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Diversity and pathogenicity of *Fusarium* spp. isolated from cultivated sorghum stems and roots in eastern Australia

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Abstract

Stalk and root rots of cultivated sorghum (*Sorghum bicolor*) are caused by several *Fusarium* species worldwide. This study evaluated *Fusarium* diversity, pathogenicity and population structure amongst 212 isolates obtained from 169 sorghum plants from commercial field crops in eastern Australia. Sequences of translation elongation factor-1α (*tef-1α*), RNA polymerase II largest subunit (*rpb1*), RNA polymerase II second largest subunit (*rpb2*) and calmodulin (*cmdA*) were used to construct multilocus phylogenies that enabled the identification of 16 *Fusarium* species in *Fusarium chlamydosporum* species complex (FCSC), *Fusarium fujikuroi* species complex (FFSC), *Fusarium incarnatum-equiseti* species complex and *Fusarium oxysporum* species complex (FOSC). The majority of isolates ($n = 171$) belonged to FFSC. The pathogenicity of 17 selected isolates was determined by artificial inoculation of sorghum seedlings and completing Koch's postulates. Isolates of species in FFSC were significantly ($p < 0.05$) more aggressive as root pathogens in sorghum seedlings than isolates of other species tested and widely distributed across all sampling sites. Amongst the 35 isolates of FOSC, 26 belonged to *Fusarium cili*, which is only known as an endophyte from healthy roots of *Rosa roxburghii* in China. *Fusarium sporodochiale* (in FCSC) and *Fusarium contaminatum* (in FOSC) are reported as sorghum seedling root rot pathogens for the first time.

KEYWORDS
Fusarium fujikuroi species complex, phylogenetic analysis, stalk rot

1 | INTRODUCTION

Sorghum (*Sorghum bicolor*) is the fifth most important cereal crop in the world with a range of applications as a dietary staple for humans as well as feed for livestock (Dahlberg et al., 2012). *Fusarium* species are ubiquitous and much studied as crop pathogens responsible for significant diseases in a wide range of crops, including sorghum (Leslie & Summerell, 2008; Niehaus et al., 2016). Diseases of sorghum caused by *Fusarium* species, especially stalk and root rots and head blight, are amongst the most economically important sorghum diseases (Thakur et al., 2007). *Fusarium* species also affect sorghum productivity through reduced grain yield and/or reduced marketability of harvested grain due to mycotoxin contamination (Astoreca et al., 2019).

Sorghum stalk and root rot reduces grain fill and head size and causes early plant lodging by internal shredding of lower nodes that

are typically discoloured pink or reddish-brown (Figure 1). *Fusarium* species are widespread across Australian sorghum fields, where stalk and root rots are an increasing threat to sorghum production (Kelly et al., 2017). Although data is not available on the actual economic losses in Australia, yield loss is probably in the range of 4%–50%, as reported in the United States (Tesso et al., 2004). *Fusarium* inoculum levels have continuously increased due to the adoption of conservation tillage farming practices and/or the increased use of sorghum as a rotational crop for the management of crown rot in wheat. Further increases in *Fusarium* diseases on sorghum have been predicted with climate change (Kelly et al., 2017). Petrovic et al. (2009) reported differences in diversity and relative abundance of *Fusarium* species associated with sorghum diseases in two agroclimatic regions in eastern Australia.

Fusarium contains almost 900 phylogenetic species in about 20 species complexes (see FUSARIOID-ID database; Crous et al., 2021). Of these, the *Fusarium fujikuroi* species complex (FFSC) is the largest, with more than 60 phylogenetic species (Costa, Saleh, et al., 2021; Yilmaz et al., 2021). *Fusarium* species in the FFSC are often associated with sorghum, and some produce fumonisins, a group of mycotoxins responsible for the contamination of sorghum grains (Corallo et al., 2023). In Australia, *Fusarium* species in the FFSC have been isolated at higher frequencies in association with sorghum diseases than isolates in other species complexes (Kelly et al., 2017; Petrovic et al., 2009). *Fusarium thapsinum* (in FFSC) was one of the dominant pathogens responsible for stalk rot and head blight across the three major sorghum-growing regions in Australia in 2009–2011, based on morphology and phylogenetic analyses of the *tef-1α* region (Kelly et al., 2017).

FIGURE 1 Stem of *Sorghum bicolor* showing symptoms of Fusarium stalk rot at lower nodes with internal shredding and typical pink or reddish-brown tissue discolouration.

Fusarium taxonomy and species identification has traditionally been confusing and complex (Costa, Saleh, et al., 2021; Yilmaz et al., 2021). Increasingly, a phylogenetic approach has been adopted for species identification and classification in *Fusarium*, as demonstrated by the formal taxonomic description based primarily (or entirely) from sequences of 31 Fusaria in 2023 (see MycoBank; Crous et al., 2004).

Characterizing the diversity of the locally dominant species associated with sorghum stalk rot is a critical step in the implementation of sustainable and enduring control measures that prevent yield losses and reduce the risk of mycotoxin contamination in sorghum grain. This is the first study in Australia to use multigene phylogenetic analyses to identify accurately and evaluate the relative abundance of *Fusarium* species associated with sorghum stalk and root rot in eastern Australia.

2 | MATERIALS AND METHODS

2.1 | Sample collection and fungal isolation

Sorghum plants showing symptoms of root and/or stem rot, and asymptomatic plants, were sampled from eight sorghum fields at four locations in Queensland (Qld) and one in northern New South Wales (NSW) (Table S1). Stem and root samples were collected during the 2018 growing season when plants were at physiological maturity. Between 18 and 69 plants were randomly sampled from each site, independently of whether symptoms of root and/or stem rot were expressed, with a minimum of 4 m between samples.

