50} entre 0,01 e 1,00 µg/mL. Houve diferença na sensibilidade entre os 10 isolados sensíveis (0,10 µg/mL) e 10 menos sensíveis (7,27 µg/mL). Não foram constatados custos de adaptabilidade de isolados menos sensíveis em relação a crescimento micelial, temperatura ótima para crescimento micelial, esporulação, sensibilidade osmótica e virulência em frutos de mamão (BANDEIRA, 2016).

Utilizando os isolados menos sensíveis e sensíveis, foi investigada a base molecular da resistência a difenoconazol e as potenciais penalidades de adaptabilidade. Estudos com frutos destacados de mamão revelaram que isolados com valores de CE_{50} de 6,07 e 6,28 µg/mL não foram controlados efetivamente por difenoconazole na dose comercial, mas reduziram a virulência e a capacidade de crescer em temperaturas variando de 12 a 32 °C, indicando a ocorrência que custos de adaptabilidade. Resistência cruzada foi observada entre difenoconazol e propiconazol (LI *et al*., 2020).

A sensibilidade de 154 isolados de Botryosphaeriaceae obtidos de mangueira no

Nordeste brasileira foi avaliada em relação aos fungicidas tiofanato metílico e tiabendazol. As espécies incluíam *Botryosphaeria dothidea* (Moug.) Ces. & De Not., *Fusicoccum fabicercianum* S.F. Chen, Pavlic, M.J. Wingf. & X.D. Zhou, *L. hormozganensis*, *L. iraniensis*, *L. pseudotheobromae*, *L. theobromae*, *L. viticola*, *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips e *Pseudofusicoccum stromaticum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. Os isolados exibiram valores de CE_{50} variando de 0,03 a 6,86 μg/mL e de 0,0001 a 10,70 μg/mL para tiabendazol e tiofanato metílico, respectivamente. Foi constatada resistência cruzada entre os dois fungicidas MBCs. Os isolados menos sensíveis demostraram estabilidade na resistência após 10 gerações e não evidenciaram custos de adaptabilidade. Ambos os fungicidas foram capazes de controlar as infecções em frutos de manga causadas pelos isolados sensíveis e menos sensíveis (SANTOS *et al*., 2019).

Uma coleção de 138 isolados de *L. theobromae* coletados em pomares de mangueira na província de Hainan (China) foram analisados quanto a sensibilidade a difenconazol e investigados os mecanismos moleculares associados à resistência a esse fungicida. Os valores de CE_{50} variaram de 0,01 a 13,72 μg/mL. Difenoconazol mostrou resistência cruzada positiva com tebuconazol, mas não com fungicidas pertencentes a outros grupos (carbendazim, piraclostrobina, fludioxonil, bromotalonil ou iprodiona) (WANG *et al*., 2021).

A sensibilidade aos fungicidas carbendazim, difenoconazol, iprodione, prochloraz e piraclostrobina foi investigada em 224 isolados de *L. theobromae* coletados em cultivos de mangueira na província de Hainan. A maioria dos isolados foi sensível a difenoconazol, procloraz e iprodiona, enquanto as frequências de resistência foram de 24,11%, 15,18% e 1,34%, respectivamente. As frequências de resistência para carbendazim e piraclostrobina foram de 71,43% e 57,14%, respectivamente. Foi constatada resistência multifungicida a um, dois, três ou quatro fungicidas, com frequências de 29,02%, 60,27%, 7,14%, 0,45%, respectivamente (YANG *et al*., 2021).

Isolados de *L. theobromae* (n = 139) coletados de mangueira na província de Hainan, foram analisados em relação a sensibilidade a procloraz. Os valores de CE_{50} variaram de 0,0006 a 16,4131 μg/mL. No total, 21 isolados foram categorizados como resistentes a procloraz. Os isolados resistentes pulverizados com procloraz não foram efetivamente controlados. O crescimento micelial e a germinação de conídios de isolados resistentes diminuíram, sugerindo custo de adaptabilidade (WANG *et al*., 2021).

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CAPÍTULO II

ARTIGO 1

Sensitivity and fitness of isolates of five *Lasiodiplodia* species from Brazilian northeast fruit orchards to the fungicides imazalil and tebuconazole

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Sensitivity and fitness of isolates of five *Lasiodiplodia* species from Brazilian northeast fruit orchards to the fungicides imazalil and tebuconazole

Raquel Maria da Silva[1] • Jadson Araújo da Silva[2] • Cosma Amanda Alves Pereira[3] • Germana Vitória Ricarto Moreira[3] • Wilson Pereira da Silva[3] • Ivna Ribeiro Salmito Melo[3] • Sami Jorge Michereff[3] • Kamila Câmara Correia[3]

[1] Postgraduate Program in Plant Protection, Federal University of Alagoas, 57100-000 Rio Largo, Alagoas, Brazil

[2] Postgraduate Program in Phytopathology, Federal Rural University of Pernambuco, 52171-900 Recife, Pernambuco, Brazil

[3] Center for Agricultural Sciences and Biodiversity, Federal University of Cariri, 63133-610 Crato, Ceará, Brazil

(✉) Kamila Câmara Correia
kamila.correia@ufca.edu.br

Abstract

Although *Lasiodiplodia theobromae* is the predominant species, other species, such as *L. brasiliensis, L. euphorbiaceicola, L. hormozganensis, L. iraniensis,* and *L. pseudotheobromae*, are also associated with important fruit diseases in Northeastern Brazil. There is limited information on the fungicide sensitivity and fitness of isolates from these other species. This study aimed to assess the sensitivity and fitness of five *Lasiodiplodia* species isolates from fruit orchards in Northeastern Brazil to the fungicides imazalil and tebuconazole. Thus, the sensitivity of 153 *Lasiodiplodia* spp. isolates from grape, mango, papaya, and red mombin was assessed by determining the effective concentration needed to inhibit 50% of mycelial growth (EC_{50}). Subsequently, five isolates with the lowest (sensitive-S) and highest (less sensitive-LS) EC_{50} values for imazalil and tebuconazole were analyzed for sensitivity stability, fungicide efficacy in controlling S and LS isolates on detached fruits, and fitness components, including mycelial growth, osmotic sensitivity, and virulence. The isolates showed differences in sensitivity to fungicides and sensitivity to imazalil (mean EC_{50} = 2.36 μg mL^{-1}) was lower than that to tebuconazole (mean EC_{50} = 0.15 μg mL^{-1}). The EC_{50} values of the five LS isolates ranged

from 7.30 to 21.31 μg mL^{-1} for imazalil and from 0.44 to 1.37 μg mL^{-1} for tebuconazole. Cross-sensitivity was detected between imazalil and tebuconazole. Regarding resistance stability, only LS isolates for imazalil showed a significant increase in sensitivity after 10 successive subcultures. All isolates successfully infected and caused lesions on grapes, mango, papaya, and red mombin fruits in the absence of fungicide treatment. The control efficacy of LS isolates to imazalil and tebuconazole in mango, papaya, and red mombin was significantly lower than that of S isolates. No fitness cost was observed for LS isolates to imazalil and tebuconazole, and these isolates demonstrated a greater ability to grow under salt stress compared to S isolates. Monitoring the occurrence and frequency of *Lasiodiplodia* isolates with low sensitivity to imazalil and tebuconazole is essential for managing fungicide resistance and enhancing disease control in grape, mango, papaya, and red mombin orchards in Northeastern Brazil.

Keywords Botryosphaeriaceae • Fruit diseases • DMI fungicide • Fungicide resistance • Reduced sensitivity • Sensitivity stability • Control effectiveness

Introduction

Tropical fruit trees play an essential role in food, job and income generation, and in promoting development in Brazil (Fonseca 2022). In 2023, Brazil was the world's third-largest fruit producer, surpassed only by China and India (FAOSTAT 2025). Some fruits have great economic prominence because they are part of the export agenda, such as grapes (*Vitis vinífera* L.), mango (*Mangifera indica* L.) and papaya (*Carica papaya* L.) (ABRAFRUTAS 2025). However, other fruits are destined only for the domestic market, such as red mombin (*Spondias purpurea* L.), and do not appear in the production statistics, but are of socioeconomic importance to small farmers (Fonseca et al. 2017).

Tropical fruit trees are affected by many diseases. Still, in Northeastern Brazil, those caused by *Lasiodiplodia* Ellis & Everh. species are prominent in grapevine (Correia et al. 2016; Nascimento 2023), mango (Marques et al. 2013), papaya (Netto et al. 2014), and red mombin (Souza 2022). Although *L. theobromae* (Pat.) Griffon & Maubl. is the most prevalent species, other species are frequent in causing diseases in tropical fruit trees, such as *L. brasiliensis* M.S.B. Netto, M.W. Marques & A.J.L. Phillips, *L. euphorbiaceicola* A.R. Machado & O. L. Pereira, *L. hormozganensis* Abdollahz., Zare & A.J.L. Phillips, *L. iraniensis* Abdollahzadeh, Zare & A.J.L. Phillips, and *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous (Coelho et al. 2022; Correia et al. 2016; Costa et al. 2010; Coutinho et al. 2017; Marques et al. 2013;

Nascimento 2023; Netto et al. 2014, 2017; Santos et al. 2019; Souza 2022). The disease symptoms induced by *Lasiodiplodia* vary depending on the host and the affected tissue, including dieback, stem canker, gummosis, decline, root rot, and fruit stalk rot in post-harvest (Correia et al. 2016; Coutinho et al. 2017; Marques et al. 2013; Netto et al. 2014; Souza, 2022).

To minimize losses caused by *Lasiodiplodia* in tropical fruit trees, integrated management measures are used, including actions during the production phase in the field and postharvest. Among these measures, chemical control is one of the alternatives. The only *Lasiodiplodia* species with fungicides registered for use in tropical fruit trees is *L. theobromae*. Only the active ingredients difenoconazole (triazole) and copper hydroxide (inorganic) + copper oxychloride (inorganic) are registered for control of this fungi in mango. In papaya, the active ingredients registered are flutriafol (triazole) and thiabendazole (benzimidazole), the latter exclusively for postharvest application. No fungicides are registered to control *L. theobromae* in grapevines and red mombin (AGROFIT 2025). However, other fungicides belonging to the demethylation-inhibiting group - DMIs (difeconazole, flutriafol, imazalil, tebuconazole and tetraconazole) are registered and applied in grapevine, mango, and papaya orchards in Northeast Brazil aiming at the control of other diseases (AGROFIT 2025; Cavalcante et al. 2014; Li et al. 2020; Pereira et al. 2012; Santos et al. 2019; Silva et al. 2022). Among these, imazalil belongs to the chemical group of imidazoles and is used in the postharvest treatment of fruits, while tebuconazole is a triazole used in field applications aiming at the control of foliar and fruit diseases (AGROFIT 2025).

Tropical fruit growers in Brazil have encountered difficulties in efficiently managing diseases caused by *Lasiodiplodia* due to the reduced number of fungicides registered for these crops and the low efficacy demonstrated by some products over the years (Batista et al. 2016; Cavalcante et al. 2014; Pereira et al. 2012; Santana et al. 2007; Santos et al. 2019). The reduction in sensitivity to fungicides is one of the most critical factors in reducing the effectiveness of chemical control. This reduction in sensitivity is more frequent with fungicides with a single-site mode of action (Hollomon 2015). DMIs are examples of fungicides with specific mode of action, because they inhibit the sterol 14α-demethylase, a critical enzyme in the sterol biosynthesis pathway, resulting in an imbalance between lipid membranes, with inhibition of phospholipids and accumulation of free fatty acids that reach levels of toxic to fungi (Ziogas & Malandrakis 2015). The DMI fungicides are considered a medium risk for developing pathogen resistance (FRAC 2024).

The competitiveness of fungicide-resistant isolates within a pathogen population is influenced not only by selection pressure but also by their fitness (Melnyk et al. 2015). While

resistant isolates gain a competitive advantage under fungicide pressure, fitness costs may limit their prevalence in the population when the fungicide is not present (Mikaberidze & McDonald 2015).

Although imazalil and tebuconazole are not registered for the control of *Lasiodiplodia* in tropical fruit trees in Brazil, populations of this fungus are commonly exposed to these DMIs when applied for the control of other diseases in the field and postharvest. All DMI fungicide sensitivity studies in Brazil involved only *L. theobrome* (Bandeira 2014; Li et al. 2020; Pereira et al. 2012). Investigating the sensitivity and fitness of these populations is essential for consciously implementing management measures. Thus, the objectives of this study were: (i) to evaluate the sensitivity to the imazalil and tebuconazole fungicides of five *Lasiodplodia* species isolates collected from commercial fruit orchards in the Brazilian Northeast; (ii) to verify the occurrence of cross-sensitivity between the fungicides; (iii) to evaluate the stability of fungicide sensitivity in sensitive and less sensitive isolates; (iv) to assess the effectiveness of the fungicides to control sensitive and less sensitive isolates in detached fruits; and (v) to verify the relationship between the sensitivity to these fungicides and the fitness-related variables.

Materials and methods

Isolates

A total of 153 isolates of *Lasiodiplodia* including the species *L. brasiliensis* (40), *L. euphorbiaceicola* (13), *L. hormozganensis* (20), *L. iraniensis* (40), and *L. pseudotheobromae* (40) were obtained from the Culture Collection of Phytopathogenic Fungi of Cariri (CFC) at the Federal University of Cariri (Crato, Ceará, Brazil) (Table 2; Supplementary Table 1). The isolates originated from grapevine (89), mango (11), papaya (16), and red mombin (37) orchards located in the Brazilian Northeast (Fig. 1) and were previously identified by phylogenetic inference (Correia et al. 2016; Costa et al. 2010; Marques et al. 2012; Netto et al. 2014; Souza 2022). Only the orchards of red mombim did not receive any fungicide applications.

