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Fusarium-Induced Diseases of Tropical Perennial Crops

Mango Malformation Disease and the Associated *Fusarium* Species

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ABSTRACT

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Mango malformation disease (MMD) occurs in Asia, Africa, and the Americas and was first reported in India in 1891. The vegetative form of MMD was first reproduced in 1966 with *Fusarium moniliforme* and the floral form with isolates of *F. moniliforme* var. *subglutinans* from both vegetative shoots and floral tissue. The fungi were subsequently recognized as *F. subglutinans*. In 2002, a new species, *F. mangiferae*, was established based on nuclear and mitochondrial DNA sequences; it included strains of *F. subglutinans* from Egypt, Florida, Israel, Malaysia,

and South Africa, some of which had been shown to cause MMD by artificial inoculation. At least three additional taxa have been associated with MMD: *F. sterilihyphosum* from Brazil and South Africa, and *Fusarium* sp. nov. and *F. proliferatum* (teleomorph: *Gibberella intermedia*) from Malaysia. To date, Koch's postulates have not been completed with them. In the future, gene sequencing will be essential to identify the *Fusarium* spp. that are associated with MMD. Work remains to be done on the morphology, sexual compatibility, pathogenicity, and toxigenicity of these taxa.

Additional keywords: *G. fujikuroi* species complex, mating population, vegetative compatibility group.

Malformation disease (MMD) of mango, *Mangifera indica* L., was first reported in India in 1891 (20). It is found elsewhere in Asia (Israel, Malaysia, and Pakistan), Africa (Egypt, South Africa, Sudan, Swaziland, and Uganda), and the Americas (Brazil, El Salvador, Mexico, Nicaragua, the United States, and Venezuela) (4,5,9,16,20,21,23). We review, briefly, the disease and recent developments on the identity of the associated *Fusarium* spp.

THE DISEASE

Symptoms. MMD causes inflorescences to shorten, thicken and branch, increases flower number and size, increases the number of male flowers, and causes sterility or abortion in the remaining hermaphroditic flowers (Fig. 1) (9,20,21). Leaves may also develop within the inflorescence (phyllody) (Fig. 2). The symptoms of malformed mango inflorescences are associated with quantitative and qualitative alterations of cytokinins (29,30). The mycotoxins diacetoxyscirpenol and T-2 toxin have been reported to occur in malformed mango tissue from which *F. moniliforme* Sheldon (also referred to as *F. moniliforme* Sheldon var. *subglutinans* Wollenweber & Reinking) was isolated in India (6,7), but this is unlikely and has not been confirmed (12) (*F. moniliforme*, used previously for at least six distinct taxa, is no longer used [24]).

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Vegetative malformation also occurs, particularly in young trees in nurseries (Fig. 3). Shoots from apical and axillary buds are misshapen and have shortened internodes with dwarfed and narrow leaves (20,21).

Etiology. Several fungi are associated with MMD, all of which are in the *Gibberella fujikuroi* species complex (i.e., *F. subglutinans* sensu lato). To date, only one of these taxa has been shown to cause MMD.

Koch's postulates were first completed in 1966 by Summanwar et al. (28) with *F. moniliforme*. In 1974, the floral form of MMD was induced with strains of *F. moniliforme* var. *subglutinans* that were isolated from both vegetative shoots and floral tissue; it was concluded that both forms of the disease are caused by the same pathogen (31). This fungus was subsequently recognized as *F. subglutinans* (Wollenweber & Reinking) Nelson, Toussoun & Marasas (14,21) and shown to cause mango malformation in Egypt (8), South Africa (11), Florida (22), and Israel (5).

In 2002, a new species, *F. mangiferae* Britz, Wingfield & Marasas, was established for 29 phylogenetically related strains from Egypt, Florida, Israel, Malaysia, and South Africa (4); it included strains of *F. subglutinans* that previously had been shown by artificial inoculation to cause mango malformation, including the ex-holotype strain MRC 7559 from Israel (also 506/2) (5).

F. mangiferae does not form microconidial chains and chlamydospores but does produce polyphialides (4). Small microscopic differences distinguish it from other taxa in the *G. fujikuroi* species complex (4,15). *F. mangiferae* can also be differentiated morphologically from two other species that are associated with MMD (Table 1).

Based on DNA sequence data (17,18,26,27), *F. mangiferae* is related to a lineage that includes *F. fujikuroi* Nirenberg, *F. proliferatum* (Matsushima) Nirenberg, and *F. sacchari* (E. J. Butler)

W. Gams (Fig. 4); clade 2 corresponds to the so-called “Asian Clade” of O’Donnell et al. (17). Based on combined sequence data for five genes, the closest known relative of *F. mangiferae* is an isolate from tropical rainforest soil in Papua-New Guinea (18).