Fusarium species were isolated from symptomatic or asymptomatic sorghum tissues following the isolation method described by Petrovic et al. (2009) with slight modifications. The outer leaves and leaf sheaths were removed from stems. Root and crown tissues were cleaned under running tap water. Tissue segments (4–5 mm^2) were excised from the inner tissues of either roots, crowns or second stem nodes, after surface sterilization in sodium hypochlorite (1.2% NaOCl) for 1 min and two rinses with sterile distilled water. Tissue samples were air dried on sterile filter paper, and then placed on plates of peptone pentachloronitrobenzene (PCNB) agar (6 g peptone, 0.4 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 1 g PCNB [Sigma–Aldrich], 14 g agar, 1 L deionized water) supplemented with 100 μg/mL streptomycin to promote growth of *Fusarium* (Leslie & Summerell, 2008). The PCNB plates were incubated at 23°C for 1 week and hyphal tips from colony margins were transferred aseptically onto half-strength potato dextrose agar (1/2 × PDA) (Difco Laboratories) plates to obtain pure cultures. If colonies with different cultural characteristics emerged from the same tissue, hyphal tips from representative colonies were transferred onto separate PDA plates. Single-spore or

hyphal tip cultures were obtained for all *Fusarium* isolates (Leyronas et al., 2012).

2.2 | DNA extraction and PCR amplification

Isolates were grown for 10–12 days on 1/2×PDA before harvesting 50–100 mg of mycelium. DNA was extracted with a Wizard Genomic DNA Purification Kit following the protocol of the supplier (Promega) after tissue homogenization at a speed of 6 m/s for 20 s (FastPrep-24 5G; MP-Biomedicals). The precipitated DNA was resuspended in 50 µL Tris-EDTA buffer. All DNA samples were stored at 4°C after determination of the concentration of DNA extracted using a Qubit 3 Fluorometer (ThermoFisher Scientific).

Several gene loci were amplified by PCR for molecular characterization of *Fusarium* isolates. Partial sequences of *tef-1α* (primers EF1 and EF2) and RNA polymerase II second largest subunit, *rpb2* (primers 5F2 and 7cR) were amplified for all isolates (Laurence et al., 2016; Lombard, Van Doorn, & Crous, 2019). The calmodulin (*cmdA*) (primers CAL228 and CAL2RD) and/or RNA polymerase II largest subunit *rpb1* (primers F7 and G2R) (Lombard, Van Doorn, & Crous, 2019; Xia et al., 2019) loci were additionally amplified if *tef-1α* and *rpb2* were not adequate to confirm species identification.

Each PCR mixture (total volume of 20 µL) consisted of 1×Hot Start Taq Master Mix (New England Biolabs), 0.2 µM of each primer and 10 ng genomic DNA. An additional 0.5 mM of $MgCl_2$ was added to promote amplification if sequences failed to amplify in the initial PCR. The primer sequences and conditions used for the PCRs are listed in Table S2. After amplification, the quality of the amplicon was determined on 1% agarose gel electrophoresis stained with GelRed (Biotium) by examination under UV light with a GelDoc system (Vilber). The PCR products were purified and sequenced at Macrogen Inc. (Seoul, South Korea).

2.3 | Phylogenetic analysis

Geneious Prime v. 2022.2.2 (Biomatters Ltd) was used to trim sequences manually prior to assembly and alignment as described below. New sequences were deposited in GenBank and accession numbers were obtained (Table S3).

A multigene phylogenetic approach was used to compare sequences generated in this study with ex-type reference sequences of known *Fusarium* spp. Trees were generated treating each gene region as separate partitions in maximum-likelihood (ML) and Bayesian inference (BI) analyses. ML trees were obtained with RAxML v. 8 (Stamatakis, 2014) available as a plug-in in Geneious Prime. Analyses were implemented with the GTR-GAMMA evolution model with 1000 bootstrap replicates. BI analyses for the concatenated data matrices were performed using MrBayes v. 3.2.6 (Huelsenbeck & Ronquist, 2001). The best nucleotide substitution model was determined based on Akaike information criterion (AIC) in MrModeltest v. 2.3 (Nylander, 2009) and PAUP v. 4.0b10 (Swofford, 2003). Posterior probabilities were calculated using Markov chain Monte Carlo (MCMC) with two simultaneous MCMC chains starting from random trees for 1,000,000 generations. Trees were sampled every 100 generations until the average standard deviation of split frequencies reached below 0.1. The 50% majority rule consensus tree was generated after a burn-in of 25%.

The first step of the phylogenetic analysis was to assign new isolates into *Fusarium* species complexes. Sequences of ex-type isolates or reliable reference isolates of *Fusarium* species (if ex-type sequences were not available), together with *Bifusarium dimerum* (ex-type strain CBS 108944) as the outgroup taxon, were retrieved from NCBI's GenBank nucleotide database (Table S3). The analysis included 252 sequences (212 new sequences generated in this study together with 40 representative reference species from the 20 different *Fusarium* species complexes). All reference sequences and new sequences generated in this study were aligned using MAFFT v. 7.450 in Geneious Prime for each locus separately. Alignments were then trimmed avoiding missing data at either end of alignments. The phylogenetic relationships of the representative reference species from different *Fusarium* species complexes with isolates from this study were reconstructed using a concatenated two loci data matrix of 1400 bp (*tef-1α*: 590 bp and *rpb2*: 810 bp).

Species were identified within the *Fusarium chlamydosporum* species complex (FCSC) ($n=4$), FFSC ($n=171$), *Fusarium incarnatum-equiseti* species complex (FIESC) ($n=2$) and *Fusarium oxysporum* species complex (FOSC) ($n=35$). Separate phylogenetic trees for each *Fusarium* species complex were constructed using data matrices for two, three or four loci, depending on the species complex. Species identities within FCSC were confirmed by a dendrogram constructed with a four-locus data matrix consisting of 2877 bp (*tef-1α*: 560 bp; *rbp1*: 898 bp; *rpb2*: 841 bp and *cmdA*: 578 bp). The ML and BI trees consisted of nine reference taxa defined by Lombard, Van Doorn, and Crous (2019) and *F. concolor* (NRRL 13459) as the outgroup. The best-fit nucleotide substitution models for each gene as selected by AIC were GTR+G for *rpb2* and *tef-1α*, SYM+I for *cmdA* and SYM+G for *rpb1*. The dendrogram for FFSC was constructed using a concatenated data matrix of 1339 bp, which included the partial gene sequences of *tef-1α* (519 bp) and *rpb2* (820 bp), and reference sequences from 63 species currently recognized in FFSC (Costa, Saleh, et al., 2021; Yilmaz et al., 2021) with *F. nirenbergiae* (ex-type strain CBS 840.88) as the outgroup taxon. The best-fit nucleotide models selected by AIC for BI tree were SYM+G for *tef-1α* and GTR+I+G for *rpb2*. For FIESC, the concatenated data matrix (2032 bp) consisted of partial gene sequences of *tef-1α* (639 bp), *rpb2* (839 bp), and *cmdA* (554 bp) with 41 taxa belonging to this complex (Xia et al., 2019) and *F. concolor* (ex-type strain NRRL 13459) as outgroup taxon for ML and BI phylogenies (selected AIC models for *tef-1α* and *rpb2* was GTR+I+G and for *cmdA* was SYM+G). ML and BI trees for FOSC were constructed with a concatenated data matrix (1360 bp)

