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Fusarium guttiforme and *F. mexicanum* Causing Shoot Blight in Yerba Mate in Southern Brazil

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ABSTRACT

Yerba mate (*Ilex paraguariensis* [St. Hil.]) is one of the main non-timber forest products in the southern region of Brazil, playing a role of great social, economic and environmental importance. Commercial yerba mate consists of leaves and young branches of the plant, traditionally consumed as tea and chimarrão. Its extract is also used in food, cosmetics and cleaning products. In 2021 and 2022, shoot blight was observed in seedlings and adult plants of yerba mate in Paraná and Rio Grande do Sul states, Brazil. The disease is characterised by dark-coloured necrotic lesions, which begin at the tip of the shoots and, in some cases, progress downwards along the shoot, causing its death. The objective of this work was to elucidate the aetiology of this disease. The causal agents of the disease were isolated from symptomatic shoots of yerba mate, and the isolates obtained were characterised using morphology and multilocus molecular phylogeny (RNA polymerase II subunit, elongation factor 1-α and β-tubulin genes). Inoculation was carried out on detached leaves and shoots, under controlled conditions and on adult plants in the field. The isolates obtained proved pathogenic when inoculated, causing symptoms similar to those observed in the field. The multilocus molecular phylogeny identified two species: *Fusarium guttiforme* and *F. mexicanum*, with *F. mexicanum* being the most aggressive in pathogenicity tests. *Fusarium* cultures were reisolated, fulfilling Koch's postulates. This study represents the first report of *F. guttiforme* and *F. mexicanum* as causal agents of yerba mate shoot blight.

1 | Introduction

Yerba mate (*Ilex paraguariensis*) is a forest species native to South America found in the Southern region of Brazil, part of Paraguay and northeastern Argentina (Oliveira and Rotta 1985; Penteado Junior and Goulart 2019). This plant belongs to the Aquifoliaceae family and is characteristic of South America's subtropical mixed ombrophilous forests. It is considered a climax species capable of tolerating shade (Carvalho 2003). In addition to traditional uses like chimarrão, tereré and mate tea, yerba mate is incorporated into products such as food, cosmetics and hygiene and cleaning products (Faccin et al. 2015; Dallabrida et al. 2016). It can also be included in the diet of dairy cows (Celi 2013; Barbato et al. 2019) and lambs (Baerley et al. 2023).

The production of yerba mate is shifting from extractive to cultivation. Brazil is the leading producer of yerba mate, and in 2022, it produced 1.06 million tons of green yerba mate leaves, with 87.5% of this production coming from Paraná and Rio Grande do Sul states (Instituto Brasileiro de Geografia e Estatística [IBGE] 2023). Argentina, the second largest producer, produced

TABLE 1 | Primers and PCR amplification procedures used in this study.

Locus	Primer		PCR amplification procedures	References
	Name	Sequence (5′–3′)[a]		
tef1	EF1–1018F EF1–1620R	GAYTTCATCAAGAACATGAT GACGTTGAADCCRACRTTGTC	94°C 5 min; 40 cycles of 94°C 50 s, 50°C 50 s, 72°C 50 s; 72°C 7 min	Stielow et al. (2015)
rpb2	fRPB2–5F fRPB2–7cR	GAYGAYMGWGATCAYTTYGG CCCATRGCTTGYTTRCCCAT	95°C 5 min; 35 cycles of 94°C 30 s, 51°C 90 s, 68°C 2 min; 68°C 5 min	Liu et al. (1999)
tub2	T1 T2	AACATGCGTGAGATTGTAAGT TAGTGACCCTTGGCCCAGTTG	95°C 5 min; 35 cycles of 95°C 45 s, 52°C 45 s, 72°C 90 s; 72°C min	O'Donnell and Cigelnik (1997)

[a]*R* = A or G; W = A or T; Y = C or T.

0.83 million tons in the same year (Instituto Nacional de la Yerba Mate [INYM] 2023).

The genus *Fusarium* is of utmost importance among the pathogens that attack the crop, causing economic losses and threatening yerba mate production. In Brazil, those fungi can cause mortality in nurseries in seedlings and young plants (Grigoletti Júnior and Auer 2000; Auer and dos Santos 2016; Vargas 2020). In the field, the pathogen causes the disease known as root rot (Poletto et al. 2012; Mezzomo et al. 2018, 2021). It may also be associated with yerba mate seeds (Mireski 2018), flowers, fruits (Poletto et al. 2015) and death of shoots (Grigoletti Júnior and Auer 1996).