In vitro sensitivity assay for DMI fungicides

To quantify the sensitivity of *Lasiodiplodia* species to the fungicides, the 153 isolates were evaluated by a mycelial growth assay using a commercial formulation of imazalil (imazalil (Magnate 500 EC, 500 g L^{-1} active ingredient (a.i.), Adama Brazil, Londrina - PR, Brazil) and tebuconazole (Folicur 200 EC, 200 g L^{-1} a.i., Bayer, São Paulo - SP, Brazil). Fungicides were solubilized in sterile distilled water and added to molten (45 °C) potato dextrose agar (PDA) medium at different concentrations: 0.01, 0.05, 0.1, 0.3, 0.5, 1.0, 3.0, 5.0, 10.0, and 50.0 μg a.i. mL^{-1}. Five-mm-diameter mycelial plugs were obtained from the edge of a four-day-old colony of each isolate and transferred to PDA medium amended with the fungicides at different concentrations. Fungicide free PDA medium was used as control. Three replicates were used to evaluate each combination of isolate fungicide concentration. The cultures were incubated at 28 °C in the dark. The colony length was measured after 2 days of incubation. The percentage of mycelial growth (PMG) of the fungal was obtained by using the formula: PMG= (100*F)/C, where F corresponds to fungal growth in PDA medium containing fungicide and C is the control (fungal growth in fungicide-free PDA medium). The value was subtracted from 100 to yield the percentage of growth inhibition at each concentration of the fungicide. The effective concentration (μg/ml) of the fungicide that was able to inhibit mycelial growth by 50% (EC_{50}) was calculated for each isolate by linear regression of the mycelial growth inhibition versus the log_{10} transformation of the fungicide concentration for all isolate fungicide combinations. For each fungicide, five isolates with the lowest EC_{50} values were considered sensitive (S) and five isolates with the highest EC_{50} values were designated less sensitive (LS) (Table 3). These isolates were used to evaluate the stability of sensitivity, the effectiveness of DMIs to control diseases in grape, mango, papaya, and purple mombim fruits, and the fitness components (mycelial growth rate, osmotic sensitivity and virulence).

Cross-resistance between imazalil and tebuconazole was investigated by linear correlation analyses using two combinations. The first was determination of imazalil sensitivity using 10 tebuconazole-LS and 10 tebuconazole-S isolates; and the other combination was determination of tebuconazole sensitivity using 10 imazalil-LS and 10 imazalil-S isolates.

Stability of the sensitivity to DMI fungicides

To determine the stability of the sensitivity to imazalil and tebuconazole of S and LS isolates, 5-mm-diameter mycelial plugs were transferred to fungicide-free PDA medium every 7 days,

totalling 10 sequential transfers. Fungicide sensitivity assessments based on the mycelial growth test were performed before the first transfer (T0) and after 10 transfers (T10).

Effectiveness of DMI fungicides to control S and LS isolates infecting fruits

The in vivo effectiveness of imazalil and tebuconazole to control S and LS isolates was assessed by quantifying the disease severity on grape, mango, papaya, and purple mombim fruits treated with the commercial formulation of both fungicides prior to inoculation with mycelial plugs of the isolates. First, grape (cv. Itália), mango (cv. Tommy Atkins), papaya (cv. Golden), and purple mombim (cv. Doce Vermelha) fruits at maturation stage were surface disinfested using dishwashing, rinsed with distilled water, immersed for 5 min in 1% NaOCl, rinsed two times with distilled water and kept on a clean surface until dry. In grape and purple mombim, the epidermis of each fruit was punctured at the central area, and lesions were 2 mm in depth and made with a sterilized pin. The approach outlined by Li *et al*. (2020) was used for inoculation in mango and papaya. Two plugs measuring 5 × 2 mm (diameter × depth) were extracted from opposite sides of the fruit using a cork borer. The manufacturer-recommended doses (label rates) of fungicides for diseases control in the field (Folicur 200 EC – 1 mL L^{-1}) and postharvest (Magnate 500 EC – 0.2 mL L^{-1}) were prepared and sprayed onto the fruits using a spray bottle. Fruits untreated (control) were sprayed with sterile distilled water prior to inoculation. After 3 h, 5 x 2 mm (diameter x depth) mycelial plugs were taken from 4-day-old cultures of S and LS isolates and were transferred to each lesion site (grape and purple mombim) or inserted where the fruit plugs were removed (mango and papaya). Five replicates containing three fruits were used for each fruit species. The fruits were kept in a humid chamber at room temperature (∼28 °C). The lesion diameter (LD; mm) was measured in two perpendicular directions 3 days after inoculation across all replicates. The experiment was conducted in a randomized complete block design. Control efficacy was calculated according to the following formula: Control efficacy (%) = [(LDW – LDF) / LDW] *100, where LDW and LDF are the lesion diameter for water treatment and fungicide treatment, respectively.

Analysis of fitness components

Three fitness components were determined for S and LS isolates exposed to imazalil and tebuconazole: (i) mycelial growth rate, (ii) osmotic sensitivity, and (iii) virulence.

To evaluate the mycelial growth rate (MGR), a mycelial plug (5 mm in diameter) was removed from the margin of a 4-day-old culture of each selected isolate and transferred to a Petri plate containing fungicide-free PDA. The plates were incubated in the dark at 28 °C. Three replicates per isolate were used. The colony length was measured at 24 h and 48 h, and the average was used to calculate the MGR (mm h^{-1}).

The osmotic sensitivity was evaluated by mycelial growth in PDA medium containing NaCl. Mycelial plugs (5 mm in diameter) were removed from the margin of a 4-day-old culture of each isolate and transferred to PDA Petri plates amended with 1.0, 2.0, 4.0, 6.0, and 8.0% (wt vol^{-1}) of NaCl. Petri plates containing PDA medium without NaCl were used as a control. Three replicate plates were used for each isolate-NaCl concentration combination tested. The plates were incubated for 48 h at 28 °C in the dark. The length of each colony was measured, and the original mycelial plug diameter (5 mm) was subtracted from this measurement. The percentage of mycelial growth inhibition related to the control was calculated for all the concentrations of NaCl. The effective NaCl concentration to inhibit 50% of the mycelial growth ($EC_{50}N$) was calculated for individual isolates.

Disease severity (lesion diameter) on untreated fruits for each isolate from the in vivo imazalil and tebuconazole sensitivity assays were used as a proxy for virulence. Untreated fruits inoculated with sterile distilled water were used as negative control.

Statistical analysis

All experiments were performed twice, and the data of two independent replicates were pooled after testing the homogeneity of variance using Levene's test. In vitro sensitivity, stability of sensitivity, effectiveness of imazalil and tebuconazole in infected fruits and fitness components (mycelial growth rate, osmotic sensitivity and control efficacy) were analyzed by using Student's t-test (P=0.05). The cross-resistance between imazalil and tebuconazole was evaluated using Pearson's correlation (P=0.01). All analyses were performed using the Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA).

Results

In vitro sensitivity assay for DMI fungicides

The isolates belonging to five different *Lasiodiplodia* species (*L. brasiliensis, L. euphorbiaceicola, L. hormozganensis, L. iraniensis,* and *L. pseudotheobromae*) showed differences in the sensitivity to imazalil and tebuconazole fungicides, with EC_{50} values ranging from 0.004 to 4.0 μg mL^{-1} for most isolates (Fig. 2). Overall, the sensitivity to imazalil (mean EC_{50} = 2.36 μg mL^{-1}) was lower than to tebuconazole (mean EC_{50} = 0.15 μg mL^{-1}) (Fig. 3). The estimated EC_{50} values for imazalil ranged from 0.05 to 21.31 μg mL^{-1}, and were <0.50 μg mL^{-1} in 3.9% of the isolates, from 0.51 to 2.00 μg mL^{-1} in 55.6%, from 2.01 to 4.00 μg mL^{-1} in 29.5%, from 4.01 to 6.00 μg mL^{-1} in 5.9%, from 6.01 to 8.00 μg mL^{-1} in 2.6%, and > 8.01 μg mL^{-1} in 2.6% of the isolates (Fig. 3). For tebuconazole, the estimated EC_{50} values ranged from 0.004 to 1.37 μg mL^{-1}, and were <0.05 μg mL^{-1} in 17.6% of the isolates, from 0.06 to 0.15 μg mL^{-1} in 45.8%, from 0.16 to 0.25 μg mL^{-1} in 24.2%, from 0.26 to 0.35 μg mL^{-1} in 8.5%, from 0.36 to 0.45 μg mL^{-1} in 1.3%, and > 0.45 μg mL^{-1} in 2.6% of the isolates (Fig. 3).

The isolates were grouped based on their extreme sensitivity to the fungicides (Table 3). The EC_{50} values of the five most sensitive (S) isolates ranged from 0.05 to 0.49 μg mL^{-1} (mean 0.33 μg mL^{-1}) for imazalil. These values were significantly lower ($P \leq 0.05$) than the five least sensitive (LS) isolates, whose EC_{50} values ranged from 7.30 to 21.31 μg mL^{-1} (mean 11.08 μg mL^{-1}) (Tables 3 and 4). Among the five LS isolates to imazalil, three belonged to *L. brasiliensis* (CFC-0698, CFC-1175, and CFC-1434), one to *L. euphorbiaceicola* (CFC-1018), and one to *L. iraniensis* (CFC-1139). In terms of host origin, two isolates were obtained from grapevine (CFC-1018 and CFC-1434), one from papaya (CFC-0698), and two from red mombin (CFC-1139 and CFC-1175) (Table 3). For tebuconazole, the EC_{50} values of the five S isolates ranged from 0.004 to 0.02 μg mL^{-1} (mean 0.01 μg mL^{-1}) and were significantly ($P \leq 0.05$) lower than the five LS isolates, whose EC_{50} values ranged from 0.44 to 1.37 μg mL^{-1} (mean 0.78 μg mL^{-1}) (Tables 3 and 4). Among the five LS isolates to tebuconazole, one was identified as *L. brasiliensis* (CFC-1028), two as *L. euphorbiaceicola* (CFC-1013 and CFC-1018), and two as *L. pseudotheobromae* (CFC-1421 and CFC-1437), all of which were isolated from grapevine (Table 3).

A positive correlation was observed between the sensitivity of imazalil and tebuconazole, both in imazalil combination isolates (r=0.7880, P=0.0001; Fig. 4A) and in tebuconazole combination isolates (r=0.6250, P=0.0032; Fig. 4B), indicating a cross-sensitivity.

Stability of the sensitivity to DMI fungicides

No significant change was observed in the sensitivity of S and LS isolates to tebuconazole, as well as S isolates to imazalil, after 10 successive subcultures on fungicide-free PDA medium ($P>0.05$) (Table 5), suggesting that their sensitivity levels remained stable over consecutive growth cycles. In contrast, LS isolates to imazalil exhibited a significant increase in sensitivity after 10 successive subcultures ($P\leq0.05$) (Table 5), indicating that their sensitivity level changed over repeated growth cycles.

Effectiveness of DMI fungicides to control S and LS isolates infecting fruits

All isolates successfully infected and caused lesions on grapes, mango, papaya, and red mombin fruits in the absence of fungicide treatment. Grapes treated with imazalil or tebuconazole before inoculation with S or LS isolates showed no significant differences in disease control efficacy ($P>0.05$). The mean control efficacy of imazalil was 20.6% for S isolates and 23.3% for LS isolates, while for tebuconazole, it was 34.4% and 31.5% for S and LS isolates, respectively. The disease control efficacy of S isolates with imazalil was 44.6% in mango, 65.9% in papaya, and 33.3% in red mombin fruits. In contrast, the control efficacy of LS isolates with this fungicide was significantly lower ($P\leq0.05$), at 2.9% in mango, 5.1% in papaya, and 6.2% in red mombin fruits. For tebuconazole, the disease control efficacy of S isolates was 33.2% in mango, 84.2% in papaya, and 29.2% in red mombin fruits. Conversely, the control efficacy of LS isolates with this fungicide was significantly lower ($P\leq0.05$), measuring 2.9% in mango, 13.8% in papaya, and 10.8% in red mombin fruits (Fig. 5).

Analysis of fitness components

The S and LS isolates to imazalil and tebuconazole showed no significant differences in mycelial growth on fungicide-free medium or in virulence on grape, mango, and red mombin fruits ($P>0.05$). However, LS isolates to imazalil displayed significantly higher virulence in papaya fruits compared to S isolates. Additionally, LS isolates exhibited a greater ability to grow under salt stress than S isolates for both fungicides ($P\leq0.05$) (Table 6).

Discussion

This study is the first to examine the sensitivity of *L. pseudotheobromae, L. iraniensis, L. brasiliensis, L. euphorbiaceicola,* and *L. hormozganensis* isolates to the fungicides imazalil and tebuconazole, as well as their impact on fitness components in isolates from grapevine, mango, papaya, and red mombin orchards in the Brazilian Northeast.

The fact that isolates of *Lasiodiplodia* species exhibit lower sensitivity to imazalil than to tebuconazole is unexpected, given that imazalil is exclusively used in postharvest treatments, whereas tebuconazole is applied in the field, where fungal populations are more frequently exposed. The possible explanation for this result is that imazalil belongs to the imidazole class, while tebuconazole is a triazole; both acting as ergosterol biosynthesis inhibitors, but with structural differences that may affect efficacy against *Lasiodiplodia* isolates. Additionally, imazalil, being a fungicide with limited systemic activity, may be less effective against certain *Lasiodiplodia* isolates compared to tebuconazole, which has a broader systemic action (Ziogas & Malandrakis 2015).

