

UNIVERSIDADE FEDERAL DE VIÇOSA

**Etiology of the stem canker-disease of *Coffea canephora*: an emerging disease
in the states of Espírito Santo and Bahia (Brazil)**

Marina Faria Bracale
Doctor Scientiae

**VIÇOSA - MINAS GERAIS
2023**

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Thesis submitted to the Plant Pathology
Graduate Program of the Universidade
Federal de Viçosa in partial fulfillment of
the requirements for the degree of *Doctor
Scientiae*.

Adviser: Robert Weingart Barreto

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2023**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa**

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B796e
2023
Bracale, Marina Faria, 1993-
Etiology of the stem canker-disease of *Coffea canephora*:
an emerging disease in the states of Espírito Santo and Bahia
(Brazil) / Marina Faria Bracale. – Viçosa, MG, 2023.
1 tese eletrônica (115 f.): il. (algumas color.).

Inclui apêndices.

Orientador: Robert Weingart Barreto.

Tese (doutorado) - Universidade Federal de Viçosa,
Departamento de Fitopatologia, 2023.

Inclui bibliografia.

DOI: <https://doi.org/10.47328/ufvbbt.2025.559>

Modo de acesso: World Wide Web.

1. Café - Espírito Santo - Doenças e pragas. 2. Café - Bahia
- Doenças e pragas. 3. Cancro. 4. Genômica. I. Barreto, Robert
Weingart, 1955-. II. Universidade Federal de Viçosa.
Departamento de Fitopatologia. Programa de Pós-Graduação em
Fitopatologia. III. Título.

CDD 22. ed. 633.739

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APPROVED: December 21, 2023.

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To those who stayed with me. And to myself.

ACKNOWLEDGMENTS

To all the gods and goddesses who blessed and protected me during this journey.

To my family, for all the prayers, kind thoughts, listening ears, and open hearts.

To my friends, who were my foundation despite the distance, without whom I could never have endured.

To the special people I met during my Ph.D., who made everything more enjoyable.

To Jonas, for being my *sic mundus*.

To my advisor, Robert Barreto, for introducing me to the incredible universe of fungi and for the opportunities that shaped my path as a researcher.

To my co-advisor, Eduardo Mizubuti, for stepping in and providing help when it was most needed.

To Priscila Chaverri, for her invaluable help with this study and for giving me a broader perspective.

To the colleagues and friends I made at the *Clínica de Doenças de Plantas*, for all the knowledge shared, coffee breaks, and laughter.

To Débora Guterres, for being a true partner in crime — I could not have done this without you. Or without the beers, wine, and all our attempts at being healthy.

To José Mardgan Neto, for being not only an excellent student but also an even better friend.

To Mariela González and Efraín Escudero, for their patience with my bioinformatics struggles and for their kindness.

To the *Universidade Federal de Viçosa* (UFV), for the opportunity to complete this graduate program.

To the *Programa de Pós-Graduação em Fitopatologia* – UFV, especially to all the professors and colleagues who accompanied me through this process, for their advice, guidance, and friendship.

To the *Laboratório de Melhoramento de Plantas – Universidade Federal do Espírito Santo* (UFES), for their assistance.

This work has been sponsored by the following Brazilian research agencies: Coordination for the Improvement of Higher Education Personnel (CAPES; Financing code 001), Minas Gerais State Foundation for Research

Aid (FAPEMIG) and National Council of Scientific and Technological Development (CNPq). In addition, this study was partly funded by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Café) and by the US National Science Foundation (NSF, DEB-1638976).

*"Speak the truth, and all nature and all spirits help you with unexpected furtherance.
Speak the truth, and all things alive or brute are vouchers, and the very roots of the
grass underground there do seem to stir and move to bear you witness."*

Ralph Waldo Emerson

ABSTRACT

BRACALE, Marina Faria, D.Sc., Universidade Federal de Viçosa, December, 2023. **Etiology of the stem canker-disease of *Coffea canephora*: an emerging disease in the states of Espírito Santo and Bahia (Brazil).** Adviser: Robert Weingart Barreto.

Most diseases occurring on coffee in Brazil have been well investigated and their etiology have been fully clarified. A notable exception is that of an emerging disease that is now affecting large areas planted with *Coffea canephora* (conilon) in the states of Espírito Santo (ES) and Bahia (BA), Brazil. Losses of large stands of adult productive plants are common. The disease symptoms resemble those associated with bark disease – a common, but poorly investigated disease of *C. arabica* in Africa. Since this disease first appeared in the early 2010s, the search for a pathogen behind it was initiated. The results of the few published studies on the matter are somewhat controversial and even the choice of a common name for the disease was pending, until recently. It has been named (Portuguese name) “cancro dos ramos do cafeeiro – (*C. canephora*)” or stem canker-disease of conilon (SCC). This is regarded as the worst threat to the conilon plantations in ES and BA, a crop of major relevance for the economy of both states and Brazil too – the second world producer of conilon coffee, behind Vietnam. Many of the elite, highly productive, conilon clones are susceptible. When plants are affected by the disease, particular positions of stems or branches present some intumescence and flaking of the bark. These areas then develop cracks and finally burst to expose the heartwood. In parallel shortening of internodes and branch proliferation together with yellowing of the foliage and complete necrosis, occurs above those damaged areas, similar to the African bark disease of *C. arabica*. One additional disease symptom appearing since early stages is seen on the leaves of affected portions of the plants. Petioles become twisted and, consequently, the leaves become inverted with the adaxial side facing down and the abaxial becoming upward oriented. Disease progress can be fast and entire rows of susceptible clones are killed leaving entire rows empty in the plantations. What was once thought to be just a localized minor problem for conilon plantations became a widespread problem for this crop in ES and BA. There are two publications that may be regarded as having resolved the issue of the etiology of SCC. These involve: a) a complex of *Fusarium* spp. and b) *Lasiodiplodia iranensis*. Nevertheless, the symptomatology of the two cases differs from that of SCC. The former is described as a wilt and the latter is a dieback disease, unlike SCC. In the present study, a detailed description of the SCC

is provided, along with results from a metagenomic study aimed at providing an alternative approach to pinpointing an unidentified and elusive pathogen behind SCC.

Keywords: coffee; disease; metagenomic

RESUMO

BRACALE, Marina Faria, D.Sc., Universidade Federal de Viçosa, dezembro de 2023. **Etiologia do cancro dos ramos de *Coffea canephora*: uma doença emergente nos estados do Espírito Santo e Bahia (Brasil)**. Orientador: Robert Weingart Barreto.

A maioria das doenças que ocorrem no café no Brasil foram bem investigadas e sua etiologia foi totalmente esclarecida. Uma exceção notável é a de uma doença emergente que atualmente afeta grandes áreas plantadas com *Coffea canephora* (conilon) nos estados do Espírito Santo (ES) e Bahia (BA), Brasil. Perdas de grandes fileiras de plantas adultas produtivas são comuns. Os sintomas da doença assemelham-se claramente aos associados à “bark disease” – uma doença comum, mas pouco investigada, de *C. arabica*. Desde que a doença apareceu pela primeira vez, no início de 2010, a busca por um patógeno foi iniciada. Os resultados dos poucos estudos publicados sobre o assunto são um tanto controversos e até a escolha de um nome comum para a doença estava pendente, até recentemente. Foi denominada “cancro dos ramos do cafeeiro – (*C. canephora*)”. Essa está sendo considerada a pior ameaça às plantações de conilon no ES e BA, cultura de grande relevância para a economia desses dois estados e para o Brasil – segundo produtor mundial de café conilon, atrás apenas do Vietnã. Muitos dos clones elites e altamente produtivos de conilon são suscetíveis. Quando as plantas apresentam a doença, determinadas posições dos caules ou ramos mostram alguma intumescência e descamação da casca. Essas áreas então desenvolvem rachaduras e finalmente explodem para expor o cerne. Paralelamente, o encurtamento dos entrenós e a proliferação dos ramos, juntamente com o amarelecimento da folhagem e a necrose completa, ocorrem acima dessas áreas danificadas, à semelhança da bark disease de *C. arabica*. Um sintoma adicional dessa doença, que aparece desde os estágios iniciais, é observado nas folhas das partes afetadas das plantas. Os pecíolos tornam-se retorcidos e, conseqüentemente, as folhas ficam invertidas com o lado adaxial voltado para baixo e o abaxial orientado para cima. O progresso da doença pode ser rápido e fileiras inteiras de clones suscetíveis são mortas, deixando fileiras inteiras vazias nas plantações. O que antes era considerado apenas um pequeno problema localizado para as plantações de conilon tornou-se um problema generalizado para essa cultura no ES e BA. Existem duas publicações que podem ser consideradas como tendo resolvido a questão da etiologia de CRC. Essas envolvem: a) um complexo de *Fusarium* spp. e b) *Lasiodiplodia iranensis*. No entanto, a sintomatologia nos dois casos é diferente daquela

do CRC. A primeira é descrita como murcha e a última é uma doença que causa morte, diferentemente de CRC. No presente estudo, é fornecida uma descrição detalhada de CRC, juntamente com os resultados de um estudo metagenômico que visa fornecer uma abordagem alternativa para identificar o patógeno associado a CRC.

Palavras-chave: café; doença; metagenômica

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GENERAL INTRODUCTION

Coffee is one of the most important agricultural commodities in the world. It has immense economic relevance and generates gross revenues worth billions of dollars annually (USDA, 2023). It has great social importance as a major source of employment in coffee producing countries, and is a highly popular beverage, being one of the most consumed drinks worldwide. Grown in more than 56 countries, coffee is cultivated throughout the tropical and subtropical areas of the globe. More than 10 million tons of coffee are produced each year, with a total revenue of around US\$ 200 billion (ICO, 2021). Brazil is the world's largest producer and exporter of coffee and the second largest consumer of the product: there are 2.26 million hectares occupied with its cultivation, distributed in 15 of the 27 Brazilian states (ICO, 2021; CONAB, 2023).

Among the 124 species of *Coffea* known to science, only two are economically relevant: *Coffea arabica* (arabica coffee) grown in highland situations and *C. canephora* (conilon and robusta coffee) grown in lowland areas. It is estimated that the global production of grains of both coffee species amounts to approximately 172.8 million bags in the 2022/23 crop season (USDA, 2023). According to a recent survey (CONAB, 2023), Brazilian coffee production is estimated to reach 52.4 million bags (32.4 million bags for arabica and 18.0 million for robusta and conilon).

Conilon coffee was first introduced in Brazil in Espírito Santo state (ES) in 1912, but commercially important areas were established 60 years after the introduction of the crop, in 1972. Conilon production is expected to reach 17 million bags in the 2023 coffee season. The northern region of the state of Espírito Santo is the main production region in Brazil. Presently, the largest Brazilian conilon producers are the states of ES, Rondônia (RO) and Bahia (BA), with 65.4%, 16.7% and 13.6%, respectively. The production of 11.460 million bags (60 kg-bag), together with the average productivity of 43.8 bags per hectare puts ES in an outstanding position in the Brazilian and international performance for conilon production (CONAB, 2023). As an obvious unfolding of this fact, conilon coffee is of major importance for the economy of ES.

As for any crop, diseases are also a limiting factor for conilon production. Among the main fungal diseases of conilon are coffee leaf rust (*Hemileia vastatrix*), brown eye spot or coffee leaf spot (*Cercospora coffeicola*), anthracnose (*Colletotrichum* spp.), target leaf spot (*Corynespora cassiicola*) and Phoma leaf spot (*Boeremia coffeae*) (Ferrão *et al.*, 2017, p. 458). Two exotic fungal diseases remain of major quarantine

significance for Brazilian coffee: coffee berry disease (CBD) and coffee wilt disease (tracheomyces). Both are endemic and restricted to Africa. The former is caused by *Colletotrichum kahawae* and only affects *C. arabica*. The latter is caused by *Fusarium xylarioides* and affects arabica, canephora and some wild coffee species (Rutherford, 2006; Phiri and Baker, 2009).

Since the early 2010s, a severe disease of unknown etiology emerged in ES and southern BA leading to major losses in plantations with adult plants. The lack of knowledge of a reliable etiological agent for this important disease hampers the advance towards the recommendation of effective measures for its management. Herein an attempt was made to produce a detailed description of the disease and to elucidate its etiology using traditional tools and a metagenomic approach.

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CHAPTER I

**Overview of the stem canker-disease of conilon coffee and the quest for an
etiologiical elucidation**

ABSTRACT

Brazil, the world's largest producer and second-largest consumer of coffee, is facing a significant threat to its conilon plantations. A novel, devastating disease – stem canker-disease of conilon (SCC) is expanding its range and killing large numbers of adult plants. This disease is affecting highly productive elite coffee clones, resulting in substantial yield losses, and posing a serious threat to the sustainability of the conilon industry in Espírito Santo and Bahia states. The cause of SCC remains unknown, impeding the development of effective control strategies. However, through reasoning based on the diagnostic routine where putative causes are progressively eliminated, microbial involvement is the likely cause. Management practices are uniform for healthy and affected plants as are the environmental conditions, no pest attack is seen connected with the abnormalities and there is field evidence of disease transmission between neighboring susceptible plants. In March 2021, a survey was conducted across six geographically diverse locations in Espírito Santo. The survey involved the examination and sampling of diseased plants in selected areas of six farms, resulting in a total of 162 samples. The study confirmed that SCC is characterized by the development of cankers in the main stem and side branches of conilon coffee plants, with symptoms observable even in young plants. As the plant matures, internodes shorten, branching becomes more prominent, and bark begins to flake. In parallel petioles of leaves of diseased parts of the plants become twisted and leaves are turned upside down. Over time, the vigor of affected plants declines, leading to the death of both orthotropic and plagiotropic branches. Approximately 500 fungal isolates were obtained from the conilon samples, with the most commonly identified genera of fungi being *Colletotrichum* spp., *Fusarium* spp., *Pestalotiopsis* spp., and *Diaporthe* spp. PCR amplification of *tef* and *rpb2* was performed to explore the claim that *Fusarium* spp. could be associated with SCC. It resulted in 82 isolates belonging to *Fusarium* species complexes: *F. fujikuroi* (FFSC), *F. oxysporum* (FOSC), *F. incarnatum-equiseti* (FIESC), *F. lateritium* (FLSC), *F. solani* (FSSC), and *Albonectria*. The second claim of a possible causal agent behind SCC was *Lasiodiplodia iranensis*, and after tests involving a repetition of inoculation of healthy conilon plants with one isolate of *L. iranensis* cited in the publication on the matter, following the procedures described by the authors, no disease symptoms were produced. This indicates that further investigation is needed to understand the association between *L. iranensis* and *C. canephora*. This study underscores the urgent need for expanding the research on the etiology of SCC, with novel approaches to resolve the SCC enigma.

Keywords: *Coffea canephora*, Disease etiology, *Fusarium*, *Lasiodiplodia*.

1. INTRODUCTION

Coffee (*Coffea arabica* or arabica coffee and *Coffea canephora* or conilon and robusta coffee) is grown in more than 50 countries and holds great economic importance globally. With an annual production of more than 10 million tons and generating revenue of approximately US\$200 billion, it is considered one of the top agricultural commodities worldwide. Brazil is the largest coffee producer in the world and ranks second in terms of coffee consumption. There are around 2.26 million hectares cultivated with coffee in Brazil (ICO, 2021; CONAB, 2023). Pests and diseases have always been a major obstacle for coffee production but *C. canephora* – although producing a lower quality beverage has always been acknowledged to be more resistant to biotic stress and more productive than *C. arabica*.

In Brazil, the states of Espírito Santo (ES) and Bahia (BA) are the main conilon producers. The ES state accounts for 68% of Brazil's conilon coffee production (CONAB, 2023; USDA, 2023). Conilon is widely cultivated in ES; there are records of coffee plantations in 50 of the 78 municipalities making it a large industry. The state of BA accounts for 12.8% of the national conilon production (Figure 1, supplementary material, table S1) (CONAB, 2023). Despite the numbers, conilon plantations in ES and southern BA have been affected by a novel disease that is rapidly expanding and threatening the crop. Major yield losses have been recorded, but in addition to reducing the harvest of coffee berries, the unknown disease is also leading to extensive death of adult productive plants. Economic losses are caused by the loss of plants in full production (particularly of high-yielding clones), the reduction in the amount of coffee berries in diseased plants and the elevated costs of replanting fields lost to the disease.

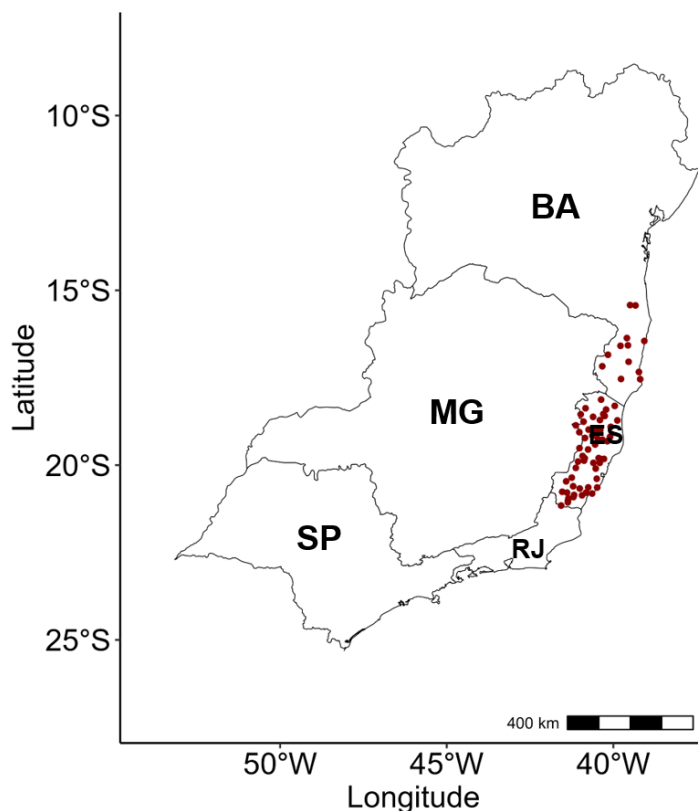


Figure 1. Municipalities engaged in conilon cultivation in the states of Espírito Santo and Bahia (Brazil).

Recent reports indicate that the spread of this novel disease has extended to the southern region of BA as well. The northern areas of ES are experiencing the worst scenario with broad losses of plant stands. The disease affects the most productive conilon clones which are informally named LB1, K61, MP3, 12V, and Verdím. There has been a significant decrease in conilon yield and reduction in plant stands because of the broad incidence of this disease on those elite coffee clones (Lima *et al.*, 2023).

The disease, referred to as stem canker-disease of conilon (SCC) (in Portuguese – cancro dos ramos do cafeeiro) is characterized by the flaking of regions of orthotropic stems, the formation of canker lesions on branches and stem, shortening of internodes, and yellowing and wilting of leaves and reduced coffee production. These symptoms ultimately lead to branch death which invariably progresses towards the lower parts of the stems leading to plant death (Figure 2). SCC remains a mysterious disease and, as with all other initially unknown pathological problems, there

is an ongoing controversy about its etiology. Regardless of the actual etiological agent of SCC, it deserves to be recognized as a major threat to the future and sustainability of the conilon industry in ES (Lima *et al.*, 2023).



Figure 2. Collapsed *Coffea canephora* individual showing advanced symptoms of SCC (note individual on the right showing early symptoms of the disease).

Coincidentally with the expansion of the disease in ES and BA, two publications have appeared in the literature reporting diseases of conilon in ES. The first proposed that a combination of species of *Fusarium* would be involved in a “wilt” namely: *F. decemcellulare*, *F. lateritium* and *F. solani* (Belan *et al.*, 2018). The second claims that *Lasiodiplodia iranensis* is behind a dieback disease of conilon (Ramos *et al.*, 2023). However, given the uncertainties regarding the symptomatology described in both articles, it is difficult to ascertain whether those publications were dealing with SCC. The finding of *Fusarium* spp. associated with coffee wilt generated worries that a novel

disease equivalent to the African tracheomycosis would have appeared in coffee plantations in Brazil.

Coffee Wilt Disease (CWD), also referred to as 'Fusarium wilt' or 'tracheomycosis', impacts arabica and canephora, along with wild coffee species. It is a vascular wilting disease caused by *F. xylarioides*. Rutherford (2006) reported CWD as a serious problem in eastern Africa, causing considerable damage to *C. canephora* in Uganda and the Democratic Republic of Congo. It also affected *C. canephora* in the Kagera region, northwest Tanzania. After almost twenty years since the last major outbreaks, CWD has disappeared silently of the radar (Peck and Boa, 2023). The early signs of CWD include yellowing, folding, and inward curling of the leaves. The leaves become limp, dry up, turn brown, and eventually fall off. Affected branches may become black-brown or blackish and dry up as well. These symptoms begin on one side of the tree but quickly spread to the entire tree. Infected trees also exhibit premature red berries that appear to ripen early due to water shortage caused by blocked xylem vessels from fungal invasion. Swollen bark with vertical or spiral cracks near the base of the trunk is also characteristic in affected trees along with blue-black discoloration directly beneath it.

Nevertheless, fortunately, after six years of Belan's publication, no evidence of a serious wilt outbreak has emerged in ES.

SCC has a far greater similarity to another endemic disease of coffee in Africa, namely coffee bark disease (BD). This common, but poorly investigated coffee disease, seems to be specific to *C. arabica* and is said to be caused by *Fusarium stilboides*. Nevertheless, this requires confirmation as the disease has been studied in the pre-molecular area and well before the large and still ongoing (and controversial) reappraisal of *Fusarium*. Bark disease has a confusing background. Holliday (1980) summarized different forms that the symptoms of the disease appear: on suckers, main stem and at or at least near the soil level. On suckers, sunken lesions form and a constriction on the stem appears, leading to stem girdling, leaves wilt. On the main stem, sunken and cankerous lesions are formed; bark becomes rough and death of the parts of the plant above the lesion may occur. At the collar level, there is a distinct constriction and eventual death of the entire plant with a purplish discoloration in the wood. Overall, bark disease involves the formation of lesions at specific points of the trunk and branches that start with the flaking of the external parts of the bark which fall off and lead to the formation of cankers, the loss of the cambium and exposure of the

heartwood and girdling of stem and trunk and progressive decline of plants (Siddiqi and Corbett, 1963; Rutherford, 2006).

Although Belan *et al.* (2018) did not claim that the disease they reported was the same as SCC, Ramos *et al.* (2023) claimed to have found the actual cause of SCC. The affirmation by Ramos *et al.* (2023) that *Lasiodiplodia iranensis* is the etiological agent of SCC was regarded here as requiring confirmation because no mention was made of the typical epidermal flaking and burst of the bark which are diagnostics for the disease. Instead, the fungus was reported as being present on tissues of stems that became necrotic in dieback episodes in the field.

Studies have shown that members of the genus *Lasiodiplodia* can infect plants through various means, including buds, lenticels, stomata, and wounds. Intriguingly, these infections may remain dormant within plant tissues before visible signs of damage such as canker and dieback arise (Sulaiman *et al.*, 2012; Maid *et al.*, 2018; Berraf-Tebbal *et al.*, 2020). This phenomenon has been observed in cacao plants and strawberries affected by *L. theobromae* (Yildiz *et al.*, 2014; Mehl *et al.*, 2017; Huda-Shakirah *et al.*, 2022). Furthermore, *Lasiodiplodia* infects a wide variety of hosts and has been correlated with stressing environmental conditions for cultivated plants such as extreme temperature and low humidity levels, which are prevalent in the coffee-growing regions of ES.

Finding the true cause of SCC is a puzzle in need to be resolved. The lack of information regarding its causal agent is a serious limiting factor that prevents delineating efficient strategies for controlling or mitigating the disease. The logical and routine process of diagnosis where “putative causes are progressively eliminated by a logical deductive process” as described by Waller *et al.* (1998) was followed and described herein in attempting to resolve the SCC puzzle.

2. MATERIAL AND METHODS

To perform the standard diagnosis exercise where “putative causes are progressively eliminated by a logical deductive process” as described by Waller *et al.* (1998), the following aspects were covered as described below.

2.1 Gathering supporting evidence for diagnosis in the field

Ancillary information on environmental conditions at the region of occurrence of SCC (climate and soil type), crop history, crop management and plant material (type of clones resistant vs. susceptible, plant age and source) were already available for each of the farms selected for inclusion in the work (data not provided here).

2.2 Observation and recording of symptoms and signs

A survey was conducted in six selected areas in ES. The selection of these locations aimed to cover a range of geographic regions within the area of occurrence of SCC in the state. The survey took place in March 2021 under the guidance of a plant pathologist from Incaper (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural), Dr. Inorbert de Melo Lima, who has been following the progress of disease and its distribution for several years. Selected areas of six farms were visited for disease examination, symptom recordings and sample collections.

The survey included conilon farms in the municipalities of Linhares, Aracruz, Jaguaré, and São Mateus (Table 1, Figure 3). To ensure an accurate comparison, only areas planted with the susceptible LB1 conilon clone were considered in this study. Clone A1 was included as a control group since it was previously thought to be resistant to the alleged disease due to no observed symptoms at that time.

Table 1. Sampling and observation localities for stem canker of conilon (SCC) in the state of Espírito Santo (Brazil).