In 2021 and 2022, symptoms of shoot necrosis and death were observed in cultivated yerba mate across several plantations in the states of Paraná and Rio Grande do Sul, affecting seedlings (in nurseries), young and adult plants (in the field), as well as in natural populations of *Ilex paraguariensis*. These symptoms were similar to those described by Grigoletti Júnior and Auer (1996). This study aimed to isolate and identify the causal agent of yerba mate shoot blight.

2 | Materials and Methods

2.1 | Fungal Isolates

Samples of symptomatic shoots and leaves of seedlings and clonal matrices were collected from nurseries in Colombo, Paraná state. Adult plant samples were collected from the field in União da Vitória, Paraná state and Venâncio Aires, Rio Grande do Sul state. Samples were collected from 10 diseased plants from each location and sent to the Forest Pathology Laboratory at Embrapa Forestry, Colombo, Paraná state. Typical symptoms of the disease were characterised by dark-coloured necrotic lesions, originating at the tip of the shoots and, in some cases, progressing downward along the shoot, ultimately leading to its death.

Direct and indirect isolation methods were employed to obtain isolates of the pathogen. Moist chambers using Gerbox boxes with moistened blotting paper were utilised for direct isolation. Indirect isolation involved disinfecting the material with 70% alcohol for 30 s and sodium hypochlorite solution (1%) for 40 s, followed by plating on potato dextrose agar medium (PDA) supplemented with chloramphenicol (40 ppm) and ampicillin (80 ppm), routinely used at the Forest Pathology Laboratory. The cultures were purified through single-spore isolation.

From eight isolates of *Fusarium* spp. obtained, the three most aggressive isolates were identified at the species level. They were F2A and F2B from Colombo (25°19′13.81″ S, 49°9′31.28″ W) and F3 from Venâncio Aires, Rio Grande do Sul (29°33′34.03″ S, 52°13′26.57″ W). The remaining isolates were not sequenced due to lack of time and resources. They have been preserved in the Collection of Forest Fungi and Oomycetes, Embrapa Forestry.

2.2 | PCR and Sequencing

Genomic DNA was extracted from cultures grown for 7 days on PDA medium in Petri dishes incubated at 24°C under a 12-h photoperiod. The extraction procedure utilised the Loccus Extracta Kit Fast-viral DNA/RNA (Loccus do Brasil, Cotia, SP, Brazil) following the manufacturer's recommendations. Three partial sequences from elongation factor 1-alpha (*tef1*), β-tubulin (*tub2*) and the second largest subunit of RNA polymerase (*rpb2*) genes were amplified and sequenced. The primer pairs and PCR conditions used are detailed in Table 1. The 25 μL PCR reaction mixture consisted of 2.5 μL buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.8 μM of each primer (forward and reverse), 1 U of GoTaq DNA Polymerase and 20–40 ng of DNA. The PCR products were separated on a 2% agarose gel, stained with Sybrsafe and examined under ultraviolet light. The amplified fragments were purified with Exo/Sap (Thermo Fisher Scientific). Fragment sequencing was conducted on a Genetic Analyser 3500xL, pop7, 50 cm capillary at GoGenetic (Curitiba, Brazil).

2.3 | Phylogenetic Analysis

Electropherograms were evaluated, and consensus strands were obtained using the SeqAssem program. These consensus strands were then compared with reference sequences available in Fusarioid-ID (Crous et al. 2021), Fusarium MLST (http://fusarium.mycobank.org) and NCBI/GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The most relevant sequences of the *Fusarium* species found were used in this study and are listed in Table 2.

Sequence alignment for each gene region was conducted using the MEGA version X program (Kumar et al. 2018), with

TABLE 2 | GenBank accession numbers and culture collection of *Fusarium* strains used in phylogenetic analysis.