In this study, the mean EC_{50} value of *Lasiodiplodia* isolates for imazalil was 2.36 μg mL^{-1}, which is high compared to the mean EC_{50} reported for this fungicide in *L. theobromae* from papaya in Brazil (1.74 μg mL^{-1}) (Pereira et al. 2012), and in *L. euphorbiaceicola* (1.44 μg mL^{-1}), *L. mahajangana* (1.50 μg mL^{-1}), and *L. theobromae* (1.21 μg mL^{-1}) from avocado in China (Chen et al. 2024). On the other hand, the mean EC_{50} value of the isolates for tebuconazole of this study (0.15 μg mL^{-1}) is low compared to the mean EC_{50} reported for this fungicide in *L. theobromae* from papaya in Brazil (0.63 μg mL^{-1}) (Pereira et al. 2012), and *L. euphorbiaceicola* (0.46 μg mL^{-1}), *L. mahajangana* (0.53 μg mL^{-1}), *L. theobromae* (0.38 μg mL^{-1}), and *L. pseudotheobromae* (0.67 μg mL^{-1}) from avocado in China (Chen et al. 2024). Differences in susceptibility to the fungicides imazalil and tebuconazole among *Lasiodiplodia* isolates from different regions or countries can be attributed to several factors, including genetic and agricultural management aspects. Different *Lasiodiplodia* populations may present natural genetic variations that affect their susceptibility to the fungicides (Brent & Hollomon 2007), and distinct species within the genus *Lasiodiplodia* may have different levels of sensitivity to fungicides, due to variations in target genes or resistance mechanisms (Chen et al. 2024). Furthermore, regions with more intensive use of some fungicides may exert greater selective pressure on fungal populations, favoring the selection of less sensitive isolates (Brent & Hollomon 2007; Hollomon 2015).

Considerable differences were observed between the extremes of sensitivity to the analyzed fungicides, particularly imazalil. Among the LS isolates with low sensitivity to imazalil, two (CFC-1139 and CFC-1175) were obtained from red mombin, a fruit that was not exposed to fungicide treatment, even during postharvest. In general, fungicide resistance or reduced sensitivity is associated to mutations in genes encoding fungicide targets (qualitative resistance) or to various mechanisms that are induced by sub-lethal fungicide stress. These mechanisms result in different and varying levels of resistance (quantitative fungicide resistance) (Deising et al. 2008; De Miccolis Angelini et al. 2015). However, the LS of *Lasiodiplodia* isolates from red mombin to imazalil may be attributed to their natural resistance. This type of resistance refers to the inherent ability of certain species or populations of fungi to tolerate a specific fungicide without prior exposure. This occurs due to natural genetic traits, such as the absence of fungicide's molecular target, efficient metabolic degradation mechanisms, or structural barriers that prevent the action of the compound (Brent & Hollomon 2007; Lucas et al. 2015).

The identification of the isolate (CFC-1018) with a high EC_{50} value for imazalil (21.31 $\mu g\ mL^{-1}$) compared to the other isolates may indicate the occurrence of resistant individuals to the fungicide in the *Lasiodiplodia* populations. DMI fungicides inhibit sterol C-14 α-demethylation of 24-methylenedihydrolanosterol, a key precursor of ergosterol in fungi. Various resistance mechanisms have been identified, including mutations in target genes and activation of alternative enzymatic pathways (Ma & Michailides 2005; Ziogas & Malandrakis 2015). Consequently, resistance to these fungicides can result from the interaction of multiple genes, leading to a quantitative insensitivity response to DMIs. This interaction is characterized by gradual increases in resistance with each mutation, which are not typically detectable in the field in the short term (Brent & Hollomon 2007; Deising et al., 2008; De Miccolis Angelini et al. 2015; Ma & Michailides, 2005; Ziogas & Malandrakis 2015). Therefore, detecting isolates with low sensitivity to the fungicide within the fungal population is crucial, as observed in this study with the isolate CFC-1018 for imazalil. Over time, this genotype may become predominant due to selection pressure from the continuous use of the fungicide (Deising et al. 2008; De Miccolis Angelini et al. 2015; Ma & Michailides, 2005). Another important aspect to consider is that, even when the resistance risk is low, as observed for tebuconazole in this study, proper fungicide management can help prevent the emergence of low sensitivity isolates within the field population.

The cross-sensitivity between imazalil and tebuconazole observed in this study is a common phenomenon among DMI fungicides. Since all DMIs share the same mode of action,

mutations or adaptive mechanisms that reduce sensitivity to one fungicide can often lead to resistance against others within the same chemical class (Ziogas & Malandrakis 2015)

The level of sensitivity of the *Lasiodiplodia* isolates S and LS to tebuconazole, as well as S isolates to imazalil, was maintained after 10 successive subcultures on free-fungicide medium. In contrast, LS isolates to imazalil exhibited a increase in sensitivity after 10 successive subcultures. Stability of fungicide resistance (or low sensitivity) is defined as the ability of the pathogen to retain the same level of fungicide insensitivity after successive generations of either exposure or no exposure to the target fungicide (Vega & Dewdney 2014). The stability of resistance is particularly crucial to judge whether growers can expect to reuse the fungicides in question after their withdrawal for some years (Ishii 2015). The findings of this study support the observation that the stability of DMI resistance (or low sensitivity) is variable (Ishii 2015), since the LS isolates to tebuconazole and imazalil presented different behaviors. Although isolates with moderate to high resistance levels to DMIs often show increased sensitivity over time (Ishii 2015), this pattern was observed only in LS isolates to imazalil, not in LS isolates to tebuconazole.

Although a significant difference was observed in the EC_{50} values of S and LS isolates for imazalil and tebuconazole, both fungicides effectively controlled S and LS isolates of *Lasiodiplodia* in artificially infected grape. In contrast, control efficacy tests on mango, papaya, and red mombin fruits confirmed the in vitro results, as both imazalil and tebuconazole were unable to control LS isolates. Therefore, the label rate of imazalil was less effective in controlling LS isolates with EC_{50} values between 7.30 and 21.31 μg mL^{-1}, while the label rate of tebuconazole was less effective against LS isolates with EC_{50} values between 0.44 and 1.37 μg mL^{-1} on fungicide-treated mango, papaya, and red mombin fruits. Presumably, isolates with EC_{50} values exceeding 21.31 μg mL^{-1} and 1.37 μg mL^{-1} are also more difficult to control using the label rates of imazalil and tebuconazole, respectively, compared to sensitive isolates.

The analysis of fitness-related variables, including mycelial growth rate and virulence in grape, mango, and red mombin, revealed no significant differences between S and LS isolates exposed to imazalil and tebuconazole, except for assay in papaya fruits, where LS isolates to imazalil demonstrated greater virulence. Thus, the findings suggest no evidence of a fitness cost for LS isolates to imazalil and tebuconazole. Fitness is a important factor in determining an isolate's ability to survive in the field. Resistant (or low-sensitive) isolates with little or no fitness cost are more likely to persist. In contrast, resistant isolates with a fitness cost are less likely to prevail, and in the absence of selection pressure, such as fungicide application, their prevalence may decrease or even vanish (Li et al. 2020; Mikaberidze & McDonald, 2015).

In this study, *Lasiodiplodia* isolates with LS to imazalil and tebuconazole exhibited increased growth ability under salt stress and may suggest a broader stress resistance phenotype that could contribute to their survival in treated orchards or other environments with high osmotic stress conditions. The potential adaptive advantage conferred by salt stress tolerance in fungicide-LS *Lasiodiplodia* isolates poses a challenge for disease control strategies. If low sensibility to imazalil and tebuconazole is associated with enhanced stress tolerance, these isolates may have a competitive advantage in environments where osmotic stress is prevalent, such as saline soils or drought-prone regions. This could lead to the persistence of LS populations even in the absence of fungicide selection pressure, making resistance management more complex.

The low sensitivity to imazalil and tebuconazole observed in some isolates in this study is particularly significant given that neither fungicide is currently registered for controlling *Lasiodiplodia*-induced diseases in grape, mango, papaya, or red mombin in Brazil. This resistance may have developed due to the exposure of *Lasiodiplodia* populations to fungicides commonly used for managing other diseases.

The evidence from this study suggests the potential existence of *Lasiodiplodia* isolates resistant to imazalil and tebuconazole. Therefore, further research is necessary to investigate the molecular mechanisms associated with their reduced sensitivity to these fungicides.

Our findings highlight the importance of regular monitoring to assess the occurrence and frequency of *Lasiodiplodia* isolates with low sensitivity to imazalil and tebuconazole. This approach is crucial for managing fungicide resistance and ensuring more effective disease control in grape, mango, papaya, and red mombin orchards in northeast of Brazil.

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Declarations

Conflicts of interest The authors declare no conflicts of interest.

ORCID

Raquel Maria da Silva (0000-0002-5503-2072)
Jadson Araújo da Silva (0000-0002-8571-3477)
Cosma Amanda Alves Pereira (0000-0002-3816-6407)
Germana Vitória Ricarto Moreira (0009-0000-5473-194X)
Wilson Pereira da Silva (0000-0002-7184-0328)
Ivna Ribeiro Salmito Melo (0000-0003-3515-0415)
Sami Jorge Michereff (0000-0002-2156-3502)
Kamila Câmara Correia (0000-0001-9113-9064)

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Table 2 List of isolates of *Lasiodiplodia* species from Brazilian northeast fruit orchards used in this study

Lasiodiplodia species	Host	Isolate code*
L. brasiliensis	Grapevine	CFC-0458; CFC-0468; CFC-1005; CFC-1012; CFC-1015; CFC-1019; CFC-1028; CFC-1035; CFC-1044; CFC-1051; CFC-1372; CFC-1405; CFC-1408; CFC-1415; CFC-1418; CFC-1424; CFC-1433; CFC-1434; CFC-1449; CFC-1450; CFC-1456; CFC-1459; CFC-1460
	Mango	CFC-0829; CFC-0832; CFC-0535
	Papaya	CFC-0536; CFC-0543; CFC-0565; CFC-0568; CFC-0571; CFC-0590; CFC-0626; CFC-0698; CFC-0706
	Red mombin	CFC-1154; CFC-1160; CFC-1163; CFC-1175; CFC-1176
L. euphorbiaceicola	Grapevine	CFC-0433; CFC-0448; CFC-0455; CFC-0473; CFC-1013; CFC-1018; CFC-1031; CFC-1395
	Red mombin	CFC-1132; CFC-1133; CFC-1134; CFC-1135; CFC-1136
L. hormozganensis	Grapevine	CFC-0443; CFC-1033; CFC-1369; CFC-1371; CFC-1373; CFC-1374; CFC-1440; CFC-1441; CFC-1445; CFC-1451; CFC-1454; CFC-1455; CFC-1461
	Mango	CFC-0814; CFC-0815; CFC-0816
	Papaya	CFC-0544; CFC-0545; CFC-0546; CFC-0547
L. iraniensis	Grapevine	CFC-1004; CFC-1008; CFC-1014; CFC-1016; CFC-1025; CFC-1032; CFC-1034; CFC-1417; CFC-1446; CFC-1463
	Mango	CFC-0819; CFC-0821; CFC-0843
	Red mombin	CFC-1126; CFC-1128; CFC-1131; CFC-1137; CFC-1139; CFC-1143; CFC-1144; CFC-1146; CFC-1148; CFC-1151; CFC-1156; CFC-1157; CFC-1158; CFC-1159; CFC-1161; CFC-1162; CFC-1164; CFC-1165; CFC-1166; CFC-1167; CFC-1168; CFC-1169; CFC-1170; CFC-1172; CFC-1173; CFC-1174; CFC-1177
L. pseudotheobromae	Grapevine	CFC-0438; CFC-0457; CFC-0469; CFC-0522; CFC-0527; CFC-1009; CFC-1023; CFC-1024; CFC-1037; CFC-1039; CFC-1040; CFC-1041; CFC-1043; CFC-1045; CFC-1046; CFC-1047; CFC-1050; CFC-1383; CFC-1384; CFC-1385; CFC-1388; CFC-1389; CFC-1392; CFC-1394; CFC-1396; CFC-1397; CFC-1403; CFC-1404; CFC-1411; CFC-1421; CFC-1425; CFC-1436; CFC-1437; CFC-1442; CFC-1443
	Mango	CFC-0824; CFC-0826; CFC-0827
	Papaya	CFC-0623; CFC-0624

*Isolate code from the Culture Collection of Phytopathogenic Fungi of Cariri (CFC) at the Federal University of Cariri (Crato, Ceará, Brazil)

Table 3 List of isolates of *Lasiodiplodia* species from fruit orchards selected in this study showing the lowest (sensitive - S) and highest (less sensitive - LS) EC_{50} values for imazalil and tebuconazole

Lasiodiplodia species	Isolate code*	Host	EC_{50} (μg a.i. mL^{-1})			
			Imazalil		Tebuconazole	
			S	LS	S	LS
L. brasiliensis	CFC-0698	Papaya	–	7.30	–	–
	CFC-0706	Papaya	0.30	–	0.02	–
	CFC-0832	Mango	0.32	–	–	–
	CFC-1028	Grapevine	–	–	–	0.44
	CFC-1175	Red mombin	–	8.10	–	–
	CFC-1434	Grapevine	–	9.33	–	–
L. euphorbiaceicola	CFC-1013	Grapevine	–	–	–	1.37
	CFC-1018	Grapevine	–	21.31	–	1.05
L. hormozganensis	CFC-0443	Grapevine	0.05	–	–	–
L. iraniensis	CFC-0819	Mango	0.47	–	–	–
	CFC-1008	Grapevine	–	–	0.004	–
	CFC-1139	Red mombin	–	9.37	–	–
	CFC-1170	Red mombin	0.49	–	–	–
L. pseudotheobromae	CFC-0438	Grapevine	–	–	0.009	–
	CFC-0527	Grapevine	–	–	0.01	–
	CFC-1421	Grapevine	–	–	–	0.59
	CFC-1425	Grapevine	–	–	0.005	–
	CFC-1437	Grapevine	–	–	–	0.46

*Isolate code from the Culture Collection of Phytopathogenic Fungi of Cariri (CFC) at the Federal University of Cariri (Crato, Ceará, Brazil)

Source: elaborated by the author (2025)

Table 4 Sensitivity to imazalil and tebuconazole of sensitive and less sensitive isolates of *Lasiodiplodia* species from fruit orchards

Isolate class*	EC_{50} (µg a.i. mL^{-1})**	
	Imazalil	Tebuconazole
Sensitive	0.33 (0.05–0.49) b	0.01 (0.004–0.02) b
Less sensitive	11.08 (7.30–21.31) a	0.78 (0.44–1.37) a

