UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FCA
FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

LUCAS ANTONIO BENSO

PATOGENICIDADE E DANOS ISOLADOS DE *Ceratocystis fimbriata* E OUTROS PATÓGENOS ASSOCIADOS À DOENÇAS DO CAULE DO EUCALIPTO

Botucatu
2023

LUCAS ANTONIO BENSO

PATOGENICIDADE E DANOS ISOLADOS DE *Ceratocystis fimbriata* E OUTROS PATÓGENOS ASSOCIADOS À DOENÇAS DO CAULE DO EUCALIPTO

Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutor em Ciência Florestal.

Orientador: Prof. Dr. Edson Luiz Furtado

Botucatu

2023

B474p

Benso, Lucas Antonio
PATOGENICIDADE E DANOS ISOLADOS DE Ceratocystis fimbriata E OUTROS PATÓGENOS ASSOCIADOS À DOENÇAS DO CAULE DO EUCALIPTO / Lucas Antonio Benso. – Botucatu, 2023
109 p. : tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu
Orientador: Edson Luiz Furtado

1. Murcha vascular. 2. Lesão caulinar. 3. Podridão do cerne. 4. Resistência. 5. Fungos fitopatogênicos. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agronômicas, Botucatu. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Botucatu

CERTIFICADO DE APROVAÇÃO

Título:

PATOGENICIDADE E DANOS ISOLADOS DE Ceratocystis fimbriata L OUTROS PATÓGENOS ASSOCIADOS À DOENÇAS DO CAULE DO EUCALIPTO

AUTOR: LUCAS ANTONIO BENSO
ORIENTADOR: EDSON LUIZ FURTADO

Aprovado como parte das exigências para obtenção do Título de Doutor em Ciência Florestal, pela Comissão Examinadora:

Prof. Dr. EDSON LUIZ FURTADO (Participaçao Presencial)
Protecao Vegetal / Faculdade de Ciencias Agronomicas de Botucatu UNESP

Dr. BRUNO CESAR ROSSINI (Participaçao Presencial)
Instituto de Biotecnologia / Instituto de Biotecnologia - UNESP

Dr. LEO ZIMBACK (Participaçao Presencial)
Unidade Botucatu / Instituto Florestal de São Paulo

Prof. Dr. WILLIAN BUCKER MORAES (Participaçao Virtual)
Centro de Ciências Agrárias / Universidade Federal do Espirito Santo

Profa. Dra. LEILA CRISTIANE DELMADI (Participaçao Virtual)
Engenharia Florestal / Universidade do Estado de Mato Grosso - UNEMAT

Botucatu, 15 de dezembro de 2023.

Dedico esse manuscrito aos

meus pais, Bernadete e Antonio,

por todo apoio,

E a minha companheira,

Júlia,

por toda parceria e carinho

AGRADECIMENTOS

Agradeço à Faculdade de Ciências Agronômicas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, das etapas de ensino e pesquisa. Campus de Botucatu / SP, pelo suporte na realização das etapas de ensino e pesquisa.

Aos professores e funcionários do programa de Pós graduação em Ciência Florestal e o Departamento de Proteção Vegetal, em especial, ao professor Edson Luiz Furtado. À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Projeto 001) pela concessão da bolsa de estudo.

Um agradecimento especial aos moradores e agregados da República Nós Travamos; Busca Pé, Calango Exótico, Cocielo, Fernando, Gambito, Nayara, Magayver, Miyage, Pepa Killer, Saboia, Sommelier e Sugar, por ajudarem a formar quem eu sou hoje.

Aos companheiros do Laboratório de Patologia Florestal do Departamento de Proteção Vegetal; Bruno, Caroline, Lisandro, Luiz Tadeu, Rodrigo e Yerly, pelo auxílio durante o projeto e pelo companheirismo ao longo do tempo.

A empresa Veracel, por colaborar imensamente nesse projeto. Ao Rafael e Marlon Michel, pois sem eles esse trabalho não seria possível.

À minha parceira Júlia por todo seu amor e compreensão.

E a todos que não foram citados, mas que ajudaram direta e indiretamente nesse trabalho.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil – CAPES – Código de financiamento 001.

Gratidão!

Os homens devem moldar seu caminho. A partir do momento em que você vir o caminho em tudo o que fizer, você se tornará o caminho.

Miyamoto Musashi

RESUMO

A ocorrência de patologias caulinares está entre os principais fatores que reduzem a produtividade de plantios de eucalipto. Dentre essas doenças, merecem destaque as lesões caulinares, murchas vasculares e podridões do cerne, devido a sua dificuldade de manejo e potencial danoso. Dessa forma, o objetivo desse trabalho foi estudar a gama de microrganismos associados a patologias caulinares em eucalipto e os possíveis danos associados a incidência da murcha de ceratocystis. Esse estudo foi realizado em plantios de eucalipto localizados no sul da Bahia, onde foram feitas coletas de amostras de tecidos vegetais, de árvores apresentando diferentes patologias caulinares. Das amostras coletadas, foram isolados microrganismos por meio de métodos de iscas e em meio de cultivo. Os isolados de *Ceratocystis fimbriata* sensu lato estudados foram identificados por meio de sequenciamento gênico das regiões ITS e Bt1, e caracterizados quanto a sua morfologia, temperatura ótima de crescimento e agressividade em diferentes clones. Foi observado que clones de eucalipto, que se mostraram resistente contra determinado isolado de *Ceratocystis*, podem se mostrar susceptíveis quando inoculados com outro isolado, dificultando assim a seleção de materiais resistentes. Em ensaios em campo, foi observado que a incidência da murcha de ceratocystis em árvores de eucalipto em idade de corte (7 anos) resultou em queda no crescimento volumétrico de até 10,2%, contudo, sem alterar o rendimento final de celulose. Esse resultado provavelmente está relacionado a relativa baixa porcentagem de volume colonizado pelo patógeno nas árvores amostradas (<3,0%; v/v). Outros microrganismos associados a patologias caulinares foram também identificados ao nível de gênero, por meio de ferramentas moleculares, e foram caracterizados quanto a produção de lacase, reação em KOH e agressividade em mudas de eucalipto. A partir desses ensaios, foi possível identificar novos microrganismos associados a sintomas de lesões caulinares, podridões do cerne e descoloração do alburno em eucalipto, ainda sem relato na literatura, e compreender sua associação com os sintomas observados nas árvores amostradas. De forma geral, o atual trabalho expande o atual conhecimento sobre patologias caulinares em eucalipto.

Palavras-chave: *Ceratocystis*; lesões caulinares; cancro; podridão do cerne; murcha vascular; celulose

ABSTRACT

The occurrence of trunk pathologies is among the main factors that reduce the productivity of eucalypt plantations. Among these diseases, stem lesions, vascular wilt and heartwood rot stand out, due to the difficulty of management and potential damage. Therefore, the objective of this work was to study the range of microorganisms associated with eucalypt trunk pathologies and the possible damage associated with the incidence of ceratocystis wilt. This study was carried out in eucalypt plantations located in southern Bahia, where plant tissue samples were collected from trees presenting different trunk pathologies. From the collected samples, microorganisms were isolated using bait methods and in culture medium. The *Ceratocystis fimbriata* sensu lato isolates studied were identified through gene sequencing of the ITS and Bt regions, and characterized regarding their morphology, optimal growth temperature and aggressiveness in different clones. It was observed that *Eucalyptus* clones resistant to a certain *Ceratocystis* isolate may prove susceptible when inoculated with another isolate, making the selection of resistant materials difficult. In field trials, it was observed that the incidence of ceratocystis wilt in *Eucalyptus* trees at cutting age (7 years) resulted in a reduction in volumetric growth of up to 10.2%, however, without changing the final cellulose yield. This result is probably related to the relatively low percentage of volume colonized by the pathogen in the sampled trees (<3.0%; v/v). Other microorganisms associated with trunk pathologies were also identified at the genus level, using molecular tools, and were characterized in terms of laccase production, reaction to KOH and aggressiveness in eucalypt seedlings. From these tests it was possible to identify new microorganisms associated with symptoms of stem lesions, heartwood rot and sapwood discoloration in *Eucalyptus*, not yet reported in the literature, and to understand their association with the symptoms observed in the sampled trees. In general, this work expands current knowledge about *Eucalyptus* trunk pathologies.

Keywords: *Ceratocystis*; trunk lesions; canker; heartwood rot; vascular wilting; cellulose

SUMÁRIO

INTRODUÇÃO GERAL

Eucalipto é o nome popular dado a árvores relacionadas a três gêneros de espécies arbóreas, *Eucalyptus*, *Corymbia* e *Angophora,* todos pertencem à família Myrtaceae e são originários da Oceania, mais marcadamente da Austrália (FLORES, 2016; MACPHAIL; THORNHILL, 2016; THORNHILL et al., 2019). No Brasil, os dois primeiros gêneros são os mais cultivados, sendo estabelecidos na forma de plantios comerciais, destinados, principalmente, para produção de madeira (DA FONSECA et al., 2010; FLORES, 2016). O lenho produzido por essas árvores pode ser usado na construção civil, fabricação de painéis e móveis, produção de carvão vegetal e extração de celulose visando principalmente a indústria do papel (DA FONSECA et al., 2010; IBÁ, 2020; 2021). Além do potencial madeireiro, algumas espécies desses gêneros são utilizadas também como fonte de pólen e néctar para o setor apícola (WOLFF; SCHNELL; SCHUHLI, 2021) e de biomassa vegetal para extração de óleo essencial (DA SILVEIRA; LAZZAROTTO, 2021).

No Brasil, essa árvore foi introduzida no final do século XIX, nos estados do Rio Grande do Sul, Rio de Janeiro e São Paulo, principalmente com finalidade ornamental (FLORES, 2016). Atualmente, o principal uso do eucalipto é na produção de celulose, sendo o Brasil classificado, no ano de 2020, como maior exportador dessa matéria prima, arrecadando, nesse ano, 9,8 bilhões de dólares (DA FONSECA et al., 2010; IBÁ, 2020; 2021). Os principais destinos dessa exportação são a China e o Estados Unidos, compreendendo 48% e 16% do total de celulose exportado, respectivamente (IBÁ, 2021). O principal gênero de eucalipto utilizado para produção de celulose é o *Eucalyptus* (DA FONSECA et al., 2010), cujos plantios, no Brasil, em 2020, cobriam 7,47 milhões de hectares plantados visando o setor industrial (IBÁ, 2021).

Por conta de sua ampla variabilidade genética, abrangendo mais de 800 espécies (FLORES, 2016), o gênero *Eucalyptus* pode ser cultivado em praticamente todos os estados brasileiros (IBÁ, 2021), abrangendo regiões que apresentam climas que variam entre o tropical, semiárido e equatorial. De acordo com a condição ambiental em que será estabelecido o plantio, pode-se selecionar espécies ou cruzamentos mais adequados ao local escolhido, resultando em maior produtividade. No ano de 2020 os estados de Minas Gerais, São Paulo, Mato Grosso do Sul, Bahia

e Rio Grande do Sul apresentavam a maior área plantada dessa cultura no território nacional, abrigando também importantes empresas florestais (IBÁ, 2021).

A produtividade média anual do eucalipto, no ano de 1965, era de apenas 10m^3/ha, contudo, no ano de 2020, esse valor saltou para 36,8m^3/ha, podendo ser superior a 60,0m^3/ha em algumas áreas (ALFENAS et al., 2009; DA FONSECA et al., 2010). Esse aumento de produtividade se deve principalmente aos programas de melhoramento genético, que deram início à realização de cruzamentos interespecíficos e da seleção de genótipos superiores, e da realização de clonagem em larga escala; possibilitando o estabelecimento de plantios homogêneos e altamente produtivos. Um considerável destaque pode ser dado ao cruzamento de *Eucalyptus grandis* x *Eucalyptus urop*hylla, popularmente chamado de Urograndis, que une características de ambas as espécies, como o alto crescimento da primeira espécie e a rusticidade, maior densidade da madeira e capacidade de rebrota da segunda (ALFENAS et al., 2009; DA FONSECA et al., 2010). Esse híbrido é um dos mais cultivados no Brasil.

O eucalipto utilizado para produção de celulose pode ser cortado em ciclos que variam de 6 a 7 anos, que, dependendo da área e do clone, pode ser reduzido para até 5 anos (SKORUPA; BEHLING; PORFIRIO-DA-SILVA, 2021). Uma vez estabelecido o plantio para essa finalidade, o mesmo pode ser conduzido em regime de talhadia, permitindo que a floresta seja restabelecida através da condução das brotações após o corte raso. Essa forma de manejo reduz os custos de implantação em até 50% (SKORUPA; BEHLING; PORFIRIO-DA-SILVA, 2021). Plantios estabelecidos visando à produção de madeira podem ter ciclos que ultrapassam 12 anos (DA FONSECA et al., 2010; SKORUPA; BEHLING; PORFIRIO-DA-SILVA, 2021).

De forma geral, o cultivo do eucalipto é visto como alternativa para redução da pressão de exploração das florestas nativas, principalmente para a produção de carvão vegetal (PINTO JÚNIOR; SILVEIRA, 2021). Essa árvore pode também ser utilizada como fonte de renda em pequenas propriedades (DE OLIVEIRA et al., 2021) e de sombra em sistemas silvipastoris (SKORUPA; BEHLING; PORFIRIO-DA-SILVA, 2021). Seu uso pode ser feito também na recuperação de solos, por ser uma árvore rústica, auxiliando no aumento da fertilidade do solo, pela incorporação de matéria

orgânica em profundidade, e na prevenção de erosão (DE OLIVEIRA et al., 2021; MAIA et al., 2021).

Devido aos potenciais danos causados e à dificuldade de manejo, as patologias caulinares e radiculares estão entre os principais problemas fitossanitários na cultura do eucalipto em condição de campo (ALFENAS et al., 2009; AUER; SANTOS; FURTADO, 2016; FERREIRA; MILANI, 2012). Seus sintomas se manifestam principalmente devido a inativação dos tecidos condutores das árvores, mais marcadamente nas patologias caulinares, e nos tecidos associados a sustentação e absorção de água e nutrientes, como raízes finas e grossas, nas patologias radiculares (AMORIM; REZENDE; BERGAMIN FILHO, 2018).

As patologias caulinares em árvores de eucalipto podem ser classificadas, de forma simplista, em lesões caulinares, murchas vasculares e podridões do cerne. As lesões caulinares são associadas à necrose nos tecidos da casca, incluindo a periderme, floema secundário e câmbio vascular, e o lenho. Os tecidos doentes passam a apresentar alteração na sua coloração, se tornando escurecidos, e perdem suas funções fisiológicas normais (FERREIRA; MILANI, 2012). Por conta desse processo, a árvore afetada pode apresentar queda no seu desenvolvimento, sintomas de deficiência nutricional, queda da folhagem, formação de brotações adventícias, murcha e, em casos extremos, seca e morte (AUER; SANTOS; FURTADO, 2016). Patógenos caulinares podem também se aproveitar de condições pré-existentes de estresse para infectar seus hospedeiros, como déficit hídrico, deficiência de nutricional (mais notadamente de boro) e injúrias (BIGGS, 1992; FERREIRA; MILANI, 2012). Árvores que conseguem sobreviver à colonização por esses patógenos se recuperam por meio da formação de novos tecidos sob a região lesionada (FERREIRA; MILANI, 2012). Esse processo de cicatrização se dá a partir das bordas do ferimento, através da formação do calo cicatricial. A combinação do calo cicatricial e da lesão caulinar, ou seja, a lesão em recuperação, recebe o nome de cancro, palavra essa que dá nome a diversas patologias caulinares na área florestal (BIGGS, 1992; FERREIRA; MILANI, 2012). Caso a lesão venha a afetar as regiões em crescimento ativo das plantas, como ponteiros e ramos, acarretando a sua morte, a patologia pode receber o nome de seca de ponteiro ou ramos, respectivamente (AUER; SANTOS; FURTADO, 2016; FERREIRA; MILANI, 2012).

O cancro basal de chrysoporthe era uma das doenças de maior importância econômica na eucaliptocultura na década de 50. Contudo, com o advento da realização de cruzamentos interespecíficos e da clonagem, tornou-se uma patologia de comparativo baixo impacto econômico (ALFENAS et al., 2009; AUER; SANTOS; FURTADO, 2016; FERREIRA; MILANI, 2012). Os agentes causais são espécies pertencentes ao gênero *Chrysoporthe*, como *C. cubensis*, *C. doradensis*, *C. deuterocubensis* e *C. austroafricana* (AUER; SANTOS; FURTADO, 2016; DAHALI et al., 2021; GRYZENHOUT et al., 2005; SOARES et al., 2018; RAUF et al., 2020). Essa doença incide em árvores a partir do quinto mês de plantio, que passam a apresentar sintomas de lesões necróticas ao longo do caule, com maior predominância no terço inferior, junto ao colo da árvore. Plantas severamente afetadas podem ser aneladas, resultando na sua morte (AUER; SANTOS; FURTADO, 2016). Árvores doentes que conseguem sobreviver apresentam descolamento da casca em tiras na região lesionada do caule, com posterior desenvolvimento de tecidos de cicatrização, levando a formação de cancros típicos (FERREIRA; MILANI, 2012). Esses cancros tipicamente apresentam formato elipsóide, com abundante exsudação de kino (AUER; SANTOS; FURTADO, 2016; FERREIRA; MILANI, 2012). Os sintomas são mais severos nas fases iniciais do plantio (AUER; SANTOS; FURTADO, 2016).

Os cancros de *Botryosphaeria* e *Teratosphaeria* são tidos como patologias caulinares de significativa importância econômica na cultura do eucalipto, afetando principalmente materiais genéticos susceptíveis (AUER; SANTOS; FURTADO, 2016; AYLWARD et al., 2019; BARRADAS et al., 2019). A primeira doença é associada a mais de 30 espécies de fungos pertencentes à família Botryosphaeriaceae e aos gêneros *Botryosphaeria*, *Diplodia*, *Lasiodiplodia*, *Neofusicoccum* e *Pseudofusicoccum* (BARRADAS et al., 2019; CROUS et al., 2009; LI et al., 2018; 2020; PILLAY et al., 2013; PAVLIC-ZUPANC et al., 2017). Essa patologia está tipicamente associada a condições de estresse, principalmente deficiência de boro. Os sintomas dessa doença se manifestam na forma de necrose da casca em ramos e caules, principalmente em tecidos jovens. Tecidos ainda verdes infectados passam a apresentar escurecimento, indicando necrose, podendo resultar em seca de ramos e ponteiros. Tecidos com crescimento secundário apresentam trincamento e descolamento da casca, com formação de cancro típico (AUER; SANTOS; FURTADO, 2016). O cancro de *Teratosphaeria* é associado a *Teratosphaeria zuluensis* e *T. gauchensis*, sendo que

seus sintomas se manifestam na forma de pequenas lesões ao longo do caule, podendo afetar também ramos levando a sua seca (AYLWARD et al., 2019). Em ambas as patologias, se observa a presença de extravasamento de kino do local afetado e a formação de frutificações características dos patógenos. Como consequência dessas doenças, temos a redução do desenvolvimento das árvores afetadas, tornando-as mais susceptíveis à quebra pelo vento, devido à fragilização do caule, e à deformação do tronco em árvores com sintomas de seca do ponteiro e/ou formação de brotações laterais (AUER; SANTOS; FURTADO, 2016; AYLWARD et al., 2019; BARRADAS et al., 2019).

As murchas vasculares em espécies florestais são associadas a patógenos que colonizam os tecidos do xilema secundário, resultando na sua obstrução, e, consequentemente, na interrupção do fluxo de seiva do xilema, que parte do sistema radicular e é translocada para parte aérea (AMORIM; REZENDE; BERGAMIN FILHO, 2018; AUER; SANTOS; FURTADO, 2016). Como consequência, observa-se sintomas reflexos semelhantes aos ocasionados pelas lesões caulinares, contudo com sintomas internos distintos, tipicamente se manifestando na forma de descoloração do lenho no formato de estrias radiais (AMORIM; REZENDE; BERGAMIN FILHO, 2018; AUER; SANTOS; FURTADO, 2016; FERREIRA; MILANI, 2012). Na cultura do eucalipto, dois tipos de etiologias são associados a murchas vasculares, sendo elas a de origem bacteriana, ocasionada por *Erwiinia psidii*, *Ralstonia solanacearum* e *Ralstonia pseudosolanacearum* (ARRIEL et al., 2014; AUER; SANTOS; FURTADO, 2016; CAIRES et al., 2019; CARSTENSEN et al., 2017; COUTINHO et al., 2011; COUTINHO; WINGFIELD, 2017; FONSECA et al., 2013; FREITAS, 2020), e fúngica, ocasionada por diversas espécies pertencentes ao gênero *Ceratocystis*, com destaque para *Ceratocystis fimbriata* (CHEN et al., 2013; DE BEER et al., 2014; KAMGAN NKUEKAM et al., 2008; 2011; LIU et al., 2015; RODAS et al., 2008; ROUX et al., 2004). Como característica diagnóstica, para distinguir entre ambas as etiologias, recomenda-se observar a presença de exsudação de pús bacteriano no lenho doente recém cortado (ARRIEL et al., 2014; AUER; SANTOS; FURTADO, 2016; FERREIRA; MILANI, 2012).

A podridão do cerne no eucalipto é associada a fungos com comportamento saprofítico, que infectam o cerne das árvores afetadas por meio de ferimentos ou raízes e ramos e ponteiros mortos (FERREIRA; MILANI, 2012). Nessa cultura, os

sintomas de podridão foram associados a fungos pertencentes aos filos Ascomycota e Basidiomycota. Dentre os agentes causais, podemos citar *Pycnoporus sanguineus*, *Inonotus rheades*, *Ganoderma* sp., *Peniophora* sp. e *Pestalotiopsis* sp. (AUER; DOS SANTOS; HALFELD-VIEIRA, 2012; DE ALONSO et al., 2007). Os seus sintomas variam de acordo com a gama de enzimas produzidas por esses microrganismos, podendo a podridão ocasionada ser do tipo branca, caso produzam tanto celulases quanto lignases, ou do tipo parda, caso produzam majoritariamente celulase (BLANCHETTE, 2000; DE ALONSO et al., 2007). A podridão do tipo branca é a mais comum, fazendo com que a madeira adquira coloração clara e apresenta severa depreciação de suas características mecânicas, tornando-a semelhante a isopor (DE ALONSO et al., 2007). Por conta desses microrganismos não atuarem em tecidos vivos das árvores, existe relutância em classificar esses fungos como patógenos. Entretanto, é importante salientar que, mesmo não interferindo na fisiologia normal da planta afetada, esses fungos podem reduzir a resistência mecânica do tronco da árvore infectada, devido à degradação do cerne, tecido que passa a ter função de sustentação, podendo torná-la mais predisposta à quebra pelo vento (AUER; DOS SANTOS; HALFELD-VIEIRA, 2012). Por não ocasionarem danos diretos às árvores, os microrganismos tipicamente associados à podridão do cerne são ainda pouco estudados.

As podridões radiculares afetam principalmente os tecidos associados à absorção de água e nutrientes, resultando em sintomas reflexos de murcha e seca das árvores doentes (AMORIM; REZENDE; BERGAMIN FILHO, 2018). Na cultura do eucalipto, *Phytophthora* spp., *Pythium* sp.; *Ganoderma* spp. e *Phellinus noxius* estão entre os agentes causais dessa patologia, podendo *Rigidoporus microporus*, *Tinctoporellus epimiltinus* e *Trametes* sp. estarem também associados (AGUSTINI et al., 2014; AUER; DOS SANTOS; HALFELD-VIEIRA, 2012; AUER; SANTOS; FURTADO, 2016; UMAMI; PARKER; ARNDT, 2021). Os fungos relatados podem formar estruturas reprodutivas na superfície dos tecidos colonizados, tipicamente na forma de crostas ou de orelhas de pau, das quais são formados e liberados esporos sexuados. Esses microrganismos, além de causarem a necrose dos tecidos radiculares, podem causar também podridões do lenho, com destaque para a do tipo branca (AGUSTINI et al., 2014; AUER; DOS SANTOS; HALFELD-VIEIRA, 2012). No tecido colonizado por esses patógenos é possível observar a formação de linhas,

também chamadas de “pseudosclerotia”, de coloração característica de acordo com o fungo associado, que correspondem a estruturas compostas por células hipertrofiadas com acúmulo de melanina, visando bloquear a colonização por microrganismos competidores. Essas linhas tipicamente delimitam zonas de degradação na madeira (LOPEZ-REAL, 1975; MORRIS, et al., 2021). Árvores afetadas por podridões radiculares podem se tornar mais predispostas à queda pelo vento, devido a fragilização do sistema radicular.

As doenças caulinares e radiculares estão entre os principais problemas fitossanitários na cultura do eucalipto, contudo ainda demandam mais estudos, principalmente no que tange os métodos de manejo. A principal medida de manejo empregada atualmente, na área florestal, é o uso de variedades/clones resistentes. Contudo, observa-se que diversas dessas patologias, devido à variabilidade genética dos patógenos associados e à pressão de seleção por genótipos mais agressivos, podem vencer e/ou driblar os mecanismos de defesa das árvores classificadas como resistentes, levando, assim, a uma “validade” da resistência das plantas selecionadas (AUER; SANTOS; FURTADO, 2016; FERREIRA et al., 2010; OLIVEIRA et al., 2015). Outras medidas devem ser testadas e, caso se mostrem eficazes, aplicadas em condição de campo.

Fungos do gênero *Ceratocystis* são pertencentes à família Ceratocystidaceae e à ordem Microascales. Esse gênero foi criado em 1890 para abrigar *C. fimbriata*, identificado como causador da podridão negra da batata doce nos Estados Unidos (DE BEER et al., 2014; MARIN-FELIX et al., 2017). Atualmente, inclui outras espécies, sendo importantes causadoras de doenças em espécies florestal e agronômicas, associadas a sintomas de podridões de órgãos de reserva, frutos e plântulas, lesões caulinares e descoloração da madeira (DE BEER et al., 2014; HALSTED, 1890; VALDETARO et al., 2019). Nesse gênero, são relatadas também associações com besouros que podem atuar como vetores (HULCR; STELINSKI, et al., 2016).

Na cultura do eucalipto, são relatadas as espécies *Ceratocystis fimbriata*, *C. pirilliformis*, *C. atrox*, C. cercfabiensis, *C. corymbiicola*, *C. eucalypticola* e *C. neglecta* (BARNES et al., 2003; CHEN et al., 2013; DE BEER et al., 2014; KAMGAN NKUEKAM et al., 2008; 2011; ROUX et al., 2004; VAN WYK et al., 2007; 2012; RODAS et al., 2008; LIU et al., 2015). Outras espécies, anteriormente classificadas como pertentes

ao gênero *Ceratocystis*, foram também relatadas nessa cultura, como *Davidsoniella eucalypti* (sin. *C. eucalypti*), *Huntiella chinaeucensis* (sin. *C. chinaeucensis*), *H. cryptoformis* (sin. *C. decipiens*), *H. tyalla* (sin. *C. tyalla*), *H. oblonga* (sin. *C. oblonga*), *H. salinaria* (sin. *C. salinaria*) e *H. savannae* (sin. *C. savannae*). O gênero *Ceratocystis* é a forma sexuada de fungos do gênero *Thielaviopsis* (sin. *Chalara*) (CHEN et al., 2013; HEATH et al., 2009; KAMGAN NKUEKAM et al., 2008; 2011; KILE et al., 1996). Por mais que essas espécies tenham sido relatadas em árvores de eucalipto, ainda é incerto o seu potencial em causar danos nessa cultura.