consisting of *rpb2* (813 bp), and *tef-1α* (547 bp). Thirty-one taxa recently defined by Maryani et al. (2018) and Lombard, Sandoval-Denis, et al. (2019) were included with *F. foetens* (ex-type strain CBS 110286) as the outgroup. The best-fit model by AIC for BI tree was SYM + I + G for both genes.

2.4 | *Fusarium* species frequency

The isolation frequencies for each *Fusarium* species complex or individual species were calculated by dividing the number of isolates recovered for each species/species complex by the total number of *Fusarium* isolates. The statistical differences between isolation frequencies were determined by a nonparametric test (one-sample chi-square test) at 0.05 significance level, hypothesizing that there was no difference between calculated frequencies (null hypothesis), using SPSS (IBM statistics, v. 26).

2.5 | Pathogenicity test

The pathogenicity of 17 *Fusarium* spp. representative of the diversity of isolates found in this study were tested in a pathogenicity assay. These included seven isolates in FFSC (BRIP 75152, BRIP 75159, BRIP 75158, BRIP 75146, BRIP 75148, BRIP 75161 and CCH300), three in FCSC (BRIP 75147, BRIP 75149 and BRIP 75150), two in FIESC (BRIP 70770 and BRIP 70756), and five in FOSC (BRIP 75313, BRIP 75154, BRIP 75162, BRIP 75157 and BRIP 70744). The aggressiveness of isolates was assessed by a test tube assay as described by Kelly et al. (2017) with slight modifications. Sterile 50 mL Falcon tubes containing 2 g of sterilized vermiculite were inoculated with an agar plug of the selected *Fusarium* isolate; controls were not inoculated with *Fusarium*. Two successive layers of sterilized vermiculite (1 g each) were then added to tubes before and after planting one seed of *S. bicolor* 'Resolute'. The tubes were then moistened with sterile distilled water and the lids closed. The seeds were surface sterilized in 1.2% NaOCl for 1 min (Kelly et al., 2017) before planting. Tubes were incubated on a laboratory bench under ambient conditions (23–24°C) until signs of development of above-ground plant parts were seen (4–5 days). Test tubes with young seedlings were transferred to a glasshouse for a further 5–6 days. Seedlings (10–12 days old) were washed under running tap water to remove vermiculite and rated visually for disease severity (tissue discolouration and/or rotting) on a 0–10 scale, where 0 was a healthy root system with no discolouration and 10 was >90% of the root system discoloured (root rot). A randomized block design with three replicates was used and the experiment was repeated three times. Differences in pathogenicity between species were determined at $p < 0.05$ by a nonparametric test (independent samples, Kruskal–Wallis) with SPSS (IBM Statistics, v. 26). Pathogen reisolations were done by plating surface-sterilized root tissues of 18 randomly selected seedlings from each of the replicated 17 treatments showing root rot symptoms or control, onto 1/2 × PDA supplemented with streptomycin (100 μg/mL).

TABLE 1 Frequencies of *Fusarium* species isolated from sorghum sampled from different locations in northern New South Wales and Queensland, Australia.

Complex	Species	*n*	Total isolation frequency (%)
FFSC	*F. thapsinum*[a]	92	43.4*
	F. verticillioides[a]	21	9.9
	F. andiyazi[a]	20	9.4
	F. nygamai[a]	19	9.0
	F. annulatum[a]	17	8.0
	F. subglutinans	1	0.5
	F. madaense	1	0.5
	Subtotal	171	80.7*
FOSC	*F. cili*	26	12.3
	F. irdabamae	4	1.9
	F. contaminatum	3	1.4
	F. queenslandicum	1	0.5
	F. wimaladesilvae	1	0.5
	Subtotal	35	16.5*
FCSC	*F. atrovinosum*	3	1.4
	F. sporodochiale	1	0.5
	Subtotal	4	1.9
FIESC	*F. nanum*	1	0.5
	F. clavum	1	0.5
	Subtotal	2	0.9
Total		212	100

Abbreviations: FFSC, *Fusarium fujikuroi* species complex; FOSC, *Fusarium oxysporum* species complex; FCSC, *Fusarium chalmydosporum* species complex; FIESC, *Fusarium incarnatum-equiseti* species complex.
[a]Species included in the statistical analysis. (Due to inadequate numbers the other species were not included in the statistical analysis).
*Significantly different values between isolation frequencies of *Fusarium* species complex or individual species as determined by chi-square test ($p < 0.05$).

3 | RESULTS

3.1 | Species diversity and frequency

A total of 212 *Fusarium* isolates were recovered from 169 symptomatic or asymptomatic sorghum plants collected from eight fields in Qld and northern NSW (Table S1). Based on the multilocus phylogenies, 16 known species were identified within FCSC, FFSC, FIESC and FOSC (Table 1). Most isolates belonged to FFSC ($n = 171$, 80.7% frequency), followed by FOSC (Figure 2a). Species belonging to FCSC and FIESC were recovered at significantly ($p < 0.05$) lower frequencies (<2%; Table 1). The four-locus phylogeny (*tef-1α*, *rpb1*, *rpb2* and *cmdA*) assigned four isolates to two species, *F. atrovinosum* and *F. sporodochiale* in FCSC (Figure 3). In FFSC, *F. thapsinum* (92 isolates), *F. verticillioides* (21), *F. andiyazi* (20), *F. nygamai*, (19) and *F. annulatum* (17) were recovered at higher frequencies than other species (Figure 2b, Table 1), while two species were only recovered once, *F. subglutinans* (1) and *F. madaense* (1) (Figure S1, Table 1).