Species name	Culture collection[a]	Substrate	Country	GenBank accession number[b]			References
				tef1	*rpb2*	*tub2*	
F. ananatum	CBS 118517	*Ananas comosus*	South Africa	KU604417	MN534229	MN534090	Al-Hatmi et al. (2016) Yilmaz et al. (2021)
	CBS 118516^T	*Ananas comosus*	South Africa	MT010996	LT996137	MN534089	Yang et al. (2020) Yilmaz et al. (2021)
F. anthophilum	NRRL 25214	*Hippeastrum* sp.	Germany	MN193857	MN193885	KF466436	Laraba et al. (2019) Proctor et al. (2013)
	CBS 119858	*Triticum aestivum*	USA	MN533990	MT010965	MN534091	Yilmaz et al. (2021) Yang et al. (2020)
F. begoniae	NRRL 25300^T	*Begonia elatior*—hybrid	Germany	MN193858	MN193886	U61543	Laraba et al. (2019) O'Donnell et al. (1998)
	CBS 452.97	*Begonia elatior*—hybrid	Germany	MT010998	MN534243	MN534101	Yang et al. (2020) Yilmaz et al. (2021)
F. brevicatenulatum	NRRL 25447	*Striga asiatica*	Madagascar	MN193859	MN193887	JABEEJ010000136	Laraba et al. (2019) Kim et al. (2020)
	CBS 404.97^T	*Striga asiatica*	Madagascar	MN533995	MT010979	MN534063	Yilmaz et al. (2021) Yang et al. (2020)
F. bulbicola	NRRL 22947	Plant	Germany	JAAOAH010000458	JAAOAH010000912	KU171779	Kim et al. (2020) Brown and Proctor (2016)

(Continues)

TABLE 2 | (Continued)

Species name	Culture collection[a]	Substrate	Country	GenBank accession number[b]			References
				tef1	*rpb2*	*tub2*	
F. dlaminii	CBS 119860^{T}	Plant debris in the soil	South Africa	MW401995	KU171701	MW402195	Yilmaz et al. (2021) Brown and Proctor (2016)
F. ficicrescens	CBS 125177	*Ficus carica*—fruits	Iran	—	MN534281	MN534071	Yilmaz et al. (2021)
	CBS 125178^{T}	*Ficus carica*—fruits	Iran	KU604452	MT010977	MT011061	Al-Hatmi et al. (2016) Yang et al. (2020)
F. fracticaudum	CBS 137234^{PT}	*Pinus maximinoi*—stem	Colombia	PDNT01000006	PDNT01000004	KJ541048	Wingfield et al. (2018) Herron et al. (2015)
F. guttiforme	CBS 409.97^{T}	*Ananas comosus*	Brazil	MT010999	MT010967	MT011048	Yang et al. (2020)
	F3	*Ilex paraguariensis*	Brazil	PQ032374	PQ032377	PQ032380	This study
F. marasasianum	CMW 25512	*Pinus tecunumanii*	Colombia	JAJEQZ010000008	MN534249	MN534113	Wingfield et al. (2022)
F. mexicanum	NRRL 53147^{T}	*Mangifera indica*—diseased vegetative tissues	Mexico	JAAOAM010000080	JAAOAM010000138	GU737494	Kim et al. (2020) Otero-Colina et al. (2010)
	NRRL 47473	*Mangifera indica*—diseased inflorescence	Mexico	—	LR792615	GU737308	Otero-Colina et al. (2010)
	F2A	*Ilex paraguariensis*	Brazil	PQ032375	PQ032378	PQ032381	This study
	F2B	*Ilex paraguariensis*	Brazil	PQ032376	PQ032379	PQ032382	This study
F. ophioides	CBS 118515	*Panicum maximum*	South Africa	—	MN534298	MN534120	Yilmaz et al. (2021)
	CBS 118514	*Panicum maximum*	South Africa	—	MN534302	MN534117	Yilmaz et al. (2021)
F. pininemorale	CMW 25243^{T}	*Pinus tecunumanii*—stem canker	Colombia	NFZR01000006	NFZR01000003	MN534115	Wingfield et al. (2017)

(Continues)

TABLE 2 | (Continued)