A identificação das espécies dentro do gênero *Ceratocystis*, por meio de características taxonômicas, é pouco confiável devido à semelhança morfológica entre os membros desse gênero, levando à formação de complexos de espécie crípticas. Os complexos atualmente reconhecidos dentro desse gênero são: *C. fimbriata*, *C. paradoxa*, *C. coerulescens*, *C. moniliformis* e outros complexos compostos por espécies conhecidas somente pelas formas assexuadas, abrangendo os gêneros *Thielaviopsis* e *Ambrosiella* (DE BEER et al., 2014; WINGFIELD et al., 2013). O uso de ferramentas moleculares é uma forma segura de distinção entre espécies no gênero *Ceratocystis*, contudo deve-se atentar às regiões gênicas e aos primers utilizados (DE BEER et al., 2014; FOURIE et al., 2015). Segundo recomendação de alguns autores, a descrição de novas espécies, dentro desse gênero, deve ser acompanhada por ensaios de compatibilidade entre colônias do isolado que se deseja classificar, em relação às outras espécies de *Ceratocystis*, e de especialização por determinados hospedeiros (BAKER et al., 2003; DE BEER et al., 2014; ENGELBRECHT; HARRINGTON, 2005; HARRINGTON et al., 2014).

O complexo *C. fimbriata* é um dos mais estudados por abranger espécies de grande importância agronômica e florestal (DE BEER et al., 2014; FOURIE et al., 2015). Dentro desse complexo, podem ser encontrados quatro diferentes clados: América Latina, Africano, Asiático e Norte Americano (BAKER et al., 2003; DE BEER et al., 2014; FERREIRA; ALFENAS; MAFIA, 2013; MBENOUN et al., 2013). No clado da América Latina estão incluídas as espécies *C. fimbriata sensu stricto*, *C. cacaofunesta* e *C. platani* (BAKER et al., 2003; ENGELBRECHT; HARRINGTON, 2005; DE BEER et al., 2014). Anteriormente, as duas últimas espécies citadas eram classificadas como subespécies de *C. fimbriata*. Contudo, com o uso de ferramentas moleculares, teste de compatibilidade entre isolados e dados sobre a especialização

dos hospedeiros, concluiu-se que consistiam de espécies distintas (BAKER et al., 2003; DE BEER et al., 2014; ENGELBRECHT; HARRINGTON, 2005). Dentro desse clado, regiões gênicas rotineiramente utilizadas para distinção entre espécies fúngicas, como a ITS-rDNA, são pouco confiáveis, apresentando resolução suficiente somente para distinção entre clados, não entre espécies (DE BEER et al., 2014; FERREIRA; ALFENAS; MAFIA, 2013; FOURIE et al., 2015; HARRINGTON et al., 2014). Para isso, foram desenvolvidos primers que exploram regiões gênicas que ofereçam melhor resolução para distinção entre espécies dentro desse complexo, como as regiões MS204 e RPBII, sendo recomendado o seu uso junto à região ITS-rDNA (FOURIE et al., 2015; MARIN-FELIX et al., 2017). As sequências obtidas após o sequenciamento dessas regiões são concatenadas e utilizadas na construção de árvores filogenéticas, junto a outras sequências obtidas de isolados, disponíveis em bancos de dados, como o GENBANK, que serão utilizados para comparação e agrupamento.

C. fimbriata s.s. é tida como espécie tipo dentro do gênero *Ceratocystis* (MARINCOWITZ et al., 2020), merecendo destaque por conta dos danos e as perdas provocadas em plantios de espécies agronômicos e florestais. Esse fungo forma colônias de crescimento lento em meio de cultivo artificial, de coloração amarronzada ao cinza ou marrom-oliva, quando madura, com pouco micélio aéreo, sendo esse submerso, e ampla formação de conídios, aleuroconídios, peritécios e ascósporos. Os conídios são hialinos, unicelulares, com paredes lisas, e recebem o nome de endoconídios por se formarem no interior de conidióforos, do tipo fiálide, podendo ser dois tipos. O primeiro tipo tem formato cilíndrico, enquanto o segundo, menos comum, formato barriliforme, tipicamente se formando em cadeias. Os endoconidióforos apresentam formato lageniforme, se formando lateralmente nas hifas, de forma agregada ou solitária, com 1-7 septos, e coloração que varia do hialino ao marrom claro. Os aleuronídios são do tipo clamidósporos, atuando como esporos de resistência, apresentando parede celular espessa e coloração que varia de hialina ao marrom escuro, e formato globoso a piriforme (ENGELBRECHT; HARRINGTON, 2005; FERREIRA; ALFENAS; MAFIA, 2013; OLIVEIRA et al., 2015). Esses esporos se formam de forma solitária ou em cadeias curtas, na extremidade de conidióforos que se desenvolvem lateralmente em hifas, com 1-10 septos (FERREIRA; ALFENAS; MAFIA, 2013; OLIVEIRA et al., 2015). Os peritécios se formam superficialmente, com

base, de formato globoso, submerso no substrato. A coloração dos peritécios varia de marrom escuro a preto. Na extremidade do rostro dessa estrutura, que apresenta a mesma coloração do restante do peritécio, se formam hifas ostiolares, sem septos, hialinas e de paredes lisas, onde é possível observar a presença de uma massa de coloração creme a alaranjada, sendo composta por ascósporos embebidos em uma matriz mucilaginosa. Esses esporos se formam no interior dos peritécios, em ascos evanescentes, que se dissolvem antes da maturação dessa estrutura. Os ascósporos são hialinos, unicelulares e com ornamentação, tornando o formato desses esporos semelhantes ao de chapéu coco (ENGELBRECHT; HARRINGTON, 2005; FERREIRA; ALFENAS; MAFIA, 2013; OLIVEIRA et al., 2015).

Por ser uma espécie cosmopolita, *C. fimbriata* é relatada em uma grande gama de hospedeiros em diversos países do mundo. Dentre as espécies agronômicas e florestais relatadas como hospedeiras desse patógeno, podemos citar a acácia (*Acacia mearnsii*), andiroba (*Carapa guianensis*), atemóia (*Annona cherimola* x *A. squamosa*), batata doce (*Ipomoea batatas*), eucalipto (*Eucalyptus* spp.), gmelina (*Gmelina arborea*), kiwi (*Actinidia deliciosa*), manga (*Mangifera indica*), maracujá (*Passiflora edulis*), mogno africano (*Khaya senegalensis*) e teca (*Tectona grandis*) (FIRMINO et al., 2012; 2013; 2017; FIRMINO; TOZZE JUNIOR; FURTADO, 2012; HALFELD-VIEIRA, 2012; MÉNDEZ-ÁLVAREZ et al., 2020; MORRIS; WINGFIELD; DE BEER, 1993; OLIVEIRA et al., 2015; 2016; PAUL et al., 2018; PIVETA et al., 2016).

Na cultura do eucalipto, a murcha de ceratocystis foi relatada pela primeira vez em 1975, em *Eucalyptus tereticornis*, estando associada a sintomas de murcha e seca de plantas em campo, apesar de não estar publicada esta ocorrência (FERREIRA et al., 2006). *C. fimbriata* é tido como principal agente causal dessa doença no Brasil (AUER; SANTOS; FURTADO, 2016; OLIVEIRA et al., 2015). Esse patógeno sobrevive no solo, na forma de esporos de resistência do tipo clamidósporo, ou em restos de cultura como saprofíticos (FERREIRA et al., 2011; 2013; MARIN-FELIX et al., 2017). Em condição de campo, a infecção das árvores ocorre principalmente por meio do sistema radicular e/ou colo da árvore suscetível (FERREIRA et al., 2013; LAIA; ALFENAS; HARRINGTON, 2000). A causa dos sintomas de murcha e seca da parte aérea é a colonização do sistema vascular da planta afetada pelo patógeno, levando à obstrução parcial ou total do xilema secundário, devido ao crescimento do fungo no interior dos vasos desse tecido (DA SILVA et al., 2020; FERREIRA; MAFFIA;

FERREIRA, 2005; FERREIRA et al., 2006; FIRMINO et al., 2015; MAFIA et al., 2013). Como consequência, ocorre o impedimento do transporte de solutos para os tecidos superiores da planta, resultando na murcha e seca da copa (DA SILVA et al., 2020; FERREIRA et al., 2006; PIMENTA et al., 2017). Esse processo é melhor observado em indivíduos em que o fungo coloniza boa parte do diâmetro do caule e em períodos de seca, quando o déficit hídrico é maior. Nessa condição, as plantas doentes, por transportarem uma quantidade menor de água para sua copa devido à obstrução do xilema, acabam por apresentar sintomas mais severos (DA SILVA et al., 2020; PIMENTA et al., 2017).

No interior dos vasos do xilema de árvores de eucalipto infectadas, o fungo *C. fimbriata* forma conídios, esporos de resistência e hifas que acabam por obstruir essas células. As estruturas do patógeno podem ser facilmente observadas em cortes histológicos (DA SILVA et al., 2020; FERREIRA; MAFFIA; FERREIRA, 2005; FIRMINO et al., 2015; MAFIA et al., 2013). Os esporos de resistência formados provavelmente estão associados à sobrevivência do patógeno no solo ou em restos de cultura (FERREIRA et al., 2011; 2013; MARIN-FELIX et al., 2017). Na superfície dos tecidos colonizados, é possível observar a formação de peritécios escuros, de rostro longo, com a liberação de uma massa gelatinosa de coloração levemente alaranjada ou amarelada, contendo os ascósporos do patógeno (AUER; SANTOS; FURTADO, 2016; FERREIRA; MILANI, 2012).

Outro sintoma marcante da murcha de ceratocystis em eucalipto é a intensa formação de brotações adventícias ao longo do caule das árvores doentes. Esse sintoma é ocasionado pelo desbalanço hormonal causado pela morte e/ou declínio da copa da árvore afetada, resultando em um desequilíbrio entre auxinas e giberelinas, semelhante ao que ocorre após a ocorrência de incêndios (FERREIRA et al., 2006; FERREIRA; MILANI, 2012). Quando esse processo de brotação ocorre em árvores em que a copa quebrou com o vento, foi podada ou passou pelo corte raso, as brotações restabelecem os processos fotossintéticos da árvore, permitindo que, de certa forma, a planta recupere suas funções fisiológicas normais. No entanto, em árvores doentes devido à murcha de ceratocystis, o processo de colonização do fungo ocorre de forma ascendente e descendente através da medula, resultando assim na colonização dos tecidos em que estão presentes as brotações, e, consequentemente, ocasionando a morte das mesmas com o passar do tempo (AUER; SANTOS;

FURTADO, 2016; DA SILVA et al., 2020; FERREIRA et al., 2006; FERREIRA; MILANI, 2012).

No eucalipto, de acordo com o material genético, é possível observar o aparecimento de lesões longitudinais na casca, de coloração marrom-avermelhada, partindo do colo ou ocorrendo ao longo do tronco. Com o progresso do processo infeccioso, essas lesões aumentam de tamanho e se tornam sulcadas, resultando na formação de um cancro típico. Esses sintomas são indícios de danos ao câmbio vascular, floema e feloderme, podendo haver exsudação de gomas através dos ferimentos (FERREIRA et al., 2006; FERREIRA; MILANI, 2012). Ao contrário do que ocorre com patógenos foliares, a planta apresentando sintomas de seca devido à murcha de ceratocystis mantém suas folhas retidas na copa por longos períodos, mesmo estas já estando secas (AUER; SANTOS; FURTADO, 2016; FERREIRA et al., 2006; FERREIRA; MILANI, 2012). Esse sintoma é semelhante ao que ocorre com podridões radiculares, devendo se tomar cuidado para não confundir ambas as patologias.

Em viveiros de eucalipto, esse patógeno pode causar danos em todas as fases de formação das mudas, desde as minicepas em jardins clonais destinadas à produção de estacas para enraizamento, até em mudas na fase de aclimatização a céu aberto; causando murcha e seca de minicepas e mudas e podridões de estacas. Mudas contaminadas podem servir como fonte de inóculo, levando o fungo *C. fimbriata* para áreas livres do patógeno, ou introduzindo genótipos mais agressivos nesses locais (FERREIRA et al., 2011). Árvores que se contaminaram ainda na fase de muda, aparentemente, apresentam sintomas mais rapidamente após o plantio do que aquelas infectadas em condição de campo (FERREIRA et al., 2011; FERREIRA; ALFENAS; MAFIA, 2013).

Nos tecidos internos de indivíduos de eucalipto afetados por *C. fimbriata*, é possível observar a descoloração dos tecidos do xilema secundário no formato de estrias radiais escuras, que partem da medula, se direcionam até o câmbio vascular (AUER; SANTOS; FURTADO, 2016; FERREIRA et al., 2006; FERREIRA; MILANI, 2012). A coloração das estrias pode estar associada à colonização dos raios pelo fungo e pelo acúmulo de fenóis oxidados nesses tecidos (DA SILVA et al., 2020). Esses raios servem como um acesso do fungo à medula, na qual, uma vez colonizada,

permite que o fungo atinja diferentes alturas da árvore (AUER; SANTOS; FURTADO, 2016; FERREIRA et al., 2006; FERREIRA; MILANI, 2012). A infecção da medula é facilitada por conta deste ser o primeiro tecido inativado durante o processo de desenvolvimento do lenho, oferecendo pouca resposta de defesa frente ao processo de colonização (FERREIRA et al., 2006).

Um dos mecanismos de reação de defesa de espécies arbóreas contra a infecção por patógenos caulinares é o de compartimentalização do lenho. Esse processo é realizado por uma série de mecanismos que permitem isolar o patógeno no lenho através da formação de novos tecidos e a alteração química de tecidos já existentes, visando tornar os mesmos impróprios ao desenvolvimento do patógeno (BIGGS, 1992; DA SILVA et al., 2020; FERREIRA; MILANI, 2012). No caso da murcha de ceratocystis em eucalipto, a formação de tiloses, acúmulo de géis, compostos fenólicos, oxalato de cálcio e materiais amorfos, lignificação das células e produção de determinadas enzimas, são vistas como reações de defesa contra a colonização do agente causal, sendo mais intensas e rápidas em materiais resistentes do que em susceptíveis (DA SILVA et al., 2018; 2020).

As tiloses são resultantes da hipertrofia das células do parênquima adjacentes aos elementos de vaso, que crescem no interior dos mesmos através das pontuações, resultando, assim, na obstrução parcial ou total dessas células (CLÉRIVET et al., 2000; DA SILVA et al., 2020; DUCHESNE; HUBBES; JENG, 1992). Essa estrutura é capaz de reduzir ou impedir o crescimento ascendente do fungo nos tecidos vasculares, sendo formada em maior abundância e velocidade em indivíduos de eucalipto resistentes à murcha de ceratocystis (DA SILVA et al., 2020; FIRMINO et al., 2015; PIMENTA et al., 2017; TUMURA; PIERE; FURTADO, 2012).

A metodologia tipicamente empregada em estudos que visam avaliar a resistência de determinados clones ou espécies de eucalipto, frente à murcha de ceratocystis, consiste da inoculação do fungo em tecidos caulinares vivos de mudas ou árvores de eucalipto, em condição de casa de vegetação ou campo, com posterior avaliação do tempo necessário para manifestação dos sintomas e/ou severidade, através, principalmente, da medição do comprimento do tecido colonizado e dos sintomas externos apresentados (DA SILVA et al., 2018; 2020; FIRMINO et al., 2013; PIMENTA et al., 2017; TUMURA; PIERE; FURTADO, 2012; ZAUZA et al., 2004). Pode

ser quantificada também a interferência da doença no crescimento da planta, comparando plantas inoculadas e não inoculadas na mesma condição ambiental (PIMENTA et al., 2017). As inoculações podem ser feitas via injeção no caule de uma determinada concentração de esporos, produzidos em meio artificial, ou por meio da inoculação do próprio meio de cultivo colonizado pelo patógeno em ferimentos abertos (DA SILVA et al., 2018; 2020; FIRMINO et al., 2013; PIMENTA et al., 2017; TUMURA; PIERE; FURTADO, 2012; ZAUZA et al., 2004). No entanto, esses métodos são destrutivos e podem levar de 30 a 120 dias até se obter resultados em eucalipto, quando feito em mudas, podendo levar mais tempo quando o ensaio é realizado em árvores em campo. O uso de ferramentas moleculares, na identificação de genótipos de eucalipto resistentes à murcha de ceratocystis, também é visto como uma alternativa para acelerar o processo de seleção, contudo ainda são necessários mais estudos para sua aplicabilidade em projetos de melhoramento (ROSADO et al., 2016).

A detecção de *C. fimbriata* em tecidos doentes é normalmente feita através do uso de iscas de cenoura, sendo considerado como um meio semi-seletivo a esse fungo (MOLLER; DEVAY, 1968). No entanto, só se mostra eficiente quando o fungo ainda se encontra viável no tecido vegetal. Nos casos dos tecidos colonizados há muito tempo, o fungo pode não estar mais viável, resultando em seu não desenvolvimento em meios artificiais ou iscas. Para essas situações, podem ser utilizadas técnicas que visam observar a presença de sinais do patógeno nos tecidos colonizados, como as análises histológicas, que têm como objetivo a observação de estruturas características do patógeno no interior dos vasos da planta (FERREIRA, MAFFIA, FERREIRA, 2005), ou o emprego de ferramentas moleculares. Para a segunda técnica, mesmo se o fungo não estiver mais viável, ainda haverá presença do seu material genético nos tecidos colonizados, sendo possível, através do uso de primers específicos, a sua detecção por meio da técnica de PCR (DHARMARAJ et al., 2022).

Do ponto de vista epidemiológico, a murcha de ceratocystis, na cultura do eucalipto, pode ser classificada como uma doença monocíclica, ou seja, o inóculo formado não contribui para o aumento da epidemia no mesmo ciclo da cultura (AMORIM; REZENDE; BERGAMIN FILHO, 2018; FERREIRA et al., 2013; TUMURA; PIERE; FURTADO, 2012). Esse padrão de doenças é normalmente associado a patógenos habitantes do solo, cuja curva de aumento da doença se encaixa com o

modelo monomolecular (AMORIM; REZENDE; BERGAMIN FILHO, 2018; FERREIRA et al., 2013). A mortalidade por essa doença é mais expressiva até os 20 meses de plantio, podendo chegar até mais de 50% aos 84 meses, com distribuição agregada das árvores doentes. Esse padrão de distribuição espacial provavelmente está relacionado à distribuição do inóculo no solo. De acordo com a tolerância e/ou resistência da árvore em relação ao patógeno, pode-se observar árvores infectadas que permanecem vivas até o final do ciclo. Caso o plantio seja estabelecido com mudas infectadas, pode-se observar plantas sintomáticas já aos 7 a 12 meses de plantio (FERREIRA et al., 2013).

A murcha de ceratocystis em eucalipto tem impactos diretos e indiretos nas cadeias produtivas envolvidas. A queda no crescimento volumétrico das árvores em campo pode chegar a 87%, em maiores níveis de severidade da doença (MAFIA et al., 2013). Como consequência da queda no crescimento, o produtor de eucalipto, que visa o uso da lenha para produção de carvão, pode ter perdas em torno de US$ 3478.43/ha (FERNANDES et al., 2014). Já na produção de celulose, o uso de madeira colonizadas por *Ceratocystis*, por demandarem de uma maior quantidade de reagente para polpação, eleva os custos do processo em até 29,6%, diminuindo também o rendimento de celulose por m^3 de madeira, entre 13,7 e 15,2% (ARAÚJO et al., 2007; MAFIA et al., 2013). Devido ao aumento nos custos e à queda no rendimento e qualidade da celulose produzida, recomenda-se o uso da madeira colonizada para outros fins, como a queima na caldeira (ARAÚJO et al., 2007).

A única medida considerada como eficiente para o manejo em campo da murcha de ceratocystis em eucalipto é o plantio de materiais resistentes (AUER; SANTOS; FURTADO, 2016). É recomendado também o maior cuidado nas operações silviculturais, de forma que se evite ao máximo a ocorrência de ferimentos nas árvores e a transmissão do patógeno através de ferramentas e do maquinário empregado (FERREIRA et al., 2011; 2013). O plantio de mudas sadias é outra medida para o manejo de *Ceratocystis*, evitando assim a entrada desse microrganismo em locais ainda sem a ocorrência do mesmo (FERREIRA et al., 2011).

CHAPTER 1

FUNGI ASSOCIATED WITH TRUNK PATHOLOGIES IN *Eucalyptus* PLANTATIONS[1]

ABSTRACT

Eucalyptus is the most cultivated tree in Brazil, used mainly for cellulose production. However, the incidence of trunk pathologies represents a risk to the productivity of *Eucalyptus* plantations, causing mortality and reduced growth of affected trees. Therefore, in this work we carried out a survey of fungi associated with different trunk pathologies in eucalypt plantations, located in the south of Bahia, Brazil. The fungi were characterized for pathogenicity, laccase production and identified to the genus level through sequencing of the internal transcribed spacer (rRNA-ITS) gene. Genera already known to include *Eucalyptus* pathogens were found, such as *Botryosphaeria*, *Ceratocystis*, *Chrysoporthe*, *Cytospora*, *Neopestalotipsis* and *Pestalotiopsis*, but several others, not yet reported in this tree, were presented, such as *Neonothopanus* and *Scytalidum*. The range of genera that cause heartwood rot was also expanded, with emphasis on *Lentinus*, *Scytalidum* and *Xenoacremonium* as they are classified as strong laccase producers. Other genera found still have an uncertain association with this tree, such as *Neoleptodontidium*, *Phaeoacremonium* and *Trichoderma*, still needing more studies. In general, the present work expands the range of microorganisms associated with eucalypt plantations, elucidating some of the possible interactions of the isolated microorganisms with this tree.

Key words: Canker, wood decay, wood discoloration, heartwood rot, bark lesion

---

[1]Formatted according to the "Forest Pathology" submission guidelines.

1.1 Introduction

Trunk diseases are among the main phytosanitary problems in *Eucalyptus* plantation, due to the difficulty of control and their potential to reduce forest productivity. These pathologies are mainly associated with phytopathogenic fungi and bacteria (Alfenas et al., 2004; Ferreira & Milani, 2012). The main symptoms caused are wood necrosis, resulting in the inactivation of the xylem vascular system; and the formation of lesions in the bark, impairing the translocation of phloem sap. Affected woody tissues presents discoloration, becoming darkened due to colonization by the pathogen and plant defense responses. Diseased trees may show external symptoms of reduced growth, dry branches, defoliation, tree break and death. (Arriel et al., 2014; Aylward et al., 2019; Barradas et al., 2019; Carstensen et al., 2017; Ferreira et al., 2006; Ferreira & Milani, 2012; Soares et al., 2018).

Several species of trunk pathogens are reported in *Eucalyptus* plantations. Among these, members of the genera *Botryosphaeria*, *Chrysoporthe, Cystospora* and *Teratosphaeria* are related as important causal agent of stem lesions, causing symptoms at different heights of the trunk (Aylward et al., 2019; Barradas et al., 2019; Soares et al., 2018). The fungi *Ceratocystis* spp. and the bacteria *Ralstonia solanacearum* and *Erwinia psidii* are associated with vascular wilt in *Eucalyptus* (Arriel et al., 2014; Carstensen et al., 2017; Ferreira et al., 2006). Stem pathologies can affect eucalypt plants from the nursery to harvest age, with different pathogens and symptoms observed throughout the forest cycle (Alfenas et al., 2004; Ferreira & Milani, 2012).

Heartwood rot is a group of trunk pathologies normally ignored in *Eucalyptus* plantations, due to the difficulty of detection. Symptoms of this disease occurs as rot in the central region of the wood, normally affecting plantations that are more than three years old, when the trees already have developed heartwood (Auer et al., 2012; Ferreira & Milani, 2012). Woody tissues affected by rot become less resistant to mechanical efforts, making their use in sawmills unfeasible (Auer et al., 2012). This alteration occurs due to the degradation of specific wood molecules, such as cellulose and/or lignin, due to enzymes produced by pathogens, also causing changes in its color, which characterizes the type of rot. White rot results from the degradation of cellulose and lignin, while brown rot occurs mainly from cellulose (Mester et al., 2004). The causal agents are typically fungi, but they can occur in association with termites,

making the affected tree hollow and reducing the volume harvested (Auer et al., 2012; Ferreira & Milani, 2012).

In addition to pathogens, endophytic microorganisms can also inhabit the tissues of plant species, without posing any risk to them. Some of these microorganisms may be beneficial to host plants, demonstrating potential for use in agriculture, while others apparently have little impact on plant physiology (Kang et al. 2007; Melnick et al., 2008; Verma et al., 2022). The positive effects of endophytic microorganisms may be related to the secretion of beneficial secondary metabolites, such as compounds with antibiotic and antifungal function and growth-inducing molecules, such as those analogous to phytohormone (Melnick et al., 2008; Verma et al., 2022). Endophytic microorganisms may have biotechnological potential and can be used in soil decontamination and in industrial processes due to production of specific enzimes (Corrêa et al. 2014; Ijaz et al., 2016; Sharma & Rath, 2021).

The survey of pathogens in forest ecosystems is a way to monitor the occurrence of new diseases, which can be introduced together with contaminated plant material or through the adaptation of native pathogens to the plant species. In *Eucalyptus* cultivation, examples of indigenous pathogens that may have adapted to this tree after introduction in Brazil, and that have gained economic importance, are *Chrysoporthe* spp. and *Austropuccinia psidii*, causing, respectively, *Chrysoporthe* canker and myrtle rust (Mausee-Sitoe et al., 2016; Tommerup et a., 2003). New diseases can put at risk the productive capacity of cultivated forests. Thus, the objective of this work was to identify fungi associated with *Eucalyptus* trunk pathologies, and to study their interaction with this tree through the characterization of their pathogenicity and production of the enzyme laccase.

1.2 Material and methods

1.2.1 Methodology for sample collection and fungal isolation

The experiment was carried out in commercial *Eucalyptus* plantations located in the southern region of the state of Bahia, Brazil. This region has sandy to clayey soils, with medium depth and the presence of rocky outcrops. The local climate is classified as tropical, with rainfall distributed throughout the year, with precipitation ranging from 700 to 1700mm and an average daily temperature of 19 to 26ºC. The clones cultivated in the studied areas belong to the crossing of *Eucalyptus grandis* x

E. urophylla (Urograndis), established in spacings that varied between 3.0 x 2.0, 5.0 x 2.4 and 4.0 x 3.0m. Planting age ranged from 24 months to approximately 84 months (harvest age).

