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*“*Comparación genética y patogénesis de taxones de *Fusarium* asociados a la malformación del mango y de árboles neotropicales*”*

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ÍNDICE

1. RESUMEN GENERAL 4

2. SUMMARY 5

3. INTRODUCCIÓN GENERAL 7

4. JUSTIFICACIÓN 7

5. HIPÓTESIS 10

6. OBJETIVO GENERAL 10

7. OBJETIVOS ESPECÍFICOS 10

8. RESULTADOS 10

8.1. CAPÍTULO I: Design and validation of a robust multiplex polymerase chain reaction assay for *MAT* idiomorph within the *Fusarium fujikuroi* species complex. . 10
- 8.1.1. ABSTRACT 11
- 8.1.2. INTRODUCTION 11
- 8.1.3. MATERIALS AND METHODS 13
- 8.1.4. RESULTS 19
- 8.1.5. DISCUSSION 24
- 8.1.6. LITERATURE CITED 26

8.2. CAPITULO II: Novel malformation disease of rosy trumpet (*Tabebuia rosea*) and cooperwood (*Bursera ovalifolia*) trees caused by *Fusarium pseudocircinatum* in Mexico. 35
- 8.2.1. ABSTRACT 35
- 8.2.2. INTRODUCTION 36
- 8.2.3. MATERIALS AND METHODS 37
- 8.2.4. RESULTS 42
- 8.2.5. DISCUSSION 51
- 8.2.6. LITERATURE CITED 54

8.3. CAPITULO III: Diversity of *Fusarium* species associated with weeds in mango orchards in Mexico 63
- 8.3.1. ABSTRACT 63
- 8.3.2. INTRODUCTION 63
- 8.3.3. MATERIALS AND METHODS 65
- 8.3.4. RESULTS AND DISCUSSION 70
- 8.3.5. LITERATURE CITED 83

9. DISCUSIÓN GENERAL 94

10. CONCLUSIÓN GENERAL 96

11. LITERATURA CITADA 97

12. ANEXOS 100

ÍNDICE DE TABLAS

Table 1. Strain histories and results of multiplex PCR for *MAT* idiomorph targeting *MAT1-1-2* and *MAT1-2-1* in the present study. 14

Table 2. Published PCR primer pairs for *MAT* idiomorph in *Fusarium* tested in the present study. 17

Table 3. PCR primers designed and tested for multiplex amplification of *MAT* in the FFSC and FNSC in the present study. 19

Table 4. Analysis of secondary metabolites production by *Fusarium* isolates associated with malformation disease of *Bursera ovalifolia* and *Tabebuia rosea* from Guerrero and Michoacán, Mexico. 48

Table 5. Pathogenicity tests on rosy trumpet tree, mango and big-leaf mahogany seedlings using *Fusarium* strains associated with malformation disease in tropical trees from Guerrero and Michoacán, Mexico. 49

Table 6. *Fusarium* isolates obtained from malformed tissues form several weeds and mango trees Michoacán, Mexico. 71

Table *7.* Secondary metabolites production and mating type of *Fusarium* isolates from weeds and mango malformation*.* 77

ÍNDICE DE FIGURAS

Figure 1. Maps showing location of the forward and reverse *MAT1-1-2* and *MAT1-2-1* multiplex PCR primer pairs for typing *MAT* idiomorph within the *Fusarium fujikuroi* species complex. 22

Figure 2. Ethidium bromide–stained agarose gel showing 450 bp *MAT1-1-2* and 330 bp *MAT1-2-1* fragments amplified by multiplex PCR assay for *MAT* idiomorph within the FFSC and FNSC. 23

Figure 3. Map of Mexico showing seven municipalities in the state of Michoacán and two in Guerrero where malformation samples were collected from neotropical trees and mango. ... 37

Figure 4. Field symptoms of malformation on neotropical trees. 44

Figure 5. Maximum likelihood tree (ML) inferred from a four-locus dataset rooted at the midpoint. 46

Figure 6. *Tabebuia rosea* and *Swietenia macrophylla* seedlings exhibiting malformation symptoms 6 and 2 months after being inoculated, respectively. 51

Figure 7. Map of Mexico showing the three mango orchard sites in the state of Michoacán where weeds and malformation samples were collected. 67

Figure 8. Maximum likelihood tree (ML) inferred from a two-locus dataset. 75

ÍNDICE DE MATERIAL SUPLEMENTARIO

Supplementary Table 1. PCR results obtained by screening 71-isolate validation panel using published PCR assays for determining *MAT* idiomorph in *Fusarium*. 31

Supplementary Table 2. PCR results obtained from screening 48-isolate/47 species panel using the multiplex PCR assay for *MAT* idiomorph developed in the present study. 33

Supplementary Table 3. *Fusarium* isolates obtained from malformed tissues form several tropical trees in Guerrero and Michoacán, Mexico. 58

Supplementary Figure 1. ISSR fingerprint patterns of *Fusarium* isolates recovered from tropical trees with malformation disease. 57

1. RESUMEN GENERAL

El mango es un cultivo frutal de gran importancia en México y el mundo, el cual es afectado por dos enfermedades importantes que limitan su producción, la antracnosis y la malformación o "escoba de bruja" (MMD). En el ámbito mundial, la malformación del mango es causada por varias especies del género *Fusarium*; mientras que, en México, *F. mexicanum* y *F. pseudocircinatum* son las especies prevalentes. Durante colectas realizadas en la región de la cuenca Baja del Río Balsas en los estados de Michoacán y Guerrero, se detectaron árboles de 'papelillo' (*Bursera ovalifolia*), caoba de hoja ancha (*Swietenia macrophylla*), 'primavera' (*Tabebuia rosea*) y mango (*Mangifera indica*) con síntomas de malformación. *Bursera ovalifolia* y *T. rosea* son especies neotropicales descritas como componentes importantes de la vegetación en las áreas subtropicales secas de México. Los objetivos de esta parte del trabajo de investigación fueron: i) describir los síntomas observados en los árboles de *B. ovalifolia* y *T. rosea*; e ii) identificar el agente causal de la enfermedad malformación en estos dos hospedantes. Se recuperaron un total de 76 aislados de *Fusarium* de los tejidos malformados muestreados, de los cuales 64 aislados se identificaron como *F. pseudocircinatum* y 12 como *F. mexicanum*. Todos los aislados obtenidos de *S. macrophylla*, *B. ovalifolia* y *T. rosea* fueron identificados como *F. pseudocircinatum*. Postulados de Koch confirmaron que *F. pseudocircinatum* es el patógeno causante de la enfermedad en *T. rosea*. Pruebas de patogenicidad con una cepa de *F. pseudocircinatum* recuperada de tejido malformado de *B. ovalifolia* reprodujo síntomas de malformación en plántulas de *S. macrophylla* y *T. rosea*. Este es el primer reporte de malformación en *T. rosea* causada por *F. pseudocircinatum* en México.

A la fecha, no existen estudios ecológicos de las especies de *Fusarium* que causan malformación del mango en México. Por lo anterior, se desconoce el papel que pudieran tener otras especies vegetales en la supervivencia, dispersión y origen de estos patógenos. El objetivo de esta parte de la investigación fue determinar si *F. mexicanum* y *F. pseudocircinatum* son habitantes naturales en hojas y yemas en dormancia de malezas presentes en huertos de mango en Michoacán. Se muestrearon dos huertas en dos municipios, Nuevo Urecho y Buenavista Tomatlán.

Un total de 59 aislados de *Fusarium* pertenecientes a cinco complejos de especies fueron recuperados de yemas apicales y laterales de cuatro especies de malezas. Los complejos de especies de *Fusarium* identificados fueron: FFSC (*F. fujikuroi* species complex), FNSC (*F. nisikadoi* species complex), FSAMSC (*F. sambucinum* species complex), FIESC (*F. incarnatum-equiseti* species complex) y FSSC (*F. solani* species complex). Dentro del FFSC se identificaron 14 aislados de *F. mexicanum* y 4 aislados de *F. pseudocircinatum*. Estudios adicionales son necesarios para determinar el papel epidemiológico de las malezas en la MMD, lo que podría ayudar a mejorar el manejo de la enfermedad en las áreas de cultivo de mango.

Se diseñó y validó un ensayo de diagnóstico basado en la técnica de PCR multiplex para determinar el idiomorfo *MAT* dentro del FFSC. De los tres ensayos de PCR multiplex diseñados y probados, los oligonucleótidos que amplifican a *MAT1-1-2* y *MAT1-2-1* genotiparon con éxito todo el panel de validación de 71 aislados, que incluía 56 filoespecies FFSC y 4 FNSC. Aunque solamente una quinta parte de los miembros del FFSC han mostrado reproducción sexual en ensayos de laboratorio, los resultados de este trabajo sugieren que todos poseen un ciclo sexual heterotálico. Esta suposición se basa en el descubrimiento de que todos los aislados FFSC analizados a la fecha poseen idiomorfos *MAT1-1* o *MAT1-2* intactos.

Palabras clave: *escoba de bruja, micotoxinas, malezas, filogenia, mating type*

2. SUMMARY

Mango is a fruit crop of great importance in Mexico and the world, which is affected by two important diseases that limit its production, anthracnose and malformation or "witch's broom" (MMD). Worldwide, mango malformation is caused by several species of the genus *Fusarium*; while in Mexico, *F. mexicanum* and *F. pseudocircinatum* are the prevalent species. During surveys carried out in the region of the Lower Basin of the Balsas River in the states of Michoacán and Guerrero, trees of 'cooperwood' (*Bursera ovalifolia*), ‘wide-leaf mahogany’ (*Swietenia macrophylla*), 'rosy trumpet' (*Tabebuia rosea*) and mango (*Mangifera indica*), were detected with symptoms of malformation. *Bursera ovalifolia* and *T. rosea* are neotropical species described as important components of the vegetation in the dry subtropical areas of

Mexico. The objectives of this part of the research work were: i) to describe the symptoms observed in the trees of *B. ovalifolia* and *T. rosea*; and ii) identify the causative agent of the malformation disease in these two hosts. A total of 76 *Fusarium* isolates were recovered from the sampled malformed tissues, of which 64 isolates were identified as *F. pseudocircinatum* and 12 as *F. mexicanum*. All isolates obtained from *S. macrophylla*, *B. ovalifolia* and *T. rosea* were identified as *F. pseudocircinatum*. Koch postulates confirmed that *F. pseudocircinatum* is the pathogen causing the disease in *T. rosea*. This is the first report of malformation in *T. rosea* caused by *F. pseudocircinatum* in Mexico. Pathogenicity tests on *B. ovalifolia* seedlings were not carried out because germination of the collected seeds was not obtained; however, a strain of *F. pseudocircinatum* recovered from malformed tissue of *B. ovalifolia* reproduced symptoms of malformation in seedlings of *S. macrophylla* and *T. rosea*.

To date, there are no ecological studies of *Fusarium* species that cause mango malformation in Mexico. Therefore, the role of other plant species in the survival, dispersion and origin of these pathogens is unknown. The objective of this part of the investigation was to determine if *F. mexicanum* and *F. pseudocircinatum* are natural inhabitants of leaves and dormant buds of weeds present in mango orchards in Michoacán. Two orchards were sampled in two municipalities, Nuevo Urecho and Buenavista Tomatlán. A total of 59 *Fusarium* isolates belonging to five species complexes were recovered from apical and lateral buds of four weed species. The *Fusarium* species complex were: FFSC (*F. fujikuroi* species complex), FNSC (*F. nisikadoi* species complex), FSAMSC (*F. sambucinum* species complex), FIESC (*F. incarnatum-equiseti* species complex) and FSSC (*F. solani* species complex). Within the FFSC, 14 isolates of *F. mexicanum* and 4 isolates of *F. pseudocircinatum* were identified. Additional studies are necessary to determine the epidemiological role of weeds in MMD, which could help improve disease management in mango cultivation areas.

A diagnostic test based on the multiplex PCR technique was designed and validated to determine the *MAT* idiomorph within the FFSC. Of the three multiplex PCR assays designed and tested, oligonucleotides that amplify *MAT1-1-2* and *MAT1-*

2-1 successfully genotyped the entire validation panel of 71 isolates, which included 56 FFSC and 4 FNSC phylospecies. Although only one fifth of FFSC members have shown sexual reproduction in laboratory trials, the results of this work suggest that everyone has a heterothallic sex cycle. This assumption is based on the discovery that all FFSC isolates analyzed to date have intact *MAT1-1* or *MAT1-2* idiomorphs.

3. INTRODUCCIÓN GENERAL

El mango (*Mangifera indica* L.) es uno de los principales cultivos frutícolas en México. La producción nacional fue de 1,867,297 ton de fruta en 2018 (SIAP 2018). Durante el 2018, México fue uno de los principales productores y exportadores en el ámbito mundial, ocupando el 4º lugar en producción y uno de los primeros en exportación, llegando a 27 países, principalmente a los Estados Unidos y Canadá (SAGARPA 2018, SIAP 2018). Los principales estados productores de mango en México son: Guerrero (385,125 ton), Nayarit (322,387 ton), Chiapas (279,281 ton) y Sinaloa (195,969 ton) (SIAP 2018). En Michoacán se produjeron 160,572.68 ton, en una superficie sembrada de 25,337 ha, con un valor de producción de más de 941 millones de pesos durante el 2018 (SIAP 2018).

El mango pertenece a la familia botánica Anacardiaceae, la cual contiene 73 géneros de plantas distribuidas principalmente en los trópicos. El centro de origen de *M. indica* se localiza en el sudeste de Asia, región donde se presenta la mayor diversidad del género (Litz 2009). Las 69 especies del género *Mangifera* se encuentran como especies silvestres en la zona que comprende Bangladesh, Brunei, India, Indonesia, Filipinas, Myanmar, Nueva Papú Guinea, Singapur, Sri Lanka, y las islas Salomón y Carolina. Aun cuando el origen geográfico de *M. indica* no se ha establecido definitivamente, la evidencia molecular y taxonómica indica que el mango probablemente evolucionó en un área muy grande que comprende el noroeste de Myanmar, Bangladesh y el noreste de la India (Litz 2009).

