Fusarium species isolated from *Pennisetum clandestinum* collected during outbreaks of kikuyu poisoning in cattle in South Africa

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Kikuyu poisoning occurs sporadically in South Africa. It is of major economic importance, as valuable dairy cows are often poisoned by it, and once affected, the mortality rate is high. *Pennisetum clandestinum* samples were collected during eight outbreaks of kikuyu poisoning in cattle in the Eastern Cape Province of South Africa from 2008 to 2010. The kikuyu grass samples were submitted specifically for the isolation and molecular identification of *Fusarium* species, as it was recently suggested that mycotoxins synthesised by *Fusarium torulosum* could be the cause of this intoxication. Ninety-four *Fusarium* isolates were retrieved from the grass samples, of which 72 were members of the *Fusarium incarnatum/Fusarium equiseti* species complex based on morphology and phylogenetic analyses of the translation elongation factor 1α sequence data. The South African isolates from kikuyu identified as members of the *F. incarnatum/F. equiseti* species complex grouped together in six separate clades. The other isolates were *Fusarium culmorum* (n = 3), *Fusarium redolens* (n = 4) and *Fusarium oxysporum* (n = 15). Although *F. torulosum* could not be isolated from *P. clandestinum* collected during kikuyu poisoning outbreaks in South Africa, the mycotoxicosis theory is still highly plausible.

Introduction

Kikuyu poisoning, an intoxication of ruminants, is described as a ruminitis syndrome resulting in, among other manifestations, forestomach necrosis and dehydration (Bourke 2007; Kellerman *et al.* 2005). Although kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.) is usually grazed without deleterious effects, it sporadically becomes toxic (Bourke 2007; Kellerman *et al.* 2005). This usually happens when the kikuyu grass pasture has been subjected to severe stress in some form (like drought or invasion by army worm caterpillars) followed by a growth spurt during warm weather (Bryson 1982; Kellerman *et al.* 2005; Newsholme *et al.* 1983). Poisoning has been reported from New Zealand, Australia and eastern and southern Africa (Bourke 2007; Bryson 1982; Martinovich, Mortimer & Di Menna 1972).

Although kikuyu poisoning occurs periodically in South Africa, it is of major economic importance, as valuable dairy cows are often poisoned and, once affected, the mortality rate is high (Bryson 1982). After moving ruminants, especially cattle, to kikuyu pastures there is usually a latent period of 24 h or longer before clinical signs, mainly gastrointestinal and neuromuscular, are observed. Gastrointestinal signs are usually present; these include ruminal atony, distension and colic (kicking at the abdomen and grunting). Ruminal irritation and malabsorption result in the accumulation of excessive fluid in the rumen, leading to sloppy ruminal contents, which may even gush from the mouth and nose at death. Severe dehydration with sunken eyes and an unpliable skin is observed. The neuromuscular signs are distinguished by bulbar paralysis, resulting in an inability to swallow that is characterised by 'sham-drinking' and salivation. Tremors and incoordination have also been described (Bryson 1982; Kellerman *et al.* 2005; Newsholme *et al.* 1983). At necropsy, excessive and sloppy, watery ruminal contents (often bright green) are frequently observed. Hyperaemia of the submucosa and necrosis and ulceration of the forestomach mucosa may be seen macroscopically. Severe dehydration is also noticed. On microscopic examination, superficial epithelial necrosis of the rumen, reticulum and omasum are present. Characteristically there is detachment or absence of the stratum corneum, the necrosis only involves the stratum spinosum and stratum granulosum, but with preservation of the stratum basale (Newsholme *et al.* 1983).

The possibility that kikuyu poisoning is a mycotoxicosis has been mooted in the scientific literature since the first reported outbreaks (Martinovich *et al.*1972). Although various fungi that are potentially toxic to animals (such as *Myrothecium* spp.) have been cultured from kikuyu grass

collected during outbreaks, they were not consistently present and there was little evidence of heavy fungal infestation of the pastures (Bourke 2007; Kellerman *et al.* 2005; Newsholme *et al.* 1983). Thus, the mycotoxicosis theory was placed on hold. However, more recently, Australian researchers reported that an endophytic fungus, *Fusarium torulosum*, was consistently isolated from kikuyu grass collected during an outbreak (Bourke 2007; Ryley *et al.* 2007).

