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Two new species of *Fusarium* section *Liseola* associated with mango malformation

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Abstract: Mango malformation is an economically important disease of *Mangifera indica* globally. A recent DNA-based study indicated that two distinct, phylogenetic lineages previously identified as *Fusarium subglutinans* are associated with this disease in South Africa. The objective of this study was to characterize *Fusarium* isolates associated with mango malformation, including the two different *F. subglutinans* groups, based on morphological characteristics. For this purpose we examined *Fusarium* strains isolated from diseased mango inflorescences from diverse geographical origins. We also used sexual compatibility tests to determine whether sexual reproduction among the strains was possible. The morphological characters considered were shape of the conidia, presence of mono- and/or polyphialides, origin of the conidiophores from the substrate, presence of chlamydospores and the presence of sterile coiled hyphae. Three unique *Fusarium* spp. were identified. In this paper, we provide formal descriptions for the two new taxa in the section *Liseola* that we have named *F. mangiferae* and *F. sterilihyphosum*. *Fusarium mangiferae* is conspecific with strains that were previously identified as *F. subglutinans* and reported to be the causal agent of malformation in mango growing areas throughout the world. *Fusarium sterilihyphosum*, on the other hand, has been isolated only from malformed mango tissue in South Africa.

Key Words: *Gibberella fujikuroi* complex, mango, taxonomy

INTRODUCTION

Mango (*Mangifera indica* L.) malformation is an economically important disease in mango-growing areas of the world including India, Pakistan, Egypt, South Africa, Brazil, Israel, Florida, and Mexico (Kumar et al 1993, Freeman et al 1999). This disease causes abnormal development of vegetative shoots and inflorescences (Kumar et al 1993). Floral malformation is the most prominent symptom and is characterized by abnormal, thick and fleshy panicles (Varma 1983, Kumar et al 1993). Affected panicles bear no fruit, resulting in significant economic losses (Varma et al 1974, Varma 1983, Kumar et al 1993).

The etiology of mango malformation disease is controversial. Physiological abnormality, virus infections, mite infestations and fungal pathogens have been reported as the causal agents of this disease (Kumar et al 1993). Summanwar et al (1966) identified the fungal pathogen commonly associated with the disease as *Fusarium subglutinans* (Wollenweber & Reinking) Nelson, Toussoun & Marasas (= *F. moniliforme* Sheldon var. *subglutinans* Wollenweber & Reinking), residing in section *Liseola*. Freeman et al (1999) recently demonstrated that isolates identified as *F. subglutinans* induced typical mango malformation symptoms on mango trees using the isolate MRC 7559 (506/2) originally collected from mango inflorescences in Israel.

Fusarium subglutinans forms part of the *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura species complex (Leslie 1995, Britz et al 1999). *Fusarium subglutinans sensu lato* is, however, a polyphyletic taxon that has been associated with various plant hosts, each of which represents a distinct lineage in the *G. fujikuroi* complex (Leslie 1995, O'Donnell et al 1998,

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Britz et al 1999, Steenkamp et al 1999, 2000a, O'Donnell et al 2000). These lineages are difficult to distinguish using conventional morphological characters such as those proposed by Nelson et al (1983). Until relatively recently, these fungi were distinguished from each other using pathogenicity and mating studies (Leslie 1995). The different lineages representing *F. subglutinans sensu lato* are, however, readily distinguishable using DNA sequences of genes for β-tubulin, translation elongation factor EF-1α, histone *H3,* and calmodulin (O'Donnell et al 1998, Steenkamp et al 1999, 2000a, O'Donnell et al 2000).

Mango malformation in South Africa is associated with two phylogenetically distinct groups of isolates until recently referred to as *F. subglutinans* (Steenkamp et al 1999, 2000a). Based on the histone *H3* and β-tubulin gene sequences, one group of isolates represents a previously undescribed lineage in the *G. fujikuroi* complex. The second group of isolates is conspecific with isolates that were previously reported to be the causal agent of mango malformation (Steenkamp et al 2000a). The results presented by these authors also confirmed those of Viljoen et al (1997), O'Donnell et al (2000) and Leslie (pers comm), who have shown using random amplified polymorphic DNA (RAPDs), DNA sequence of several genes, and isozymes, respectively, that mango malformation is associated with two distinct species, both with morphological characters typical of *F. subglutinans*.

The aim of this study was to characterize, using morphology, *Fusarium* spp. isolated from malformation mango tissue from diverse geographical origins. For this purpose the morphological characteristics proposed by Nirenberg and O'Donnell (1998) were used. Sexual compatibility tests were also used to verify the identity of some of these *Fusarium* spp.

MATERIALS AND METHODS

Morphological and cultural studies.—*Fusarium* spp. associated with mango malformation in South Africa were isolated from mango trees in Tzaneen (Northern Province), which included the areas Letsitele (LS) and Deer Park (DP). Isolates were also collected from Nelspruit (NS), Fredenheim (FH), Malelane (ML) and Hazyview (HZ) (Mpumalanga). Other isolates used in this study were isolated from malformed mango tissue by other collectors in Florida, Egypt, Israel, Malaysia and South Africa (TABLE I). Mating tester strains (MRC 6213 and MRC 7488) for *F. circinatum* Nirenberg & O'Donnell (mating population H of the *G. fujikuroi* complex) were used in sexual compatibility tests (Britz et al 1999). All the isolates were stored in 15% glycerol at −70 C in the *Fusarium* culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and the culture collection of the Medical Research Council (MRC), P.O. Box 19070, Tygerberg, South Africa.