*Each class is composed of five isolates, selected by the lowest and highest EC_{50} values for imazalil and tebuconazole

**Values (µg a.i. mL^{-1}) are the means of two independent experiments because no heterogeneity was detected between them according to Levens's test (P>0.05). Averages followed by the same letter in the column do not differ significantly according to Student's t-test (P=0.05). Values in the parentheses represent the range of EC_{50} values

Source: elaborated by the author (2025)

Table 5 Stability of sensitivity to imazalil and tebuconazole of sensitive and less sensitive isolates of *Lasiodiplodia* species from fruit orchards based on the comparison between the initial (T_0) effective concentration required to inhibit 50% of the mycelial growth (EC_{50}) and the following 10 sequential transfers on fungicide-free medium (T_{10})

Isolate class*	EC_{50} (µg a.i. mL^{-1})**			
	Imazalil		Tebuconazole	
	T_0	T_{10}	T_0	T_{10}
Sensitive	0.33 a	0.34 a	0.01 a	0.01 a
Less sensitive	11.08 a	2.88 b	0.80 a	0.82 a

*Each class is composed of five isolates, selected by the lowest and highest EC_{50} values for imazalil and tebuconazole

**Values (µg a.i. mL^{-1}) are the means of two independent experiments because no heterogeneity was detected between them according to Levens's test ($P>0.05$). Averages followed by the same letter in the line do not differ significantly according to Student's t-test ($P=0.05$)

Source: elaborated by the author (2025)

Table 6 Fitness components between isolates of *Lasiodiplodia* species from fruit orchards that are sensitive and less sensitive to imazalil and tebuconazole

Isolate class*	MGR (mm h-^{1})**	$EC_{50}N$ (%NaCl)**	Lesion diameter (mm)**			
			Grape	Mango	Papaya	Red mombin
	Imazalil					
Sensitive	0.43 a	0.41 b	13.14 a	23.00 a	14.98 b	13.60 a
Less sensitive	0.48 a	1.15 a	13.17 a	23.47 a	19.55 a	14.75 a
	Tebuconazole					
Sensitive	0.29 a	0.67 b	13.93 a	13.56 a	13.56 a	13.48 a
Less sensitive	0.30 a	2.28 a	12.76 a	12.80 a	12.80 a	13.73 a

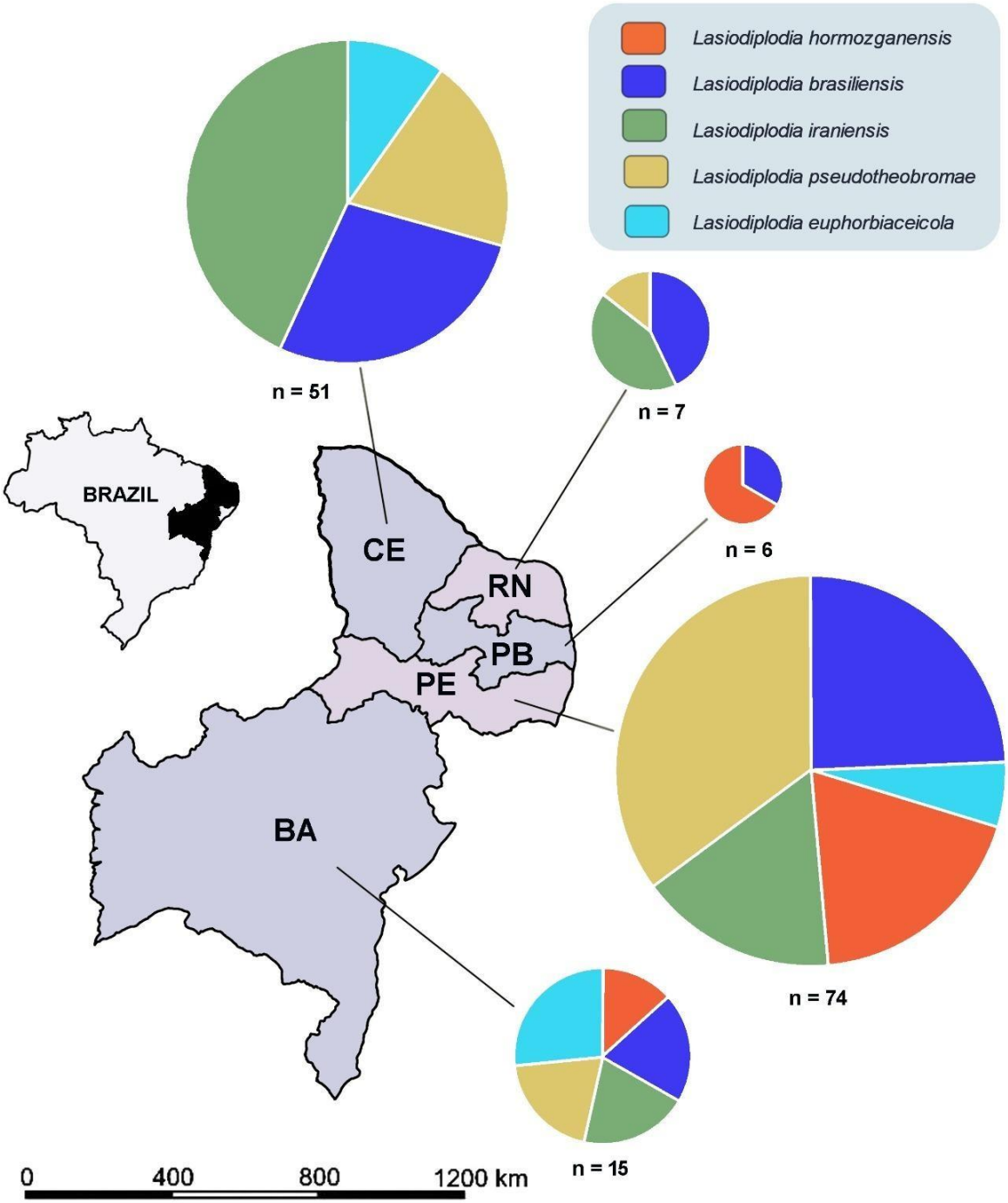
MGR: Mycelial growth rate in fungicide-free PDA medium; $EC_{50}N$: osmotic sensitivity

*Each class is composed of five isolates, selected by the lowest and highest EC_{50} values for imazalil and tebuconazole

**Values are the means of two independent experiments because no heterogeneity was detected between them according to Levene's test ($P>0.05$). Averages followed by the same letter in the column, inside each fungicide, do not differ significantly according to Student's t-test ($P=0.05$)

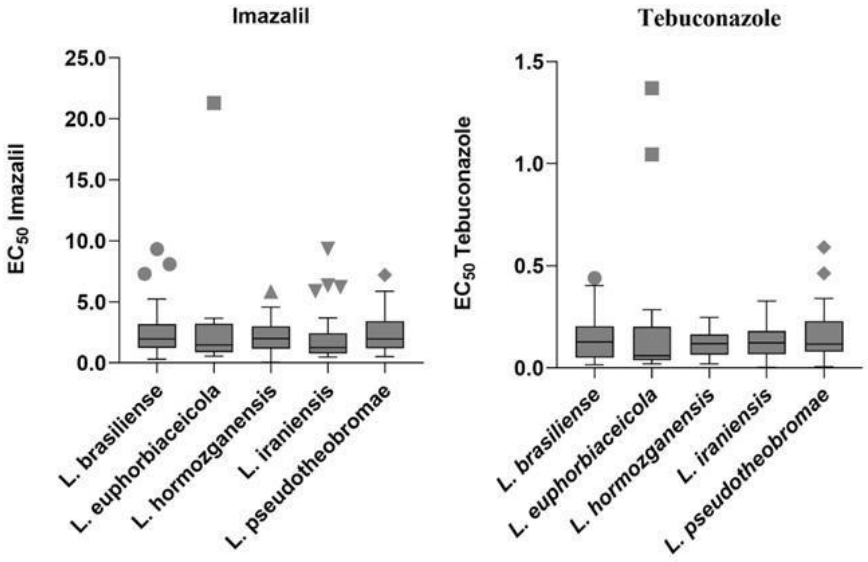
Source: elaborated by the author (2025)

Fig. 1 Sampling locations of *Lasiodiplodia* isolates associated with diseases in grape, mango, papaya, and red mombin across the states of Bahia (BA), Ceará (CE), Pernambuco (PE), and Rio Grande do Norte (RN) in Northeastern Brazil. Circles represent association frequency of each species and n is the number of isolates in each population



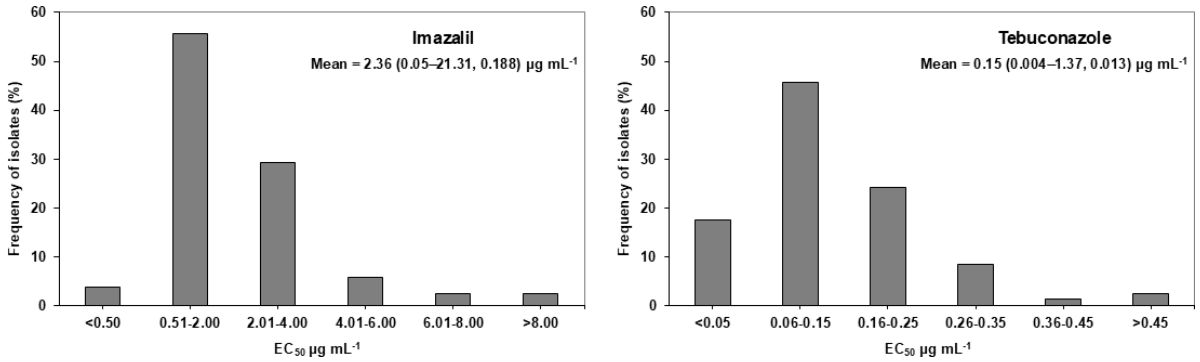
Source: elaborated by the author (2025)

Fig. 2 Box plot of EC_{50} values from isolates of *Lasiodiplodia* species from fruit orchards to imazalil and tebuconazole fungicides



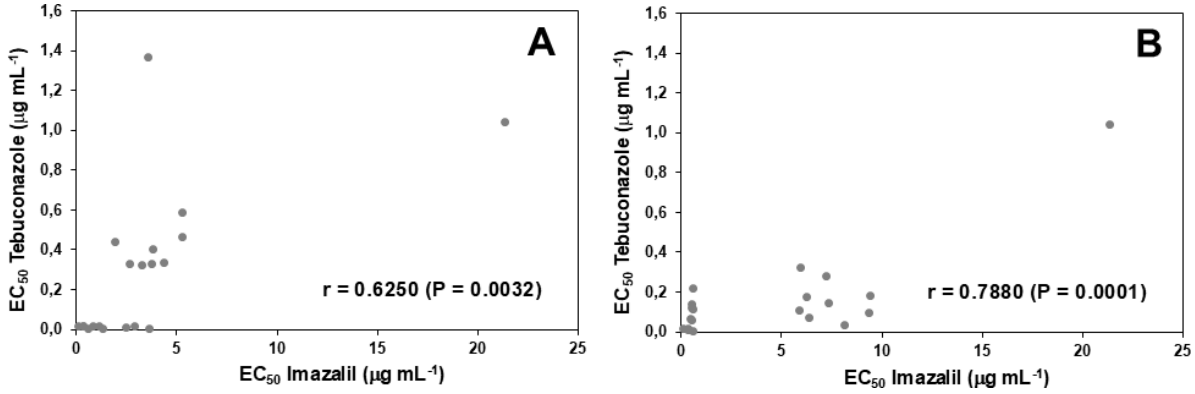
Source: elaborated by the author (2025)

Fig. 3 Frequency distribution of effective imazalil and tebuconazole concentrations required to inhibit 50% of the mycelial growth (EC_{50}) of 153 isolates of *Lasiodiplodia* species collected from Brazilian northeast fruit orchards. Values in the parentheses represent the range of EC_{50} values followed by the standard error



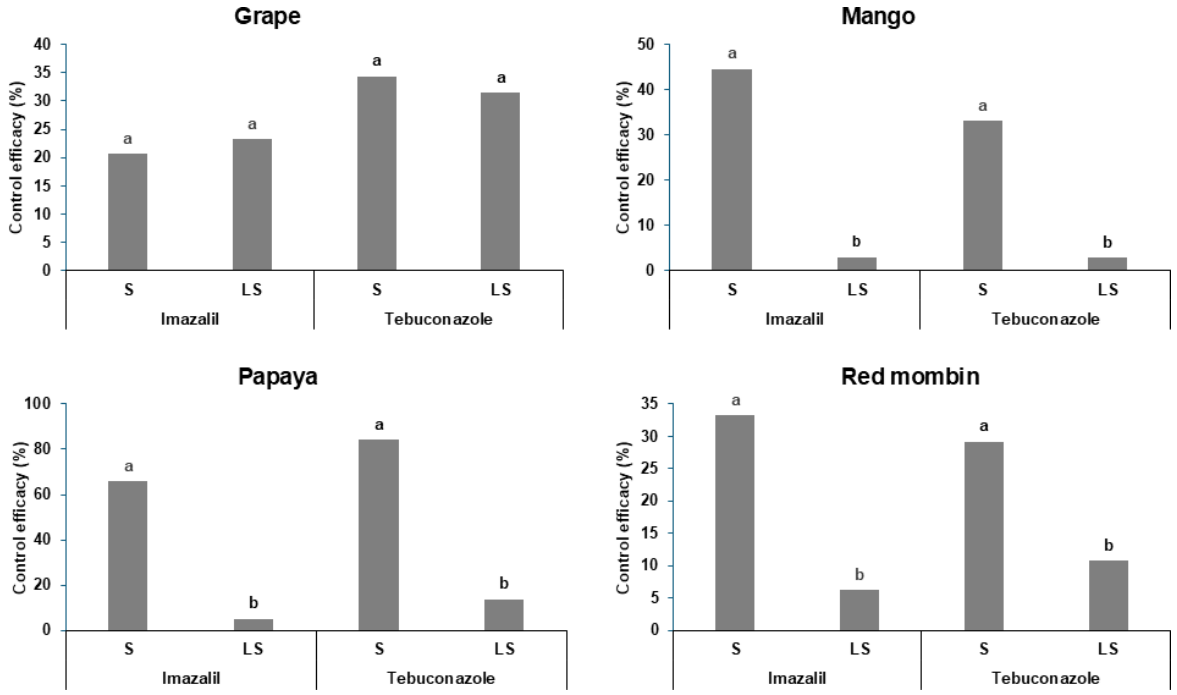
Source: elaborated by the author (2025)