Farms (identified by farmer)	Municipality (ES)	Latitude	Longitude
Edmar Tessarolo	Aracruz	-19,665922	-40,301998
Eduardo Zuccolotto	Aracruz	-19,633684	-40,241808
Renato Bianchini	Linhares	-19,562121	-40,074033
Eduardo Bortolini	Linhares	-19,409833	-40,242174
Thiago Gama	São Mateus	-18,592169	-40,100599
Ivan Catelan	Jaguaré	-18,9328	-39,9387

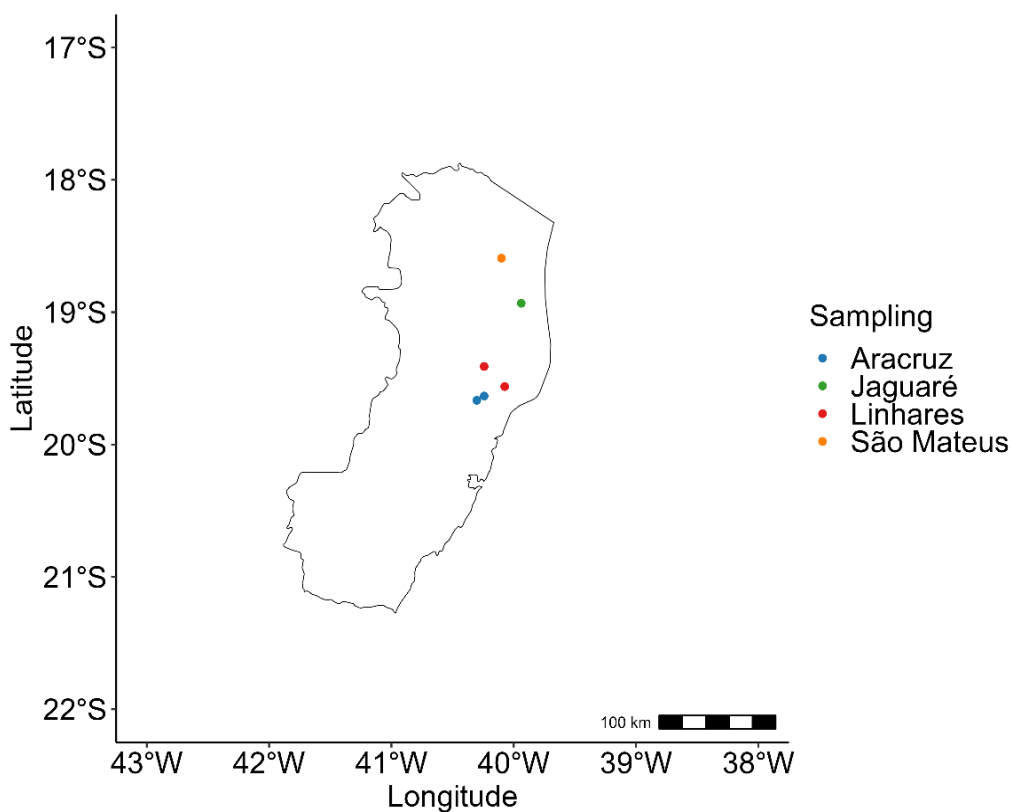


Figure 3. Locality of *Coffea canephora* sampling in Espírito Santo, Brazil.

2.3 Sample collection

Leaves, stems, and roots of conilon clones A1, considered to be resistant to SCC, and LB1, which is a high-yield elite clone, but highly susceptible to SCC were sampled. Different categories of plant source were defined, namely: 1) A1 (resistant); 2) LB1 (susceptible) asymptomatic and 3) LB1 symptomatic. Different plant parts were taken from those three sources, namely: a) leaves, b) stems and c) roots. We collected three plants per plant source and three samples of each organ from each plant. A total of 162 samples were collected.

A random set of leaves from each plant was collected such as to ensure representation from the upper, middle, and lower sections of the plants. Moreover, stems were cut from the base of each of the plant to the beginning of the internodes with lengths ranging between 50 to 70 cm. Finally, root samples were obtained by carefully digging around each plant and gently removing its secondary roots. All samples were placed in sterilized plastic bags and labeled. The samples were transported to the laboratory and stored in a refrigerator (~ 4 °C) until processing. A set of tools including sterilized scissors, a shovel, gloves, and a handsaw were used to ensure proper collection of samples without contamination.

2.4 Examination of samples and isolation of fungi

2.4.1 Examination of samples

Upon arrival in the laboratory, (Clínica de Doenças de Plantas at the Departamento de Fitopatologia at Universidade Federal de Viçosa) the samples were initially examined using a stereomicroscope Motic SMZ-140 and scanned for the observation of the possible regular association of pests or signs of a pathogen in connection with the diseased tissues. Any potential pathogen structure was mounted in microscope slides on lactoglycerol and lactofuchsin and observed under a light microscope (Olympus BX 51) equipped with differential interference contrast. Following that visual inspection, the samples went through a standard disinfestation process as described below. Additionally, some attempts at observation bacterial oozing from symptom-bearing plant tissues, as described in the literature, were also occasionally performed.

2.4.2 Isolation of endophytic fungi

Fungal endophyte isolates were obtained from selected fragments of living leaves, sapwood tissue (vascular cambium and phloem), and rootlets.

- **Leaf samples:** The leaves underwent a thorough washing with distilled water followed by a disinfestation process consisting of 1 min in ethanol (70%), 2 min in sodium hypochlorite (1%), and three rinses with sterilized water. After drying on sterile blotter paper, 0.5 cm-diameter leaf disks were cut and transferred onto plates containing potato-dextrose-agar medium (PDA) or Nash and Snyder medium (NS) (Nash and Snyder, 1962). Six leaf disks were placed in each Petri dish, and two dishes per medium were used.
- **Root samples:** Roots underwent the same cleaning and disinfestation process, and fragments approximately 1 cm long were taken from the rootlets and plated in the same media. Six fragments were placed in each Petri dish, and two dishes per medium were used.
- **Stem samples:** For stem samples, an inner portion of approximately 10 cm in length was utilized after discarding the edges and bark with a sterilized knife and handsaw. The stems were vertically cut to expose internal tissue with a sterile blade, and fragments measuring 1-2 cm from the epidermis to the cortex (excluding heartwood) were taken with a sterile scalpel and forceps and plated onto NS and PDA as described above.

All plates were kept in an incubator at 25 °C under a light regime of 12 h light/12 h dark. Each plate was visually inspected daily for the emergence of fungal colonies. The morphology and pattern of fungal growth were used as selection criteria to detect and transfer potentially pure colonies onto other plates. Small blocks were taken from the margin of such selected colonies with a sterile needle and transferred to fresh PDA plates. To ensure long-term storage viability, isolates were preserved either in tubes containing potato carrot-agar (PCA) in a refrigerator (~ 4 °C) and regularly transferred onto new tubes or in cryovials containing preserving glycerol solution (15%) in an ultrafreezer (-80 °C) (Alfenas; Mafia, 2016).

2.4.3 *Fusarium* spp. from conilon

Special attention was given to any isolates recognized as belonging to *Fusarium*. Such a priority was given because of both the earlier published possible indications (Belan *et al.*, 2018) of the involvement of *Fusarium* spp. in a conilon disease in ES and the recognized similarities of SCC symptoms and the *Fusarium*-induced African bark disease.

2.5 DNA extraction and PCR amplification

Among the 198 isolates obtained from stem samples, 82 were provisionally considered to be *Fusarium*-like and subjected to preliminary molecular analysis. Pure cultures of each representative isolate were obtained by excising the hyphal tips of colonies formed on the medium and transferring these to fresh plates. Later, these isolates were grown for four days in PDA medium, and the mycelia mass was dried in sterilized paper.

Total genomic DNA was extracted by using Wizard[®] Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions. The extracted DNA was used for polymerase chain reaction (PCR) to amplify the translation elongation factor 1-alpha (*tef*) with EF1 (5'-ATGGGTAAGGARGACAAGAC-3') and EF2 (5'-GGARGTACCAGTSATCATGTT-3') primers (O'Donnell *et al.*, 1998). This region is often treated as the "barcode-like region" for *Fusarium* spp. and is key for the study of most '*Fusarium* species' groups (O'Donnell *et al.*, 2015; Boutigny *et al.*, 2019). Additionally, primers 5F2 (5'-GGGGWGAYCAGAAGAAGGC-3') and 7cR (5'-CCCATRGCT TGYTTR CCCAT-3') primers were used to amplify a portion of the RNA polymerase II 2nd largest subunit (*rpb2*) (Sung *et al.*, 2007, Liu *et al.*, 1999). PCR was performed with 12.5 µl of Dream Taq TM PCR Master Mix 2× (MBI Fermentas, Vilnius, Lithuania); 1 µl of 10 µM each forward and reverse primer; 1 µl of dimethyl sulfoxide; 5 µl of 100× (10 mg/ml) bovine serum albumin; 2 µl of genomic DNA (50 ng/µl) and 2.5 µl of nuclease-free water. Conditions were set as follows: *tef* - an initial denaturation temperature of 95 °C for 5 min, followed by 35 cycles of denaturation temperature of 95 °C for 30 s, primer annealing at 55 °C for 30 s, primer extension at 72 °C for 1 min and a final extension step at 72 °C for 1 min; *rpb2* - an initial denaturation temperature of 94 °C for 5 min, followed by 35 cycles of denaturation temperature of 94 °C for 30

s, primer annealing at 57 °C for 90 s, primer extension at 72 °C for 7 min and a final extension step at 72 °C for 10 min. The PCR products were analyzed by electrophoresis on 1% agarose gels that were stained with GelRed™ (Biotium Inc., Hayward, CA) in a 1× TAE buffer and visualized under UV light (L-Pix Touch, Loccus) to check for amplification size and purity. The amplicons were purified and sequenced by MacroGen Inc., South Korea (www.macrogen.com).

2.6 Phylogenetic analysis

DNA sequence data were analyzed in MEGA 11 (Molecular Evolutionary Genetics Analysis) v. 11.0.13 (Tamura; Stecher Kumar, 2021). Consensus sequences were generated and imported into MEGA for initial alignment and the construction of sequence datasets. All sequences and alignments were checked manually with adjustments made when necessary. Preliminary analyses involved comparing the obtained sequences with similar ones using a local BLASTn search in the GenBank database (www.ncbi.nlm.nih.gov). Reference sequences of *Fusarium* species complexes, to which the isolates belong, were retrieved from GenBank to form a dataset for phylogenetic analysis based on the isolates described in the studies of Geiser *et al.* (2005), Sandoval-Denis *et al.* (2018), Lombard *et al.* (2019), Xia *et al.* (2019), Crous *et al.* (2021), and Yilmaz *et al.* (2021) (Supplementary material, table S2).

Bayesian inference (BI) analyses employing a Markov Chain Monte Carlo (MCMC) method were performed. Initially, jModeltest2 v. 2.1.6 (Darriba *et al.*, 2012) was used to determine the best parameters of the models for each gene region and species complex. Once the likelihood scores were calculated, the model was selected according to the Akaike Information Criterion (AIC), applying a substitution model accordingly for each *Fusarium* species complex. The phylogenetic analysis of the *tef* and *rpb2* alignment was carried out using the CIPRES web portal (Miller *et al.*, 2010) using the MrBayes program v. 3.2.7a (Ronquist; Huelsenbeck, 2001). Four MCMC chains were run simultaneously, starting from random trees for 10,000,000 generations. The trees were sampled every 1,000th generation and the first 2.5% trees were discarded as the burn-in phase of each analysis. The posterior probabilities were determined from a majority-rule consensus tree that was generated from the remaining trees. Maximum Likelihood (ML) analysis was performed using RAxML-HPC v. 8.2.12

(Stamatakis, 2014) implemented in the Cipres Portal. The resulting trees were visualized in FigTree (Rambaut, 2009) and exported to Inkscape v. 0.91 (www.inkscape.org) for editing of the layout. Bayesian inference posterior probability above 0.9 and ML bootstrap values above 70% were used for describing branch support.

2.7 Double-checking the *Lasiodiplodia iranensis* conilon dieback and SCC connection hypothesis

To verify the pathogenicity of *Lasiodiplodia iranensis* to *C. canephora* and to observe closely the symptoms produced on infected conilon, one of the original isolates COAD 3536 (Ramos *et al.*, 2023), cited in the publication and deposited in the local culture collection Coleção Octávio de Almeida Drummond (Universidade Federal de Viçosa) was inoculated onto healthy conilon coffee plants identified as belonging to two susceptible clones: LB1 and MP3. Twenty-one plants were used in these infectivity/disease symptom observation attempts (Table 2).

Table 2. Number, clone and age of conilon plants used for the observation attempt of disease symptoms caused by inoculating *Lasiodiplodia iranensis* (COAD 3536).

Number of conilon plants	Clone	Age
6	LB1	cuttings from seedlings
5	LB1	Seedlings
5	LB1	2 years old
5	MP3	4 years old

Four-year-old plants were inoculated in mature woody parts of the stem with *ca.* 1 cm diam or more). Wounds were produced with a flamed metal hole punch and removal of the bark at those points to expose the heartwood to allow the direct contact of inoculum to the inner tissue of the plants. The inoculum consisted of a mycelial plug; 1 cm diameter disks taken from COAD 3536 colonies formed on PDA after seven days grown at 25°C. Each wound received a disk taken with the metal punch from the culture and placed with a sterile metal forceps directly on each hole. Covering each inoculated hole, a sterile water-soaked cotton pad was placed around the stem and the whole area was then covered with plastic film. A PDA disk without mycelia was used to inoculate the control plants in all tests. Plants were kept in the laboratory, under

ambient conditions (temperature ranging from 23 to 32 °C) to be monitored for signs of infection and disease development. We also kept a set of plants on a greenhouse bench at 25 ± 2 °C and irrigated regularly. Each inoculation test was conducted three times. Cuttings were evaluated by the presence or absence of symptoms four days after inoculation and seedlings after 10 days, as described by Ramos *et al.* (2023).

2.8 Diagnose

An exercise was conducted of a combination of analysis of the evidences generated in this work with information available in the literature, to eliminate putative, but unlikely causes for SCC and to reduce the number of possibilities towards a manageable number of options for the etiology of this disease.

3. RESULTS AND DISCUSSION

3.1 Observation and recording of symptoms and signs

A recently published bulletin by Lima *et al.* (2023) includes detailed information and illustrations about SCC. These are combined below with our observations and images to provide a more complete description of SCC.

SCC is primarily characterized by the development of cankers in both the main stem and side branches of conilon coffee plants (Figure 4). The appearance of flaking bark areas can be observed at few to numerous positions of woody parts. Even young plants (up to 12 months) may show early signs of SCC. Additionally, there may be constriction and girdling of the stems.

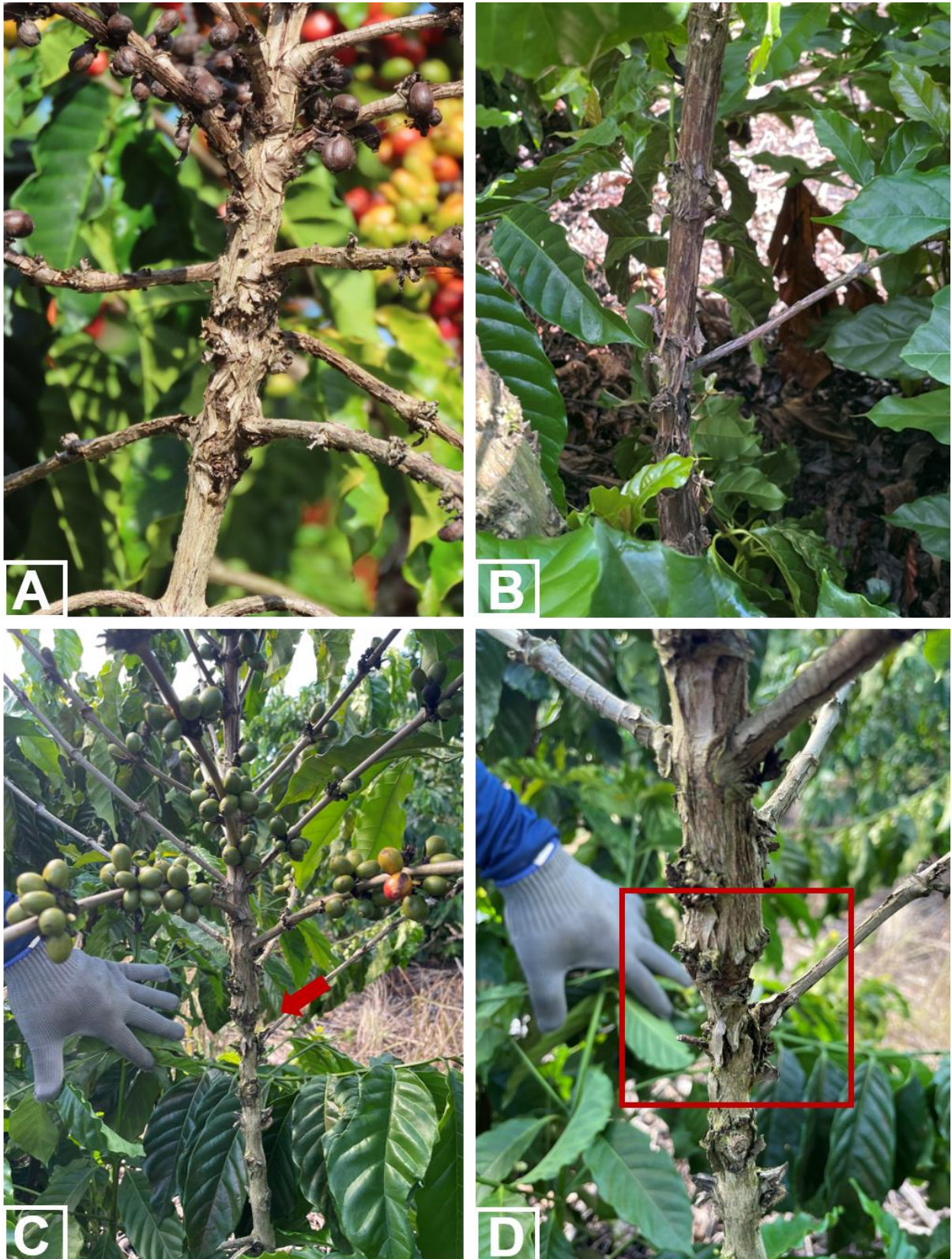


Figure 4. Orthotropic (vertical) and plagiotropic (horizontal or lateral) branches of *Coffea canephora* showing stem canker (A and B) and annealing (C arrow, zoom in D). Photo A by Inorbert de Melo Lima. Source: Lima *et al.* (2023).

As the plant matures, internodes become shorter and branching becomes more prominent. Branches also present flaking of the bark (Figure 5).

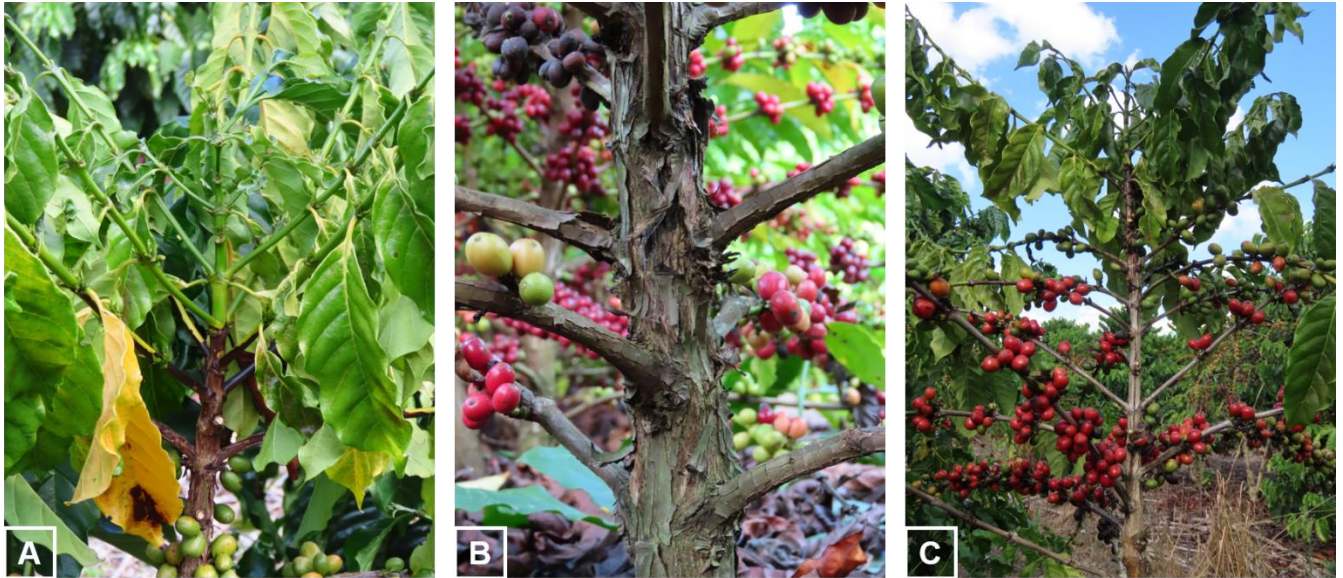


Figure 5. Shortening of internodes of *C. canephora* as a result of SCC. A: shortening of internodes and wilting of leaves; B: flaking of the bark in branches; C: productive plant presenting symptoms. Photo B by Inorbert de Melo Lima. Source: Lima *et al.* (2023).

Foliage yellowing and petiole twisting – with consequent leaf upturning - on SCC diseased plants (Figure 6).

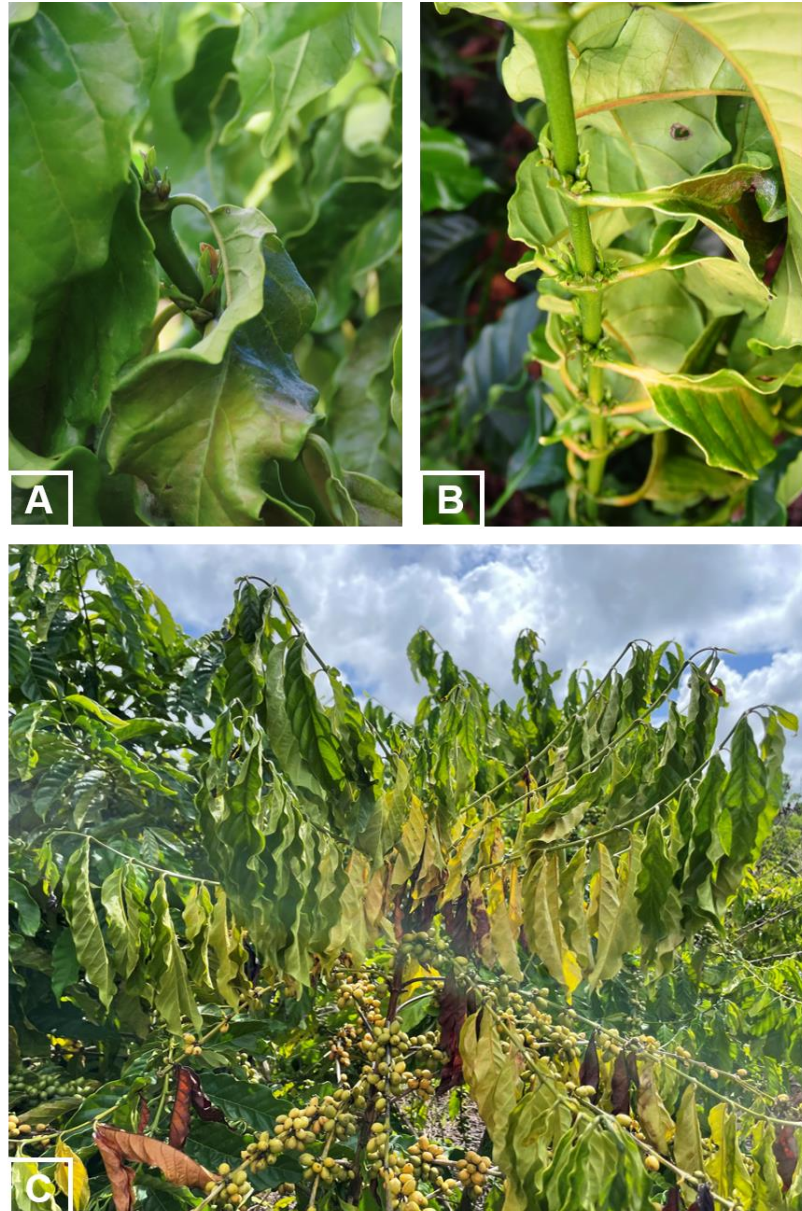


Figure 6. Yellowing of leaves and twisting of petioles of *C. canephora*. A: twisted petiole in close; B: abaxial side of leaves turning upwards; C: wilting and yellowing of leaves. Photo A and B by Inorbert de Melo Lima.

Over time, the vigor of affected plants gradually declines (Figure 7). This is an expected outcome from the wilting and yellowing of leaves. Consequently, both orthotropic and plagiotropic branches eventually die. Although apparently healthy new

shoots tend to emerge below the areas where orthotropic branches have died, this “recovery effort” of plants fails because infected plants invariably die, since these new shoots are actually asymptomatic.

No arthropod attack or signs of pathogens were observed in direct connection with the SCC symptoms in the field at any of the sites/farms.



Figure 7. *Coffea canephora*. A: diseased plant, presenting symptoms of SCC; B: healthy plant.

The planting of different clones in alternating rows is a common practice in conilon coffee cultivation (Ferrão *et al.*, 2019, p. 332). The disease often spreads along the row of susceptible clones, leading to the loss of entire rows and a significant reduction of plant stands. As a result, growers have been removing these affected clones from their fields. This has resulted in significant losses and increased production costs (Figure 8). Currently, the infected seedlings do not show any visible symptoms, and there are no established methods to identify the pathogen in the seedling stage to prevent future losses (Lima *et al.*, 2023).