Mango inflorescence clusters collected in South Africa were surface sterilized with 70% ethanol for 2 min and washed with sterile deionized water for 1 min. Single malformed flowers were removed from the sterilized cluster and plated onto a *Fusarium* selective medium (Nash and Snyder 1962). After incubation for 5 d at 25 C, small agar pieces overgrown with mycelium were taken from the edges of the colonies and transferred to 90 mm diameter Petri dishes containing carnation leaf agar (CLA) (Fisher et al 1982). After incubation on CLA at 25 C for 7 d, single conidial isolates were prepared and stored in 15% glycerol at −70 C.

To stimulate culture and conidial development, *Fusarium* isolates (TABLE I) were transferred to CLA (Fisher et al 1982) and KCl agar (Nelson et al 1983). Cultures were incubated at 23 C under fluorescent and cool-white light with a 12 h photoperiod. After 10 to 14 d of incubation, the following morphological characters were examined: shape of the conidia, presence of mono- and/or polyphialides, origin of the conidiophores from the substrate, presence of chlamydospores and sterile coiled hyphae (Nirenberg and O'Donnell 1998). Secondary characteristics such as growth rate and colony color (Rayner 1970) were determined on potato dextrose agar (PDA) after incubation at 25 C in the dark (Nelson et al 1983) for the two newly described species. Each isolate was plated onto three different PDA plates and the growth rate was determined over a period of 10 d. This entire procedure was repeated once more. A one way ANOVA was done to determine whether growth rate differed significantly for the different isolates. Colony color was determined after 14 d using the color coding system of Rayner (1970). Fifty measurements were made of all diagnostic morphological characters. The measurements are indicated as minimum, mean, and maximum.

Mating type and sexual compatibility tests.—The mating type (*MAT*-1 or *MAT*-2) of all the isolates included in this study were determined using the PCR-based method described by Steenkamp et al (2000b). Only isolates with opposite mating types were crossed within each species and between species using the method described by Klittich and Leslie (1988) with some modifications (Britz et al 1999). Covert et al (1999) found that a lower incubation temperature increased sexual fertility among isolates of *G. circinata* Nirenberg & O'Donnell. We, therefore, used an incubation temperature of 17 C for our crosses. Sexual crosses have already been performed within and between isolates of the two new species in a previous study (Steenkamp et al 2000a), and the present study served to confirm those results using a more clearly defined collection of isolates. Since the morphological characteristics of some isolates were similar to those of *F. circinatum*, all isolates (TABLE I) were crossed with the standard tester strains (MRC 6213 and MRC 7488) for *G. circinata* (anamorph: *F. circinatum*).

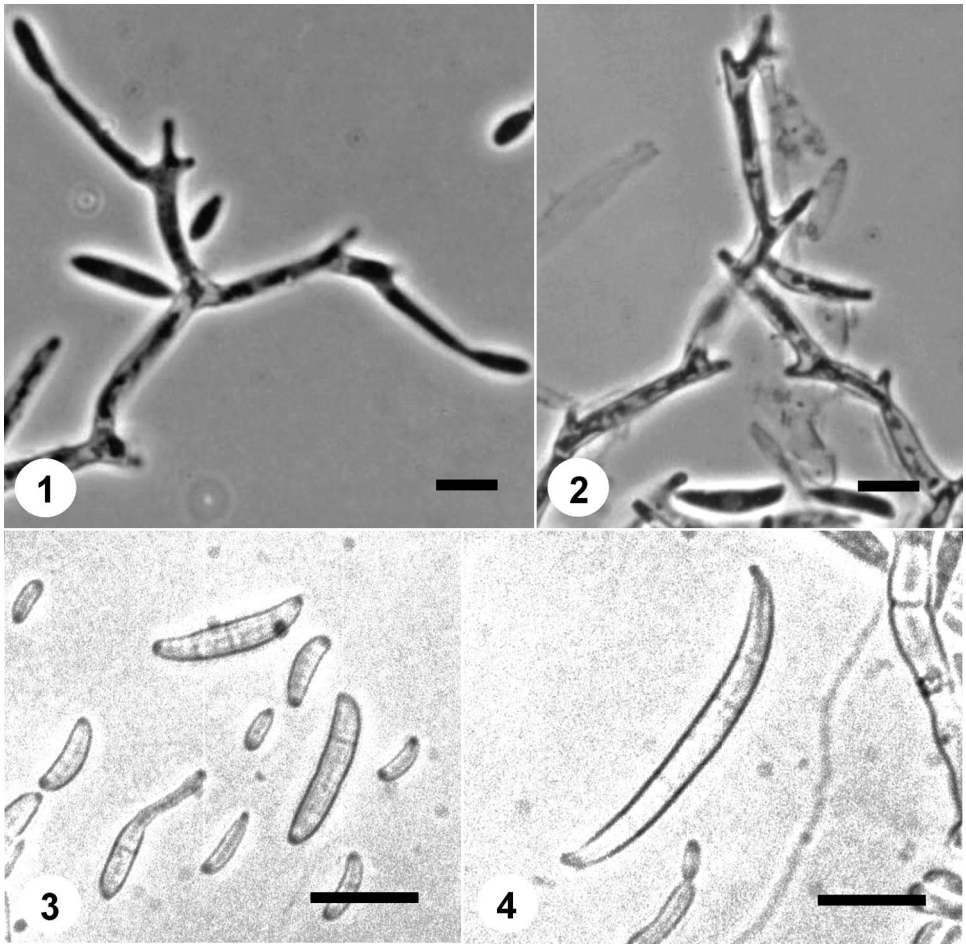
TABLE I. *Fusarium* species isolated from malformed mango tissue