Entre los principales problemas que afectan la producción de esta fruta a nivel nacional se encuentran diversas plagas, la deficiente planeación de nuevos huertos, el mal manejo y daños por enfermedades. Las enfermedades que destacan por su importancia son: pudrición texana [*Phymatotrichopsis omnívora* (Duggar) Henneber], antracnosis [*Colletotrichum gloesporioides* (Penz.) Penz. & Sacc.)], cenicilla (*Oidium mangiferae* Berthet), cancro del tronco (*Phytophthora parasitica* Dastur), y la comúnmente llamada “escoba de bruja” o “malformación”, que es causada por varias especies del hongo *Fusarium* Link (Marasas et al. 2006, Ploetz and Freeman 2009).

La malformación del mango es una de las principales enfermedades que afecta a este cultivo en el campo en México. En algunas regiones de Michoacán, la alta incidencia y severidad de la enfermedad (Vega-Piña y Miranda-Salcedo 1993), han ocasionado que los agricultores sustituyan huertas de mango afectadas por malformación por otros cultivos como cítricos. La reducción directa en producción ocurre por la malformación de los brotes florales, mientras que la malformación de los brotes vegetativos afecta indirectamente al reducir la superficie foliar disminuyendo la fotosíntesis (Ploetz 2003). En México, se han descrito y detectado dos especies de *Fusarium* en la mayoría de las regiones productoras del país, *F. mexicanum* T. Aoki, S. Freeman, Otero-Colina, Rodríguez-Alvarado, Fernández-Pavía, Ploetz & O’Donnell (Rodríguez-Alvarado et al. 2008, Otero-Colina et al. 2010) y *F. pseudocircinatum* Nirenberg & O’Donnell (Freeman et al. 2014).

Estudios realizados en la región centro occidente de México indican que *F. mexicanum* es el patógeno prevalente ocasionando malformación en huertas de mango (Betancourt-Resendes et al. 2012). Este patógeno ha sido detectado en Colima, Guerrero, Jalisco, Michoacán y Morelos. Mientras que *F. pseudocircinatum* ha sido reportado en Campeche y Guerrero. Síntomas de la enfermedad han sido observados en Chiapas, Nayarit, Sinaloa y Veracruz. Por otra parte, la diversidad genética que las poblaciones de *F. mexicanum* presentan en Colima, Jalisco y Michoacán es baja, lo cual sugiere que este patógeno se encuentra como poblaciones clonales con una ausencia de reproducción sexual (Rodríguez-Alvarado et al. 2012).

En un estudio llevado a cabo para determinar las fuentes de inóculo de la malformación en huertas de mango (Soto-Plancarte et al. 2015), se detectaron plantas de mango de 1 a 2 años de edad con síntomas de malformación en la mayoría de los viveros comerciales visitados en Michoacán. *Fusarium mexicanum* fue detectado en los tejidos malformados de todas las plantas muestreadas. Análisis genéticos indicaron que las cepas aisladas de los viveros son una población clonal, idénticas genéticamente a cepas aisladas previamente de árboles de mango en Michoacán. Lo anterior indica que este patógeno está siendo diseminado a nuevas plantaciones o huertas establecidas por medio de plantas infectadas (Soto-Plancarte et al. 2015).

Durante el estudio de Soto-Plancarte et al. (2015) también se analizaron otras especies de plantas como una posible fuente de inóculo (datos no publicados). Los resultados del estudio mostraron que varios taxones de *Fusarium* estaban asociados con los tejidos malformados o asintomáticos de diversas plantas: malformación de caoba (*Swietenia macrophylla* King), 'árbol de primavera' (*Tabebuia rosea* Gomes ex DC.) y 'papelillo' (*Bursera* sp. Jacq. ex L.), y en yemas en dormancia de *Senna* sp. Mill*.*

4. JUSTIFICACIÓN

La malformación del mango es la principal enfermedad de este cultivo en huertas comerciales en diversas zonas de México. Dos especies de *Fusarium* han sido detectadas causando la malformación del mango en el país. Además, se han detectado taxones de este género asociados con los tejidos malformados de otras especies vegetales en la región centro occidente de México. Varias interrogantes surgen de estos hallazgos; i) Se desconocen las relaciones filogenéticas que existen entre las especies del género *Fusarium* causantes de malformación en el mango, y los fusaria detectados en tejidos malformados de árboles y en malezas encontrados en áreas productoras de este frutal; ii) No se tiene información sobre si ocurre flujo de cepas o poblaciones de *Fusarium*, entre árboles de mango y árboles neotropicales con malformación, iii) Una hipótesis plausible es que plantas silvestres en/o adyacentes a huertas de mango, podrían ser infectadas de manera quiescente por cepas patogénicas de *Fusarium,* y funcionar como reservorios del agente causal que induce la malformación en árboles de mango. Las pérdidas económicas que ocasiona esta enfermedad ameritan conocer más acerca de los aspectos ecológicos, filogenéticos y estructura de poblaciones de estos patógenos, para establecer mejores medidas de manejo de esta enfermedad.

5. HIPÓTESIS

Fusarium mexicanum y *F. pseudocircinatum* causantes de malformación en mango, también causan malformación en árboles neotropicales.

Las especies de *Fusarium* causantes de malformación en mango infectan de manera latente a malezas.

6. OBJETIVO GENERAL

Identificar filogenéticamente a las especies de *Fusarium* asociadas a tejidos malformados de árboles neotropicales y los fusaria que infectan latentemente a plantas silvestres.

7. OBJETIVOS ESPECÍFICOS

- Diseñar oligonucleótidos para la determinación del tipo de apareamiento dentro del complejo *Fusarium fujikuroi*.
- Determinar la identidad filogenética de especies de *Fusarium* asociadas a tejidos malformados de mango, árboles neotropicales (*Bursera ovalifolia*, *Swietenia macrophylla* y *Tabebuia rosea*), y a los fusaria que infectan latentemente a plantas silvestres (*Desmodium procumbens*, *Senna occidentalis*, *S. uniflora* y *Sida rhombifolia*).
- Analizar la patogenicidad de aislados de *Fusarium* obtenidos de malformaciones de mango y árboles neotropicales.

8. RESULTADOS

8.1. CAPÍTULO I: Design and validation of a robust multiplex polymerase chain reaction assay for *MAT* idiomorph within the *Fusarium fujikuroi* species complex.

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8.1.1. ABSTRACT

We discovered that published polymerase chain reaction (PCR) assays for determining mating type (*MAT*) idiomorph failed to genotype some of the *Fusarium fujikuroi* species complex (FFSC) isolates recovered from *Mangifera indica* (mango), *Swietenia macrophylla* (big-leaf mahogany), *Annona muricata* (soursop), *Bursera* sp., and *Tabebuia* sp. in Mexico. Thus, the primary objective of this study was to design and validate a robust multiplex PCR–based diagnostic for typing *MAT* within the FFSC. To accomplish this objective, we mined the *MAT1-1* or *MAT1-2* locus from the genomes of 60 FFSC isolates, representing 56 phylospecies, and from four species in its sister group, the *F. nisikadoi* species complex (FNSC). Bioinformatic searches were facilitated by targeting DNA lyase (*SLA2*) and apurinic endonuclease (*APN1*), the genes that flank the *MAT* locus in *Fusarium*. As expected, three genes were identified within *MAT1-1* (*MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3*) and two in *MAT1-2* (*MAT1-2-1* and *MAT1-2-9*), using the ab initio prediction tool AUGUSTUS. Of the three multiplex PCR assays we designed and tested, the one targeting *MAT1-1-2* and *MAT1-2-1* successfully genotyped the entire 71-isolate validation panel, which included 56 FFSC and 4 FNSC phylospecies. By contrast, the published PCR assays we tested produced positive genotypes for only 46.5–59% of the 71-isolate validation panel, but only when they were run as a uniplex assay. Although only one-fifth of the FFSC/FNSC are known to reproduce sexually, our results suggest that if they possess a sexual cycle, it is heterothallic (self-sterile).

8.1.2. INTRODUCTION

The ascomycete genus *Fusarium* (Nectriaceae, Hypocreales) is estimated to comprise over 300 phylogenetically distinct species, representing one of the most important groups of mycotoxigenic plant pathogens (Aoki et al. 2014). As presently circumscribed, multilocus phylogenetic analyses support *Fusarium* as a monophyletic group comprising 23 genealogically exclusive species complexes and several monotypic lineages (Laurence et al. 2011; O'Donnell et al. 2013; Zhou et al. 2018). Of these, members of the species-rich *Fusarium fujikuroi* species complex (FFSC) pose one of the greatest threats to global agricultural biosecurity due to the large number of economically important plant diseases that they cause and the plethora of mycotoxins that they produce (O'Donnell et al. 1998; Kvas et al. 2009). FFSC toxins that pose the greatest threat to food safety and human health include fumonisins (Proctor et al. 2004, 2013) and moniliformin, enniatins, bikaverin, and beauvercin (Munkvold 2017; O'Donnell et al. 2018).

When fusarial toxins contaminate food and feed, it is often deemed unsafe for consumption by humans and other animals (Marasas et al. 1984). Notable plant diseases induced by members of the FFSC include corn ear rot and seedling blight caused by *F. verticillioides* (Gai et al. 2018); foolish seedling disease, or bakanae-byo, of rice caused by *F. fujikuroi* (Aoki et al. 2014); pitch canker of *Pinus* spp. caused by *F. circinatum* (Correll et al. 1991); and mango malformation caused by *F. mangifera* in India and Spain (Zheng and Ploetz 2002; Marasas et al. 2006; Crespo et al. 2012), *F. tupiense* in Brazil (Lima et al. 2012), and *F. mexicanum* in Mexico (Otero-Colina et al. 2010). The latter species and *F. pseudocircinatum* were recently reported to induce a novel malformation-like disease of big-leaf mahogany (*Swietenia macrophylla*) in Michoacán, Mexico (Santillán-Mendoza et al. 2018). Because plant pathogens with the ability to reproduce both sexually and asexually pose the greatest threat to overcoming control methods based on fungicides and host resistance (McDonald and Linde 2002), numerous studies have investigated the reproductive mode and genetic architecture of the mating type (*MAT*) locus within the FFSC (Kerényi et al. 1999; Steenkamp et al. 2000; Martin et al. 2011). Although considerable research has focused on discovering and characterizing biological species in the FFSC via laboratory crosses, teleomorphs have only been reported for approximately one-fifth of the 60 phylogenetically distinct species within this clade (see MycoBank, http://www.mycobank.org/MB/). Where known, fertile perithecia were only formed when FFSC strains of the opposite mating type (i.e., *MAT1-1* and *MAT1-2*) were paired, which is consistent with a self-sterile or heterothallic sexual reproductive mode. Comparative phylogenetic and molecular evolutionary analyses of the mating type locus in 11 biological species with a known sexual cycle in the FFSC revealed that three genes are present in the *MAT1-1* idiomorph (*MAT1-1-1*, *MAT1-1-*

2, and *MAT1-1-3*) and two within *MAT1-2* (*MAT1-2-1* and *MAT1-2-3*). The *MAT1-2-3* gene was first discovered by Martin et al. (2011); however, it was recently renamed *MAT1-2-9* because it is not orthologous with *MAT1-2-3* in *Coccidioides immits* (Wilken et al. 2017). Results of uni- and/or multiplex polymerase chain reaction (PCR) assays for typing *MAT* idiomorph within the FFSC have revealed that isolates were either *MAT1-1* or *MAT1-2* (Kerényi et al. 1999, 2004; Steenkamp et al. 2000; Wallace and Covert 2000), which suggests that members of this species complex only possess a heterothallic sexual reproductive mode. The *MAT* PCR assays reported in the aforementioned studies were tested on one species within the FFSC (Wallace and Covert 2000; Kerényi et al. 2004), seven biological species (Kerényi et al. 1999), or 23 phylospecies (Steenkamp et al. 2000). However, these assays have not been tested on two-thirds of the known FFSC, including the novel phylospecies that have been discovered and described during the two decades since these *MAT* idiomorphs were published (see MycoBank, http://www.mycobank.org/MB/). Therefore, the present study was initiated based on our discovery that published *MAT* PCR assays failed to genotype some of the FFSC isolates recovered from *Mangifera indica* (mango), *Swietenia macrophylla* (big-leaf mahogany), *Bursera* sp., and *Tabebuia* sp. in Mexico (Montoya-Martínez et al. unpubl.). The objectives of this research were to design and validate a robust multiplex PCR–based diagnostic for typing *MAT* within the FFSC and to assess how broadly they worked across the phylogenetic breadth of *Fusarium*. To accomplish these objectives, we mined the *MAT1-1* or *MAT1-2* locus from the genomes of 60 FFSC isolates, representing 56 phylospecies, and from four species in the *F. nisikadoi* species complex (FNSC), which is sister to the FFSC. Of the four multiplex PCR primer pairs that we designed and tested, the ones that targeted *MAT1-1-2* and *MAT1-2-1* were the only ones that worked universally on the FFSC/FNSC.

8.1.3. MATERIALS AND METHODS

Fungal strains and genomic DNA. A total of 77 FFSC and 4 FNSC isolates, comprising 56 and 4 species, respectively, were included in this study. The isolates were analyzed separately as design, test, and validation panels (Table 1). *MAT1-1* or

MAT1-2 sequences that were mined from the 64-isolate design panel, which comprised 56 FFSC and 4 FNSC species (GenBank accessions MK926663–MK926726), were used to develop three PCR assays for *MAT* idiomorph within the FFSC. The robustness of these assays was initially evaluated by screening a 20-isolate test panel comprising 11 phylogenetically diverse species within the FFSC, including mating type testers for six biological species (designated A–F; Table 1), and *F. miscanthi* and *F. nisikadoi* within the FNSC. The assays were further evaluated by screening a 71-isolate evaluation panel (67 FFSC and 4 FNSC isolates), comprising 56 FFSC and 4 FNSC species (Table 1). This panel was also used to evaluate the robustness of three published PCR assays for *MAT* idiomorph (Kerényi et al. 1999, 2004; Steenkamp et al. 2000; Wallace and Covert 2000). All of the isolates included in this study are available for distribution upon request from the Agricultural Research Service Culture Collection (NRRL; https://nrrl.ncaur.usda.gov/). Isolates are archived in NRRL as lyophilized preparations or stored cryoprotected in 15% glycerol in the vapor phase at 175° C in liquid nitrogen freezers. Total genomic DNA was isolated from yeast-malt cultures using a cetyltrimethylammonium bromide (CTAB; Sigma-Aldrich, St. Louis, Missouri) protocol (Gardes and Bruns 1993) or a ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, California) as previously described (Laraba et al. 2017). Based on prior research that indicated that DNA concentration is an important variable for multiplex PCR success (Steenkamp et al. 2000), genomic DNA was quantified with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts), and then ~10 ng of DNA was included in each PCR reaction.