During the summers and late summers of 2008–2010 various reports were received of large numbers of cattle dying as a result of kikuyu poisoning in the Eastern Cape Province of South Africa (F. van Niekerk, Humansdorp, personal comm., 2009). Sods of kikuyu grass (roots and base of plant) were collected during eight outbreaks; in five outbreaks, the clinical diagnosis was confirmed by the characteristic histopathology of lesions in the forestomach. The kikuyu grass was submitted particularly for the isolation and molecular identification of *Fusarium* species. The specific objective was to verify if *F. torulosum* was also consistently present in kikuyu grass during outbreaks of intoxication in South Africa. If this endophytic fungus is always present in poisonous kikuyu grass on two different continents, it could direct future research to identify an aetiological agent.

Methods

Isolations

From 2008 to 2010, sods of kikuyu grass were collected from various localities in the Eastern Cape Province during eight outbreaks of kikuyu poisoning in cattle. Isolations were made from leaf and stem material. Small pieces (4 mm) of plant tissue were surface disinfected with 1% sodium hypochlorite, rinsed twice with sterile water, blotted dry and plated onto *Fusarium* selective medium (20.0 g agar, 15.0 g peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 1 g PCNB, 20 mL streptomycin sulphate in 1 L water) in Petri dishes (Nelson, Tousson & Marasas 1983). Petri dishes were incubated for 7–10 days at 25 °C under cool-white fluorescent light. The plates were checked regularly and all colonies with typical *Fusarium* morphology were transferred to half-strength potato dextrose agar (PDA) (Merck, Germany). Single-spore isolates were preserved and retained in the culture collection of the National Collections of Fungi, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council (ARC-PPRI), Pretoria, South Africa. All the single-spored isolates from *P. clandestinum* were used in the morphological comparison. Isolates were grown on PDA and synthetic low-nutrient agar (SNA) (Nirenberg 1976). Colony colour and morphology were compared with that stipulated by Leslie and Summerell (2006). Morphological characters were described from structures produced on SNA, except the macroconidia morphology. Ten measurements per isolate of the macroconidia and microconidia were also taken and the averages computed.

DNA extraction and amplification

Isolates were grown on half-strength PDA at 25 °C for 7 days. DNA was isolated using the DNeasy plant mini extraction kit (Qiagen, Valencia, CA) and following the manufacturer's protocol after the mycelium was placed in Eppendorf tubes and ground with approximately 10 µg sterile, chemically treated sand.

Extracted DNA was used as the template in polymerase chain reactions (PCR). Part of the translation elongation factor (TEF) 1-α gene was amplified using the primer set EF1 (5′-CGAATCTTTGAACGCACATTG-3′) and EF2 (5′-CCGTGTTTCAAGACGGG-3′) (O'Donnell, Cigelnik & Nirenberg 1998). The PCR consisted of 1 x SuperTherm *Taq* reaction buffer with $MgCl_2$, dNTPs (250 µM each), primers (0.2 µM each), template DNA (25 ng) and SuperTherm *Taq* polymerase (0.5 U) (Southern Cross, South Africa). The PCR conditions for the TEF gene region were amplified by initial denaturation at 94 °C for 2 min. This was followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and elongation at 72 °C for 1 min, with a final elongation step at 72 °C for 5 min (Jacobs *et al.* 2010). The resulting PCR amplicons were visualised on a 1% agarose gel under ultraviolet (UV) light and purified using a QIAquick PCR Purification kit (QIAGEN, Hilden, Germany).

DNA sequencing and sequence comparisons

DNA sequences were determined from PCR amplicons using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase (Applied Biosystems, Warrington, United Kingdom) using the primers EF1 and EF2. Sequences generated in the present study have been deposited in GenBank (Table 1).