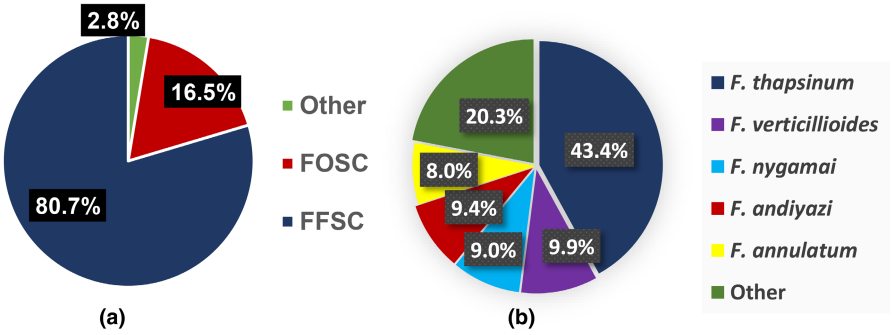

FIGURE 2 Recovery frequencies of *Fusarium* species collected from commercial sorghum fields in five different locations in northern New South Wales and Queensland, Australia. (a) Recovery frequency of the *Fusarium fujikuroi* species complex (FFSC), *Fusarium oxysporum* species complex (FOSC) and other species complexes; (b) frequency of *Fusarium* species belonging to the FFSC.

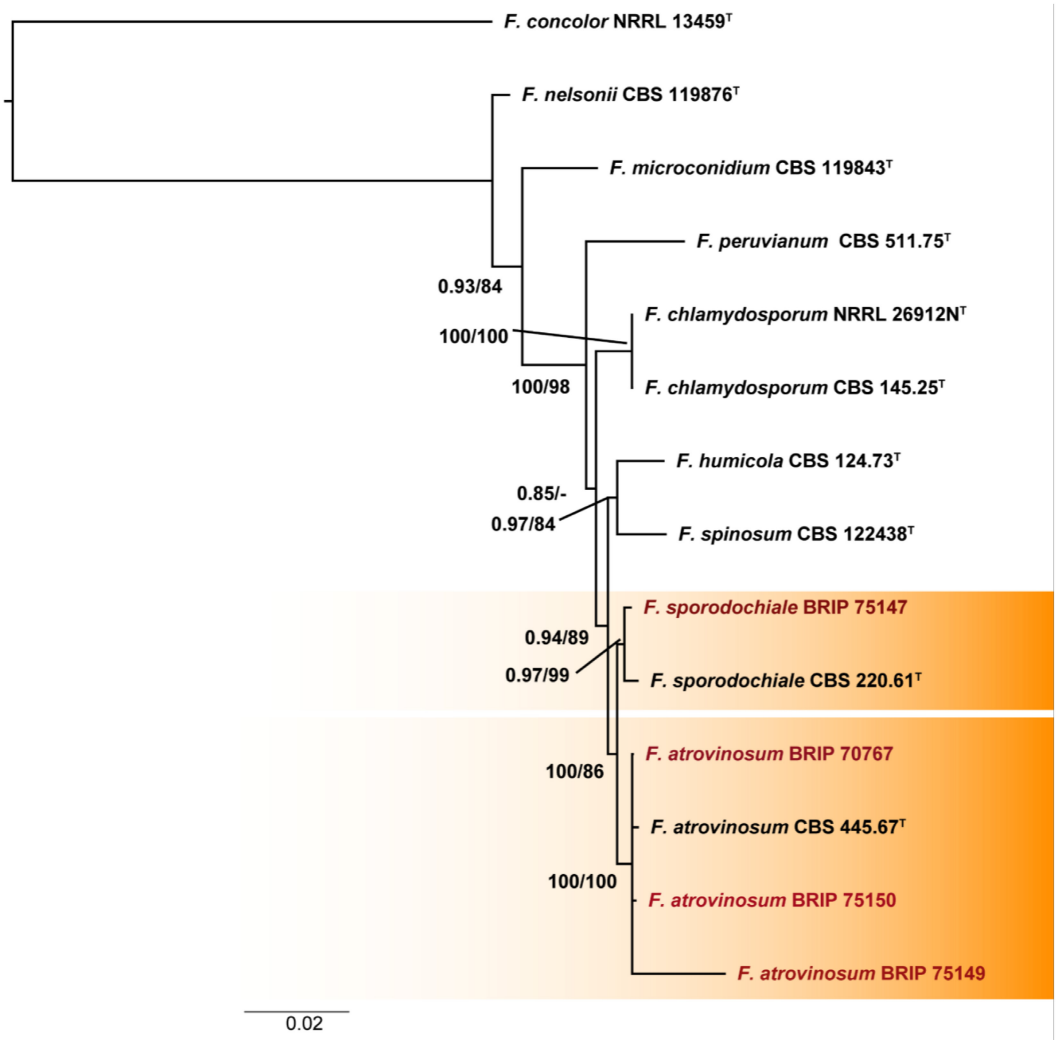

FIGURE 3 Maximum-likelihood phylogenetic tree inferred from a concatenated alignment including partial sequences of *tef-1α*, *rpb1*, *rpb2*, and *cmdA* with *Fusarium concolor* (ex-type strain NRRL 13459) as the out-group, depicting the phylogenetic relatedness of four *Fusarium* isolates (BRIP 75149, BRIP 70767, BRIP 75150 and BRIP 75147) from this study with reference species within the *Fusarium chlamydosporum* species complex (FCSC). Maximum-likelihood bootstrap (<70%) and Bayesian posterior probability values (<0.80) are shown at the internodes. The sequences from ex-type and ex-epitype strains are indicated with ^{T} and ^{ET}, respectively.