Table 1. Strain histories and results of multiplex PCR for *MAT* idiomorph targeting *MAT1-1-2* and *MAT1-2-1* in the present study.

Species	Strain #[a]	Design Panel[b]	Test Panel (P/A-F)[c]	Validation Panel[d]	Clade[e]	Origin[f]	*MAT*[g]
F. acutatum	13308	+		+	African	India	2
F. agapanthi	54463	+		+	American	Australia	2
F. andiyazi	31727	+		+	African	Ethiopia	2
F. anthophilum	25214	+		+	American	Germany	1
F. bactridioides	20476	+		+	American	AZ, USA	1

F. begoniae	25300	+		+	American	Germany	1
F. brevicatenulatum	25447	+		+	African	Madagascar	2
F. bulbicola	13618	+		+	American	Germany	1
F. circinatum	25332			+	American	GA, USA	2
F. circinatum	53756			+	American	Spain	2
F. coixii	66233	+		+	African	Australia	2
F. commune	28387	+	P	+	FNSC	USA	2
F. concentricum	25181	+	P	+	Asian	Costa Rica	1
F. denticulatum	25311	+		+	African	LA, USA	2
F. dlaminii	13164	+		+	African	South Africa	1
F. fracticaudium	CBS 137233	+		+	American	Columbia	2
F. fractiflexum	43689	+		+	Asian	Unknown	2
F. fujikuroi	13566	+		+	Asian	Taiwan	2
F. fujikuroi	13998	+		+	Asian	Taiwan	2
F. fujikuroi	22059		C+		Asian	Lab cross	2
F. fujikuroi	22060		C-		Asian	Lab cross	1
F. globosum	25190	+		+	Asian	Japan	1
F. globosum	26131	+		+	Asian	South Africa	2
F. guttiforme	22945	+		+	American	England	2
F. konzum	53919	+		+	American	KS, USA	1
F. lactis	25200			+	African	CA, USA	1
F. lyrante	54252	+		+	FNSC	Australia	1
F. mangifera	25226	+	P	+	Asian	India	2
F. marasasianum	CBS 137237	+		+	American	Columbia	2
F. mexicanum	53147	+	P	+	American	Mexico	1
F. miscanthi	26231	+		+	FNSC	Denmark	1
F. mundagurra	66235	+		+	African	Australia	1
F. musae	25059	+		+	African	Honduras	2
F. napiforme	25196	+		+	African	South Africa	2
F. nisikadoi	25179	+	P	+	FNSC	Japan	2
F. nygamai	66327	+		+	African	USA	1
F. parvisorum	CBS 137236	+		+	American	Columbia	1
F. phyllophilum	13617	+		+	African	Italy	1
F. pininemorale	CBS 137240	+		+	American	Columbia	1
F. proliferatum	22057		D+		Asian	Lab cross	2
F. proliferatum	22058		D-		Asian	Lab cross	1
F. proliferatum	66686	+		+	Asian	Italy	1
F. proliferatum	66687	+		+	Asian	Spain	2
F. pseudoanthophilum	25211	+		+	African	Zimbawe	2
F. pseudocircinatum	36939	+	P	+	African	Unknown	1

F. pseudonygamai	13592	+		+	African	Nigeria	2
F. ramigenum	25208	+		+	African	CA, USA	1
F. sacchari	22043		B+		Asian	Lab cross	2
F. sacchari	22044		B-		Asian	Lab cross	1
F. sacchari	66326	+		+	Asian	USA	2
F. siculi	CBS 142422	+		+	Asian	Italy	2
F. sororula	CBS 137242	+		+	American	Columbia	1
F. sterilihyphosum	25623	+		+	American	South Africa	1
F. subglutinans	20981		E-		American	IL, USA	1
F. subglutinans	22016		E+		American	IL, USA	2
F. subglutinans	66333	+		+	American	NM, USA	1
F. subglutinans	66698			+	American	KS, USA	2
F. temperatum	25622	+	P	+	American	South África	2
F. temperatum	66707			+	American	USA	2
F. thapsinum	22048		F+		African	Lab cross	1
F. thapsinum	22049	+	F-	+	African	Lab cross	2
F. tjaetaba	66243	+		+	African	Australia	1
F. tupiense	53984	+		+	American	Brazil	2
F. udum	22114			+	African	India	2
F. udum	22949	+		+	African	Germany	1
F. udum	25194	+		+	African	India	2
F. verticillioides	20955	+	P	+	African	CA, USA	2
F. verticillioides	20956		A+	+	African	CA, USA	1
F. verticillioides	20984		A-		African	IN, USA	2
F. werrikimbe	54250	+		+	American	Australia	2
F. xylarioides	25486	+		+	African	Ivory Coast	2
Fusarium sp.	25303	+		+	Asian	Japan	1
Fusarium sp.	43304	+		+	African	LA, USA	1
Fusarium sp.	47473	+		+	American	Mexico	1
Fusarium sp.	52700	+		+	FFSC	Unknown	2
Fusarium sp.	62710	+		+	African	Guyana	2
Fusarium sp.	62721	+		+	African	Guyana	1
Fusarium sp.	66330	+		+	African	IL, USA	2
Fusarium sp.	66332	+		+	African	IL, USA	2
Fusarium sp.	66890	+		+	African	Guyana	2
Fusarium sp.	13601	+		+	American	South Africa	1

[a] CBS = Westerdijk Fungal Biodiversity Institute (formerly the CBS-KNAW Fungal Biodiversity Centre), Utrecht, the Netherlands; 5-digit numbers are from ARS Culture Collection (NRRL), ARS-NCAUR, Peoria, IL, USA.

[b] *MAT* loci were mined from the genomes of 64 isolates representing the design panel.

[c] The test panel included 20 phylogenetically divergent members of the FFSC/FNSC, which were screened initially to assess robustness of the PCR assays for *MAT* idiomorph. P = phylogenetically diverse set of 8 species, representing the African, Asian and American clades of the FFSC and its sister group the FNSC. A-F = mating type tester strains for six species within the FFSC.

[d] The 71-isolate validation panel was used to identify the most robust PCR assay for *MAT* idiomorph.
[e] Clades as defined in O'Donnell et al. (2013).
[f] Four pairs of mating type testers were obtained from lab crosses.
[g] Mating type idiomorph: 1 = *MAT1-1*, 2 = *MAT1-2*.

Assessment of published PCR assays for genotyping *MAT* idiomorph in the FFSC. The 71-isolate evaluation panel was used to assess the utility of published PCR primer pairs (Table 2) and protocols for typing *MAT* idiomorph within the FFSC (Kerényi et al. 1999, 2004; Steenkamp et al. 2000; Wallace and Covert 2000). The Steenkamp et al. (2000) protocol was run as uni- and multiplex amplifications, whereas the latter two assays were only run as a uniplex PCR following the published protocols (Supplementary Table 1).

Table 2. Published PCR primer pairs for *MAT* idiomorph in *Fusarium* tested in the present study.

Locus	Gene	Primer pair	Amplicon (bp)	Sequence (5′-3′)[a]	Reference
MAT1-1	*MAT1-1-1*	GFmat1a	~200	GTTCATCAAAGGGCAAGCG	Steenkamp et al. 2000
		GFmat1b		TAAGCGCCCTCTTAACGCCTTC	
MAT1-2	*MAT1-2-1*	Gfmat2c	~800	AGCGTCATTATTCGATCAAG	
		GFmat2d		CTACGTTGAGAGCTGTACAG	
MAT1-1	*MAT1-1-1*	fusALPHAfor	~200	CGCCCTCTKAAYGSCTTCATG	Kerényi et al. 2004
		fusALPHArev		GGARTARACYTTAGCAATYAGGGC	
MAT1-2	*MAT1-2-1*	fusHMGfor	~260	CGACCTCCCAAYGCYTACAT	
		fusHMGrev		TGGGCGGTACTGGTARTCRGG	
MAT1-1	*MAT1-1-3*	MAT1p2	~380	AGAAACTGACTGATACATCAAGGGG	Wallace and Covert 2000
		MAT1p3		TCATAAGAAGTGTTGAAGGAATCACAG	
MAT1-2	*MAT1-2-1*	GcHMG1	~190	CTTTACCGTAAGGAGCGTCACCAT	Covert et al. 1999
		GcHMG2		TGATCCGCCATCTGCTTGTAGAGT	

[a] K = G or T; R = A or G; S = G or C; Y = C or T.

Design and validation of a novel PCR assay for *MAT* idiomorph in the FFSC. The strategy we employed to develop a robust PCR assay for *MAT* idiomorph was to conduct BLASTn bioinformatic searches of the 64-isolate design panel genomes (Kim et al. 2017), using genes that flank the *MAT* locus. These included searches for a DNA lyase (*SLA2*) and an apurinic endonuclease (*APN1*), which flank the *MAT* locus in *Fusarium* (Martin et al. 2011; Hughes et al. 2014) and several other filamentous ascomycetes (Dyer et al. 2016). *SLA2* and *APN1* nucleotide sequences mined from the genome of *F. pseudocircinatum* NRRL 36939 (GenBank MK926663) were used to conduct BLASTn searches of the design panel genomes, using CLC Bio Genomics Workbench (CLC Bio, Qiagen, Aarhus, Denmark). After contigs that contained the *MAT* locus in all 64-design panel isolates were identified, the AUGUSTUS server (http:// augustus.gobics.de) was used to determine whether the idiomorph was *MAT1-1* or *MAT1-2*. Once determined, nucleotide sequences of the three genes at *MAT1-1* (*MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3*) and two at *MAT1-2* (*MAT1-2-1* and *MAT1-2-9*) were aligned using Sequencher 5.4.6 (Gene Codes, Ann Arbor, Michigan), and conserved regions in all of these except *MAT1-2-9* were used to design PCR primer pairs that were conserved across the FFSC and FNSC (Table 3). These four PCR primer pairs were initially tested on a 20-isolate test panel that included (i) a phylogenetically diverse set of eight species (identified by P in Table 1), representing the African (*F. pseudocircinatum* NRRL 36939, *F. verticillioides* NRRL 20955), Asian (*F. concentricum* NRRL 25181, *F. mangifera* NRRL 25226), and American (*F. mexicanum* NRRL 53147, *F. temperatum* NRRL 25622) clades of the FFSC and its sister group, the FNSC (*F. commune* NRRL 28387, *F. nisikadoi* NRRL 25179); and (ii) mating type tester strains for six species within the FFSC (identified by A–F in Table 1). Uniplex PCR reactions were performed in 30 µL volumes, each containing 15 µL of DNA (0.7 ng/µL), 0.6 µL of a deoxynucleoside triphosphate mix (10 mmol/µL; DNTP100; Sigma-Aldrich, St. Louis, Missouri), 1.5 µL of 50 mM MgSO4, 0.75 µL of each *MAT1-1* or *MAT1-2* oligonucleotide pair (10 pmol/µL; see Table 3), 3 µL of 10× High Fidelity buffer (Invitrogen Life Technologies, Carlsbad, California), 0.12 µL of Platinum Taq DNA Polymerase High Fidelity (5 U/µL), and deionized water to 30 µL. The initial denaturation was conducted at 94°C for 90 s, followed by 40 cycles of 94°C for 30 s, 55°C for 40 s, and 68°C for 1 min. A final extension was performed at 68°C for 5 min. Multiplex PCR conditions were the same as in the uniplex, except that reactions contained 0.75 µL of both *MAT1-1* and *MAT1-2* oligonucleotide pairs (10 pmol/µL). Amplicons were sized in 2% agarose gels run in 1× TAE buffer pH 8.0 (40 mM Tris, 20 mM acetic acid, 1 mM ethylenediaminetetraacetic acid [EDTA]), using the EZ load 100 bp molecular weight marker (Bio-Rad, Hercules, California), followed by staining with ethidium bromide and visualization over an ultraviolet (UV) transilluminator (Fotodyne, Hartland, Wisconsin). Sanger sequencing confirmed the identity of the amplicons following published protocols that included BLASTn queries of GenBank (O'Donnell et al. 2015). Although the primary focus of this study was to develop and validate a PCR assay for *MAT* idiomorph within the FFSC, we also tested the *MAT1-1-2* and *MAT1-2-1* primer pairs on a 48-isolate/47- species panel that spanned the phylogenetic breadth of *Fusarium* to assess how broadly they worked within this genus (Supplementary Table 2; O'Donnell et al. 2013).

Table 3. PCR primers designed and tested for multiplex amplification of *MAT* in the FFSC and FNSC in the present study.

Locus	Gene	Amplicon (bp)[a]	Primer pair	Sequence (5′-3′)[b]	Tm (°C)
MAT1-1	*MAT1-1-1*	781	M111f1	TYGACCTGDTCGGTCATGAG	56.2
			M111r1	GGAATGAACGAARCGATTTCC	53.3
MAT1-1	*MAT1-1-2*	465	M112f1	GAAGAAGCTYCTHGTCAGATC	52.2
			M112r1	GCAGTYGACATGAADGGGAGAG	57.4
MAT1-1	*MAT1-1-3*	619	M131f1	GTCCTCTRGGYGTTCTCATG	54.9
			M131r1	CARTAAGCTTGAATCTCRGG	50.7
MAT1-2	*MAT1-2-1*	330	M121f2a	ARYCATTTTCATTCACCGTCC	53.5
			M121r2	GGCGBCGCTCMGAAGGAC	62.1

[a] Amplicon size determined using sequence data.
[b] B = C or G or T; D = A or G or T; H = A or C or T; M = A or C; R = A or G; S = G or C; Y = C or T.