Species name	Culture collection[a]	Substrate	Country	GenBank accession number[b]			References
				tef1	*rpb2*	*tub2*	
F. sacchari	FRC R-6865	Unknown	Unknown	KU171729	KU171709	KU171789	Brown and Proctor (2016)
	NRRL 66326	Unknown	USA	MN193868	MN193896	JABSTH010000001	Laraba et al. (2019) Kim et al. (2020)
F. sororula	FCC 5425	Seedlings—*Pinus tecunumanii*	Colombia	JACWFA010000005	JACWFA010000007	JACWFA010000001	van der Nest et al. (2021)
F. sterilihyphosum	NRRL 25623^{T}	*Mangifera indica*—malformed inflorescence	South Africa	MN193869	MN193897	AF160316	Laraba et al. (2019) O'Donnell et al. (2000)
F. succisae	NRRL 13298	Plant	Germany	KU171732	KU171712	JAAOAW010000314	Brown and Proctor (2016) Kim et al. (2020)
F. succisae	CBS 219.76^{ET}	*Succisa pratensis*- flowers	Germany	—	MW402766	U34419	Yilmaz et al. (2021) O'Donnell et al. (1998)
F. venenatum	A 3/5	—	—	NC038012	LN649230	NC038015	King (2014)[c]

Abbreviations: ET, ex-epitype specimen; PT, ex-paratype specimen; T, ex-type specimen.
[a](NRRL) USDA Agricultural Research Service Culture Collection, Peoria (Illinois), EUA. (FCR) *Fusarium* Research Center collection at Pennsylvania State University, University Park, USA. (CBS) Culture collection of the Central Bureau voor Schimmelcultures, Utrecht, Netherlands. (CMW) The working collection of FABI, University of Pretoria, South Africa.
[b]*rpb2* = RNA polymerase second largest subunit; *tef1* = translation elongation factor 1-alpha; *tub2* = Beta-tubulin.
[c]Direct submission in GenBank.

manual adjustments when necessary. Phylogenetic analyses were performed using Maximum Likelihood for individual genes. For the combined dataset, both Maximum Likelihood and Bayesian Inference algorithms were applied. All analyses were conducted through the Cipres Science Gateway portal (Miller et al. 2011). The RAxML hpc Blackbox tool was used for 1000 bootstrap replicates for Maximum Likelihood. For Bayesian Inference, the MrBayes on XSEDE tool was used, with two parallel runs, four chains of 10,000,000 generations (of which the initial 25% were discarded) and a sampling frequency of 1000. The trees were visualised in Figtree v.1.4.4 and edited in Inkscape 1.1.1.

2.4 | Morphological Characterisation

Fusarium isolates were characterised by colony colour and pigmentation diffused in the medium following the methodology described by Nelson et al. (1983), using Natural PDA medium (infusion of 250 g of peeled and cooked white potatoes, 20 g of dextrose and 20 g of agar, with the final volume adjusted to 1 L). The morphological characteristics were evaluated according to Leslie and Summerell (2006), as follows: characterisation of conidiogenous cells as mono- or polyphialidic on Synthetic Nutrient-poor Agar (SNA); formation of chains in Potassium Chloride Agar (KCl); formation of sporodochia on Carnation Leaf-Piece Agar (CLA), with variations in photoperiod and light (cold light, 12 h photoperiod or continuous black light); formation of chlamydospores in Soil Agar (SA) medium. Macroconidia and microconidia were characterised by sporodochia and cultures grown in SNA medium, respectively. The most common number of septa is given in parentheses.

2.5 | Pathogenicity Test

The pathogenicity test was conducted twice on detached leaves from the Yari clone and detached shoots from the EC25 clone of adult *I. paraguariensis* plants.

Initially, yerba mate leaves and shoots, obtained from adult plants, underwent a disinfestation process in a sodium hypochlorite solution (0.5%) for 120 s for leaves and 50 s for shoots, followed by rinsing in ultrapure sterile water. Then, wounds were made in the epidermis of the central region of the leaves using a cork borer (3 mm in diameter) and at the tip of the shoots using a scalpel. Seven leaves were inoculated in each assay at two points and each shoot inoculated at only one point. A PDA plug containing the actively growing isolate was deposited onto each wound.

The inoculated leaves were placed in Petri dishes lined with two sheets of blotting paper moistened with purified and sterilised water and sealed with PVC film. The shoots were placed in test tubes containing 3.5 mL of purified and sterilised water, with the tube ends partially sealed with cotton pads. Both leaves and shoots were secured with a small cotton pad (at the leaf petiole and base of the shoot). The leaves and shoots were incubated in a growth chamber set at 24°C ± 2°C, with a 12-h photoperiod (cool light). The leaves were incubated for 4 days, and the shoots were incubated for 6 days.