The partial sequence data for TEF were compared against both the *Fusarium* multilocus sequence typing (MLST) database and the *Fusarium* database (Geiser *et al.* 2004). Complete dataset and alignments for previously published phylogenetic relationships within the *Fusarium incarnatum/Fusarium equiseti* species complex (FIESC) were used (O'Donnell *et al.* 2009). Gaps were treated as missing data in the subsequent analysis. Phylogenetic analysis was based on parsimony using PAUP 4.0* (Swofford 2002). Heuristic searches were done with random addition of sequences (100 replicates), tree bisection-reconnection (TBR) branch swapping, and MULPAR effective and MaxTrees set to auto-increase. Phylogenetic signal in the data sets ($g1$) was assessed by evaluating tree length distributions over 100 randomly generated trees (Hillis & Huelsenbeck 1992). The consistency (CI) and retention (RI) indices were determined for the TEF data set. Phylogenetic trees were rooted with *Fusarium concolor* as the monophyletic sister outgroup to the rest of the taxa. Bootstrap analyses were performed to determine branching point confidence intervals (1000 replicates) for the most parsimonious trees generated for the TEF data set (also see Jacobs & Van Heerden 2012).

Results

Isolations

Ninety-four *Fusarium* isolates were retrieved from grass samples collected from camps with reported kikuyu poisoning during eight outbreaks from 2008 to 2010. The *Fusarium* isolates consisted of *Fusarium culmorum* (*n* = 3), *Fusarium redolens* (*n* = 4), *Fusarium oxysporum* (*n* = 15) and *Fusarium* species within the FIESC (*n* = 72) based on morphology (Table 1).

The *F. equiseti* isolates were characterised by the absence of microconidia and macroconidia with elongated apical cells. Chlamydospores were present in all isolates. The

TABLE 1: *Fusarium* isolates obtained from *Pennisetum clandestinum* collected during kikuyu poisoning outbreaks in the Eastern Cape Province of South Africa.

Species	PPRI culture number[a]	Origin	Collection date	GenBank accession number
FIESC	10220	Humansdorp	15 April 2009	JQ286022
FIESC	10221	Humansdorp	15 April 2009	JQ286058
FIESC	10223	Humansdorp	15 April 2009	JQ286059
FIESC	10224	Humansdorp	15 April 2009	JQ286060
FIESC	10225	Humansdorp	15 April 2009	JQ286053
FIESC	10226	Humansdorp	15 April 2009	JQ286050
FIESC	10240	Humansdorp	11 March 2008	JQ286027
FIESC	10391	Humansdorp	08 April 2010	JQ286042
FIESC	10393	Humansdorp	08 April 2010	JQ286033
FIESC	10395	Humansdorp	08 April 2010	JQ286029
FIESC	10400	Humansdorp	08 April 2010	JQ286052
FIESC	10401	Humansdorp	08 April 2010	JQ286028
FIESC	10403	Humansdorp	08 April 2010	JQ286032
FIESC	10413	Humansdorp	08 April 2010	JQ286048
FIESC	10415	Humansdorp	08 April 2010	JQ286026
FIESC	10416	Humansdorp	08 April 2010	JQ286046
FIESC	10420	Humansdorp	08 April 2010	JQ286034
FIESC	10422	Humansdorp	08 April 2010	JQ286023
FIESC	10477	Humansdorp	24 February 2010	JQ286031
FIESC	10478	Humansdorp	06 March 2010	JQ286041
Fusarium culmorum	10217	Humansdorp	15 April 2009	N/I
Fusarium culmorum	10222	Humansdorp	15 April 2009	N/I
Fusarium culmorum	10397	Humansdorp	08 April 2010	N/I
Fusarium oxysporum	10227	Humansdorp	11 March 2008	N/I
Fusarium oxysporum	10396	Humansdorp	08 April 2010	N/I
Fusarium oxysporum	10404	Humansdorp	08 April 2010	N/I
Fusarium oxysporum	10407	Humansdorp	08 April 2010	N/I
Fusarium oxysporum	10423	Humansdorp	08 April 2010	N/I
Fusarium redolens	10408	Humansdorp	08 April 2010	N/I
Fusarium redolens	10412	Humansdorp	08 April 2010	N/I
FIESC	10451	Kareedouw	16 May 2010	JQ286064
FIESC	10452	Kareedouw	16 May 2010	JQ286065
FIESC	10455	Kareedouw	16 May 2010	JQ286054
FIESC	10457	Kareedouw	16 May 2010	JQ286020
FIESC	10461	Kareedouw	16 May 2010	JQ286021
FIESC	10466	Kareedouw	16 May 2010	JQ286025
FIESC	10470	Kareedouw	16 May 2010	JQ286024
FIESC	10471	Kareedouw	16 May 2010	JQ286039
FIESC	10522	Kareedouw	16 May 2010	JQ286019
FIESC	10525	Kareedouw	16 May 2010	JQ286088
FIESC	10526	Kareedouw	16 May 2010	JQ286067
FIESC	10530	Kareedouw	16 May 2010	JQ286068
Fusarium oxysporum	10454	Kareedouw	16 May 2010	N/I
Fusarium oxysporum	10521	Kareedouw	16 May 2010	N/I
Fusarium oxysporum	10523	Kareedouw	16 May 2010	N/I
Fusarium oxysporum	10524	Kareedouw	16 May 2010	N/I
Fusarium oxysporum	10527	Kareedouw	16 May 2010	N/I
Fusarium oxysporum	10528	Kareedouw	16 May 2010	N/I
Fusarium oxysporum	10529	Kareedouw	16 May 2010	N/I
Fusarium redolens	10459	Kareedouw	16 May 2010	N/I
Fusarium redolens	10464	Kareedouw	16 May 2010	N/I
FIESC	10390	Somerset-East	22 April 2010	JQ286038