The sampled living trees were classified into three types of trunk pathologies, based on external and internal symptoms:

- Stem lesion (Figure 1a): presence of necrosis of secondary phloem tissues, with or without bark detachment or exposure of internal wood tissues;

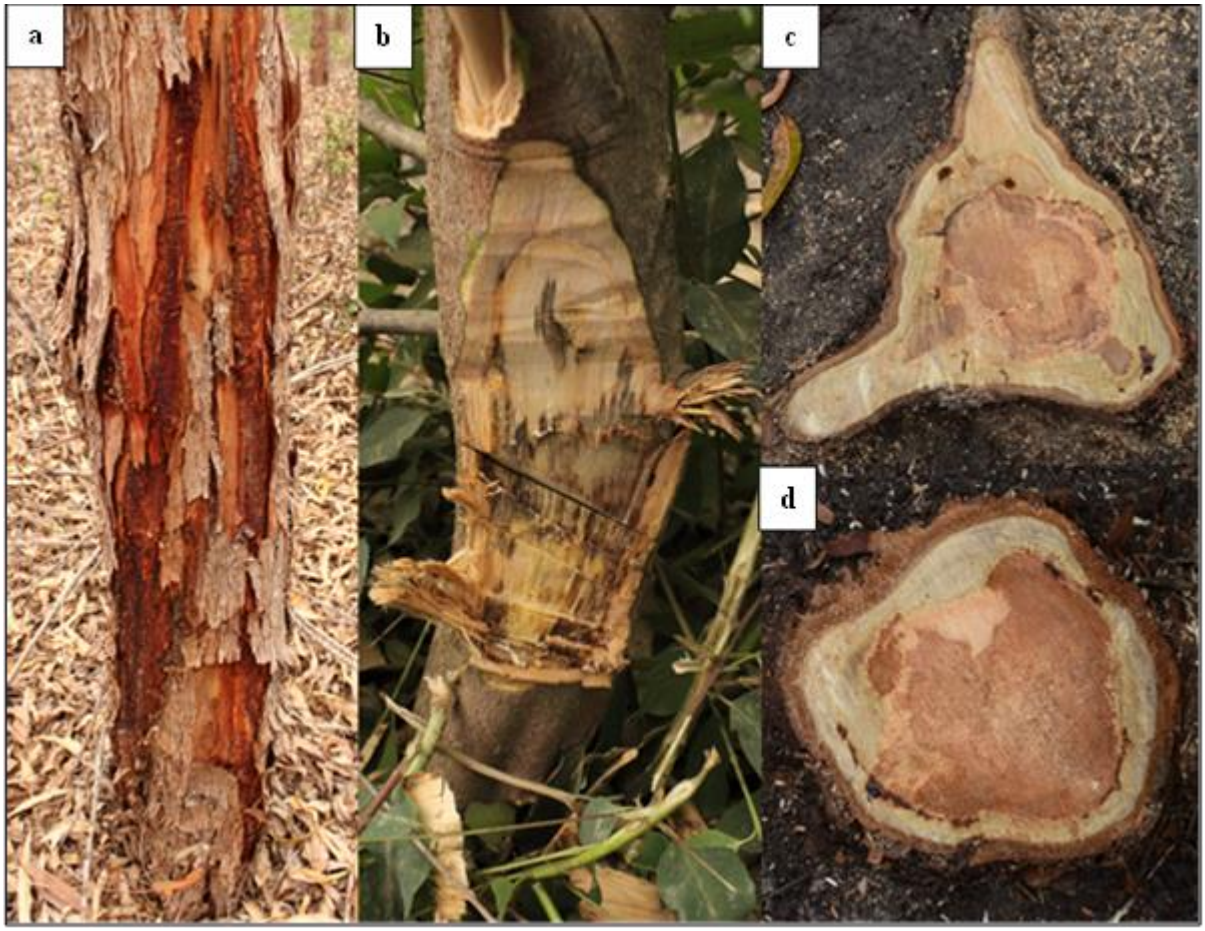
- Sapwood discoloration (Figure 1b): sapwood showing symptoms of darkening, indicating colonization by microorganisms, without the presence of large bark lesions;

- Heartwood rot (Figure 1c, d): discoloration in the heartwood region, involving loss of mechanical resistance. The only type of rot observed was the white type.

Trees showing symptoms of stem lesion and sapwood discoloration were initially selected in field based on the manifestation of external symptoms of dry foliage, defoliation and the presence of adventitious shoots. However, some of the trees that showed symptoms of sapwood discoloration were externally asymptomatic, requiring the bark to be removed using a machete. Heartwood rot was detected in trees that had been planted for more than five years, making it necessary to fell the trees with a chainsaw.

Bark and wood fragments were collected from symptomatic tissues and stored in paper bags until analysis. Under laboratory conditions, indirect isolation of the samples was carried out, through sequential washing for 2 minutes with 70% alcohol, 2% hypochlorite and sterilized distilled water, with subsequent deposition in petri dishes containing PDA culture medium (39g/L) with addition of streptomycin sulfate (50mg/L). In order to detect *Ceratocystis* sp., the carrot bait method was used when appropriate (Moller & Devay, 1968). We monitor the development of fungal colonies daily, purifying them. The fungal isolates obtained were stored in the mycological collection of the Microbiology and Forest Pathology Laboratory at UNESP/FCA.

Figure 1. Symptoms manifested by *Eucalyptus* trees from which samples were collected aiming for indirect fungal isolation. **a.** Stem lesion; **b.** Sapwood discoloration; **c, d.** Heartwood rot.



1.2.2. Characterization of the isolates obtained

All isolates obtained were characterized regarding their pathogenicity and lignin degradation capacity, through the production of laccase; enabling a better understanding of their interaction with the trees. The isolates were grouped based on the symptoms of the trees from which they were isolated, reactivity with KOH and morphological characteristics of the colonies and spores formed, when found. For each grouping, one isolate was selected as representative and its identification was carried out through sequencing of the rDNA-ITS region.

1.2.3 Pathogenicity test

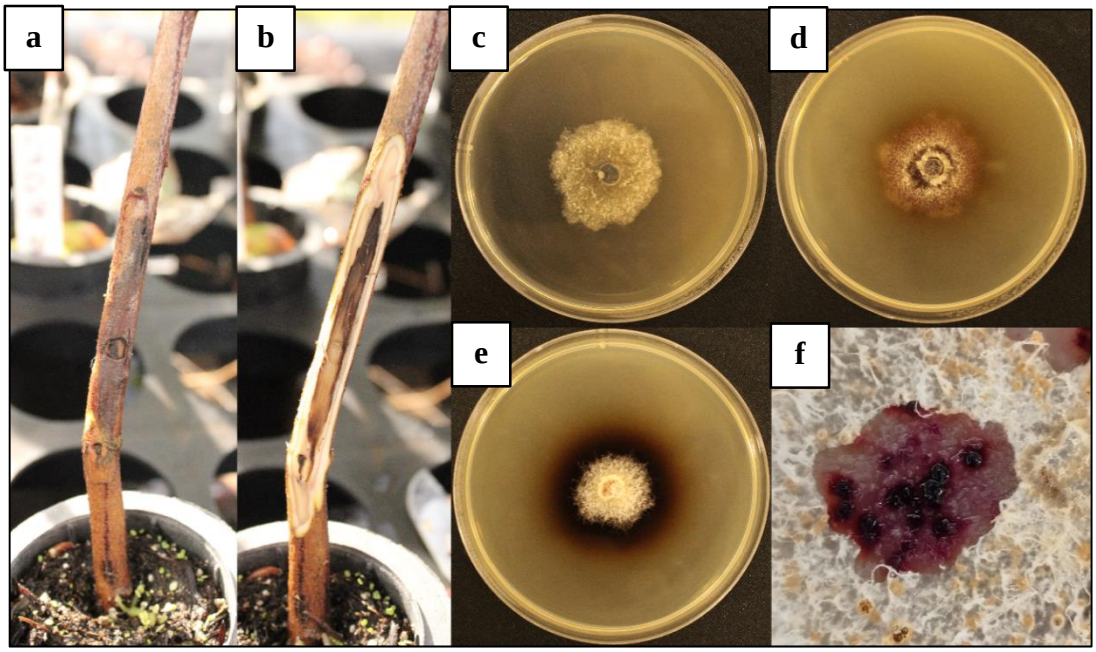
The fungal isolates obtained were cultivated in PDA culture medium for 10 days, under constant temperature of 25ºC and alternating photoperiod (12/12). The formed colonies were inoculated in *Eucalyptus* seedlings, maintained for 90 days in 390cm^3 pots, containing substrate based on vermiculite, peat and carbonized rice husks, with the addition of 200g/L of MAP and 200g/L of osmocote (NPK; 15:09:12). The seedlings remain during this period in a greenhouse, under periodic irrigation. The chosen clone belongs to the crossing of *E. grandis* x *E. urophylla* and, According to reports from those responsible for eucalypt plantation, it showed a higher incidence of trunk pathologies in field conditions in the study area. Inoculations were performed by opening a wound, with the aid of a 5mm diameter cork borer, 5cm above the ground line, in order to expose the internal woody tissues. A disk of culture medium, colonized by the fungus, of the same diameter was placed in the injured area. The inoculated site was covered with cotton soaked in distilled water, both previously sterilized, and wrapped with parafilm, reducing desiccation. As control, seedlings were inoculated with disks of fungus-free culture medium. Treated plants were kept in the same greenhouse in which they grew. After 90 days, the length of the external (bark) and internal (wood) lesions (Figure 2a, b) of the inoculated seedlings was measured and woody fragments were collected for isolation of the inoculated microorganisms. Only reisolated fungi were considered pathogenic. The experiment was carried out in a completely randomized design, with 8 seedlings (replications) being inoculated per fungal isolate.

1.2.4. Laccase production

The capacity of the isolates to produce laccase was determined by the qualitative method of the Bavendamm reaction (Etheridge, 1957; Smit et al., 1996). For this, the fungi were cultivated for 10 days in PDA culture medium. From the edge of the formed colonies, mycelium discs with a diameter of 5 mm were collected, with the aid of a cork borer. These discs were transferred to the center of 90 x 15mm petri dishes containing 15ml of MEA culture medium (15g of malt extract and 15g of agar/L), with the addition of 5g of tannic or gallic acid/L. To avoid agar hydrolysis, the phenols were autoclaved separately and incorporated into the MEA medium at the time of plating. The plates containing the fungus were kept for five days at a constant temperature of

28ºC and in the dark. After this period, the formation of amber colored halos was evaluated, indicating the degradation of phenols by enzyme laccase. Fungi were classified as non-producers (np), weak producers (+) and strong producers (++), according to the size and intensity of halo staining (Figure 2c, d, e). The test was carried out in a completely randomized design, with three replications (plates) per isolate.

Figure 2. External lesions on the bark (**a**) and internal lesions on the wood (**b**) of seedlings inoculated with pathogenic fungi (LPFMC-3). Absence of Bavendamm reaction in the isolate that does not produce laccase (LPFMC-1) in medium containing gallic acid (**c**) and formation of a halo by the isolate that are weak producer (LPFMC-7; **d**) and strong producer (LPFMC-10; **e**). Reaction of the LPFMC-4 isolate to KOH, showing purple color (**f**).



1.2.5 Morphological characterization and KOH reaction

The fungal isolates were initially identified at the genus or family level according to the morphological characteristics of the spores and colonies formed in PDA culture medium. For this, the fungi were cultivated under a constant temperature of 25ºC and alternating photoperiod, for 7 to 20 days, according to the growth of the colony. Characteristics such as color, type of mycelium and growth rate were observed. When formed in colonies, micro and macro reproductive structures of the isolates were observed under the microscope, in terms of size, shape and color. For characterization

of KOH reaction, drops of 3% (w/v) potassium hydroxide (KOH) solution were deposited on the formed colonies and color changes were observed after 5 minutes (Figure 2f).

1.2.6 Molecular identification

To identify the isolates selected as representatives of the clusters, DNA extraction was realized from the mycelium collected from colonies formed in culture medium, using the DNA extraction kit TransDirect® Plant Tissue PCR Kit (Transgenbiotech, China), following the recommendations of the manufacturer. Amplification of the internal transcribed spacer (rDNA-ITS) region was performed using primers ITS1F and ITS4, following the author's polymerase chain reaction (PCR) recommendations (Gardes & Bruns, 1993). The sequencing of the isolates was performed on ABI3500 Genetic Analyzer (Applied Biosystems), using BigDye™ Terminator v3.1 Cycle Sequencing Kit, according to manufacturer's instructions. The resulting sequences were edited in BioEdit 7.0.5.3 (Hall, 1999) and compared to another at the National Center for Biotechnology Information (NCBI) database. The rRNA-ITS sequences were deposited in GenBank. Phylogenetic analysis was conducted using the Maximum Likelihood method in MEGA11 (Tamura et al., 2021).

1.3 Results

1.3.1 Isolation and identification of fungi

In total, 127 fungal isolates were obtained from trunk tissues of 67 *Eucalyptus* trees, of different ages, showing symptoms of stem lesions, sapwood discoloration or heartwood rot. According to the morphological characteristics, the isolates were grouped into twenty-one different morphological taxa, including KOH reaction, each one representing a distinct genus. Its identification was carried out through sequencing of the rDNA-ITS region of an isolate selected as representative of each morphological taxon. The sequences obtained were compared with others from Genbank, obtaining 97.3 to 100.0% similarity with the associated species (Table 1). In the KOH reaction test, only the genera *Chrysoporthe* and *Coniochaeta* were positive, showing purple and green color, respectively, in contact with this reagent.

The fungal genera obtained varied in incidence and diversity according to the type of symptom of the *Eucalyptus* trees from which they were isolated (Table 1). Of

the trees that showed symptoms of sapwood discoloration, the greatest diversity of genera was isolated, totaling 15, with the highest incidence of *Botryospharia* (52.2%), followed by *Neoleptodontidium* (47.8%) and *Ceratocystis* (43.5%). Of the plants that showed heartwood rot, eight genera were isolated, with a greater abundance of *Lasiodiplodia* (36.8%), and of those with stem lesions, eight genera were also reported, with emphasis on *Botryosphaeria* (32.0%) and *Neoleptodontidium* (28.0%). The genera *Chrysoporthe* and *Trichoderma* were the only ones isolated from trees showing all three types of symptoms.

1.3.2 Phylogenetic relationship analysis

The sequences obtained after sequencing the rDNA-ITS region were submitted to Genbank, aiming to obtain accession numbers. Other sequences of fungal isolates, with high similarity values, were obtained from the Genbank database, and were used to create a phylogenetic tree, enabling the analysis of the phylogenetic affiliations of the isolated fungi (Figure 3). The 21 genera obtained belong to 15 families, with emphasis on Botryosphaeriaceae, Nectriaceae and Pestalotiopsisdaceae, as they cover more than one genus. The genus Neoleptodontidium still has an uncertain family. The families found can be classified into 12 orders, including Agaricales (2 families), Amphisphaeriales (1), Botryosphaeriales (1), Calosphaeriales (1), Coniochaetales (1), Diaporthales (2), Hypocreales (2), Leotiomycetidae (1), Microascales (1), Polyporales (2), Togniniales (1) and Xylariales (2). Of the genera obtained, 17 belong to the phylum Ascomycota, while the others belong to the phylum Basidiomycota.

1.3.3 Pathogenicity of fungal isolates

The isolates obtained presented different levels of aggressiveness, with those that could be reisolated from the inoculated tissues being considered pathogens (Table 2). *Eucalyptus* seedlings inoculated with isolates belonging to the genera *Ceratocystis* (5.12cm) and *Neonothopanus* (4.65cm) showed a greater extent of internal lesions. All tested isolates of the genus *Ceratocystis* showed pathogenicity. Fungi from the genera *Botryosphaeria* (1.80cm) and *Neonothopanus* (1.65cm) were associated with greater external lesions on the bark of inoculated seedlings. None of the isolates obtained from trees with symptoms of heartwood rot showed pathogenicity, while, of the 15 genera originating from sapwood discoloration, 11 include pathogens (73.3%). In lesions on

the stem, only three of the seven isolated genera included isolates with pathogenic potential (42.9%).

Table 1. Identification and frequency of fungi isolated from *Eucalyptus* presenting different types of trunk pathologies.

Representative Isolates	Genus identification	Accession number	Tree symptoms	Frequency	Closest related species	Similarity (%)
LPFMC-1	*Botryosphaeria*	OR708588	Stem lesion	32.0% (8/25)	*Botryosphaeria dothidea* KU360149	99.8%
			Sapwood discoloration	52.2% (12/23)		
LPFMC-2	*Castanediella*	OR708589	Sapwood discoloration	13.0% (3/23)	*Castanediella couratarii* NR_145250	100.0%
LPFMC-3	*Ceratocystis*	OR708590	Sapwood discoloration	39.1% (9/23)	*Ceratocystis fimbriata* MN788669	98.8%
LPFMC-4	*Chrysoporthe*	OR708591	Stem lesion	12.0% (3/25)	*Chrysoporthe cubensis* MH858337	99.6%
			Sapwood discoloration	21.7% (5/23)		
			Heartwood rot	31.6% (6/19)		
LPFMC-5	*Coniochaeta*	OR708592	Heartwood rot	5.3% (1/19)	*Coniochaeta* sp. MZ648895	97.5%
LPFMC-6	*Cytospora*	OR708593	Sapwood discoloration	8.7% (2/23)	*Cytospora eucalypticola* KY051849	99.6%
LPFMC-7	*Fusarium*	OR708594	Sapwood discoloration	4.3% (1/23)	*Fusarium oxysporum* MH311044	100.0%
			Heartwood rot	5.3% (1/19)		
LPFMC-8	*Jattaea*	OR708595	Stem lesion	4.0% (1/25)	*Jattaea leucospermi* EU552127	98.6%
LPFMC-9	*Lasiodiplodia*	OR708596	Heartwood rot	36.8% (7/19)	*Lasiodiplodia pseudotheobromae* KY052959	99.8%
LPFMC-10	*Lentinus*	OR708597	Heartwood rot	21.1% (4/19)	*Lentinus berteroi* MK036389	99.8%
LPFMC-11	*Marasmius*	OR708598	Sapwood discoloration	4.3% (1/23)	*Marasmius cladophyllus* MF039218	100.0%
LPFMC-12	*Neofusicoccum*	OR708599	Sapwood discoloration	8.7% (2/23)	*Neofusicoccum parvum* EU860378	99.8%
LPFMC-13	*Neoleptodontidium*	OR708600	Stem lesion	28.0% (7/25)	*Neoleptodontidium aquaticum* OQ990116	97.3%
			Sapwood discoloration	47.8% (11/23)		
LPFMC-14	*Neonothopanus*	OR708601	Sapwood discoloration	4.3% (1/23)	*Neonothopanus* sp. MT611019	99.0%
LPFMC-15	*Neopestalotiopsis*	OR708602	Stem lesion	12.0% (3/25)	*Neopestalotiopsis clavispora* MK288127	99.8%
			Sapwood discoloration	26.1% (6/23)		
LPFMC-16	*Pestalotiopsis*	OR708603	Stem lesion	8.0% (2/25)	*Pestalotiopsis microspora* MMK801280	100.0%
			Sapwood discoloration	39.1% (9/23)		
LPFMC-17	*Phaeoacremonium*	OR708604	Stem lesion	12.0% (3/25)	*Phaeoacremonium parasiticum* MT706008	100.0%
LPFMC-18	*Phlebia*	OR708605	Sapwood discoloration	8.7% (2/23)	*Phlebia* sp. KP096361	99.7%
LPFMC-19	*Scytalidium*	OR708606	Sapwood discoloration	8.7% (2/23)	*Scytalidium lignicola* MF671825	99.6%
			Heartwood rot	15.8% (3/19)		
LPFMC-20	*Trichoderma*	OR708607	Stem lesion	12.0% (3/25)	*Trichoderma harzianum* OP237474	99.7%
			Sapwood discoloration	13.0% (3/23)		
			Heartwood rot	26.3% (5/19)		
LPFMC-21	*Xenoacremonium*	OR708608	Heartwood rot	5.3% (1/19)	*Xenoacremonium recifei* CBS54189	99.6%

1.3.4 Laccase production

Of the 127 fungal isolates studied, 56 (44.1%) showed the formation of a dark brown halo, indicating the production of laccase, in a culture medium containing gallic acid, while 43 (33.9%) produced this enzyme in the same medium, however with the addition of tannic acid. Of the genera with the capacity to produce loccase in both types of phenols, *Castanediella*, *Lentinus*, *Marasmius*, *Neonothopanus*, *Neopestalotiopsis*, *Pestalotiopsis*, *Phlebia*, *Scytalidium* e *Xenoacremonium* stood out due to the formation of halos of more intense color and larger size (classified as “++”). The four genera belonging to the Basidiomycota family were considered strong producers of this enzyme, in both or one of the phenols tested. Of the eight genera originating from trees with heartwood rot, only isolates of the genus *Trichoderma* did not show laccase production, while three others were considered strong producers. For those originating from lesions on the stem, only *Neopestalotiopsis* and *Pestalotiopsis* include isolates classified as strong producers, among the seven genera found associated with this symptom. Of the 15 genera associated with sapwood discoloration, 10 formed halos in the cultivation medium, seven (*Castanediella, Marasmius, Neonothopanus, Neopestalotiopsis, Pestalotiopsis, Phlebia* and *Scytalidium*) of which were strong producers. The two types of phenols used, gallic acid and tannic acid, showed similar results for most of the genera studied, with some excesses, such as for *Chrysoporthe* and *Jattaea* due to the non-detection of the enzyme in just one of the phenols.

Figure 3. Phylogenetic tree of fungi isolated from *Eucalyptus* trees presenting different types of trunk pathologies, based on the rDNA-ITS sequence. The tree was constructed using the maximum likelihood method and bootstrap values were calculated after 1.000 replications.

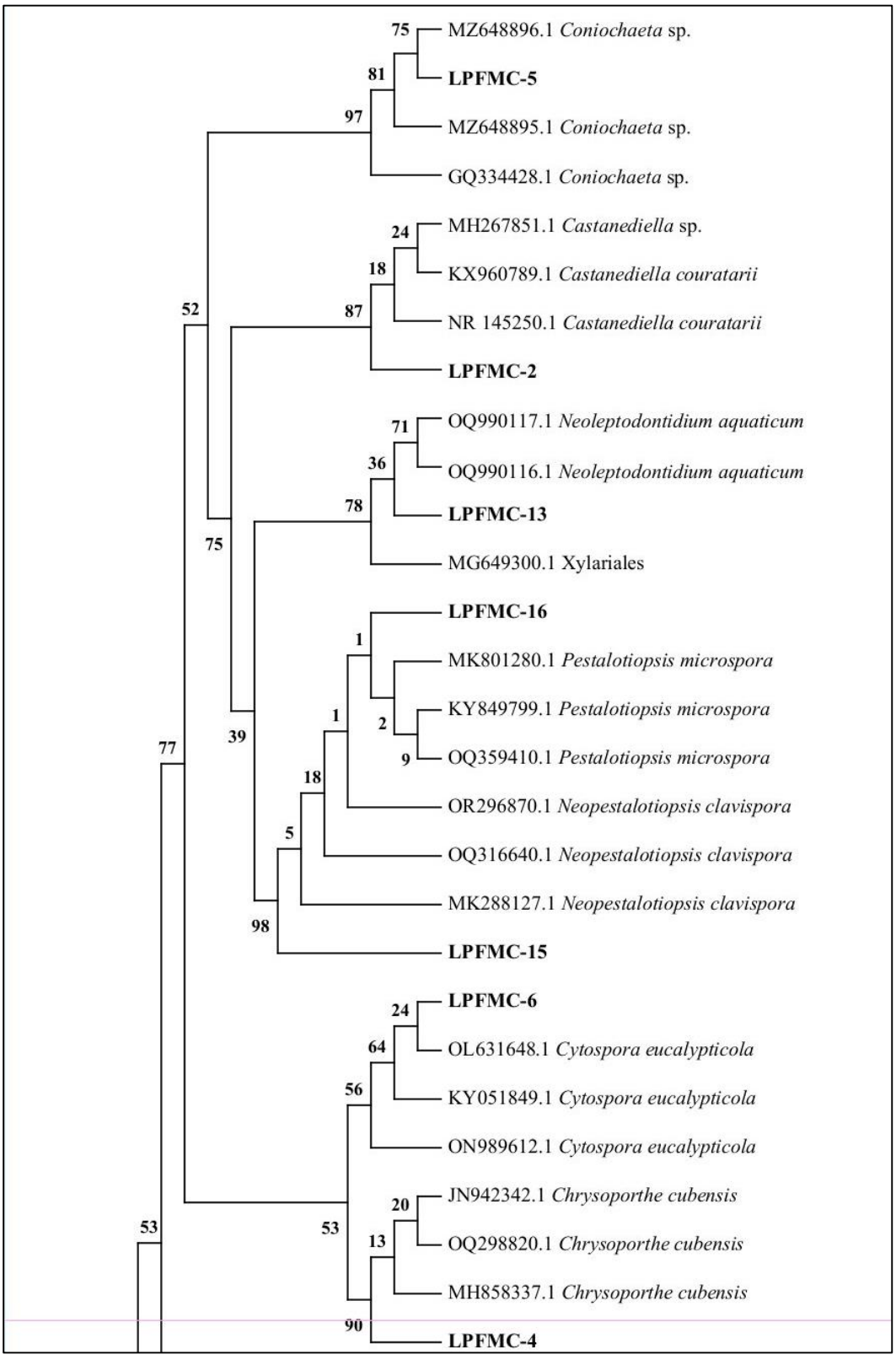


Figure 3. Continued…..