Evaluation consisted of determining the incidence and measuring the diameter of lesions on leaves and the length of lesions on shoots using a digital calliper. The experimental design was completely randomised with seven replications. Data were analysed using ANOVA and Tukey's test at a significance level of 5% with R software.

Additionally, the pathogenicity of the isolates was evaluated in the field using adult plants of the Aupaba clone, following the map pin method described by Talgø and Stensvand (2013). This method used map pins to pierce the shoot apex, introducing a portion of the isolate's aerial mycelium. The inoculated shoots were covered with a filter bag to prevent birds from removing the pin. The control group was subjected to the same procedure without fungal inoculation.

The evaluation involved observing the presence of lesions 21 days after inoculation. The pathogens were reisolated in PDA medium and identified using the same methodology described above.

3 | Results

3.1 | Field Symptoms and Pathogenicity Test

Shoot blight symptoms were observed in seedlings and adult plants of yerba mate (Figure 1). The disease is characterised by dark-coloured necrotic lesions, which initiate at the shoot tip and can progress downwards, ultimately causing shoot death (Figure 1b–d). Under high humidity conditions, signs of the pathogen were visible (Figure 1e).

Inoculation performed on detached leaves and shoots of yerba mate resulted in the formation of lesions (Figure 2). In the inoculated leaves, rapid symptom development was observed, characterised by dark-coloured necrosis. The necrosis developed circularly and symmetrically around the wound, with well-defined edges.

In detached shoots, symptoms similar to those observed in the field were noted, characterised by dark necrosis progressing from the point of inoculation toward the base of the shoot and adjacent leaves (Figure 2b,c). When inoculating shoots in the field, symptoms were characterised by the formation of dark necrosis around the inoculation point, accompanied by reduced development of the apical part of the inoculated shoots. Additionally, isolates were successfully recovered through the reisolation process on the PDA medium.

Overall, greater aggressiveness of *F. mexicanum* was observed compared to *F. guttiforme* when inoculated on leaves and detached shoots of yerba mate (Table 3).

3.2 | Identification of Pathogens

The concatenated alignment included 30 sequences, all belonging to the *F. fujikuroi* species complex, except for the outgroup, covering 1897 base pairs. Isolate F3 grouped within the *F. guttiforme* clade (63% bootstrap and 1 posterior probability),

FIGURE 1 | Characteristics of yerba mate shoot blight observed in the field, resulting from natural infection by *Fusarium* spp. (a) Healthy shoot. (b, c) Shoot blight in adult plants and (d) Seedlings. (e) Sporodochia formed on the surface of the yerba mate sprout after a humid chamber.

FIGURE 2 | Characteristics of symptoms observed in *Ilex paraguariensis*. Detached shoots, 6 days after inoculation. (a) Control. (b) *Fusarium guttiforme*, isolate F3. (c) *F. mexicanum*, isolate F2A. Shoots in the field, 21 days after inoculation. (d) Control. (e) *F. guttiforme*, isolate F3. Detached leaves, 4 days after inoculation. (f) Control. (g) *F. guttiforme*, isolate F3. (h) *F. mexicanum*, isolate F2B.

supported solely by the concatenated analysis (Figure 3). Isolates F2A and F2B clustered with the *F. mexicanum* clade, with statistical support of 68% bootstrap and 0.9 posterior probability.

Morphologically, isolate F3 closely resembled the description of the type species of *F. guttiforme* (Nirenberg and O'Donnell 1998). Colonies on natural PDA medium exhibited white cottony aerial mycelium and partially dark violet on the reverse. Pigmentation diffused in the medium was not observed. Microconidia were abundant in the aerial mycelium, grouped in false heads, usually on monophialides but occasionally on polyphialides, hyaline, oval to obovoid in shape, (0-)0-1-septate. Orange-coloured sporodochia formed only on yerba mate leaves. Macroconidia were slender, straight to slightly curved, with a usually foot-shaped basal cell and a beak-shaped apical cell, 3–5-septate. Chlamydospores were not observed (Figure 4a–f).

Likewise, isolates F2A and F2B were similar to the description of the type species of *F. mexicanum* (Otero-Colina et al. 2010), with minor variations. Colonies on natural PDA medium displayed cottony aerial mycelium, ranging from light purple to white. The reverse side of the colony appeared white to pale purple at the

TABLE 3 | Pathogenicity of *Fusarium* spp. strains on the leaves and shoots of yerba mate.