Figure 8. Aerial view of planting of *C. canephora* clones in rows and eradication of lines of clones that showed susceptibility to SCC (arrows). Photo by Renato Corrêa Taques. Source: Lima *et al.* (2023).

3.2 Examination of samples and isolation from conilon samples

No evidence of bacterial oozing or darkening of tissues, even in highly damaged SCC-lesioned stems, was observed. Bacterial isolates were only seen emerging from root samples (not investigated here).

Approximately 500 fungal isolates were obtained from the samples of conilon brought from the field. The isolation process was laborious, and the identification process began as soon as colonies started to grow. Numerous isolates did not sporulate in culture and, based on microscope examinations were ranked as “unidentified” since mycelium-only colonies of fungi are impossible to name. A tentative provisional identification at the generic level was generated to produce a “workable-practical” informal grouping of isolates (Table 3). This was acknowledged as temporary and requiring further morphological and molecular analyses.

Table 3. Tentative naming at the generic level of isolates obtained from leaves, stem and roots of *Coffea canephora* (conilon) samples (either healthy or diseased).

Tentative naming at generic level	Number of isolates per plant part		
	Leaves	Stem	Roots
<i>Acremonium</i>	0	5	0
<i>Aspergillus</i>	3	1	0
<i>Cylindrocarpon</i>	0	1	0
<i>Cladosporium</i>	2	1	0
<i>Colletotrichum</i>	54	16	0
<i>Clonostachys</i>	0	13	0
<i>Diaporthe</i>	0	22	0
<i>Fusarium</i>	32	82	89
<i>Melanographium</i>	0	1	0
<i>Pestalotiopsis</i>	6	15	0
<i>Phoma</i>	12	0	0
<i>Symplicium</i>	0	2	0
N. id. ¹	57	39	36

¹ Not identified. Representing the assemblage of isolates that did not sporulate in culture. These were kept for later studies.

The seemingly most commonly identified genera of fungi obtained were *Colletotrichum*, *Fusarium*, *Pestalotiopsis*, and *Diaporthe*. These fungal genera are known to include species that can cause plant diseases, but these are very large and ecologically diverse genera occupying a vast range of niches and habitats. All include taxa that are endophytes or saprophytes rather than pathogens on different plant tissues.

Endophytes are widely found in various plant species across different ecosystems, including land, freshwater, and marine plants. They can exhibit different relationships with their host plants such as latent pathogens, mutualists, commensals, temporary residents or inactive saprotrophs (Veloso *et al.*, 2023).

The genus *Colletotrichum*, belonging to the family Glomerellaceae, is recognized as one of the most significant fungal pathogenic genera in plant pathology. Anthracnose, caused by various species of *Colletotrichum*, poses a serious threat to economically important crops. While certain strains of *Colletotrichum* can exist as symptomless endophytes within living plant tissues, numerous other non-pathogenic species function as endophytes (Asad *et al.*, 2023).

Fusarium is a large genus (over 300 spp.) that includes saprophytes, plant pathogens, non-pathogenic endophytes, mycoparasites and even some opportunistic human pathogenic species. It is known as one of the most prevalent genera among endophytic fungi, with approximately 70 species known to have endophytic capability. This biological plasticity influences their biology, interactions with other organisms, and secondary metabolism (Ahmed *et al.*, 2023).

From their study, Bongiorno *et al.* (2015) found that the majority (72%) of leaf endophytes that they isolated from coffee plants were identified as *Colletotrichum* spp.. Similarly, Vega *et al.* (2009) obtained 843 isolates of fungal endophytes from various parts of coffee plants namely leaves, roots, stems, and berries. They found that *Colletotrichum* spp. and *Fusarium* spp. were among the most prevalent taxa. In our study, we also found a large frequency of *Colletotrichum* (19%) and *Fusarium* (31%) among the isolates of fungal endophytes obtained from leaves and stems. Lu *et al.* (2022) isolated 100 strains of *Colletotrichum* but *Fusarium*, *Diaporthe* and *Pestalotiopsis* were also detected in their study on endophytic fungi associated with coffee leaves in China. These findings suggest that *Colletotrichum* and *Fusarium* are common endophytic fungi in various plant species, including coffee (Ma *et al.*, 2019; Cao *et al.*, 2019; Azuddin *et al.*, 2022).

In general, *Pestalotiopsis* is mostly regarded by plant pathologists to be a saprophyte or weak pathogen. It is, for instance, of no known relevance as a coffee pathogen. Nevertheless, some *Pestalotiopsis* species are known to cause severe damage to plants (Wu *et al.*, 2022), canker and dieback being the worst of them (Singh and Devi 2007; Espinoza *et al.*, 2008; Chen *et al.*, 2013; Yun *et al.*, 2015; Hernández-Ceja *et al.*, 2021). On the other hand, there are examples of species, such as *Pestalotiopsis microspora* which is regarded as of interest for the biological control of plant pathogens (Lu *et al.*, 2022).

Diaporthe pathogens are commonly found on cultivated and wild plants. Some species have been demonstrated to cause blights, cankers, diebacks, melanose, and stem-end rots (Udayanga *et al.*, 2014). Additionally, *Diaporthe* has been identified as a seemingly benign endophyte in grapevines (Manawasinghe *et al.*, 2019), *Citrus* spp. (Dhong *et al.*, 2021) and from coffee plants (Lu *et al.*, 2022). In previous studies, *Diaporthe* has been found as an endophytic fungus in different plant hosts. Silva *et al.* (2017) identified various species of *Diaporthe*, along with *Fusarium* and *Glomerella*, in the endophytic communities of *Paullinia cupana* roots and seeds. These genera were also previously detected in phyllosphere endophytic communities (Sia *et al.*, 2013).

The ecological role of endophytic fungi, such as *Colletotrichum*, *Fusarium*, *Pestalotiopsis* and *Diaporthe*, is complex and multifaceted. These endophytic fungi can have both positive and negative effects on their host plants. On one hand, they may promote plant growth and enhance the plant's ability to tolerate stress by producing growth-promoting substances, improving nutrient uptake, and inducing systemic resistance against pathogens. For example, some strains of *Colletotrichum* and *Fusarium* have been shown to stimulate plant growth and increase crop yields in various agricultural systems. However, certain taxa and populations of these endophytic fungi can change into a pathogenic status under specific circumstances, leading to the development of diseases in their host plants (Asad *et al.*, 2023).

3.3 *Fusarium* spp. from conilon

PCR amplification of *tef* and *rpb2* was successful for species complexes, however, successive attempts of *rpb2* amplification failed for a few isolates. Sequence length after trimming low-quality end regions varied from 580 to 652 bp for *tef*, and 843 to 920 bp for *rpb2*. Each of these sequences was submitted as a query in BLASTn

searches to verify their affinity with *Fusarium* spp. before their inclusion in the subsequent phylogenetic analysis. This included *Fusarium* isolates of taxa belonging to different species complexes such as *F. fujikuroi* (FFSC), *F. oxysporum* (FOSC), *F. incarnatum-equiseti* (FIESC), *F. lateritium* (FLSC), *F. solani* (FSSC), and *Albonectria* (previously *F. decemcellulare*).

Out of the 82 *Fusarium* isolates that were analyzed, the most frequent species complex was FFSC, comprising 40 isolates. This was followed by 20 isolates of FOSC, 10 isolates of FLSC, and 8 isolates of FIESC. Additionally, there were 3 occurrences of *F. solani* and *Albonectria* as a single isolate (Table 4).

Table 4. Isolates belonging to different *Fusarium* species complexes obtained from samples of *Coffea canephora* from northern Espírito Santo (Brazil).

Number of isolates	Blast ID	<i>Fusarium</i> species complex
2	<i>F. concentricum</i>	FFSC
1	<i>F. decemcellulare</i>	<i>Albonectria</i>
8	<i>F. incarnatum/ F. equiseti</i>	FIESC
10	<i>F. fujikuroi</i>	FFSC
10	<i>F. lateritium</i>	FLSC
25	<i>F. pseudocircinatum</i>	FFSC
3	<i>F. proliferatum</i>	FFSC
20	<i>F. oxysporum</i>	FOSC
3	<i>F. solani</i>	FSSC

Phylogenetic analysis using Maximum Likelihood and Bayesian Inference were implemented to elucidate isolates' identity. The concatenated alignment consisted of 17 operational taxonomic units (OTU's) for *Albonectria*, 195 OTUs for FFSC, 78 OTUs for FIESC, 31 OTUs for FLSC, 75 OTUs for FOSC, and 85 OTUs for FSSC, including the outgroup sequence specific to each species complex (Supplementary material, table S2).

The resulting trees of Bayesian Inference (BI) and Maximum Likelihood (ML) analysis agreed on topology, however, some clades showed lower support values in ML (Supplementary material, figures S1-S7). We consider a node strongly supported when BI posterior probability was higher than 0.90 and ML bootstrap value was higher than 70%.

Fusarium lateritium species complex – FLSC

The majority (6/7) of isolates belonging to FLSC fell into one major clade, Clade I. This clade includes the isolate FRC L-402, which was isolated from coffee bark in Malawi, and was identified as *F. stilboides*. The type species originally described as causing bark disease in coffee is the isolate FRC L-405, which falls into Clade IIA. The remaining isolate of this complex obtained in our study, MFB-CC-78, grouped with isolates belonging to Clade IIB, FRC L-82 and FRC L-120. Those isolates were identified as *F. lateritium/stilboides* and *F. lateritium*, respectively (Figure 9).

Fusarium lateritium species complex

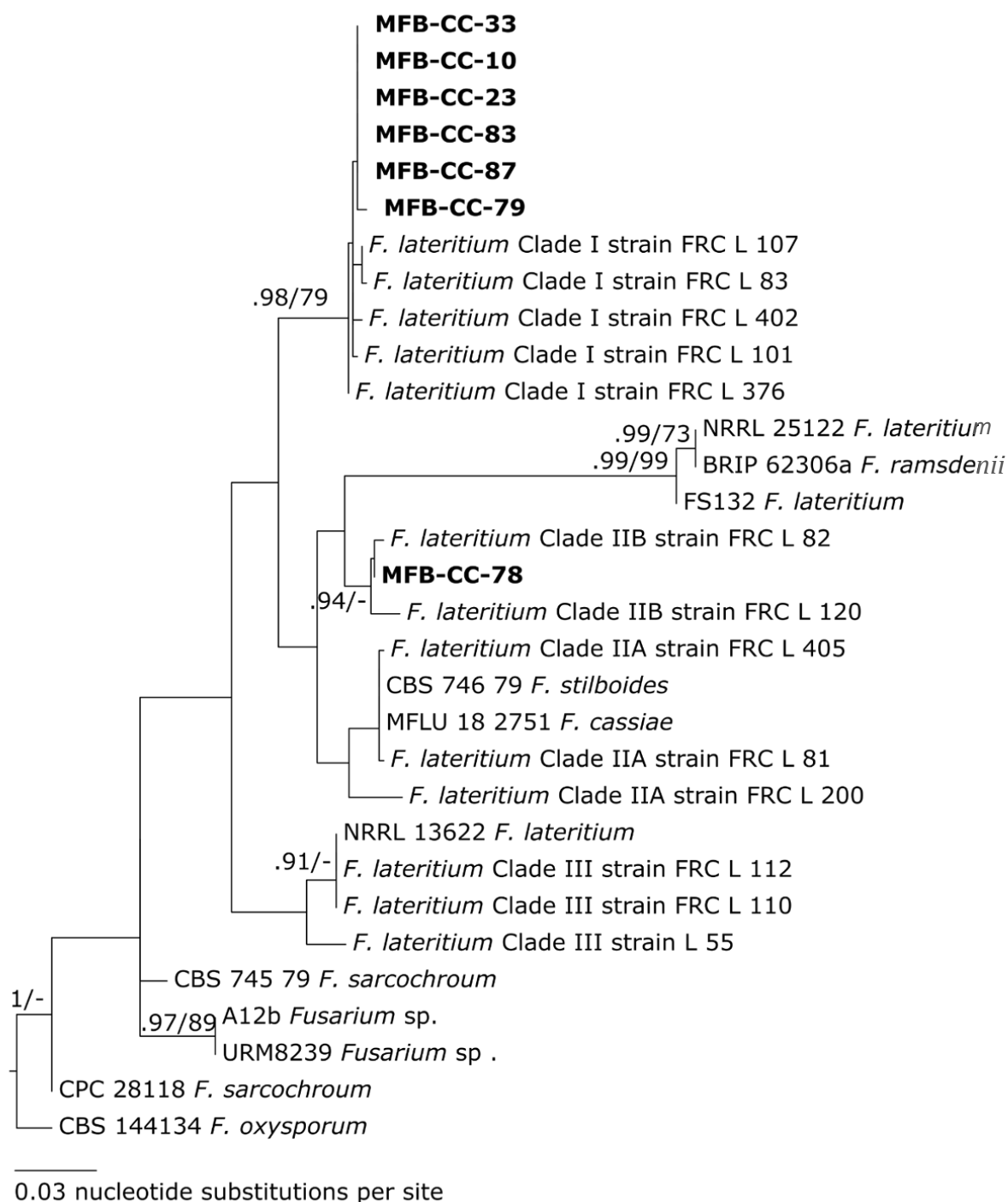


Figure 9. Maximum likelihood phylogram of the *Lateritium* clade fusaria primarily from coffee, based on the *tef* gene region. Bootstrap values (70% shown) and posterior probability (0.90). The tree was rooted with *F. oxysporum* CBS 144134. The isolates obtained in this study are emphasized in bold.

Geiser *et al.* (2005) elected the use of “Lateritium clade” in reference to those taxa that appeared to be monophyletic in their study. The term clade refers to a piece of a phylogeny that includes an ancestral lineage and all the descendants of that ancestor (Baum, 2008). Many taxonomic studies, which rely on morphological characteristics to classify organisms, have observed that certain groups of closely related species, in addition to specific characteristics that evolved from a common ancestor, have provided evolutionary proximity between these species. This occurs in classifications of *Fusarium* species (Taylor *et al.*, 2000; Watanabe *et al.*, 2011).

Members of the Lateritium clade have been associated to coffee diseases (Geiser *et al.*, 2005). *Fusarium stilboides* is known to cause bark and fruit rots in citrus and coffee. Some authors considered this species as a synonym of *F. lateritium*, making it challenging to distinguish between *F. stilboides* and certain isolates of *F. lateritium* within the Lateritium clade (Snyder and Hansen, 1945; Nelson *et al.*, 1983).

Comparative studies of phylogenetics and phylogenomics transformed our comprehension of species boundaries, and evolutionary connections in *Fusarium*. Phylogenetic recognition of species based on genealogical concordance has consistently revealed the considerable constraints of morphological and biological identification methods in *Fusarium*, leading to a significant increase in species identification within the genus (Taylor *et al.*, 2000; Waalwijk *et al.*, 2018).

The lack of current studies regarding the bark disease of coffee makes it challenging to accurately distinguish between *F. stilboides* and isolates of *F. lateritium*. Relying solely on morphological characteristics may not be enough to determine species boundaries anymore. There is an urgent need for additional molecular studies to elucidate the connections among FLSC.

3.4 Double-checking the *Lasiodiplodia iranensis* conilon dieback and SCC connection hypothesis

The steps described in Ramos *et al.* (2023) were carefully repeated. COAD 3536, the isolate of *L. iranensis* obtained from *C. canephora* by those authors and tested on healthy conilon plants, was recovered from the local culture collection where it had been deposited and, after the procedures described earlier were followed no disease symptoms were observed regardless of the form of inoculation and kind of conilon material involved and the length of the observation period.

Tests involving fresh cuttings, as described in Ramos *et al.* (2023), were evaluated at four and seven days after inoculation (dai). No indications of mycelial colonization were observed. By seven dai, the cuttings started to perish due to excessive moisture. The damp cottons were removed after 48 hours. There was no clear explanation in the original publication about the procedure for avoiding the desiccation of the stems during the experiment and, after some failed attempts, we chose to keep a moist filter paper with a plastic grid cover to prevent contact with the cuttings. The experiment was repeated with less water and no water maintenance to assess the influence of humidity in the formation of symptoms in the cuttings, but the same results were obtained (Figure 10).

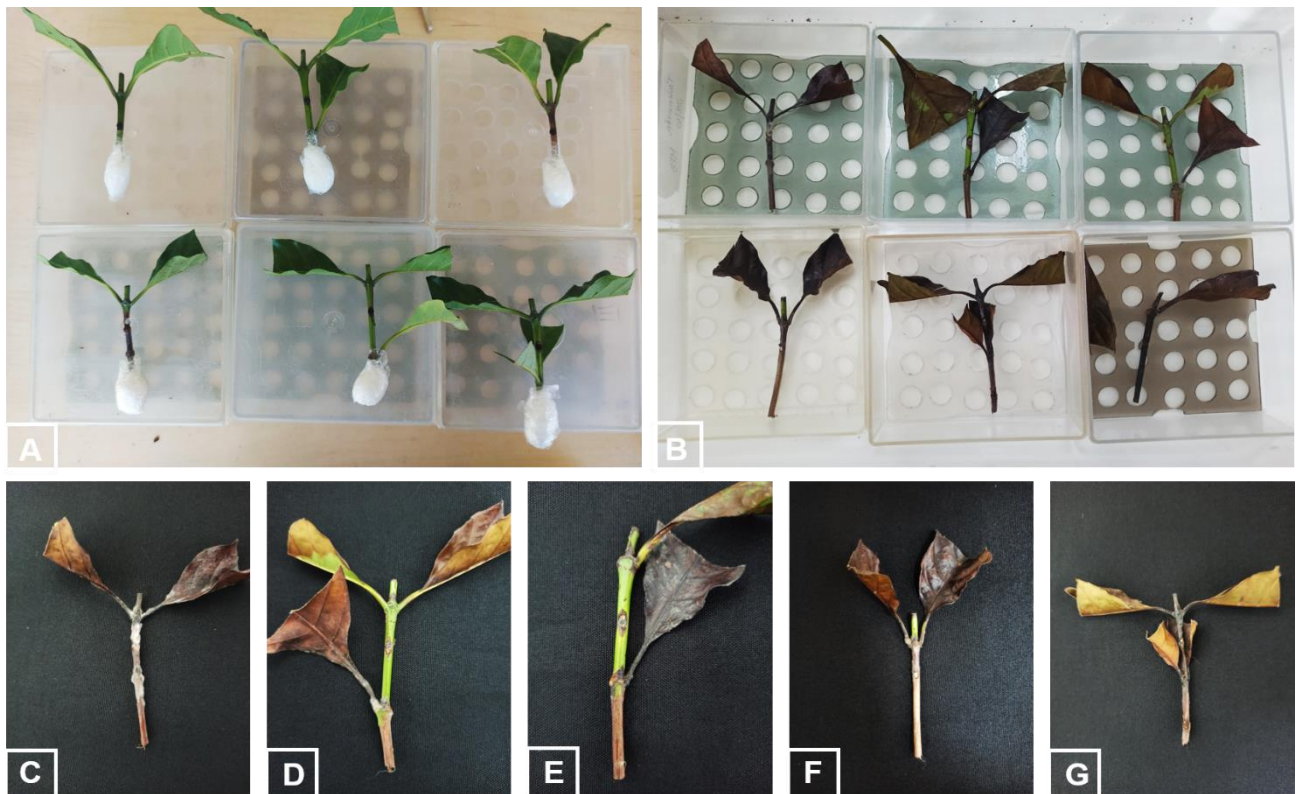


Figure 10. Testing of *Lasiodiplodia iranensis* (COAD 3536) in cuttings of *C. canephora*. A: Cuttings inoculated with a mycelial disk and covered with a damp cotton. B: Cuttings after seven days of inoculation. C: control treatment showing only saprophytic colonization. D-G: cuttings presenting no signs of *L. iranensis*.

It's important to note that green stem cuttings, which are still herbaceous and have not yet developed woody tissues, deteriorated more quickly than cuttings with woody stem tissues. This observation indicates that the age and maturity of the plant material could potentially impact susceptibility to *Lasiodiplodia* infection.

The experiments on seedlings were carried out in both a greenhouse environment and laboratory settings, where they were not exposed to direct sunlight and the temperature ranged from 23 to 32 °C. The seedlings were assessed at 10, 30, and 45 dai. Throughout all these periods, there were no observable symptoms related to *Lasiodiplodia* infection in the inoculated seedlings (Figure 11). The experiment was repeated three times but there were no observed symptoms or signs of infection by the pathogen.



Figure 11. Inoculation of *C. canephora* seedlings with *L. iranensis* (COAD 3536).

Based on our tests, it appears that the isolate COAD 3536 of *L. iraniensis* did not show pathogenicity or cause any visible symptoms in *C. canephora* plants. These findings suggest that *L. iranensis* may not be the causal agent of SCC, or that other factors, such as environmental conditions or interactions with other microorganisms, are necessary for the development of disease symptoms.

3.5 Diagnose

Possible causes of SCC are listed below and commented.

- *Abiotic*

Although known for many years, the disease appears to be restricted to northern ES and southern BH. This requires confirmation since published results of consistent monitoring of the disease is still lacking. It seems, nevertheless, unlikely that environmental stress would be causing the disease selectively on some clones but not others, and only in recent years. Management procedures adopted in SCC-affected and SCC-free farms are the same (I. M. Lima, pers. comm.), excluding, in principle, an abiotic management-related cause for SCC. Different conilon clones growing side by side, and subjected to the same climatic conditions, irrigation, fertilization and general management, respond differently as either immune or susceptible, a good evidence that a biotic cause is responsible for SCC. It is, nevertheless, possible, that an environmental or management condition is playing a role by favoring biotic damage.

- *Pests*

Neither our observations in the field or in the lab nor any report from farmers or scientists investigating the disease indicate a connection between attack of arthropods or even macrofauna with SCC.

- *Nematodes*

Nematodes attack may be important in conilon plantations. Nevertheless, no evidence from our observation of roots of SCC diseased plants nor any earlier (unpublished) surveys have indicated a gall nematode-SCC connection.

- *Bacteria*

The possibility of a biotrophic/fastidious bacterium or bacterial-like agent involved in SCC etiology can't be excluded at this stage, but the absence of vascular necrosis and bacterial ooze from the blasted stems of SCC infected conilon suggests that necrotrophic bacteria are not involved in SCC etiology.

- *Fungi*

Lasiodiplodia iranensis

Although we failed to confirm that COAD 3536 is pathogenic to conilon, this may have resulted from loss of virulence of this isolate in culture or some particular methodological detail that was not met during our experiments. We, nevertheless, interpret the involvement of *L. iranensis* differently from Ramos *et al.* (2023). While in their publication it is implied that conilon canker on a broad range of planted areas is caused by *L. iranensis*, the illustrations in their publication only include dieback symptoms. Additionally, the images presented and the isolates utilized in their study were all from a single field in the municipality of Santa Teresa (ES). The presence of the black dots on necrotic tissues presented in Ramos *et al.* (2023) was not observed in any of the samples collected in our study. Additionally, none of the isolates obtained from diseased tissues were recognized as belonging to *L. iranensis*. It is possible that *L. iranensis* is the etiological agent of a dieback disease with no relation to SCC. Key symptoms of SCC such as foliar inversion and bark blasting were not documented in association with infection by *L. iranensis*. It is reasonable, until proven otherwise, to consider SCC as not having *L. iranensis* as its etiological agent.

Fusarium spp.

The symptoms of SCC bear some resemblance to those of two other coffee diseases: tracheomyces or coffee vascular wilt and bark disease. Interestingly, both are *Fusarium*-induced diseases. The former is caused by *Fusarium xylarioides* and the latter by *Fusarium stilboides* (Li *et al.*, 2022). *Fusarium xylarioides*. Once thought to be restricted to Africa, was recently reported from China, although not causing a coffee disease, but instead causing root and stem rot on *Aloe vera* (Zhu *et al.*, 2021). This suggests a broader distribution of *F. xylarioides* in the Old World, but a restricted occurrence of the disease on coffee – possibly indicating pathological variants below the species level for *F. xylarioides*. Coffee vascular wilt is characterized by a progressive development of yellowing, withering leaves that eventually develop brown necrotic lesions. This process can start from any part of the plant but spreads throughout, causing affected leaves to curl, dry up, and fall off. The initial symptoms are seen on the coffee stem where fungi colonize and block vascular bundles, resulting

in blue-black stains due to the host's response. No such signs have ever been found on SCC-diseased plants and none of the *Fusarium* spp. isolates obtained in this study were close to *F. xylarioides*. *Fusarium* spp.-complex. According to Belan *et al.* (2018), the occurrence of Fusarium wilt of *C. canephora* in ES was linked to three *Fusarium* species: *F. decemcellulare*, *F. lateritium*, and *F. solani*. The symptoms of Fusarium wilt in the *C. canephora* plants described in their study includes wilting, leaf yellowing, defoliation, darkening of the vascular tissue, drying, and death of plagiotropic and orthotropic branches and ultimately leading to plant death. This discovery attracted significant attention among coffee growers and researchers in Brazil as it was the first report potentially connecting a candidate pathogen with SCC. Some extension workers, agronomists and farmers promptly addressed the disease on *C. canephora* as "fusariosis". We believe this to be a questionable choice.

Belan *et al.* (2018) publication included a preliminary molecular study that they claimed to have led to the identification of three different *Fusarium* species which would have been involved in conilon wilt. Nevertheless, this relied solely on a Blast search that compared their accessions with those in GenBank. Unfortunately, their sequences have not even been deposited disallowing any further checking of their results. Strangely, after six years no further studies on this important possibility of an etiology for SCC have been published.