ADDITIONAL SPECIES ASSOCIATED WITH MMD

***F. sterilihyphosum* Britz, Wingfield & Marasas.** This species was described based on 17 isolates from malformed mango tissue in the Tzaneen area of South Africa (4). It was subsequently recovered from malformed mango trees in Brazil (32).

F. sterilihyphosum can be differentiated from *F. mangiferae* by its shorter, three- to five-septate macroconidia and the production of sterile coiled hyphae in the aerial mycelium (Table 1). In the latter characteristic, *F. sterilihyphosum* resembles *F. circinatum* Nirenberg & O’Donnell emend Britz, Coutinho, Wingfield & Marasas and *F. pseudocircinatum* O’Donnell & Nirenberg.

Based on DNA sequences (18,26), *F. sterilihyphosum* falls in clade 3 (Fig. 4), which corresponds to the “American Clade” of O’Donnell et al. (17). This clade includes *F. guttiforme* O’Donnell & Nirenberg, *F. circinatum* (teleomorph: *G. circinata* Nirenberg & O’Donnell emend Britz, Coutinho, Wingfield & Marasas), and *F. subglutinans* sensu stricto (*G. subglutinans* Nelson, Toussoun & Marasas). The five-gene phylogeny of O’Donnell et al. (18) suggests that the closest known relative of *F. sterilihyphosum* is a strain from wood in Venezuela.

***Fusarium* sp. nov.** This taxon was reported by Britz et al. (4), but not described formally since only three isolates, all from malformed mango tissue in Malaysia, were available. The species

differs from *F. mangiferae* and *F. sterilihyphosum* by having conidiogenous cells with more than three openings and relatively short three- to five-septate macroconidia (Table 1). Currently, its phylogenetic relatedness to other *Fusarium* species is not known.

Other species. The role that other MMD-associated species play in the development of the disease is not known. Isolates of *F. proliferatum* (*G. intermedia* (Kuhlman) Samuels, Nirenberg & Seifert) have been recovered from malformed mango trees in Malaysia (10), but their pathogenicity has not been determined. Reports that *F. oxysporum* Schlecht emend. Snyder & Hansen causes MMD (1) either result from a misidentification of the pathogen (20) or indicate that a new, chlamyospore-producing taxon is involved; an independent identification of this fungus and confirmation of its causal role are needed.

Sexual compatibility and genetic diversity. Fifty-five isolates of *F. subglutinans* from malformed mango trees in Florida belonged in a single vegetative compatibility group (VCG) (19); several of these isolates were subsequently identified as *F. mangiferae*. No teleomorph was produced in crosses between isolates from Florida and Egypt (20). A single VCG amongst isolates from Florida was also reported by Shaw et al. (25), but five VCGs were found in Egypt, nine in Malaysia, and two in South Africa. None of these isolates produced a teleomorph in crosses. However, 10 isolates of *F. proliferatum* from Malaysia formed the *G. intermedia* teleomorph (10).

In a survey in Egypt, Ploetz et al. (23) detected four VCGs among 45 isolates of *F. mangiferae*; 39 of these isolates were sexually incompatible with testers of the B, C, and D mating populations of the *G. fujikuroi* species complex, respectively *G. sacchari*



Fig. 1. Malformed inflorescence of mango cv. Haden (Florida).

Summerell & Leslie, *G. fujikuroi* (Sawada) Ito in Ito & K. Kimura, and *G. intermedia*. Ten of these isolates did not produce perithecia in crosses with each other.

A recent study indicated that genetic diversity is limited in *F. mangiferae* in Florida, Egypt, India, Israel, and South Africa (32). Six VCGs and three random amplified polymorphic DNA (RAPD) profiles were identified among 71 isolates of *F. mangiferae* that were tested, but four of the six VCGs were characterized by a single RAPD profile. Thus, populations of this pathogen probably reproduce clonally.

Britz et al. (4) used a polymerase chain reaction (PCR)-based method to determine mating type (MAT-1 and MAT-2). In *F. mangiferae*, 27 isolates from Egypt, Israel, Florida, and South Africa were MAT-2 and two from Malaysia were MAT-1, whereas in *F. sterililyphosum*, 14 isolates were MAT-1 and three were MAT-2. When isolates of opposite mating type were crossed, sexual compatibility was not observed within and between the two species (4).

Management. New mango plantings should be established with noninfected nursery stock. Scion material should not be taken from an infected orchard and nurseries should never be established in affected orchards (this is a common practice in Egypt [21]). A PCR assay for identifying *F. mangiferae* has been reported and is being developed for use in a quarantine diagnostic kit (32). In infected orchards, symptomatic tissues should be removed from trees and burned. By doing this for two or three consecutive seasons, MMD can be reduced to insignificant levels. Integrated management with pruning and acaricide and fungicide sprays has been recommended in Mexico (16).