8.1.4. RESULTS

Assessment of published PCR assays for *MAT* idiomorph in the FFSC. The utility of several published PCR assays for determining *MAT* idiomorph within the FFSC was assessed by testing them on a 71- isolate evaluation panel. When used in a uniplex PCR following published protocols, these assays successfully amplified a portion of *MAT1-1* or *MAT1-2* from 46.5% (33/71; Wallace and Covert 2000), 51% (36/71; Steenkamp et al. 2000), and 59% (42/71; Kerényi et al. 2004) of the isolates tested (Supplementary Table 1). The Wallace and Covert (2000) *MAT1-2* primer set outperformed the *MAT1-1* primer set; the failure rate was 90.3% (28/31) for the *MAT1-1* set and 25% (10/40) for the *MAT1-2* set. The reverse was obtained with the Steenkamp et al. (2000) primer sets; the failure rate was 16.1% (5/31) for the *MAT1-1* set and 75% (30/40) for the *MAT1-2* set. By contrast, no difference was observed for the Kerényi et al. (2004) primer sets; the failure rate was 45.2% (14/31) for the *MAT1-1* set and 37.5% (15/40) for the *MAT1-2* set.

Design and validation of a novel PCR assay for *MAT* idiomorph in the FFSC. Bioinformatic searches of the 64-isolate FFSC/FNSC design panel genomes using CLC Bio Genomics Workbench (Table 1) together with those performed using the online tool AUGUSTUS (http://bioinf.uni-greifswald.de/webaugustus/; Stanke et al. 2004) revealed that 30 and 34 of the genomes, respectively, possessed a *MAT1-1* or *MAT1-2* idiomorph. The AUGUSTUS-based analyses further indicated that no *MAT* genes resided outside of the *MAT* locus and that none of the isolates possessed both idiomorphs. All but 10 of the 64 *MAT* loci, flanking *SLA2* and *APN1* genes, were found to reside on a single contig, and when the 2–3 overlapping contigs in the 10 *Fusarium* genomes were assembled using CLC Bio Genomics Workbench, the sequence spanning *SLA2* and *APN1* was determined to be complete in all 64 isolates. The 30 *MAT1-1* and 34 *MAT1-2* idiomorphs ranged in length from 4106 to 4197 bp and 3099 to 3146 bp, respectively. As expected, three *MAT* genes were identified within *MAT1-1* (*MAT1-1-3*, *MAT1-1-2*, and *MAT1-1-1*) and two within *MAT1-2* (*MAT1-2-1* and *MAT1-2-9*). No other open reading frames were identified within the *MAT* idiomorphs.

Alignments of the 30 *MAT1-1* nucleotide sequences were used to design one PCR primer pair within conserved regions of *MAT1-1-3*, *MAT1-1-2* (Fig. 1), and *MAT1-1-1* (Table 3). Similarly, the 34 aligned *MAT1-2* nucleotide sequences were used to identify conserved regions in *MAT1-2-1*, which were used to design a PCR primer pair (Fig. 1; Table 3). When the three multiplex PCR primers were tested on the 20-isolate test panel (Table 1), the multiplex PCR primer pair that targeted *MAT1-1-2* and *MAT1-2-1* (Fig. 1) was selected for subsequent analyses because it successfully genotyped all of the strains tested (data not shown). Sanger sequencing of the 465 bp *MAT1-1-2* and 330 bp *MAT1-2-1* amplicons verified that the PCR primers amplified these genes. Analysis of a range of DNA and annealing temperatures indicated that these parameters were critical variables for success of the PCR assay, as previously reported (Steenkamp et al. 2000). For the assay reported here, the optimal DNA concentration was ~10 ng per 30 µL PCR reaction and the optimal annealing temperature was 55°C. The latter temperature was the only one tested between 51 and 57°C that successfully typed all of the strains in the design panel. Further testing of the *MAT1-1-1* and *MAT1-2-1* primer pairs on the full 71-isolate validation panel revealed that they successfully typed all of the FFSC/FNSC strains for *MAT i*diomorph (Fig. 2). The *MAT1-1-2* (M112f1 × M112r1) and *MAT1-2-1* (M121f2a × M121r2) multiplex PCR assay uniformly yielded amplicons that were 465 or 330 bp in length, respectively. Only one of the two idiomorphs was amplified in all of the isolates except for *Fusarium* sp. NRRL 66332 from Illinois corn where portions of both idiomorphs were amplified (Fig. 2, lane 44 identified by asterisk). However, only the *MAT1-2* idiomorph was detected in the whole genome of *Fusarium* sp. NRRL 66332. To assess whether *Fusarium* sp. NRRL 66332 was heterokaryotic for *MAT*, or the strain was a mixture of two isolates with different mating type idiomorphs, we obtained 20 single-conidium isolates of the strain and subjected them to our multiplex *MAT* assay. The results of this assay revealed that all 20 isolates were *MAT1-2*.

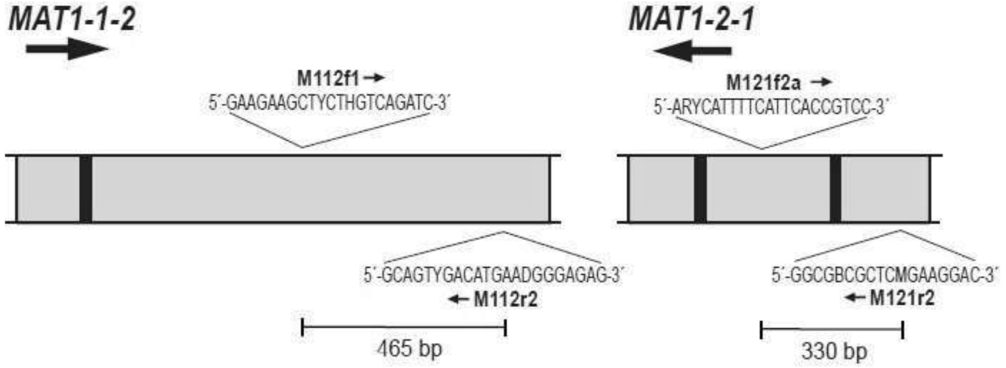

Figure 1. Maps showing location of the forward and reverse *MAT1-1-2* and *MAT1-2-1* multiplex PCR primer pairs for typing *MAT* idiomorph within the *Fusarium fujikuroi* species complex. The *MAT1-1-2* and *MAT1-2-1* amplicons were 465 and 330 bp, respectively, based on w whole genome and Sanger sequencing. The coding region of *MAT1-1-2* and *MAT1-2-1* are interrupted by 1 and 2 introns (identified by black rectangle), respectively. Bold black arrows are used to indicate the direction of *MAT* gene transcription.

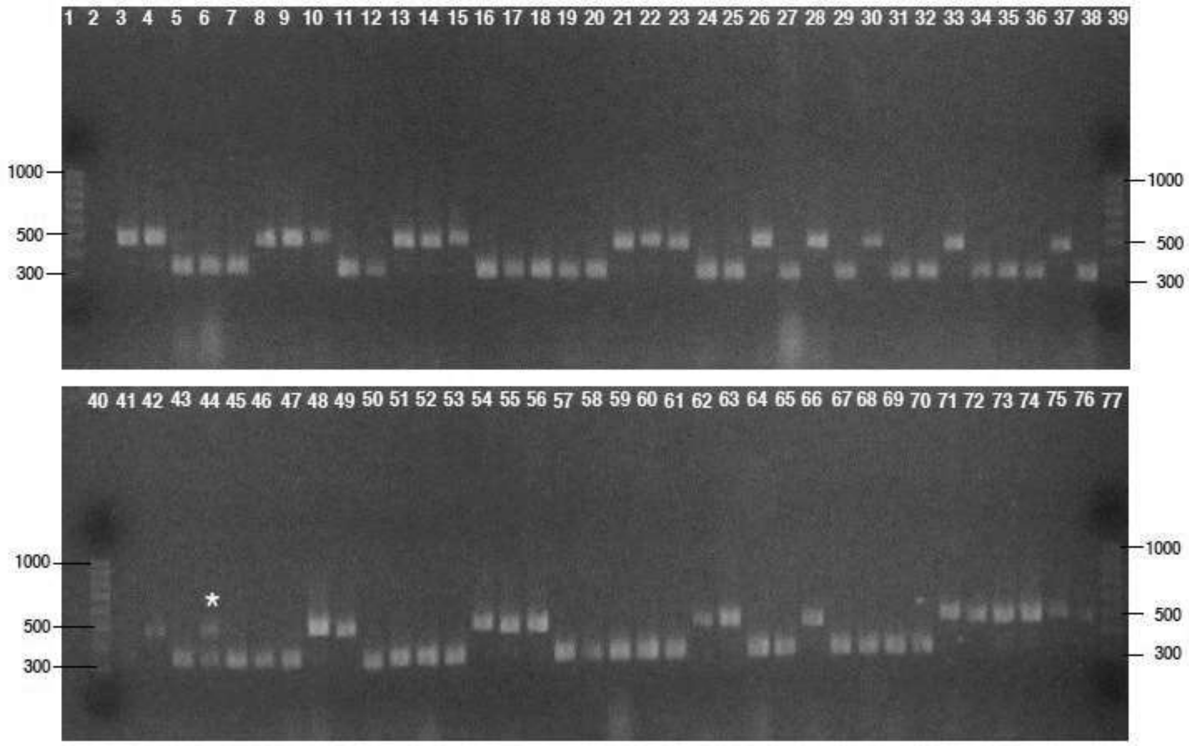

Figure 2. Ethidium bromide–stained agarose gel showing 450 bp *MAT1-1-2* and 330 bp *MAT1-2-1* fragments amplified by multiplex PCR assay for *MAT* idiomorph within the FFSC and FNSC. Lanes 1, 39, 40, and 77 = EZ load 100 bp Molecular Ruler (Bio-Rad); lanes 2 and 41 = no DNA negative controls. Lanes: 3 = *F. parvisorum;* 4 = *F. nygamai;* 5 = *F. nisikadoi;* 6 = *F. napiforme;* 7 = *F. musae;* 8 = *F. mundagurra;* 9 = *F. miscanthi;* 10 = *F. mexicanum;* 11 = *F. marasasianum;* 12 = *F. mangifera;* 13 = *F. lyrante;* 14 = *F. lactis;* 15 = *F. konzum;* 16 = *F. commune;* 17 = *F. guttiforme;* 18 = *F. coixii;* 19 = *F. globosum;* 20 = *F. circinatum;* 21 = *F. globosum;* 22 = *F. bulbicola;* 23 = *Fusarium* sp. NRRL 62721*;* 24 = *F. brevicatenulatum;* 25 = *F. fujikuroi;* 26 = *F. begoniae;* 27 = *F. fujikuroi;* 28 = *F. bactridioides;* 29 = *F. fractiflexum;* 30 = *F. anthophilum;* 31 = *F. fracticaudium;* 32 = *F. andiyazi;* 33 = *F. dlaminii;* 34 = *F. circinatum;* 35 = *F. denticulatum;* 36 = *F. agapanthi;* 37 = *F. concentricum;* 38 = *F. acutatum;* 42 = *Fusarium* sp. NRRL 25303; 43 = *Fusarium* sp. NRRL 66890; 44 = *Fusarium* sp. NRRL 66332; 45 = *Fusarium* sp. NRRL 66330; 46 = *Fusarium* sp. NRRL 62710; 47 = *Fusarium* sp. NRRL 52700; 48 = *Fusarium* sp. NRRL 47473; 49 = *Fusarium* sp. NRRL 43304; 50 = *F. xylarioides;* 51 = *F. werrikimbe;* 52 = *F. subglutinans;* 53 = *F. verticillioides;* 54 = *F. sterilihyphosum;* 55 = *F. udum;* 56 = *F. sororula;* 57 = *F. udum;* 58 = *F. siculi;* 59 = *F. udum;* 60 = *F. sacchari;* 61 = *F. tupiense;* 62 = *F. ramigenum*; 63 = *F. tjaetaba;* 64 = *F. pseudonygamai;* 65 = *F. thapsinum;* 66 = *F. pseudocircinatum;* 67 = *F. temperatum;* 68 = *F. pseudoanthophilum;* 69 = *F. temperatum;* 70 = *F. proliferatum;* 71 = *Fusarium* sp. NRRL 62710; 72 = *F. proliferatum;* 73 = *F. verticillioides;* 74 = *F. pininemorale;* 75 = *F. subglutinans;* 76 = *F. phyllophilum. Fusarium* sp. NRRL 66332 (lane 44, identified by asterisk) was the only strain where both idiomorphs were PCR amplified; however, when 20 single-conidium isolates were typed, all were *MAT1-2*. See Table 1 for a complete listing of NRRL strain numbers and histories.

Thus, results of the *MAT* assay indicate that the FFSC and FNSC analyzed possess a *MAT1-1* or *MAT1-2* idiomorph. Therefore, if members of these species' complexes possess a sexual cycle, it is heterothallic (self-sterile). Lastly, although the primary objective of this study was to design and validate a robust multiplex PCR assay for typing *MAT* within the FFSC, we tested this assay on a 48-isolate/47-species panel that spanned the phylogenetic breadth of the genus to assess how broadly they worked within *Fusarium* (Supplementary Table 2; O'Donnell et al. 2013). Positive genotypes were only obtained for three near relatives of the FFSC/FNSC, and these included *MAT1-1* in *F. oxysporum* NRRL 20433 and NRRL 34936 and *MAT1-2* in *F. foetens* NRRL 38302 and *F. hostae* NRRL 29889.

8.1.5. DISCUSSION

The present study was prompted by our discovery that published PCR assays for identifying *MAT* idiomorph (Kerényi et al. 1999, 2004; Steenkamp et al. 2000; Wallace and Covert 2000) failed to work on some of our FFSC isolates. Using aligned *MAT1-1* and *MAT1-2* idiomorph sequences mined from 64 *Fusarium* genomes, we designed and validated a multiplex PCR assay that successfully typed 100% of the 71-isolate validation panel for *MAT* idiomorph within the FFSC and FNSC. By contrast, the Steenkamp et al. (2000) PCR assay performed poorly when multiplexed, and when the three assays were run as a uniplex, positive genotypes were only obtained for 46.5–59% of the 71- isolate validation panel. Of the 23 genealogically exclusive species complexes currently recognized within the *Fusarium* (Laurence et al. 2011; O'Donnell et al. 2013; Zhou et al. 2018), the FFSC contains the largest number of described phylospecies, together with several that are unnamed. Because approximately one-third of the currently recognized FFSC were discovered during the past 20 years (http://www.mycobank.org/MB/), knowledge of this novel genetic diversity was not available at the time the published *MAT* PCR assays we evaluated were designed and tested.