Species	Origin	MRC no[a]	Other no[b]	Original source[c]
Fusarium sp.	Malaysia	8079	KSU-X4379	B. Salleh
	Malaysia	8070	KSU-X4381	B. Salleh
	Malaysia	8071	KSU-X2330	B. Salleh
F. mangiferae	South Africa (ex-paratype)	2730	KSU-X3873	F. Wehner
	South Africa (ex-paratype)	3477	KSU-X3875	C. Crookes
	South Africa	3478	KSU-X3876	C. Crookes
	South Africa	3479	KSU-X3877	C. Crookes
	South Africa	8077	FCC 1551, ML3-1	H. Britz
	South Africa	8078	FCC 1537, FH1-8	H. Britz
	South Africa	8079	FCC 1542, FH1-16	H. Britz
	South Africa	8080	NS1-1	H. Britz
	South Africa	8081	NS1-9	H. Britz
	South Africa	8082	FCC 1547, ML1-9	H. Britz
	South Africa	8083	FCC 1548, ML2-1	H. Britz
	South Africa	8084	FH1-73	H. Britz
	South Africa	8085	FH1-6	H. Britz
	South Africa	8086	FCC 1545, ML1-6	H. Britz
	South Africa	8087	FCC 1546, ML1-8	H. Britz
F. mangiferae	Florida, USA	7034		R. Ploetz
	Florida, USA	7035		R. Ploetz
	Florida, USA	7038		R. Ploetz
	Florida, USA	7039		R. Ploetz
	Florida, USA	8088	KSU-X4079, FRC-M3622	R. Ploetz
	Egypt	8089	KSU-X4706	I. Mausour
	Egypt	8090	KSU-X4702	I. Mausour
	Egypt	8091	KSU-X4700	I. Mausour
	Israel (ex-holotype)	7559	FCC 73, KSU 11781, 506/2	S. Freeman
	Israel	7560	FCC 74	S. Freeman
	Israel	7561	FCC 80	S. Freeman
	Israel	7562	FCC 81	S. Freeman
	Malaysia	8092	KSU-X4382	B. Salleh
	Malaysia	8093	KSU-X4384	B. Salleh
F. sterilihyphosum	South Africa (ex-holotype)	2802	KSU-X3874, NRRL 25623	J. Darvas
	South Africa	7602	FCC 1367, A1-2	H. Britz
	South Africa	7605	FCC 1398, A20-1	H. Britz
	South Africa	7606	A33-1	H. Britz
F. sterilihyphosum	South Africa	8094	D2-1	H. Britz
	South Africa (ex-paratype)	8095	FCC 1315, KSU 11783, A40-1	H. Britz
	South Africa	8096	FCC 1143, E6-1	H. Britz
	South Africa	8099	FCC 1563, HZ1-9	H. Britz
	South Africa	8100	DP3-5	H. Britz
	South Africa (ex-paratype)	8101	FCC 1286, KSU 11782, A26-1	H. Britz
	South Africa	8102	FCC 1478, B12-1	H. Britz
	South Africa	8103	C6-1	H. Britz
	South Africa	8104	FCC 1555, HZ1-1	H. Britz
	South Africa	8105	ML2-10	H. Britz
	South Africa	8106	DP3-7	H. Britz
	South Africa	8107	FCC 1632, DP3-9	H. Britz
	South Africa	8108	FCC 1557, HZ1-3	H. Britz

[a] MRC = Culture collection of the Medical Research Council, Tygerberg, South Africa.

[b] KSU-X = Kansas State University culture collection, Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA; FRC = *Fusarium* Research Center, Pennsylvania State University, USA; NRRL = Northern Regional Research Laboratory, Peoria, Illinois, USA; FCC = *Fusarium* culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa with original numbers indicated as follows: A, B, C, D, E = Isolates from different orchards from Letsitele, South Africa, HZ = Isolates from Hazyview, South Africa, ML = Isolates from Malelane, South Africa, DP = Isolates from Deer Park, South Africa, FH = Isolates from Fredenheim farm, Nelspruit, South Africa, NS = Isolates from Nelspruit, South Africa.

[c] B. Salleh collected isolates KSU-X2330, 4379, 4381, 4382 and 4384 in Malaysia; Ibrahim Mausour collected KSU-X4079, 4700, 4702 and 4706 in Egypt; C. Crookes collected isolates MRC 3477–3479 in KwaZulu-Natal, South Africa; S. Freeman collected MRC 7559–7562 in Isreal; R. Ploetz collected isolates MRC 7034–7035, 7038–7039 in Florida, USA.



FIGS. 1–4. *Fusarium mangiferae*. 1. Branched conidiophores bearing polyphialides with 3 conidiogenous openings (scale bar: 5 μm). 2. Branched conidiophores bearing mono- and polyphialides (scale bar: 5 μm). 3. Microconidia (scale bar: 15 μm). 4. Macrocondium (scale bars: 15 μm).