Fig. 4 Cross-resistance between imazalil and tebuconazole in *Lasiodiplodia* species from fruit orchards evaluated by imazalil less sensible (A) and tebuconazole less sensible isolates (B). Each point represents one isolate (n = 20)



Source: elaborated by the author (2025)

Fig. 5 Control efficacy on grape, mango, papaya, and red mombin fruits treated with imazalil and tebuconazole prior to inoculation with sensitive (S) and less sensitive (LS) isolates of *Lasiodiplodia* species collected from Brazilian northeast fruit orchards. Columns with same letter, inside each fungicide, do not differ significantly according to Student's t-test (P=0.05)



Source: elaborated by the author (2025)

Supplementary Table 1 List of isolates of *Lasiodiplodia* species from Brazilian northeast fruit orchards used in this study

Isolate code*	*Lasiodiplodia* species	Municipality – State**	Original host
CFC-0433	*L. euphorbiaceicola*	Casa Nova - BA	Grapevine
CFC-0438	*L. pseudotheobromae*	Petrolina - PE	Grapevine
CFC-0443	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-0448	*L. euphorbiaceicola*	Juazeiro - BA	Grapevine
CFC-0455	*L. euphorbiaceicola*	Casa Nova - BA	Grapevine
CFC-0457	*L. pseudotheobromae*	Petrolina - PE	Grapevine
CFC-0458	*L. brasiliensis*	Petrolina - PE	Grapevine
CFC-0468	*L. brasiliensis*	Petrolina - PE	Grapevine
CFC-0469	*L. pseudotheobromae*	Casa Nova - BA	Grapevine
CFC-0473	*L. euphorbiaceicola*	Petrolina - PE	Grapevine
CFC-0522	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-0527	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-0535	*L. brasiliensis*	São José do Mipibú - RN	Papaya
CFC-0536	*L. brasiliensis*	Santa Rita - PB	Papaya
CFC-0543	*L. brasiliensis*	Conde - PB	Papaya
CFC-0544	*L. hormozganensis*	Conde - PB	Papaya
CFC-0545	*L. hormozganensis*	Conde - PB	Papaya
CFC-0546	*L. hormozganensis*	Conde - PB	Papaya
CFC-0547	*L. hormozganensis*	Conde - PB	Papaya
CFC-0565	*L. brasiliensis*	Quixeré - CE	Papaya
CFC-0568	*L. brasiliensis*	Quixeré - CE	Papaya
CFC-0571	*L. brasiliensis*	Quixeré - CE	Papaya
CFC-0590	*L. brasiliensis*	Goiana - PE	Papaya
CFC-0623	*L. pseudotheobromae*	Goiana - PE	Papaya
CFC-0624	*L. pseudotheobromae*	Goiana - PE	Papaya
CFC-0626	*L. brasiliensis*	Mossoró - RN	Papaya
CFC-0698	*L. brasiliensis*	Petrolina - PE	Papaya
CFC-0706	*L. brasiliensis*	Petrolina - PE	Papaya
CFC-0814	*L. hormozganensis*	Petrolina - PE	Mango
CFC-0815	*L. hormozganensis*	Petrolina - PE	Mango
CFC-0816	*L. hormozganensis*	Juazeiro - BA	Mango
CFC-0819	*L. iraniensis*	Ipanguaçu - RN	Mango
CFC-0821	*L. iraniensis*	Ipanguaçu - RN	Mango
CFC-0824	*L. pseudotheobromae*	Juazeiro - BA	Mango
CFC-0826	*L. pseudotheobromae*	Ipanguaçu - RN	Mango
CFC-0827	*L. pseudotheobromae*	Juazeiro - BA	Mango
CFC-0829	*L. brasiliensis*	Afonso Bezerra - RN	Mango
CFC-0832	*L. brasiliensis*	Petrolina - PE	Mango
CFC-0843	*L. iraniensis*	Ipanguaçu - RN	Mango
CFC-1004	*L. iraniensis*	Petrolina - PE	Grapevine
CFC-1005	*L. brasiliensis*	Petrolina - PE	Grapevine
CFC-1008	*L. iraniensis*	Casa Nova - BA	Grapevine
CFC-1009	*L. pseudotheobromae*	Petrolina - PE	Grapevine
CFC-1012	*L. brasiliensis*	Casa Nova - BA	Grapevine
CFC-1013	*L. euphorbiaceicola*	Casa Nova - BA	Grapevine
CFC-1014	*L. iraniensis*	Petrolina - PE	Grapevine
CFC-1015	*L. brasiliensis*	Casa Nova - BA	Grapevine
CFC-1016	*L. iraniensis*	Petrolina - PE	Grapevine
CFC-1018	*L. euphorbiaceicola*	Petrolina - PE	Grapevine

CFC-1019	*L. brasiliensis*	Petrolina - PE	Grapevine
CFC-1023	*L. pseudotheobromae*	Petrolina - PE	Grapevine
CFC-1024	*L. pseudotheobromae*	Petrolina - PE	Grapevine
CFC-1025	*L. iraniensis*	Petrolina - PE	Grapevine
CFC-1028	*L. brasiliensis*	Casa Nova - BA	Grapevine
CFC-1031	*L. euphorbiaceicola*	Petrolina - PE	Grapevine
CFC-1032	*L. iraniensis*	Casa Nova - BA	Grapevine
CFC-1033	*L. hormozganensis*	Casa Nova - BA	Grapevine
CFC-1034	*L. iraniensis*	Casa Nova - BA	Grapevine
CFC-1035	*L. brasiliensis*	Petrolina - PE	Grapevine
CFC-1037	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1039	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1040	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1041	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1043	*L. pseudotheobromae*	Russas - CE	Grapevine
CFC-1044	*L. brasiliensis*	Russas - CE	Grapevine
CFC-1045	*L. pseudotheobromae*	Russas - CE	Grapevine
CFC-1046	*L. pseudotheobromae*	Russas - CE	Grapevine
CFC-1047	*L. pseudotheobromae*	Russas - CE	Grapevine
CFC-1050	*L. pseudotheobromae*	Russas - CE	Grapevine
CFC-1051	*L. brasiliensis*	Russas - CE	Grapevine
CFC-1126	*L. iraniensis*	Juazeiro do Norte - CE	Red mombin
CFC-1128	*L. iraniensis*	Juazeiro do Norte - CE	Red mombin
CFC-1131	*L. iraniensis*	Juazeiro do Norte - CE	Red mombin
CFC-1132	*L. euphorbiaceicola*	Crato - CE	Red mombin
CFC-1133	*L. euphorbiaceicola*	Crato - CE	Red mombin
CFC-1134	*L. euphorbiaceicola*	Crato - CE	Red mombin
CFC-1135	*L. euphorbiaceicola*	Crato - CE	Red mombin
CFC-1136	*L. euphorbiaceicola*	Crato - CE	Red mombin
CFC-1137	*L. iraniensis*	Santana do Cariri - CE	Red mombin
CFC-1139	*L. iraniensis*	Santana do Cariri - CE	Red mombin
CFC-1143	*L. iraniensis*	Santana do Cariri - CE	Red mombin
CFC-1144	*L. iraniensis*	Santana do Cariri - CE	Red mombin
CFC-1146	*L. iraniensis*	Barro - CE	Red mombin
CFC-1148	*L. iraniensis*	Crato - CE	Red mombin
CFC-1151	*L. iraniensis*	Moreilândia - PE	Red mombin
CFC-1154	*L. brasiliensis*	Moreilândia - PE	Red mombin
CFC-1156	*L. iraniensis*	Moreilândia - PE	Red mombin
CFC-1157	*L. iraniensis*	Moreilândia - PE	Red mombin
CFC-1158	*L. iraniensis*	Moreilândia - PE	Red mombin
CFC-1159	*L. iraniensis*	Moreilândia - PE	Red mombin
CFC-1160	*L. brasiliensis*	Moreilândia - PE	Red mombin
CFC-1161	*L. iraniensis*	Moreilândia - PE	Red mombin
CFC-1162	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1163	*L. brasiliensis*	Mauriti - CE	Red mombin
CFC-1164	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1165	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1166	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1167	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1168	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1169	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1170	*L. iraniensis*	Mauriti - CE	Red mombin
CFC-1172	*L. iraniensis*	Barro - CE	Red mombin
CFC-1173	*L. iraniensis*	Barro - CE	Red mombin
CFC-1174	*L. iraniensis*	Barro - CE	Red mombin

CFC-1175	*L. brasiliensis*	Juazeiro do Norte - CE	Red mombin
CFC-1176	*L. brasiliensis*	Caririaçu - CE	Red mombin
CFC-1177	*L. iraniensis*	Caririaçu - CE	Red mombin
CFC-1369	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-1371	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-1372	*L. brasiliensis*	Petrolina - PE	Grapevine
CFC-1373	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-1374	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-1383	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1384	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1385	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1388	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1389	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1392	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1394	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1395	*L. euphorbiaceicola*	São Vicente Férrer - PE	Grapevine
CFC-1396	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1397	*L. pseudotheobromae*	São Vicente Férrer - PE	Grapevine
CFC-1403	*L. pseudotheobromae*	Machados - PE	Grapevine
CFC-1404	*L. pseudotheobromae*	Machados - PE	Grapevine
CFC-1405	*L. brasiliensis*	Machados - PE	Grapevine
CFC-1408	*L. brasiliensis*	Barbalha - CE	Grapevine
CFC-1411	*L. pseudotheobromae*	Barbalha - CE	Grapevine
CFC-1415	*L. brasiliensis*	Barbalha - CE	Grapevine
CFC-1417	*L. iraniensis*	Barbalha - CE	Grapevine
CFC-1418	*L. brasiliensis*	Barbalha - CE	Grapevine
CFC-1421	*L. pseudotheobromae*	Barbalha - CE	Grapevine
CFC-1424	*L. brasiliensis*	Barbalha - CE	Grapevine
CFC-1425	*L. pseudotheobromae*	Barbalha - CE	Grapevine
CFC-1433	*L. brasiliensis*	Barbalha - CE	Grapevine
CFC-1434	*L. brasiliensis*	Mauriti - CE	Grapevine
CFC-1436	*L. pseudotheobromae*	Mauriti - CE	Grapevine
CFC-1437	*L. pseudotheobromae*	Mauriti - CE	Grapevine
CFC-1440	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-1441	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-1442	*L. pseudotheobromae*	Petrolina - PE	Grapevine
CFC-1443	*L. pseudotheobromae*	Petrolina - PE	Grapevine
CFC-1445	*L. hormozganensis*	Petrolina - PE	Grapevine
CFC-1446	*L. iraniensis*	Petrolina - PE	Grapevine
CFC-1449	*L. brasiliensis*	Petrolina - PE	Grapevine
CFC-1450	*L. brasiliensis*	Lagoa Grande - PE	Grapevine
CFC-1451	*L. hormozganensis*	Lagoa Grande - PE	Grapevine
CFC-1454	*L. hormozganensis*	Lagoa Grande - PE	Grapevine
CFC-1455	*L. hormozganensis*	Lagoa Grande - PE	Grapevine
CFC-1456	*L. brasiliensis*	Lagoa Grande - PE	Grapevine
CFC-1459	*L. brasiliensis*	Lagoa Grande - PE	Grapevine
CFC-1460	*L. brasiliensis*	Lagoa Grande - PE	Grapevine
CFC-1461	*L. hormozganensis*	Lagoa Grande - PE	Grapevine
CFC-1463	*L. iraniensis*	Lagoa Grande - PE	Grapevine

*Isolate code from the Culture Collection of Phytopathogenic Fungi of Cariri (CFC) at the Federal University of Cariri (Crato, Ceará, Brazil)

** BA = Bahia, CE = Ceará, PB = Paraíba, PE = Pernambuco e RN = Rio Grande do Norte
Source: elaborated by the author (2025)

CAPÍTULO III

ARTIGO 2

Thiabenazole and thiophanate-methyl sensitivity in five *Lasiodiplodia* species isolated from fruit orchards in Northeast Brazil

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Thiabenazole and thiophanate-methyl sensitivity in five *Lasiodiplodia* species isolated from fruit orchards in Northeast Brazil

Raquel Maria da Silva[1] • Jadson Araújo da Silva[2] • Walisson Ferreira da Silva[1] • Rafael Luis de Oliveira[3] • Damiana Andressa Alves Pereira[3] • Rodrigo Marques da Costa[2] • Sami Jorge Michereff[3] • Kamila Câmara Correia[3]

[1] Postgraduate Program in Plant Protection, Federal University of Alagoas, 57100-000 Rio Largo, Alagoas, Brazil

[2] Postgraduate Program in Phytopathology, Federal Rural University of Pernambuco, 52171-900 Recife, Pernambuco, Brazil

[3] Center for Agricultural Sciences and Biodiversity, Federal University of Cariri, 63133-610 Crato, Ceará, Brazil

(✉) Kamila Câmara Correia
kamila.correia@ufca.edu.br

Abstract

The Northeast region of Brazil is an important producer and exporter of tropical fruits. Diseases caused by *Lasiodiplodia* species are particularly significant in this region, affecting crops such as grapevine, mango, papaya, and red mombin. While *L. theobromae* is the most prevalent species, others like *L. brasiliensis*, *L. euphorbiaceicola*, *L. hormozganensis*, *L. iraniensis*, and *L. pseudotheobromae* are also associated with these diseases. Chemical control is frequently employed in grapevine, mango, and papaya orchards to manage various diseases; however, there is limited information on the fungicide sensitivity and fitness of isolates from these lesser-known *Lasiodiplodia* species. This study aimed to evaluate the sensitivity and fitness of five *Lasiodiplodia* species isolates from fruit orchards in Northeastern Brazil to the fungicides thiabendazole and thiophanate-methyl. The sensitivity of 153 *Lasiodiplodia* spp. isolates from grape, mango, papaya, and red mombin to thiabendazole and thiophanate-methyl was evaluated by determining the effective concentration required to inhibit 50% of mycelial growth (EC_{50}). Five isolates, representing the highest sensitive (S) and lowest sensitive (LS) based on EC_{50} values for both fungicides, were then selected for further analysis. These isolates were assessed

for sensitivity stability, fungicide efficacy in controlling S and LS isolates on detached fruits, and fitness components, including mycelial growth, osmotic sensitivity, and virulence. In general, sensitivity to thiophanate-methyl (mean EC_{50} = 0.34 μg/ml) was greater than to thiabendazole (mean EC_{50} = 1.15 μg/ml). No cross-sensitivity was found between fungicides. Isolates S and LS to both fungicides showed stability of sensitivity levels throughout consecutive growth cycles. Grapes treated with thiabendazole, and red mombin treated with either thiabendazole or thiophanate-methyl, showed no significant reduction in disease severity compared to untreated fruits. Fitness costs were observed in thiabendazole LS isolates, as they exhibited lower virulence in papaya fruits compared to S isolates. Thiophanate-methyl LS isolates demonstrated a greater capacity for growth under salt stress than S isolates. The findings of this study suggest the potential presence of *Lasiodiplodia* isolates from grape, mango, papaya, and red mombin orchards in Northeastern Brazil that are resistant to thiabendazole and thiophanate-methyl.