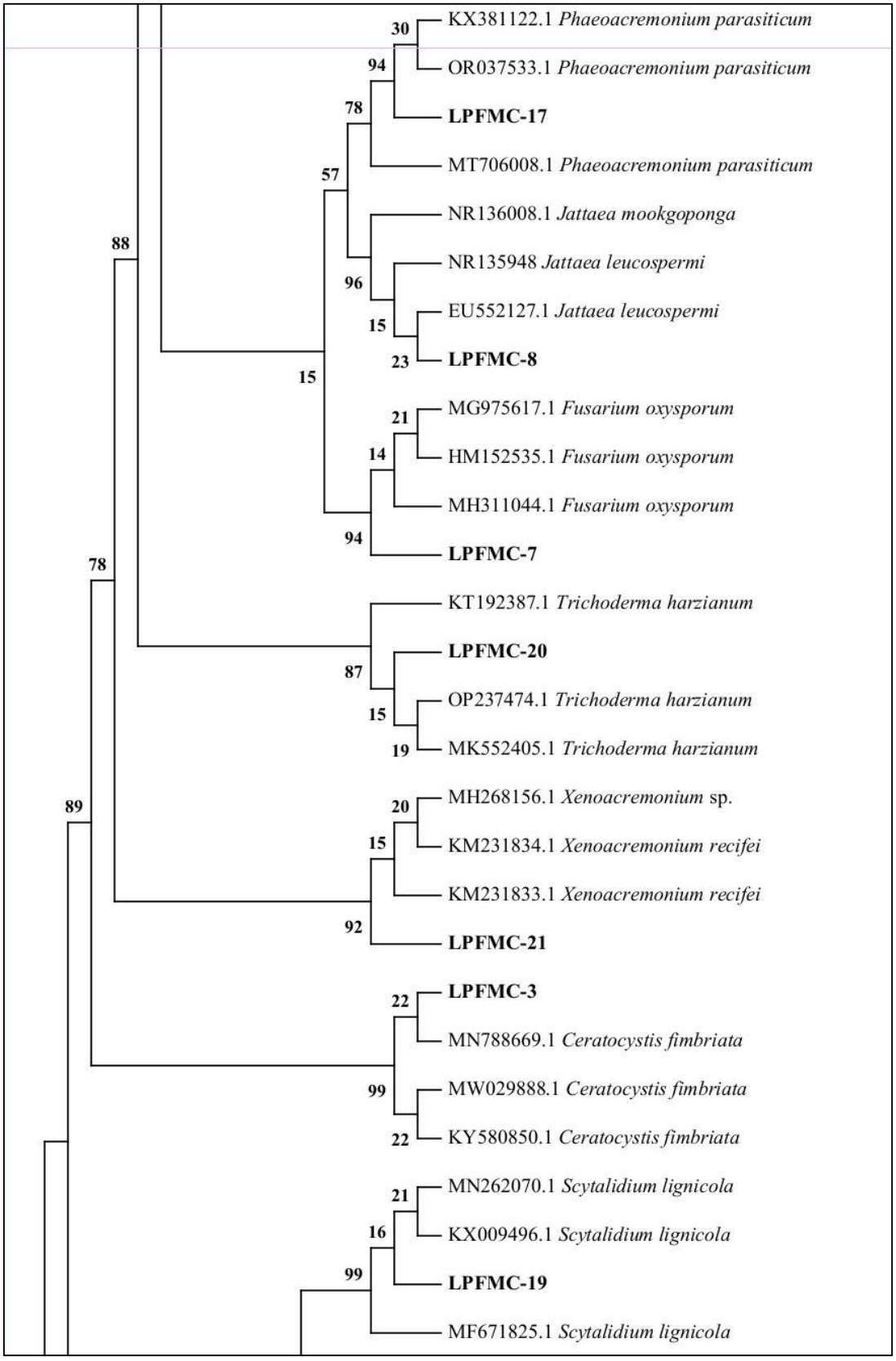


Figure 3. Continued…..

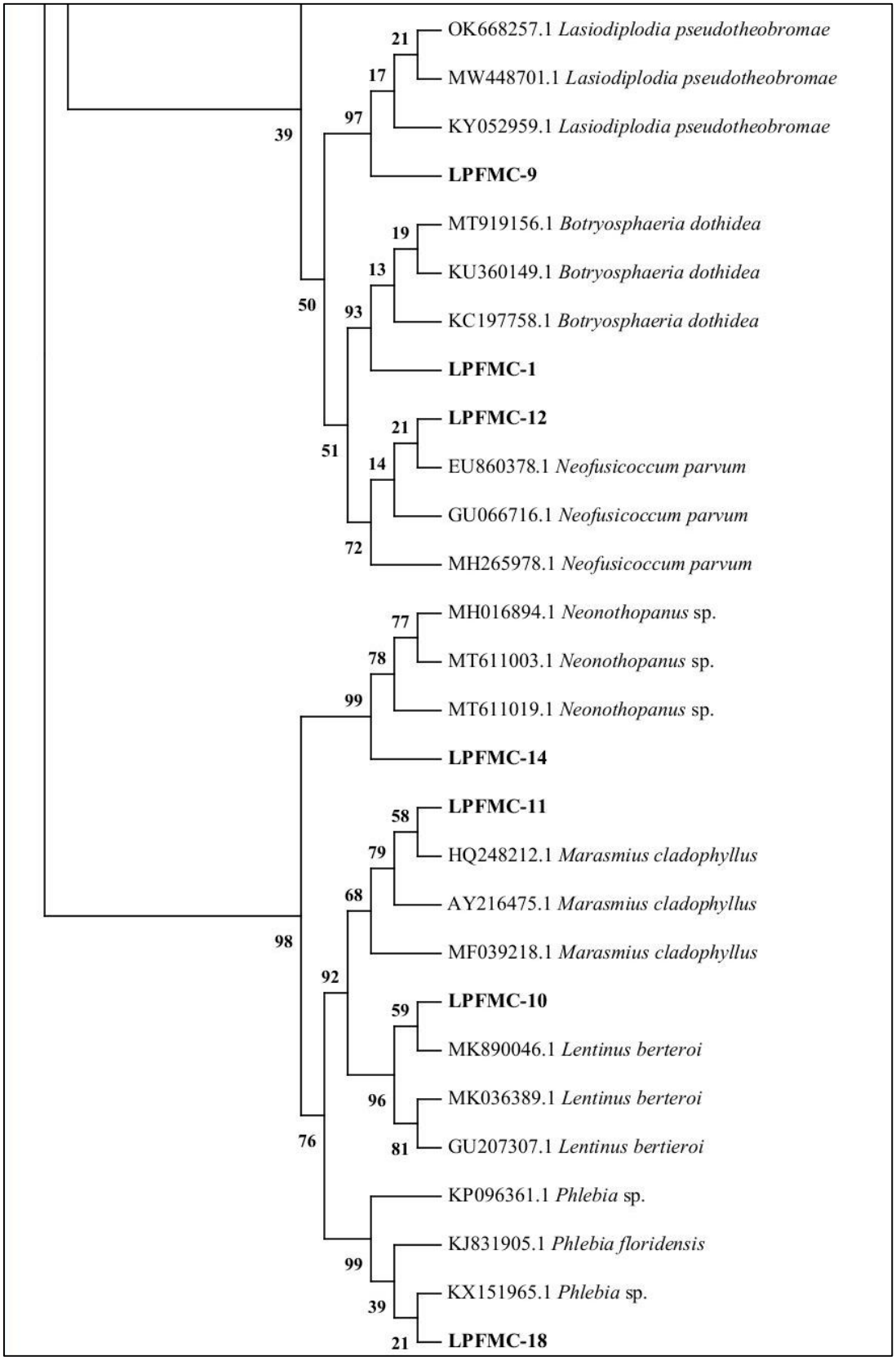


Table 2. Characterization of fungal isolates obtained from *Eucalyptus* trees presenting different trunk pathologies.

Genus identification	Tree symptoms	Aggressiveness (cm)		KOH reaction	Bavendamm reaction	
		External lesion	Internal lesion		Ag	At
Botryosphaeria	Stem lesion	NP[1] - 1.80	NP - 3.68	-	np[2]	np
	Sapwood discoloration	NP - 1.20	NP - 2.45	-	np	np
Castanediella	Sapwood discoloration	NP - 0.66	NP - 1.32	-	++	+ - ++
Ceratocystis	Sapwood discoloration	0.66 - 4.25	2.20 - 5.12	-	np	np
Chrysoporthe	Stem lesion	NP - 0.62	NP - 1.20	+	+	np
	Sapwood discoloration	0.50 - 0.66	1.18 - 1.40	+	+	np
	Heartwood rot	NP	NP	+	+	np
Coniochaeta	Heartwood rot	NP	NP	+	+	+
Cytospora	Sapwood discoloration	NP - 0.65	NP - 1.15	-	np	np
Fusarium	Sapwood discoloration	0.64	1.22	-	+	+
	Heartwood rot	NP	NP	-	+	+
Jattaea	Stem lesion	NP	NP	-	np	+
Lasiodiplodia	Heartwood rot	NP	NP	-	np	np
Lentinus	Heartwood rot	NP	NP	-	+ - ++	++
Marasmius	Sapwood discoloration	0.68	1.18	-	+	++
Neofusicoccum	Sapwood discoloration	NP	NP	-	+	+
Neoleptodontidium	Stem lesion	NP	NP	-	np	np
	Sapwood discoloration	NP	NP	-	np	np
Neonothopanus	Sapwood discoloration	1.64	4.65	-	++	++
Neopestalotiopsis	Stem lesion	NP - 0.72	NP - 1.16	-	+ - ++	+ - ++
	Sapwood discoloration	NP - 1.10	NP - 1.62	-	+ - ++	+ - ++
Pestalotiopsis	Stem lesion	NP	NP	-	++	++
	Sapwood discoloration	NP - 0.50	NP - 1.14	-	++	+ - ++
Phaeoacremonium	Stem lesion	NP	NP	-	np	np
Phlebia	Sapwood discoloration	NP	NP	-	++	++
Scytalidium	Sapwood discoloration	NP - 0.80	NP - 2.88	-	++	++
	Heartwood rot	NP	NP	-	+ - ++	++
Trichoderma	Stem lesion	NP	NP	-	np	np
	Sapwood discoloration	NP	NP	-	np	np
	Heartwood rot	NP	NP	-	np	np
Xenoacremonium	Heartwood rot	NP	NP	-	++	++

[1]NP: non pathogenic; [2]np: non-producer of enzyme laccase

1.4 Discussion

The present work shows that several genera of fungi can be associated with *Eucalyptus* trees showing symptoms of stem lesions, sapwood discoloration and heartwood rot, but not all of them appear to harbor pathogens which can act as primary or opportunistic. According to other studies, in diseased tissues, in addition to pathogens, endophytic or saprophytic fungi can also be found (Corrêa et al., 2014; Crous et al., 2019; Hong et al., 2016; Mester et al., 2004; Verma et al., 2022). In our study, different genera of pathogens may be associated with the same symptom, establishing different etiologies for the same pathology or, in cases where different pathogens occur in the same tree, there may be synergistic action between them. The effect of co-infection is reported in species of the Botryosphaeriaceae family and may result in increased severity of the symptoms caused (Whitelaw-Weckert et al., 2013).

Among the genera found in this study, which harbored isolates with pathogenic potential, *Botryosphaeria*, *Ceratocystis*, *Chrysoporthe*, *Cystospora*, *Neopestalotiopsis* and *Pestalotiopsis* were previously reported in *Eucalyptus* trees (Alfenas et al., 2004; Ferreira & Milani, 2012). The genera *Botryosphaeria*, *Chysoporthe* and *Cytospora* comprise important pathogens in this tree, associated mainly with symptoms of stem lesions (Alfenas et al., 2004; Ferreira & Milani, 2012). The first two genera mentioned can remain in woody tissues as latent pathogens, waiting for environmental stress conditions to initiate infections (Marsberg et al., 2017; Mausee-Sitoe et al., 2016). In *Eucalyptus* plantations in Brazil, *Ceratocystis fimbriata* is reported as the causal agent of ceratocystis wilt, involving the manifestation of symptoms of sapwood discoloration and death of the affected tree (Ferreira & Milani, 2012). In the present study, all tested isolates belonging to the *Ceratocystis* genus were pathogenic and caused the greatest lesions to the inoculated seedlings. *Neopestalotiopsis* and *Pestalotiopsis* comprise pathogens that cause foliar lesions on *Eucalyptus* under field and nursery conditions, but have been isolated from trees that exhibit sapwood discoloration and stem lesions due to ceratocystis wilt (Crous et al., 2019; Ferreira et al., 2006). As isolates from these genera have comparatively low pathogenic potential in the present study, they probably act as opportunists, infecting tissues previously colonized by other pathogens or stressed plants. However, in other tree species, fungi from these genera are considered important stem pathogens (Maharachchikumbura et al., 2016).

The Bavendamm reaction allows visual detection of laccase production by microorganisms in culture media (Etheridge, 1957; Smit et al., 1996). This enzyme is associated with the primary metabolism of fungi that degrade lignin, a molecule responsible for the integrity and mechanical resistance of woody tissues (Etheridge, 1957). In the present work, all basiodiomycetes were classified as strong laccase producers (++), while the smallest part of ascomycotes were classified as weak producers (+). Among the strong producers, the genus *Pestalotiospsis* is the only one cited in the literature as causing heartwood rot in *Eucalyptus*, with potential use for degradation of stumps (De Alonso et al., 2007). *Lentinus*, *Marasmius* and *Neonothopanus* are reported as saprophytes in leaf litter (Tagger et al., 1998; Vieira et al., 2022; Wu et al., 2018). *Neopestalotiopsis* and *Pestalotiopsis* as stem pathogens in woody species (Maharachchikumbura et al., 2016) and *Castanediella*, *Phlebia*, *Scytalidium* and *Xenoacremonium* as wood decay fungi (Fackler et al., 2006; Hong et al., 2023; Jusino et al., 2015; Lin et al., 2019; Markakis et al., 2017).

The heartwood of woody species has higher levels of lignin and extractives, in addition to having its vessels obstructed by tylosis, making colonization by unadapted microorganisms difficult (De Micco et al., 2016). Isolates such as *Botryosphaeria* and *Ceratocystis*, which behaved as pathogens, did not show the ability to produce laccase or were isolated from trees with heartwood rot, indicating their probable inability to colonize this tissue. None of the fungi isolated from heartwood rot showed pathogenic potential in *Eucalyptus* seedlings, which may indicate their specificity for this tissue, especially genera that were isolated only from trees with this symptom. Genera that include isolates with the capacity to produce laccase, but originating only from trees with stem lesions and sapwood discoloration, were not isolated from heartwood tissues with rot, probably due to the need not only for this enzyme for colonization of the heartwood.

Other isolates, without pathogenic potential or laccase production, are probably endophytic fungi, contaminants or decomposers, but without the ability to degrade lignin. *Trichoderma* is reported to be a cosmopolitan genus, colonizing a wide range of substrates and acting as an endophyte in several plant species (Jaklitsch, 2009). In diseased tissues, microorganisms of this genus can infect others fungi (Vinale et al. 2009). Fungi from the genera *Lasiodiplodia* and *Phaeoacremonium* are reported as pathogens in plants, but can act as endophytes in woody species (Li et al. 2016;

Mostert et al., 2005; Whitelaw-Weckert et al., 2013). Endophytic fungi can offer a series of benefits to plants, producing compounds that improve plant physiology and increase resistance to stress (Gouda et al., 2016). *Neoleptodontidium* spp are isolated from the water of hydroponic systems and from decaying wood (Crous et al., 2023).

In general, the present work observed the association of several fungal genera with eucalypt presenting trunk pathologies, with several of them being reported for the first time in this tree. The increase in the range of pathogens was most notable in the symptoms of heartwood rot, with new genera related to this pathology, as *Lentinus*, *Scytalidium* and *Xenoacremonium*. Several of the isolated genera still have an uncertain association with *Eucalyptus* tree, requiring further studies.

References

Alfenas, A. C., Zauza, E. A. V., Mafia, R. G., & Assis, T. F. (2004). Clonagem e Doenças do Eucalipto. Universidade Federal de Vicosa.

Arriel, D. A. A., Fonseca, N. R., Guimarães, L. M. S., Hermenegildo, P. S., Mafia, R. G., Borges Júnior, N., De Souza, H. P., & Alfenas, A. C. (2014). Wilt and die-back of *Eucalyptus* spp. caused by *Erwinia psidii* in Brazil. *Forest Pathology*, 44, 4, 255-265. https://doi.org/10.1111/efp.12087

Auer, C. G., Dos Santos, A. F., & Halfeld-Vieira, B. de A. A. (2012). Podridão do cerne em árvores vivas no Brasil. *Tropical Plant Pathology*, S37.

Aylward, J., Roets, F., Dreyer, L. L., & Wingfield, M. J. (2019). *Teratosphaeria* stem canker of *Eucalyptus*: two pathogens, one devastating disease. *Molecular Plant Pathology*, 20, 1, 8-19. https://doi.org/10.1111/mpp.12758

Barradas, C., Pinto, G., Correia, B., Jesus, C., & Alves, A. (2019). Impact of *Botryosphaeria*, *Diplodia* and *Neofusicoccum* species on two *Eucalyptus* species and a hybrid: From pathogenicity to physiological performance. Forest Pathology, 49, 2. https://doi.org/10.1111/efp.12493

Carstensen, G. D., Venter, S. N., Wingfield, M. J., & Coutinho, T. A. (2017). Two *Ralstonia* species associated with bacterial wilt of *Eucalyptus*. *Plant Pathology*, 66, 3, 393-403. https://doi.org/10.1111/ppa.12577

Corrêa, R. C. G., Rhoden, S. A., Mota, T. R., Azevedo, J. L., Pamphile, J. A., De Souza, C. G. M., Polizeli, M. de L. T. de M., Bracht, A., & Peralta, R. M. (2014). Endophytic fungi: expanding the arsenal of industrial enzyme producers. *Journal of Industrial Microbiology and Biotechnology*, 41, 10, 1467-1478, https://doi.org/10.1007/s10295-014-1496-2

Crous, P. W., Wingfield, M. J., Cheewangkoon, R., Carnegie, A. J., Burgess, T. I., Summerell, B. A., Edwards, J., Taylor, P. W. J., & Groenewald, J. Z. (2019). Foliar pathogens of eucalypts. *Studies in Mycology*, 94, 125-298. https://doi.org/10.1016/j.simyco.2019.08.001

Crous, P.W., Akulov, A., Balashov, S., Boers, J., Braun, U., Castillo, J., Delgado, M. A., Denman, S., Erhard, A., Gusella, G., Jurjević, Ž., Kruse, J., Malloch, D. W., Osieck, E. R., Polizzi, G., Schumacher, R. K., Slootweg, E., Starink-Willemse, M., van Iperen, A. L., Verkley, G. J. M., & Groenewald, J. Z. (2023). New and Interesting Fungi. *Fungal Systematics and Evolution*, 11, 1, 109-156. https://doi.org/10.3114/fuse.2023.11.09

De Micco, V., Balzano, A., Wheeler, E. A., & Baas, P. (2016). Tyloses and gums: a review of structure, function and occurrence of vessel occlusions. *IAWA Journal*, 37, 2, 186-205. https://doi.org/10.1163/22941932-20160130

Etheridge, D. (1957). Differentiation of White- and Brown-Rot Fungi by an Oxidase Reaction. *Nature*, 179, 921-922. https://doi.org/10.1038/179921a0

Fackler, K., Gradinger, C., Hinterstoisser, B., Messner, K., & Schwanninger, M. (2006). Lignin degradation by white rot fungi on spruce wood shavings during short-time solid-state fermentations monitored by near infrared spectroscopy. *Enzyme and Microbial Technology*, 39, 1476-1483. https://doi.org/10.1016/j.enzmictec.2006.03.043

Ferreira, F. A., & Milani, D. (2012) Diagnose Visual e Controle das Doenças Abióticas e Bióticas do Eucalipto no Brasil. Universidade Federal de Vicosa.

Ferreira, F. A., Maffia, L. A., Barreto, R. W., Demuner, N. L., & Pigatto, S. (2006). Sintomatologia da murcha de *Ceratocystis fimbriata* em eucalipto. *Revista Árvore*, 30, 2, 155-162. https://doi.org/10.1590/S0100-67622006000200001

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2, 113-118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gouda, S., Das, G., Sen, S. K., Shin, H-S., & Patra, J. K. (2016). Endophytes: a treasure house of bioactive compounds of medicinal importance. *Frontiers in Microbiology*, 7, 1-8. https://doi.org/10.3389/fmicb.2016.01538

Hall, T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symp Series*, 41, 95-98.

Hong, Y., Tan, J. Y., Xue, H., Chow, M. L., Ali, M., Ng, A., Leong, A., Yeo, J., Koh, S. M., Tang, M. S. Y., Lee, Y. Y., Choong, A. M. F., Lee, S. M. L., Ponti, R. D., Chan, P. M., Lee, D., Wong, J. Y., Mutwil, M., & Fong, Y. K. (2023). A Metagenomic Survey of Wood Decay Fungi in the Urban Trees of Singapore. *Journal of Fungi*, 9, 4. https://doi.org/10.3390/jof9040460

Ijaz, A., Iqbal, Z., & Afzal, M. (2016). Remediation of sewage and industrial effluent using bacterially assisted floating treatment wetlands vegetated with *Typha domingensis*. *Water Science and Technology*, 74, 9, 2192–2201. https://doi.org/10.2166/wst.2016.405

Jaklitsch, W. M. (2009). European species of *Hypocrea* Part I. The green-spored species. *Studies in Mycology*, 63, 1-91. https://doi.org/10.3114/sim.2009.63.01

Jusino, M. A., Lindner, D. L., Banik, M. T., & Walters, J. R. (2015). Heart rot hotel: fungal communities in red-cockaded woodpecker excavations. *Fungal ecology*, 14, 33-43. https://doi.org/10.1016/j.funeco.2014.11.002

Kang, S. H., Cho, H-S., Cheong, H., Ryu, C-M., Kim, J. F., & Park, S. H. (2007). Two bacterial entophytes eliciting both plant growth promotion and plant defense on pepper (*Capsicum annuum* L.). *Journal of Microbiology and Biotechnology*, 17, 96-103.

Li, J., Xue, Y., Yuan, J., Lu, Y., Zhu, X., Lin, Y., & Liu, L. (2016). Lasiodiplodins from mangrove endophytic fungus *Lasiodiplodia* sp. 318#. *Natural Product Research*, 30, 7, 755-760. https://doi.org/10.1080/14786419.2015.1062762

Lin, C-G., Bhat, D. J., Liu, J-K., Hyde, K. D., & Wang, Y. (2019). The genus *Castanediella*. *MycoKeys*, 51, 1-14. https://doi.org/10.3897/mycokeys.51.32272

Maharachchikumbura, S. S. N., Larignon, P., Hyde, K. D., Al-Sadi, A. M., & Liu, Z-Y. (2016). Characterization of *Neopestalotiopsis*, *Pestalotiopsis* and *Truncatella* species

associated with grapevine trunk diseases in France. Phytopathologia Mediterranea 55, 3, 380-390. https://doi.org/10.14601/Phytopathol_Mediterr-18298

De Alonso, S. K., Da Silva, A. G., Kasuya, M. C. M., De Barros, N. F., Cavallazzi, J. R. P., Bettucci, L., Lupo, S., & Alfenas, A. C. (2007). Isolamento e seleção de fungos causadores da podridão-branca da madeira em florestas de *Eucalyptus* spp. com potencial de degradação de cepas e raízes. *Revista Árvore*, 31, 1, 145-155. https://doi.org/10.1590/S0100-67622007000100016

Markakis, E. A., Kavroulakis, N., Ntougias, S., Koubouris, G. C., Sergentani, C. K., & Ligoxigakis, E. K. (2017). Characterization of Fungi Associated With Wood Decay of Tree Species and Grapevine in Greece. *Plant Disease*, 101, 11, 1929-1940. https://doi.org/10.1094/PDIS-12-16-1761-RE

Marsberg, A., Kemler, M., Jami, F., Nagel, J. H., Postma-Smidt, A., Naidoo, S., Wingfield, M. J., Crous, P. W., Spatafora, J. W., Hesse, C. N., Robbertse, B., & Slippers, B. (2017). *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Molecular Plant Pathology*, 18, 477-488. https://doi.org/10.1111/mpp.12495

Mausse-Sitoe, S. N. D., Rodas, C. A., Wingfield, M. J., Chen, S., & Roux, J. (2016). Endophytic Cryphonectriaceae on native Myrtales: Possible origin of *Chrysoporthe* canker on plantation-grown *Eucalyptus*. *Fungal Biology*, 120, 827-835. https://doi.org/10.1016/j.funbio.2016.03.005

Melnick, R. L., Zidack, N. K., Bailey, B. A., Maximova, S. N., Guiltinan, M., & Backman, P.A. (2008). Bacterial endophytes: *Bacillus* spp. from annual crops as potential biological control agents of black pod rot of cacao. *Biological Control*, 46, 46-56. https://doi.org/10.1016/j.biocontrol.2008.01.022

Mester, T., Varela, E., & Tien, M. (2004). Wood Degradation by Brown-Rot and White-Rot Fungi. In Kück, U. (eds) *The Mycota*, Vol 2 (pp. 355-368). Springer. https://doi.org/10.1007/978-3-662-07426-8_17

Moller, W. J., & Devay, J. E. (1968). Carrot as a species-selective medium for *Ceratocystis fimbriata*. *Phytopathology*, 58, 123-124.

Mostert, L., Groenewald, J. Z., & Summerbell, R. C. (2005). Species of *Phaeoacremonium* associated with infections in humans and environmental reservoirs

in infected woody plants. *Journal of Clinical Microbiology*, 43, 4, 1752-1767. https://doi.org/10.1128/JCM.43.4.1752-1767.2005

Sharma, P., & Rath, S.K. (2021). Potential applications of fungi in the remediation of toxic effluents from pulp and paper industries. In Sharma, V. K., Shah, M. P., Parmar, S., & Kumar, A. (Eds) *Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nanotechnology* (pp. 193–211). Academic Press. https://doi.org/10.1016/B978-0-12-821925-6.00010-1

Smit, S. W., Wingfield, B. D., & Wingfield, M. J. (1996). Reduction of Laccase Activity and Other Hypovirulence-Associated Traits in dsRNA-Containing Strains of *Diapothe ambigua*. *Phytopathology*, 86, 1311-1316. https://doi.org/10.1094/Phyto-86-1311

Soares, T. P. F., Ferreira, M. A., Mafia, R. G., Oliveira, L. S. S., Hodges, C. S., & Alfenas, A. C. (2018). Canker disease caused by *Chrysoporthe doradensis* and *C. cubensis* on *Eucalyptus* sp. and *Tibouchina* spp. in Brazil. *Tropical Plant Pathology*, 43, 314-322. https://doi.org/10.1007/s40858-018-0238-9

Tagger, S., Pe´rissol, C., Gil, G., Vogt, G., & Le Petit, J. (1998). Phenoloxidases of the white-rot fungus *Marasmius quercophilus* isolated from an evergreen oak litter (*Quercus ilex* L.). *Enzyme and Microbial Technology*, 23, 372-379. https://doi.org/10.1016/S0141-0229(98)00062-3

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38, 3022-3027. https://doi.org/10.1093/molbev/msab120

Tommerup, I. C., Alfenas, A. C., & Old, K. M. (2003). Guava rust in Brazil – a threat to *Eucalyptus* and other Myrtaceae. *New Zealand Journal of Forestry Science*, 33, 420-428.

Verma, A., Shameem, N., Jatav, H. S., Sathyanarayana, E., Parray, J. A., Poczai, P., & Sayyed, R. Z. (2022). Fungal Endophytes to Combat Biotic and Abiotic Stresses for Climate-Smart and Sustainable Agriculture. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.953836

Vieira, M. B. B., Oliveira, I. C., De Oliveira, M. Das D. A., Da Costa Júnior, J. S., Andrade, T. De J. A. Dos S., Feitosa, C. M., Rai, M., Lima, N. M., & Silva, D. Da C.