RESULTS

Morphological and cultural studies.—*Fusarium* isolates from trees suffering from mango malformation in Malaysia, Egypt, Israel, South Africa, and Florida were separated into three different groups based on morphological characters defined by Nirenberg and O'Donnell (1998). Based on the morphological characters that were used, each of these groups represented new species in the *G. fujikuroi* complex. Of the three groups, two are clearly discrete taxa based both on morphological characteristics and sequencing data (Steenkamp et al 2000a). These two taxa are represented by an extensive group of isolates and we elect to describe them as new species in *Fusarium* section *Liseola*.

Fusarium mangiferae Britz, Wingfield et Marasas sp. nov. FIGS. 1–5

Coloniae in agaro PDA apud 25 C 3.4 mm per diem crescentes. Mycelium aerium floccosum, album, infra roseololuteum ad atropurpureum. Conidiophora in agaro CLA erecta vel prostrata, simplicia vel ramosa, cellulae conidiogenae mono- et polyphialides, usque ad 30 × 3 μm. Hyphae steriles absentes. Microconidia in capitulis falsis, hyalina, plerumque obovoidea, subinde ovata vel allantoidea, plerumque 0-septata, subinde 1-septata, 4.3–9.0–18.4 × 1.7–2.4–3.3 μm. Sporodochia praesentia, alba ad aurantiaca. Macroconidia hyalina, falcata, gracilia, leniter curvata, tenuitunicata, cellula basali pedicellata, cellula apicali leniter curvata, 3–5-septata, 43.1–51.8–61.4 × 1.9–2.3–3.4 μm. Clamydosporae absentes.

HOLOTYPUS. Cultura exsiccata in agaro CLA ex MRC 7559, sejuncta a inflorescentis malformatis *Mangifera indica*, Volcani Center, Bet Dagan, Israel, 1993, S. Freeman (PREM 57299).

Colonies on PDA with average growth rate of 3.4 mm/d at 25 C. Aerial mycelium white, floccose. Reverse of colonies sometimes rosy buff (17″f) to dark purple (65k). Conidiophores on aerial mycelium originating erect and prostrate from substrate. Conidiophores sympodially branched bearing mono- and polyphialides (FIGS. 1, 2). Polyphialides have 2–5 conidiogenous openings (FIGS. 1, 2). Phialides on

the aerial conidiophores mono- and polyphialidic, up to 30.0 μm long and 3 μm wide. Sterile hyphae absent. Microconidia variable in shape, obovoid conidia the most abundant type, oval to allantoid conidia occurring occasionally (FIG. 3). Microconidia mostly 0-septate with 1-septate conidia occurring less abundantly, 0-sepate: 4.3–9.0–14.4 × 1.7–2.4–3.3 μm. Sporodochia present, cream (19′f) and orange (15b). Macroconidia long and slender, usually 3–5 septate (FIG. 4): 43.1–51.8–61.4 × 1.9–2.3–3.4 μm. Chlamydospores absent.

Etymology. *Mangiferae* (L. gen) indicating the species association with the genus *Mangifera* L.

Specimens examined. ISRAEL. Bet Dagan, Volcani center plantation, Mango malformation inflorescence on *M. indica*, 1993, *S. Freeman* 506/2 (PREM 57299, HOLOTYPE; MRC 7559, ex-holotype); Ginosar, inflorescence malformation of *M. indica* cultivar Kent, 1998, *S. Freeman* 34 (MRC 7560); Sde Nitzar, inflorescence malformation of *M. indica*, 1998, *S. Freeman* 41 (MRC 7561); Bene Dror, inflorescence malformation of *M. indica* cultivar Keitt, 1998, S. *Freeman* 86 (MRC 7562). SOUTH AFRICA. MPUMALANGA: Nelspruit, inflorescence malformation of *M. indica*, 1982, *F. Wehner* MRC 2730 (PREM 57300, PARATYPE; KSU 3873, ex-paratype); inflorescence malformation of *M. indica*, 1998, *H. Britz* NS1–1 (MRC 8080); inflorescence malformation of *M. indica*, 1998, *H. Britz* NS1–9 (MRC 8081); Nelspruit, Fredenheim, inflorescence malformation of *M. indica*, 1998, *H. Britz* FH1–6 (MRC 8085); inflorescence malformation of *M. indica*, 1998, *H. Britz* FCC 1537-FH1–8 (MRC 8078); inflorescence malformation of *M. indica*, 1998, *H. Britz* FCC 1542-FH1–16 (MRC 8079); inflorescence malformation of *M. indica*, 1998, *H. Britz* FH1–73 (MRC 8084). MPUMALANGA: Malelane, inflorescence malformation of *M. indica*, 1998, *H. Britz* FCC 1551 = ML3–1 (MRC 8077); inflorescence malformation of *M. indica*, 1998, *H. Britz* FCC 1547 = ML1–9 (MRC 8082); inflorescence malformation of *M. indica*, 1998, *H. Britz* FCC 1545 = ML1–6 (MRC 8086); inflorescence malformation of *M. indica*, 1998, *H. Britz* FCC 1546 = ML1–8 (MRC 8087); inflorescence malformation of *M. indica*, 1998, *H. Britz* FCC 1548 = ML2–1 (MRC 8083); KWAZULU-NATAL: inflorescence malformation of *M. indica*, 1984, *C. Crookes* MRC 3477 (PREM 57301, PARATYPE; KSU-X3875, ex-paratype); inflorescence malformation of *M. indica*, 1984, *C. Crookes* MRC 3478 (KSU-X 3876); inflorescence malformation of *M. indica*, 1984, *C. Crookes* MRC 3479 (KSU-X 3877). EGYPT. Inflorescence malformation of *M. indica*, *Ibrahim Mausour*, KSU-X4706 (MRC 8089); inflorescence malformation of *M. indica*, *Ibrahim Mausour* KSU-X4702 (MRC 8090); inflorescence malformation of *M. indica*, *Ibrahim Mausour* KSU-X4700 (MRC 8091). USA. FLORIDA: Dade County, Miami, inflorescence malformation of *M. indica* cultivar Keitt, 1994, *R. Ploetz* FS16 (MRC 7034); inflorescence malformation of *M. indica* cultivar Keitt, 1994, *R. Ploetz* FS23 (MRC 7035); inflorescence malformation of *M. indica* cultivar Keitt, 1994, *R. Ploetz* FS55 (MRC 7038); inflorescence malformation of *M. indica* cultivar Keitt, 1994, *R. Ploetz* MRC 7039; inflorescence malformation of *M. indica*, *R. Ploetz* KSU-X4079 = FRC-M3622 (MRC 8088). MALAYSIA. Inflorescence malformation of *M. indica*, *Baharuddin Salleh* KSU-X4382 (MRC 8092); inflorescence malformation of *M. indica*, *Baharuddin Salleh* KSU-X4384 (MRC 8093).