F. thapsinum was the dominant species in FFSC with an isolation frequency of 43% ($p < 0.05$) (Figure 2b). Isolates identified as *F. thapsinum*, *F. verticillioides*, *F. andiyazi*, *F. nygamai* and *F. annulatum* in FFSC were mostly recovered from stems, crowns and roots. Two isolates were identified as *F. nanum* and *F. clavum* in the FIESC, using a three-locus phylogeny (*tef-1α*, *rpb2* and *cmdA*; Figure 4). In

the phylogenetic tree inferred from the concatenated gene alignment for FOSC, 35 isolates were identified comprising *F. contaminatum* ($n=3$ isolates), *F. queenslandicum* ($n=1$), *F. irdabamae* ($n=4$), *F. wimaladesilvae* ($n=1$) and *F. cili* ($n=26$) (Figure 5).

Of the 212 isolates recovered, 78 isolates were from asymptomatic sorghum tissues. None of the frequently isolated pathogenic species were exclusively recovered from symptomatic or asymptomatic tissues. However, a significantly ($p<0.05$) higher number of isolates of *F. thapsinum* and *F. verticillioides* were recovered from symptomatic tissues (data not shown).

3.2 | Pathogenicity and aggressiveness

Thirteen of 17 isolates of *Fusarium* caused root rot of sorghum seedlings characterized by stunted growth and/or reddish-to-brown

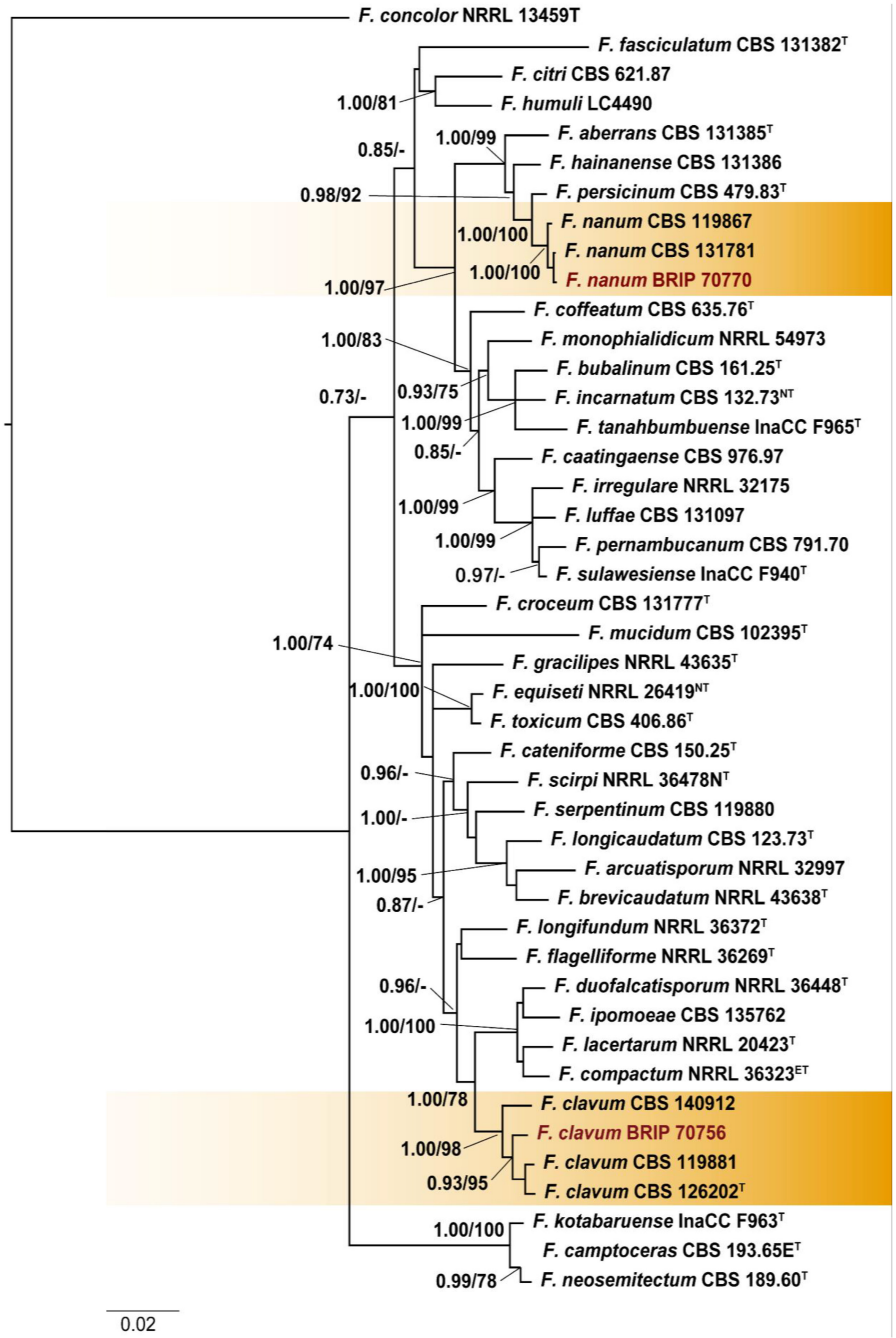

FIGURE 4 Maximum-likelihood phylogenetic tree of *Fusarium incarnatum-equiseti* species complex (FIESC) inferred from a concatenated alignment including partial sequences of *tef-1α*, *rpb2*, and *cmdA* with *Fusarium concolor* ex-type strain NRRL 13459 as the out-group. Two isolates BRIP 70756 and BRIP 70770 from this study clustered with *F. clavum* and *F. nanum*, respectively. Maximum-likelihood bootstrap (<70%) and Bayesian posterior probability values (<0.80) are shown at the internodes. The sequences from ex-type and ex-epitype strains are indicated with ^{T} and ^{ET}, respectively.

discolouration of the roots (Figure 6) in the pathogenicity tests. All isolates used in the inoculations were reisolated from diseased roots completing the requirement of Koch's postulates for causality. *Fusarium* was not isolated from control plants. Average disease severity ratings induced by the 13 isolates on seedlings of *S. bicolor* 'Resolute' ranged from 1 to 7. All seven species belonging to the FFSC were highly aggressive in inducing root symptoms ($p < 0.05$; Figure 6). The highest disease severity rating (7) was recorded with an isolate of *F. thapsinum* (BRIP 75152) from FFSC (Figures 6 and 7). Disease severity ratings for isolates in FIESC, FCSC and FOSC were significantly lower than isolates in FFSC. Four isolates, *F. clavum* (BRIP 70756) in FIESC, *F. cili*, (BRIP 70744 and BRIP 75157) and *F. irdabamae* (BRIP 75313) in FOSC, were nonpathogenic.