FIESC, *Fusarium incarnatum/Fusarium equiseti* species complex; PPRI, Plant Protection Research Institute; N/I, not included in phylogenetic analysis.
a, PPRI culture collection of the National Collections of Fungi, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa.

Table 1 continues on next page →

TABLE 1 (continues...): *Fusarium* isolates obtained from *Pennisetum clandestinum* collected during kikuyu poisoning outbreaks in the Eastern Cape Province of South Africa.

Species	PPRI culture number[a]	Origin	Collection date	GenBank accession number
FIESC	10392	Somerset-East	22 April 2010	JQ286043
FIESC	10394	Somerset-East	22 April 2010	JQ286047
FIESC	10398	Somerset-East	22 April 2010	JQ286035
FIESC	10406	Somerset-East	22 April 2010	JQ286061
FIESC	10409	Somerset-East	22 April 2010	JQ286049
FIESC	10411	Somerset-East	22 April 2010	JQ286062
FIESC	10417	Somerset-East	22 April 2010	JQ286030
FIESC	10418	Somerset-East	22 April 2010	JQ286036
FIESC	10419	Somerset-East	22 April 2010	JQ286063
FIESC	10472	Somerset-East	22 April 2010	JQ286037
FIESC	10475	Somerset-East	22 April 2010	JQ286066
FIESC	10476	Somerset-East	22 April 2010	JQ286056
FIESC	10511	Somerset-East	22 April 2010	JQ286044
FIESC	10512	Somerset-East	22 April 2010	JQ286045
FIESC	10513	Somerset-East	22 April 2010	JQ286051
FIESC	10514	Somerset-East	22 April 2010	JQ286057
FIESC	10515	Somerset-East	22 April 2010	JQ286085
FIESC	10517	Somerset-East	22 April 2010	JQ286055
FIESC	10518	Somerset-East	22 April 2010	JQ286040
FIESC	10519	Somerset-East	22 April 2010	JQ286086

FIESC, *Fusarium incarnatum/Fusarium equiseti* species complex; PPRI, Plant Protection Research Institute; N/I, not included in phylogenetic analysis.
a, PPRI culture collection of the National Collections of Fungi, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa.