Commentary. A dried culture to serve as holotype has been deposited at the Plant Protection Research Institute, Pretoria, South Africa (PREM 57299). Exholotype cultures have been deposited in the culture collection of the South African Medical Research Council, Tygerberg, South Africa (MRC 7559) and the department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA (KSU 11781). Exparatype cultures have been deposited as MRC 2730 (KSU-X3873) and MRC 3477 (KSU-X3875).

Fusarium sterilihyphosum Britz, Marasas & Wingfield, sp. nov. FIGS. 5–8

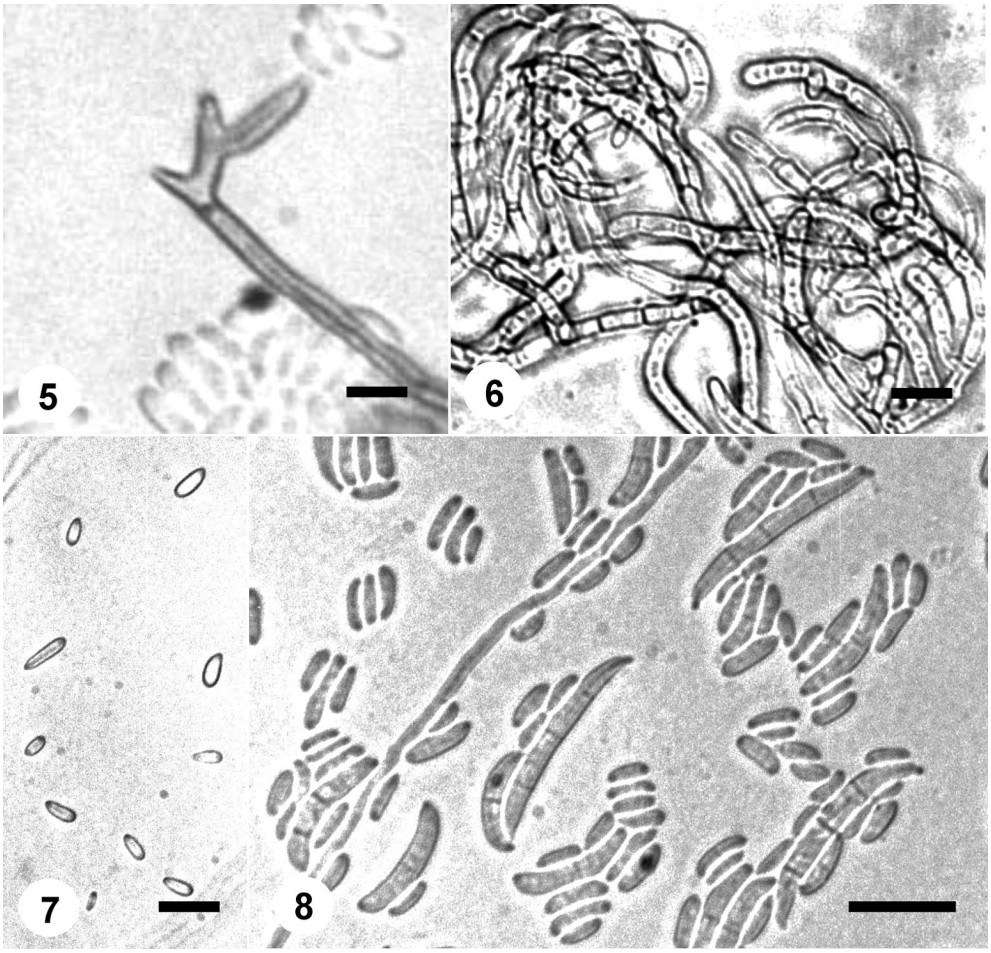
Coloniae in agaro PDA apud 25 C 4.8 mm per diem crescentes. Mycelium aerium floccosum, subalbum, infra giseoroseum ad pallido- purpureum. Conidiophora in agaro CLA erecta vel prostrata, simplicia vel ramosa, cellulae conidiogenae mono- et polyphialides, usque ad 30 × 3 μm. Hyphae steriles circinatae praesentes. Microconidia in capitulis falsis, hyalina, plerumque obovoidea, subinde ovata vel allantoidea, plerumque 0-septata, subinde 1-septata, 4.5–8.8–24.2 × 1.6–2.6–3.5 μm. Sporodochia raro praesentia. Sporodochia raro praesentia, alba ad aurantiaca. Macroconidia hyalina, falcata, gracilia, leniter curvata, tenuitunicata, cellula basali pedicellata, cellula apicali leniter curvata, 3–5-septata, 28.4–37.1–47.1 × 2.4–3.2–4.1 μm. Clamydosporae absentes.