Keywords Botryosphaeriaceae • Fruit diseases • MBC fungicide • Fungicide resistance • Fitness

Introduction

The fruit production sector is a prominent segment in the national economy, as it drives Brazil towards economic and global integration, being an emerging country in commercial expansion and one of the largest agricultural producers in the world (Carvalho et al. 2024). Mango (*Mangifera indica*), papaya (*Carica papaya*), and grapevine (*Vitis vinifera*) are important cash crops in this country, as they have relevant representation in the export commodities (ABRAFRUTAS 2025). But there is also a significant production of fruits destined exclusively for the domestic market, such as the red mombin (*Spondias purpurea*), which are socially and economically important for the subsistence of small producers (Fonseca et al. 2017).

Tropical fruit trees are susceptible to numerous diseases, with those caused by *Lasiodiplodia* species being particularly significant in Northeastern Brazil, especially in grapevine, mango, papaya, and red mombin. While *L. theobromae* is the most prevalent species, other species such as *L. brasiliensis*, *L. euphorbiaceicola*, *L. hormozganensis*, *L. iraniensis*, and *L. pseudotheobromae* are also frequently associated with diseases in these crops (Correia et al. 2016; Marques et al. 2013; Nascimento 2023; Netto et al. 2014; Souza 2022).

Species of *Lasiodiplodia* can induce a range of symptoms in plants, depending on the host and environmental conditions. Some of the most common symptoms in perennial fruits crops include stem canker, gummosis, dieback, and postharvest fruit decay (Coutinho 2017).

Chemical control has been frequently used in grapevine, mango, and papaya orchards in Northeastern Brazil to control various diseases (AGROFIT 2025; Cavalcante et al. 2014; Chen et al. 2020; Li et al. 2020; Pereira et al. 2012; Santos et al. 2019; Silva et al. 2022). Only the active ingredients difenoconazole (triazole; demethylation inhibitor group – DMI) and the combination of copper hydroxide + copper oxychloride (both inorganic group) are registered for *Lasiodiplodia* control in mango. In papaya, flutriafol (triazole, DMI) and thiabendazole (benzimidazole; methyl benzimidazole carbamate group - MBC) are registered, with the latter being exclusively approved for postharvest application. No fungicides are currently registered for the control of *Lasiodiplodia* in grapevines and red mombin (AGROFIT, 2025). While thiabendazole is the only fungicide of the MBC group registered for postharvest treatment of fruits (AGROFIT, 2025), another MBC group fungicide, thiophanate-methyl, is registered and commonly applied to fruit orchards in Northeast Brazil to control of various diseases in grapevine, mango, and papaya (Cavalcante et al. 2014; Chen et al. 2020; Santos et al. 2019; Silva et al. 2022).

In some situations, farmers have warned about the low effectiveness of products over the years, especially some systemic fungicides. A decline in fungicide sensitivity is one of the key factors compromising the effectiveness of chemical disease control in plants. This decrease in sensitivity is frequently observed following the introduction of fungicides with a single mode of action (Ceresini et al. 2024; Corkley, Fraaije and Hawkins 2022; Hollomon 2015; Yin et al. 2023). MBCs are fungicides with a single-site mode of action, acting directly on the β-tubulin genes, disrupting mitosis and, consequently, the development of fungi (Young 2015). Fungicides of this group are considered high risk for developing pathogen resistance (FRAC 2024).

Although thiophanate-methyl is not used in the field to control *Lasiodiplodia*, populations of this fungus are exposed to the fungicide. This incidental exposure of populations to thiophanate-methyl provides the selective pressure for fungicide resistance to evolve.

Fungicide resistance is a stable and heritable adaptation in fungi that leads to a reduced sensitivity to fungicide doses that were previously effective for control (Hollomon 2015). However, resistance may come with a fitness cost (Hawkins and Fraaije 2018; Mikaberidze and Mcdonald 2015). Fitness refers to a fungal isolate's ability to grow, reproduce, survive, and cause disease. Key fitness components assessed in plant pathogen populations to determine the

cost of resistance include mycelial growth, reproductive potential, and virulence (Mikaberidze and Mcdonald 2015; Milgroom 2015).

Limited research has been conducted on the sensitivity of *Lasiodiplodia* isolates from mango, papaya, and grapevine orchards from Northeastern Brazil to thiabendazole and thiophanate-methyl. The sensitivity of 120 isolates of *L. theobromae* from papaya to thiabendazole was evaluated, revealing that 8.4% of the isolates exhibited no growth inhibition at the highest tested concentration (100 μg a.i./ml), indicating resistance to the fungicide. However, no evidence of fitness costs was observed in these isolates (Pereira et al. 2012). In another study, the sensitivity of 109 isolates of *L. theobromae* from papaya to thiophanate-methyl was assessed, showing that 20.2% of the isolates were resistant, with EC_{50} values exceeding 300 μg/ml. Resistant isolates displayed a significantly lower sporulation capacity compared to sensitive isolates, suggesting a fitness cost associated with resistance (Cavalcante et al. 2014).

Only one study was performed with other species of *Lasiodiplodia*, besides *L. theobromae*. In this study, the sensitivity of 154 *Botryosphaeriaceae* isolates from mango, including *Lasiodiplodia hormozganensis* (5 isolates), *L. iraniensis* (14), *L. pseudotheobromae* (13), and *L. theobromae* (29), was assessed for thiabendazole and thiophanate-methyl. EC_{50} values ranged from 0.03 to 6.86 μg/ml for thiabendazole and from 0.0001 to 10.70 μg/ml for thiophanate-methyl. A significant positive correlation was observed between the sensitivity of isolates to both fungicides. The less sensitive isolates retained their reduced sensitivity after 10 generations without exhibiting fitness costs compared to sensitive isolates. Both fungicides effectively controlled infections caused by sensitive and less sensitive isolates similarly (Santos et al. 2019).

The study of fungicide sensitivity and fitness in populations of *Lasiodiplodia* species, aside from *L. theobromae*, is crucial for developing effective disease management strategies. Therefore, the aims of this study were: (a) to assess the sensitivity of five *Lasiodiplodia* species isolates, collected from grapevine, mango, papaya, and red mombin orchards in Brazil's Northeast, to the fungicides thiabendazole and thiophanate-methyl; (b) to investigate the presence of cross-sensitivity between these fungicides; (c) to examine the stability of fungicide sensitivity in both sensitive and less sensitive isolates; (d) to evaluate the fungicides' effectiveness in controlling both sensitive and less sensitive isolates on detached fruits; and (e) to explore the relationship between fungicide sensitivity and fitness-related factors.

Materials and methods

Fungal isolates

A total of 153 isolates of *Lasiodiplodia* were obtained from the Culture Collection of Phytopathogenic Fungi of Cariri (CFC) at the Federal University of Cariri (Crato, Ceará, Brazil), representing five species: *L. brasiliensis* (40), *L. euphorbiaceicola* (13), *L. hormozganensis* (20), *L. iraniensis* (40), and *L. pseudotheobromae* (40). The isolates were obtained from grapevine (89), mango (11), papaya (16), and red mombin (37) orchards located in the Brazilian Northeast (Table 7). Grapevine, mango, and papaya orchards received at least one spray application with thiophanate-methyl at two consecutive seasons before fruit sampling and isolation. Red mombin orchards were not sprayed with fungicides. The isolates were previously identified by phylogenetic inference based on the partial sequences of the elongation factor 1-α gene (EF1-α) and complete sequence of the internal transcript space (ITS) (Correia et al. 2016; Costa et al. 2010; Marques et al. 2013; Netto et al. 2014; Souza 2022). Stock cultures were stored in potato dextrose agar (PDA) slants at 5 °C in the dark.

In vitro sensitivity to thiabendazole and thiophanate-methyl and

Commercial formulation of thiabendazole (Tecto SC, 485 g a.i./kg, Syngenta Crop Protection, São Paulo, SP, Brazil) and thiophanate-methyl (Cercobin 700 WP, 700 g/kg active ingredient (a.i.), Iharabras, Sorocaba, SP, Brazil) were evaluated by a mycelial growth assay. Fungicides were solubilized in sterile distilled water and added to molten (45 °C) PDA at final concentrations of 0.01, 0.5, 1, 2, 4, 8 and 16 μg a.i./ml for thiophanate-methyl, and 0.01, 0.05, 0.1; 0.3, 0.5, 1, 3 e 5 μg a.i./ml for tiabendazole. Mycelial plugs (5 mm in diameter) were removed from the margins of 4-day-old cultures of *Lasiodiplodia* isolates and transferred to the PDA plates amended with the different fungicide concentrations. Petri plates containing medium without fungicide were used as controls. Three replicates of each isolate were used to test each fungicide concentration and control. After a 48-h incubation period at 30 °C in the dark, the length of each colony was measured, and the original mycelial plug diameter (5 mm) was subtracted from this measurement. The percentage of mycelial growth inhibition related to the control was calculated for all the fungicide concentrations. The effective fungicide concentration (μg/ml) to inhibit 50% of mycelial growth (EC_{50}) was calculated for individual isolates by linear regression of the mycelial growth inhibition versus the $\log_{10}$ transformation

of the fungicide concentration for all isolate × fungicide combinations. Frequency distributions of the isolates between the intervals of EC_{50} values were established. The five most-sensitive (S) and the five least-sensitive (LS) isolates were grouped and used in subsequent tests (Table 8).

The investigation of cross-sensitivity between thiabendazole and thiophanate-methyl was conducted by linear correlation analyses through two combinations. The first combination involved assessing thiabendazole sensitivity with 10 thiophanate-methyl-LS and 10 thiophanate-methyl-S isolates. The second combination focused on evaluating thiophanate-methyl sensitivity using 10 thiabendazole-LS and 10 thiabendazole-S isolates.

Stability of the sensitivity to thiabendazole and thiophanate-methyl

The stability of the sensitivity of S and LS isolates to thiophanate-methyl and thiabendazole was evaluated by assessing the mycelial growth after 10 sequential transfers. Mycelial plugs (5mm in diameter) were transferred to fungicide-free PDA every 7 days, and EC_{50} was calculated before the first transfer (T0) and after 10 transfers (T10). The EC_{50} was calculated as described above.

Efficacy of thiabendazole and thiophanate-methyl in controlling S and LS isolates in fruits

The efficacy of thiophanate-methyl and thiabendazole to control S and LS isolates of *Lasiodiplodia* was evaluated in fruits of grape (cv. Itália), mango (cv. Tommy Atkins), papaya (cv. Golden), and red mombin (cv. Doce Vermelha) at maturation stage. The fruits were washed in distilled water containing dishwashing liquid to eliminate adhering materials from the surface and rinsed abundantly with distilled water. Subsequently, the surface was disinfected by immersing the fruit in 1% NaOCl for 5 min, followed by two rinses with distilled water, and the fruit were kept on a clean surface until dry. In grape and red mombin, the fruit epidermis was punctured at the central area using a sterilized pin, creating lesions 2 mm deep. In mango and papaya, two plugs measuring 5 mm in diameter and 2 mm in depth were removed from opposite sides of the fruit using a cork borer, as described by Lin *et al*. (2020). Doses of each fungicide were prepared according to the manufacturer's recommended field rates in Brazil and sprayed onto the fruit using a spray bottle. Cercobin 700 WP was applied at 1 g/l for mango, papaya, and red mombin, where a dose of 0.7 g/l was used for grape. Tecto SC was applied at a uniform dose of 1 ml/l across all fruits. Control fruits were sprayed with sterile distilled water.

Fruits were allowed to dry for 3 h at 28 °C. The same size mycelium plugs taken from the margins of 4-day-old colonies of S and LS isolates were placed upside down on each lesion site (grape and red mombin) or inserted where the fruit plugs were removed (mango and papaya). Five replicates containing three fruits were used for each fruit species. After inoculation, the plastic boxes were sealed with plastic bags to keep the relative humidity close to 100% at room temperature (~28 °C). Lesion diameters (LD; mm) were measured after 3 days using a digital caliper. The experiment was conducted twice independently in a randomized complete block design.

Fitness-related variables

The following fitness components were determined for thiophanate-methyl and thiabendazole LS and S isolates of *Lasiodiplodia*: (a) mycelial growth rate, (b) osmotic sensitivity, and (c) virulence. All fitness assays were conducted using five LS and five S isolates to the fungicides. The experiments were conducted twice independently in a randomized complete block design.