colony colour ranged from white to brownish with age. Isolates identified as *F. incarnatum* were characterised by morphology typical of *Fusarium semitectum*. These included macroconidia with a curved apical cell and pyriform to obovate microcondia borne on monophialides and polyphialides. No chlamydospores were observed in these cultures. The *F. oxysporum* isolates were characterised by purple colonies, with chlamydospores forming after 4 weeks. The macroconidia were straight to slightly curved, whilst the microconidia were oval to elliptical, borne on short monophialides. *Fusarium redolens* isolates formed oval to cylindrical microconidia on monophialides, whilst the macroconidia were thick-walled with hooked apical cells. Isolates of *F. culmorum* were characterised by the absence of microconidia and robust, short, thick-walled macroconidia.

DNA extraction and amplification

Amplicons of the TEF gene region were 640 bp in size.

DNA sequencing and sequence comparisons

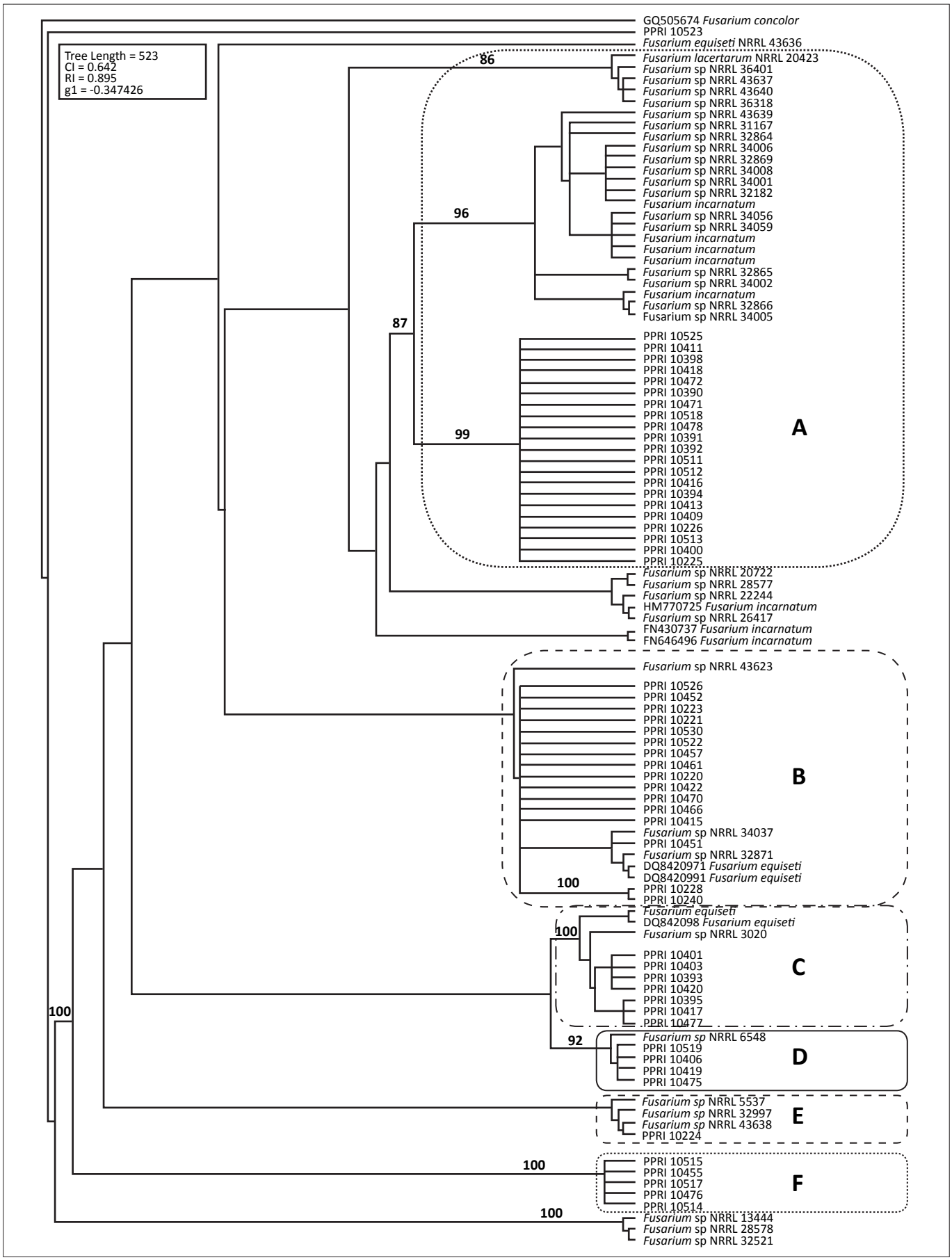
Parsimony analysis of the TEF gene region was performed to determine the phylogenetic placement of the kikuyu isolates within the FIESC. Alignment of the TEF data set by inserting gaps resulted in a total of 587 characters used in the comparison of the different species. All parsimony-uninformative and constant characters were excluded, resulting in 164 parsimony-informative characters. Heuristic searches on the data set generated one most parsimonious tree.