HOLOTYPUS. Cultura exsiccata in agaro CLA ex MRC 2802, sejuncta a inflorescentis malformatis *Mangifera indica*, Letsitele, Tzaneen, Northern Province, South Africa, 1982, J. M. Darvas (PREM 57302).

Colonies on PDA with average growth rate of 4.8 mm/d at 25 C. Aerial mycelium almost white (′1). Reverse of colonies straw to grayish rose (3″f) and light purple (63i). Conidiophores on aerial mycelium erect, occasionally prostrate. Conidiophores sympodially branched bearing mono- and polyphialides (FIG. 5). Phialides on aerial conidiophores mono- and polyphialidic, up to 30.0 μm long and 3 μm wide. Sterile hyphae present (FIG. 6). Microconidia obovoid, oval to allantoid, 0-septate conidia abundant, 1-septate conidia less common (FIG. 7, 8): 0-septate: 4.5–8.8–14.2 × 1.6–2.6–3.5 μm. Sporodochia seldom present, cream (19′f) to orange (15b). Macroconidia slightly beaked apical cells, a footlike basal cell, 3–5 septate (FIG. 8), 28.4–37.1–47.1 × 2.4–3.2–4.1 μm. Chlamydospores absent.

Etymology. *Sterilihyphosum* (L. adj) refers to the presence of sterile hyphae in mycelium.

Specimens examined. SOUTH AFRICA. NORTHERN PROVINCE: Tzaneen, Letsitele area, Mango malformation



FIGS. 5–8. *Fusarium sterilihyphosum*. 5. Conidiophores bearing polyphialides with 3 conidiogenous openings (scale bar: 5 μm). 6. Sterile coiled hyphae (scale bar: 10 μm). 7. Microconidia (scale bar: 10 μm). 8. Microconidia with 0–1 septa and 3-septate macroconidia (scale bars: 20 μm).

inflorescence on *M. indica*, 1982, *J. M. Darvas* MRC 2802 = NRRL 25623 (PREM 57302, HOLOTYPE; KSU-X3874, ex-holotype); mango malformation of *M. indica*, 1997, *H. Britz* A33–1 (MRC 7606); mango malformation of *M. indica*, 1997, *H. Britz* FCC 1315 = A40–1 (PREM 57303, PARATYPE; MRC 8095, ex-paratype); mango malformation of *M. indica*, 1997, *H. Britz* FCC 1367 = A1–2 (MRC 7602); mango malformation of *M. indica*, 1997, *H. Britz* FCC 1398 = A20–1 (MRC 7605); mango malformation of *M. indica*, 1997, *H. Britz* FCC 1286 = A26–1 (PREM 57304, PARATYPE; MRC 8101, ex-paratype); mango malformation of *M. indica*, 1997, *H. Britz* FCC 1478 = B12–1 (MRC 8102); mango malformation of *M. indica*, 1997, *H. Britz* C6–1 (MRC 8103); mango malformation of *M. indica*, 1997, *H. Britz* D2–1 (MRC 8094); mango malformation of *M. indica*, 1997, *H. Britz* FCC 1146 = E6–1 (MRC 8096); Deer Park, Mango malformation of *M. indica*, 1998, *H. Britz* DP3–5 (MRC 8100); mango malformation of *M. indica*, 1998, *H. Britz* DP3–7 (MRC 8106); mango malformation of *M. indica*, 1998, *H. Britz* FCC 1632 = DP3–9 (MRC 8107). MPUMALANGA: Hazyview, mango malformation of *M. indica*, 1998, *H. Britz* FCC 1563 = HZ1–9 (MRC 8099); mango malformation of *M. indica*, 1998, *H. Britz* FCC 1555 = HZ1–1 (MRC 8104); mango malformation of *M. indica*, 1998, *H. Britz* FCC 1557 = HZ1–3 (MRC 8108); Malelane, mango malformation of *M. indica*, 1998, *H. Britz* ML2–10 (MRC 8105).

Commentary. A dried culture to serve as holotype has been deposited at the Plant Protection Research Institute, Pretoria, South Africa (PREM 57302). Ex-holotype culture specimens have been deposited in the culture collection of the South African Medical Research Council, Tygerberg, South Africa (MRC 2802) and the department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA (KSU-X3874). Ex-paratype cultures have been deposited as MRC 8095 (KSU 11783) and MRC 8101 (KSU 11782).