Although fusarium wilt in *C. canephora* was purportedly linked with *Fusarium* spp., there is still a need for additional clarifications for the initial findings of Belan *et al.* (2018). The symptoms of SCC are different. No typical wilting occurs. No darkening of the vascular tissues of diseased coffee plants was ever found and inoculated plants in Belan *et al.*'s study did not develop the typical bark blasting or twisting of petioles observed on conilon plants in the field. The wilt of *C. canephora* is likely an entirely different disease of rare occurrence, or even an experimental aberration. No report of conilon wilt in the fields of ES, deserving to be addressed as "fusariosis", has emerged since their publication.

Fusarium stilboides

As for bark disease, it can cause cankers and necrotic lesions on the bark of the coffee tree, leading to dieback and eventually death of the affected branches, but seems to be known only to affect only *C. arabica* (Siddiqi and Corbet 1963; Li *et al.*,

2022). Nevertheless, the significant occurrence of Clade I FLSC among our isolates raises the possibility of a potential form of conilon bark disease at play and deserves further detailed investigation. At this time, all the inoculations of *Fusarium* spp. isolates conducted during this work failed to reproduce any SCC symptoms on test plants known to belong to susceptible clones. Bark disease in Africa remains a poorly studied disease and, unfortunately, even the identity of the species involved remains somewhat obscure.

Both coffee vascular wilt and bark disease are challenges for coffee growers. The wilt disease had been particularly detrimental to Arabica coffee production in Ethiopia, Uganda, the Democratic Republic of Congo, and Tanzania, resulting in a significant yield loss estimated to be around 30-40% in Ethiopia alone (Rutherford, 2006; Mulatu *et al.*, 2023). These diseases are primarily spread through wind, rain, and human activities, with pathogens entering the plant through wounds. Managing these coffee diseases is crucial for the survival and productivity of coffee plantations (Li *et al.*, 2022).

- *Undetermined non-culturable pathogen*

SCC appears in the conilon plantations firstly on susceptible groups and progressively spreads to neighboring plants in the row. Possible means of transmission following such a pattern would be through root anastomosis, through the extension of rhizomorphs of an *Armillaria*-like fungus or through pruning or harvesting tools contaminated with the pathogen. All such options would involve a transmissible pathogen. Several competent researchers have already attempted to elucidate the etiology of SCC in various labs and using traditional procedures. Here we have also attempted to use optic microscopy and standard isolation techniques to obtain a culturable fungus from the diseased tissues that would be specific to these but absent from healthy tissues. Most isolates obtained were of fungi, but some bacteria were also isolated (not investigated here). Nevertheless, such exercise did not yield a convincing result. No consistent dominant fungal isolate was obtained solely, or mostly, from diseased stems as would be expected in case a culturable species of pathogenic fungus was involved in the etiology. No darkening of vascular tissues that might be an indication of the growth of a vascular wilt-fungus or bacteria was observed here or by any earlier study or reported to us by other plant pathologists who have studied SCC.

The diagnosis of SCC remains a pending matter. The evidence obtained in this study combined with the examination of the published information and the experience of the practitioners in the field at ES allowed us to exclude several possibilities. The more likely chances seem now to fall on the involvement of a non-culturable pathogen. This might be a mechanically transmitted virus, a fastidious bacterium or some more elusive obscure kind of pathogen such as a prion. Less likely seems the involvement of a biotrophic fungus, since the best-known biotrophs are easily recognized by their external sporulation such as in rusts, powdery mildews and mildews. Nevertheless, since the focus of this work was the possibility of a fungus, since this is the largest group of plant pathogens, we next attempted to further verify the possibility of a fungus being involved using metagenomic tools. Methodology and results will be discussed in chapter 2.

Controversy on the elucidation of the etiology of diseases is commonplace for every novel epidemic. This is true for human diseases such as “mad cow-disease”, AIDS or, more recently, Covid-19. It should also be expected for significant plant diseases such as SCC. What is now broadly accepted as the true etiology for those human diseases took time until a consensus is reached. SCC is a phytopathological example of a disease demanding an elucidation of its etiology.

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CHAPTER II

**A metagenomic approach to elucidate the etiology of an emerging biotic stress
on *Coffea canephora***

ABSTRACT

Biotic stresses can impact cultivated plants, especially in monoculture settings. In the case of perennial crops like conilon coffee (*Coffea canephora*), there is evidence of a new disease called stem-canker of conilon in Brazil. Though efforts to isolate microorganisms associated with SCC have not fulfilled Koch's postulates, it is reasonable to consider the role of endophytic fungi in its development and investigate their potential pathogenicity and risks to coffee plants is necessary. Diagnostic methods like metagenomic sequencing could be promising for identifying unknown pathogens associated with *C. canephora* and assessing their risk to crop production. The recent outbreak of SCC in Espírito Santo (ES) and Bahia states is an urgent call for an investigation into the latent pathogenicity of endophytic fungi in *C. canephora*. There is a need for further research to identify and understand this unknown pathogen to develop strategies for disease management and prevention, ensuring the future sustainability of the conilon industry in the region. In March 2021, a survey was conducted across six locations in ES state. Leaves, stems, and roots of conilon clones A1, considered to be resistant to SCC, and LB1, an elite clone highly susceptible to SCC were sampled. Different categories regarding the status of the sampled plants were established: 1) Presence/absence of symptoms: A1 (resistant) - asymptomatic; LB1 (susceptible) asymptomatic and symptomatic; 2) Plant part affected or not by the problem: leaves, stems and roots. We collected three plants per category per farm to serve as repetitions. A total of 162 samples were collected. DNA was extracted and sequenced for shotgun metagenomic analyses. Statistical analyses were performed using R environment and R packages. Alpha and beta diversity were estimated for species richness, along with non-metric multidimensional scaling analyses and relative abundance. The study found that while there were no significant differences in the abundance of species to indicate a specific cause of SCC, it was possible to narrow down the list of potential causal agents. The microorganisms colonizing conilon plant organs differed significantly, with leaves having the lowest species richness and stems and roots showing higher diversity. There was also a noticeable variation in the relative abundance of OTUs in stem samples from asymptomatic and symptomatic plants, as well as among symptomatic samples based on age. Based on curated entries in reference databases and known associations with similar diseases, *Fusarium* sp. is identified as the likely fungus associated with SCC.

Key words: *Coffea canephora*, Disease, *Fusarium* spp., Shotgun sequencing.

1. INTRODUCTION

Occasionally, biotic stresses emerge in cultivated plants. Probably, the high genetic homogeneity of plants grown in monoculture over extensive acreages year after year may be related to the evolution of pests (*sensu lato*) that can severely compromise yield and negatively alter the economy of agriculturally-based clusters. For perennial crops, such as coffee (*Coffea* spp.), this problem can be exacerbated given that the long lifetime of the plantations enhances the chances of emergence of new problems.

Recently, there has been mounting evidence for the emergence of a disease in conilon coffee (*Coffea canephora*) plantations in Espírito Santo (ES) and in Bahia (BA) states in Brazil. Nevertheless, to date, the cause of this disease is still under debate. This "anomaly" has been putatively associated with biotic stress, thus an emerging "disease" may be involved and has been initially designated as the stem-canker of conilon (SCC). Herein, this disease will be considered as the biotic stress and its symptoms are characterized by the presence of cankers on branches and stems, internode shortage, yellowing and wilting of leaves, leading to reduced coffee production and branch death. This disease poses a significant threat to the future sustainability of the conilon industry in ES and BA (Lima *et al.*, 2023).

Several attempts were made to isolate microorganisms associated with SCC, but none provided convincing evidence of the fulfillment of Koch's postulates (Belan *et al.*, 2018; Ramos *et al.*, 2023).

Given the lack of evidence for the involvement of plant pathogenic fungi, it may be reasonable to hypothesize the role of endophytic fungi in SCC development. Endophytic fungi are functionally vital members of the plant microbiome, colonizing various plant species without causing visible damage or disease symptoms (Udo *et al.* 2022). Endophytes are microorganisms that "for all or part of their life cycle invade asymptomatic infections entirely within plant tissues, but cause no symptoms of disease" (Wilson, 1995). They are considered the main source of bioactive compounds and secondary metabolites with potential applications in agriculture, pharmaceuticals, environmental cleaning, and the food industry. Endophytic fungi have been found in practically every tissue and organ of vascular plants, including *Coffea canephora* (Vega *et al.*, 2010; Asad *et al.*, 2023). To date, most of the research on endophytic

fungi associated with *C. canephora* has focused on their beneficial roles in plant growth promotion and disease protection (Xing *et al.*, 2022).

The high biodiversity of fungal endophytes in coffee plants suggests that many have no specific role in the plant. However, some can significantly impact its health and growth. Further investigation is needed into the potential pathogenicity of these fungi and whether they pose any risks to the coffee plant, particularly for *C. canephora* (Li *et al.*, 2022; Tran, 2022; Asad *et al.*, 2023).

Studies have shown that specific endophytic fungi, like *Trichoderma flagellatum*, strongly inhibit various pathogens including *Fusarium* spp., *Alternaria alternata*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum* (Asad *et al.*, 2023). Additionally, volatile compounds produced by other endophytic fungi, such as *Muscodor* spp. and *Simplicillium* spp., completely or partially inhibit different phytopathogens (Vega *et al.*, 2010; Valente *et al.*, 2013; Monteiro *et al.*, 2020).

The diagnostic methods currently used for screening, monitoring, and identifying microorganisms vary in their accuracy. Traditional methods have drawbacks as they are labor-intensive, slow, and require expertise. Molecular identification is often combined with traditional approaches to address these limitations by amplifying and sequencing specific genes. However, molecular techniques can only detect well-characterized microorganisms and cannot identify new species unknown to science (Nilsson *et al.*, 2019; Piombo *et al.*, 2021).

Metagenomic-based methods have the advantage of identifying all microorganisms in a single analysis, making it suitable for detecting pathogens. High-throughput sequencing (HTS) is effective for monitoring and identifying plant pathogens, providing comprehensive information on both harmful and naturally-occurring microorganisms. This information helps distinguish organisms, as well as track disease outbreaks. Shotgun metagenomics involves sequencing all DNA present in a given sample (Piombo *et al.*, 2021). Combining HTS with bioinformatics allows for rapid and accurate characterization of microorganisms at the strain level (Esposito *et al.*, 2016; Baldrian *et al.*, 2022).

Metagenomic sequencing approaches allowed the detection of the fungus *Calonectria pseudonaviculata*, the causal agent of boxwood (*Buxus* spp.) blight disease (Yung *et al.*, 2022), *Shaeropsis sapinea* and other pathogens in pine trees (Blumenstein *et al.*, 2021). Thus, applying metagenomic sequencing could be a

promising strategy to identify potential unknown pathogens associated with *C. canephora* and assess the risks they pose to crop production.

The recent outbreak of SCC in ES and BA is an urgent call for an investigation into the latent pathogenicity of endophytic fungi in *C. canephora*. This discovery has sparked the need for further research to identify and understand this unknown pathogen to develop strategies for disease management and prevention, ensuring the future sustainability of the conilon industry in the region. More extensive research is needed to elucidate the specific roles of endophytic fungi in the pathology of *C. canephora*, including their potential pathogenic effects and the mechanisms underlying their interaction with the coffee plants. To address this novel problem, our study aimed to investigate the presence and abundance of endophytic fungi in *C. canephora*, with a specific focus on identifying this unknown pathogen.

2. MATERIAL AND METHODS

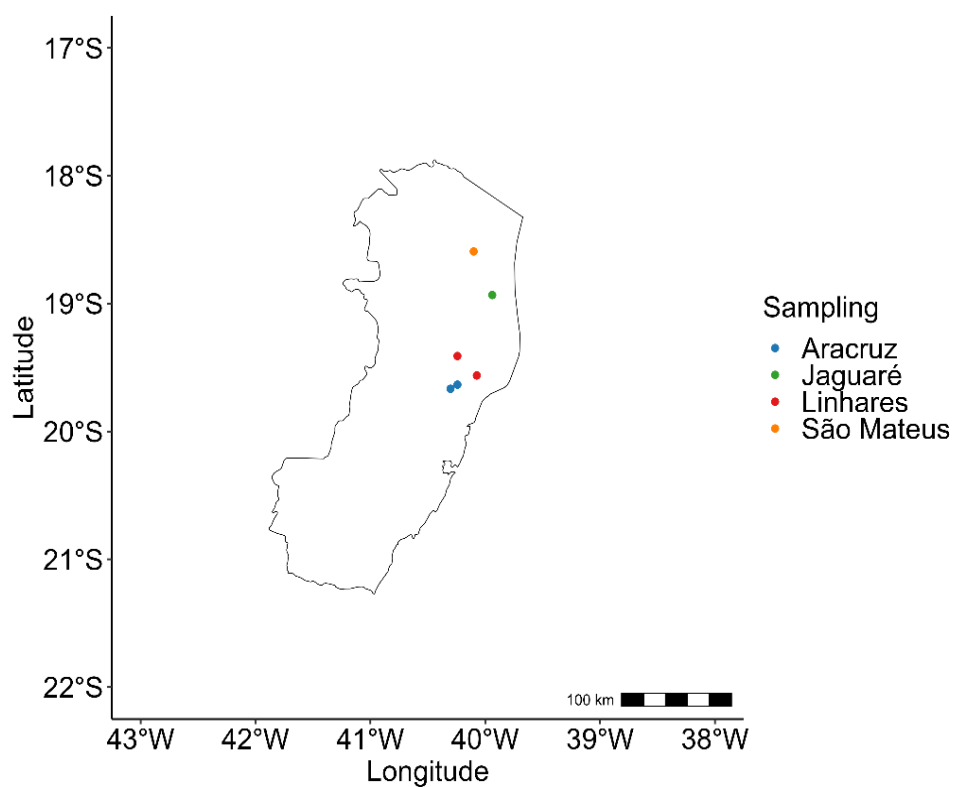
2.1 Sampling

A survey was conducted across six locations in ES state, Brazil. The survey occurred in March 2021 under the supervision of a plant pathologist from INCAPER (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural), Dr. Inorbert de Melo Lima, who is familiar with the disease and its distribution.

The survey involved conilon farms at the municipalities of Linhares, Aracruz, Jaguaré, and São Mateus (Table 1). To ensure an accurate comparison, only areas planted with the LB1 conilon clone were used in the research. Clone A1 was included as a control group since it was previously believed to be resistant to the reported disease based on the absence of observed symptoms at that time.

Table 1. Locality of *Coffea canephora* sampling in Espírito Santo, Brazil.

	Farm	Municipality (ES)	Latitude	Longitude
1	Edmar Tessarolo	Aracruz	-19,665922	-40,301998
2	Eduardo Zuccolotto	Aracruz	-19,633684	-40,241808
3	Renato Bianchini	Linhares	-19,562121	-40,074033
4	Eduardo Bortolini	Linhares	-19,409833	-40,242174
5	Thiago Gama	São Mateus	-18,592169	-40,100599
6	Ivan Catelan	Jaguaré	-18,9328	-39,9387

Figure 1. Locality of *Coffea canephora* sampling in Espírito Santo, Brazil.

2.2 Sample collection

Leaves, stems, and roots of conilon clones A1, considered to be resistant to SCC, and LB1, which is a high-yield elite clone, but highly susceptible to SCC were sampled. Different categories regarding the status of the sampled plants were established: 1) Presence/absence of symptoms: A1 (resistant) - asymptomatic, LB1 (susceptible) asymptomatic and symptomatic; 2) Plant part affected or not by the problem: leaves, stems and roots. We collected three plants per category per farm to serve as repetitions. A total of 162 samples were collected:

- 54 samples from clone A1 (18 samples each of leaf, stem and root);
- 54 samples from clone LB1 asymptomatic (18 samples each of leaf, stem and root);
- 54 samples from clone LB1 symptomatic (18 samples each of leaf, stem, and root).

A random set of leaves from each plant was collected, such as to ensure representation from the upper, middle, and lower sections of the plants. Moreover, stem segments ranging from 50 to 70 cm long were cut from the base of the plant up to the first internode. Root samples were carefully obtained by digging around each plant and gently removing its secondary roots. All samples were then placed in sterilized plastic bags and labeled. The samples were transported to the laboratory and stored in a refrigerator (~4°C) until further processing. Sterilized scissors, shovel, gloves along with a handsaw were utilized during sample collection for proper handling without contamination concerns.

2.3 Disinfestation process

Leaf and root samples:

Leaves and roots were thoroughly washed with distilled water, followed by a disinfestation process involving 1 min in ethanol (70%), 2 min in sodium hypochlorite (1%), and three rinses with sterilized water. After drying on sterile blotter paper, approximately 1 g of 0.5 cm leaf disks and 1 g of 1 cm-long root fragments were cut and transferred to Falcon tubes and stored in ultrafreezer -80 °C until DNA extraction.

Stem samples:

For stem samples, a central section of about 10 cm in length was used after removing the outer parts and bark using sanitized tools. The stems were sliced vertically to reveal inner tissue with a sterile blade, and 1 g of pieces measuring 1-2 cm from the surface to the cortex (excluding heartwood) were collected using sterilized scalpels and forceps, then placed into Falcon tubes and stored in ultrafreezer -80 °C until DNA extraction.

2.4 DNA extraction

The samples were lyophilized for five days and then macerated using a TissueLyser II sample disruptor until achieving a fine powder consistency: two minutes for leaves, six minutes for stems, and five minutes for roots. Once the maceration was completed, the samples were combined in bulks consisting of sample repetitions and homogenized according to the design by plant age, presence of symptoms, and part of the plant (Table 2). This process resulted in a subset of 18 pools that we made in triplicates, totalizing 54 samples for DNA extraction and sequencing.

Table 2. Sample pool design used for processing data.

Sample	Plant organ	Clone	Susceptibility	Age (years old)
P01	leaves	A1	resistant	≤ 4
P02	leaves	A1	resistant	> 4
P03	leaves	LB1	asymptomatic	≤ 4
P04	leaves	LB1	asymptomatic	> 4
P05	leaves	LB1	symptomatic	≤ 4
P06	leaves	LB1	symptomatic	> 4
P07	stem	A1	resistant	≤ 4
P08	stem	A1	resistant	> 4
P09	stem	LB1	asymptomatic	≤ 4
P10	stem	LB1	asymptomatic	> 4
P11	stem	LB1	symptomatic	≤ 4
P12	stem	LB1	symptomatic	> 4
P13	roots	A1	resistant	≤ 4
P14	roots	A1	resistant	> 4
P15	roots	LB1	asymptomatic	≤ 4
P16	roots	LB1	asymptomatic	> 4
P17	roots	LB1	symptomatic	≤ 4
P18	roots	LB1	symptomatic	> 4

The DNA extraction process took place at the Laboratório de Melhoramento Genético Vegetal, at Universidade Federal do Espírito Santo – Alegre, ES. It followed the total genomic DNA extraction protocol for plants with a high concentration of polysaccharides, with some modifications (Doyle; Doyle 1990). To check DNA integrity, 1% agarose gel electrophoresis was used and quantification was performed using a NanoDrop® 2000 spectrophotometer (Life Technologies Invitrogen). The extracted samples were then sent to Novogene Corporation Inc., Sacramento, CA for shotgun metagenomics sequencing. All downstream processes, including library preparation, were conducted by the company.

2.5 Processing data

Systems preparation and initial data analysis were carried out using the Cluster, at the Universidade Federal de Viçosa, which is equipped with 1 AMD Opteron™ Processor 6376 (16 M cache, 2.3 GHz and 32 cores), 64 GB of RAM, 100 TB of storage capacity and 24 calculation nodes. All analyses were performed on a computer equipped with an Intel® Core™ i5-7200 U- 7th generation processor, 8 GB of RAM.

The raw sequencing data obtained from Novogene Corporation Inc. was processed using bioinformatics tools and pipelines. A 200 GB compressed file with total genomic DNA sequences was received containing files in FASTAq format. Samples were unzipped and trimmed for bad or low-quality ends using 'seqtk' (<https://github.com/lh3/seqtk>). A host database was created for *C. canephora* by downloading its reference genome sequence from the Coffee Genome Hub (coffee-genome-hub.southgreen.fr/) (Denoëud *et al.*, 2014).

Furthermore, the trimmed reads were mapped to the *C. canephora* genome, and host-originated reads were identified and removed using Bowtie2 software (Langmead; Salzberg, 2012). The metagenomes were then mapped to the host reads and assembly of un-mapped reads was performed using 'samtools' and 'bedtools' (Li *et al.*, 2009; Quinlan; Hall, 2010).

The metagenomes were assembled with metaSPAdes. Taxonomic information of the metagenes was determined using the 'nr_euk' database created from Kaiju (https://kaiju-idx.s3.eu-central-1.amazonaws.com/2023/kaiju_db_nr_euk_2023-05-10.tgz), where each sequencing read was assigned to a taxon in the NCBI taxonomy dataset (Menzel *et al.*, 2016; Nurk *et al.*, 2017).

After taxonomic assignment, samples were filtered for missing data and repetitions were merged according to their original DNA extraction pool design (Table 2).

2.6 Statistical analysis

The statistical analyses were performed in R software v.4.1.2 (R Core Team, 2021) using various packages: *phyloseq* v.1.40.0 (McMurdie; Holmes, 2013), *vegan* v.6-2 (Oksanen, 2022), *DESeq2* v.1.34.0 (Love, 2014), *microbiome* v.1.16.0 (Lahti;

Shetty, 2022), *ggplot2* (Wickham, 2016), *tidyverse* (Wickham *et al.*, 2019) and *dplyr* (Wickham *et al.*, 2023).

Diversity analysis

A *phyloseq* object was created using the '*phyloseq*' package. An operational taxonomic unit (OTU) table was composed with the frequencies of reads that each taxon presented in each sample. The metadata used to identify the variables was the same as the pool design (Table 2). The identity of the OTUs were listed in the taxonomic table. The analyses of conilon microbiome were divided into three stages:

1. Diversity and Composition of Microbial Communities

Diversity, richness, and evenness were evaluated for each sample to check for repeatability considering archaeal, bacterial and fungal communities. This was done by calculating the Observed richness, and the Shannon diversity index using the '*diversity*' function in the microbiome package. Venn diagram was used to represent the number of taxa associated with each plant organ (leaves, stem and roots) and susceptibility category (resistant, asymptomatic and symptomatic).

2. Mycobiome of C. canephora

Based on our assumption that SCC is likely caused by a fungal pathogen, the specific fungal composition of *C. canephora* microbiome was analyzed separately to determine the diversity, richness, and evenness of the microbiome using the Shannon and InvSimpson diversity indexes. NMDS based on Bray-Curtis distance was conducted considering the samples and plant organs. The relative abundance was estimated for the top 20% genera considering the susceptibility categories.

3. Mycobiome in stem samples

The relative abundance was estimated for the top 30% more abundant species considering the susceptibility categories. Furthermore, differential abundance analysis was performed using the DESeq2 package to identify

significant differences in the abundance of fungal taxa between asymptomatic and symptomatic stem samples.

3. RESULTS

The shotgun metagenomic sequencing data resulted in 195,732 OTUs. After filtering for missing data and removing duplicates, the final dataset was composed of 2,716 fungal taxa. The two categories of samples used to assess the distribution of OTUs were: plant parts (root, stem, and leaves) and susceptibility (susceptible and resistant). Among the plant parts, the richness of OTUs in leaves was the lowest with only 11 unique OTUs, while the highest proportion of taxa was observed in the root samples with 15,041 OTUs. Moreover, the analysis showed 396 OTUs shared among the different plant parts (Figure 2).

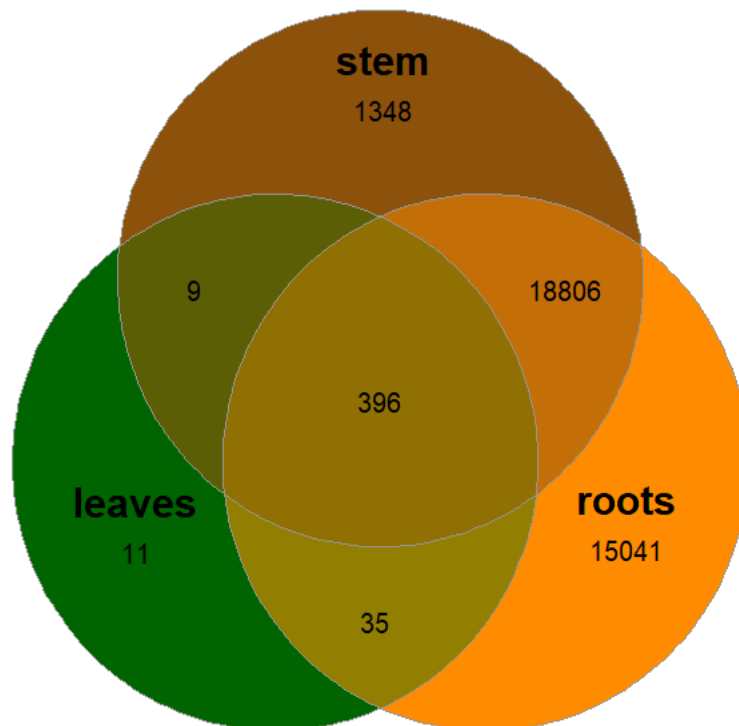


Figure 2. Venn's diagram of operational taxonomic units shared among leaves, stem and root samples of *C. canephora*.

The analysis of the susceptibility category revealed that the resistant samples had the lowest number of unique OTUs with 1,954, while the asymptomatic samples had the highest number with 2,473 OTUs (Figure 3).