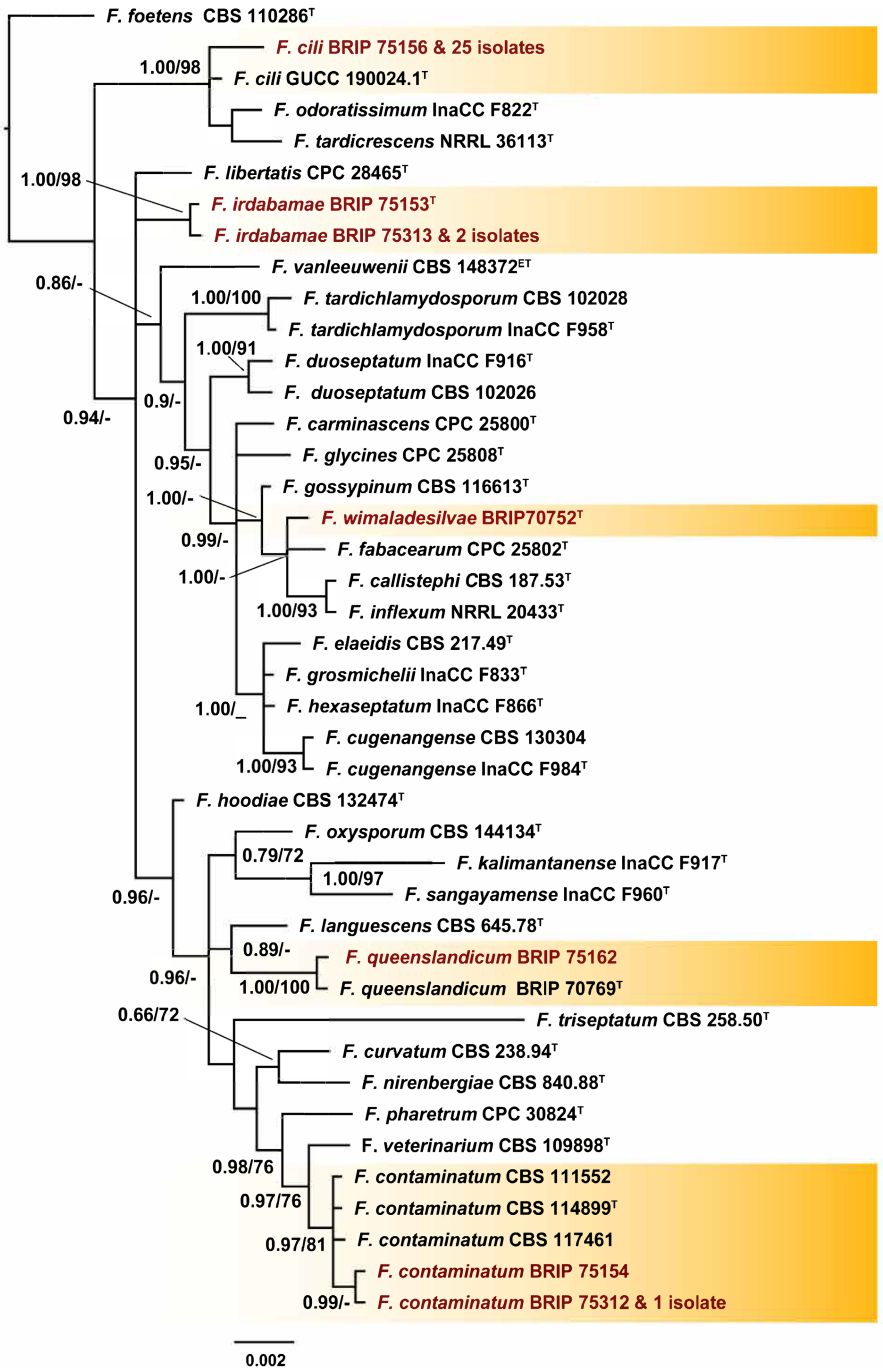

FIGURE 5 Maximum-likelihood phylogenetic tree constructed from a concatenated alignment including partial sequences of *tef-1α*, *rbp1* and *rbp2*, with *Fusarium foetens* (CBS 110286 strain) as the out-group, depicting the phylogenetic relatedness of 36 *Fusarium* isolates from this study (shown in red) with reference species within the *Fusarium oxysporum* species complex (FOSC). Maximum-likelihood bootstrap values (<70%) and Bayesian posterior probability values (<0.80) are shown at the internodes. The sequences from ex-type and ex-epitype strains are indicated with ^{T} and ^{ET}, respectively.