Of the three *Fusarium* species found associated with mango malformation, two have been described as new in this study. The third group included three isolates (KSU-X4379, KSU-X4381 and KSU-X2330)

TABLE II. Distinguishing characteristics described by Nirenberg and O'Donnell (1998) of isolates of *Fusarium subglutinans sensu lato* as well as characteristics observed for isolates in this study

	F. subglutinans sensu lato								New species		
Morphological characteristics[a]	*F. begoniae*	*F. bulbicola*	*F. circinatum*	*F. concentricum*	*F. guttiforme*	*F. pseudocircinatum*	*F. sacchari*	*F. subglutinans*	*F. mangiferae*	*F. sterilihyphosum*	Fusarium sp.
Microconidia obovoid	+	+	+	+	+	+	−	−	+	+	+
Microconidia oval to allantoid/or fusoid	+	+	(+)	+	−	+	+	+	+	+	+
Sterile coiled hyphae	−	−	+	−	−	+	−	−	−	+	−
Conidiophore originated erect	−	−	+	+	+	?	−[b]	+[b]	+	+	+
Conidiophore originated prostrate	+	+	−	−	+	+	+[b]	+[b]	+	+	−
Conidiogenous openings: ≤3	+	+	−	−	−	−	−[b]	+	−	−	+
Conidiogenous openings: ≥3	−	−	+	+	+	+	+[b]	−	+	+	−
Microconidia 0-septate, occasionally 1-septate	+	+	+	+	+	+	+[c]	−[c]	+	(+)	+
Microconidia 0-3 septate	−	−	−	−	−	−	−[c]	+[c]	−	−	+
Macroconidia 3-septate	+	−	+	−	+	+	+[c]	−[c]	−	−	−
Macroconidia 3-5 septate	−	+	−	+	−	−	−[c]	+[c]	+	+	+
Host specific	+	+	+	?	+	?	+	+	?	?	?

[a] + indicates the presence of the characteristic, (+) indicates that the character is not present in all isolates of the species, − indicates the absence of the characteristic and ? indicates that the characteristic has not been reported.

[b] Morphological characteristic identified from *F. sacchari* isolates (FRC-M941, 943) from sugarcane in India (MP-B) and *F. subglutinans* isolates (BBA 11157 from Iran and FRC-M3696 from St. Elmo, Illinois).

[c] Morphological characteristics obtained from Gerlach and Nirenberg (1982).

that were collected from malformed mango tissue in Malaysia. These isolates have sparse aerial mycelium. Their aerial mycelial conidiophores emerge directly from the substrate hyphae (referred to as erect). The polyphialides of these isolates have fewer than 3 conidiogenous openings. Microconidia are borne in false heads and are obovoid in shape, predominantly without septa but with 1-septate conidia occurring occasionally. Macroconidia are short 19.3–24.8–29.5 × 1.3–2.0–3.0 μm and 3–5 septate. Chlamydospores and sterile coiled hyphae are absent (TABLE II). We believe that this fungus also represents a new taxon, but the collections are insufficient in number to justify a formal description at this stage.

The newly described species, *F. mangiferae* and *F. sterilihyphosum* had different growth rates on PDA at 25 C. *Fusarium mangiferae* had a slower growth rate than *F. sterilihyphosum*, but variation in growth rate among isolates of both species was observed. The one way ANOVA indicated that the growth rate did not differ significantly when the growth rate of all the isolates of both species were analyzed ($P > 0.001$). Colony color of the two species was the same.

Mating type and sexual compatibility tests.—All isolates of the undescribed *Fusarium* species represented by only 3 isolates were of the *MAT*-1 mating type. Most of the *F. mangiferae* isolates were *MAT*-2 except for two isolates from Malaysia (MRC 8092 and MRC 8093) that were *MAT*-1. Both mating types were identified amongst *F. sterilihyphosum* isolate. The majority of these isolates were *MAT*-1 and isolates MRC 8101, MRC 8104 and MRC 8105 were *MAT*-2.

Isolates of *F. mangiferae* and *F. sterilihyphosum* of opposite mating type were sexually incompatible when crossed within each species and between the two species. None of the other *Fusarium* isolates (TABLE I) were sexually compatible with the tester strains of mating population H.

DISCUSSION

In this study we have shown that at least two distinct *Fusarium* spp. are associated with mango malformation symptoms, namely *F. mangiferae* and *F. sterilihyphosum*. *Fusarium mangiferae* was previously shown to be the causal agent of mango malformation and *F. sterilihyphosum* is associated with similar disease symptoms in South Africa. A third taxon was also identified, but our collections are insufficient in number to justify describing the fungus. Furthermore, the fungus does not occur in South Africa, and has not been a primary focus of our investigation.

The results of this study, together with those of Steenkamp et al (2000a), have shown that mango malformation in South Africa is associated with two distinct species, *F. mangiferae* and *F. sterilihyphosum*.

Fusarium sterilihyphosum has only been isolated from malformed mango tissue in South Africa. The histone *H3* and β-tubulin gene sequences for isolates of *F. mangiferae* are similar to those of *F. subglutinans* strains NRRL 25226 and MRC 7559 (Steenkamp et al 2000a), which were previously reported to be the causal agent of mango malformation (Freeman et al 1999).