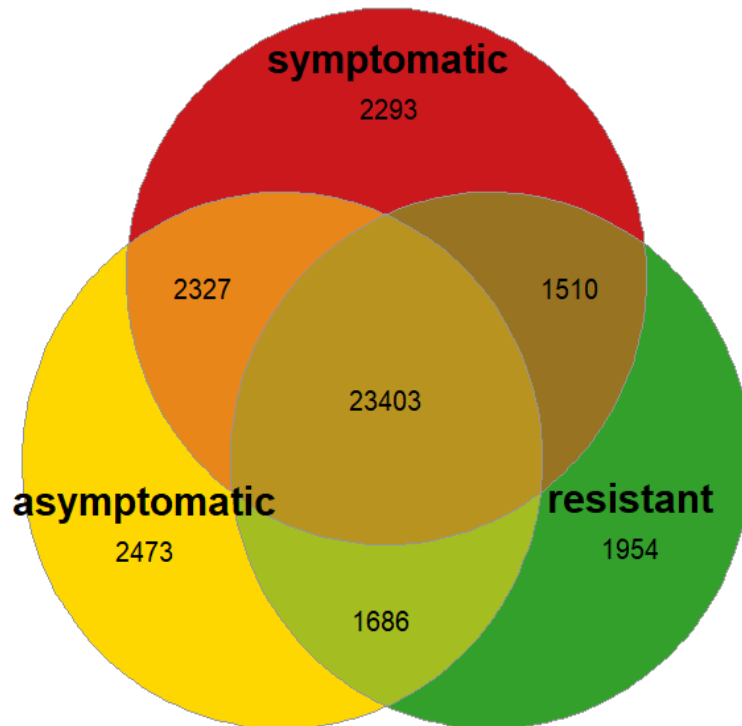


Figure 3. Venn's diagram of operational taxonomic units shared among the susceptibility categories of *C. canephora* samples collected.

1. Diversity and Composition of Microbial Communities

Estimation of species richness based on unique taxa per sample per plant organ demonstrated that roots exhibited the highest species richness, followed by stems and leaves (Figure 4). The analysis of diversity in the *C. canephora* microbiome indicated that although there was not a high number of OTUs in the leaves, these organs harbored different fungal, bacterial, and archaeal taxa, in all the examined samples (Figure 5).

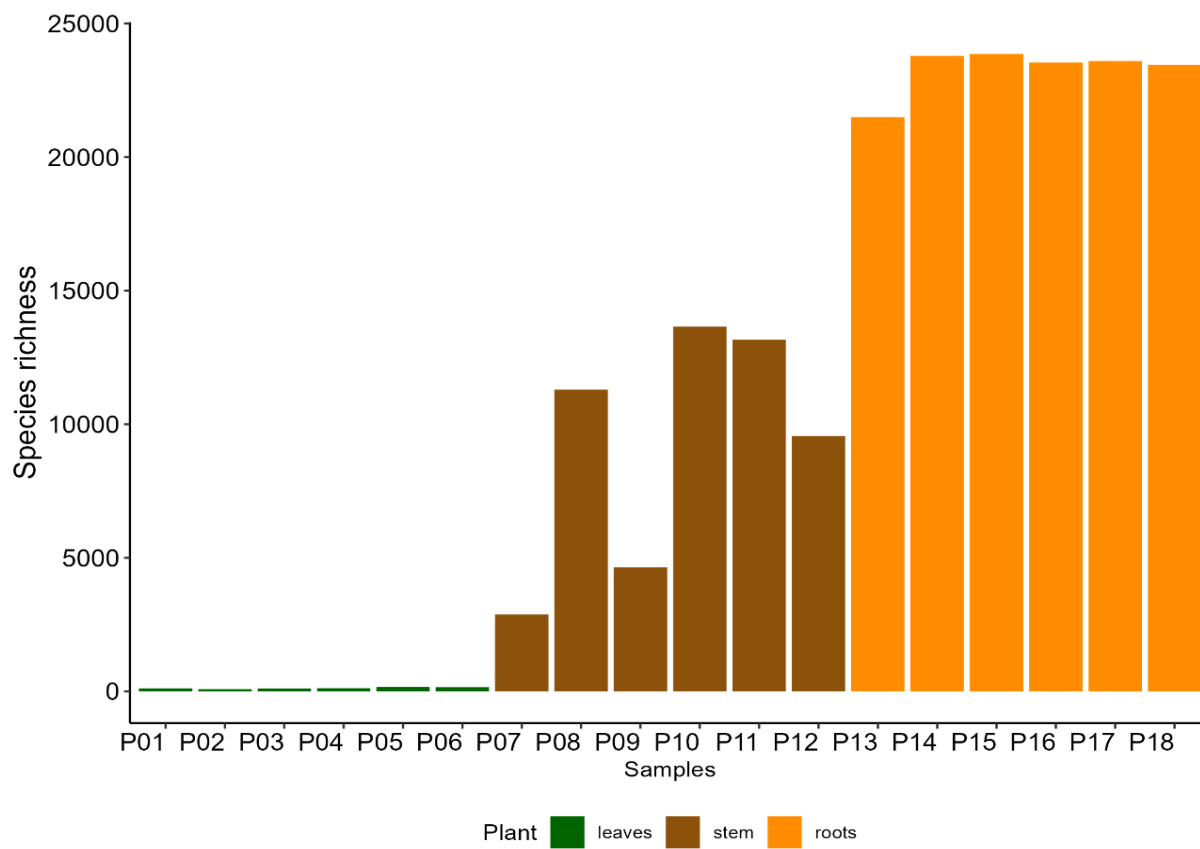


Figure 4. Species richness based on leaves, stem and root samples of *C. canephora*.

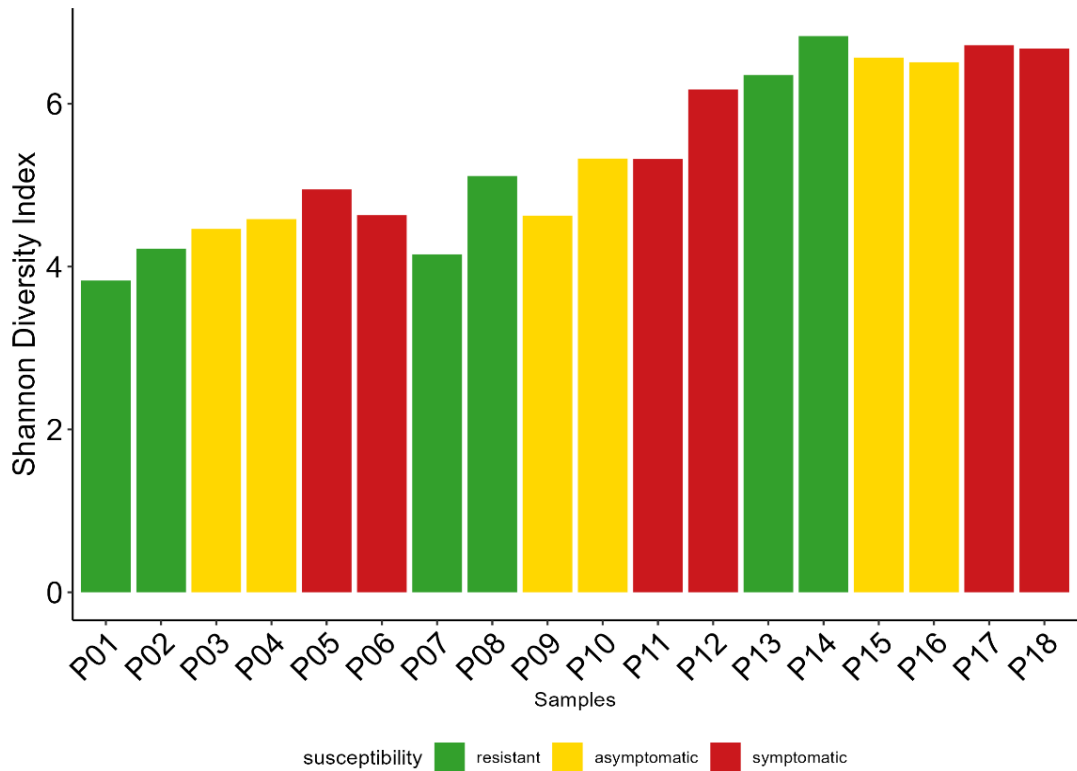


Figure 5. Shannon diversity index based on the susceptibility category.

The analysis of the *C. canephora* microbiome relative abundance indicated Ascomycota as the predominant phylum, followed by Bacteroidota, Discosea and Pseudomonadota. These findings suggest that the microbial communities in *C. canephora* are diverse but consistently dominated by the same few phyla across different plant parts and susceptibility categories (Figure 6).

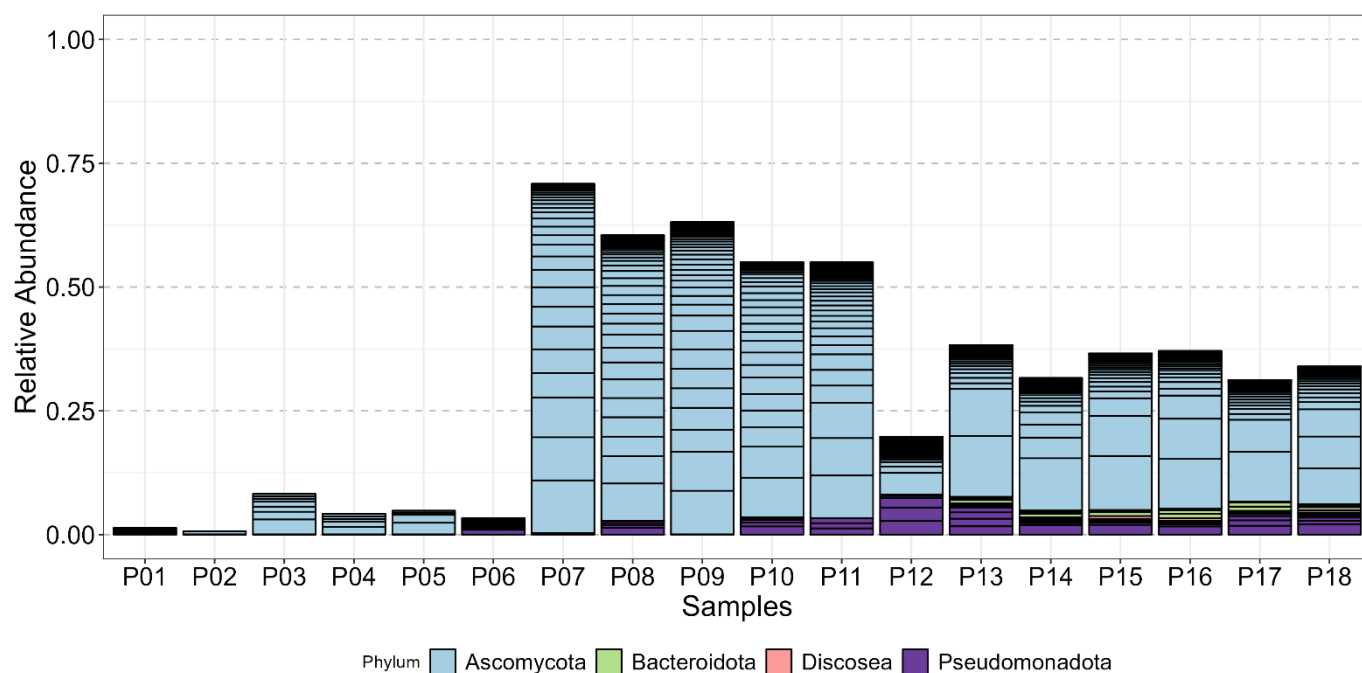


Figure 6. Relative abundance of the main phyla present in the samples of *C. canephora* microbiome.

2. Mycobiome of *C. canephora*

The fungal diversity analysis of the *C. canephora* microbiome revealed a high number of fungal taxa in the samples analyzed. The observed species richness of fungi was highest in the root samples, followed by the stem and leaf samples. Overall, the results suggest that the plant organs of *C. canephora* harbor diverse and distinct microbial communities, with the roots exhibiting the highest diversity. Shannon diversity index showed relatively low fungal diversity in leaf samples, while stem and root samples exhibited higher fungal diversity, regardless of susceptibility category. Inverted Simpson index displayed a more variable distribution of fungal species in leaf and stem samples and a more even distribution in root samples. For stem samples, a larger fluctuation in fungal diversity was observed compared to leaf and root samples (Figure 7).

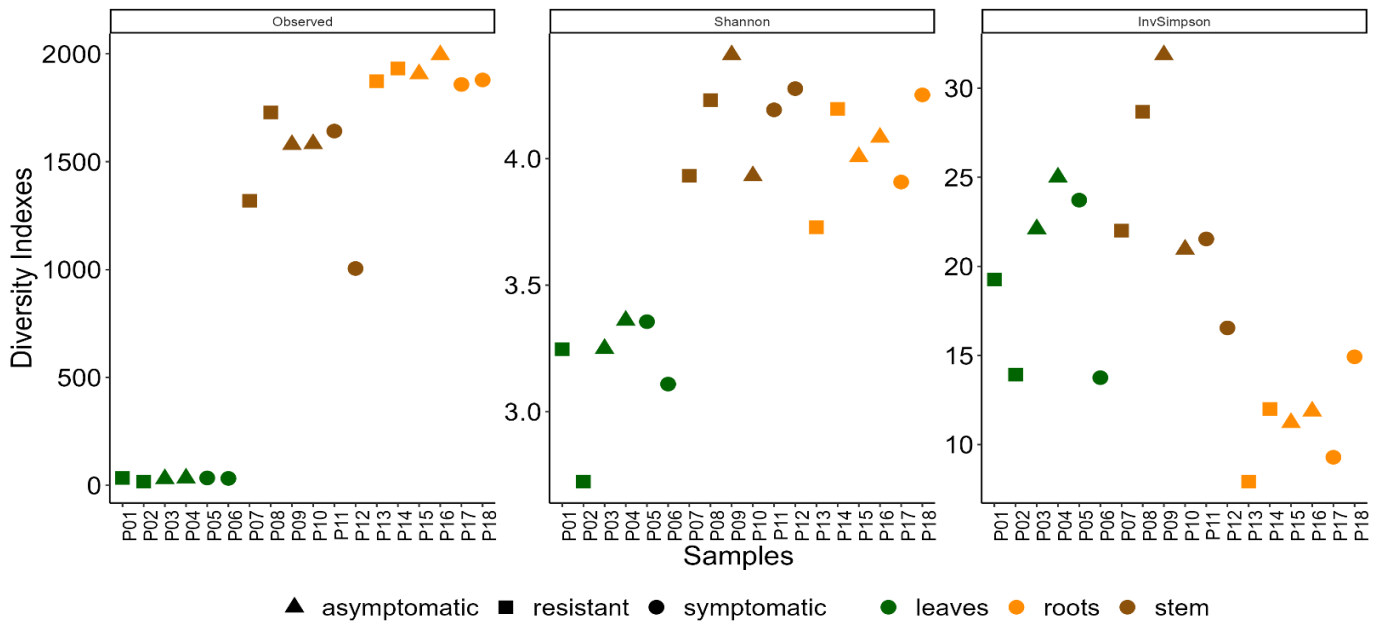


Figure 7. Observed richness, Shannon and InvSimpson diversity indices of fungal communities of *C. canephora* mycobiome, represented by plant part and susceptibility categories.

NMDS analyses indicated that the fungal communities in leaf, stem, and root samples of *C. canephora* were distinct from each other, further highlighting the variability and uniqueness of the fungal communities across different plant organs within the microbiome (Figure 8).

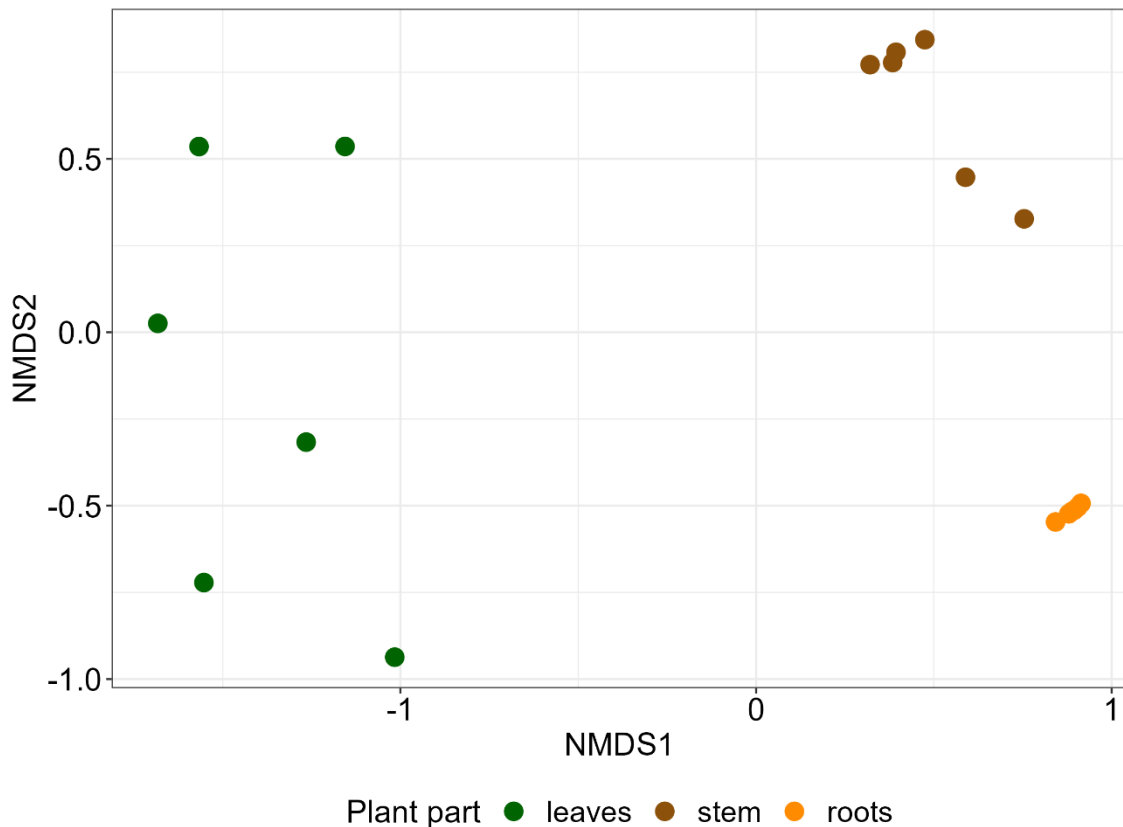


Figure 8. Non-metric multidimensional scaling analysis demonstrated the composition of fungal communities in leaf, stem, and root samples of *C. canephora*.

3. *Mycobiome in stem samples*

In stem samples, the fungal communities were found to vary based on the susceptibility category and age of the plant. The resistant samples P06 and P07 showed no distinct fungal communities among different age groups, suggesting a stable and consistent fungal composition. The asymptomatic sample P08 also displayed a similar pattern, indicating a consistent fungal community regardless of plant age, grouping together with the resistant samples. On the other hand, the asymptomatic sample P09 and the symptomatic samples P11 and P12 exhibited more variability in their fungal communities based on the age of the plant. The most diverse sample in terms of fungal species composition was P11, which had the highest number of unique fungal taxa compared to other stem samples (Figure 9).

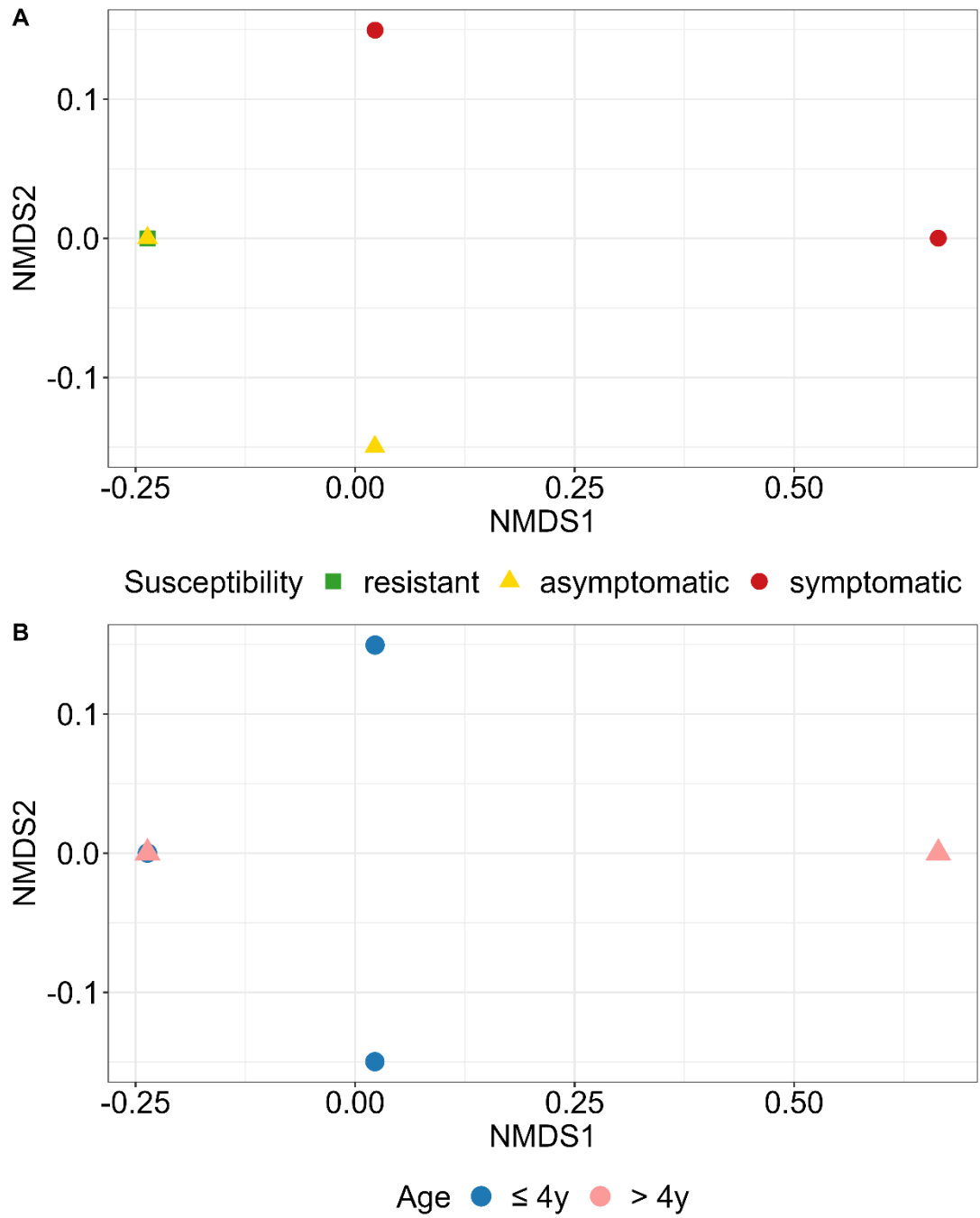


Figure 9. NMDS analysis based on susceptibility category and age of the plant. A: display of stem samples according to susceptibility category; B: display of stem samples according to age of the plant.

When comparing asymptomatic and symptomatic stem samples (P09 to P12), 28 out of 60 species were found to be unique to the symptomatic samples. These included the majority of accessions of *Fusarium* spp., *Rhinocladiella mackenziei*, *Phialophora attinorum*, *Exophiala* spp., *Cyphellophora europaea*, *Neopestalotiopsis cubana*, *Clonostachys* spp., *Chaetothryales* spp., *Capronia* spp., and *Botryobasidium botryosum* (Figure 10, supplementary material table S11).