In contrast to prior analyses of *MAT* structure and evolution in *Fusarium* that employed labor-intensive PCR amplifications and Sanger sequencing via primer walking (Yun et al. 2000; O'Donnell et al. 2004; Martin et al. 2011; Hughes et al. 2014), our bioinformatics approach targeted the *SLA2* and *APN1* genes that flank the *MAT* locus in *Fusarium* and several other filamentous ascomycetes (Martin et al. 2011; Dyer et al. 2016). As reported in prior studies (Laraba et al. 2017; Jacobs-

Venter et al. 2018; Zhouet al. 2018), our BLASTn searches of *Fusarium* genomes (Kim et al. 2017), using *SLA2* and *APN1* nucleotide sequences of *F. pseudocircinatum* as the queries, easily identified contigs on which the *MAT* locus was located. Analyses of the FFSC idiomorph nucleotide sequences conducted with the ab initio prediction tool AUGUSTUS (Stanke et al. 2004) revealed that the complement of *MAT* gene orthologs within the 30 *MAT1-1* and 34 *MAT1-2* idiomorphs was identical to that reported for other heterothallic fusaria (Martin et al. 2011; Hughes et al. 2014). Our results, and those of Hughes et al. (2014), suggest that the recently discovered *MAT1-2-9* gene may be a conserved feature of the *MAT1-2* idiomorph within *Fusarium*. However, whether *MAT1-2-9* is essential for sexual reproduction within the FFSC and other heterothallic fusaria remains to be determined because *MAT1-2-9* null strains of *Fusarium graminearum* can successfully complete a sexual cycle (Kim et al. 2012, reported as *MAT1-2-3*). Although only one-fifth of the FFSC have been shown to reproduce sexually, our findings suggest that they all may possess a heterothallic sexual cycle. This assumption is based on the discovery that all of the FFSC isolates analyzed to date possess intact *MAT1-1* or *MAT1-2* idiomorph, coupled with the fact that in silico translations of the five *MAT* genes suggests that most if not all may be functional (Martin et al. 2011).

The ability to type fusaria for mating type, as reported in prior studies, made it possible to (i) replace the arbitrary +/− terminology previously used for mating type testers with the precise *MAT* idiomorph (Arie et al. 1999; Kerényi et al. 1999), (ii) only set laboratory crosses between *MAT1-1* and *MAT1-2* isolates (e.g., Scauflaire et al. 2011), (iii) demonstrate by screening ascospore progeny that sexual reproduction had occurred (Covert et al. 2007; Zhou et al. 2018), and (iv) determine whether putatively heterothallic fusaria are segregating for *MAT* at various geographic scales (Laraba et al. 2017; Jacobs-Venter et al. 2018). Determining whether a pathogen possesses the ability to reproduce sexually informs plant disease management because recombining pathogens pose a much greater risk for overcoming control methods based on fungicides and host resistance (McDonald and Linde 2002).

Results of the *MAT* assay indicate that the FFSC and FNSC analyzed possess a *MAT1-1* or *MAT1-2* idiomorph. Therefore, if members of these species' complexes possess a sexual cycle, it is heterothallic (self-sterile). Lastly, although the primary objective of this study was to design and validate a robust multiplex PCR assay for typing *MAT* within the FFSC, we tested this assay on a 48-isolate/47-species panel that spanned the phylogenetic breadth of the genus (Supplementary Table 2; O'Donnell et al. 2013). Positive genotypes were only obtained for three near relatives of the FFSC/FNSC, and these included *MAT1-1* in *F. oxysporum* NRRL 20433 and NRRL 34936 and *MAT1-2* in *F. foetens* NRRL 38302 and *F. hostae* NRRL 29889. Similarly, although Steenkamp et al. (2000) reported that their *MAT* assay successfully typed the 23 FFSC species tested, and two *MAT1-1* isolates of *F. oxysporum,* it did not work on members of the *F. tricinctum* and *F. sambucinum* species complexes. The *MAT1-1* and *MAT1-2* uniplex PCR assays developed by Kerényi et al. (2004) worked, respectively, on some isolates in three or four of the species complexes outside the FFSC. However, our results show that they cannot be used to type all fusaria for *MAT*, including over 20 species in the FFSC. Therefore, future research employing the same bioinformatics approach as reported here will focus on developing universal PCR primer pairs for typing *MAT* idiomorph across the phylogenetic breadth of *Fusarium*.

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Supplementary Table 1. PCR results obtained by screening 71-isolate validation panel using published PCR assays for determining *MAT* idiomorph in *Fusarium* (Kerényi et al. 2004; Steenkamp et al. 2000; Wallace and Covert 2000).

Species	Strain #[a]	Wallace and Covert 2000[b]	Kerényi et al. 2004[b]	Steenkamp et al. 2000[b]	Steenkamp et al. 2000[c]
F. acutatum	13308	2	2	2	2
F. agapanthi	54463	2	-	-	-
F. andiyazi	31727	-	2	-	-
F. anthophilum	25214	-	-	1	1
F. bactridioides	20476	-	-	1	-
F. begoniae	25300	-	1	1	-
F. brevicatenulatum	25447	2	-	2	-
F. bulbicola	13618	-	1	1	-
F. circinatum	25332	2	-	-	-
F. circinatum	53756	2	2	-	-
F. coixii	66233	2	-	-	-
F. commune	28387	-	2	-	-
F. concentricum	25181	-	-	1	-
F. denticulatum	25311	-	-	2	-
F. dlaminii	13164	-	1	1	1
F. fracticaudium	CBS 137233	-	-	-	-

F. fractiflexum	43689	-	2	-	-
F. fujikuroi	13566	2	2	-	-
F. fujikuroi	13998	2	2	-	-
F. globosum	25190	-	1	1	1
F. globosum	26131	-	2	-	-
F. guttiforme	22945	2	2	2	-
F. konzum	53919	-	1	1	1
F. lactis	25200	-	-	1	1
F. lyrante	54252	-	-	-	-
F. mangifera	25226	2	2	-	-
F. marasasianum	CBS 137237	2	2	-	-
F. mexicanum	53147	-	1	1	-
F. miscanthi	26231	-	1	1	-
F. mundagurra	66235	-	-	-	-
F. musae	25059	2	-	-	-
F. napiforme	25196	2	2	2	-
F. nisikadoi	25179	-	-	2	-
F. nygamai	66327	-	-	1	1
F. parvisorum	CBS 137236	-	1	1	1
F. phyllophilum	13617	-	-	1	1
F. pininemorale	CBS 137240	-	-	1	1
F. proliferatum	66686	-	1	1	1
F. proliferatum	66687	2	-	-	-
F. pseudoanthophilum	25211	2	-	2	-
F. pseudocircinatum	36939	-	-	-	-
F. pseudonygamai	13592	2	2	2	-
F. ramigenum	25208	1	1	1	-
F. sacchari	66326	2	2	2	-
F. siculi	CBS 142422	2	2	-	-
F. sororula	CBS 137242	-	-	1	1
F. sterilihyphosum	25623	1	1	1	-
F. subglutinans	66333	-	1	1	-
F. subglutinans	66698	-	2	2	2
F. temperatum	25622	2	-	-	-

F. temperatum	66707	2	2	-	-
F. thapsinum	22049	2	2	-	-
F. tjaetaba	66243	-	-	-	-
F. tupiense	53984	2	2	-	-
F. udum	22114	-	-	-	-
F. udum	22949	-	1	1	-
F. udum	25194	2	2	-	-
F. verticillioides	20955	2	2	-	-
F. verticillioides	20956	-	1	-	-
F. werrikimbe	54250	2	2	-	-
F. xylarioides	25486	2	2	-	-
Fusarium sp.	25303	-	1	1	1
Fusarium sp.	43304	-	-	1	1
Fusarium sp.	47473	-	-	1	-
Fusarium sp.	52700	2	2	-	-
Fusarium sp.	62710	-	-	-	-
Fusarium sp.	62721	-	1	1	1
Fusarium sp.	66330	2	2	-	-
Fusarium sp.	66332	2	-	-	-
Fusarium sp.	66890	2	-	-	-
Fusarium sp.	13601	1	1	1	1

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[a] CBS = Westerdijk Fungal Biodiversity Institute (formerly the CBS-KNAW Fungal Biodiversity Centre), Utrecht, the Netherlands; 5-digit numbers are from ARS Culture Collection (NRRL), ARS-NCAUR, Peoria, IL, USA.

[b] Uniplex PCR assay.

[c] Multiplex PCR assay.

Supplementary Table 2. PCR results obtained from screening 48-isolate/47 species panel using the multiplex PCR assay for *MAT* idiomorph developed in the present study.

Complex[a]	**Species**[b]	**NRRL #**[c]	**Origin**	***MAT***[d]
albidum	*F. albidum*	22152	Jamaica	–
babinda	*F. babinda*	25539	Australia	–
buharicum	*F. buharicum*	13371	Iran	–
buharicum	*F. sublunatum*	13384	Costa Rica	–
burgessii	*F. beomiforme*	25174	New Caledonia	–
buxicola	*F. buxicola*	36148	Belgium	–
buxicola	*F. cyanostomum*	54603	France	–

chlamydosporum	*F. nelsonii*	13338	Australia	–
chlamydosporum	*Fusarium* sp. (FCSC 2-a)	13444	Australia	–
concolor	*F. concolor*	13459	South Africa	–
concolor	*F. anguioides*	25385	China	–
decemcellulare	*F. decemcellulare*	13412	Dominican Republic	–
decemcellulare	*F. albosuccineum*	20459	Venezuela	–
dimerum	*F. nectrioides*	20689	Honduras	–
dimerum	*F. dimerum*	20691	Romania	–
fujikuroi	*F. pseudocircinatum*	36939	Unknown	1
fujikuroi	*F. verticillioides*	20955	CA, USA	2
heterosporum	*F. graminum*	20692	Ethiopia	–
heterosporum	*F. heterosporum*	20693	Netherlands	–
incarnatum-equiseti	*F. sciripi*	13402	Australia	–
incarnatum-equiseti	*F. equiseti*	20697	Chile	–
lateritium	*F. lateritium*	13622	LA, USA	–
lateritium	*F. stilbioides*	20429	Nyasaland	–
Neonectria	*Neonectria candidum*	20485	Germany	–
Neonectria	*Neonectria neomacrospora*	22505	Canada	–
NONE	*F. verrucosum*	22566	Venezuela	–
NONE	*F. setosum*	36526	French Guiana	–
NONE	*F. foetens*	38302	Chile	2
oxysporum	*F. oxysporum*	20433	Germany	1
oxysporum	*F. oxysporum*	34936	Spain	1
redolens	*F. redolens*	22901	Canada	–
redolens	*F. hostae*	29889	SC, USA	2
sambucinum	*F. sporotrichioides*	3299	USA	–
sambucinum	*F. longipes*-1	13368	Australia	–
sambucinum	*F. sambucinum*	22187	England	–
sambucinum	*F. pseudograminearum*	28062	Australia	–
sambucinum	*F. graminearum*	31084	MI, USA	–
solani	*F. neocosmosporiellum*	22436	South Africa	–
solani	*F. virguliforme*	31041	IL, USA	–
solani	*F. falciforme*	43529	FL, USA	–
solani	*F. solani* f. sp. *pisi*	45880	USA	–
staphyleae	*F. staphyleae*	22316	NJ, USA	–
staphyleae	*F. zealandicum*	22465	New Zealand	–
torreyae	*F. torreyae*	54149	FL, USA	–
tricinctum	*F. torulosum*	22748	Netherlands	–
tricinctum	*F. tricinctum*	26892	Finland	–

ventricosum	*F. ventricosum*-1	13953	Germany	–
ventricosum	*F. ventricosum*-3	20846	Germany	–

[a] Clades as defined in O'Donnell et al. (2013). *Neonectria* is a near relative of *Fusarium*.

[b] Multilocus haplotype of a phylospecies in the *Fusarium chlamydosporum* species complex (FCSC) is indicated in parentheses; *F. longipes*-1 and *F. ventricosum*-1 and -3 are used to distinguish phylogenetically distinct species.

[c] NRRL = Agricultural Research Service Culture Collection, NCAUR, ARS-USDA, Peoria, IL, USA.

[d] Mating type idiomorph: 1 = *MAT*1-1, 2 = *MAT*1-2, –= no amplicon.

8.2. CAPITULO II: Novel malformation disease of rosy trumpet (*Tabebuia rosea*) and cooperwood (*Bursera ovalifolia*) trees caused by *Fusarium pseudocircinatum* in Mexico.

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8.2.1. ABSTRACT

Cooperwood and rosy trumpet are neotropical tree species that are important components of the vegetation in the dry subtropical areas in Mexico. Surveys conducted in the Balsas River Lower Basin region in the states of Michoacán and Guerrero, detected cooperwood, rosy trumpet tree, big-leaf mahogany and mango trees with malformation symptoms at 23 sites. All the diseased trees sampled in this study were detected in public urban gardens, backyard home gardens and suburban areas alongside highways. The aim of this work was to describe these previously unrecognized diseases on cooperwood and rosy trumpet trees and identify their causal agents. A total of 76 fusaria were recovered from the malformed tissues sampled, of which 64 isolates were identified as *Fusarium pseudocircinatum* and 12 isolates as *F. mexicanum*. Koch's postulates on rosy trumpet tree seedlings showed that *F. mexicanum* and *F. pseudocircinatum*, pathogens that cause malformation on big-leaf mahogany and mango, also induce malformation on rosy trumpet. This is the first report of rosy trumpet malformation disease caused by *F. pseudocircinatum* in Mexico. A *F. pseudocircinatum* isolate recovered from malformed tissues of cooperwood trees, was able to induce malformation symptoms on inoculated big-leaf mahogany and rosy trumpet seedlings.

8.2.2. INTRODUCTION

Santillán-Mendoza et al. (2018) recently described a mango malformation like disease (MMD) on big-leaf mahogany (BLMMD, *Swietenia macrophylla* King) caused by *F. pseudocircinatum* O'Donnell & Nirenberg and *F. mexicanum* T. Aoki, S. Freeman, Otero-Colina, Rodríguez-Alvarado, Fernández- Pavía, Ploetz & O'Donnell in Mexico. Koch's postulates confirmed that these two pathogens induced vegetative malformation symptoms on big-leaf mahogany seedlings similar to those observed in the field. Symptoms on affected buds included an increase in size, development of numerous, clustered small ramifications with shortened internodes and smaller than normal leaves (Santillán-Mendoza et al. 2018). In addition, *F. pseudocircinatum* has been reported to cause MMD in Mexico (Freeman et al. 2014) and the Dominican Republic (García-López et al. 2016).