Fusarium mangiferae has been isolated from mango malformation symptoms in various geographical areas, such as South Africa, Florida, Egypt, India, Israel and Malaysia. *Fusarium mangiferae* is morphologically most similar to *F. concentricum* Nirenberg & O'Donnell and *F. guttiforme* Nirenberg & O'Donnell. *Fusarium concentricum* has long, slender, 3–4 septate macroconidia similar to those produced by *F. mangiferae*, which has sympodially branched conidiophores in contrast to the branched conidiophores of *F. concentricum*. *Fusarium guttiforme* can be distinguished from *F. mangiferae* based on the presence of the uniformly obovoid microconidia and 3-septate macroconidia that are shorter in length than those of *F. mangiferae*. The occasional production of 3-septate macroconidia in *F. guttiforme* isolates (MRC 7539, MRC 6784, and MRC 6785) found in the present study was also observed by Viljoen et al (1997). Nirenberg and O'Donnell (1998) did not refer to the macroconidial characteristics in their description of *F. guttiforme*.

Fusarium sterilihyphosum has been isolated only from South Africa. This species is morphologically similar to *F. mangiferae*, but can be distinguished from *F. mangiferae*. *Fusarium sterilihyphosum* has shorter 3–5 septate macroconidia, faster growth rate on PDA at 25 C than *F. mangiferae* and produces sterile coiled hyphae. *Fusarium sterilihyphosum* is most closely related to *F. guttiforme* based on histone gene sequence (Steenkamp et al 2000a). Morphologically, *F. sterilihyphosum* resembles *F. circinatum* and *F. pseudocircinatum* O'Donnell & Nirenberg. These three species all produce sterile coiled hyphae. However, macroconidia are long, slender, and 3–5 septate in *F. sterilihyphosum*, while shorter 3-sepate macroconidia are produced in both *F. circinatum* and *F. pseudocircinatum*.

Both *F. sterilihyphosum* and *F. mangiferae* are morphologically distinct from species belonging to *F. subglutinans sensu lato* occurring on various host plants, including *F. begoniae* Nirenberg & O'Donnell, *F. bulbicola* Nirenberg & O'Donnell, *F. circinatum* (MP-H), *F. guttiforme*, *F. concentricum*, *F. pseudocircinatum*, *F. sacchari* (Butler) W. Gams (MP-B), and *F. subglutinans sensu stricto* (MP-E) (TABLE II). *Fusarium mangiferae* and *F. sterilihyphosum* can also be distinguished from each other based on morphological characteristics. Sterile coiled hyphae and shorter 3–5 septate macroconidia produced by *F. sterilihyphosum* distinguish it from *F. mangiferae*. *Fusarium mangiferae* had a slower growth rate than *F. sterilihyphosum* on PDA at 25 C. However, growth rate is a secondary morphological characteristic and no significant difference ($P > 0.001$) among isolates of both species was observed. Furthermore, secondary characteristics are generally not used in species descriptions in view of the variability within populations and/or the instability of these characters (Gerlach and Nirenberg 1982, Nelson et al 1983, Nirenberg and O'Donnell 1998).

Both mating types (*MAT*-1 and *MAT*-2) were identified in *F. mangiferae* and *F. sterilihyphosum* isolates. This is in contrast to the Steenkamp et al (2000a) study, where isolates of each of these species included only a single mating type. In the present study, isolates of the two species having opposite mating type were sexually incompatible.

Likewise, isolates of *F. mangiferae* and *F. sterilihyphosum* of different mating types failed to cross with each other. This failure to produce sexual crosses could be explained by sterility, female-sterility of isolates or unfavorable conditions for crosses to occur (Perkins 1994, Leslie 1995). At this stage, there is thus no evidence to suggest that sexual outcrossing is occurring within or between these two fungi from mango.

Mango is native to Asia, eastern India, Burma, and the Andaman Islands, and mango malformation was first reported over a century ago in India (Kumar et al 1993). *Fusarium mangiferae* isolates from South Africa, United States, Israel, Malaysia, and Egypt grouped into the so-called 'Asian clade' of O'Donnell et al (1998) based on histone *H3* and β-tubulin gene sequences (O'Donnell et al 1998, 2000, Steenkamp et al 2000a). *Fusarium mangiferae* from different geographical areas was most probably introduced from India (Zheng & Ploetz 2002), which would explain the presence of *F. mangiferae* isolates grouping in the 'Asian clade'. *Fusarium sterilihyphosum* isolates from mango malformation symptoms in South Africa grouped into the so-called 'American clade' (O'Donnell et al 2000, Steenkamp et al 2000a). Based on its phylogenetic position, O'Donnell et al (2000) speculated that *F. sterilihyphosum* (MRC 2802 = NRRL 25623) originated from mango that was imported into South Africa from South America. Vegetative malformation has been reported in Mexico (Noriega-Cantu et al 1999). These Mexican isolates produced sterile coiled hyphae and grouped also in the 'American clade' based on β-tubulin gene sequences, like *F. sterilihyphosum* isolates from South Africa (David M. Geiser pers comm). Clearly, further

investigations with strains from South America would be required to test the hypothesis that *F. sterilihyphosum* isolates in South Africa originated in South America.

The fact that three distinct taxa are found associated with mango malformation symptoms emphasizes a serious problem regarding the etiology of mango malformation disease. *Fusarium mangiferae* has been unequivocally indicated as the causal agent of mango malformation (Freeman et al 1999). It is, however, not known whether *F. sterilihyphosum* or the undescribed *Fusarium* sp. are also able to cause disease on mango trees. Their role in the etiology of mango malformation disease clearly requires further intensive study.

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