Mycelial growth rate. Five-millimeter-diameter plugs were taken from the edge of 4-day-old colonies and transferred to PDA plates. Each isolate was plated to three replicate plates and incubated in the dark at 28 °C. The colony length was measured at 24 h and 48 h, and the average was used to calculate the mycelial growth rate (mm/h).

Osmotic sensitivity. Five-millimeter-diameter plugs were taken from the expanding margins of 4-day-old colonies grown on PDA and transferred to PDA plates amended with 0 (control), 1, 2, 4, 6, and 8% (w/v) NaCl. Each isolate was plated to three replicates plates at each NaCl concentration and incubated at 28 °C in the dark. The length measurements were taken from the colonies after 48 h of incubation. The percentage of mycelial growth inhibition compared with the control was calculated for all NaCl concentrations. The concentration of the NaCl that inhibited fungal development by 50% ($EC_{50}N$) was determined for each individual isolate by linear regression of the mycelial growth inhibition versus the $\log_{10}$ transformation of the NaCl concentration.

Virulence. Disease severity (lesion diameter) on untreated fruits for each isolate from the in vivo thiophanate-methyl and thiabendazole sensitivity assays were used as a proxy for virulence. Untreated fruits inoculated with sterile distilled water were used as negative control.

Statistical analysis

The data of two independent experiments were pooled after testing the homogeneity of variance using Levene's test. In vitro sensitivity, stability of sensitivity, fungicide assay in fruit, and fitness components (mycelial growth rate, osmotic sensitivity and virulence) were analyzed by using Student's t-test (P=0.05). Cross-resistance between thiophanate-methyl and thiabendazole was analyzed by Pearson's correlation (P = 0.01). All analyses were performed using the Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA).

Results

In vitro sensitivity to thiabendazole and thiophanate-methyl

Isolates from five distinct species of *Lasiodiplodia* collected from grapevine, mango, papaya, and red mombin orchards in Northeastern Brazil exhibited varying sensitivity to the fungicides thiabendazole and thiophanate-methyl, with EC_{50} values ranging from 0.01 to 2.0 μg/ml for most isolates (Fig. 6). In general, sensitivity to thiophanate-methyl (mean EC_{50} = 0.34 μg/ml) was greater than to thiabendazole (mean EC_{50} = 1.15 μg/ml) (Fig. 7). The estimated EC_{50} values for thiabendazole ranged from 0.01 to 4.47 μg/ml and were <0.50 μg/ml in 8.5% of isolates, 0.51 to 1.00 μg/ml in 39.2%, 1.01 to 1.50 μg/ml in 34.0%, 1.51 to 2.0 μg/ml in 11.8%, and >2.00 μg/ml in 6.5% of isolates (Fig. 7). For thiophanate-methyl, estimated EC_{50} values ranged from 0.01 to 2.14 μg/ml, and were <0.25 μg/ml in 49.7% of isolates, 0.26 to 0.50 μg/ml in 26.8%, 0.51 to 0.75 μg/ml in 20.3%, 0.76 to 1.00 μg/ml in 0.7%, and >1.01 μg/ml in 2.6% of isolates (Fig. 7).

The isolates were classified according to their extreme sensitivity to the fungicides (Table 8). The EC_{50} values for the five most sensitive (S) isolates to thiabendazole ranged from 0.01 to 0.17 μg/ml, with a mean of 0.11 μg/ml. These values were significantly lower (P≤0.05) than those of the five least sensitive (LS) isolates, whose $EC5_0$ values ranged from 3.19 to 4.47 μg/ml, with a mean of 4.02 μg/ml (Tables 8 and 9). Among the LS isolates for thiabendazole, two were identified as *L. brasiliensis* (CFC-0829 and CFC-1015), one as *L. iraniensis* (CFC-0819), and two as *L. pseudotheobromae* (CFC-0827 and CFC-1421). Regarding host origin, two isolates came from grapevine (CFC-1015 and CFC-1421), while three were from mango (CFC-0819, CFC-0827, and CFC-0829) (Table 8). For thiophanate-methyl, the EC_{50} values of the five S isolates ranged from 0.01 to 0.03 μg/ml (mean 0.02 μg/ml), which were significantly lower (P≤0.05) than those of the five LS isolates, with EC_{50} values ranging from 0.99 to 2.14 μg/ml (mean 1.41 μg/ml) (Tables 8 and 9). All five LS isolates for thiophanate-methyl were

identified as *L. brasiliensis*, including two from grapevine (CFC-1034 and CFC-1463) and three from red mombin (CFC-1128, CFC-1151, and CFC-1157) (Table 8).

No significant correlation was found between the sensitivity of the isolates to thiabendazole and thiophanate-methyl, whether in the thiabendazole combination isolates (r=-0.0472, P=0.8432) or the thiophanate-methyl combination isolates (r=-0.2304, P=0.3284), indicating no cross-sensitivity.

Stability of the sensitivity to thiabendazole and thiophanate-methyl

The sensitivity of S and LS isolates to thiabendazole and thiophanate-methyl remained unchanged after 10 successive subcultures on fungicide-free PDA medium (P>0.05) (Table 10), indicating that their sensitivity levels remained stable across consecutive growth cycles.

Efficacy of thiabendazole and thiophanate-methyl in controlling S and LS isolates in fruits

Grapes treated with thiabendazole, and red mombin treated with either thiabendazole or thiophanate-methyl before inoculation with S and LS isolates of *Lasiodiplodia* showed no significant reduction in disease severity (lesion diameter) compared to untreated fruits (P>0.05). A similar result was observed for mango fruits treated with thiabendazole and inoculated with LS isolates. In contrast, papaya fruits treated with either thiabendazole or thiophanate-methyl, as well as grape and mango fruits treated with thiophanate-methyl before inoculation with S and LS isolates, exhibited a significant reduction in disease severity compared to untreated fruits (P≤0.05). A comparable effect was also observed in mango treated with thiabendazole and inoculated with S isolates (Table 11).

Fitness-related variables

The S and LS isolates to thiabendazole and thiophanate-methyl exhibited no significant differences in mycelial growth on fungicide-free medium or in virulence on grape, mango, and red mombin fruits (P>0.05). Similarly, no significant differences were observed in osmotic sensitivity between S and LS isolates to thiabendazole. However, LS isolates to thiabendazole showed significantly lower virulence in papaya fruits compared to S isolates. Moreover, thiophanate-methyl LS isolates demonstrated a greater capacity for growth under salt stress than S isolates (P≤0.05) (Table 12).

Discussion

This study evaluated the sensitivity of *L. pseudotheobromae, L. iraniensis, L. brasiliensis, L. euphorbiaceicola,* and *L. hormozganensis* isolates, collected from grapevine, mango, papaya, and red mombin orchards in Northeastern Brazil, to the fungicides thiabendazole and thiophanate-methyl, along with their impact on fitness components.

It is unexpected that *Lasiodiplodia* species isolates exhibit greater sensitivity to thiophanate-methyl than to thiabendazole, considering that thiabendazole is used exclusively in postharvest treatments, while thiophanate-methyl is applied in the field, where fungal populations are more frequently exposed. These results contrast with findings from *L. hormozganensis, L. iraniensis,* and *L. pseudotheobromae* isolates from mango orchards in Northeastern Brazil, where EC_{50} values were higher for thiophanate-methyl than for thiabendazole (Santos et al. 2019).

In this study, the mean EC_{50} value of *Lasiodiplodia* isolates for thiophanate-methyl was 0.34 μg/mL, which is relatively low compared to the mean EC_{50} reported for this fungicide in in *L. euphorbiaceicola* (0.89 μg/mL), *L. mahajangana* (0.91 μg/mL), *L. theobromae* (0.91 μg/mL), and *L. pseudotheobromae* (1.13 μg/mL) from avocado in China (Chen et al. 2024). Variations in susceptibility to thiophanate-methyl among *Lasiodiplodia* isolates from different regions or countries can be influenced by multiple factors, including genetic differences and agricultural management practices. Natural genetic variation within *Lasiodiplodia* populations may impact their sensitivity to fungicides (Brent and Hollomon 2007), and distinct species within the genus may exhibit different levels of susceptibility due to variations in target genes or resistance mechanisms (Chen et al. 2024). Additionally, regions with more intensive fungicide use may impose greater selective pressure on fungal populations, promoting the emergence of less sensitive isolates (Brent and Hollomon 2007; Hollomon 2015).

Significant differences were observed between the extremes of sensitivity to the analyzed fungicides. The five LS isolates to thiabendazole were collected from grapevine (2 isolates) and mango (3 isolates), both fruit crops with intensive fungicide use. In contrast, among the five LS isolates to thiophanate-methyl, two originated from grapevine and three from red mombin, a fruit that had not been exposed to fungicide treatments, even during postharvest. The low sensitivity of isolates from red mombin to thiophanate-methyl may be due to their natural resistance. This type of resistance is the innate ability of certain fungal species or populations to withstand a specific fungicide without previous exposure. It arises from natural

genetic traits, such as the absence of fungicide's molecular target, efficient metabolic degradation, or structural barriers that inhibit the compound's action (Brent and Hollomon 2007; Lucas et al. 2015).

In this study, no cross-sensitivity was observed between thiabendazole and thiophanate-methyl, despite cross-resistance or cross-sensitivity being common among fungicides in the MBC group due to similar resistance mechanisms (Ishii 2015; Ma and Michailides 2005; Young 2015). Cross-resistance between these fungicides has been reported in populations of *L. theobromae* from papaya (Cavalcante et al. 2014; Pereira et al. 2012) and Botryosphaeriaceae from mango (Santos et al. 2019) collected in the Brazilian Northeast.

The *Lasiodiplodia* isolates, both S and LS to thiabendazole and thiophanate-methyl, maintained stable sensitivity to these fungicides, showing no significant changes in sensitivity levels after 10 successive subcultures on a fungicide-free medium. These results align with findings from studies on Botryosphaeriaceae populations in the Brazilian Northeast (Cavalcante et al. 2014; Chen et al. 2020; Santos et al. 2019) and further support the observation that resistance stability is a common characteristic of MBC fungicides (Ishii 2015).The stability of fungicide resistance (low sensitivity) refers to a pathogen's ability to retain the same level of insensitivity across successive generations, regardless of whether the fungicide is present or absent (Vega and Dewdney 2014). This stability is particularly important in determining whether growers can effectively reuse a fungicide after discontinuing its use for several years (Ishii 2015).

At the labeled rate, thiabendazole effectively controlled LS isolates of *Lasiodiplodia* in artificially infected papaya fruit, while thiophanate-methyl successfully controlled LS isolates in grape, mango, and papaya. In contrast, efficacy tests showed that thiabendazole failed to control LS isolates in grape, mango, and papaya, and thiophanate-methyl was ineffective in red mombin fruit, aligning with the in vitro results. These findings provide strong evidence of resistance to these fungicides.

Information on the fitness components of fungicide-resistant and sensitive fungi is valuable for preventing resistance development and guiding effective disease management strategies (Ma and Michailides 2005; Mikaberidze and McDonald 2015; Milgroom 2015). This study evaluated three fitness-related variables to compare S and LS isolates to thiabendazole and thiophanate-methyl: mycelial growth rate, osmotic sensitivity, and virulence. Significant differences between S and LS isolates were observed in only two cases. In the first, LS isolates resistant to thiabendazole exhibited lower virulence in papaya fruits compared to S isolates, suggesting a fitness cost for LS isolates. These results support the observation that resistance

can have a pleiotropic effect, leading to reduced fitness and a decline in the frequency of non-sensitive (or low-sensitive) isolates when fungicide selection pressure is removed in the field (Ma and Michailides 2005). In the second case, LS isolates to thiophanate-methyl exhibited a greater ability to grow under salt stress compared to S isolates. The adaptive advantage of salt stress tolerance in fungicide-LS isolates presents a challenge for disease control strategies, as this combination may enhance survival, persistence, and competitive dominance within fungal populations.

The findings of this study suggest the possible presence of *Lasiodiplodia* isolates from grape, mango, papaya, and red mombin orchards in northeast of Brazil resistant to thiabendazole and thiophanate-methyl. Therefore, regular monitoring is essential to evaluate the occurrence and frequency of *Lasiodiplodia* isolates with low sensitivity to these fungicides. Additionally, further research is necessary to uncover the molecular mechanisms responsible for their reduced sensitivity.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Institutional review board Not applicable.

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Conflict of interest The authors declare no conflict of interest.