In addition of the detection floral and vegetative malformation symptoms on big-leaf mahogany and mango trees (Otero-Colina et al. 2010; Santillán-Mendoza et al. 2018), during our 2010, 2011, 2015, 2016 and 2018 surveys of neotropical trees conducted in the Balsas River Lower Basin region, which included areas of the state of Michoacán and Guerrero, we detected similar symptoms on two economically important neotropical trees, *Bursera ovalifolia* (Schltdl.) Engl. (cooperwood) and *Tabebuia rosea* (Bertol.) DC. (rosy trumpet tree). The resin of cooperwood is utilized by indigenous people of tropical America in ceremonial events as incense, and as glue, while the soft and light wood is used extensively for crafts and toys (Vázquez-Yanes et al. 1999a). Wood of rosy trumpet tree is used for a diversity of purposes from furniture to crafts and packaging. It is also commonly utilized as an ornamental and shade tree in parks, gardens and for property boundaries (Vázquez-Yanes et al. 1999b).

Thus, given this background, the objectives of the current study were to (i) isolate the putative etiological agents from floral and vegetative malformation tissues of *B. ovalifolia* and *T. rosea* and characterize them using PCR-based molecular markers and multilocus molecular phylogenetic data, (ii) assess the ability of the isolates to produce mycotoxins and other biologically active secondary metabolites *in vitro*, (iii) predict sexual reproductive mode by screening isolates for mating type idiomorph, and (iv) complete Koch's postulates on *T. rosea, M. indica* and *S. macrophylla*.

8.2.3. MATERIALS AND METHODS

Sampling sites. Surveys were conducted in the Lower Balsas River Basin in the states of Guerrero and Michoacán in order to assess whether economically important tropical trees (big-leaf mahogany, cooperwood and rosy trumpet tree) exhibited malformation symptoms. Seven municipalities of the state of Michoacán (i.e., Apatzingán, Gabriel Zamora, Huetamo, La Huacana, Nuevo Urecho, San Lucas, and Tiquicheo) and two in Guerrero (i.e., Coyuca de Catalán and Pungarabato were surveyed during 2010, 2011, 2015, 2016 and 2018 (Supplementary Table S3, Fig. 3).

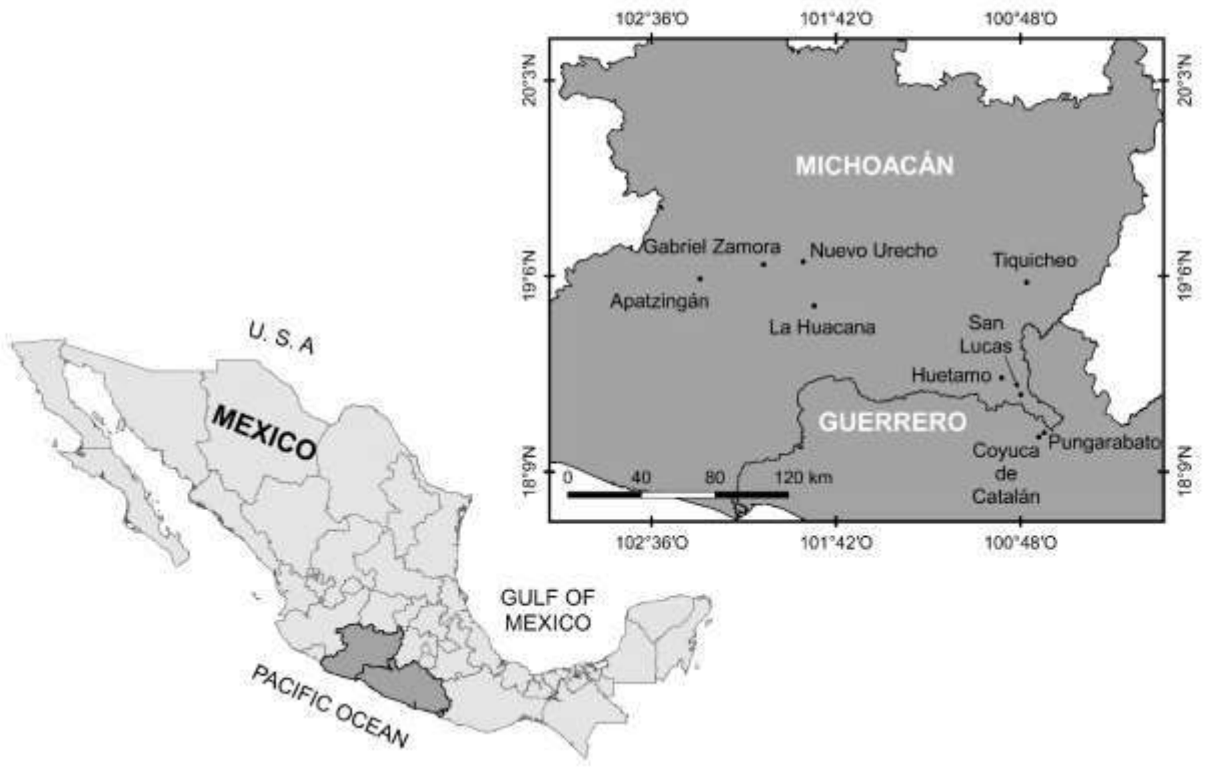

Figure 3. Map of Mexico showing seven municipalities in the state of Michoacán and two in Guerrero where malformation samples were collected from neotropical trees and mango.

Fungal isolation. Malformations were cut from the trees using garden scissors sterilized with 70% alcohol. One to four malformations per tree were collected

(Supplementary Table S1). The samples were kept in labeled plastic bags in an ice chest and transported to the lab for processing immediately or stored at 4°C for no more than 5 days before processing. The protocol used to isolate fusaria from the malformed tissues was described by Santillán-Mendoza et al. (2018) with the following modification. Isolates were purified by transferring a hyphal tip taken directly from the colonies emerging from the tissue explants to synthetic low nutrient agar (SNA, Nirenberg and O'Donnell 1998). The purified isolates were cultured alternating 12 h periods of cool-white fluorescent light and black light at 25°C for 7 to 10 days to induce sporulation. The isolates were stored at −80°C in 15% glycerol and deposited in the Agricultural Research Service Culture (NRRL), Peoria, IL, where they are available upon request (https://nrrl.ncaur.usda.gov/).

DNA extraction and genotyping of *Fusarium* isolates. *Fusarium* isolates were incubated at 25°C for 10 days on Petri plates containing PDA covered with a cellophane disk. Genomic DNA was extracted from the mycelial mats that colonized the cellophane discs following a CTAB protocol previously described (Leslie and Summerell 2006) with slight modifications. Ground mycelium was extracted with 500 μl of CTAB buffer that contained 10 μl of β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), 10 μl of 1% polyvinylpyrrolidone (Sigma-Aldrich), and incubated at 65°C for 90 min. DNA pellets were dried in a speed-vacuum concentrator (Concentrator 5301, Eppendorf, Hauppauge, NY), suspended in 80 μl of TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA), and then stored at -20°C. DNA concentration was determined in a spectrophotometer (NanoDrop 2000c, Thermo Scientific, Waltham, MA), and diluted to obtain a concentration of 30 ng/μl.

Fusarium isolates were initially genotyped by PCR amplification of internal simple sequence repeats (ISSR), following a protocol that utilized four oligonucleotides as previously described (Liu et al. 2014): 891 [HVH$(TG)_7$], 809 [$(AG)_8G$], 812 [$(GA)_8A$] and 835 [$(AG)_8YC$]. Banding patterns were compared to those of fusaria that cause mango malformation: *F. mangiferae* (CML409), *F. sterilihyphosum* (CML280) and *F. tupiense* (CML345) (Coleção Micológica de Lavras [CML]), and *F. mexicanum* (MXMIC-698, MICMW-32.13a) (Soto-Plancarte et al.

2015; Santillán-Mendoza et al. 2018). Strains of *F. pseudocircinatum* (MICMW-26.6, MICMW-30.2) previously described as causal agents of malformation of big-leaf mahogany trees (Santillán-Mendoza et al. 2018) were also included in the analysis. PCR reactions were carried out in an Eppendorf Mastercycler Gradient (Eppendorf, Hauppauge, NY), where each reaction contained 6.3 µl of GoTaq Hot Start (Promega, Madison, WI), 6.25 pmol of one ISSR oligonucleotide, 1 µl of genomic DNA (30 ng/µl) and 3.7 µl of molecular biology grade water. PCR conditions included an initial denaturation step at 95°C for 2 min, followed by 35 cycles of 95°C for 1 min, 48-53ºC (depending on the oligonucleotide used) for 1 min, 72°C for 2 min, a final extension at 72°C for 5 min, followed by a 4°C soak. The amplified DNA products were electrophoresed in 1.5% agarose gels supplemented with ethidium bromide (0.5 µg/ml) and visualized over a UV transilluminator (High Performance UV Transilluminator UVP, Lab-Tech, Guadalajara, MX).

Phylogenetic analysis of *Fusarium* isolates*.* Genomic DNA was isolated from yeast-malt cultures using a hexadecyl trimethyl-ammonium bromide (CTAB, Sigma-Aldrich, St. Louis, MO) protocol (Gardes and Bruns 1993). Portions of translation elongation factor 1-α (*TEF1*), RNA polymerase largest (*RPB1*) and second largest subunits (*RPB2*) and β-tubulin (*β-tub*) were PCR amplified and sequenced as previously described (O'Donnell and Cigelnik 1997; O'Donnell et al. 2010). Amplicons were sequenced with BigDye 3.1 (Applied Biosystems, Foster City, CA), purified with BigDye Xterminator, and run on an ABI 3730 genetic analyzer. Sequence chromatograms were edited with Sequencher 5.2.4 (Gene Codes Corp., Ann Arbor, MI), exported as NEXUS files, and aligned with MUSCLE in SeaView ver. 4.3.0 (Edgar 2004; Gouy et al. 2010) prior to conducting partitioned maximum likelihood (ML) phylogenetic analyses with IQ-TREE 1.6.10 (Nguyen et al. 2015 http://www.iqtree.org/). ModelFinder (Kalyaanamoorthy et al. 2017) was used to identify the best-fit models of molecular evolution for each partition based on the Bayesian information criterion scores (Chernomor et al. 2016). DNA sequences were deposited in the National Center for Biotechnology Information GenBank under accession numbers MN242841-MN242905, MN242947-MN242981, MN724928-MN724931 and MN724938-MN724980.

Secondary metabolite production. To assess their ability to produce biologically active secondary metabolites *in vitro*, 30 *Fusarium* isolates were inoculated onto solid cracked corn kernel cultures (50 g cracked corn + 22 mL water; Aoki et al. 2015). The cultures were incubated for 28 d in the dark, at which time 10 g of culture material was extracted with 50 mL acetonitrile-water, 86:14 (v/v). The filtered extracts were analyzed by high-performance liquid chromatography–mass spectrometry (LC-MS), using a Dionex model U3000 liquid chromatography system (Thermo Scientific, Waltham, Massachusetts) and a QTRAP 3200 mass spectrometer (AB SCIEX, Concord, Ontario, Canada). The LC-MS analysis was conducted with the full scan in positive ionization mode to observe a wide range of metabolites and in negative-mode ionization specifically for the detection of the mycotoxin moniliformin. Positive-mode tandem mass spectrometry was used to assay for production of fumonisin FB1, beauvericin, bikaverin, aurofusarin, methylbostrycoidin and fusaric acid (Busman 2017; Busman et al. 2012; O'Donnell et al. 2018).

Mating type determination. Screening for mating type idiomorph was accomplished by using two diagnostic primer pairs: M112f1 × M112r1 for *MAT1-1* and M121f2a × M121r2 for *MAT1-2* following a published multiplex PCR protocol (Montoya-Martínez et al. 2019). Amplicons were separated in 2% agarose gels run in 1X TAE buffer pH 8.0 (40 mM Tris, 20 mM acetic acid, 1 mM EDTA), sized using an EZ load 100 bp molecular weight marker (Bio-Rad, Hercules, CA), and then visualized over a UV trans-illuminator (Fotodyne Inc.; Hartland, WI) after staining with ethidium bromide.

Pathogenicity tests. Mature fruits of rosy trumpet tree were collected from trees in Coyuca de Catalán, Gro. Once the seeds were extracted from the fruits and the hyaline wing was removed, the seeds were stored at 25°C. Immature fruits from 'Criollo' mango trees were obtained in Gabriel Zamora, Mich. After the pericarp was removed, the seeds were stored at 4°C for 24 to 48 h before the pathogenicity test was initiated. Big-leaf mahogany seeds collected from Nuevo Urecho and Gabriel Zamora, Mich. were processed as described by Santillán-Mendoza et al. (2018). Big-leaf mahogany, mango and rosy trumpet tree seeds were incubated separately in plastic containers with sterile distilled water-soaked paper towels under an alternating cycle of 8 h cool-white fluorescent light and 16 h darkness at 25°C. Most seeds of rosy trumpet tree germinated in 7 days. After two weeks, seedlings with fully developed cotyledon leaves were used in the pathogenicity tests. Mango seeds germinated after 10 to 14 days. Twenty-four days old mango seedlings were selected for the pathogenicity tests. Big-leaf mahogany seeds germinated after 7 to 10 days. Twenty-three day old big-leaf mahogany seedlings were used for the pathogenicity tests.

Fusarium isolates obtained from different hosts in this study (Table 5) and *F. mexicanum* strain MXMIC-698 (Soto-Plancarte et al. 2015), previously characterized as pathogenic to big-leaf mahogany seedlings (Santillán-Mendoza et al. 2018), were cultivated on SNA at 25°C under cool-white and black fluorescent lights with alternating 12 h periods of light and darkness for 10 days. Following a procedure previously described (Santillán-Mendoza et al. 2018), rosy trumpet tree, mango and big-leaf mahogany seedlings were submerged separately in a conidial suspension of each isolate for 18 h at 25°C. The concentration of the conidial suspensions was 2.6 x 10^6 conidia per ml. Control plants were immersed in sterile distilled water.