Isolate	Leaf* assay 1		Leaf* assay 2		Shoot*	
	Diseased/ Inoculated site (%)	Lesion diameter (mm)	Diseased/ Inoculated site (%)	Lesion diameter (mm)	Diseased/ Inoculated site (%)	Lesion diameter (mm)
F. mexicanum (F2A)	100	10.9 A	100	5.24 B	100	11.5 A
F. mexicanum (F2B)	100	11.4 A	100	6.75 A	100	9.0 B
F. guttiforme (F3)	8,5	5.7 B	100	4.85 B	100	5.46 C
Control	0	0	0	0	0	0
CV (%)	—	15	—	22	—	11

Note: Means followed by different letters in columns differ using Tukey's comparison test ($p < 0.05$). *Seven leaves were inoculated in each assay at two points and each shoot inoculated at only one point.
Abbreviation: CV, coefficient of variation.

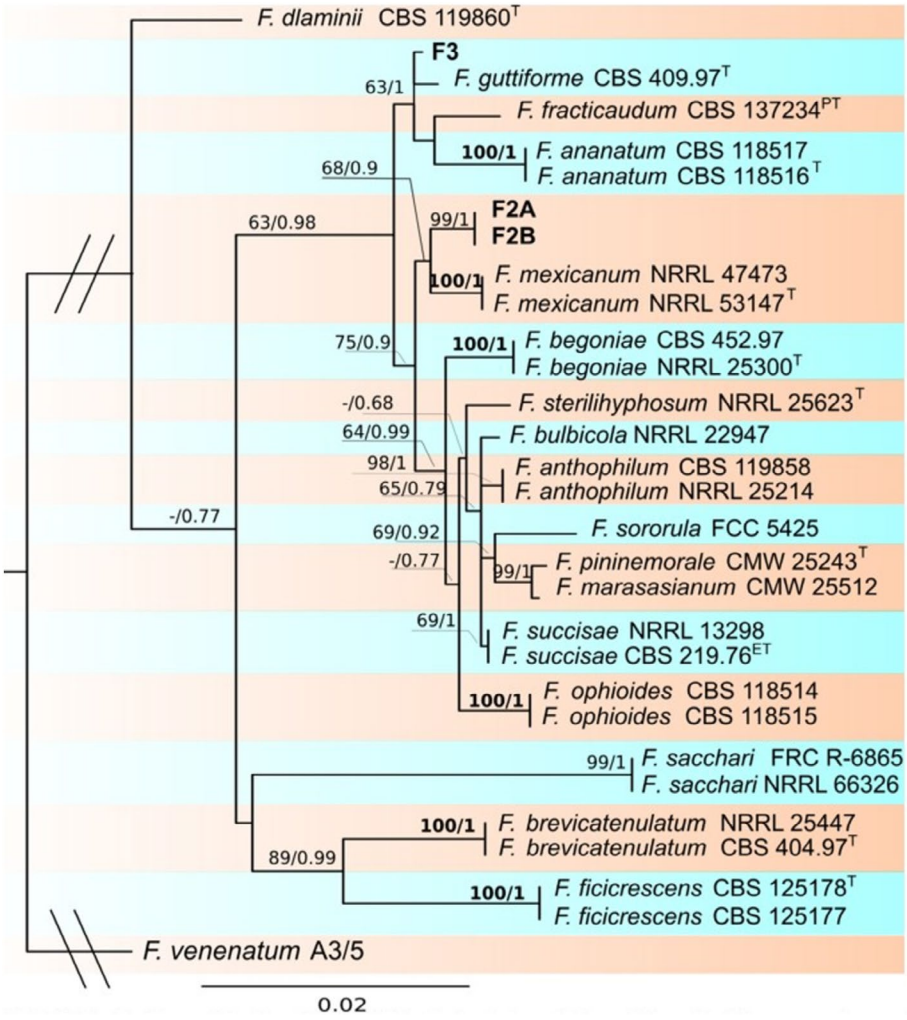

FIGURE 3 | Maximum-likelihood tree (ML) obtained from tef1, rpb2 and tub2 gene regions of species from the *Fusarium fujikuroi* species complex. *F. venenatum* (A3/5) was used as an outgroup. Bayesian posterior probability values (BIPP) ≥ 0.65 and ML bootstrap values (BS) ≥ 50%, are shown at the branches. ET, ex-epitype; PT, ex-paratype; T, ex-type. Isolates from this study are given in bold.