In the TEF data set (Figure 1), the South African isolates from kikuyu identified as members of the FIESC grouped together in six separate clades (A–F), of which four were supported by high bootstrap values (> 85%). The kikuyu isolates clustered with five of the 28 phylogenetic lineages in the FIESC, namely FIESC MLST 5b, 5e, 6a, 10a and 12a (O'Donnell *et al.* 2009). Isolates from these MLSTs originated from cereals, human and mammalian samples. Two unique clusters not associated with any of the included phylogenetic lineages in the FIESC were also observed amongst the South African isolates from kikuyu. Based on the Basic Local Alignment Search Tool (BLAST) results (Table 2) from the two selected databases, the South African isolates clustered with seven of the 28 phylogenetic lineages in the FIESC, namely: FIESC MLST 1a, 5c, 5f, 6a, 10a, 12a and 22a (O'Donnell *et al.* 2009); again, these samples are associated with cereal, human and mammalian origin. The analyses have FIESC MLST 6a, 10a and 12a in common.

Discussion

Contrary to the findings in Australia, *F. torulosum* was not isolated from the *P. clandestinum* collected during eight outbreaks of kikuyu poisoning in South Africa. Nonetheless, a number of other *Fusarium* species were isolated from kikuyu grass collected from toxic pastures during 2008 to 2010.

The genus *Fusarium* is recognised for the taxonomic difficulties associated with it and, based on the classification system used, the total number of species in this genus has ranged from 9–1000 (Summerell & Leslie 2011). *Fusarium* isolates from toxic kikuyu grass pastures in South Africa mainly represent species in the FIESC. The DNA sequence comparisons based on the TEF gene formed the most important basis for distinguishing these *Fusarium* species. The TEF 1 α gene was also the most informative of the four gene regions used by O'Donnell *et al.* (2009) to distinguish the 28 phylogenetic species in the FIESC. The grouping of the South African isolates from *P. clandestinum* with five of these species, based on sequence data for the TEF gene, confirms that the majority of the *Fusarium* isolates obtained from *P. clandestinum* associated with kikuyu poisoning in cattle form part of the FIESC.



Bootstrap values above 50% (percentages of 1000 bootstrap replicates) are indicated in bold above the branches of the tree. The dotted lines indicate the different clades.

FIGURE 1: Phylogenetic tree produced using parsimony of the translation elongation factor gene with *Fusarium concolor* as outgroup.

TABLE 2: Molecular identification of *Fusarium* isolates based on translation elongation factor sequence data.