(2022). A review on bioluminescent fungus *Neonothopanus gardneri*. *Research, Society and Development*, 11, 5. https://doi.org/10.33448/rsd-v11i5.28009.

Vinale, F., Ghisalberti, E. L., Sivasithamparam, K., Marra, R., Ritieni, A., Ferracane, R., Woo, S., & Lorito, M. (2009). Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. *Letters in Applied Microbiology*, 48, 6, 705-711. https://doi.org/10.1111/j.1472-765X.2009.02599.x

Whitelaw-Weckert, M. A., Rahman, L., Appleby, L. M., Hall, A., Clark, A. C., Waite, H., & Hardie, W. J. (2013). Co-infection by Botryosphaeriaceae and *Ilyonectria* spp. fungi during propagation causes decline of young grafted grapevines. *Plant Pathology*, 62, 1226-1237. https://doi.org/10.1111/ppa.12059

Wu, B., Xu, Z., Knudson, A., Carlson, A., Chen, N., Kovaka, S., LaButti, K., Lipzen, A., Pennachio, C., Riley, R., Schakwitz, W., Umezawa, K., Ohm, R. A., Grigoriev, I. V., Nagy, L. G., Gibbons, J., & Hibbett, D. (2018). Genomics and Development of *Lentinus tigrinus*: A White-Rot Wood-Decaying Mushroom with Dimorphic Fruiting Bodies. *Genome Biology and Evolution*, 10, 12, 3250-3261. https://doi.org/10.1093/gbe/evy246

CHAPTER 2

CHARACTERIZATION OF CERATOCYSTIS WILT ON *Eucalyptus* AND ITS CAUSAL AGENT[1]

ABSTRACT

Ceratocystis fimbriata sensu lato (s.l.) is an important pathogen complex in woody species, being associated with symptoms of wilt, trunk lesions and death of infected plants. In *Eucalyptus* plantations, this pathogen can reduce the productivity of affected plants, in addition to causing mortality in the first years of planting. The objective of this work was to evaluate the symptoms and characterize isolates of *C. fimbriata* (s.l.) associated with *Eucalyptus* plantations in southern Bahia, Brazil. In this study we observed that external symptoms manifested mainly in trees with up to 36 months of planting, however asymptomatic diseased trees were found until the end of the cycle (7 years). Symptoms of wilt, wood discoloration, formation of adventitious shoots and bark lesions, drying of branches and death have been observed in diseased trees. The discoloration of the wood began in the root system, which is probably the point of penetration of the causal agent. The isolates obtained from these trees were identified as belonging to the species *C. fimbriata* (s.l.), presenting similar morphological characteristics and optimum growth temperatures ranging from 23.9 to 24.9ºC. *Eucalyptus* clones inoculated with these pathogens showed different levels of resistance. Clones resistant to a certain isolate may be susceptible when infected by another. This variation in aggressiveness among *Ceratocystis* isolates makes the selection of resistant clones difficult. The present work improves the understanding of ceratocystis wilt in *Eucalyptus* and its causal agent.

Key words: vascular wilt, xylem discoloration, canker, root infection, variability

---

[1]Formatted according to the "Australasian Plant Pathology" submission guidelines.

1.1 Introduction

The genus *Ceratocystis* includes important species of phytopathogenic fungi, capable of infecting forest and agronomic crops (de Beer et al. 2014). *C. fimbriata* sensu lato (s.l.) stands out for its wide range of hosts and damage caused. Different symptoms may be related to this pathogen according to the host. Symptoms of fruit rot and damping-off are reported in passion fruit (*Passiflora edulis*) and andiroba (*Carapa guianensis*), respectively (Firmino et al. 2016; Valdetaro et al. 2019). Rot of storage root occurs in sweet potato (*Ipomea batatas*) and carrot (*Daucus carota*) and in taro corms (*Colocasia esculenta*) (Halsted 1890; Harrington et al. 2005; Xu et al. 2020). In woody species, *C. fimbriata* is associated mainly with symptoms of vascular wilt and trunk lesions, being reported in *Acacia* spp., african mahogany (*Khaya senegalensis*), coffee (*Coffea arabica*), eucalypt (*Eucalyptus* spp.), *Gmelina arborea*, kiwi (*Actinidia deliciosa*), loquat (*Eriobotrya japonica*), mango (*Manguifera indica*), pequi (*Caryocar brasiliensis*), pomegranate (*Punica granatum*), rubber tree (*Hevea brasiliensis*), sycamore (*Platanus* spp.), teak (*Tectona grandis*) and yerba mate (*Ilex paraguariensis*) (da Silva et al. 2017; de Brito et al. 2021; Engelbrecht and Harrington 2005; Firmino et al. 2012, 2017; Heath et al. 2009; Li et al. 2014a; Marin et al. 2003; Méndez-Álvarez et al. 2020; Oliveira et al. 2015, 2016; Piveta et al. 2013; Somasekhara 1999). According to the species, the occurrence of *Ceratocystis* species can be a limiting factor for the cultivation of susceptible varieties or clones (Arriel et al. 2016; Ferreira et al. 2013; Oliveira et al. 2015).

Ceratocystis fimbriata (s.l.) is considered the causal agent of *Eucalyptus* ceratocystis wilt in Brazil (Ferreira et al. 2011; Oliveira et al. 2015). This fungus is also reported as associated with this pathology in Uruguay, China, South Africa, Pakistan, Democratic Republic of Congo and Uganda (Alam et al. 2017; Barnes 2003; Heath et al. 2009; Li et al. 2014b; Roux et al. 2000). Other species of this genus are listed as causal agents in other countries, such as *C. eucalypticola* in South Africa (van Wyk et al. 2012). The symptoms of ceratocystis wilt in *Eucalyptus* usually start with wilting of the canopy, followed by progressive drying of the foliage and death of the plant. The internal woody tissues of the diseased tree show discoloration in the form of radial streaks, which starts from the base of the tree (Ferreira et al. 2006). Surviving in the soil as chlamydospore-like resistance structures and as a saprobe in crop debris, *Ceratocystis* infects *Eucalyptus* mainly through the root system and collar region

(Ferreira et al. 2006; Harrington 2013). Infection can also occur through injuries to the trunk, caused during silvicultural practices or other external factors (Ferreira et al. 2006, 2013).

Forests established with susceptible clones in areas with *C. fimbriata* (s.l.) inoculum in the soil can result in reductions of more than 50% in the final stand of *Eucalyptus* forests (Ferreira et al. 2013). Diseased trees can exhibit a reduction in their growth of up to 87% at higher severity levels (Mafia et al. 2013). Wood from diseased trees also has a lower basic density and a higher content of extractives, resulting in an increase of up to 29.6% in the costs of pulping, due to the higher consumption of reagents (Mafia et al. 2013). Reduces from 13.7 to 15.2% in the final pulp yield per m^3 of processed wood can also be observed (Mafia et al. 2013). Due to the increase in costs and reduced yield, it is recommended to use wood from diseased trees for other purposes, such as burning it in the factory's boiler to generate energy.

The management of ceratocystis wilt in *Eucalyptus* is made mainly through the use of resistant clones. However, *C. fimbriata* (s.l.) is native to Brazilian soils and presents high genetic variability and variation in its aggressiveness according to the population (Ferreira et al. 2010, 2011; Oliveira et al. 2015). These differences between populations result in different interactions with eucalyptus clones, impacting the severity of symptoms manifested and making it difficult to select clones that behave as resistant in different locations (Oliveira et al. 2015). Thus, this study aimed to characterize the symptoms manifested by diseased *Eucalyptus* trees due to ceratocystis wilt and its causal agent in southern Bahia. In this region, a high incidence of the disease was reported (Oliveira et al. 2015). We also evaluated the pathogen's development at different temperatures and its aggressiveness against different *Eucalyptus* clones.

1.2 Materials and methods

1.2.1 Studied area

Field studies and material collections were carried out in clonal *Eucalyptus* plantations located in the southern region of the state of Bahia, Brazil. The cultivated clones belonged to the hybrid *Eucalyptus urophylla* x *E. grandis*, popularly known as Urograndis. According to information obtained from the company responsible for the

plantations, the clones showed different levels of resistance to ceratocystis wilt, with some clones classified as resistant and others as susceptible. The plantations were destined mainly to the production of cellulose, with spacing that varied between 3.0 x 2.0, 4.0 x 1.9 and 4.0 x 3.0m, and with different planting ages. The soil in the region is sandy to clayey, of medium depth and with an abundance of rocky outcrops. The climate in the region is tropical, with rainfall distributed throughout the year or dry winter. Average daily temperature varies from 19ºC to 26ºC, while annual precipitation from 700mm, in the driest location, to 1700mm in the rainiest.

1.2.2 Characterization of symptoms and detection of the pathogen

The trees affected by ceratocystis wilt were initially identified by the typical external symptoms of the disease, such as presence of adventitious shoots, wilting, trunk lesion, dry branches or defoliation. Plants that appeared to be long time dead were not examined because of colonization by saprofitic organisms. Once the diseased trees were identified, internal symptoms of discoloration were observed with the aid of a machete or chainsaw. We also expose the superficial root system (<50cm) of some young diseased trees (approximately 12 months of planting) with the aid of directed water jets. Woody samples were collected from diseased trees, proceeding with isolation using the carrot sandwich bait method and purification on plates containing PDA culture medium (Potato Dextrose Agar; 4%; w/v), plus streptomycin sulfate (100mg/L). The plates and baits were kept at 25ºC and alternating photoperiod (12/12). The isolates were identified according to the area of collection (Table 1) and were preserved in glycerol at -20°C.

1.2.3 Characterization of isolates

Of the isolates collected in southern Bahia, eight from several areas were randomly selected. In addition to these isolates, two others, obtained in the state of São Paulo and regularly used in inoculation trials, were included in the study. The selected isolates were characterized according to their morphological characteristics in laboratory conditions. For this, petri dishes (90x15 mm) containing 15ml of PDA medium received 5mm diameter discs, collected from the edge of plates containing the same medium and colonized by *Ceratocystis* isolates. The plates were kept at 25ºC and alternating photoperiod for 16 days. After this time, the colonies were characterized according to their colors and formation of perithecia. For each isolate,

30 reproductive structures of each type were classified in terms of shape, colors and size.

The isolates were also characterized for their growth at different temperatures. For this, we followed the same methodology used in the morphological characterization test, however, incubating the colonies at temperatures ranging from 15 to 35ºC, with intervals of 5ºC. Colony diameters were measured every two days in two perpendicular directions and the average of both was obtained by subtracting the 5mm value corresponding to the disc size. Evaluations continued until the first isolate reached the edge of the plate. The essay was conducted in a completely randomized design, with five replications per treatment. The optimum temperature for growth of the isolates was determined by polynomial regression in the statistical program R.

1.2.3 Aggressiveness of isolates on eucalyptus clones

The aggressiveness of the isolates was evaluated in three *Eucalyptus* clones, cultivated in the south of Bahia, derived from the crossing of *E. urophylla* x *E. grandis*. Seedlings of these clones were formed in 70ml pots under nursery conditions over three months. These seedlings were planted in pots with volume of 2.0L, filled with substrate based on vermiculite, peat and carbonized rice husk (CarolinaSoil), supplemented with 150g of osmocote (NPK; 15:09:12) and 200g of MAP for each 20L of substrate. The seedlings were kept in a greenhouse under periodic irrigation for 90 days, reaching a minimum basal diameter of 1.0cm. Inoculation was performed by opening a wound 10cm above the seedling collar, with the aid of a sterilized cork borer with diameter of 5mm. A disk of PDA medium colonized by the *Ceratocystis* isolates, of the same diameter as the wound, was deposited in the injured area. The inoculated site was covered with cotton soaked in distilled water, both sterilized, and involved with parafilm, aiming to form a humid chamber, favoring the development of the inoculated pathogen. Plants that received only pathogen-free culture medium were maintained as controls. The evaluation of the experiment was realized three months after inoculation, measuring the length of the outer lesion on the bark and the inner one on the wood/pith. Samples were collected from inoculated plants to reisolate the pathogen. This experiment was conducted in a completely randomized design, with 10 replications (seedlings) per treatment (clone x isolate). The data were submitted to the Skott-Knott test ($p < 0.05$) in the R statistical program.

1.2.4 Molecular identification of *Ceratocystis* isolates

Genomic DNA was extracted from mycelium obtained from 7-day-old colonies using the Trans Direct® Plant Tissue PCR kit (Transgenbiotech, China), following the manufacturer's instructions. The internal transcribed spacer region (rDNA-ITS) and beta-tubulin 1 (Bt1) loci were amplified with the primer sets ITS1F/ITS4 (Gardes and Bruns 1993) and BT1a/BT1b (Glass and Donaldson 1995), respectively. PCR conditions were employed as detailed in the referenced literature.

Table 1. Identification and collection sites of the studied *Ceratoystis* isolates.

Isolates	City/State	Coordinates (Latitude; Longitude)	Host	Age of planting (Years)
BH012	Belmonte/BA	-16.107078; -39.331136	*E. urophylla* x *E. grandis*	1
BH014	Belmonte/BA	-16.105231; -39.345710	*E. urophylla* x *E. grandis*	4
BH015	Eunápolis/BA	-16.094639; -39.414665	*E. urophylla* x *E. grandis*	1
BH021	Eunápolis/BA	-16.248229; -39.723723	*E. urophylla* x *E. grandis*	3
BH023	Santa Cruz Cabrália/BA	-16.210576; -39.272746	*E. urophylla* x *E. grandis*	1
BH028	Santa Cruz Cabrália/BA	-16.210981; -39.273253	*E. urophylla* x *E. grandis*	3
BH030	Itapebi/BA	-16.012699; -39.587297	*E. urophylla* x *E. grandis*	1
BH031	Itagimirim/BA	-16.077095; -39.604788	*E. urophylla* x *E. grandis*	2
SP052	Botucatu/SP	-22.845002; -48.434991	*Eucalyptus* sp.	2
SP101	Lençóis Paulista/SP	-22.552891; -48.830222	*E. urophylla*	1

1.3 Results

1.3.1 Symptoms of ceratocystis wilt

Observed symptoms varied in frequency and severity according to tree age and clone resistance. External symptoms of wilting, defoliation and dry branches were more frequent in young trees (Fig. 1a), with less than 36 months of planting. In older plantations, these external symptoms were less frequent. The formation of adventitious shoots occurred both in young and adult plantations, however, in older plantations (>36 months); this symptom was also related to other occurrences of environmental stress, not always indicating that the tree is diseased. In this situation, the shoots were distributed along the entire tree. In young plantations, in the studied areas, most of the trees that presented adventitious shoots, mainly in the basal third or close to the collar, presented internal symptoms associated with ceratocystis wilt. The formation of adventitious shoots was more frequent in diseased trees from clones previously classified as susceptible. In some plants, it was possible to observe the death of the main stem, with adventitious shoots assuming apical dominance (Fig. 1b). With time, the lateral shoots also dried up, resulting in the complete death of the tree. Longitudinal cankers and minicankers were observed mainly in young trees (Fig. 1c), up to two years old, with characteristic discoloration of the wood below the damaged site and formation of kino pockets. Stem deformations have also been observed in diseased young trees (Fig. 1d). According to our observations in the field, the external symptoms of diseased plants appeared after three months of planting, mainly in places where we observed a high incidence of the disease in the previous cycle. In some of these sites, incidences of over 50% of ceratocystis wilt have been reported in previous cycles. Diseased trees were distributed with an aggregated pattern in the plantations.

As internal symptom in diseased tree, we observe discoloration of the wood in a radial pattern in cross-section, which started from the pith and went to the bark. Cankers and stem deformations were observed where the dark staining reached the bark. In trees in which the main stem died and adventitious shoots assumed apical predominance, a new layer of healthy wood, visually free of the pathogen, surrounded the wood with discoloration. Over time, this new layer of wood also showed discoloration, originating from the internalized diseased wood (Fig. 2a), resulting in wilting and death of adventitious shoots, as already mentioned. In old plantations, with

more than four years of age, we observed the presence of asymptomatic trees, mainly in resistant clones, showing discoloration of the wood, but without the manifestation of external symptoms. In this situation, the discoloration was concentrated mainly in the oldest region of the sapwood or heartwood, while the youngest portion of the wood, in greater physiological activity, was healthy (Fig. 2b). It was also possible to observe the association of heartwood rot (Fig. 2b) and termites (Fig. 2c) with diseased wood. In trees at the end of the cycle, five to seven years after planting, symptoms of discoloration in a radial pattern were observed in the heartwood of asymptomatic trees (Fig. 2d), but it was not possible to isolate the pathogen from these tissues.

Root discoloration was observed in trees with approximately 12 months of planting, after exposing the roots with the aid of water jets (Fig. 2e). In these trees, the discoloration of the wood was concentrated mainly in the basal region of the diseased trees, starting from the root system, which presented discoloration similar to that of the trunk. We observed that the infection could come from just one root, totally necrotic or still alive, but with internal symptoms of discoloration, while other trees had several diseased roots. The infection was mainly found in roots up to 50 cm deep. In the analyzed areas, there was an abundance of cultural remains from previous cycles.

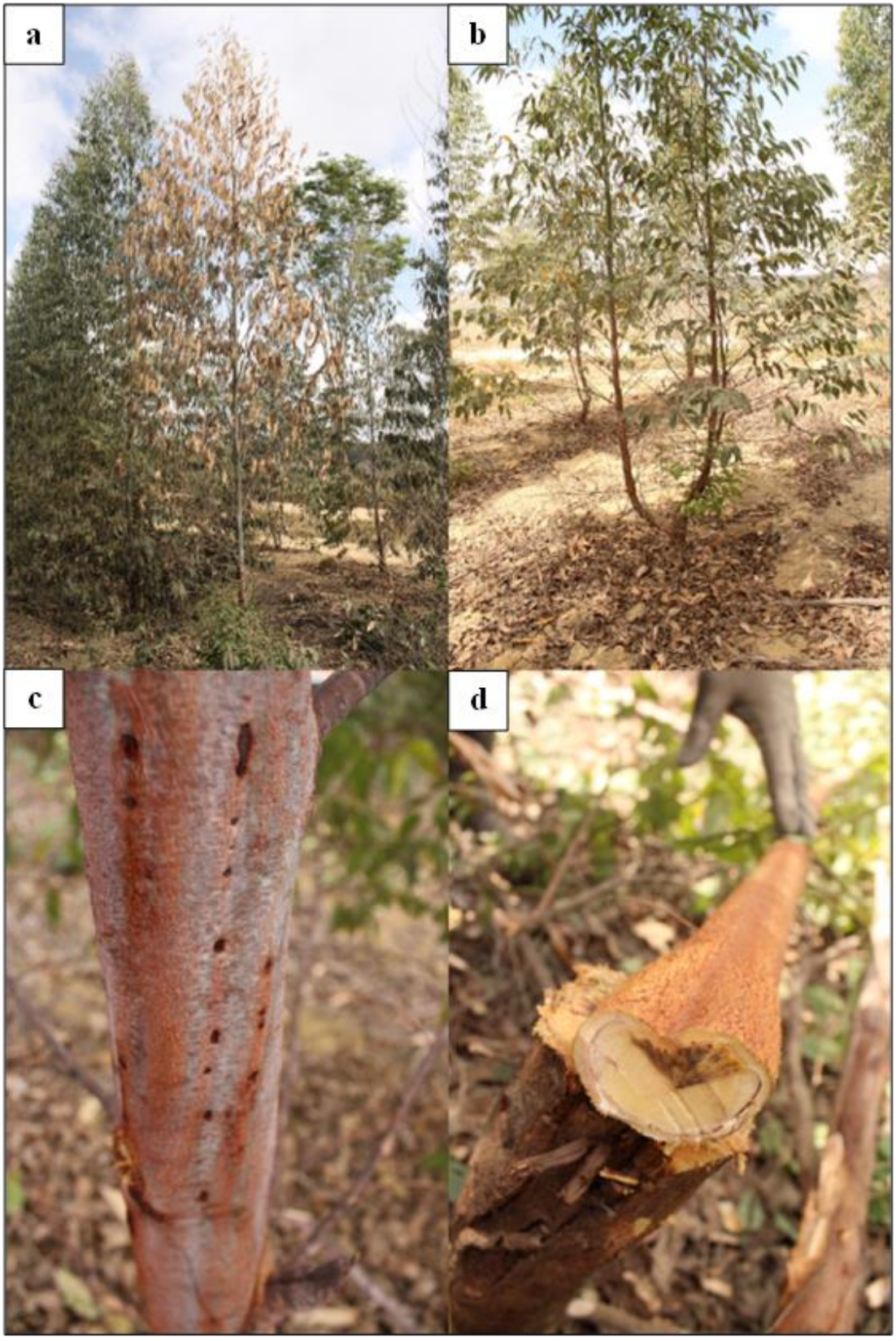


Figure 1. External symptoms of ceratocystis wilt in eucalyptus trees. **a.** Dry folliages and death of the tree. **b.** Loss of apical dominance. **c.** Longitudinal minicanker. **d.** Stem deformation associated with vascular cambium necrosis caused by *Ceratocystis*.

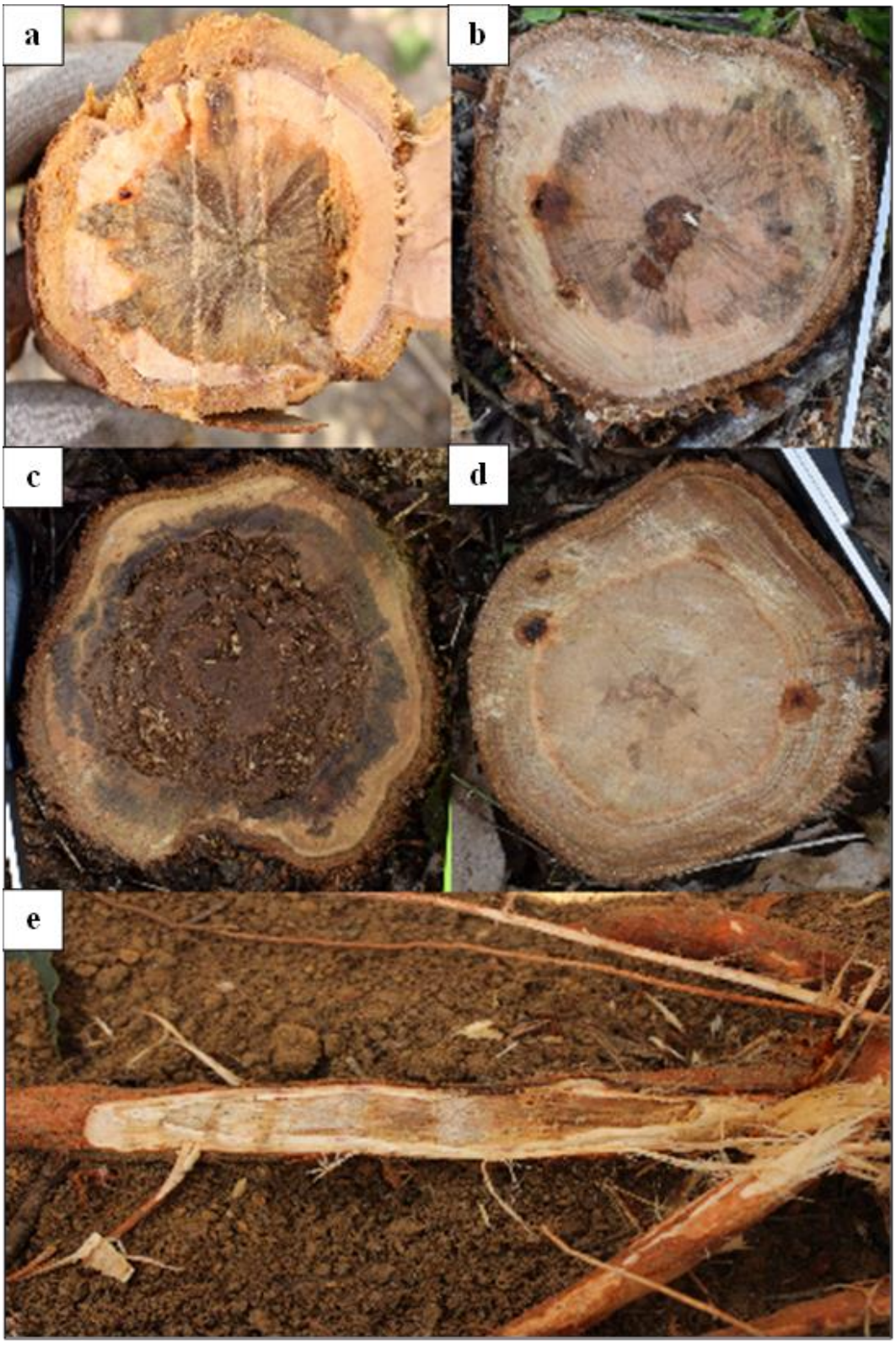


Figure 2. Internal symptoms of ceratocystis wilt in *Eucalyptus* trees. **a.** Darkening in streak pattern. Association of termites (**b**) and heart rot (**c**) with wood colonized by *Ceratocystis*. **d**. Symptoms of discoloration affecting the heartwood. **e**. Internal discoloration in root of *Eucalyptus* trees infected by *Ceratocystis*.

1.3.2 Pathogenicity of *Ceratocystis* isolates

The tested isolates showed the ability to infect and colonize the inoculated plants, leading to the manifestation of typical symptoms of the disease, such as discoloration of the wood and lesions on the bark. The severity of the symptoms varied according to the clone and isolate (Table 3). Clone EC187 proved to be the most resistant to ceratocyst wilt under the conditions tested, and showed the smallest size of internal and external lesions for most of the inoculated isolates. Clones EC121 and EC090 showed similar values of internal injury for most isolates, but clone EC121 showed greater external lesion. Inoculation with the BH012 isolate led to the manifestation of the largest external and internal lesions, while the BH015 isolate caused the smallest lesions. The other isolates showed intermediate aggressiveness in the inoculated clones. During the period of the experiment, no symptoms of wilting, drying of branches and formation of adventitious shoots were observed. It was possible to reisolate the pathogen from the inoculated seedlings, thus proving the association of the *Ceratocystis* isolates with the manifested symptoms.

1.3.3 Molecular and morphological characterization of *Ceratocystis* isolates

The sequences obtained from sequencing the gene regions ITS and Bt1 showed similarity of 99 to 100% with *C. fimbriata* (s.l.) sequences in GenBank, from different origins. Based on these results, the isolates were classified as belonging to the *C. fimbriata* complex.