centre. No diffuse pigmentation was observed in the medium. Abundant microconidia in aerial mycelium, grouped in false heads, on mono- and polyphialides, hyaline, predominantly ellipsoidal and occasionally allantoid in shape, (0-)0–1-septate. Cream-coloured sporodochia with a slight orange hue formed only on yerba mate leaves. Macroconidia were slender, slightly curved, generally wider in the middle of their length, and narrowing toward both ends, with a usually foot-shaped basal cell and a beak-shaped apical cell, 3–6-septate. Chlamydospore were not observed (Figure 3g–l).

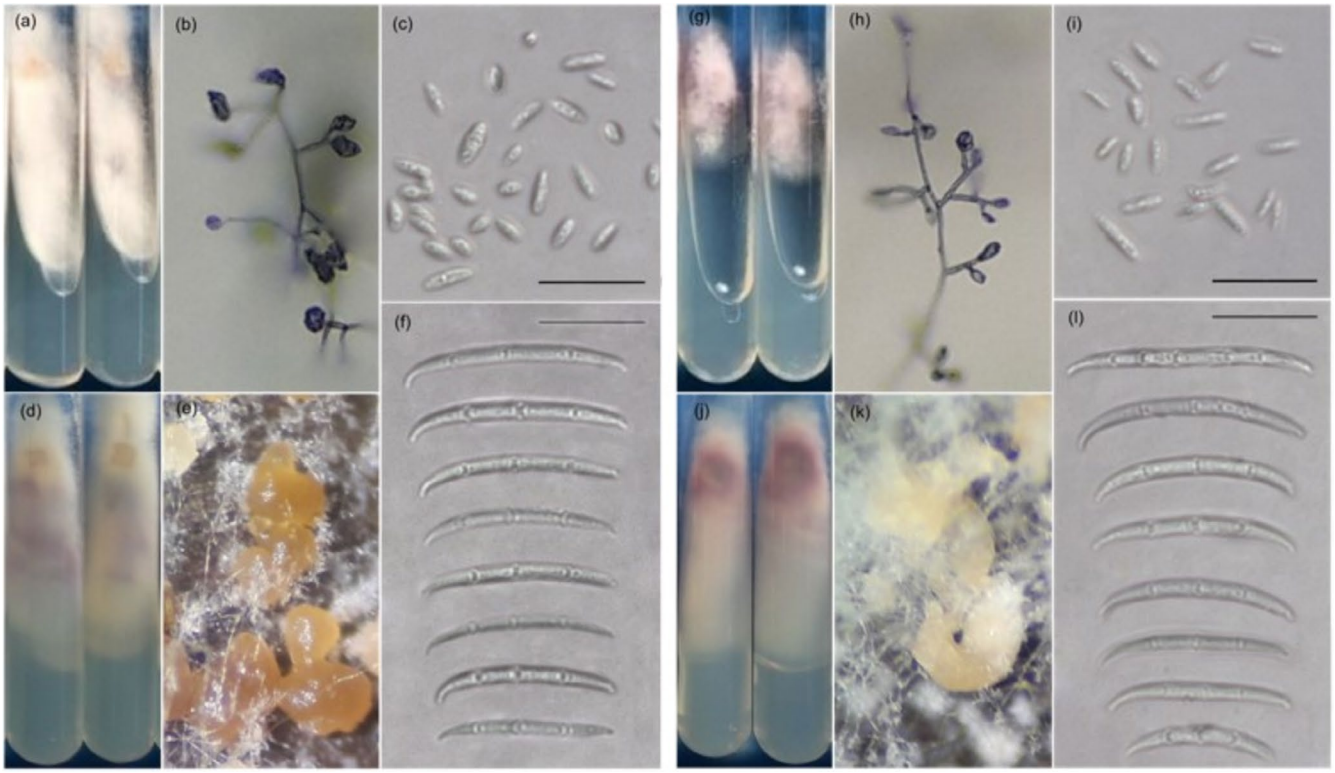

FIGURE 4 | Cultural and morphological characteristics of aerial mycelium and colour of *Fusarium guttiforme* isolate F3: (a–f); *F. mexicanum* isolates F2A and F2B: (g–l). (a, g) The upper view of a colony on PDA after 7 days. (d, j) The reverse view of a colony on PDA after 14 days. (b, h) Microconidia clustering in false heads at the tip of mono- and polyphialides in the aerial mycelium. (c, i) Microconidia. (e, k) Sporodochia formed on the surface of inoculated yerba mate leaves after 14 days. (f, l) Macroconidia. Scale bar: 20 μm.