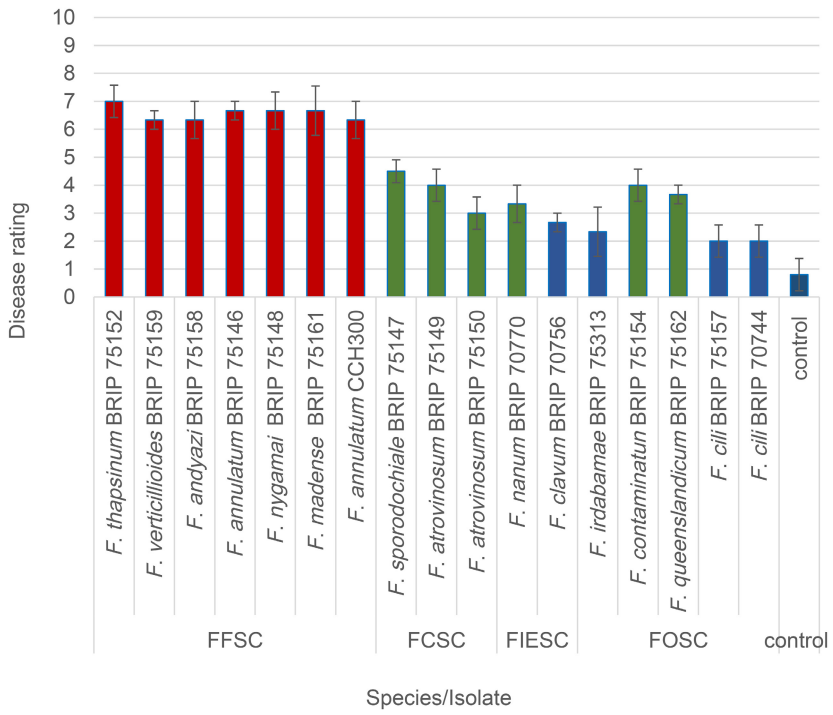

FIGURE 6 Differences in severity of root disease symptoms caused by 17 representative species/isolates belonging to four species complexes: *Fusarium fujikuroi* species complex (FFSC), *Fusarium chlamydosporum* species complex (FCSC), *Fusarium incarnatum-equiseti* species complex (FIESC) and *Fusarium oxysporum* species complex (FOSC) on 10-day-old seedlings of *Sorghum bicolor* 'Resolute'. Bars in different colours are significantly different from each other ($p < 0.05$).

FIGURE 7 Root symptoms (dark red to black discolouration of diseased roots) caused by *Fusarium thapsinum* (BRIP 75152) (a) and *Fusarium andiyazi* (BRIP 75158) (b) on 10-day-old seedlings of *Sorghum bicolor* 'Resolute' compared to the healthy root system in the control (c).

4 | DISCUSSION

Fusarium species in at least six species complexes have been reported to cause sorghum stalk rot (Kelly et al., 2017; Lombard et al., 2022; Petrovic et al., 2009). In general, species in the FFSC have often been isolated and recorded as sorghum pathogens that cause stalk rot, root rot, sheath blight and head blight (Félix-Gastélum et al., 2022; Pena et al., 2019). Previous surveys of sorghum stalk rot in Australia identified species in FFSC, FCSC, FOSC and FIESC as well as *Fusarium sambusinum* species complex (FSAMSC) and *Fusarium solani* species complex (FSSC) (Table 2; Kelly et al., 2017; Petrovic et al., 2009). Overall, species belonging to FFSC were recovered in high frequencies from stalks of sorghum, with recovery frequencies of individual species varying depending on the year of surveying.

We found that *F. thapsinum* (FFSC) was the most frequently isolated species from sorghum samples that we collected and examined in Qld and NSW. *F. thapsinum* is also the most important pathogen associated with sorghum diseases worldwide and is frequently recovered from both symptomatic (Diakite et al., 2022; Jardine & Leslie, 1992) and asymptomatic (Thio et al., 2021) sorghum plants.

Fusarium verticillioides was the second most recovered species in our study. Previous studies (Kelly et al., 2017; Petrovic et al., 2009) have also indicated that *F. verticillioides* may be an important pathogen of sorghum crops in eastern Australia. We showed that one isolate of *F. verticillioides* caused severe root rot in sorghum seedlings. *F. verticillioides* is a common pathogen of maize and millet worldwide (Leslie et al., 2005; Yilmaz et al., 2021). Our study indicated that *F. verticillioides* may be an important pathogen of sorghum crops in

TABLE 2 *Fusarium* species isolated from sorghum stalks in Australia: A comparison of our results with two previous surveys.

Complex	Species	Isolation frequency		
		2018 (this study)[a]	2008–2012 (Kelly et al., 2017)[a]	2003 (Petrovic et al., 2009)[b]
FFSC	*F. thapsinum*	43.4	46.9	33.4
	F. verticillioides	9.9	0	1.0
	F. andiyazi	9.4	36.5	38.5
	F. nygamai	9.0	0	4.6
	F. annulatum	8.0	2.7	3.8
	F. subglutinans	0.5	0	1.0
	F. madaense	0.5	0	0
FCSC		1.9	0	6.8
FIESC		0.9	12.2	10.0
FOSC		16.5	1.7	0.3
FSSC		0	0	0.3
FSAMSC		0	0	0.1

Abbreviations: FFDSC, *Fusarium fujikuroi* species complex; FCSC, *Fusarium chlamydosporum* species complex; FIESC, *Fusarium incarnatum-equiseti* species complex; FOSC, *Fusarium oxysporum* species complex; FSSC, *Fusarium solani* species complex; FSAMSC, *Fusarium sambucinum* species complex.

[a]Samples collected from southern Queensland and northern New South Wales.

[b]Samples collected from northern grain belt of eastern Australia.

eastern Australia as found in previous studies (Kelly et al., 2017; Petrovic et al., 2009).

Fusarium annulatum was commonly isolated from sorghum samples in our survey, which is reported to be associated with a range of tropical grasses (Yilmaz et al., 2021), including sorghum in eastern Australia (as *F. proliferatum*) (Kelly et al., 2017; Petrovic et al., 2009).

Fusarium nygamai was first collected from sorghum in Australia in 1986 (Burgess & Trimboli, 1986) and was subsequently found on maize, millet, bean, cotton and rice in some tropical countries (Balmas et al., 2000; Costa, Saleh, et al., 2021). *F. nygamai* was recovered from sorghum stalks in 2003 (Petrovic et al., 2009) at an isolation frequency similar to that found in our study (9%). However, *F. nygamai* was not found in eastern Australia in surveys conducted in 2008–2011 (Kelly et al., 2017).

In previous surveys conducted in Australia, *F. andiyazi* was isolated at significantly higher frequencies than *F. verticillioides*, *F. annulatum* or *F. nygamai* (Kelly et al., 2017; Petrovic et al., 2009). In our study, *F. andiyazi* was widely distributed at low frequency (9%), which may be due to variable environmental conditions (e.g., available moisture, temperature) at different sampling sites in Qld and NSW (Petrovic et al., 2009).