The studied isolates showed similar morphological characteristics. The colonies formed were dark brown to black in color, with little aerial mycelium and an aroma similar to ripe fruit. The conidia were of two types. The first type, also called primary, has a cylindrical shape, forming in structures of the “phialid” type, with the shape of bottle, measuring 15.6 (8.5-33.9) x 4.2µm (2.7 – 7.0). The second type, secondary or doliform, is barrel-shaped, occurs in a chain and measure 8.6 (4.8 - 14.9) x 5.2µm (2.8 – 8.64). Both conidia were unicellular and hyaline. We also observe the presence of resistance spores of the chlamydospore type, also called aleuroconidia, with a shape ranging from globose to pear, solitary or in short chains, from light brown to brown, with dimensions 13.7 (9.3 – 19.7) x 9.62µm (5.4 – 13.5). Perithecia formed on the surface of the colonies had globular base, 165.7 (90.5 – 231.4) x 153.83µm (83.1 – 236.2). The neck shows the same color of the base, 274.9 (122.6 – 624.1) x 22.0µm

(12.1 – 33.1), with the presence of aseptate ostiolar hyphae and hyaline to light brown at the end of the rostrum, measuring 37.7µm (12.4 – 77.5). From the ostiole, abundant cream-colored exudation was observed, consisting of ascospores embedded in a mucilaginous matrix. Ascospores measure 4.4 (2.9 – 6.6) x 6.2µm (4.4 – 8.4), and were hyaline, unicellular and hat-shaped, formed in evanescent asci at the base of the perithecium.

Table 3. Length of external and internal lesions manifested by *Eucalyptus* clones inoculated with *Ceratocystis* isolates.

	External lesion			Internal lesion		
Isolates	**EC187**	**EC121**	**EC090**	**EC187**	**EC121**	**EC090**
BH012	1.31aC	12.67aA	6.39cB	10.22aB	18.43aA	11.26aB
BH014	0.78aB	8.61bA	1.76dB	2.95bB	12.03cA	5.14bB
BH015	0.83aB	4.58cA	2.26dB	1.67bB	6.80eA	6.07bA
BH021	0.83aB	7.65bA	9.36bA	3.10bC	7.97eB	12.35aA
BH023	0.71aB	5.49cA	6.30cA	2.03bB	6.11eA	6.87bA
BH028	0.80aB	9.76bA	11.39aA	1.83bB	12.34cA	13.28aA
BH030	0.85aB	8.05bA	6.29cA	3.35bB	9.90dA	11.79aA
BH031	0.99aC	8.89bA	5.64cB	1.57bC	15.63bA	10.21aB
SP052	1.64aC	3.94cB	6.18cA	5.16bB	9.80dA	12.46aA
SP101	0.96aB	4.81cA	1.68dB	2.79bB	7.86eA	6.80bA

[1]Equal capital letters indicate that there was no significant difference between the means presented in the columns using the Skott-Knott test ($p < 0.05$). [2]Equal lowercase letters indicate that there was no significant difference between the means presented in the lines by the Skott-Knott test ($p < 0.05$). [3]Statistical analyzes were carried out individually for external and internal lesions.

1.3.4 Temperature in the growth of isolates

The isolates grew at all temperatures tested, but with minimal growth at 35ºC (Table 4). The optimal temperature of isolates from the state of Bahia was 23.9 to 24.9ºC, while that of São Paulo was 24.0 to 24.1ºC. The isolated BH014 had the highest optimal development temperature, while BH031 had the lowest.

1.4 Discussion

The isolates were identified as belonging to the *C. fimbriata* complex, based on the morphological characteristics and the grouping in the phylogenetic tree. This species is considered the causal agent of ceratocystis wilt of *Eucalyptus* in Brazil (Oliveira et al. 2015). *C. fimbriata* (s.l.) is a natural inhabitant of the soils of the Brazilian Atlantic Forest and Cerrado biomes, naturally infecting plants from these biomes, such as pequi and andiroba (da Silva et al. 2017; Ferreira et al. 2011; Valdetaro et al. 2019). By surviving in the soil, this fungus infects *Eucalyptus* trees mainly in the root system, leading to its necrosis and inactivation (Ferreira et al. 2006; Harrington 2013). From the point of infection, the fungus colonizes the plant's vascular system upwards, resulting in the decline of the aerial part and in a hormonal imbalance between roots/aerial parts. As a consequence of this imbalance, occurs the formation of adventitious shoots along the trunk of the diseased tree (Ferreira et al. 2006). Based on field observations, adventitious shoot formation symptoms may not be a diagnostic symptom in trees older than three years, as this symptom may also indicate other forms of stress, such as water stress, fire or cold temperatures (Ferreira and Milani 2012). Colonization of secondary xylem cells by *Ceratocystis* can increase resistance to water flow, reducing the arrival of sap to the upper third, resulting in symptoms of wilting and drying of branches (da Silva et al. 2018; Ferreira et al. 2006).

Table 4. Optimal growth temperature of the *Ceratocystis* isolates originated from *Eucalyptus* plantations.

Isolate	Regression	R^2 (%)	Optimum temperature (°C)
BH012	$MG = -4.351 + 0.4642T - 0.009691T^2$	96.7	24.0
BH014	$MG = -4.711 + 0.4599T - 0.009245T^2$	97.9	24.9
BH015	$MG = -4.536 + 0.4934T - 0.01037T^2$	96.2	24.1
BH021	$MG = -5.247 + 0.5372T - 0.01097T^2$	90.5	24.5
BH023	$MG = -5.378 + 0.5575T - 0.01146T^2$	93.5	24.3
BH028	$MG = -4.684 + 0.4798T - 0.009819T^2$	92.7	24.4
BH030	$MG = -3.686 + 0.4148T - 0.008796T^2$	97.2	24.6
BH031	$MG = -4.344 + 0.4697T - 0.009833T^2$	87.7	23.9
SP052	$MG= -4.052 + 0.4340T- 0.009020T^2$	94.5	24.1
SP101	$MG = -5.077 + 0.5385T - 0.01121T^2$	97.7	24.0

MG = Mycelial growth; T = temperature in ^oC

The occurrence of termites in *Eucalyptus* trees with symptoms of ceratocystis wilt has already been reported (Ferreira and Milani 2012), observing the formation of galleries in colonized wood. However, it has not been determined whether there is any association between the occurrence of termites and the incidence or severity of ceratocystis wilt. In living *Eucalyptus* trees, with more than two years old, is reported the occurrence of heartwood termites, penetrating through the root system (Wilcken et al. 2002). According to our observations, *Ceratocystis* causes root death and termites can use these tissues to penetrate diseased trees. Heartwood rot is caused by fungi in *Eucalyptus*, which degrade complex molecules in the wood. These microorganisms penetrate through wounds and dead roots and branches (Ferreira and Milani 2012). Probably, in diseased trees whose heartwood rot was associated with wood colonized by *Ceratocystis*, dead roots due to the incidence of the pathogen served as a penetration point for degrading microorganisms.

We observed different levels of aggressiveness according to the clone x isolate interaction. Some isolates showed low to intermediate aggressiveness in one clone, while in another they behaved as aggressive. According Oliveira et al. (2015), isolates of *C. fimbrita* (s.l.) from geographically distant *Eucalyptus* plantations show different levels of aggressiveness according to the clone. However, we noticed in this study that geographically close populations can also present varied aggressiveness. This result exemplifies the difficulty in selecting clones resistant to ceratocystis wilt, since even geographically close plantations can be affected by different genotypes of the pathogen. This variability observed among isolates may be related to the fact that the areas studied belong to the Atlantic Forest biome, probably one of the centers of diversity for *C. fimbriata* (s.l.) (Ferreira et al. 2011; Valdetaro et al 2019). *C. fimbriata* (s.l.) populations may remain geographically isolated and may undergo specialization by local hosts, resulting in genotypic variation between populations (Baker et al. 2003). Native hosts generally have greater resistance to this pathogen than exotic ones, and sequential cultivation of the same genetic material can select for more aggressive isolates against a specific clone (Baker et al. 2003; Ferreira et al. 2010). Consequently, different populations may show different levels of aggressiveness against the same host. Despite this, we did not observe differences in aggressiveness between the isolates tested in the south of Bahia and in the state of São Paulo.

We also found that EC187 clone was resistant to all tested isolates, indicating that some resistance mechanisms may be nonspecific against certain pathogen genotypes. Oliveira et al. (2015) recommend that artificial inoculations be made with isolates of *C. fimbriata* (s.l.) with proven pathogenicity in previous trials and which, preferably, have shown high aggressiveness against several clones. The use of pathogen genotypes with low aggressiveness or unknown aggressiveness can lead to incorrect estimates of the resistance of the tested clone (Oliveira et al. 2015).

The evaluation of the external lesion in the seedlings can help in the process of selection of resistant clones. In the present work, it was observed that the clone EC121 and EC090 presented similar values of internal lesion, however the clone EC090 presented smaller external lesions for most of the isolates. Thus, aiming at decision-making, clone EC090 behaved as more resistant. Bark lesions arise due to colonization of the vascular cambium and secondary phloem, resulting in its necrosis (Ferreira et al. 2006; Silva et al. 2020). In response to infection and colonization, *Eucalyptus* plants can produce phenols, PR proteins, amorphous material, phytoalexins and by deposition of gels, calcium oxalate and tyloses in their vessels. According to Silva et al. (2020), resistant clones have a faster and more intense reaction against colonization by *C. fimbriata* (s.l.), resulting in less severe symptoms.

The isolates studied were morphologically indistinguishable and had an optimal growth temperature close to the range of 24 to 26ºC, described as ideal for the development of *C. fimbriata* (s.l.) (Kile 1993; Oliveira et al. 2015). Due to the morphological similarity between species belonging to the *C. fimbriata* complex, sequencing of specific genetic regions, pathogenicity assays and/or intersterility tests are recommended (de Beer et al. 2014; Fourie et al. 2015; Engelbrecht and Harrington 2005). In the present work, to identify isolates of *C. fimbriata* (s.l.), we sequenced different genomic regions.

In addition to identifying and characterizing the isolates, the present work also explored the symptoms manifested by diseased *Eucalyptus* trees, at different ages, and the association with other pathologies and pests. These descriptions are likely to assist professionals and researchers in accurately diagnosing ceratocystis wilt in *Eucalyptus* plantations.

Acknowledgements

We thank CAPES (Project 001) and the company Veracel Celulose S/A for making this work possible.

References

Alam MW, Rehman A, Iqbal M, Saira M, Aslam S, Muhammad S, Hameed A, Gleason ML (2017) First Report of *Ceratocystis fimbriata* Causing *Eucalyptus* Wilt in Pakistan. Plant Disease 101(6):1050. https://doi.org/10.1094/PDIS-12-16-1703-PDN

Arriel DAA, Guimarães LMdaS, de Resendec MDV, Lima Neto FP, Silva DFSHS, de Siqueira DL, Alfenas AC (2016) Genetic control of resistance on *Mangifera indica* to Ceratocystis wilt. Scientia Horticulturae 211:312-316. http://dx.doi.org/10.1016/j.scienta.2016.09.001

Barnes I (2003) *Ceratocystis fimbriata* infecting *Eucalyptus grandis* in Uruguay. Australasian Plant Pathology 32:361-366. https://doi.org/10.1071/AP03032

Baker CJ, Harrington TC, Krauss U, Alfenas AC (2003) Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata*. Phytopathology 93(10):1274-1284. http://dx.doi.org/10.1094/PHYTO.2003.93.10.1274.

de Beer ZW, Duong TA, Barnes I, Wingfield BD, Wingfield MJ (2014) Redefining *Ceratocystis* and allied genera. Studies In Mycology 79:187-219. http://dx.doi.org/10.1016/j.simyco.2014.10.001.

de Brito NM, Duarte HSS, Auer CG, Wendling I, Alfenas RF, Dos Santos ÁF (2021) Aggressiveness and screening for host responses in yerba mate (*Ilex paraguariensis*) clones to a wilt disease caused by *Ceratocystis fimbriata*. Forest Pathology 51(3):1-8. https://doi.org/10.1111/efp.12682

da Silva AC, Cândido TS, Sales NLP, Harrington TC, Alfenas AC (2017) First Report of Ceratocystis Wilt Caused by *Ceratocystis fimbriata* on *Caryocar brasiliense* Trees in Brazil. Plant Disease, 101(10):1822. https://doi.org/10.1094/PDIS-03-17-0376-PDN

da Silva AC, Silva FMdeO, Milagre JC, Omena-Garcia RP, Abreu MC, Mafia RG, Nunes-Nesi A, Alfenas AC (2018) Eucalypt plants are physiologically and metabolically

affected by infection with *Ceratocystis fimbriata*. Plant Physiology and Biochemistry 123:170-179. https://doi.org/10.1016/j.plaphy.2017.12.002

Engelbrecht CJ, Harrington TC (2005) Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. Mycologia 97(1):57-69. https://doi.org/10.3852/mycologia.97.1.57.

Ferreira FA, Maffia LA, Barreto RW, Demuner NL, Pigatto S (2006) Sintomatologia da murcha de *Ceratocystis fimbriata* em eucalipto. Revista Árvore 30:155-162. https://doi.org/10.1590/S0100-67622006000200001

Ferreira EM, Harrington TC, Thorpe DJ, Alfenas AC (2010) Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis fimbriata* in Brazil. Plant Pathology 59:721-735. https://doi.org/10.1111/j.1365-3059.2010.02275.x

Ferreira MA, Harrington TC, Alfenas AC; Mizubuti ESG (2011) Movement of Genotypes of *Ceratocystis fimbriata* Within and Among Eucalyptus Plantations in Brazil. Phytopathology 101:1005-1012. https://doi.org/10.1094/PHYTO-01-11-0015

Ferreira FA, Milani D (2012) Visual Diagnosis and Control of Abiotic Eucalyptus Diseases in Brazil. UFV, Viçosa, pp 26-61

Ferreira MA, Harrington TC, Gongora-Canul CC, Mafia RG, Zauza EAV, Alfenas AC (2013) Spatial–temporal patterns of Ceratocystis wilt in *Eucalyptus* plantations in Brazil. Forest Pathology 43:153-164. https://doi.org/10.1111/efp.12013

Firmino AC, Tozze Júnior HJ, Furtado EL (2012) First report of *Ceratocystis fimbriata* causing wilt in *Tectona grandis* in Brazil. New Disease Reports, 25:24. https://doi.org/10.5197/j.2044-0588.2012.025.024

Firmino AC, Fischer IH, Antonio GL, De Novaes QS, Tozze Júnior HJ, Furtado EL (2016) Characterization of *Ceratocystis fimbriata* from passion fruits. Arquivos do Instituto Biológico 83:1-7. https://doi.org/10.1590/1808-1657000982014

Firmino AC, Matos MAS, Tozze Jr HJ, Barretto VCM (2017) First report of *Ceratocystis fimbriata* causing wilt on *Khaya senegalensis*. New Disease Reports 35:35. http://dx.doi.org/10.5197/j.2044-0588.2017.035.035

Fourie A, Wingfield MJ, Wingfield BD, Barnes I (2015). Molecular markers delimit cryptic species in *Ceratocystis* sensu stricto. Mycological Progress 14, 1020. https://doi.org/10.1007/s11557-014-1020-0

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Glass NL, Donaldson GC (1995) Development of Primer Sets Designed for Use with the PCR to amplify Conserved Genes from Filamentous Ascomycetes. Applied and Environmental Microbiology, 61, 1323-1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Halsted BD (1890) Some fungous diseases of sweet potato. The black rot. New Jersey Agricultural Experiment Station Bulletin 76:7-14.

Harrington TC, Thorpe DJ, Marinho VLA, Furtado EL (2005) First report of black rot of *Colocasia esculenta* caused by *Ceratocystis fimbriata* in Brazil. Fitopatologia Brasileira 30:88-89. https://doi.org/10.1590/S0100-41582005000100017

Harrington TC (2013) Ceratocystis diseases. In: Infectious Forest diseases. Ed. by Gonthier, P.; Nicolotti, G. Wallingford, UK: CAB International.

Heath RN, Wingfield MJ, Wingfield BD, Meke G, Mbaga A, Roux J (2009) *Ceratocystis* species on *Acacia mearnsii* and *Eucalyptus* spp. in eastern and southern Africa including six new species. Fungal Diversity 34:41-67.

Kile, G. A., 1993: Plant diseases caused by species of Ceratocystis sensu stricto and Chalara. In: Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity. Ed. by Wingfield, M. J.; Seifert, K. A.; Webber, J. A.. St. Paul, MN: APS Press, pp. 173–183.

Li J, Gao JM, Han YH, Sun YX, Huang Q (2014a) First Report of *Ceratocystis fimbriata*-Caused Wilt of *Eriobotrya japonica* in China. Plant Disease 98(9):1270. https://doi.org/10.1094/PDIS-12-13-1290-PDN.

Li J, Zhang Y, Xu KC, Yang JY, Han YH, Sun YX, Huang Q (2014b) First Report of Wilt of *Eucalyptus* Caused by *Ceratocystis fimbriata* in China. Plant Disease 98(12):1744-1744. https://doi.org/10.1094/PDIS-06-14-0580-PDN

Mafia RG, Ferreira MA, Zauza EAV, Silva JF, Colodette JL, Alfenas AC (2013) Impact of Ceratocystis wilt on eucalyptus tree growth and cellulose pulp yield. Forest Pathology 43:379-385. https://doi.org/10.1111/efp.12041

Marin M, Castro B, Gaitan A, Preisig O, Wingfield BD, Wingfield MJ (2003) Relationships of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. Journal of Phytopathology 151:395-405. https://doi.org/10.1046/j.1439-0434.2003.00738.x

Méndez-Álvarez D, Cândido TS, Alfenas AC, Murillo O, Badilla, Y, Alfenas RF (2020) First report of *Ceratocystis fimbriata* causing wilt on *Gmelina arborea* in Costa Rica. Forest Pathology 50(5):1-4. https://doi.org/10.1111/efp.12628

Oliveira LSS, Guimarães LMS, Ferreira MA, Nunes AS, Pimenta LVA, Alfenas AC (2015) Aggressiveness, cultural characteristics and genetic variation of *Ceratocystis fimbriata* on *Eucalyptus* spp. Forest Pathology 45(6):505-514. https://doi.org/10.1111/efp.12200

Oliveira LSS, Damacena MB, Guimarães LMS, Siqueira DL, Alfenas AC (2016) *Ceratocystis fimbriata* isolates on *Mangifera indica* have different levels of aggressiveness. European Journal of Plant Pathology 145:847-856. https://doi.org/10.1007/s10658-016-0873-2

Piveta G, Alfenas AC, Muniz MFB, Valdebenito-Sanhueza RM, Ferreira MA (2013) *Ceratocystis fimbriata* kiwi (*Actinidia deliciosa*) in Southern Brazil. Revista Brasileira de Fruticultura 35(2):665-669. https://doi.org/10.1590/S0100-29452013000200040

Roux J, Wingfield MJ, Bouillet JP, Wingfield BD, Alfenas AC (2000) A serious new wilt disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. Forest Pathology 30:175-184. https://doi.org/10.1046/j.1439-0329.2000.00202.x

Silva AC, Betancourth BML, Ferreira DC, Elerati TL, Rodrigues FÁ, Alfenas AC (2020) Responses of resistant and susceptible hybrid clones of *Eucalyptus urophylla* ×

Eucalyptus grandis to infection by *Ceratocystis fimbriata*. Annals of Forest Science 77:45. https://doi.org/10.1007/s13595-020-00932-6

Somasekhara YM (1999) New Record of *Ceratocystis fimbriata* Causing Wilt of Pomegranate in India. Plant Disease 83(4):400. https://doi.org/10.1094/PDIS.1999.83.4.400B

Tamura K, Stecher G, Kumar S (2021) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120

Valdetaro DCOF, Harrington TC, Oliveira LSS, Guimaraes LMS, McNew DL, Pimenta LVA, Gonçalves RC, Schurt DA, Alfenas AC (2019) A host specialized form of *Ceratocystis fimbriata* causes seed and seedling blight on native *Carapa guianensis* (andiroba) in Amazonian rainforests. Fungal Biology 123:170-182. https://doi.org/10.1016/j.funbio.2018.12.001

Van Wyk M, Roux J, Nkuekam GK, Wingfield BD, Wingfield MJ (2012) *Ceratocystis eucalypticola* sp. nov. from *Eucalyptus* in South Africa and comparison to global isolates from this tree. IMA Fungus 3(1):45-58. https://doi.org/10.5598/imafungus.2012.03.01.06

Wilcken CF, Raetano CG, Forti LC (2002) Termites pests in Eucalyptus forests of Brazil. Sociobiology 40(1):179-190.

Xu K, Li J, Yang X, Zhang R, Li X, Xie M, Huang Q (2020) Postharvest rot on carrot caused by *Ceratocystis fimbriata* and *Chalaropsis thielavioides* (≡ *Thielaviopsis thielav*ioides) in China. Journal of General Plant Pathology 86:322–325. https://doi.org/10.1007/s10327-020-00919-1

CHAPTER 3

GROWTH AND PULP PRODUCTION OF EUCALYPTUS TREES AFFECTED BY CERATOCYSTIS WILT [1]

ABSTRACT

Ceratocystis wilt is one of the most harmful diseases affecting eucalyptus crops and is associated with the fungus *Ceratocystis fimbriata* sensu lato in Brazil. This pathology leads to the obstruction of the vascular system in infected trees, resulting in symptoms such as branch wilting and death. The wood affected by the pathogen exhibits discoloration and changes in its chemical composition, which can impact the pulping process. Therefore, the objective of this work is to investigate the impact of ceratocystis wilt on growth and the pulping process when sampling whole diseased trees. For this, healthy and diseased eucalyptus trees at the harvest age of 7 years were cut down, and their dendrometric variables (diameter at breast height, height and volume) and disease severity (basal discoloration area and volume of discolored tissue) were measured. All tree trunks, classified into different severity classes, were sampled individually, peeled, chipped and subjected to Kraft cooking (Kappa number 18±0.5). Diseased trees, under the evaluated condition, exhibited an average volume of 2.5% with discoloration and a reduction in the volumetric growth of 18.1%. The wood from diseased trees showed no significant differences in the Kraft cooking variables when compared to healthy trees. This result suggests that ceratocystis wilt reduces the volumetric growth of affected trees; however, due to the low proportion of symptomatic wood observed, the utilization of wood from diseased trees does not influence pulping and can be used in the pulp production process.

Key words: Vascular wilt, root infection, cellulose, wood discoloration, xylem colonization

---

[1]Formatted according to the "Forest Pathology" submission guidelines.

1.1 Introduction

Eucalypts (*Eucalyptus* spp. and *Corymbia* spp.) are the most cultivated trees in Brazil, with a planted area of 7.51 million hectares in 2021 (IBA, 2022). The main cultivation system employed is monoculture, utilizing vegetatively propagated plants thar are genetically identical. This clonal propagation process has enabled the multiplication of phenotypically superior genotypes, characterized by higher productivity, adequate physical and chemical wood properties, straight trunks, stress tolerance and resistance to diseases and pests (Alfenas et al., 2009). In Brazil, *Eucalyptus* wood is primarily utilized in pulp production for export (IBA, 2022).

The Kraft cooking is the primary industrial pulping method employed for pulping eucalyptus wood, enabling the extraction of cellulose. This method involves transforming eucalyptus trunks into chips, followed by the addition of a sodium hydroxide and sodium sulfide solution, and subjecting it to a temperature of 170ºC for approximately 2 hours (Bajpai & Bajpai, 1992). As a result, constituents of wood, mainly lignin, are solubilized. The residual cellulosic pulp still contains residues of lignin, resulting in a light brown color. Depending on the desired final product, the cellulosic pulp can undergo a bleaching process using chlorine-based compounds and their oxides to achieve lighter colors (Bajpai & Bajpai, 1992).

Eucalyptus trees, like any other crop, can be affected by various diseases, with stem diseases being particularly significant due to the damage caused and the challenges associated with their control. These diseases compromise forest productivity, causing reductions in tree growth and final forest stand due to mortality. Stem lesions and vascular wilt are the main symptoms observed, which can be caused by either bacterial or fungal pathogens (Alfenas et al., 2009). In eucalyptus plantations, important causal agents of stem lesions include *Chrysoporthe* spp., *Botryosphaerea* spp., and *Teratosphaeria* spp. (Aylward et al., 2019; Gryzenhout et al., 2006; Nakabonge et al., 2006; Pérez et al., 2010; Rodas et al., 2009). Vascular wilts in eucalyptus are associated with fungi of the genus *Ceratocystis* and bacteria such as *Erwinia psidii* and *Ralstonia* spp. (Carstensen et al., 2017; Coutinho et al., 2011; Oliveira et al., 2015).

In Brazil, ceratocystis wilt in eucalyptus plantation is caused by the fungus *Ceratocystis fimbriata* sensu lato (s.l) (Ferreira et al., 2006; Oliveira et al., 2015). This

fungus can survive in the form of resistance structures, known as chlamydospores, or in crop debris in the soil. The infection primarily occurs in the root system (Ferreira et al., 2006; 2013). Internal symptoms are mainly characterized by discoloration in a radial streaks pattern, progressing upward in the wood from the point of infection (Ferreira et al., 2006). The presence of fungal structures in the vessels of the secondary xylem colonized by *Ceratocystis* results in increased resistance to sap flow, leading to external symptoms such as wilting, defoliation, and drying of branches. These symptoms can ultimately lead to the death of the affected plant (Ferreira et al., 2006; Da Silva et al., 2018). Bark lesions, kino pocket formation and the development of adventitious shoots can also be observed in diseased trees (Ferreira et al., 2006).

Management of ceratocystis wilt in eucalyptus plantations primarily involves planting resistant clones. However, due to the high genetic variability of *C. fimbriata* s.l., clones that exhibit resistance against certain genotypes in one region may behave as susceptible in other locations (Oliveira et al., 2015). This variation in aggressiveness between populations makes it challenging to select universally resistant clones. Additionally, the limited spatial dispersal of *C. fimbriata* s.l. can result in geographically isolated populations of the pathogen, leading to speciation by specific hosts. Continuous cultivation of the same clone in the same area can lead to the selection of more aggressive genotypes against the genetic material (Baker et al., 2003; 2005).

As a direct damage, eucalyptus trees affected by ceratocystis wilt can experience significant growth reduction of up to 87%, with an incidence rate exceeding 50% in the planted population (Ferreira et al., 2013; Mafia et al., 2013). Wood of diseased trees also have a lower density, which may reduce their value for use as lumber. Utilizing wood with 50% of its volume colonized by the fungus results to a 23.8% increase in the consumption of reagents during the pulping process, along with a 13.7% reduction in cellulose yield per cubic meter of wood (Mafia et al., 2013). As a result of the decreased yield and increased costs, it is recommended to redirect highly colonized wood from diseased trees to other purposes, such as charcoal production or energy generation in the industry (Fernandes et al., 2014). The increase in costs and lower cellulose yield can be attributed to the elevated in extractives and lignin content in the wood of diseased trees, which occurs in response to infection by the pathogen. Additionally, the obstruction of the xylem vessels in wood colonized by *Ceratocystis*

reduce the infiltration of pulping reagents, leading to a reduction in the efficiency of the process (Da Silva et al., 2018; Mafia et al., 2013; Silva et al., 2020).