4 | Discussion

This study demonstrated that the fungi *F. guttiforme* and *F. mexicanum* are pathogenic to yerba mate, causing necrotic lesions on detached leaves and shoots and on shoots of adult yerba mate plants. Previous studies have found associations between unidentified *Fusarium* species and yerba mate shoots in Brazil without conducting pathogenicity tests (Paula et al. 2018; Auer et al. 2021) and Argentina (Gutiérrez et al. 2021). This is the first report of these species causing diseases in yerba mate worldwide.

Fusarium mexicanum was originally described by Otero-Colina et al. (2010) in Mexico, causing mango malformation (*Mangifera indica* L.). On the other hand, *F. guttiforme* was described by Nirenberg and O'Donnell et al. (1998) causing Fusarium basal rot in pineapple (*Ananas comosus* L. Merril). Further studies including more isolates may elucidate the variability within the *F. mexicanum* clade, as observed in this study.

The species identified in this study, *F. mexicanum* and *F. guttiforme*, belonged to the *F. fujikuroi* species complex. It is important to note that other species within this complex may also be involved in the aetiology of this disease (Piassetta 2022). Similarly, the *F. graminearum* complex may be involved, given its frequent association with seeds (Mireski 2018; Souza et al. 2019; Vargas 2020). Additionally, *F. solani* and *F. oxysporum* species complexes have been associated with seedling damage and root rot in yerba mate (Grigoletti Júnior and Auer 2000; Poletto et al. 2012; Auer and dos Santos 2016). The involvement of these fungi in yerba mate shoot blight cannot be ruled out. However, further studies are required to address this issue.

This study identified these fungi through multilocus molecular phylogeny using rpb2, tef1 and tub2 genes (Yilmaz et al. 2021; Crous et al. 2021). Cultural and morphological characteristics were also examined to complement the molecular identification. Their pathogenic association was also confirmed.

Species of the *F. fujikuroi* complex are known to cause diseases in important tree plant species, such as mango (Liew et al. 2016) and pecan (Lazarotto et al. 2014). In mango trees in Australia, seven species within the *F. fujikuroi* species complex were identified in association with mango malformation (Liew et al. 2016). In pecan in Southern Brazil, the *F. fujikuroi* species complex has been isolated from inflorescences and roots and was pathogenic when inoculated (Lazarotto et al. 2014).

Shoot blight commonly occurs in cultivated yerba mate (Paula et al. 2018; Auer et al. 2021); additionally, we observed this disease in natural *Ilex paraguariensis* populations. However, the epidemiological factors influencing disease development, such as humidity and temperature, remain largely unexplored. The future of forest sustainability will largely depend on our ability to deal with the growing threat of pests and pathogens. This will require more efficient global coordination and optimised use of the best available identification tools. In this context, accurately identifying pathogens using DNA-based technologies emerges as a crucial component (Wingfield et al. 2015). Future studies could involve analysis of a larger number of isolates collected

from broader geographic areas to enhance understanding of genetic and pathogenic variability among these pathogens.

Author Contributions

Valdomiro Bilenki Junior carried out sample collection, pathogen isolation, morphological characterisation, DNA sequence analysis, phylogenetic analysis and pathogenicity tests, as well as data analysis and manuscript writing. Paula Cristina dos Santos Rodrigues deposited the obtained sequences in GenBank. Celso Garcia Auer provided methodological suggestions, reviewed the text and supported practical laboratory issues. Dauri José Tessmann reviewed the text, assisted in performing the phylogenetic analyses and interpreting the results. Tiago Miguel Jarek assisted in the morphological characterisation of the isolates. Álvaro Figueredo dos Santos obtained the resources needed for sequencing the isolates, organised the work and reviewed the manuscript. All authors discussed the results, provided critical feedback and contributed to the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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