One isolate each of *F. madaense* and *F. subglutinans* (both in FFSC) was obtained in our study. *F. madaense* and *F. subglutinans* are both known to cause sorghum root and stem rots in Australia (Gunasinghe et al., 2023; Kelly et al., 2017). Three species, *F. nanum* (FIESC), *F. clavum* (FIESC) and *F. atrovinosum* (FCSC), found in our study have been previously isolated from sorghum in Australia and identified as potential sorghum pathogens (Boonmee et al., 2021; Xia et al., 2019). In Australia, *F. atrovinosum* was first recorded on wheat in 1919 (Lombard, Van Doorn, & Crous, 2019) and on sorghum in 2021 (Boonmee et al., 2021). Isolates in FIESC, FCSC and FOSC not identified at species level were previously recovered from sorghum stalks and panicles in Australia (Kelly et al., 2017; Petrovic et al., 2009). Three species, *F. sporodochiale* (FCSC), *F. contaminatum* and *F. cili* (FOSC), identified in our study have not been previously reported from Australia nor from sorghum anywhere in the world. In our study, FOSC isolates were either nonpathogenic or weak pathogens of sorghum seedlings in comparison to the other species tested. Species in FOSC associated with sorghum may be endophytes or saprobes (Kelly et al., 2017; Petrovic et al., 2009).

The isolate (BRIP 75147) of *F. sporodochiale* (FCSC) that we obtained from a diseased sorghum stalk in Qld induced root discolouration on sorghum seedlings in the pathogenicity tests. *F. sporodochiale* has been previously found in South Africa in soil from a termite nest (Lombard, Van Doorn, & Crous, 2019) as well as from the healthy inflorescence of *Syzygium cordatum* (Mkandawire et al., 2022), but never previously as a pathogen on sorghum.

Fusarium species in FOSC have often been reported as both pathogens that cause root and crown rots in sorghum (Idris et al., 2007) and as nonpathogenic endophytes with potential biocontrol capabilities (Rebeka et al., 2013). *F. contaminatum* was previously found in contaminated dairy products and fruit juice from Germany and the Netherlands (Lombard, Sandoval-Denis, et al., 2019). However, our study is the first to show that *F. contaminatum* is a plant pathogen that can cause root rot on sorghum seedlings. *F. queenslandicum* was first reported from a dead sorghum stalk in Qld (Boonmee et al., 2021) and the isolate from this study (BRIP 75162) collected from a diseased sorghum root was able to cause seedling root discolouration in the seedling pathogenicity tests. *F. cili* was first recorded as an endophyte of *Rosa roxburghii* roots (Zhang et al., 2023). The representative

isolates of *F. cili* (BRIP 75157 and BRIP 70744) recovered from sorghum crown tissues in our study did not induce seedling root rot symptoms.

In FFSC, we isolated *F. andiyazi*, *F. annulatum*, *F. nygamai*, *F. thapsinum* and *F. verticillioides* from healthy-looking sorghum tissues. These species are known to colonize the tissues of several plants without producing symptoms under environmental conditions not conducive to disease development (Blacutt et al., 2018; Potshangbam et al., 2017). Thio et al. (2021) reported a diverse population of endophytic *Fusarium* species, dominated by *F. thapsinum* (69%), from different tissues of healthy-looking sorghum plants. Some *Fusarium* species can switch between the roles of asymptomatic endophyte and necrotrophic pathogen, by regulating the pathways involved with host interactions, development and survival, for example, *F. verticillioides* in maize tissues (Blacutt et al., 2018). Many *Fusarium* species that colonize sorghum as either pathogens or endophytes (Gerling et al., 2022) are associated with latent infections of Australian native grasses (Costa, Melo, et al., 2021), which aids their survival and distribution. The ability of weed species in Australia to serve as alternative hosts and increase inoculum levels of *Fusarium* species pathogenic to sorghum is not known.

In our study, multiple *Fusarium* species were recovered from one sorghum tissue sample. *Fusarium* species are well known for causing mixed infections in sorghum (Chala, 2019; Kelly et al., 2017) or other crop species such as maize (Leyva-Madrigal et al., 2015). For example, the disease severity of naturally occurring ear rot in maize is highly variable because initial infections are caused by a mixture of locally available *Fusarium* species and subsequently, one species dominates at the later stage of disease development (Leyva-Madrigal et al., 2015). Further studies are needed to determine whether individual species survive as endophytes and/or co-exist as pathogens in mixed infections, which may affect the severity of plant disease at later stages of host development.

Fusarium andiyazi has been reported as less aggressive than *F. thapsinum* in sorghum seedling pathogenicity tests (Leslie et al., 2005) and similarly aggressive in stalk rot resistance evaluation trials of adult sorghum plants (Kelly et al., 2017; Tesso et al., 2004). In our seedling pathogenicity assay, we observed that both *F. andiyazi* and *F. thapsinum* caused similar levels of root rot. Our isolates in FIESC and FCSC were pathogenic on sorghum roots but were significantly less aggressive than isolates in FFSC, as found by Kelly et al. (2017). In Australia, *F. oxysporum* has previously been reported as a sorghum root colonizer, together with several other *Fusarium* spp. (Kelly et al., 2017; Petrovic et al., 2009).

In conclusion, we found 16 *Fusarium* species in four *Fusarium* species complexes on sorghum in eastern Australia. Species in FFSC were widely distributed in sorghum crops, with *F. thapsinum* the most frequently isolated. Some isolates of *F. andiyazi*, *F. annulatum*, *F. madaense*, *F. nygamai*, *F. thapsinum* and *F. verticillioides* were highly aggressive root rot pathogens in the sorghum seedling assays. *F. sporodochiale* (in FCSC) and *F. contaminatum* (in FOSC) are reported as sorghum seedling root rot pathogens for the first time.

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DATA AVAILABILITY STATEMENT

The data support the findings of this study are available through three supplementary tables and a figure, and from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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