The use of 20% of wood colonized by *Ceratocystis* negatively impacts the pulping process, progressively increasing as the proportion of diseased wood increases (Mafia et al., 2013). However, in a field survey, we observed that at the end of the forest cycle (7 years), diseased trees exhibited considerably lower values of wood showing discoloration when considering the total volume of the affected tree. Thus, the objective of this study was to investigate the impact of ceratocystis wilt on both the growth and the pulping process by sampling entire trees at the harvest age.

1.2 Materials and methods

1.2.1 Study area

The sampled trees were grown in monoclonal commercial plantations, spaced at 5.0 x 2.4m, with an approximate density of 770 plants/ha. The cultivated clone used belongs to the *E. grandis* x *E. urophylla* cross, which was classified as susceptible to ceratocystis wilt based on the high observed mortality rate (about 30% in certain areas) under field conditions. The plantation was located in the city of Belmonte, Bahia, Brazil, at coordinates: latitude -16.098676/longitude -39.330219. The plantation was situated in the Atlantic Forest biome, characterized by an equatorial climate with no distinct dry season (Af; Thorthwaite, 1948). At the time of sampling and evaluations, the plantation was at harvest age, with 7 years of establishment. The presence of diseased trees affected by ceratocystis wilt had been previously observed in the studied area.

1.2.2 Sampling method

In this planting, we conducted two sampling sessions. The first sampling aimed to study the internal colonization of diseased trees, while the second sampling focused on evaluating the damage caused by ceratocystis wilt.

During the first sampling, we systematically selected 50 trees affected by ceratocystis wilt by sampling trees in plantation rows. These trees were cut at the base (10-15 cm) and classified as either diseased or healthy based on the internal symptoms observed in the wood. Among the diseased trees, those showing wood discoloration similar to ceratocystis wilt were further sectioned at regular intervals of 50 cm, starting

from the base and continuing up to the height where tissue discoloration was present. Dendrological parameters such as height, diameter at breast height, and volume were measured for the diseased trees during this sampling. The volume of the trees was calculated using a specific pre-established equation for the clone under study. We captured photos of the exposed faces of the wood using a Nikon D5300 camera with a resolution of 24.2 MP. The camera was positioned at a distance of 50 cm from the wood. To measure the area of discoloration on the exposed wood, as well as the area of healthy tissue, we utilized the ImageJ software (Schneider et al., 2012). A graduated ruler was placed next to the exposed wood at the time of the photo to serve as a reference for measurements. The data obtained served as the basis for estimating the height of pathogen colonization and the volume of colonized wood in the trunk sections. The volume calculation was performed using the formula for partial cone volume (Liu et al., 2019). By applying this formula to each trunk section, we obtained the volume of discoloration, and subsequently, the volumes of all sections were summed to obtain the total volume of colonized wood. In order to confirm the association of *Ceratocystis* sp. with the observed symptoms of wood discoloration, we performed the isolation of the causal agent using the carrot bait method (Moller & De Vay, 1968) for approximately 20% of the symptomatic sampled trees.

The second sampling was conducted within the same planting area, where a plot consisting of 250 plants arranged in 10 rows of 25 trees in sequence was established. All the trees in this plot were cut at a height of 10-15 cm, and photographs of the exposed wood were taken for later measurement of the area of discolored and healthy woody tissue. Diseased trees were identified based on internal symptoms of radial wood discoloration, which are characteristic of ceratocystis wilt. These trees were then classified into two severity classes: Class 1 (<25.0% discoloration) and Class 2 (25.1% - 51.0% discoloration). Dendrometric parameters were collected from both healthy (Class 0) and diseased trees in order to compare the impact of the disease on the growth of the clone. As the number of plants analyzed at different severity levels varied, the data were subjected to a non-parametric Kruskal-Wallis test ($p < 0.05$) using the Minitab for Windows software (Version 17; Minitab Inc., State College, PA). The experiment was conducted in a completely random design.

1.2.3 Impact on the pulping process

In the plot of 250 trees, five plants were randomly selected to represent each severity class (**Fig. 1**): 0 (healthy - 0% discoloration), 1 (<25.0% discoloration), and 2 (25.1% - 51.0% discoloration) among the diseased trees. The selected trees had their discoloration volume estimated by cutting the trunk at regular intervals of 50 cm, as previously described, and dendrometric data were collected. Wood samples from each selected tree were processed separately based on their severity classes. First, the trunk sections were chipped, and only chips with appropriate dimensions were selected and retained for further analysis. The selected chips, with a thickness ranging from 2 to 8 mm, were homogenized, dried in the shade, and stored in bags until analysis. Subsamples were collected from each tree for determination of basic density using the hydrostatic balance method, as well as for analysis of extractive content in ethanol/toluene and hot water. The chips were subjected to a Kraft cooking simulation under specific conditions, including a liquor/wood ratio of 4:1, cooking liquor sulphidity of 25%, maximum temperature of 170°C, time to reach maximum temperature of 90 minutes, time at maximum temperature of 50 minutes, and a target Kappa number of 18±0.5 for the unbleached pulp. For each sample, the yield and alkali load required to achieve the target Kappa number were recorded. The wood consumption (m^3) for the production of one ton of air-dried pulp (TSA) was determined. The experiment was conducted using a completely randomized design, with five replications per severity class. The data were analyzed using Tukey's test ($p < 0.05$) in the statistical software Minitab for Windows (Version 17).

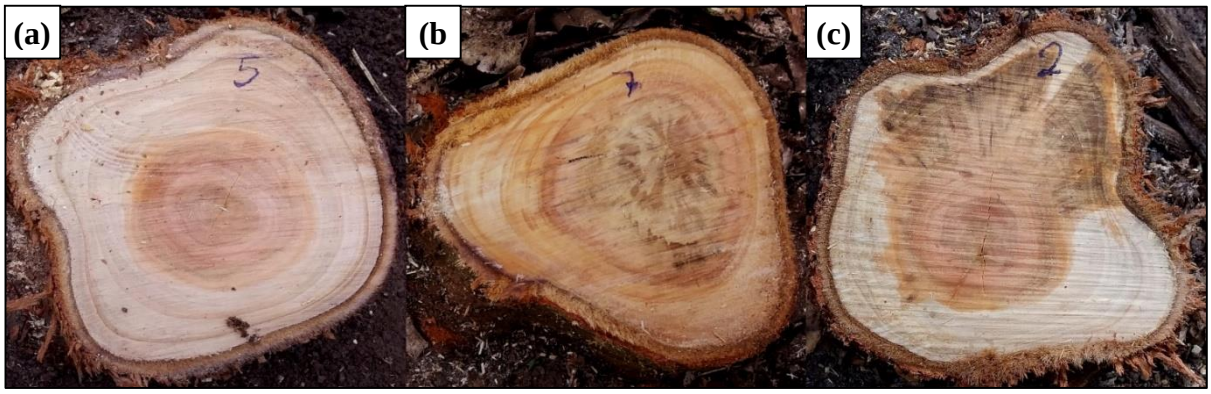


Figure 1. Internal woody tissues, from sampled eucalyptus trees, with different severity classes of basal discoloration due to ceratocystis wilt. (a) Class 0 (healthy); (b) Class 1 (<25.0% discoloration); (c) Class 2 (25.1% - 51.0%).

1.3 Results

1.3.1 Internal symptoms of diseased trees

In the studied area, we observed an incidence of 18.8% of internal symptoms related to ceratocystis wilt among the evaluated trees. Although symptoms of wilting and drying of branches were not observed, some planting failures were noticed, suggesting possible mortality during the early stages of the plantation. The isolation of *Ceratocystis* sp. from trees displaying symptoms of wood discoloration (**Fig. 2**) confirmed the association of this pathogen with the observed symptoms.

The diseased trees of the studied clone exhibited an average height of discoloration of 1.92m, with a range from 0.5m to 13.0m. The majority of the sampled trees (76%) had a colonization height of less than 2.0m (**Fig. 3**). On average, the discoloration volume of the diseased trees was 0.0078m^3, which accounted for approximately 2.5% of the total volume of the trees (**Fig. 4**). The highest observed volume of colonized tissue was 7.1%, while the lowest was 0.3%. The majority of the trees (54%) had less than 2.0% of their wood volume colonized by the pathogen.

1.3.2 Impact on growth

Diseased trees exhibited significant reductions in height ($p = 0.032$), diameter at breast height ($p = 0.012$), and volume ($p = 0.019$) compared to healthy trees (Table 1). The most pronounced reduction in development was observed in severity Class 2, although there was no statistical difference from Class 1 in diameter at breast height and volume. The height of trees in Class 1 was statistically similar to that of healthy trees but higher than that of trees in Class 2. The volume of diseased plants was 18.1% lower, resulting from a reduction in height and diameter at breast height by 4.8% and 10.2%, respectively.

1.3.3 Impact on physical and chemical properties of eucalyptus wood and on the pulping process

The severity classes studied showed statistically significant differences in terms of the percentage of discoloration volume, with Class 2 having the highest percentage ($p < 0.01$; Table 2). However, there were no statistically significant differences in basic density ($p = 0.441$) and extractive contents ($p = 0.280$) among the collected eucalyptus

wood from different severity classes. Similarly, there were no statistical differences observed in the depured yield of the pulping process (p = 0.772), demand for alkaline load (p = 0.433), and wood consumption (p = 0.865) between wood from diseased and healthy trees (**Table 3**).

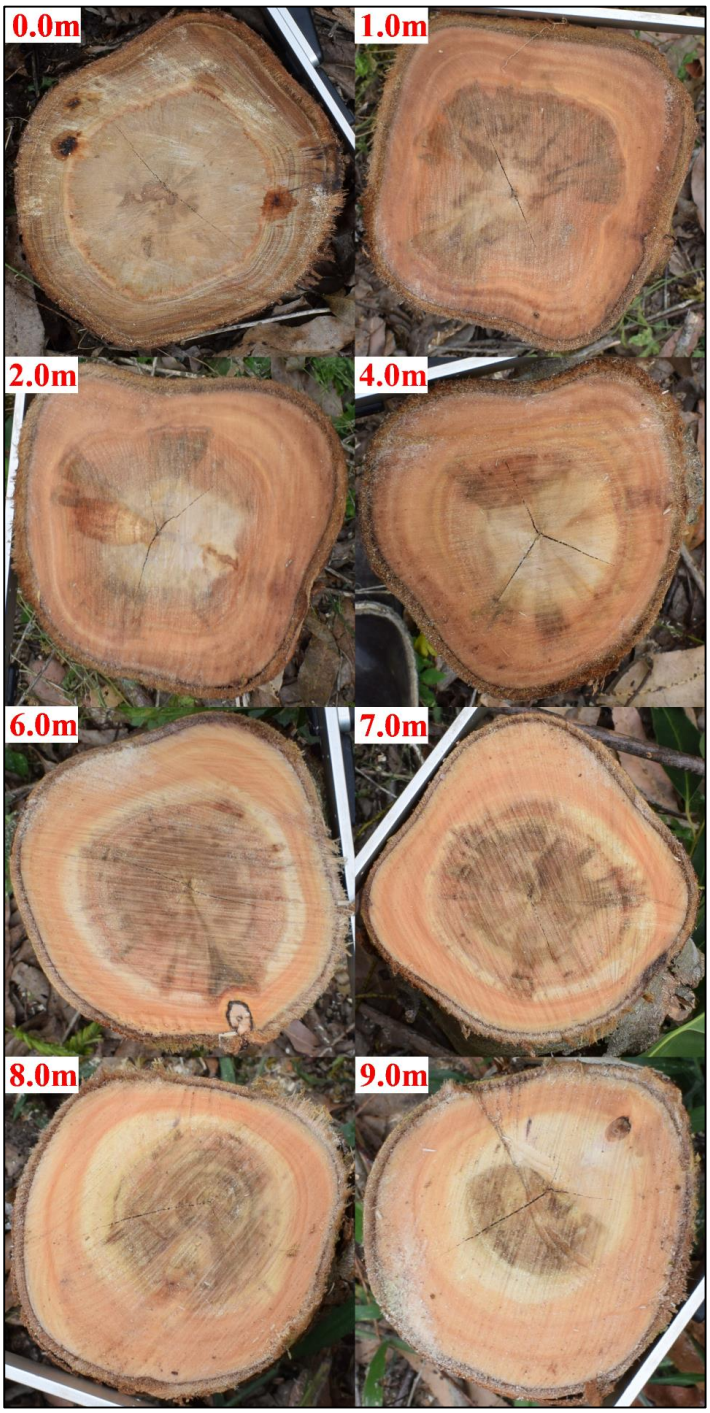


Figure 2. Wood discoloration symptoms in a *Eucalyptus* tree due to colonization by *Ceratocystis* sp. at different heights.

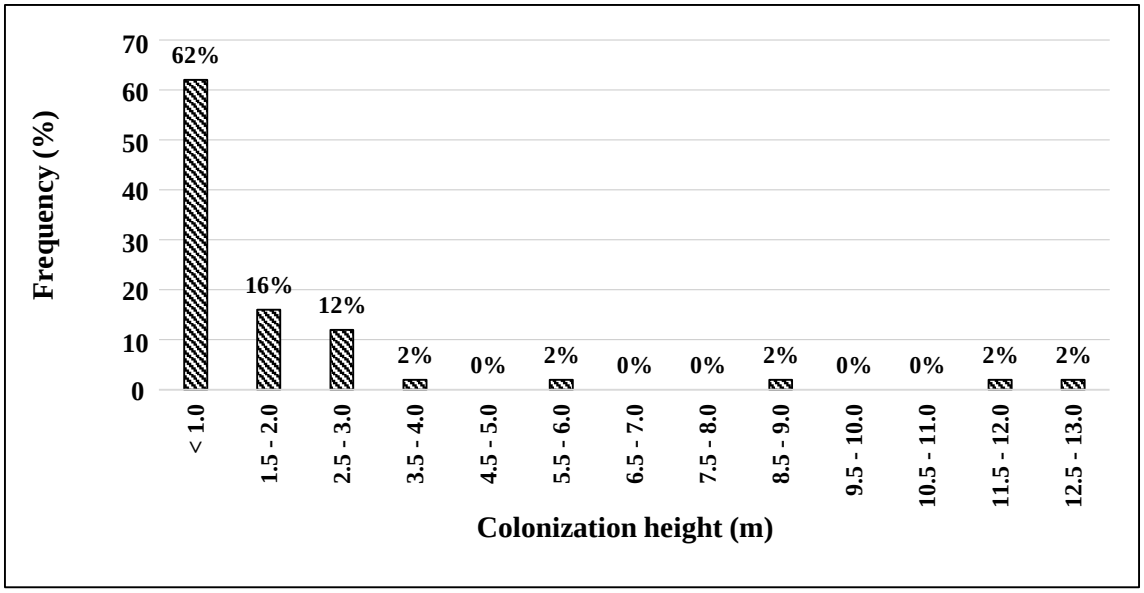


Figure 3. Frequency (n = 50) of heights of colonization by *Ceratocystis* sp. in the studied *Eucalyptus* plantation.

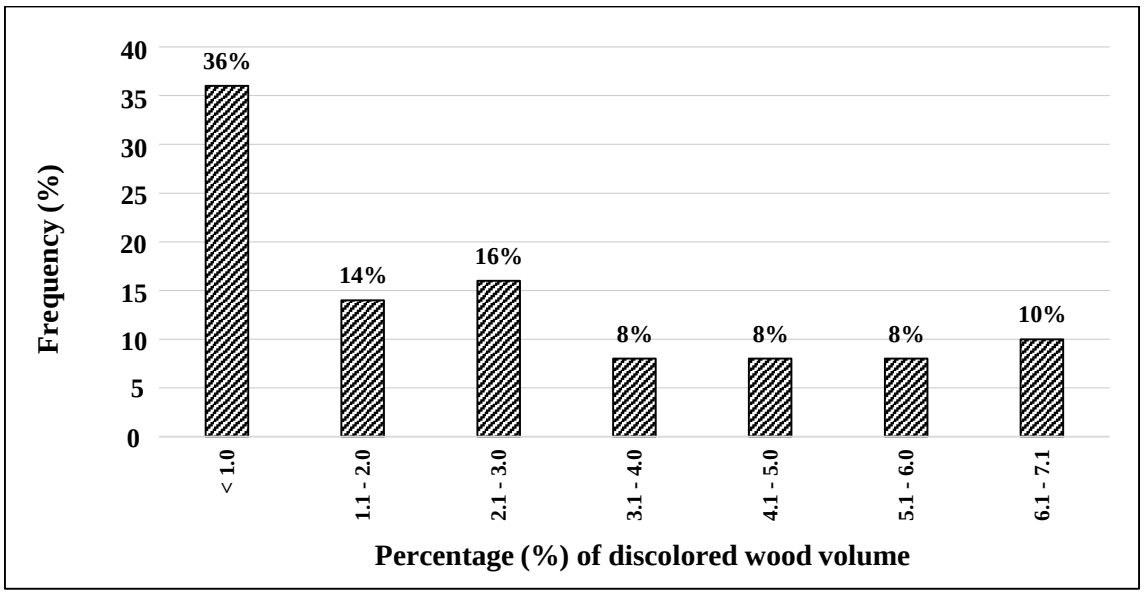


Figure 4. Frequency distribution (n = 50) of wood colonization percentages by *Ceratocystis* sp. in the studied *Eucalyptus* plantation.

Table 1. Impact of ceratocystis wilt, at different levels of severity, on the growth of *Eucalyptus* trees.

Class of severity	DBH[1] (cm)	Height (m)	Volume (m^3)
Class 0 (Healthy) (n = 203)	18.4 **a**[2]	26.9 **a**	0.3084 **a**
Class 1 0.1 - 25.0% (n = 30)	16.9 **b**	26.1 **a**	0.2679 **b**
Class 2 25.1 – 51.0% (n = 17)	16.5 **b**	25.6 **b**	0.2526 **b**

[1]Diameter at breast height. [2]Medians within columns followed by different letters are significantly different from each other using Kruskal-Wallis test ($p < 0.05$).

Table 2. Influence on the physical and chemical properties of eucalyptus wood (n = 5) at different levels of discoloration caused by ceratocystis wilt.

Class of severity	Percentage (%) of discoloration volume	Basic density (Kg/m^3)	Extractives content (%)		
			Alcohol-toluene	Hot water	Total extractives
Class 0 (Healthy)	-	454.2 **a**	1.49 **a**	1.07 **a**	2.56 **a**
Class 1 0.1 - 25.0%	0.34 **a***	449.2 **a**	1.37 **a**	0.98 **a**	2.35 **a**
Class 2 25.1 – 51.0%	2.63 **b**	456.6 **a**	1.59 **a**	1.01 **a**	2.59 **a**

*Mean within columns followed by different letters are significantly different from each other using Tukey's test ($p < 0.05$).

Table 3. Pulping results using *Eucalyptus* wood (n = 5) with different percentages of volume affected by *Ceratocystis* sp.

Class of severity	Percentage (%) of discoloration volume	Alkali load (%)	Depured yield (%)	Wood consumption (m^3/TSA)
Class 0 (Healthy)	-	18.00 **a**	52.56 **a**	4.19 **a**
Class 1 0.1 - 25.0%	0.34 **a***	17.64 **a**	52.90 **a**	4.21 **a**
Class 2 25.1 – 51.0%	2.63 **b**	18.06 **a**	52.46 **a**	4.18 **a**

*Mean within columns followed by different letters are significantly different from each other using Tukey's test ($p < 0.05$).

1.4 Discussion

Ceratocystis wilt affects the physiology of adult trees, compromising the process of sap translocation, which in turn results in damage to the development of diseased trees (Mafia et al., 2013; Da Silva et al., 2018). Due to the effects of the disease in the studied area, diseased trees in the highest severity class grew significantly less than the healthy ones, with a volume reduction of 18.1%. The reduction in growth that we observed was lower than that reported by Mafia et al. (2013), which was 87%, likely attributed to the resistance of the clones under study and/or the aggressiveness of the pathogen genotype present in the area. Resistant genotypes manifest more intense and faster defense reactions against ceratocystis wilt than susceptible ones (Silva et al., 2020). However, due to the aggressiveness variability among different *C. fimbriata* s.l. populations, *Eucalyptus* clones that behave as resistant in a given location may be susceptible to pathogen genotypes from other regions (Oliveira et al., 2015). This difference between populations may be related to their limited spatial dispersion mechanisms, resulting in the population isolation of this pathogen and specialization by local hosts (Baker et al., 2003; 2005; Ferreira et al., 2010; 2011).

Based on our observations, no significant differences were observed in the volumetric growth of trees in severity classes 1 and 2, associated with discoloration from ceratocystis wilt. Mafia et al. (2013) obtained a similar result, not observing any statistical difference in volumetric growth between diseased trees with different colonization height classes. Since *Ceratocystis* infects eucalyptus trees through the

root system (Ferreira et al., 2006), resulting in its necrosis, the level of root inactivation may be a more appropriate variable to be correlated with the impact on the growth of diseased trees. However, evaluating roots under field conditions is extremely costly, in addition to requiring further studies to confirm its association with losses.

In the studied area, we observed that the diseased trees showed basal discoloration of up to 57%, mainly concentrated around the pith region. The most external region of the sapwood, which is in greater physiological activity, remained healthy in the diseased trees, explaining the lack of obvious external symptoms (Fig. 1). Due to the susceptibility of the studied clone, it is likely that the trees showing a high volume of wood colonization by the pathogen died in the initial years of planting. The observed failures in the studied plantation, along with results from other studies indicating that the mortality of diseased trees is higher within the first 20 months of planting (Ferreira et al., 2013), support this hypothesis. Based on our observations in the study area, diseased trees with internal symptoms can survive until the end of the cultivation cycle, but their productivity is significantly lower than that of healthy trees.

In response to infection by *Ceratocystis*, eucalyptus trees can interrupt or delay the colonization of the pathogen in the vascular tissues through the formation of tyloses, gels and amorphous compounds inside the vessels, in addition to tissue lignification and accumulation of PR-proteins and phytoalexins (Silva et al., 2020). The defense responses against ceratocystis wilt, involving the obstruction of vessel cells, can make the impregnation process of *Eucalyptus* wood with an alkaline solution difficult during the Kraft pulping process (Mafia et al., 2013). Affected wood also has lower basic density and higher extractive content (Fernandes et al., 2014; Mafia et al., 2013). Compounds that accumulate in response to infection and tissue lignification increase the demand for reagents during the pulping process and reduce its efficiency, requiring a greater volume of wood to obtain the same weight of pulp. The use of eucalyptus wood with 50% of its volume showing discoloration due to colonization by *Ceratocystis* reduces the cellulose yield by 13.7% and increases the required alkaline load by 23.8% (Mafia et al., 2013). However, in our current study, we found that diseased trees at the harvesting age showed wood discoloration volume values of up to 7.1%, with most cases not exceeding 2.5%. When we sampled the entire trees, the impact of the volume of infected wood tended to be less significant due to the relatively small proportion of diseased wood compared to healthy wood. As a result, we did not

observe any notable differences in the analyzed variables when comparing wood from healthy and diseased trees. This suggests that, under the conditions studied, diseased trees exhibit similar characteristics to healthy ones, including basic density, extractive content, final pulp yield, and the demand for reagents during the pulping process.

In addition to ceratocystis wilt, other diseases are reported to cause reductions in the productivity of eucalyptus plantations. The incidence of basal chrysoporthe canker can reduce growth in 77.1% of four-year-old trees when the lesion compromises the wood and vascular cambium. However, this pathology did not significantly alter the levels of extractives and lignin in the wood of diseased plants or significantly interfere with the Kraft cooking process (Souza et al., 2010). Similarly, Ralstonia wilt can decrease the volumetric growth of eucalyptus trees at 30 months of age by 81.6%, affecting 72.4% of the trees in the evaluated plantation. The use of wood from diseased trees due to Ralstonia wilt in the pulping process results in reduced pulp yield (Ferreira et al., 2017). Like ceratocystis wilt, these stem diseases compromise the natural physiological processes of the host plant, reducing tree growth, and affecting its chemical composition.

The production of bleached pulp is the main destination for eucalyptus wood in Brazil. However, the incidence of ceratocystis wilt can increase the demand for planted area to supply the cellulose production chain due to reduced productivity per hectare caused by tree mortality and decreased growth. In addition to direct damage, these pathogens can change the chemical composition of the affected wood, increasing the levels of extractives and lignins and reducing the basic density. Therefore, colonized wood demands a higher alkaline load and has a lower yield in the pulping process (Mafia et al., 2013). In the present work, we observed a reduction in the growth of diseased trees due to ceratocystis wilt, however, we did not find any impact of this pathology on the pulping process and on the final yield of the pulp. This result indicates that the interference of chemical alterations of diseased wood on pulping is not detectable when whole trees are sampled, thus diluting the possible impact of colonized wood due to the comparatively low proportion of diseased wood in the studied trees. Consequently, diseased trees, under the condition studied, can be harvested and used in pulp production without causing losses to the process.

Acknowledgements

We thank CAPES (Project 001) and the company Veracel Celulose S/A for making this work possible.

References

Alfenas, A. C., Zauza, E. A. V., Mafia, R. G., & De Assis, T. F. (2009). Clonagem e doenças do eucalipto (2nd. Ed.). Viçosa, MG, Brasil: Editora UFV.

Aylward, J., Roets, F., Dreyer, L. L., & Wingfield, M. J. (2019) *Teratosphaeria* stem canker of eucalyptus: two pathogens, one devastating disease. Molecular plant pathology, *20*, p. 8-19.

Bajpai, P., & Bajpai, P. K. (1992). Biobleaching of Kraft Pulp. Process biochemistry, *27*, 319-325.

Baker, C. J., Harrington, T. C., Krauss, U., & Alfenas, A. C. (2003). Genetic variability and host specialization in the latin american clade of *Ceratocystis fimbriata*. Phytopathology, *93*(10), 1274-1284.

Baker, C. J., & Harrington, T. C. (2005). Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. Mycologia, *97*(1), 57-69.

Carstensen, G. D., Venter, S. N., Wingfield, M. J., & Coutinho, T. A. (2017). Two *Ralstonia* species associated with bacterial wilt of *Eucalyptus*. Plant pathology, *66*(3), 393-403.

Coutinho, T. A., Brady, C. L., Van Der Vaart, M., Venter, S. N., Telechea, N., Rolfo, M., Perez, C., & Wingfield, M. J. (2011). A new shoot and stem disease of *eucalyptus* species caused by *Erwinia psidii*. Australasian plant pathology, *40*, 55-60.

Da Silva, A. C., Silva, F. M. De O., Milagre, J. C., Omena-Garcia, R. P., Abreu, M. C., Mafia, R. G., Nunes-Nesi, A., & Alfenas, A. C. (2018). Eucalypt plants are physiologically and metabolically affected by infection with *Ceratocystis fimbriata*. Plant physiology and biochemistry, *123*, 170-179.