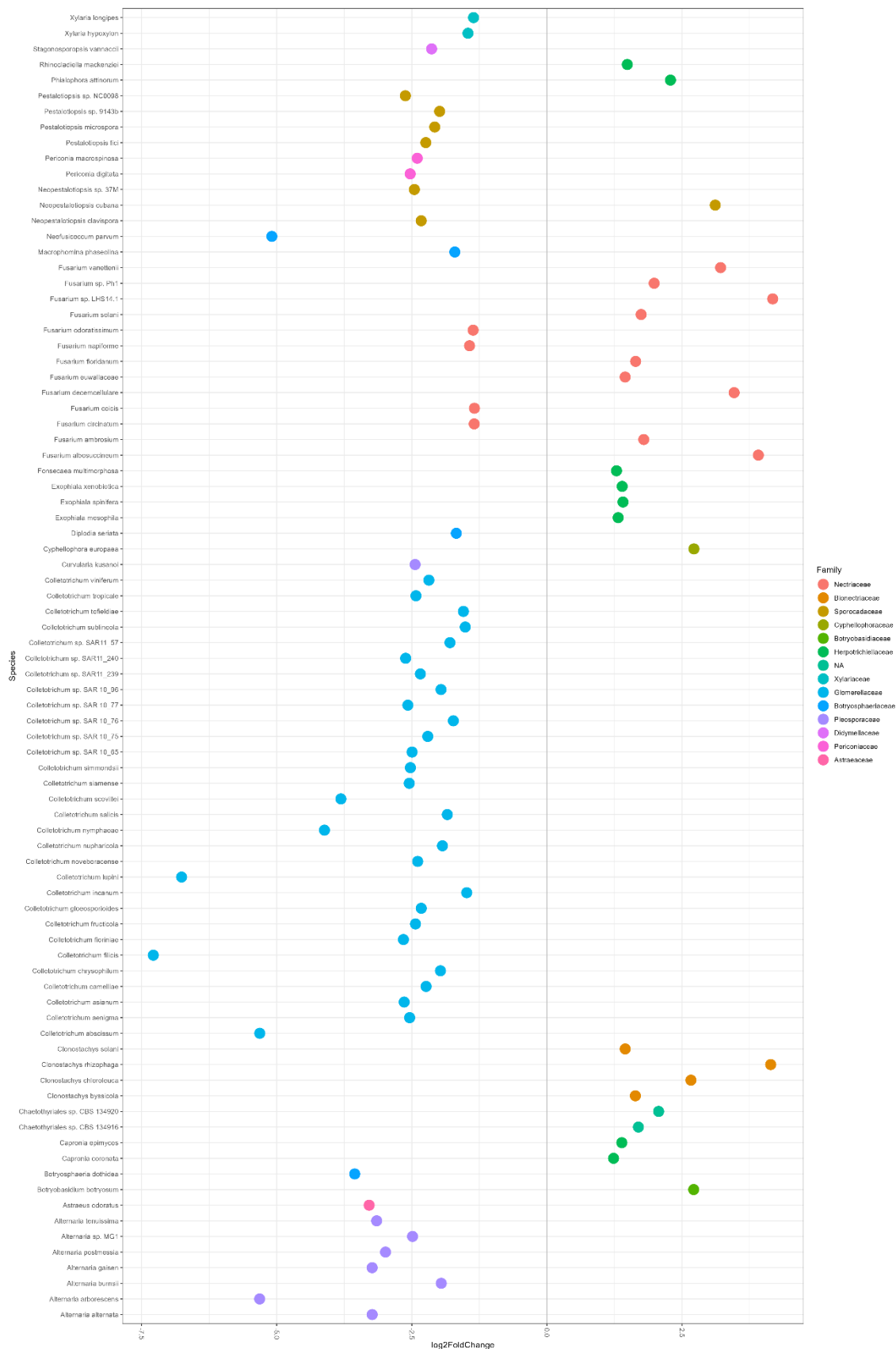


Figure 10. Differential abundance of fungal species in asymptomatic and symptomatic stem samples of *C. canephora* based on the $\log_2\text{FoldChange}$ statistic. Colored dots represent the families each of the species belong to.

When splitting the symptomatic samples according to the age of the plant, 20 out of 32 species were found to be unique to plants over 4 years old. Of those, *Cladophialophora bantiana* and two *Fusarium* spp. were found exclusively in plants less than 4 years old. *Neopestalotiopsis cubana* and *Fusarium fujikuroi* were found exclusively in 4-year-old plants or older. These findings suggest that the fungal communities in the stem samples of *C. canephora* are influenced by both the susceptibility category and the age of the plant (Figure 11, supplementary material table S12).



Figure 11. Fungal species present in symptomatic samples of *C. canephora* according to the age of the plant.

4. DISCUSSION

Why a biotic stress, a disease in specific, seems to be the best explanation of the "anomaly" observed in conilon plants in ES and southern BA states in Brazil? Several clues point to a microbial origin for this condition as there are no other obvious explanations. Moreover, the anomaly follows a specific pattern: it begins seemingly randomly in a susceptible plant and then appears to transmit its symptoms to nearby healthy ones. The management methods in the fields remain consistent for both symptomatic and healthy plants, and with no observed pests matching the occurrence of the problem, it suggests potential involvement of microbial transmission. The nature of the symptoms is another indicator of a possible microbial disease - wilting and yellowing leaves, twisting petioles, and cankerous lesions on stems and branches are classic signs of microbial involvement. Additionally, the occurrence of this anomaly in previously considered resistant clones indicates that a new pathogen or variation of an existing pathogen may be at play.

Plenty are the examples of plant diseases caused by phytopathogenic fungi, such as *Fusarium* spp. (Sandoval-Denis *et al.*, 2018; Zamora-Ballesteros *et al.*, 2019; Zakaria, 2023), *Ceratocystis* spp. (de Beer *et al.*, 2014; Tsopelas *et al.*, 2017; Barnes *et al.*, 2018); *Diaporthe* spp. (Mostert *et al.*, 2001; Guarnaccia *et al.*, 2018), *Diplodia* spp. (Punithalingam; Waller, 1973; Jiao *et al.*, 2014; Hlaiem *et al.*, 2022; Bhat *et al.*, 2023), *Botryosphaeria* spp. (Van Niekerk *et al.*, 2004; Slippers and Wingfield, 2007; Yang *et al.*, 2017; Pan *et al.*, 2022), and many others, that lead to similar symptoms in various crops worldwide. The spread of this anomaly in the conilon plants in ES and southern BA aligns with characteristic symptoms seen in fungal infections.

The analysis of diversity of prokaryotes, bacteria and archaea, did not provide strong support for the involvement of these microbes with SSC. While Bacteroidota, Discosea, and Pseudomonadota were found to be among the most prevalent phyla, in 50% of the samples Ascomycota was identified as the predominant phylum. Despite that fact, SSC characteristic symptoms suggest a more prominent role of fungal pathogens rather than prokaryotes in the observed conilon plants, as stated above.

Overall, there were no highly contrasting differences regarding the abundance of species that could point to a specific causal agent of SSC, nevertheless, it was possible to reduce the list of potential candidates of etiological agents.

The conilon plant organs had marked differences regarding colonizing microorganisms. Leaves had the lowest species richness while stems and roots are apparently habitats of higher diversity. A notable variation in the relative abundance of OTUs was observed when comparing stem samples from asymptomatic and symptomatic plants, as well as among symptomatic samples based on age.

Rhinocladiella spp., *Phiallophora* spp., *Fonsecaea* spp., *Exophiala* spp., *Cyphellophora* spp., *Chaetothyrales* spp., and *Capronia* spp. present in the symptomatic stem samples are opportunistic pathogens affecting humans and animals (Crous *et al.*, 2007; de Hoog *et al.*, 2011; Feng *et al.*, 2014; Shen *et al.*, 2020; Pádua *et al.*, 2022). They have also been reported to cause sooty blotch and flyspeck disease in certain fruit crops or have associations with ants (Grabowski, 2007; Gao *et al.*, 2015; Sánchez *et al.*, 2019; Bizarría *et al.*, 2021; Quan *et al.*, 2022). There is a possibility that some of these fungi may also parasitize plants as saprophytes, but further discussion on this topic is beyond the scope of this study.

Colletotrichum spp., *Fusarium* spp., *Alternaria* spp., *Pestalotiopsis* spp., and *Neopestalotiopsis* spp. are among the most common fungal pathogens causing plant diseases worldwide and were found mainly in the asymptomatic stem samples. Although eleven *Fusarium* spp. had a unique presence in the symptomatic samples. The identification of various fungal taxa in the symptomatic samples indicates the potential involvement of phytopathogenic fungi in causing stem canker disease in conilon plants.

Diplodia seriata, first found in asymptomatic samples, was also present in symptomatic plants less than 4-years-old. This fungus is responsible for causing canker, dieback, fruit rot and leaf spots on economically significant forest and horticultural species like apples, pine trees, and grapevines (Bhat *et al.*, 2023).

Alternaria is a diverse genus of fungal species responsible for a range of plant diseases such as leaf spots and cankers (Malathrakis, 1986). In our study, we discovered *A. ventricosa* and *A. arborescens* in stem samples younger than 4 years old. These fungi have been linked to post-harvest rot in European pears (DeShields and Achala, 2021), leaf blotch and defoliation of *Celtis julianae* in China (Liao *et al.*, 2023), as well as necrotic leaf spots in *Pereskia aculeata* from Brazil (Hahn *et al.*, 2021). However, despite the significance of *Alternaria* spp. as a plant pathogen, none of these symptoms matched the observed stem canker disease in conilon plants.

Colletotrichum abscissum took a similar course. It was mainly present in the asymptomatic samples, but also in symptomatic samples less than 4-years-old. The fungus is responsible for postbloom fruit drop and is considered one of the most serious citrus diseases in Brazil. It survives without asymptotically on vegetative tissue (Savi *et al.*, 2021; Goulin *et al.*, 2023).

Fungal pathogens found in the samples of plants over 4-years-old included *Neopestalotiopsis cubana*, which has been reported as the cause of leaf fall disease of rubber trees in Thailand (Thaochan *et al.*, 2020) and brown spots on the leaves of *Ixora chinensis* from Malaysia (Khoo *et al.*, 2022). Another significant fungal pathogen present was *Fusarium fujikuroi*, known to cause various plant diseases such as fusarium wilt in crops like rice, maize, and bananas with devastating effects on agricultural production and yield (Yilmaz *et al.*, 2021).

F. fujikuroi is also a species complex that consists of multiple genetically distinct groups or lineages, each with its own host range and pathogenic capabilities (Yilmaz *et al.*, 2021). Among its members is *F. xylarioides*, a soil-borne fungal pathogen regarded as the causal agent of fusarium wilt disease (CWD) in coffee plants. CWD originally appeared as a destructive disease affecting *C. canephora* crops in West and Central Africa from the 1920s to 1960s, resurfacing in the 1970s and rapidly spreading throughout the 1990s and 2000s. Around the same time, there were also reports of its occurrence in Ethiopia on arabica coffee (Rutherford, 2006; Peck *et al.*, 2021). After nearly two decades since the last significant outbreaks, CWD has quietly disappeared from the radar. Early indications of CWD include leaf yellowing, folding, and inward curling. The leaves then become floppy, dry up, turn brown, and eventually drop off. Affected branches may also darken to black-brown or blackish and shrivel. These symptoms initially appear on one side of the tree but quickly spread throughout it. Infected trees also display prematurely ripening red berries due to water scarcity resulting from fungal invasion blocking xylem vessels. Swollen bark with vertical or spiral cracks near the trunk base is another characteristic feature in affected trees along with blue-black discoloration directly beneath it (Peck and Boa, 2023). Although there was no darkening of vessels observed in the samples collected in our study, CWD presents some resemblance to SCC's symptoms.

In that regard, the bark disease of coffee caused by *Fusarium stilboides*, although not present in our metagenomic study, is another important disease affecting coffee plants caused by a *Fusarium* species. Its symptoms involve the formation of

lesions at specific points of the trunk and branches that start with the flaking of the external parts of the bark which fall off and lead to the formation of cankers, the loss of the cambium and exposure of the heartwood and girdling of stem and trunk and progressive decline of plants (Siddiqi; Corbett, 1963; Rutherford, 2006).

Interestingly enough, all data seems to indicate a *Fusarium* involvement in SCC etiology and its resemblance to coffee wilt Disease and bark disease warrants further investigation into the role of *Fusarium* species in causing the disease in conilon plants.

The metagenomic approach used in the present study broadened the options of analysis given that it allows for the detection of yet uncultivated microorganisms associated with a disease. Despite its limitations including sample size, potential biases in DNA extraction, the reference database, and bioinformatic processing, the traditional methods of microbial isolation and culturing have their limitations too - intensive labor, time consuming activities and potential risk of miss important specific taxa. Along with the fact that metagenomic analysis allows for the detection of rare or low abundance microbes that may play a role in disease etiology, it offers a more comprehensive understanding of the microbial community associated with the disease (Piombo *et al.*, 2021; Baldrian *et al.*, 2022).

Considering only curated entries in the reference databases and the genus/species known to be associated with diseases of similar symptomatology and pathogenesis, the best evidence gathered so far points to the involvement of *Fusarium* sp. as the associated fungus with SCC.

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SUPPLEMENTARY MATERIAL

Table S1. Municipalities engaged in conilon cultivation in the state of Espírito Santo (ES) and Bahia (BA) in relation of planted area until 2017.

Municipality	State	Planted area (ha)	Latitude	Longitude
Afonso Cláudio	ES	5517	-20.0789475	-41.1254351
Águia Branca	ES	5461	-18.9844626	-40.7428347
Água Doce do Norte	ES	2395	-18.5473513	-40.9799144
Alegre	ES	2136	-20.7632702	-41.5337962
Alfredo Chaves	ES	3708	-20.6376213	-40.7525287
Alto Rio Novo	ES	1384	-19.0584494	-41.0201604
Anchieta	ES	1261	-20.8121688	-40.636727
Apiacá	ES	340	-21.1561284	-41.5674594
Aracruz	ES	5243	-19.8224433	-40.2812643
Atílio Vivácqua	ES	1203	-20.9152943	-41.199084
Baixo Guandu	ES	2090	-19.5134388	-41.0154194
Barra de São Francisco	ES	5021	-18.7569647	-40.8938483
Boa Esperança	ES	5417	-18.5398554	-40.3009198
Cachoeiro de Itapemirim	ES	1191	-20.8535945	-41.1777506
Castelo	ES	1529	-20.6044812	-41.2059016
Colatina	ES	1976	-19.5527337	-40.7578803
Conceição da Barra	ES	273	-18.5945522	-40.2584005
Conceição do Castelo	ES	366	-20.3574309	-41.247391
Ecoporanga	ES	1419	-18.3730676	-40.835178
Fundão	ES	2156	-19.9336623	-40.407621
Governador Lindenberg	ES	9785	-19.2501392	-40.4661242
Guarapari	ES	1047	-20.640295	-40.4842826
Ibiraçu	ES	1952	-19.8307425	-40.371462
Iconha	ES	3918	-20.78686	-40.8165919
Itaguaçu	ES	4373	-19.8031361	-40.858508
Itapemirim	ES	603	-21.0105659	-41.3612248
Itarana	ES	1647	-19.8721078	-40.8781042
Jaguaré	ES	14669	-18.9055078	-40.0774762
Jerônimo Monteiro	ES	2133	-20.7870245	-41.3896608
João Neiva	ES	1212	-19.798575	-40.4394942
Laranja da Terra	ES	2480	-19.8986208	-41.0599886
Linhares	ES	17245	-19.3156373	-40.1791975
Mantenópolis	ES	688	-18.8624507	-41.1250253
Marilândia	ES	8135	-19.4108129	-40.5460819

Mimoso do Sul	ES	5294	-21.0629471	-41.3660855
Montanha	ES	3827	-18.1264771	-40.3668721
Muniz Freire	ES	1202	-20.4641077	-41.4154603
Muqui	ES	4873	-20.9517249	-41.3486079
Nova Venécia	ES	13727	-18.7098323	-40.4026823
Pancas	ES	11012	-19.2238152	-40.8519583
Pedro Canário	ES	1379	-18.3060714	-39.9576817
Pinheiros	ES	6416	-18.4110434	-40.2170517
Rio Bananal	ES	18207	-19.2723685	-40.3293021
Rio Novo do Sul	ES	2631	-20.8635753	-40.9394391
Santa Leopoldina	ES	2655	-20.0996953	-40.5288336
Santa Teresa	ES	5144	-19.9356105	-40.5984873
São Domingos do Norte	ES	5916	-19.1536053	-40.6283972
São Gabriel da Palha	ES	7985	-19.0204801	-40.5353031
São Mateus	ES	14907	-18.7204845	-39.8792519
São Roque do Canaã	ES	3628	-19.7379163	-40.9227351
Sooretama	ES	8804	-19.1902843	-40.0988755
Vargem Alta	ES	2702	-20.6693075	-41.008477
Viana	ES	498	-20.3893301	-40.4976197
Vila Pavão	ES	4057	-18.6204261	-40.6082954
Vila Valério	ES	12028	-18.9988424	-40.3919764
Alcobaça	BA	1264	-17.5411955	-39.1949621
Camacan	BA	1055	-15.4218827	-39.4971831
Eunápolis	BA	4147	-16.3649627	-39.5932137
Guaratinga	BA	1698	-16.5867097	-39.7852878
Itabela	BA	4242	-16.572891	-39.5609714
Itamaraju	BA	9134	-17.0436704	-39.5407426
Itanhém	BA	237	-17.1717446	-40.3324895
Jucuruçu	BA	1019	-16.8432525	-40.1609053
Porto Seguro	BA	4858	-16.4482687	-39.069963
Prado	BA	4131	-17.3354963	-39.2325834
Santa Luzia	BA	1153	-15.4319122	-39.335269
Teixeira de Freitas	BA	1930	-17.5358987	-39.769441

Source: IBGE (2017).

Table S2.1. GenBank accession numbers of DNA sequences of *Fusarium* species complexes used in the phylogenetic analyses.

Species	Collection number	GenBank accession number	
		<i>tef</i>	<i>rpb2</i>
<i>Fusarium oxysporum</i> species complex			
<i>Fusarium callistephi</i>	CBS 187.53T	MH484966	MH484875
<i>F. carminascens</i>	CPC 25800T	MH485028	MH484937
<i>F. carminascens</i>	CPC 25792	MH485025	MH484934
<i>F. contaminatum</i>	CBS 114899T	MH484992	MH484901
<i>F. contaminatum</i>	CBS 117461	MH485002	MH484911
<i>F. cugenangense</i>	InaCC F984T	LS479757	LS479308
<i>F. cugenangense</i>	CBS 620.72	MH484970	MH484879
<i>F. curvatum</i>	CBS 238.94T	MH484984	MH484893
<i>F. curvatum</i>	CBS 141.95	MH484985	MH484894
<i>F. duoseptatum</i>	CBS 102026	MH484987	MH484896
<i>F. duoseptatum</i>	InaCC F916T	LS479688	LS479239
<i>F. elaeidis</i>	CBS 217.49	MH484961	MH484870
<i>F. elaeidis</i>	CBS 218.49	MH484962	MH484871
<i>F. fabacearum</i>	CPC 25802T	MH485030	MH484939
<i>F. fabacearum</i>	CPC 25803	MH485031	MH484940
<i>F. foetens</i>	NRRL 31852T	AY320087	MW928825
<i>F. glycines</i>	CPC 25808T	MH485033	MH484942
<i>F. glycines</i>	CPC 25804	MH485032	MH484941
<i>F. gossypinum</i>	CBS 116613T	MH485000	MH484909
<i>F. gossypinum</i>	CBS 116612	MH484999	MH484908
<i>F. grosnichelii</i>	InaCC F833T	LS479744	LS479295
<i>F. grosnichelii</i>	InaCC F848	LS479786	LS479338
<i>F. hexaseptatum</i>	InaCC F866T	LS479805	LS479359
<i>F. hoodiae</i>	CBS 132474T	MH485020	MH484929
<i>F. hoodiae</i>	CBS 132476	MH485021	MH484930
<i>F. inflexum</i>	NRRL 20433T	AF008479	JX171583
<i>F. kalimantanense</i>	InaCC F917 = Indo 110	LS479690	LS479241
<i>F. kalimantanense</i>	InaCC F918 = Indo115	LS479695	LS479246
<i>F. languescens</i>	CBS 645.78T	MH484971	MH484880

<i>F. languescens</i>	CBS 300.91	MH484982	MH484891
<i>F. libertatis</i>	CPC 28465T	MH485035	MH484944
<i>F. libertatis</i>	CPC 25788	MH485024	MH484933
<i>F. nirenbergiae</i>	CBS 840.88T	MH484978	MH484887
<i>F. nirenbergiae</i>	CBS 130300	MH485016	MH484925
<i>F. odoratissimum</i>	InaCC F822ET	LS479828	LS479386
<i>F. oxysporum</i>	CBS 144134ET	MH485044	MH484953
<i>F. oxysporum</i>	CBS 144135	MH485045	MH484954
<i>F. pharetrum</i>	CPC 30824T	MH485043	MH484952
<i>F. pharetrum</i>	CPC 30822	MH485042	MH484951
<i>F. phialophorum</i>	InaCC F971ET	LS479741	LS479292
<i>F. sangayamense</i>	InaCC F960ET	LS479732	LS479283
<i>F. tardichlamydosporum</i>	CBS 102028	MH484988	MH484897
<i>F. tardichlamydosporum</i>	InaCC F958T	LS479729	LS479280
<i>F. tardicrescens</i>	NRRL 36113T	LS479665	LS479217
<i>F. triseptatum</i>	CBS 258.50 = NRRL 36389T	MH484964	MH484873
<i>F. triseptatum</i>	CBS 116619	MH485001	MH484910
<i>F. veterinarianum</i>	CBS 109898 = NRRL 36153T	MH484990	MH484899
<i>F. veterinarianum</i>	NRRL 62547	MH485040	MH484949
<i>F. redolens</i>	NRRL 25600	MT409453	MT409443
Species			
Collection number		GenBank accession number	
		<i>tef</i>	<i>rpb2</i>
<i>Fusarium incarnatum-equiseti</i> species complex			
<i>F. aberrans</i>	CBS 131385T	MN170445	MN170378
<i>F. aberrans</i>	CBS 119866	MN170444	MN170377
<i>F. arcuatisporum</i>	LC12147	MK289584	MK289739
<i>F. arcuatisporum</i>	NRRL 32997	GQ505624	GQ505802
<i>F. brevicaudatum</i>	NRRL 43694	GQ505668	GQ505846
<i>F. brevicaudatum</i>	NRRL 43638T	GQ505665	GQ505843
<i>F. bubalinum</i>	CBS 161.25 = NRRL 26918T	MN170448	MN170381
<i>F. caatingaense</i>	URM 6779 T	LS398466	LS398495
<i>F. caatingaense</i>	URM 6776	LS398463	LS398492
<i>F. cateniforme</i>	CBS 150.25T	MN170451	MN170384
<i>F. citri</i>	LC6896T	MK289617	MK289771
<i>F. citri</i>	LC4879	MK289615	MK289768

<i>F. clavum</i>	CBS 126202T	MN170456	MN170389
<i>F. clavum</i>	NRRL 34032	GQ505635	GQ505813
<i>F. coffeatum</i>	CBS 635.76T	MN120755	MN120736
<i>F. coffeatum</i>	CBS 430.81	MN120756	MN120737
<i>F. compactum</i>	NRRL 36323T	GQ505648	GQ505826
<i>F. compactum</i>	NRRL 28029	GQ505602	GQ505780
<i>F. croceum</i>	CBS 131777T	MN170463	MN170396
<i>F. croceum</i>	CBS 131788	MN170464	MN170397
<i>F. duofalcatisporum</i>	NRRL 36448T	GQ505652	GQ505830
<i>F. duofalcatisporum</i>	NRRL 36401	GQ505651	GQ505829
<i>F. equiseti</i>	NRRL 26419NT	GQ505599	GQ505777
<i>F. equiseti</i>	NRRL 20697	GQ505594	GQ505772
<i>F. fasciculatum</i>	CBS 131382T	MN170473	MN170406
<i>F. fasciculatum</i>	CBS 131383	MN170474	MN170407
<i>F. flagelliforme</i>	NRRL 36269T	GQ505645	GQ505823
<i>F. flagelliforme</i>	NRRL 6548	GQ505589	GQ505767
<i>F. gracilipes</i>	NRRL 43635T	GQ505662	GQ505840
<i>F. guilinense</i>	LC12160T	MK289594	MK289747
<i>F. hainanense</i>	LC11638T	MK289581	MK289735
<i>F. hainanense</i>	NRRL 26417	GQ505598	GQ505776
<i>F. humuli</i>	CQ1039T	MK289570	MK289724
<i>F. humuli</i>	CQ1032	MK289568	MK289722
<i>F. incarnatum</i>	CBS 132.73T	MN170476	MN170409
<i>F. incarnatum</i>	CBS 132907	MN170477	MN170410
<i>F. ipomoeae</i>	LC12165T	MK289599	MK289752
<i>F. ipomoeae</i>	LC12164	MK289598	MK289751
<i>F. irregulare</i>	LC7188T	MK289629	MK289783
<i>F. irregulare</i>	NRRL 31160	GQ505607	GQ505785
<i>F. lacertarum</i>	NRRL 20423T	GQ505593	JX171581
<i>F. lacertarum</i>	NRRL 36123	GQ505643	GQ505821
<i>F. longicaudatum</i>	CBS 123.73T	MN170481	MN170414
<i>F. longifundum</i>	NRRL 36372T	GQ505649	GQ505827
<i>F. luffae</i>	LC12167T	MK289601	MK289754
<i>F. luffae</i>	CQ1038	MK289569	MK289723
<i>F. monophialidicum</i>	NRRL 54973	MN170483	MN170416
<i>F. mucidum</i>	CBS 102395T	MN170485	MN170418
<i>F. mucidum</i>	CBS 102394	MN170484	MN170417
<i>F. multiceps</i>	NRRL 43639T	GQ505666	GQ505844
<i>F. nanum</i>	LC12168T	MK289602	MK289755
<i>F. nanum</i>	NRRL 32868	GQ505617	GQ505795
<i>F. neoscirpi</i>	NRRL 26922T	GQ505601	GQ505779
<i>F. pernambucanum</i>	URM 7559 T	LS398489	LS398519
<i>F. pernambucanum</i>	URM 6801	LS398483	LS398513

<i>F. persicinum</i>	CBS 479.83T	MN170495	MN170428
<i>F. persicinum</i>	CBS 131780	MN170496	MN170429
<i>F. scirpi</i>	NRRL 36478NT	GQ505654	GQ505832
<i>F. scirpi</i>	CBS 448.84	MN170498	MN170431
<i>F. serpentinum</i>	CBS 119880	MN170499	MN170432
<i>F. sulawesiense</i>	InaCC F940T	LS479443	LS479855
<i>F. sulawesiense</i>	CBS 163.57	MN170501	MN170434
<i>F. tanahbumbuense</i>	InaCC F965T	LS479448	LS479863
<i>F. tanahbumbuense</i>	CBS 131009	MN170506	MN170439
<i>F. toxicum</i>	CBS 406.86T	MN170508	MN170441
<i>F. toxicum</i>	CBS 219.63	MN170507	MN170440
<i>F. concolor</i>	NRRL 13994	MH742650	MH742569