Fig. 3. A vegetatively malformed mango seedling from a nursery beneath malformed trees (Giza, Egypt).



Fig. 2. Phyllody of mango cv. Haden panicle (Apatzing, Mexico).

DISCUSSION

Mango malformation is a serious disease in many areas where this important crop is grown. Despite this fact, relatively little is

TABLE 1. Differential morphological characteristics of three *Fusarium* species associated with mango malformation^a

Morphological characteristics	<i>F. mangiferae</i>	<i>F. sterilihyphosum</i>	<i>Fusarium</i> sp.
Growth rate (mm/day at 25°C)	3.4	4.8	?
Sterile coiled hyphae	-	+	-
Conidiophores prostrate	+	+	-
Macroconidial length	43–61 µm	28–47 µm	19–29 µm

^a Adapted from Britz et al. (4). + Indicates the presence and - the absence of a characteristic, and ? indicates that the characteristic has not been reported.

known about the disease. The recent discovery that several *Fusarium* spp. are associated with MMD is intriguing. However, only *F. mangiferae* has been shown to cause MMD. More work is needed to understand the roles that are played by the other species.

Advances in molecular phylogenetics have made it possible to characterize two species that are associated with MMD, *F. mangiferae* and *F. sterilihyphosum*, and to differentiate them from each other. DNA sequence data have also made it possible to distinguish these species from morphologically similar lineages of the *G. fujikuroi* species complex that are associated with other host-specific diseases such as pitch canker of pine, caused by *F. circinatum* (*G. circinata*), and fusariosis of pineapple, caused by *F. guttiforme*. Previously, pathogenicity tests were required to identify some of these taxa.

Crosses with MAT-1 and MAT-2 tester strains of species in the *G. fujikuroi* species complex also yield valuable information, e.g.,