Fernandes, B. V., Zanuncio, A. J. V., Furtado, E. L., & Andrade, H. B. (2014). Damage and loss due to *Ceratocystis fimbriata* in *Eucalyptus* wood for charcoal production. Bioresources, *9*(3), 5473-5479.

Ferreira, F. A., Maffia, L. A., Barreto, R. W., Demuner, N. L., & Pigatto, S. (2006). Sintomatologia da murcha de *Ceratocystis fimbriata* em eucalipto. Revista Árvore, *30*, 155-162.

Ferreira, E. M., Harrington, T. C., Thorpe, D. J., & Alfenas, A. C. (2010). Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis fimbriata* in Brazil. Plant pathology, *59*, 721-735.

Ferreira, M. A., Harrington, T. C., Alfenas, A. C., & Mizubuti, E. S. G. (2011). Movement of genotypes of *Ceratocystis fimbriata* within and among eucalyptus plantations in Brazil. Phytopathology, *101*, 1005-1012.

Ferreira, M. A., Harrington, T. C., Gongora-Canul, C. C., Mafia, R. G., Zauza, E. A. V. & Alfenas, A. C. (2013). Spatial–temporal patterns of ceratocystis wilt *in Eucalyptus* plantations in Brazil. Forest pathology, *43*, 153-164.

Ferreira, M. A., Mafia, R. G., & Alfenas, A. C. (2017). *Ralstonia solanacearum* decreases volumetric growth of trees and yield of kraft cellulose of *Eucalyptus* spp. Forest pathology, *48*(1).

Industria brasileira de árvores – Ibá. (2022). Relatório anual - 2022.

Gryzenhout, M., Rodas, C. A., Mena Portales, J., Clegg, P., Wingfield, B. D., & Wingfield, M. J. (2006). Novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* and a new *Chrysoporthe* species from Colombia. Mycological research, *110*, 833–845.

Liu, J., Feng, Z., Mannan, A., Khan, T. U., & Z. Cheng. (2019). Comparing Non-Destructive Methods to Estimate Volume of Three Tree Taxa in Beijing, China. Forests, *10*(2), 92.

Mafia, R. G., Ferreira, M. A., Zauza, E. A. V., Silva, J. F., Colodette, J. L., & Alfenas, A. C. (2013). Impact of ceratocystis wilt on eucalyptus tree growth and cellulose pulp yield. Forest pathology, *43*, 379-385.

Moller, W. J., & DeVay, J. E. (1968). Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. Phytopathology, 58, 123–124.

Nakabonge, G., Roux, J., Gryzenhout, M., & Wingfield, M. J. (2006). Distribution of *Chrysoporthe* canker pathogens on *Eucalyptus* and *Syzygium* spp. In Eastern and Southern Africa. Plant disease, *90*, 734-740.

Oliveira, L. S. S., Guimarães, L. M. S., Ferreira, M. A., Nunes, A. S., Pimenta, L. V. A., & Alfenas, A. C. (2015). Aggressiveness, cultural characteristics and genetic variation of *Ceratocystis fimbriata* on *Eucalyptus* spp. Forest pathology, *45*(6), 505-514.

Pérez, C. A., Wingfield, M. J., Slippers, B., Altier, N. A., & Blanchette, R. A. (2010). Endophytic and canker-associated Botryosphaeriaceae occurring on non-native *Eucalyptus* and native Myrtaceae trees in Uruguay. Fungal diversity, *41*, 53–69.

Rodas, C. A., Slippers, B., Gryzenhout, M., & Wingfield, M. J. (2009). Botryosphaeriaceae associated with *Eucalyptus* canker diseases in Colombia. Forest pathology, *39*(2), 110-123.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675.

Silva, A. C., Betancourth, B. M. L., Ferreira, D. C., Elerati, T. L., Rodrigues, F. Á., & Alfenas, A. C. (2020). Responses of resistant and susceptible hybrid clones of *Eucalyptus urophylla* × *Eucalyptus grandis* to infection by *Ceratocystis fimbriata*. Annals of forest science, *77*(45).

Souza, S. E., Sansigolo, C. A., Furtado, E. L., De Jesus, W. C., & Oliveira, R. R. (2010). Influence of the basal canker on *Eucalyptus grandis* wood properties and kraft pulping. Scientia Florestalis, *38*, 447-457.

Thorthwaite, C. W. (1948). An approach towards a rational classification of climate. Geographycal Review London, *38*, 55-94.

CONSIDERAÇÕES FINAIS

O atual trabalho melhora a compreensão dos danos relacionados à murcha de ceratocystis em eucalipto, assim como do agente causal no Brasil e a sintomatologia da doença. Segundo nosso resultados, árvores de eucalipto doentes devido a murcha de ceratocystis podem permanecer vivas até o final do ciclo (7 anos), sendo que a penetração do patógeno ocorreu via sistema radicular na área estudada. A murcha de ceratocystis impactou negativamente no crescimento de árvores de eucalipto doentes, contudo não foi observado impacto na polpação quando amostradas árvores inteiras de eucalipto doentes. Observamos também que fungos de diferentes gêneros podem estar associados aos mesmos sintomas caulinares em árvores de eucalipto, sendo que alguns podem estar diretamente ou indiretamente associados aos sintomas, enquanto outros podem ser endofíticos ou saprófitos.

REFERÊNCIAS

AGUSTINI, L. et al. Signs and identification of fungal root-rot pathogens in tropical *Eucalyptus pellita* plantations. **Forest Pathology**, v, 44, n. 6, p. 486-495, 2014.

ALFENAS, C.A. et al. **Clonagem e Doenças do Eucalipto**. 2. ed. Viçosa, MG: Editora da UFV, 2009.

AMORIM, L.; REZENDE, J.A.M.; BERGAMIN FILHO, A. **Manual de Fitopatologia:** Volume 1 - Princípios e Conceitos. 5. ed. São Paulo: Editora Agronômica Ceres Ltda, 2018.

ARAÚJO, J.C.P.; et al. Influência da madeira com fungo *Ceratocystis fimbriata* no processo de produção de celulose e qualidade da celulose. **O Papel**, v. 68, n. 12, p. 95-105, 2007.

ARRIEL, D.A.A. et al. Wilt and die-back of *Eucalyptus* spp. caused by *Erwinia psidii* in Brazil. **Forest Pathology**, v. 44, n. 4, p. 255-265, 2014.

AUER, C.G.; DOS SANTOS, A.F.; HALFELD-VIEIRA, B. de A. A podridão do cerne em árvores vivas no Brasil. **Tropical Plant Pathology**, v. 37, 2012.

AUER, C.G.; SANTOS, A.F.; FURTADO, E.L. Doenças do Eucalipto. *In*: AMORIM, L. et al. **Manual de Fitopatologia**: Volume 2 - Doença das plantas cultivadas. 5. ed. São Paulo: Editora Agronômica Ceres Ltda, 2016. cap. 37, p. 359-372.

AYLWARD, J. et al. *Teratosphaeria* stem canker of *Eucalyptus*: two pathogens, one devastating disease. **Molecular Plant Pathology**, v. 20, n. 1, p. 8-19, 2019.

BAKER, C.J. et al. Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata*. **Phytopathology**, v. 93, p. 1274-1284, 2003.

BARNES, I. et al. *Ceratocystis pirilliformis*, a new species from *Eucalyptus nit*ens in Australia. **Mycologia**, v. 95, n. 5, p. 865-871, 2003.

BARRADAS, C. et al. Impact of *Botryosphaeria*, *Diplodia* and *Neofusicoccum* species on two *Eucalyptus* species and a hybrid: From pathogenicity to physiological performance. **Forest Pathology**, v. 49, n. 2, 2019.

BIGGS, A.R. Anatomical and physiological responses of bark tissues to mechanical injury. *In*: BLANCHETTE, R.A.; BIGGS, A.R. **Defense mechanisms of woody plants against fungi**. Berlin: Springer Heidelberg, 1992. p. 13-40.

BLANCHETTE, R.A. A review of microbial deterioration found in archeological wood from different environments. **International Biodeterioration and Biodegradation**, v. 46, n. 3, p. 189-204, 2000.

CAIRES, N.P. et al. Host range of *Erwinia psidii* and genetic resistance of *Eucalyptus* and *Corymbia* species to this pathogen. **Forest Pathology**, v. 49, n. 4, 2019.

CARSTENSEN, G.D.; et al. Two *Ralstonia* species associated with bacterial wilt of *Eucalyptus*. **Plant Pathology**, v. 66, n. 3, p. 393-403, 2017.

CHEN, S.F. et al. Taxonomy and pathogenicity of *Ceratocystis* species on *Eucalyptus* trees in South China, including *C. chinaeucensis* sp. nov. **Fungal Diversity**, v, 58, p. 267-279, 2013.

CLÉRIVET, A. et al. Tyloses and gels associated with cellulose accumulation in vessels are responses of plane tree seedlings (*Platanus acerifo*lia) to the vascular fungus *Ceratocystis fimbriata* f. sp. *platani*. **Trees**, v. 15, p. 25-31, 2000.

COUTINHO, T.A. et al. A new shoot and stem disease of *Eucalyptus* species caused by *Erwinia psidii*. **Australasian Plant Pathology**, v. 40, p. 55-60, 2011.

COUTINHO, T.A.; WINGFIELD, M.J. *Ralstonia solanacearum* and *R. pseudosolanacearum* on *Eucalyptus*: Opportunists or Primary Pathogens? **Frontiers in Plant Science**, v. 8, 2017.

CROUS, P.W et al. Co-occurring species of *Teratosphaeria* on *Eucalyptus*. **Persoonia**, v. 22, p. 38-48, 2009.

DA FONSECA, S.M. et al. **Manual Prático de Melhoramento Genético do Eucalipto**. 1. ed. Viçosa, MG: Editora UFV, 2010.

DA SILVA, A.C. et al. Eucalypt plants are physiologically and metabolically affected by infection with *Ceratocystis fimbriata*. **Plant Physiology and Biochemistry**, v. 123, p. 170-179, 2018.

DA SILVA, A.C. et al. Responses of resistant and susceptible hybrid clones of *Eucalyptus urophylla* × *Eucalyptus grandis* to infection by *Ceratocystis fimbriata*. **Annals of Forest Science**, v. 77, 2020.

DA SILVEIRA, A.C.; LAZZAROTTO, M. Óleos essenciais de espécies de eucaliptos. *In*: OLIVEIRA, E. B. de; PINTO JUNIOR, J. E. **O eucalipto e a Embrapa: quatro décadas de pesquisa e desenvolvimento**. Colombo, PR: Embrapa Floresta, 2021. cap. 18, p. 723-750.

DAHALI, R. et al. Influence of *Chrysoporthe deuterocubensis* Canker Disease on the Physical and Mechanical Properties of *Eucalyptus urograndis*. **Forests**, v. 12, n. 5, 2021.

DE ALONSO, S.K. et al. Isolamento e seleção de fungos causadores da podridão branca da madeira em florestas de *Eucalyptus* spp. com potencial de degradação de cepas e raízes. **Revista Árvore**, v.31, n.1, p.145-155, 2007.

DE BEER, Z.W. et al. Redefining *Ceratocystis* and allied genera. **Studies in Mycology**, v. 79, p. 187-219, 2014.

DE OLIVEIRA, E.B. et al. Eucalipto para restauração florestal com renda para propriedades rurais familiares. *In*: OLIVEIRA, E. B. de; PINTO JUNIOR, J. E. **O eucalipto e a Embrapa: quatro décadas de pesquisa e desenvolvimento.** Colombo, PR: Embrapa Floresta, 2021. cap. 15, p. 667-684.

DHARMARAJ, K. et al. A New Real-Time PCR Assay for Detecting Fungi in Genus *Ceratocystis*. **Plant Disease**, v. 106, n. 2, p. 661-668, 2022.

DUCHESNE, L.C.; HUBBES, M.; JENG, R.S. Biochemistry and molecular biology of defense reactions in the xylem of angiosperm trees. *In*: BLANCHETTE, R.A.; BIGGS, A.R. **Defense mechanisms of woody plants against fungi**. Berlin: Springer Heidelberg. Berlin: Springer Heidelberg, 1992. p. 133-146.

ENGELBRECHT, C.J.; HARRINGTON, T.C. Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. **Mycologia**, v. 97, n. 1, p. 57-69, 2005.

FERNANDES, B.V. et al. Damage and Loss Due to *Ceratocystis fimbriata* in *Eucalyptus* Wood for Charcoal Production. **BioResources**, v. 9, n. 3, p. 5473-5479, 2014.

FERREIRA, E.M. et al. Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis fimbriata* in Brazil. **Plant Pathology**, v. 59, p. 721-735, 2010.

FERREIRA, E.M. et al. Spatial–temporal patterns of *Ceratocystis* wilt in *Eucalyptus* plantations in Brazil. **Forest Pathology**, v. 43, n. 2, 2013.

FERREIRA, F.A. et al. Sintomatologia da murcha de *Ceratocystis fimbriata* em eucalipto. **Revista Árvore**, v. 30, n. 2, p. 155-162, 2006.

FERREIRA, F.A.; MAFFIA, L.; FERREIRA, E. Rapid detection of *Ceratocystis fimbriata* in infected wood of eucalyptus, mango, and other woody hosts. **Fitopatologia Brasileira**, v. 30, n. 5, p. 543-545, 2005.

FERREIRA, F.A.; MILANI, D. **Diagnose Visual e Controle das Doenças Abióticas e Bióticas do Eucalipto no Brasil**. Viçosa, MG: Editora UFV, 2012.

FERREIRA, M.A. et al. Movement of Genotypes of *Ceratocystis fimbriata* Within and Among Eucalyptus Plantations in Brazil. **Phytopathology**, v. 101, n. 8, p. 1005-1012, 2011.

FERREIRA, M.A.; ALFENAS, A.C.; MAFIA, R.G. *Ceratocystis fimbriata* em espécies florestais e agronômicas no Brasil. *In:* DOS SANTOS, B.A. et al. **Patologia florestal:** desafios e perspectivas. 1. ed. São Carlos, SP: Suprema Gráfica e Editora, 2013. cap. 3. p. 75-95.

FIRMINO, A.C. et al. *Ceratocystis fimbriata* causando murcha em atemóia na região de Botucatu-SP. **Summa phytopathologica**, v. 38, n. 2, p. 1825, 2012.

FIRMINO, A.C. et al. Colonização do xilema de eucalipto por *Ceratocystis* spp. isolado de diferentes hospedeiros. **Summa Phytopathologica**, v. 41, n. 2, p. 138-143, 2015.

FIRMINO, A.C. et al. First report of *Ceratocystis fimbriata* causing fruit-rot of *Passiflora edulis* in Brazil. **New Disease Reports**, v. 27, n. 1, p. 4-4, 2013.

FIRMINO, A.C. et al. First report of *Ceratocystis fimbriata* causing wilt on *Khaya senegalensis*. **New Disease Reports**, v. 35, p. 35-35, 2017.

FIRMINO, A.C.; TOZZE JUNIOR, H.; FURTADO, E.L. First report of *Ceratocystis fimbriata* causing wilt in *Tectona grandis* in Brazil. **New Disease Reports**, v. 25, n. 1, 2012.

FLORES, T.B. *Eucalyptus* **no Brasil**: Zoneamento Climático e Guia para Identificação. Piracicaba, SP: IPEF, 2016.

FONSECA, N.R. et al. Molecular characterization of *Ralstonia solanacearum* infecting *Eucalyptus* spp. in Brazil. **Forest Pathology**, 2013.

FOURIE, A. et al. Molecular markers delimit cryptic species in *Ceratocystis* sensu stricto. **Mycological Progress**, v. 11, 2015.

FREITAS, R.G. Detection and characterization of *Ralstonia pseudosolanacearum* infecting *Eucalyptus* sp. in Brazil. **Forest Pathology**, v. 50, n. 3, 2020.

GRYZENHOUT, M. et al. *Chrysoporthe doradensis* sp. nov. pathogenic to *Eucalyptus* in Ecuador. **Fungal Diversity**, v. 20, p. 39–57, 2005.

HALFELD-VIEIRA, B. First record of *Ceratocystis fimbriata* on *Carapa guianensis.* **New Disease Reports**, v. 26, n. 1, p. 13, 2012.

HALSTED, B.D. Some fungous diseases of the sweet potato. **New Jersey Agricultural College Experiment Station Bulletin**, v. 76, p. 1-32, 1890.

HARRINGTON, T.C. et al. Intraspecific and intragenomic variability of ITS rDNA sequences reveals taxonomic problems in *Ceratocystis fimbriata* sensu stricto. **Mycologia**, v. 106, n. 2, p. 224-242, 2014.

HEATH, R.N. et al. *Ceratocystis* species on *Acacia mearnsii* and *Eucalyptus* spp. in eastern and southern Africa including six new species. **Fungal Diversity**, v. 34, p. 41-68, 2009.

HULCR, J.; STELINSKI, L.L. The Ambrosia Symbiosis: From Evolutionary Ecology to Practical Management. **Annual Review of Entomology**, v. 62, p. 285-303, 2016.

IBÁ - INDÚSTRIA BRASILEIRA DE ÁRVORES. **Indicadores de desempenho do setor nacional de árvores plantadas referentes ao ano de 2019**. Brasília: IBÁ, 2020.

IBÁ - INDÚSTRIA BRASILEIRA DE ÁRVORES. **Indicadores de desempenho do setor nacional de árvores plantadas referentes ao ano de 2020**. Brasília: IBÁ, 2021.

KAMGAN NKUEKAM G. et al. *Ceratocystis* and *Ophiostoma* species, including three new taxa, associated with wounds on native South African trees. **Fungal Diversity**, v. 29, p. 37-59, 2008.

KAMGAN NKUEKAM G. et al. *Ceratocystis* species, including two new species associated with nitidulid beetles on *Eucalyptus* in Australia. **Antonie van Leeuwenhoek**, v. 101, n. 2, p. 217-241, 2011.

KILE, G.A. et al. *Ceratocystis eucalypti* sp. nov., a vascular stain fungus from eucalypts in Australia. **Mycological Research**, v. 100, n. 5, p. 571-579, 1996.

LAIA, M. L.; ALFENAS, A.C.; HARRINGTON, T.C. Isolation, detection in soil, and inoculation of *Ceratocystis fimbriata*, causal agent of wilting, die-back and canker in *Eucalyptus*. **Fitopatologia Brasileira**, v. 25, p. 384-384, 2000.

LI, G.Q. et al. Botryosphaeriaceae from *Eucalyptus* plantations and adjacent plants in China. **Persoonia**, v. 40, p. 63-95, 2018.

LI, G.Q. et al. Variation in Botryosphaeriaceae from *Eucalyptus* plantations in YunNan Province in southwestern China across a climatic gradient. **IMA Fungus**, v. 11, n. 22, 2020.

LIU, F. et al. New *Ceratocystis* species from *Eucalyptus* and *Cunninghamia* in South China. **Antonie van Leeuwenhoek**, v. 107, p. 1451-1473, 2015.

LOPEZ-REAL, J.M. Formation of pseudosclerotia (‘zone lines’) in wood decayed by *Armillaria mellea* and *Stereum hirsutum*: I. Morphological aspects. **Transactions of the British Mycological Society**, v. 64, n. 3, p. 465-471, 1975.

MACPHAIL, M.; THORNHILL, A.H. How old are the eucalypts? A review of the microfossil and phylogenetic evidence. **Australian Journal of Botany**, v. 64, p. 579-599, 2016.

MAFIA, R.G. et al. Impact of Ceratocystis wilt on eucalyptus tree growth and cellulose pulp yield. **Forest Pathology**, v. 43, n. 5, p. 379-385, 2013.

MAIA, C.M.B.DE F. et al. Serviços ecossistêmicos e eucalipto. *In*: OLIVEIRA, E. B. de; PINTO JUNIOR, J. E. **O eucalipto e a Embrapa: quatro décadas de**

pesquisa e desenvolvimento. Colombo, PR: Embrapa Floresta, 2021. cap. 14, p. 611-666.

MARINCOWITZ, S. et al. Epitypification of *Ceratocystis fimbriata*. **Fungal Systematics and Evolution**, v. 6, n. 1, p. 289-298, 2020.

MARIN-FELIX, Y. et al. Genera of phytopathogenic fungi: GOPHY 1. **Studies in Mycology**, v. 86, p. 99-216, 2017.

MBENOUN, M. et al. Molecular phylogenetic analyses reveal three new *Ceratocystis* species and provide evidence for geographic differentiation of the genus in Africa. **Mycological Progress**, v. 13, p. 219-240, 2013.

MÉNDEZ-ÁLVAREZ, D. et al. First report of *Ceratocystis fimbriata* causing wilt on *Gmelina arborea* in Costa Rica. **Forest Pathology**, v. 50, n. 5, 2020.

MOLLER, W.J.; DEVAY, J.E. Carrot as a species-selective medium for *Ceratocystis fimbriata*. **Phytopathology**. v. 58, p. 123-124, 1968.

MORRIS, H. et al. The dark side of fungal competition and resource capture in wood: Zone line spalting from science to application. **Materials & Design**, v. 201, 2021.

MORRIS, M.J., WINGFIELD, M.J.; DE BEER, C. Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. **Plant Pathology**, v. 42, n. 5, p. 814-817, 1993.

OLIVEIRA, L.S.S. et al. Aggressiveness, cultural characteristics and genetic variation of *Ceratocystis fimbriata* on *Eucalyptus* spp. **Plant Pathology**, v. 45, n. 6, p. 505-514, 2015.

OLIVEIRA, L.S.S. et al. *Ceratocystis fimbriata* isolates on *Mangifera indica* have different levels of aggressiveness. **European Journal of Plant Pathology**, v. 145, p. 847-856, 2016.

PAUL, N.C. et al. Characterization and pathogenicity of sweet potato (*Ipomoea batatas*) black rot caused by *Ceratocystis fimbriata* in Korea. **European Journal of Plant Pathology**, v. 152, p. 833-840, 2018.

PAVLIC-ZUPANC, D. et al. Diversity, phylogeny and pathogenicity of Botryosphaeriaceae on non-native *Eucalyptus* grown in an urban environment: A case study. **Urban Forestry & Urban Greening**, v. 26, p. 139-148, 2017.

PILLAY, K. et al. Diversity and distribution of co-infecting Botryosphaeriaceae from *Eucalyptus grandis* and *Syzygium cordatum* in South Africa. **South African Journal of Botany**, v. 84, p. 38-43, 2013.

PIMENTA, L. et al. Physiological responses of *Eucalyptus* spp. hybrids to infection by *Ceratocystis fimbriata*. **Forest Pathology**, v. 47, n. 4, 2017.

PINTO JÚNIOR, J.E.; SILVEIRA, R.A. A introdução do eucalipto no Brasil pela Embrapa: bases institucionais e sua estruturação para a pesquisa com eucaliptos e corímbias. *In*: OLIVEIRA, E. B. de; PINTO JUNIOR, J. E. **O eucalipto e a Embrapa: quatro décadas de pesquisa e desenvolvimento**. Colombo, PR: Embrapa Floresta, 2021. cap. 1, p. 33-112.

PIVETA, G. et al. *Ceratocystis fimbriata* on Kiwifruit (*Actnidia* spp.) in Brazil. **New Zealand Journal of Crop and Horticultural Science**, v. 44, n. 1, p. 13-24, 2016.

RAUF, M.R.B.A. et al. Pathogenicity of *Chrysoporthe deuterocubensis* and *Myrtoporthe bodenii* gen. et sp. nov. on *Eucalyptus* in Sabah, Malaysia. **Australasian Plant Pathology**, v. 49, p. 53-64, 2020.

RODAS, C.A. et al. *Ceratocystis neglecta* sp. nov. infecting *Eucalyptus* trees in Colombia. **Fungal Diversity**, v. 28, p. 73-84, 2008.

ROSADO, C.C.G. et al. QTL mapping for resistance to *Ceratocystis* wilt in *Eucalyptus*. **Tree Genetics & Genomes**, v. 12, p. 62-72, 2016.

ROUX, J. et al. *Ceratocystis* species infecting stem wounds on *Eucalyptus grandis* in South Africa. **Plant Pathology**, v. 53, n. 4, p. 414-421, 2004.

SKORUPA, L.A.; BEHLING, M.; PORFIRIO-DA-SILVA, V. O eucalipto e os desafios para a transferência de tecnologias em sistemas de integração lavourapecuária-floresta (ILPF). *In*: OLIVEIRA, E. B. de; PINTO JUNIOR, J. E. **O eucalipto e a Embrapa: quatro décadas de pesquisa e desenvolvimento**. Colombo, PR: Embrapa Floresta, 2021. cap. 35, p. 1147-1160.

SOARES, T.P.F. et al. Canker disease caused by *Chrysoporthe doradensis* and *C. cubensis* on *Eucalyptus* sp. and *Tibouchina* spp. in Brazil. **Tropical Plant Pathology**, v. 43, p. 314-322, 2018.

THORNHILL, A.H. et al. A dated molecular perspective of eucalypt taxonomy, evolution and diversification. **Australian Systematic Botany**, v. 32, p. 29-48, 2019.

TUMURA, K.G.; PIERE, C.D.; FURTADO, E.L. Murcha por *Ceratocystis* em eucalipto: avaliação de resistência e análise epidemiológica. **Summa Phytopathologica**, v. 38, n. 1, p. 54-60, 2012.

UMAMI, M.; PARKER, L.; ARNDT, S. The impacts of drought stress and *Phytophthora cinnamomi* infection on short-term water relations in two-year-old *Eucalyptus obliqua*. **Forests**, v. 12, n. 2, p. 1-14, 2021.

VALDETARO, D.C.O.F. et al. host specialized form of Ceratocystis fimbriata causes seed and seedling blight on native *Carapa guianensis* (andiroba) in Amazonian rainforests. **Fungal Biology**, v. 123, p. 170-182, 2019.

VAN WYK, M. et al. *Ceratocystis atrox* sp. nov. associated with *Phoracantha acanthocera* infestations on *Eucalyptus grandis* in Australia. **Australasian Plant Pathology**, v. 36, p. 407-414, 2007.

VAN WYK, M. et al. *Ceratocystis eucalypticola* sp. nov. from *Eucalyptus* in South Africa and comparison to global isolates from this tree. **IMA Fungus**, v. 3, p. 45-58, 2012.

WINGFIELD, B, et al. *Ceratocystis*: emerging evidence for discrete generic boundaries. **CBS Biodiversity Series**, v. 12, p. 57-64, 2013.

WOLFF, L.F.; SCHNELL, G.; SCHUHLI. Eucaliptos e abelhas. *In*: OLIVEIRA, E. B. de; PINTO JUNIOR, J. E. **O eucalipto e a Embrapa: quatro décadas de pesquisa e desenvolvimento**. Colombo, PR: Embrapa Floresta, 2021. cap. 16, p. 685-701.

ZAUZA, E.A.V. et al. Resistance of *Eucalyptus* clones to *Ceratocystis fimbriata*. **Plant Disease**: Saint Paul, v. 88, n. 98, p. 758-760, 2004.