Species	Collection number	GenBank accession number <i>tef</i>	<i>rpb2</i>
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***Fusarium fujikuroi* species complex**

<i>F. acutatum</i>	CBS 402.97T	MW402125	MW402768
<i>F. acutatum</i>	CBS 401.97	MW402124	MW402813
<i>F. agapanthi</i>	NRRL 54463T	KU900630	KU900625
<i>F. agapanthi</i>	CBS 100193	MW401959	MW402727
<i>F. ananatum</i>	CBS 118516T	LT996091	LT996137
<i>F. ananatum</i>	CBS 118517	MN533988	MN534229
<i>F. andiyazi</i>	NRRL 31727T	MN193854	LT996138
<i>F. andiyazi</i>	CBS 119856	MN533989	MN534286
<i>F. annulatum</i>	CBS 258.54T	MT010994	MT010983
<i>F. annulatum</i>	CBS 115.97	MW401973	MW402785
<i>F. anthophilum</i>	CBS 222.76ET	MW402114	MW402811
<i>F. anthophilum</i>	CBS 119859	MN533991	MN534233
<i>F. awaxy</i>	LGMF 1930T	MG839004	MK766941
<i>F. awaxy</i>	CBS 139380	MN534058	MN534238
<i>F. babinda</i>	NRRL 25539	MH742712	JX171632
<i>F. babinda</i>	NRRL 26207	MH742691	MH742620
<i>F. bactridioides</i>	CBS 100057T	MN533993	MN534235
<i>F. bactridioides</i>	NRRL 20476	AF160290	-
<i>F. begoniae</i>	CBS 452.97T	MN533994	MN534243
<i>F. begoniae</i>	CBS 110282	MW401968	-
<i>F. brachiariae</i>	CML 3032T	MT901348	MT901314
<i>F. brachiariae</i>	CML 3163	MT901349	MT901315
<i>F. brevicatenulatum</i>	CBS 404.97T	MN533995	MN534295
<i>F. brevicatenulatum</i>	CBS 100196	MN193859	MN193887

<i>F. bulbicola</i>	NRRL 13618T	KF466415	MW402767
<i>F. caapi</i>	CML 3657T	MT901350	MT901316
<i>F. caapi</i>	CML 3658	MT901351	MT901317
<i>F. casha</i>	PPRI 21883 Ex- type	MF787261	MN605059
<i>F. casha</i>	PPRI 20462	MF787262	MN605060
<i>F. chinhoiense</i>	NRRL 25221T	MN534050	MN534262
<i>F. chinhoiense</i>	NY 001B5	MN534051	MN534263
<i>F. circinatum</i>	CBS 405.97T	MN533997	MN534252
<i>F. circinatum</i>	CBS 117843	MW401978	MW402786
<i>F. coicis</i>	NRRL 66233T	KP083251	KP083274
<i>F. concentricum</i>	CBS 450.97T	AF160282	JF741086
<i>F. concentricum</i>	FCTJX-3	MK609901	-
<i>F. curculicola</i>	PPRI 20458Ex- type	MF787266	MN605062
<i>F. curculicola</i>	PPRI 20464	MF787267	MN605063
<i>F. denticulatum</i>	CBS 407.97T	MN534000	MN534274
<i>F. denticulatum</i>	CBS 406.97	MN533999	MN534273
<i>F. dlamirii</i>	CBS 119860T	MW401995	KU171701
<i>F. dlamirii</i>	CBS 671.94	MN534004	MN534254
<i>F. echinatum</i>	CBS 146497	MW834273	MW834004
<i>F. ficicrescens</i>	CBS 125178T	KU604452	KT154002
<i>F. ficicrescens</i>	CBS 125181	KU604453	KT154003
<i>F. fracticaudum</i>	CMW 25237	KJ541058	-
<i>F. fracticaudum</i>	CMW 25245	KJ541059	-
<i>F. fractiflexum</i>	NRRL 28852T	AF160288	-
<i>F. fredkrugeri</i>	CBS 144209T	LT996097	LT996147
<i>F. fredkrugeri</i>	CBS 144495	LT996096	LT996146
<i>F. fujikuroi</i>	NRRL 13566	AF160279	JX171570
<i>F. fujikuroi</i>	CBS 221.76T	MN534010	KU604255
<i>F. globosum</i>	NRRL 26131T	KF466417	KF466406
<i>F. globosum</i>	CBS 429.97	LT746230	LT746343
<i>F. guttiforme</i>	CBS 409.97	MT010999	MT010967
<i>F. guttiforme</i>	NRRL 22945	AF160297	JX171618
<i>F. konzum</i>	CBS 119849T	LT996098	MW402733
<i>F. konzum</i>	CBS 119847	MW401990	-
<i>F. lactis</i>	CBS 411.97ET	MN193862	MN534275
<i>F. longicornicola</i>	NRRL 52706T	JF740788	JF741114

<i>F. longicornicola</i>	NRRL 52712	JF740794	JF741120
<i>F. lumajangense</i>	InaCCF872T	LS479441	LS479850
<i>F. lumajangense</i>	InaCCF993	LS479442	LS479851
<i>F. madaense</i>	CBS 146669T	MW402098	MW402764
<i>F. madaense</i>	CBS 146651	MW402096	MW402762
<i>F. mangiferae</i>	CBS 120994T	MN534017	MN534271
<i>F. mangiferae</i>	CBS 119853	MN534016	MN534270
<i>F. marasasianum</i>	CMW 25261T	KJ541063	-
<i>F. marasasianum</i>	CMW 25253	KJ541062	-
<i>F. mexicanum</i>	NRRL 53147T	GU737282	MN724973
<i>F. mexicanum</i>	NRRL 47473	GU737416	LR792615
<i>F. mundagurra</i>	RGB 5717T	KP083256	KP083276
<i>F. musae</i>	CBS 624.87T/ NRRL 25059T	FN552086	MW402772
<i>F. napiforme</i>	CBS 748.97T / NRRL 25196T	MN193863	MN534291
<i>F. nygamai</i>	CBS 749.97T	MW402151	EF470114
<i>F. nygamai</i>	CBS 119852	MW401992	MW402734
<i>F. ophioides</i>	CBS 118512T	MN534022	MN534303
<i>F. ophioides</i>	CBS 118510	MN534020	MN534301
<i>F. phyllophilum</i>	NRRL 13617T	MN193864	KF466410
<i>F. parvisorum</i>	CMW 25267T	KJ541060	-
<i>F. parvisorum</i>	CMW 25268	KJ541061	-
<i>F. pilosicola</i>	NRRL 29124T	MN534055	MN534248
<i>F. pilosicola</i>	NRRL 29123	MN534054	MN534247
<i>F. pininemorale</i>	CMW 25243	KJ541064	-
<i>F. pininemorale</i>	CMW 25244	KJ541065	-
<i>F. prieskaense</i>	CBS 146498T	MW834275	MW834007
<i>F. prieskaense</i>	CPC 30825	MW834274	MW834006
<i>F. proliferatum</i>	CBS 480.96ET	MN534059	MN534272
<i>F. pseudoanthophilum</i>	CBS 414.97T	MW402128	-
<i>F. pseudoanthophilum</i>	CBS 415.97	MW402129	-
<i>F. pseudocircinatum</i>	NRRL 22946T	AF160271	MN534277
<i>F. pseudocircinatum</i>	CBS 455.97	MN534029	MN534276
<i>F. pseudonygamai</i>	NRRL 13592T	AF160263	MN534285
<i>F. ramigenum</i>	NRRL 25208T	KF466423	KF466412
<i>F. ramigenum</i>	CBS 526.97	MN534032	MN534292

<i>F. sacchari</i>	CBS 223.76ET	MW402115	JX171580
<i>F. sacchari</i>	CBS 121683	MW402002	MW402789
<i>F. secorum</i>	NRRL 62593T	KJ189225	-
<i>F. siculi</i>	CPC 27188T	LT746214	LT746327
<i>F. siculi</i>	CPC 27189	LT746215	LT746328
<i>F. sororula</i>	CMW 25254	KJ541066	-
<i>F. sororula</i>	CMW 40578T	KJ541067	-
<i>F. sterilihyphosum</i>	NRRL 25623T	MN193869	MN193897
<i>F. sterilihyphosum</i>	NRRL 53997	GU737414	-
<i>F. subglutinans</i>	CBS 747.97NT	MW402150	MW402773
<i>F. subglutinans</i>	CBS 136481	MW402059	MW402748
<i>F. succisae</i>	NRRL 13613ET	AF160291	MW402766
<i>F. succisae</i>	CBS 187.34	MW402109	MW402810
<i>F. sudanense</i>	CBS 454.97T	MN534037	MN534278
<i>F. sudanense</i>	CBS 675.94	MN534038	MN534279
<i>F. temperatum</i>	MUCL 52463T	-	MW402776
<i>F. temperatum</i>	CBS 135538	MN534039	MN534239
<i>F. terricola</i>	CBS 483.94T	MN534042	LT996156
<i>F. thapsinum</i>	CBS 776.96T	MN534044	MN534289
<i>F. thapsinum</i>	CBS 119833	MW401987	MW402787
<i>F. tjaetaba</i>	RBG5361T	KP083263	KP083275
<i>F. tjaetaba</i>	RBG5363	KP083264	-
<i>F. tupaense</i>	CML 262T	DQ452859	-
<i>F. tupaense</i>	CML 345	DQ452861	-
<i>F. udum</i>	BBA 65058ET	KY498862	KY498875
<i>F. verticillioides</i>	CBS 218.76ET	MW402113	-
<i>F. verticillioides</i>	CBS 447.95	MW402133	MW402770
<i>F. volatile</i>	CBS 143874T	LR596007	LR596006
<i>F. xylarioides</i>	NRRL 25486T	AY707136	HM068355
<i>F. xyrophilum</i>	NRRL 62721T	MN193877	MN193905
<i>F. xyrophilum</i>	NRRL 62710	MN193875	MN193903
<i>F. werrikimbe</i>	CBS 125535T	MW928846	MN534304
<i>F. foetens</i>	CBS 110286T	AY320087	MW928825

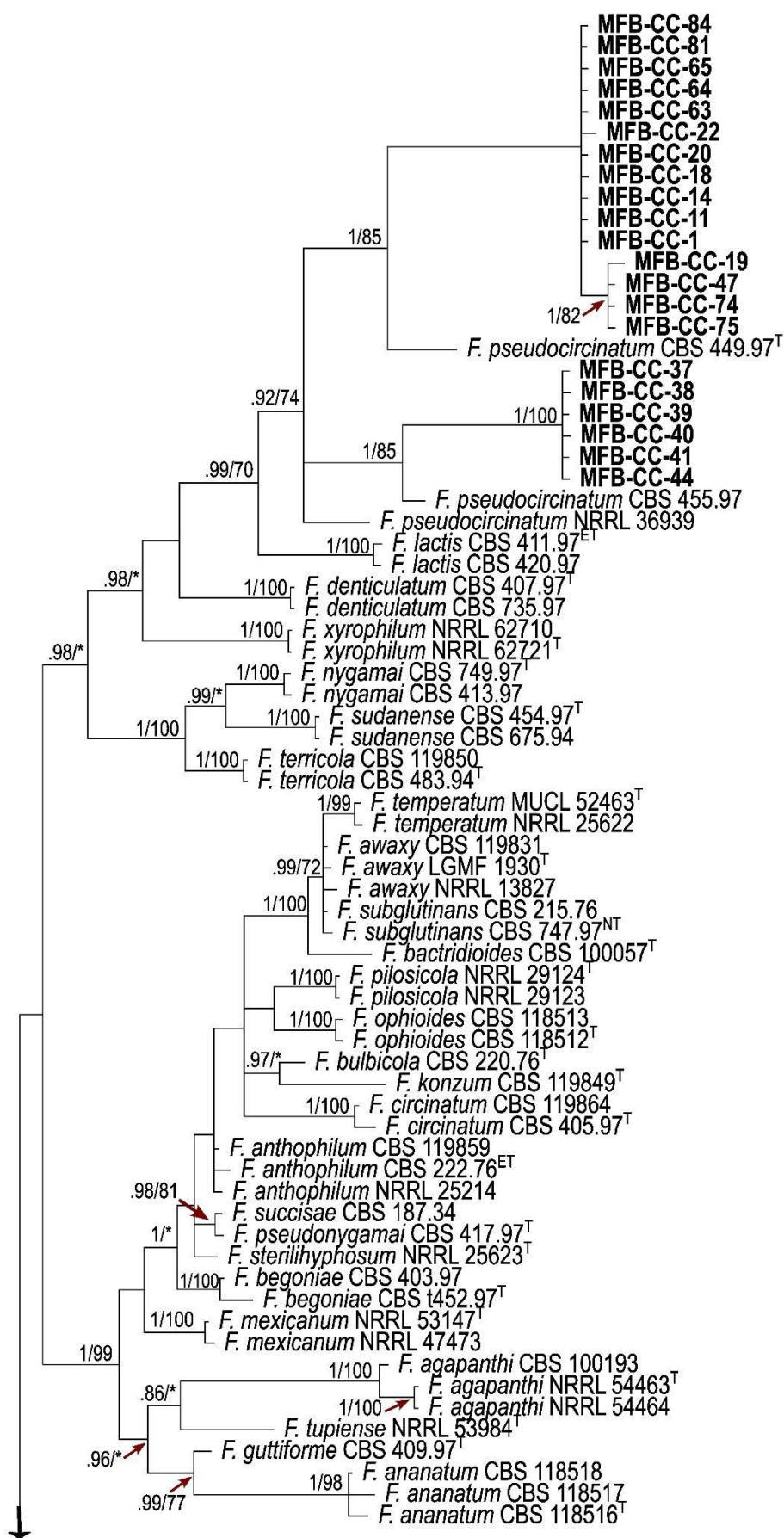
Species	Collection number	GenBank accession number <i>tef</i>	<i>rpb2</i>
<i>Fusarium solani</i> species complex			
<i>F. atrofusca</i>	NRRL 22316	AF178361	EU329502
<i>F. cicatricum</i>	CBS 125552	HM626644	HQ728153
<i>F. ampla</i>	CBS 202.32T	LR583594	LR583815
<i>F. bataticola</i>	CBS 144397	AF178343	EU329509

<i>F. bataticola</i>	CBS 144398T	AF178344	FJ240381
<i>F. borneensis</i>	CBS 145462ET	AF178352	EU329515
<i>F. brevicona</i>	CBS 204.31ET	LR583600	LR583821
<i>F. citricola</i>	CBS 146511	MW248745	MW446579
<i>F. citricola</i>	CBS 146512	MW248746	MW446580
<i>F. citricola</i>	CBS 146513T	MW248747	MW446581
<i>F. crassa</i>	CBS 144386T	LR583604	LR583823
<i>F. crassa</i>	VG211	MW248760	MW446594
<i>F. cucurbitae</i>	CBS 410.62	DQ247640	LR583824
<i>F. cucurbitae</i>	CBS 616.66T	DQ247592	LR583825
<i>F. elegans</i>	CBS 144395	AF178328	EU329496
<i>F. elegans</i>	CBS 144396ET	AF178336	FJ240380
<i>F. falciformis</i>	CBS 475.67T	LT906669	LT960558
<i>F. falciformis</i>	CBS 121450	JX435161	JX435261
<i>F. falciformis</i>	VG296	MW248761	MW446595
<i>F. haematococca</i>	CBS 119600ET	DQ247510	LT960561
<i>F. ipomoeae</i>	CBS 353.87	DQ247639	LR583831
<i>F. ipomoeae</i>	CBS 833.97	LR583611	LR583833
<i>F. keratoplastica</i>	CBS 490.63T	LT906670	LT960562
<i>F. keratoplastica</i>	CBS 144389	LR583613	LR583836
<i>F. lichenicola</i>	CBS 509.63	LR583618	LR583843
<i>F. lichenicola</i>	CBS 623.92ET	LR583620	LR583845
<i>F. martii</i>	CBS 115659ET	JX435156	JX435256
<i>F. martii</i>	MRC 2198	MH582418	MH582225
<i>F. mori</i>	CBS 145467T	AF178358	EU329499
<i>F. mori</i>	CBS 145468	AF178359	EU329493
<i>F. noneumartii</i>	CBS 115658T	LR583630	MW446618
<i>F. noneumartii</i>	VG87	MW248769	MW446619
<i>F. noneumartii</i>	VG88	MW248770	MW446620
<i>F. paraeumartii</i>	CBS 487.76T	DQ247549	LR583855
<i>F. piperis</i>	CBS 145470T	AF178360	EU329513
<i>F. protoensiformis</i>	CBS 145471T	AF178334	EU329498
<i>F. quercicola</i>	CBS 141.90T	DQ247634	LR583869
<i>F. regularis</i>	CBS 230.34T	LR583643	LR583873
<i>F. silvicola</i>	CBS 123846T	LR583646	LR583876
<i>F. solani</i>	CBS 140079ET	KT313611	KT313623
<i>F. solani</i>	VG36	MW248771	MW446621
<i>F. solani</i>	VG38	MW248772	MW446622
<i>F. stercicola</i>	CBS 142481T	LR583658	LR583887
<i>F. stercicola</i>	CBS 144388	LR583659	LR583888
<i>F. suttoniana</i>	CBS 143214T	DQ247163	EU329630
<i>F. suttoniana</i>	CBS 143224	KC808197	KC808336
<i>F. tonkinensis</i>	CBS 115.40T	LT906672	LT960564
<i>F. tonkinensis</i>	CBS 118931	LR583662	LR583891

<i>F. vasinfecta</i>	CBS 446.93	LR583670	LR583898
<i>F. vasinfecta</i>	CBS 533.65	LR583671	LR583899
<i>F. petroliphilum</i>	NRRL 22141	AF178329	EU329491
<i>F. petroliphilum</i>	CBS 224.34	JX435169	JX435269
<i>F. falciforme</i>	NRRL 43441	DQ790478	DQ790566
<i>F. neocosmosporiellum</i>	NRRL 22166	AF178350	EU329497
<i>F. neocosmosporiellum</i>	NRRL 22436	AF178348	JX171610
<i>F. cucurbiticola</i>	NRRL 22153	AF178346	EU329492
<i>F. vanettenii</i>	CBS 123669	LR583636	LR583862
<i>F. vanettenii</i>	Fs77	KY556457	KY556541
<i>F. silvicola</i>	LC5482	MW620187	MW474712
<i>F. lichenicola</i>	NRRL 32434	DQ246977	EF470161
<i>F. ambrosium</i>	NRRL 22346	FJ240350	EU329503
<i>F. ambrosium</i>	NRRL 20438	AF178332	JX171584
<i>F. suttonianum</i>	NRRL 22608	DQ246838	EU329517
<i>F. striatum</i>	NRRL 22101	AF178333	EU329490
<i>F. striatum</i>	CCLF11	MT249025	MT263727
<i>F. striatum</i>	CCLF12	MT249026	MT263728
<i>F. paranaense</i>	CML 1830	KF597797	KF680011
<i>F. paranaense</i>	CML 2022	KF597813	KF680017
<i>F. pseudensiforme</i>	NRRL 46517	DQ247512	KC691645
<i>F. pseudensiforme</i>	CBS 135557	KU711771	KU604376
<i>F. euwallaceae</i>	NRRL 54728	JQ038013	JQ038034
<i>F. euwallaceae</i>	NRRL 54722	JQ038007	JQ038028

Species	Collection number	GenBank accession number	
		<i>tef</i>	<i>rpb2</i>
<i>Fusarium lateritium</i> species complex			
<i>F. lateritium</i> Clade IIA	FRC L-81	AY707156	-
<i>F. lateritium</i> Clade IIB	FRC L-82	AY707157	-
<i>F. lateritium</i> Clade I	FRC L-83	AY707158	-
<i>F. lateritium</i> Clade I	FRC L-101	AY707163	-
<i>F. lateritium</i> Clade I	FRC L-107	AY707164	-
<i>F. lateritium</i> Clade III	FRC L-110	AY707165	-
<i>F. lateritium</i> Clade III	FRC L-112	AY707166	-
<i>F. lateritium</i> Clade IIB	FRC L-120	AY707167	-
<i>F. lateritium</i> Clade IIA	FRC L-200	AY707168	-
<i>F. lateritium</i> Clade I	FRC L-376	AY707170	-
<i>F. lateritium</i> Clade IIA	FRC L-405	AY707172	-
<i>F. lateritium</i> Clade III	L-55	AY707173	-
<i>F. lateritium</i> Clade I	FRC L-402	AY707171	-
<i>F. lateritium</i>	NRRL 25122	JF740747	-
<i>F. lateritium</i>	FS132	OR553061	-

<i>F. lateritium</i>	NRRL 13622	AY707173	-
<i>F. ramsdenii</i>	BRIP 62306a	OL332732	-
<i>F. cassiae</i>	MFLU 18-2751	MT212205	-
<i>F. sarcochroum</i>	CBS 745.79	MW834278	-
<i>F. sarcophroum</i>	CPC 28118	LT746213	-
<i>F. stilboides</i>	CBS 746.79	MW928843	-
<i>F. massalimae</i>	URM8239	MN939763	-
<i>F. massalimae</i>	A12b	MN939764	-
<i>F. oxysporum</i>	CBS 144134	MH485044	-
Species	Collection number	GenBank accession number	
		<i>tef</i>	<i>rpb2</i>
<i>Albonectria</i>			
<i>A. guizhouensis</i>	CGMCC3.25472	OR043878	-
<i>A. guizhouensis</i>	GUCC 198015.1	OR043879	-
<i>A. rigidiuscula</i>	CMW 18783	EU849670	-
<i>A. rigidiuscula</i>	FA-SHPZH01	HM625868	-
<i>A. rigidiuscula</i>	CBS 133754T	MW834269	-
<i>Neocosmospora plagianthi</i>	NRRL 22632	AF178354	-
<i>Setofusarium setosum</i>	CBS 574.94	MW834293	-
<i>S. setosum</i>	CBS 635.92T	MW834294	-