Inoculated seedlings were individually transferred into 200 ml pots filled with peat moss (Sunshine Mix 3, Sun Gro Horticulture Canada Ltd., Agawam, MA) previously sterilized at 120°C for 2 h and kept at 25°C under cool-white fluorescent light (12 h light/12 h darkness) for seven days before being moved to a greenhouse with natural lighting. Temperatures in the greenhouse during the experiments ranged from 12°C to 35°C. No fertilization or fumigation were applied. The big-leaf mahogany, rosy trumpet tree and mango pathogenicity experiments lasted 2, 6 and 7 months, respectively. *Fusarium* isolates recovered from the inoculated plants were identified by sequencing a portion of the *TEF1* gene. The experiment was conducted twice.

8.2.4. RESULTS

Field observations of malformation symptoms on big-leaf mahogany, cooperwood and rosy trumpet tree. Based on surveys conducted in the Lower Balsas River Basin in the states of Guerrero and Michoacán, we sampled cooperwood trees (N = 6), rosy trumpet trees (N = 5) and an unidentified tree in the Meliaceae (i.e., mahogany family) with floral and vegetative malformation symptoms. Additionally, big-leaf mahogany trees (N = 13) with vegetative malformation symptoms were sampled to determine if the pathogens, previously reported by Santillan-Mendoza et al. (2018), were associated with the disease in the geographic area surveyed in this work.

Four mango trees with MMD were located alongside big-leaf mahogany trees with BLMMD, in three different sites. Those mango trees were sampled to compare the identity of its associated pathogens, with those present on adjacent big-leaf mahogany trees. Samples were obtained from a total of 23 sites (Fig. 3) in seven municipalities of the state of Michoacán (Apatzingán, Gabriel Zamora, Huetamo, La Huacana, Nuevo Urecho, San Lucas, and Tiquicheo) and in two municipalities of Guerrero (Coyuca de Catalán and Pungarabato). Diseased trees were detected in public urban gardens, in backyard home gardens and in suburban areas alongside highway roads where they were planted as ornamentals and for shade. An exception was the unknown tree in the Meliaceae, which was detected in a natural area few meters away from a commercial mango orchard with 100% incidence of MMD.

Big-leaf mahogany symptoms were as described previously (Santillán-Mendoza et al. 2018). Briefly, symptomatology included enlarged malformed apical vegetative and axillary shoots, the malformed axillary shoots developed into a mass of tissue from which numerous small ramifications emerged with shortened internodes and deformed dwarfed leaves (Fig. 4). Cooperwood trees exhibited malformed apical and axillary floral and vegetative buds, the malformed panicles presented shortened internodes and compacted multiple unopened flowers, necrosis was observed on the stems and flowers of some of the affected panicles. Malformed vegetative shoots also presented necrosis on the external primordia leaves, that subsequently spread to all deformed leaves. Occasionally, healthy flowers and leaves were observed next to malformed tissue. Typically, malformations were initially green but later turned brown as they dried up prior to falling off from the branch leaving a scar (Fig. 4). Floral malformations in rosy trumpet trees involved terminal inflorescences. Malformed panicles produced multiple shoots that were tightly curled with shortened internodes, giving the deformed flowers a cauliflower appearance. A few healthy flowers and leaves, however, were observed among the malformed flower buds. Malformations remained green for a long period before finally drying up, but these remained on the branches (Fig. 4).

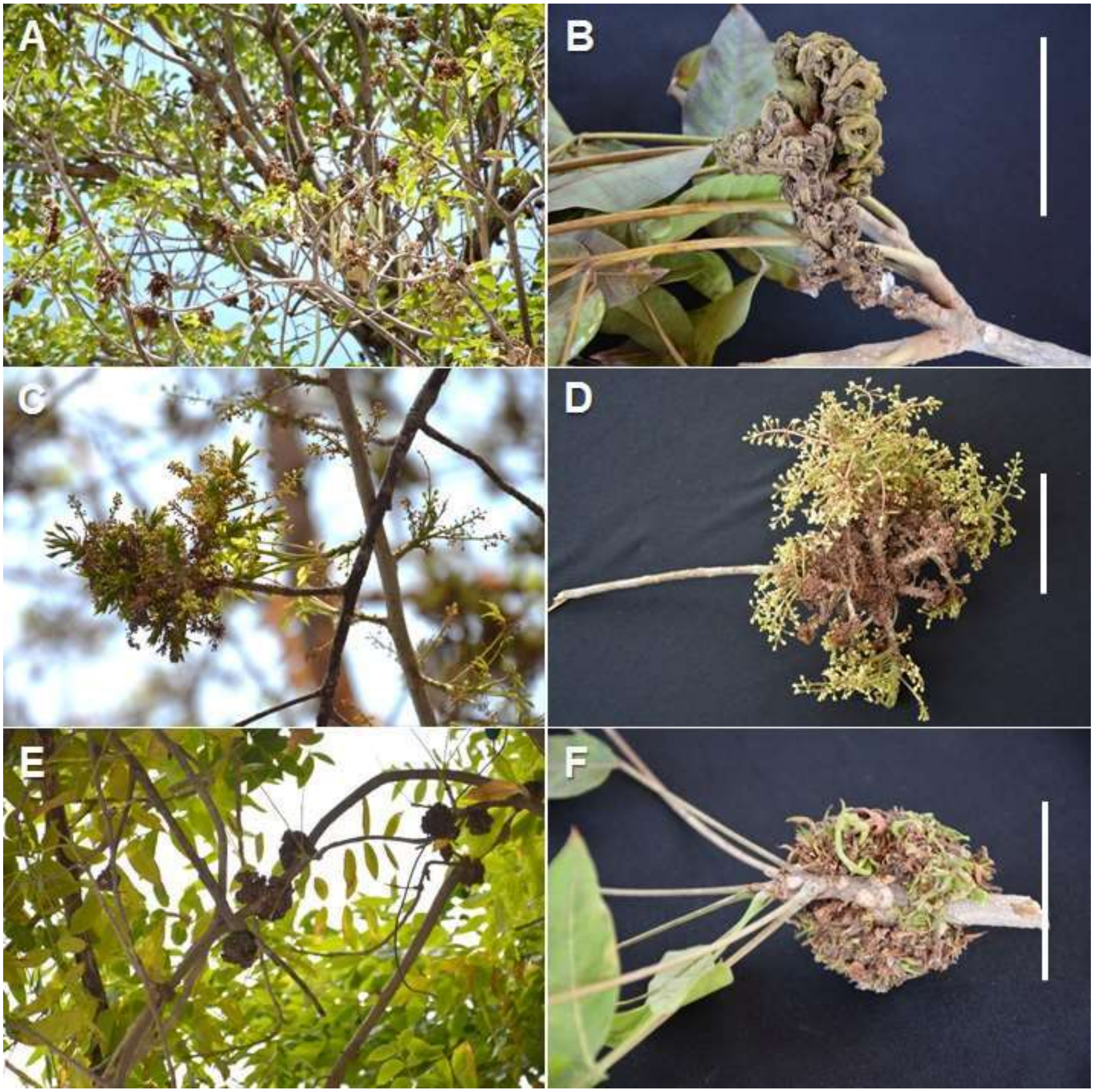

Figure 4. Field symptoms of malformation on neotropical trees. A, B) *Tabebuia rosea*, C, D) *Bursera ovalifolia* and E, F) *Swietenia macrophylla*. Bar = 5 cm.

Genotyping of *Fusarium* isolates. A total of 70 *Fusarium* isolates were obtained from four neotropical trees with malformation symptoms: big-leaf mahogany (N = 26), rosy trumpet tree (N = 20), cooperwood (N = 17) and an unidentified tree in the Meliaceae (N = 7). For comparison, six additional isolates were obtained from four mango trees with MMD in Michoacán and Guerrero (Supplementary Table S3). ISSR genotyping of the 76 isolates yielded one to 11 fragments (Supplementary Fig. S1) that ranged from 300 to 3000 bp in length. Oligonucleotide 809 generated a total of 11 genotypes with 2 to 9 bands for each genotype, oligonucleotide 812 produced 5 genotypes with 1 to 4 bands, oligonucleotide 835 generated 8 genotypes with 4 to 8 bands, and 891 oligonucleotides produced 10 genotypes with 3 to 11 bands. Based on the results reported by Santillán-Mendoza et al. (2018), 64 isolates obtained from neotropical trees with malformation symptoms were identified as *F. pseudocircinatum*, whereas the six isolates recovered from mango trees and six isolates recovered from big-leaf mahogany were identified as *F. mexicanum* (Supplementary Table S3).

Phylogenetic analysis of *Fusarium* isolates. BLASTn searches of *Fusarium* MLST database (http://www.westerdijkinstitute.nl/fusarium/), using the partial *TEF1* sequences as the queries, confirmed the ISSR identifications that 64 and 12 of the isolates, respectively, were *F. pseudocircinatum* and *F. mexicanum*. In addition, partitioned ML analyses of a 4-locus dataset conducted with IQ-tree (Nguyen et al. 2015) that included portions of *TEF1* (622 bp, 44 informative, K2P model), *β-tub* (1281 bp, 61 informative, TN+F model), *RPB1* (1651 bp, 88 informative, TNe+I model), and *RPB2* (1766 bp, 98 informative, TNe+I model) for 25 and 5 of the *F. pseudocircinatum* and *F. mexicanum* isolates, respectively, collected in the present study together with 17 previously characterized isolates of these species, including the ex-type strains *F. pseudocircinatum* NRRL 22946 and *F. mexicanum* NRRL 53147, confirmed the ISSR and BLASTn identifications (Fig. 5).

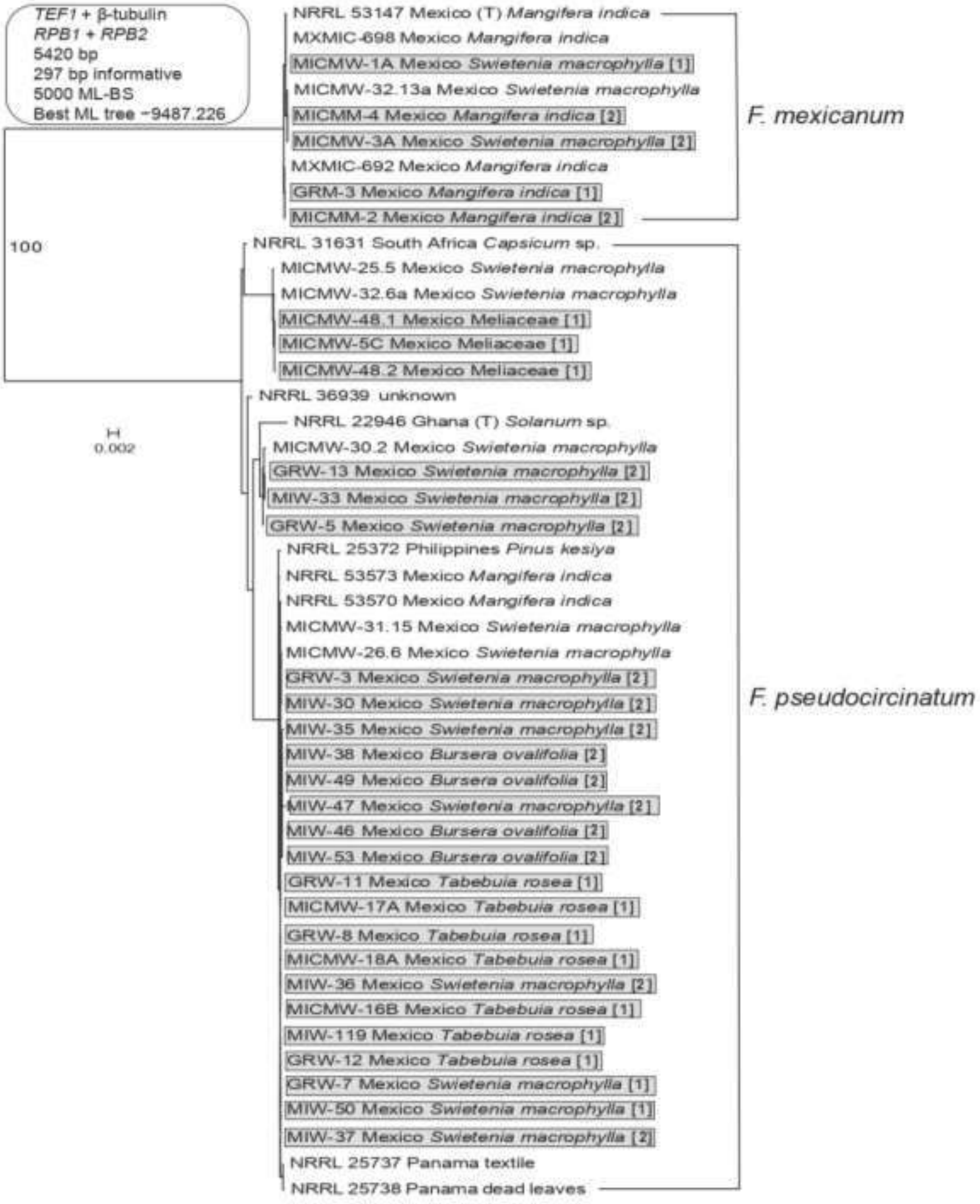

Figure 5. Maximum likelihood tree (ML) inferred from a four-locus dataset rooted at the midpoint. The ML bootstrap value (ML-BS) of 100% is based on 5,000 pseudoreplicates of the data; MICMW, MICMM, MIW, GRW and GRM = Fungal Collection, Laboratorio de Patología Vegetal, UMSNH; NRRL = Agricultural Research Service Culture Collection, Peoria, IL; T = ex-type strain.

Secondary metabolite production *in vitro*. The 30 *F. pseudocircinatum* and *F. mexicanum* isolates collected in the present surveys that were included in the multilocus phylogenetic analysis, were tested for their ability to produce mycotoxins and other secondary metabolites on a solid crack maize kernel medium (Table 5). Moniliformin production was detected in four isolates of *F. pseudocircinatum* one of *F. mexicanum*. One isolate of *F. pseudocircinatum* and one of *F. mexicanum* were confirmed to produce fusaric acid. No other metabolite analyzed was detected above productions of 1 µg/ml, but were detected above 1 ng/ml. Production of pigment bikaverin was confirmed for two isolates of *F. mexicanum* and nine of *F. pseudocircinatum*. Full-scan LC-MS analysis indicated that 11 isolates of *F. pseudocircinatum* and one of *F. mexicanum* produced beauvericin. The AZ anthraquinone pigment methylbostrycoidin was elaborated by 16 isolates of *F. pseudocircinatum* and four of *F. mexicanum*. Aurofusarin was produced by two isolates of *F. pseudocircinatum* and one of *F. mexicanum* (Table 4).