ORCID

Raquel Maria da Silva (0000-0002-5503-2072)

Jadson Araújo da Silva (0000-0002-8571-3477)

Walisson Ferreira da Silva (0009-0007-3911-3845)

Rafael Luis de Oliveira (0009-0002-4679-5649)

Damiana Andressa Alves Pereira (0000-0002-4065-8101)

Rodrigo Marques da Costa (0009-0009-2146-0229)

Sami Jorge Michereff (0000-0002-2156-3502)

Kamila Câmara Correia (0000-0001-9113-9064)

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Table 7 List of isolates of *Lasiodiplodia* species from Brazilian northeast fruit orchards used in this study

Lasiodiplodia species	Host	Isolate code*
L. brasiliensis	Grapevine	CFC-0458; CFC-0468; CFC-1005; CFC-1012; CFC-1015; CFC-1019; CFC-1028; CFC-1035; CFC-1044; CFC-1051; CFC-1372; CFC-1405; CFC-1408; CFC-1415; CFC-1418; CFC-1424; CFC-1433; CFC-1434; CFC-1449; CFC-1450; CFC-1456; CFC-1459; CFC-1460
	Mango	CFC-0829; CFC-0832; CFC-0535
	Papaya	CFC-0536; CFC-0543; CFC-0565; CFC-0568; CFC-0571; CFC-0590; CFC-0626; CFC-0698; CFC-0706
	Red mombin	CFC-1154; CFC-1160; CFC-1163; CFC-1175; CFC-1176
L. euphorbiaceicola	Grapevine	CFC-0433; CFC-0448; CFC-0455; CFC-0473; CFC-1013; CFC-1018; CFC-1031; CFC-1395
	Red mombin	CFC-1132; CFC-1133; CFC-1134; CFC-1135; CFC-1136
L. hormozganensis	Grapevine	CFC-0443; CFC-1033; CFC-1369; CFC-1371; CFC-1373; CFC-1374; CFC-1440; CFC-1441; CFC-1445; CFC-1451; CFC-1454; CFC-1455; CFC-1461
	Mango	CFC-0814; CFC-0815; CFC-0816
	Papaya	CFC-0544; CFC-0545; CFC-0546; CFC-0547
L. iraniensis	Grapevine	CFC-1004; CFC-1008; CFC-1014; CFC-1016; CFC-1025; CFC-1032; CFC-1034; CFC-1417; CFC-1446; CFC-1463
	Mango	CFC-0819; CFC-0821; CFC-0843
	Red mombin	CFC-1126; CFC-1128; CFC-1131; CFC-1137; CFC-1139; CFC-1143; CFC-1144; CFC-1146; CFC-1148; CFC-1151; CFC-1156; CFC-1157; CFC-1158; CFC-1159; CFC-1161; CFC-1162; CFC-1164; CFC-1165; CFC-1166; CFC-1167; CFC-1168; CFC-1169; CFC-1170; CFC-1172; CFC-1173; CFC-1174; CFC-1177
L. pseudotheobromae	Grapevinr	CFC-0438; CFC-0457; CFC-0469; CFC-0522; CFC-0527; CFC-1009; CFC-1023; CFC-1024; CFC-1037; CFC-1039; CFC-1040; CFC-1041; CFC-1043; CFC-1045; CFC-1046; CFC-1047; CFC-1050; CFC-1383; CFC-1384; CFC-1385; CFC-1388; CFC-1389; CFC-1392; CFC-1394; CFC-1396; CFC-1397; CFC-1403; CFC-1404; CFC-1411; CFC-1421; CFC-1425; CFC-1436; CFC-1437; CFC-1442; CFC-1443
	Mango	CFC-0824; CFC-0826; CFC-0827
	Papaya	CFC-0623; CFC-0624

*Isolate code from the Culture Collection of Phytopathogenic Fungi of Cariri (CFC) at the Federal University of Cariri (Crato, Ceará, Brazil)

Source: elaborated by the author (2025)

Table 8 List of isolates of *Lasiodiplodia* species from fruit orchards selected in this study showing the lowest (sensitive - S) and highest (less sensitive - LS) EC_{50} values for thiabendazole and thiophanate-methyl

Lasiodiplodia species	Isolate code*	Host	EC_{50} (µg a.i./ml)			
			Thiabendazole		Thiophanate	
			S	LS	S	LS
L. brasiliensis	CFC-0590	Papaya	0.01	–	–	–
	CFC-0829	Mango	–	3.89	–	–
	CFC-1005	Grapevine	–	–	0.01	–
	CFC-1015	Grapevine	–	4.36	–	–
	CFC-1456	Grapevine	0.13	–	–	–
	CFC-1459	Grapevine	–	–	0.01	–
L. hormozganensis	CFC-0443	Grapevine	0.14	–	–	–
	CFC-1445	Grapevine	–	–	0.03	–
L. iraniensis	CFC-0819	Mango	–	4.21	–	–
	CFC-1008	Grapevine	0.11	–	–	–
	CFC-1034	Grapevine	0.17	–	–	1.27
	CFC-1126	Red mombin	–	–	0.01	–
	CFC-1128	Red mombin	–	–	–	0.99
	CFC-1151	Red mombin	–	–	–	1.08
	CFC-1157	Red mombin	–	–	–	2.14
	CFC-1463	Grapevine	–	–	–	1.57
L. pseudotheobromae	CFC-0827	Mango	–	3.19	–	–
	CFC-1024	Grapevine	–	–	0.03	–
	CFC-1421	Grapevine	–	4.47	–	–

*Isolate code from the Culture Collection of Phytopathogenic Fungi of Cariri (CFC) at the Federal University of Cariri (Crato, Ceará, Brazil)

Source: elaborated by the author (2025)

Table 9 Sensitivity to thiabendazole and thiophanate-methyl of sensitive and less sensitive isolates of *Lasiodiplodia* species from fruit orchards

Isolate class*	EC_{50} (µg a.i./ml)**	
	Thiabendazole	Thiophanate-methyl
Sensitive	0.11 (0.01–0.17) b	0.02 (0.01–0.03) b
Less sensitive	4.02 (3.19–4.47) a	1.41 (0.99–2.14) a

*Each class is composed of five isolates, selected by the lowest and highest EC_{50} values for thiabendazole and thiophanate-methyl

**Values (µg a.i./ml) are the means of two independent experiments because no heterogeneity was detected between them according to Levens's test ($P > 0.05$). Averages followed by the same letter in the column do not differ significantly according to Student's t-test ($P = 0.05$). Values in the parentheses represent the range of EC_{50} values

Source: elaborated by the author (2025)

Table 10 Stability of sensitivity to thiabendazole and thiophanate-methyl of sensitive and less sensitive isolates of *Lasiodiplodia* species from fruit orchards based on the comparison between the initial (T_0) effective concentration required to inhibit 50% of the mycelial growth (EC_{50}) and the following 10 sequential transfers on fungicide-free medium (T_{10})

Isolate class*	EC_{50} (µg a.i./ml)**			
	Thiabendazole		Thiophanate-methyl	
	T_0	T_{10}	T_0	T_{10}
Sensitive	0.11 a	0.12 a	0.02 a	0.01 a
Less sensitive	4.04 a	3.91 a	1.44 a	1.46 a

*Each class is composed of five isolates, selected by the lowest and highest EC_{50} values for thiabendazole and thiophanate-methyl

**Values (µg a.i./ml) are the means of two independent experiments because no heterogeneity was detected between them according to Levens's test ($P > 0.05$). Averages followed by the same letter in the line do not differ significantly according to Student's t-test ($P = 0.05$)

Source: elaborated by the author (2025)

Table 11 Disease severity (lesion diameter) on detached grape, mango, papaya, and red mombin fruits treated with thiabendazole and thiophanate-methyl prior to inoculation with sensitive and less sensitive isolates of *Lasiodiplodia* species from fruit orchards

Isolate class*	Lesion diameter (mm)**			
	Thiabendazole		Thiophanate-methyl	
	Without fungicide	With Fungicide	Without fungicide	With Fungicide
	Grape			
Sensitive	12.98 a	10.34 a	14.84 a	9.60 b
Less sensitive	12.30 a	10.58 a	13.88 b	9.73 a
	Mango			
Sensitive	27.62 a	13.28 b	35.94 a	15.07 b
Less sensitive	27.33 a	22.51 a	33.61 a	10.15 b
	Papaya			
Sensitive	19.76 a	9.34 b	25.17 a	1.30 b
Less sensitive	14.07 a	5.34 b	21.03 a	5.55 b
	Red mombin			
Sensitive	16.38 a	16.32 a	15.46 a	15.14 a
Less sensitive	17.50 a	13.77 a	17.86 a	16.52 a

*Each class is composed of five isolates, selected by the lowest and highest EC_{50} values for thiabendazole and thiophanate-methyl

**Values (µg a.i./ml) are the means of two independent experiments because no heterogeneity was detected between them according to Levens's test ($P > 0.05$). Averages followed by the same letter in the line, inside each fungicide, do not differ significantly according to Student's t-test ($P = 0.05$)

Source: elaborated by the author (2025)

Table 12 Fitness components between isolates of *Lasiodiplodia* species from fruit orchards that are sensitive and less sensitive to thiabendazole and thiophanate-methyl

Isolate class*	MGR (mm/h)**	$EC_{50}N$ (%NaCl)**	Lesion diameter (mm)**			
			Grape	Mango	Papaya	Red mombin
	Thiabendazole					
Sensitive	0.31 a	1.11 a	12.98 a	27.62 a	19.76 a	16.38 a
Less sensitive	0.39 a	0.83 a	12.30 a	27.33 a	14.07 b	17.50 a
	Thiophanate-methyl					
Sensitive	0.36 a	0.94 b	14.84 a	35.94 a	25.17 a	15.46 a
Less sensitive	0.40 a	1.95 a	13.88 a	33.61 a	21.03 a	17.86 a

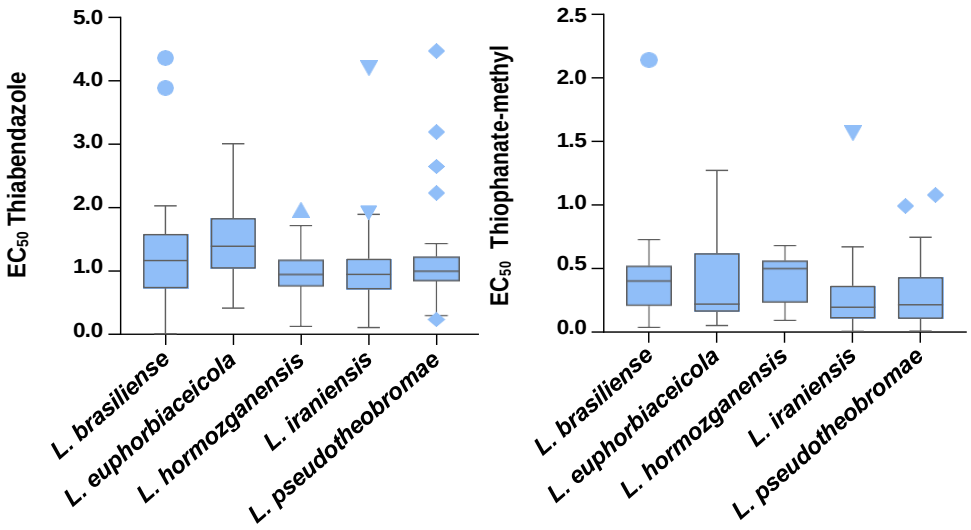
MGR: Mycelial growth rate in fungicide-free PDA medium; $EC_{50}N$: osmotic sensitivity

*Each class is composed of five isolates, selected by the lowest and highest EC_{50} values for thiabendazole and thiophanate-methyl

**Values are the means of two independent experiments because no heterogeneity was detected between them according to Levene's test ($P > 0.05$). Averages followed by the same letter in the column, inside each fungicide, do not differ significantly according to Student's t-test ($P = 0.05$)

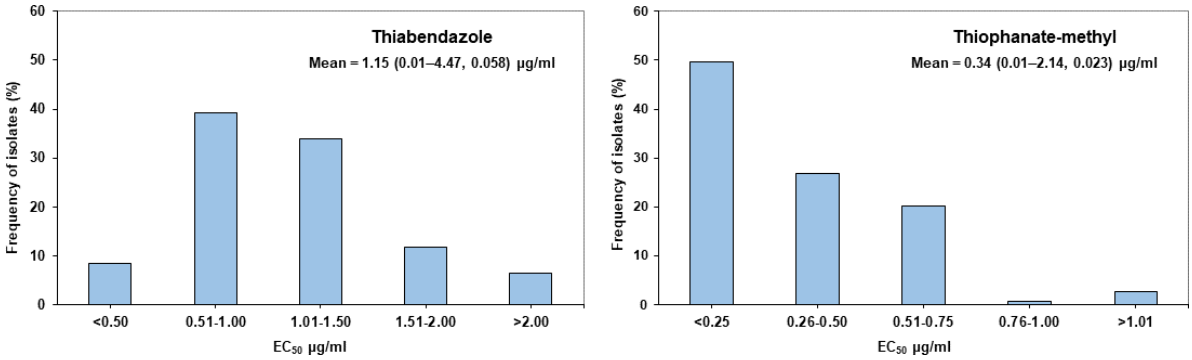
Source: elaborated by the author (2025)

Fig. 6 Box plot of EC_{50} values from isolates of *Lasiodiplodia* species from fruit orchards to thiabendazole and thiophanate-methyl fungicides



Source: elaborated by the author (2025)

Fig. 7 Frequency distribution of effective thiabendazole and thiophanate-methyl concentrations required to inhibit 50% of the mycelial growth (EC_{50}) of 153 isolates of *Lasiodiplodia* species collected from Brazilian northeast fruit orchards. Values in the parentheses represent the range of EC_{50} values followed by the standard error



Source: elaborated by the author (2025)

CONCLUSÕES GERAIS

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1. Isolados de *Lasiodiplodia brasiliensis, L. euphorbiaceicola, L. hormozganensis, L. iraniensis* e *L. pseudotheobromae* oriundos de pomares de mamão, manga, seriguela e uva do Nordeste brasileiro têm diferentes níveis de sensibilidade aos fungicidas imazalil, tebuconazol, tiabendazol e tiofanato metílico;

2. Os isolados das espécies de *Lasiodiplodia* possuem sensibilidade cruzada entre imazalil e tebuconazol, mas não entre tiabendazol e tiofanato metílico;

3. Isolados de *Lasiodiplodia* menos sensíveis (LS) ao imazalil não tem estabilidade na sensibilidade, que aumenta com ciclos sucessivos de crescimento;

4. Imazalil e tebuconazol não são eficazes no controle de isolados de *Lasiodiplodia* classificados como LS em mamão, manga e seriguela;

5. A adaptabilidade na virulência dos isolados de *Lasiodiplodia* LS aos fungicidas imazalil, tebuconazol, tiabendazol e tiofanato metílico é variável em função do hospedeiro;

6. Os isolados de *Lasiodiplodia* classificados como LS aos fungicidas têm maior adaptação à estresse salino;

7. Isolados LS a tiabendazol apresentam um custo de adaptabilidade na virulência;

8. Há fortes indícios da ocorrência de resistência a imazalil, tebuconazol, tiabendazol e tiofanato-metílico entre isolados de espécies menos conhecidas de *Lasiodiplodia* presentes em pomares de mamão, manga, seriguela e uva no Nordeste brasileiro.