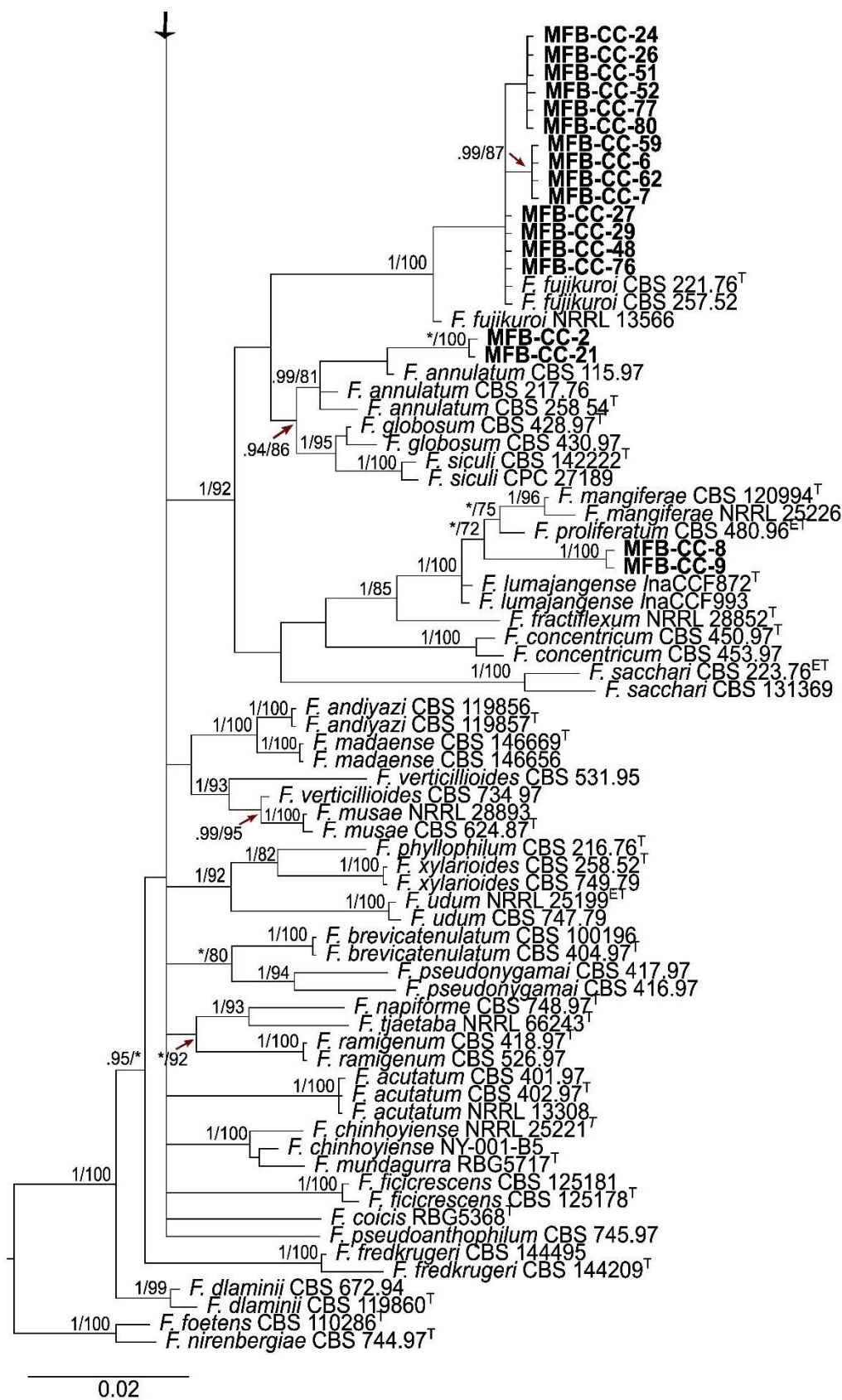
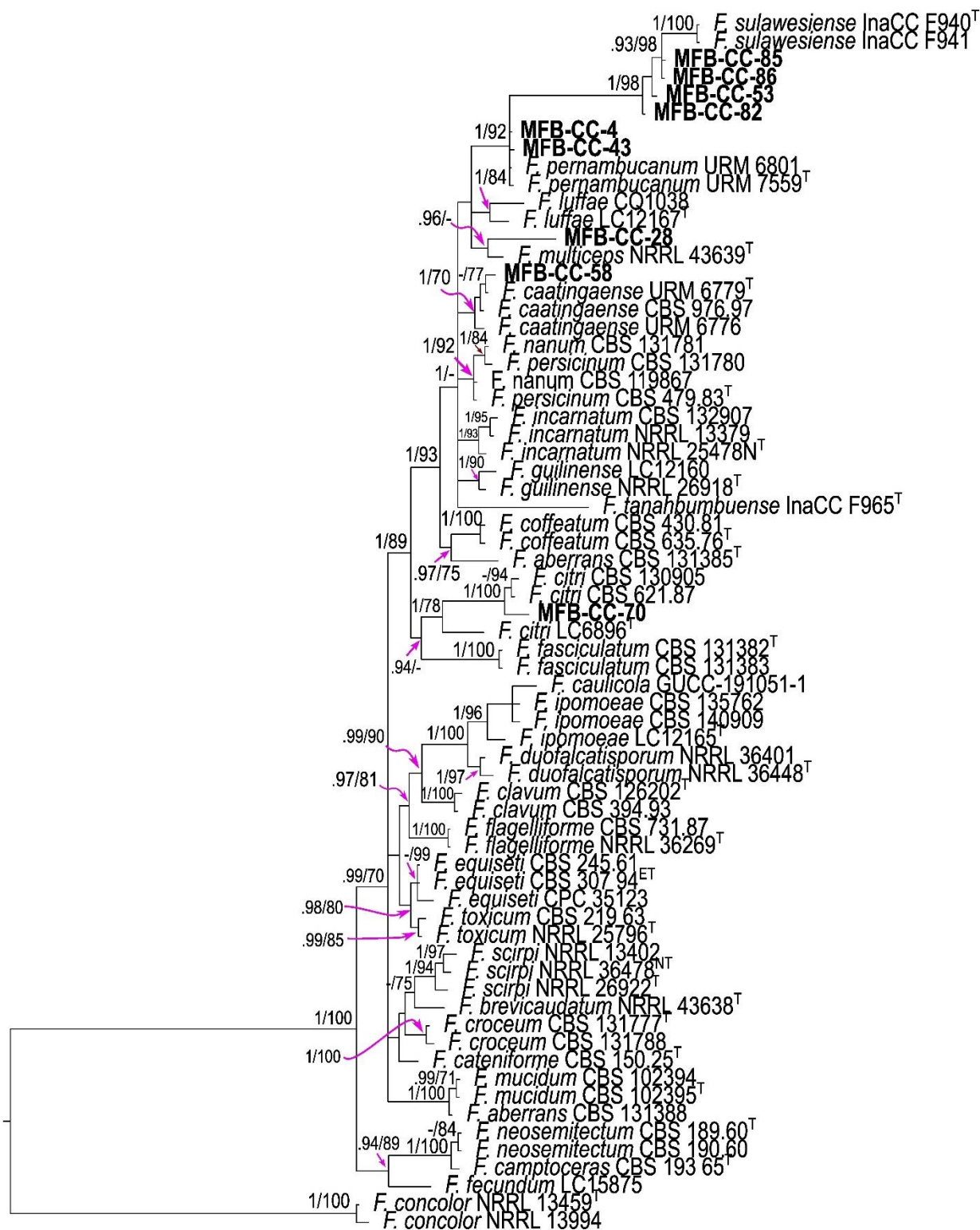


Figure S1. Phylogenetic analysis of the *rpb2* region of the *Fusarium fujikuroi* species complex using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Bootstrap support values are indicated on the branches, with values $\geq 70\%$ considered significant. Posterior probabilities (PP) of nodes are represented on the branches, with values ≥ 0.90 also considered significant. The isolates obtained in this study are emphasized in bold. The tree was rooted using CBS 744.97 *F. niremburgiae* as the outgroup.



0.02

Figure S2. Phylogenetic analysis of the *tef-1 α* and *rpb2* concatenated regions of the *Fusarium incarnatum-equiseti* species complex using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Bootstrap support values are indicated on the branches, with values $\geq 70\%$ considered significant. Posterior probabilities (PP) of nodes are represented on the branches, with values ≥ 0.90 also considered significant. The isolates obtained in this study are highlighted in bold. The tree was rooted using NRRL 13994 *F. concolor* as the outgroup.

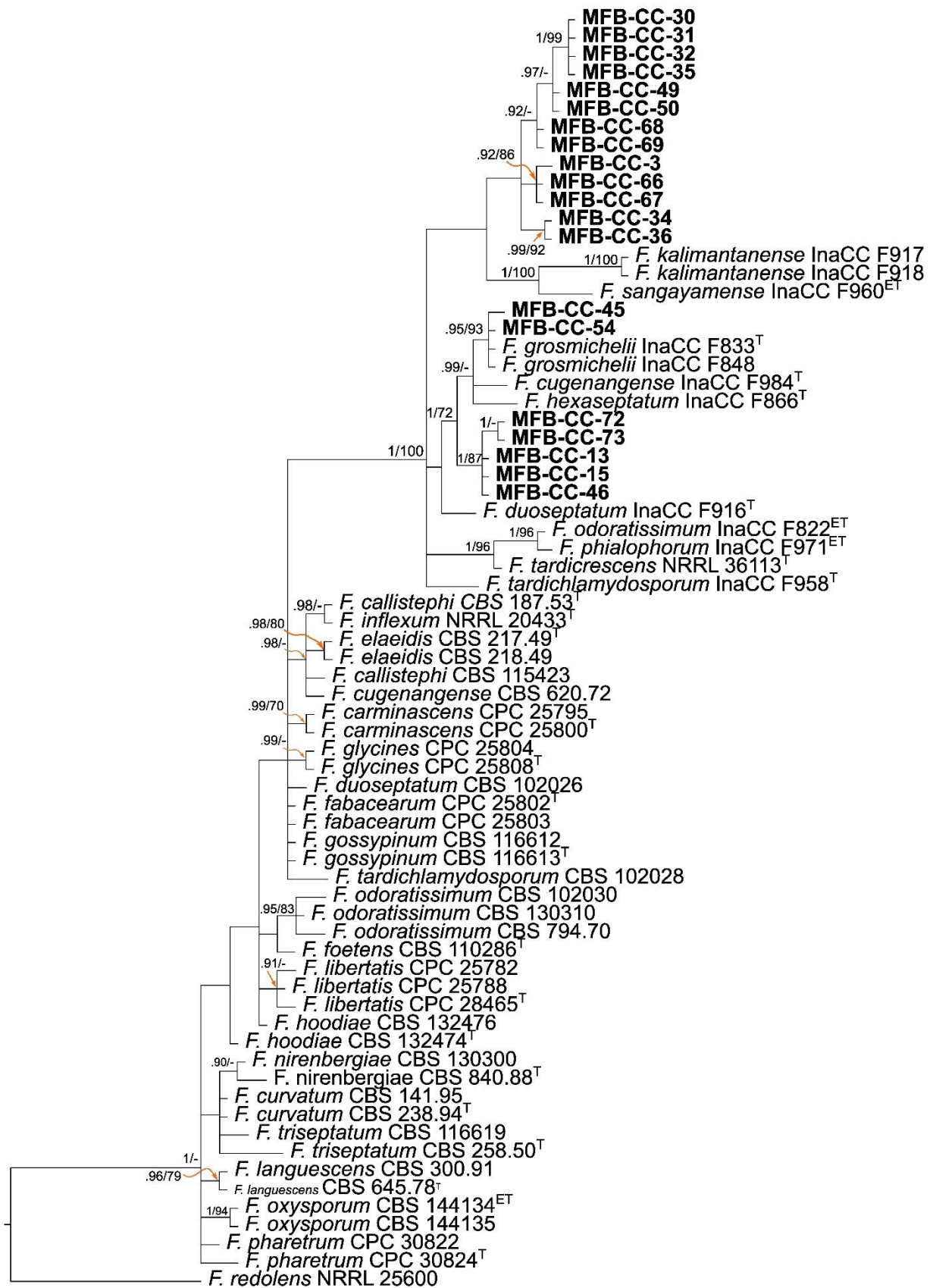


Figure S3. Phylogenetic analysis of the *tef-1 α* and *rpb2* concatenated regions of the *Fusarium oxysporum* species complex using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Bootstrap support values are indicated on the branches, with values $\geq 70\%$ considered significant. Posterior probabilities (PP) of nodes are represented on the branches, with values ≥ 0.90 also considered significant. The isolates obtained in this study are highlighted in bold. The tree was rooted using NRRL 25600 *F. redolens* as the outgroup.

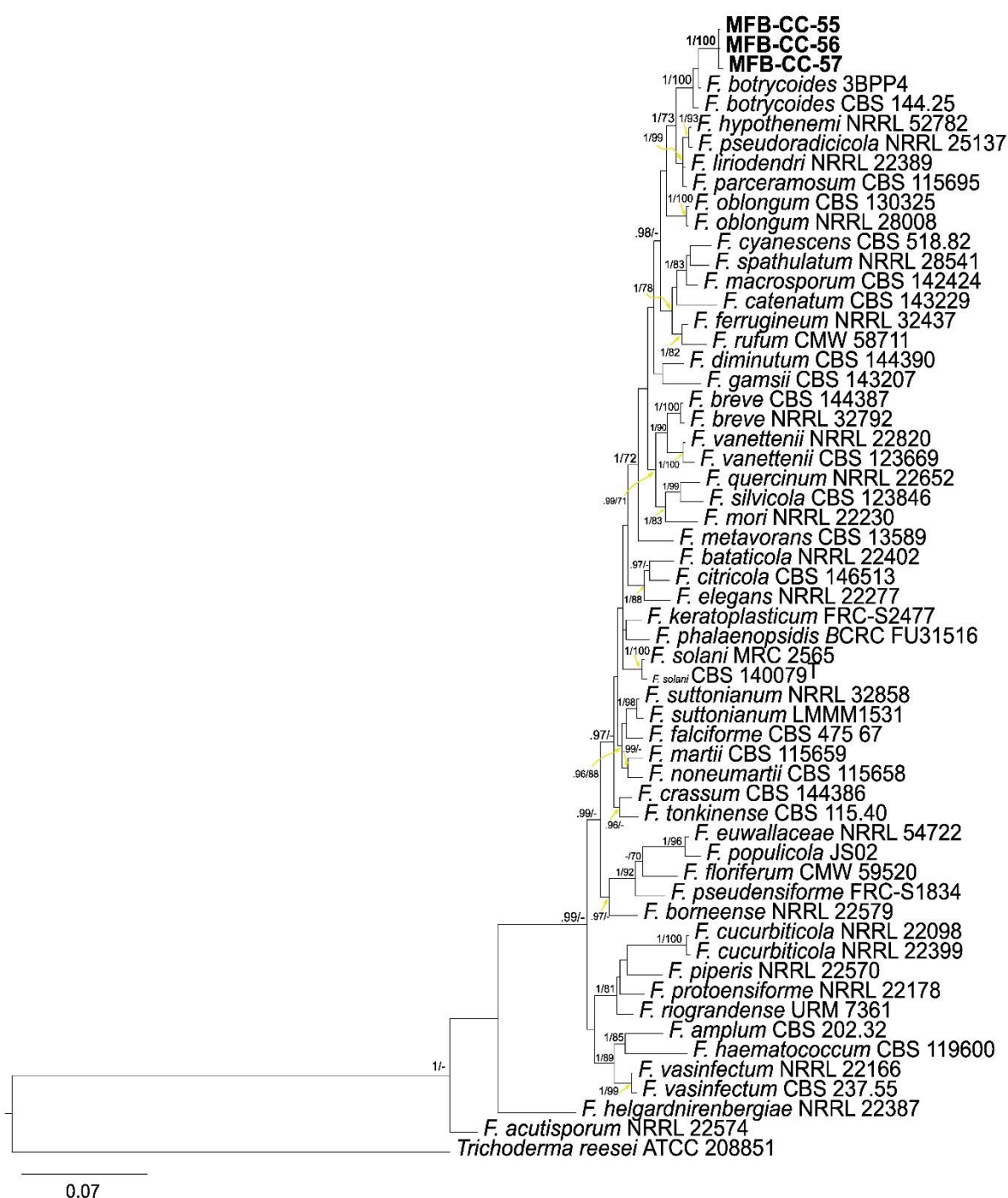


Figure S4. Phylogenetic analysis of the *rpb2* region of the *Fusarium solani* species complex using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Bootstrap support values are indicated on the branches, with values $\geq 70\%$ considered significant. Posterior probabilities (PP) of nodes are represented on the branches, with values ≥ 0.90 also considered significant. The isolates obtained in this study are highlighted in bold. The tree was rooted using ATCC 208851 *Trichoderma reesei* as the outgroup.

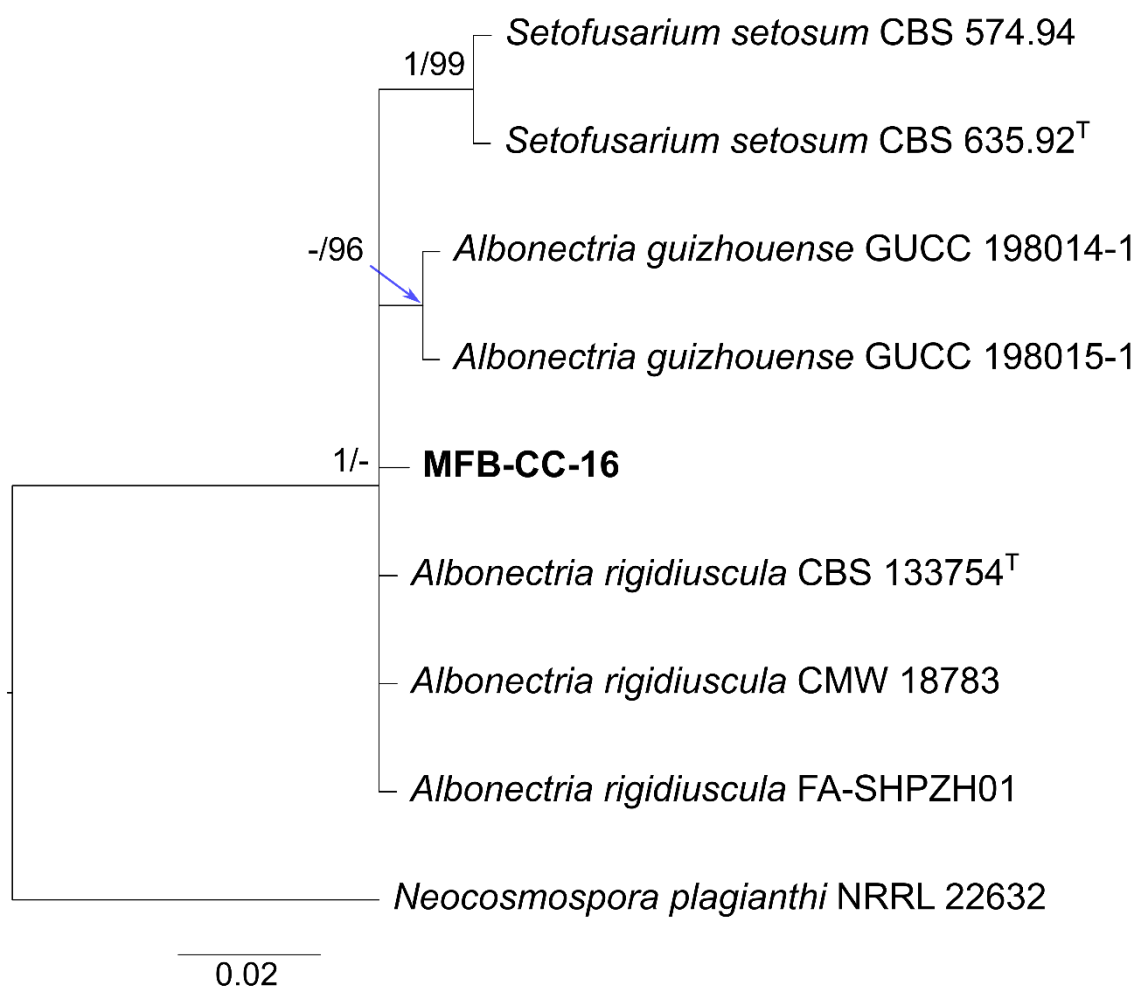


Figure S5. Phylogenetic analysis of the *tef-1 α* region of the *Albonectria* species complex using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Bootstrap support values are indicated on the branches, with values $\geq 70\%$ considered significant. Posterior probabilities (PP) of nodes are represented on the branches, with values ≥ 0.90 also considered significant. The isolate obtained in this study is highlighted in bold. The tree was rooted using *Neocosmospora plagianthi* NRRL 22632 as the outgroup.

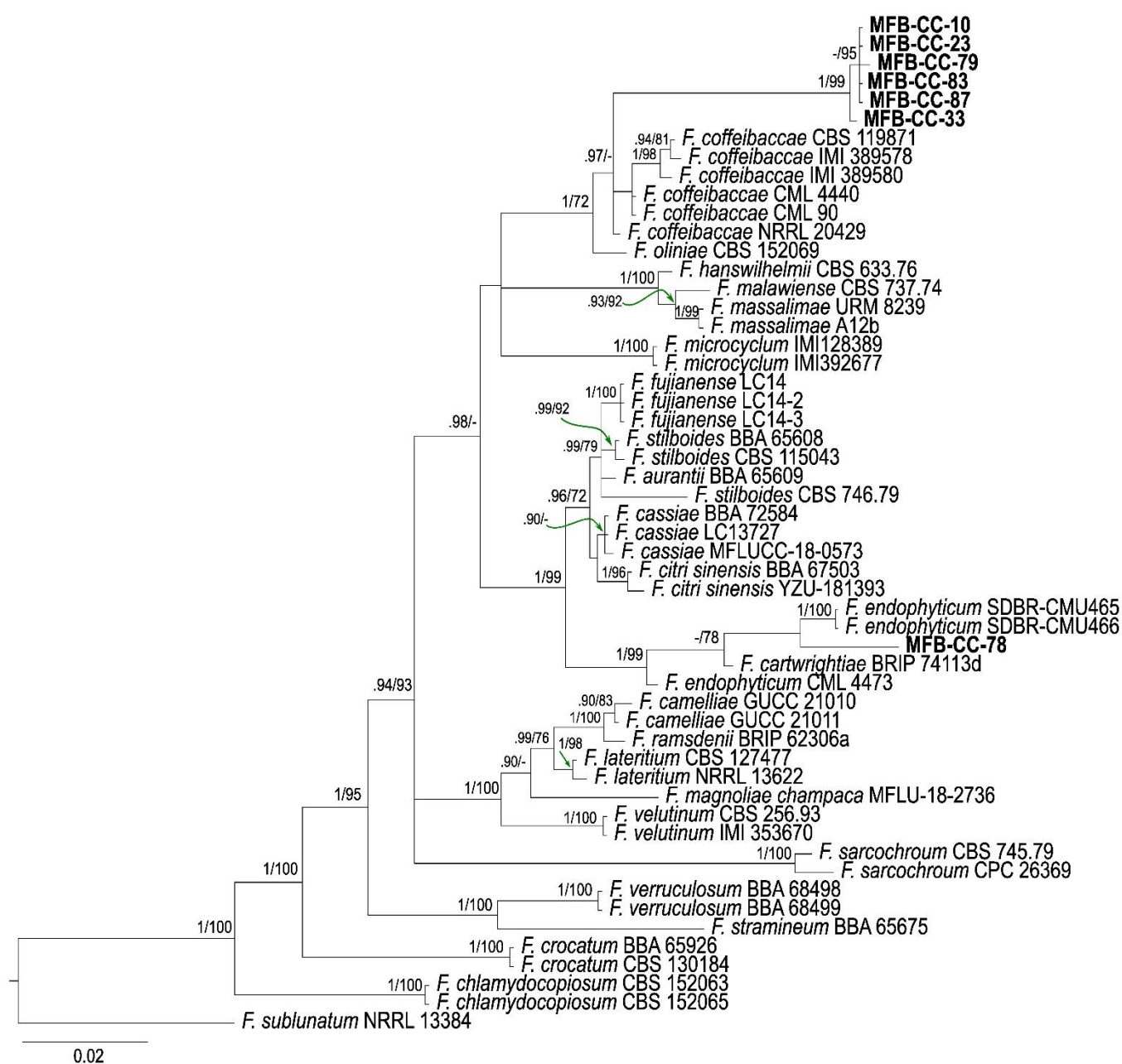


Figure S6. Phylogenetic analysis of the concatenated *tef-1 α* and *rpb2* regions of the *Fusarium lateritium* species complex using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Bootstrap support values are indicated on the branches, with values $\geq 70\%$ considered significant. Posterior probabilities (PP) of nodes are represented on the branches, with values ≥ 0.90 also considered significant. The isolates obtained in this study are highlighted in bold. The tree was rooted using NRRL 13384 *F. sublunatum* as the outgroup.

Table S3. Species list present in figure 11 of the chapter 2, by order of appearance.

Species
<i>Alternaria alternata</i>
<i>A. arborescens</i>
<i>A. burnsii</i>
<i>A. gaisen</i>
<i>A. postmessia</i>
<i>Alternaria</i> sp. MG1
<i>A. tenuissima</i>
<i>Astraeus odoratus</i>
<i>Botryobasidium botryosum</i>
<i>Botryosphaeria dothidea</i>
<i>Capronia coronata</i>
<i>C. epimyces</i>
<i>Chaetothyriales</i> sp. CBS 134916
<i>Chaetothyriales</i> sp. CBS 134920
<i>Clonostachys byssicola</i>
<i>C. chloroleuca</i>
<i>C. rhizophaga</i>
<i>C. solani</i>
<i>Colletotrichum abscissum</i>
<i>C. asianum</i>
<i>C. aenigma</i>
<i>C. camelliae</i>
<i>C. chrysophilum</i>
<i>C. filicis</i>
<i>C. fioriniae</i>
<i>C. fructicola</i>
<i>C. gloeosporioides</i>
<i>C. incanum</i>
<i>C. lupini</i>
<i>C. noveboracense</i>
<i>C. nupharicola</i>
<i>C. nymphaeae</i>
<i>C. salicis</i>
<i>C. scovillei</i>
<i>Colletotrichum</i> sp. SAR 10_65
<i>Colletotrichum</i> sp. SAR 10_75
<i>Colletotrichum</i> sp. SAR 10_76
<i>Colletotrichum</i> sp. SAR 10_77
<i>Colletotrichum</i> sp. SAR 10_96
<i>Colletotrichum</i> sp. SAR 11_239
<i>Colletotrichum</i> sp. SAR 11_240

Colletotrichum sp. SAR 11_57
C. sublineola
C. tofieldiae
C. tropicale
C. viniferum
Curvularia kusanoi
Cyphellophora europaeae
Diplodia seriata
Exophiala mesophila
E. spinifera
E. xenobiotica
Fonsecaea multimorphosa
Fusarium albosuccineum
F. ambrosium
F. circinatum
F. coicis
F. decemcellulare
F. euwallaceae
F. floridanum
F. napiforme
F. odoratissimum
F. solani
Fusarium sp. LHS14.1
Fusarium sp. Ph 1
F. vanettenii
Macrophomina phaseolina
Neofusicoccum parvum
Neopestalotiopsis clavispora
N. cubana
Neopestalotiopsis sp. 37M
Periconia digitata
P. macrospinosa
Pestalotiopsis fici
P. microspora
Pestalotiopsis sp. 9143b
Pestalotiopsis sp. NC0098
Phialophora attinorum
Rhinocladiella mackenzie
Stagonosporopsis vannaccii
Xylaria hypoxylon
X. longipes

Table S4. Species list present in figure 12 of the chapter 2, by order of appearance.

Species
<i>Alternaria arborescens</i>
<i>Alternaria</i> sp. BMP 0032
<i>A. ventricosa</i>
<i>Aspergillus aculeatinus</i>
<i>Astraeus odoratus</i>
<i>Aureobasidium melanogenum</i>
<i>Cladonia metacorallifera</i>
<i>Cladophialophora bantiana</i>
<i>C. yegresii</i>
<i>Colletotrichum abscissum</i>
<i>Curvularia kusanoi</i>
<i>Dichomitus squalens</i>
<i>Dioszegia hungarica</i>
<i>Diplodia seriata</i>
<i>Erysiphe neolycopersici</i>
<i>Fusarium fujikuroi</i>
<i>Fusarium</i> sp. LHS 14.1
<i>F. vanettenii</i>
<i>Geoglossum umbratile</i>
<i>Kwoniella shandongensis</i>
<i>Marasmius crinis-equi</i>
<i>Nadvornikia sorediata</i>
<i>Neofusicoccum parvum</i>
<i>Neopestalotiopsis cubana</i>
<i>Periconia digitata</i>
<i>P. macrospinosa</i>
<i>Periconia</i> sp. BCC 2871
<i>Rhizopus delemar</i>
<i>R. microsporus</i>
<i>R. arrhizus</i>
<i>Russula earlei</i>
<i>Spinellus fusiger</i>