Culture number	Multi Loci Sequence Type associated with the particular isolate	*Fusarium* ID database BLAST result
PPRI 10220†	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10221	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10222	CBS 417.86 *Fusarium culmorum*	*Fusarium culmorum*
PPRI 10223	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10224	FIESC (NRRL 43638; MLST type: 6-a)	FIESC
PPRI 10225	FIESC (NRRL 3020; MLST type: 10-a)	FIESC
PPRI 10226	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10227	FIESC (NRRL 3020; MLST type: 10-a)	FIESC
PPRI 10240	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10387	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10390	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10391	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10392	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10393	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10394	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10395	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10396	FIESC (NRRL 43637; MLST type: 1-a)	FIESC
PPRI 10397	*Fusarium oxysporum* species complex (NRRL 38326; MLST type: 196)	*Fusarium oxysporum*
PPRI 10398	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10400	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10401	FIESC (NRRL 3020; MLST type: 10-a)	FIESC
PPRI 10403	FIESC (NRRL 3020; MLST type: 10-a)	FIESC
PPRI 10404	*Fusarium coeruleum*, potato tuber, 'Hansa', Berlin market DQ164859	*Fusarium oxysporum*
PPRI 10406	FIESC (NRRL 31011; MLST type: 12-a)	FIESC
PPRI 10407	*Fusarium oxysporum* species complex (NRRL 38326; MLST type: 196)	*Fusarium oxysporum*
PPRI 10408	*Fusarium polyphialidicum*	*Fusarium redolens*
PPRI 10409	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10411	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10412	*Fusarium polyphialidicum*	*Fusarium redolens*
PPRI 10413	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10415	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10416	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10417	FIESC (NRRL 3020; MLST type: 10-a)	FIESC
PPRI 10418	FIESC (NRRL 3020; MLST type: 10-a)	FIESC
PPRI 10419	FIESC (NRRL 43637; MLST type: 1-a)	FIESC
PPRI 10420	FIESC (NRRL 3020; MLST type: 10-a)	FIESC
PPRI 10422	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10451	*Fusarium oxysporum* species complex (NRRL 38295; MLST type: 181)	*Fusarium oxysporum*
PPRI 10452	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10454	*Fusarium oxysporum* species complex (NRRL 38295; MLST type: 181)	*Fusarium oxysporum*
PPRI 10455	FIESC (NRRL 43637; MLST type: 1-a)	FIESC
PPRI 10457	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10459	FIESC (NRRL 45997; MLST type: 5-f)	*Fusarium redolens*
PPRI 10461	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10464	*Fusarium polyphialidicum*	*Fusarium redolens*
PPRI 10466	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10470	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10471	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10472	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10475	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10476	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10477	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10478	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10511	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10512	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10513	FIESC (NRRL 34002; MLST type: 22-a)	FIESC
PPRI 10514	FIESC (NRRL 43637; MLST type: 1-a)	FIESC
PPRI 10515	FIESC (NRRL 43637; MLST type: 1-a)	FIESC

PPRI, Plant Protection Research Institute; NRRL, Agricultural Research Service culture collection, United States Department of Agriculture, Illinois, United States of America; MLST, multi loci sequence type; CBS, culture collection of Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, Utrecht, The Netherlands; FIESC, *Fusarium incarnatum/Fusarium equiseti* species complex; BLAST, Basic Local Alignment Search Tool.
†, PPRI refers to the PPRI culture collection of the National Collections of Fungi, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council (ARC-PPRI), Pretoria, South Africa.

Table 2 continues on next page →

TABLE 2 (continues...): Molecular identification of *Fusarium* isolates based on translation elongation factor sequence data.

Culture number	Multi Loci Sequence Type associated with the particular isolate	*Fusarium* ID database BLAST result
PPRI 10516	FIESC (NRRL 43637; MLST type: 1-a)	FIESC
PPRI 10517	FIESC (NRRL 43637; MLST type: 1-a)	FIESC
PPRI 10518	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10519	FIESC (NRRL 31011; MLST type: 12-a)	FIESC
PPRI 10521	*Fusarium oxysporum* species complex (NRRL 20433; MLST type: 2)	*Fusarium oxysporum*
PPRI 10522	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10523	*Fusarium oxysporum* species complex (NRRL 20433; MLST type: 2)	*Fusarium oxysporum*
PPRI 10524	*Fusarium oxysporum* species complex (NRRL 38295; MLST type: 181)	*Fusarium oxysporum*
PPRI 10525	FIESC (NRRL 34002; MLST type: 22-a	FIESC
PPRI 10526	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10527	*Fusarium oxysporum* species complex (NRRL 38295; MLST type: 181)	*Fusarium oxysporum*
PPRI 10528	*Fusarium oxysporum* species complex (NRRL 20433; MLST type: 2)	*Fusarium oxysporum*
PPRI 10530	FIESC (NRRL 45997; MLST type: 5-f)	FIESC
PPRI 10541	FIESC (NRRL 45997; MLST type: 5-f)	FIESC