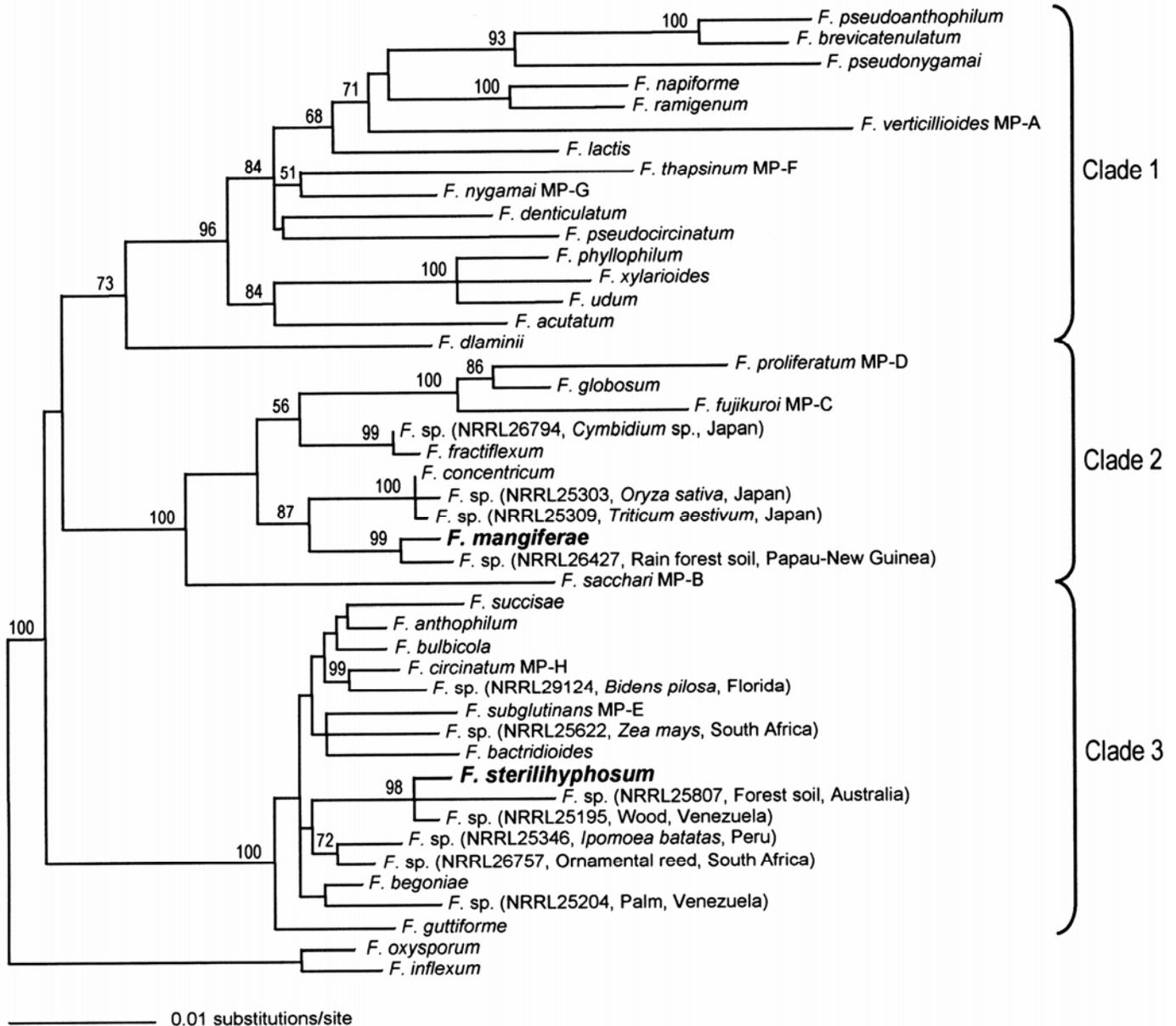


Fig. 4. A maximum likelihood phylogeny of the *Gibberella fujikuroi* species complex based on the combined DNA sequence information for translation elongation factor 1 α and β -tubulin. Clades 1 to 3 correspond to the so-called African, Asian, and American Clades, respectively, of O'Donnell et al. (17). In addition to representatives for all the formally described species in this complex, the tree also includes representatives of previously identified phylogenetic species (17,18) in clades 2 and 3 for which sequences for both genes are available. For the undescribed species, isolate number (NRRL, Northern Regional Research Laboratory, Peoria, IL), host/source, and geographic origin are indicated in parentheses. Bootstrap values >50% are indicated above the branches and *G. fujikuroi* mating populations are indicated with MP-A to MP-H.

compatible strains of *F. circinatum* will produce the teleomorph, *G. circinata* when crossed with the appropriate testers (3). In contrast, *F. mangiferae* and *F. sterilihyphosum* have not formed a teleomorph when crossed with tester strains or amongst themselves. Gene sequencing is now essential to identify these morphologically similar taxa.

Few population genetic studies have been conducted with the fungi that are associated with MMD (25,32). These studies show very little genetic variation amongst isolates of *F. mangiferae* from Florida, Egypt, India, Israel, Malaysia, and South Africa. The low numbers of VCGs that have been reported indicate clonal or near clonal populations of this species, and the limited genetic variation is consistent with asexual reproduction. It also supports the view that *F. mangiferae* has been introduced into areas such as South Africa and Israel as single genets.

The origins of the associated *Fusarium* species are unknown. Two types of mango are recognized: a subtropical group with monoembryonic seed that evolved on the Indian subcontinent, and a tropical type from Southeast Asia that has polyembryonic seed (13). Sixty-nine species of *Mangifera* are recognized, the greatest diversity of which (28 species) occurs in western Malaysia (2). Whether *F. mangiferae* evolved with mono- or polyembryonic *M. indica*, other *Mangifera* spp., or other hosts is not known. However, since the pathogen latently infects *M. indica* and is not uncommon in symptomless tissue of this host, it has probably been disseminated in infected germ plasm (19). Even less is known about the isolates of *F. proliferatum* and *Fusarium* sp. nov. that are associated with MMD in Malaysia. Much might be learned about MMD and the associated taxa by surveying mango in its centers of origin.

The origin of *F. sterilihyphosum* is also a mystery. If O'Donnell et al. (17) are correct in their assertion that it originated in the Americas, its original host(s) could not be mango. Although a higher priority would be verifying that this species causes MMD, work to determine whether *F. sterilihyphosum* evolved on mango relatives (e.g., cashew, *Anacardium occidentale* L.) or other species that are native to the western tropics is warranted.

Although DNA sequence data distinguish host-specific taxa in the *G. fujikuroi* species complex, much more work is needed on the morphology, sexual compatibility, pathogenicity, and toxigenicity of those that are associated with MMD. The morphology of *Fusarium* sp. nov. in Malaysia needs to be investigated and a formal description published. More crosses should be made between MAT-1 and MAT-2 strains of *F. mangiferae*, *F. sterilihyphosum*, and *Fusarium* sp. nov. to search for the teleomorphs. Pathogenicity studies are needed with more strains of *F. mangiferae* from different countries and it needs to be established whether *F. proliferatum*, *F. sterilihyphosum*, and *Fusarium* sp. nov. cause MMD. Finally, the secondary metabolite profiles of the associated taxa should be investigated to determine whether they produce mycotoxins, such as trichothecenes, moniliformin, and fumonisins, that may occur in mangos and present a potential threat to human health.

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