PPRI, Plant Protection Research Institute; NRRL, Agricultural Research Service culture collection, United States Department of Agriculture, Illinois, United States of America; MLST, multi loci sequence type; CBS, culture collection of Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, Utrecht, The Netherlands; FIESC, *Fusarium incarnatum/Fusarium equiseti* species complex; BLAST, Basic Local Alignment Search Tool.
†, PPRI refers to the PPRI culture collection of the National Collections of Fungi, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council (ARC-PPRI), Pretoria, South Africa.

Historically, the genus *Fusarium* has been known as a plant pathogen, but since the 1960s has also been reported to be associated with secondary metabolites responsible for *Fusarium*-related mycotoxicoses in humans and animals (Summerell & Leslie 2011). In many of the *Fusarium*-associated intoxications, the mycotoxin(s) are unknown but the toxins of veterinary importance synthesised by some of the strains include fumonisins, zearalenone and trichothecenes such as diacetoxyscirpenol, deoxynivalenol and T2-toxin (Desjardins 2006; Kellerman *et al.* 2005; Marasas, Nelson & Tousson 1984). Other lesser-known *Fusarium* mycotoxins include beauvericin, enniatins and fusarochromanone (Altomare *et al.* 1995; Bryden *et al.* 2004; Logrieco *et al.* 1998).

The mycotoxicosis theory is thus still very plausible and is supported by the periodic occurrence of kikuyu poisoning usually during warm, humid weather when fungal growth in a pasture is likely to be optimal (Martinovich *et al.*1972). *Fusarium torulosum* is known to produce mycotoxins such as wortmannin and butenolide (Bourke 2007; Ryley *et al.* 2007). Oral administration of wortmannin to rats is toxic at a dose as low as 4 mg/kg, causing gastric and myocardial haemorrhage (Gunther, Abbas & Mirocha 1989). Butenolide is reported to induce acute inflammation in the forestomachs of cattle (Tookey & Grove 1972). In addition, Brazilian researchers described a ruminitis syndrome very similar to kikuyu poisoning (Riet-Correa *et al.* 2009). Unlike kikuyu poisoning, this intoxication is caused by members of the Asteraceae family, *Baccharis coridifolia* and *Baccharis megapotamica*, but the toxins in the plants are a range of trichothecenes synthesised by soil fungi (*Myrothecium* spp.) and absorbed by the roots of the plant (Riet-Correa *et al.* 2009). If kikuyu poisoning is a mycotoxicosis, assays could be developed to be able to forecast risk and to warn farmers that intoxication may occur.

Conclusion

Although *F. torulosum* could not be isolated from *P. clandestinum* when collected during eight outbreaks of kikuyu poisoning in South Africa, various other *Fusarium* isolates (n = 94) were retrieved from the toxic kikuyu grass. These isolates were predominantly members of the FIESC; however, the possibility that kikuyu poisoning is a mycotoxicosis should be further investigated and toxic grass samples could be subjected to analytical screening for fungal metabolites.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

C.J.B. (University of Pretoria) was the principal investigator and study coordinator. M.T. (Agricultural Research Council-Plant Protection Research Institute) and A.J. (Agricultural Research Council-Plant Protection Research Institute) performed the mycological culturing and identification. A.J. was responsible for the phylogenetic analyses. All authors compiled, read and approved the final manuscript.

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