Table 4. Analysis of secondary metabolites production by *Fusarium* isolates associated with malformation disease of *Bursera ovalifolia* and *Tabebuia rosea* from Guerrero and Michoacán, Mexico.

Species	Strain	Beauvericin[a]	Aurofusarin[a]	Bikaverin[a]	Methylbostrycoidin[a]	Fusaric acid[b]	Moniliformin[b]
F. mexicanum	GRM-3	251.59	0	2.17	3.49	0.16	23.74
F. mexicanum	MICMM-2	0	0	0.68	5.41	0.08	0
F. mexicanum	MICMM-4	0	0	0.03	22.96	0.03	0
F. mexicanum	MICMW-1A	0	6.4	2.43	22.48	0.32	0
F. mexicanum	MICMW-3A	0	0	0.67	0.01	3.33	0.01
F. pseudocircinatum	GRW-3	443.28	0	0	2.93	0.24	0
F. pseudocircinatum	GRW-5	68.07	0	0	10.88	0.17	0
F. pseudocircinatum	GRW-7	0.9	0	0	0.07	0.34	0
F. pseudocircinatum	GRW-8	859.75	0	0	23.27	0.35	0.01
F. pseudocircinatum	GRW-11	4.61	0	0.36	0.02	3.13	0.08
F. pseudocircinatum	GRW-12	735.07	0	0.1	24.12	0.14	0
F. pseudocircinatum	GRW-13	234.21	0	0	18.23	0.21	18.63
F. pseudocircinatum	MIW-30	0	0	1.78	5.26	0.05	0.07
F. pseudocircinatum	MIW-33	0	0	2.9	3.02	0.1	0.04
F. pseudocircinatum	MIW-35	0	0	1.31	2.51	0.24	0.02
F. pseudocircinatum	MIW-36	510.11	0	2.67	3.87	0.12	0.01
F. pseudocircinatum	MIW-37	0	0	0.68	0.82	0.27	0.03
F. pseudocircinatum	MIW-38	0	0	1.69	2.2	0.18	0.05
F. pseudocircinatum	MIW-46	0	0	1.51	1.74	0.12	0.1
F. pseudocircinatum	MIW-47	0	0	0.06	2.66	0.02	0
F. pseudocircinatum	MIW-49	92.92	0	1.69	2.31	0.11	0.06
F. pseudocircinatum	MIW-50	49.79	0	1.71	0.03	0.28	0.01
F. pseudocircinatum	MIW-53	206.26	0	2.19	3.72	0.1	0.07
F. pseudocircinatum	MICMW-5C	0	0	0.81	0.03	0.04	92.22
F. pseudocircinatum	MICMW-16B	0	14.65	0.11	16.11	0.19	0.02
F. pseudocircinatum	MICMW-17A	0	4.26	0.01	5.32	0.07	0.01
F. pseudocircinatum	MICMW-18A	259.41	0	0.71	1.08	0.14	0
F. pseudocircinatum	MICMW-48.1	0	0	0.12	0.01	0.1	3.07
F. pseudocircinatum	MICMW-48.2	0	0	0.91	0	0.3	35.49
F. pseudocircinatum	MIW-119	0	0.02	0.41	0.22	0.08	0.05

[a] ng/ml
[b] µg/ml

Mating type determination. Mating type idiomorph was determinate for 64 isolates of *F. pseudocircinatum* and 12 of *F. mexicanum*. The multiplex PCR assay revealed that 31 of the *F. pseudocircinatum* strains were *MAT1-1* and 33 were *MAT1-2*. Of the 26 isolates of *F. pseudocircinatum* isolates from big-leaf mahogany, five were *MAT1-1* and 21 were *MAT1-2* (Supplementary Table 3). Both idiomorphs were segregating on this host in La Huacana, Michoacán and Coyuca de Catalán, Guerrero. However, *the F. pseudocircinatum* isolates from rosy trumpet tree and cooperwood were *MAT1-1* and *MAT1-2*, respectively. Of the 12 isolates of *F. mexicanum* typed, six were *MAT1-1* and six were *MAT1-2*; however, both idiomorphs were only segregating on big-leaf mahogany in La Huacana, Michoacán.

Pathogenicity tests. A selection of isolates of *F. mexicanum* and *F. pseudocircinatum* recovered from five different hosts, including mango and an unidentified tree of the Meliaceae, induced malformation symptoms on rosy trumpet tree seedlings (Table 5). All but two of the *F. pseudocircinatum* isolates from big-leaf mahogany and rosy trumpet tree were able to cause malformation symptoms on big-leaf mahogany seedlings, (Table 5). However, only three of the *F. pseudocircinatum* isolates were able to induce malformation symptoms on the mango seedlings (Table 5). In general, malformed tissues on this host were small and emerged near the cotyledon leaves or from the base of the stem near the crown (Fig. 6). Partial *TEF1* sequence data confirmed that the isolates recovered from malformed tissues were the same as those used to inoculate the plants.

Table 5. Pathogenicity tests on rosy trumpet tree, mango and big-leaf mahogany seedlings using *Fusarium* strains associated with malformation disease in tropical trees from Guerrero and Michoacán, Mexico.

Fusarium	**Strain**	**Host origin**	***Tabebuia rosea***[a]	***Mangifera indica***[b]	***Swietenia macrophylla***[c]
F. mexicanum	GRM-3	*M. indica*	3/6[d]	0/6[d]	4/5[d]
F. mexicanum	MICMW-1A	*S. macrophylla*	3/6	nd[e]	2/6
F. mexicanum	MXMIC-698[f]	*M. indica*	2/6	nd	3/5
F. pseudocircinatum	GRW-7	*S. macrophylla*	4/6	0/6	1/6

F. pseudocircinatum	GRW-8	*T. rosea*	4/6	0/6	2/6
F. pseudocircinatum	GRW-12	*T. rosea*	3/6	2/6	4/6
F. pseudocircinatum	MIW-30	*S. macrophylla*	1/6	0/6	0/5
F. pseudocircinatum	MIW-36	*S. macrophylla*	2/6	0/6	3/5
F. pseudocircinatum	MIW-46	*Bursera ovalifolia*	2/6	1/6	4/5
F. pseudocircinatum	MICMW-48.1	Meliaceae	2/6	nd	2/6
F. pseudocircinatum	MICMW-16B	*T. rosea*	4/6	1/6	0/5
F. pseudocircinatum	MICMW-18A	*T. rosea*	2/6	0/6	2/6
Mock inoculated			0/6	0/6	0/5

[a] Results 6 months after the inoculation.

[b] Results 7 months after the inoculation.

[c] Results 2 months after the inoculation.

[d] Plants with symptoms / total of inoculated plants.

[e] Not determined. Plants died soon after moving them to greenhouse conditions.

[f] Soto-Plancarte et al. 2015

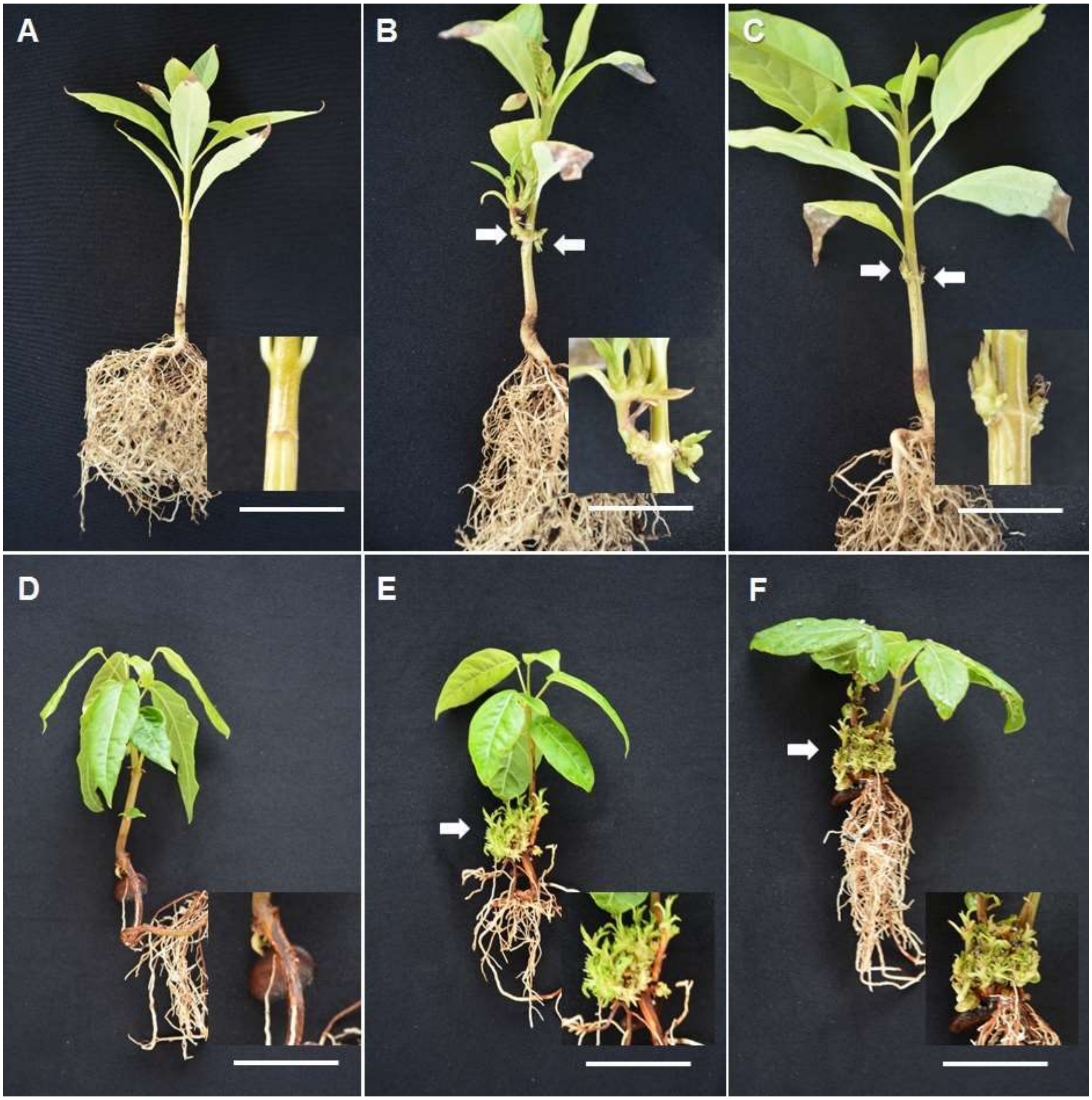

Figure 6. *Tabebuia rosea* (A, B, C) and *Swietenia macrophylla* (D, E, F) seedlings exhibiting malformation symptoms 6 (B, C) and 2 (E, F) months after being inoculated, respectively. A and D, Sterile distilled water negative controls. B and C, inoculated with *Fusarium pseudocircinatum* GRW-12 and MIW-36, respectively. E and F, inoculated with *F. pesudocircinatum* MIW-36 and MICMW-18A, respectively. Arrows indicate malformed vegetative shoots. Bar = 3 cm. Inset = ×4.

8.2.5. DISCUSSION

Fusaria isolates recovered from malformed tissues of rosy trumpet and cooperwood trees were identified as *F. pseudocircinatum*. Koch's postulates on rosy trumpet tree seedlings were successfully completed with isolates of *F.*

pseudocircinatum recovered from symptomatic rosy trumpet trees from Michoacán (MICMW-16B and MICMW-18A) and Guerrero (GRW-12 and GRW-8). Additionally, *F. pseudocircinatum* (5) and *F. mexicanum* (2) isolates recovered from malformed tissues from big-leaf mahogany, cooperwood trees, mango and an unknown Meliaceae tree, were also pathogenic in rosy trumpet tree seedlings. However, Koch's postulates on cooperwood were not completed because we were not able to produce seedlings from field collected seed (data not shown). The results of the pathogenicity tests on big-leaf mahogany seedlings showed the ability of 9 out of the 12 inoculated isolates obtained from different hosts, to produce disease. However, the tests using mango seedlings were less successful with 3 out of 12 inoculated isolates inducing disease symptoms. In general, the results of the pathogenicity tests suggested that rosy trumpet and big-leaf mahogany seedlings can be more effectively infected by *F. pseudocircinatum* and *F. mexicanum* recovered from several hosts, than mango seedlings. These results are similar to those of Santillán-Mendoza et al. (2018), where malformation symptoms were induced on big-leaf mahogany seedlings when inoculated with *F. mexicanum* isolates recovered from mango. In this sense, Goudarzi et al. (2019) found that the causal agent for the coconut malformation in Iran was *F. proliferatum*, a pathogen that also causes MMD. This show how *Fusarium* species previously reported causing MMD can also produce a similar malformation disease in other trees species.

ISSR genotyping of all the fusaria isolates recovered in this work, in combination with a BLAST analysis, confirmed that all the isolates were either *F. mexicanum* or *F. pseudocircinatum*. These results were expected since these are the only two pathogens that have been identified as the causal agent of MMD (Freeman et al. 2014; Otero-Colina et al. 2010) and BLMMD (Santillán-Mendoza et al. 2018) in Mexico. ML bootstrapping of the four-locus dataset, grouped the thirty selected isolates in two principal clades, *F. mexicanum* and *F. pseudocircinatum*. Among the *F. pseudocircinatum* clade we see three subgroups: one of these groups includes the three isolates from the unknown Meliaceae tree along with two isolates from Apatzingán from big-leaf mahogany (Santillán-Mendoza et al. 2018). The second group includes isolates obtained from big-leaf mahogany, close to the ex-type strain from Ghana (Nirenberg and O'Donnell 1998), and the other 19 *F. pseudocircinatum* isolates are included in the third group along with two isolates from mango and two from big-leaf mahogany (Freeman et al. 2014; Santillán-Mendoza et al. 2018). We found that all isolates obtained from rosy trumpet tree were *MAT1-1* and all from cooperwood tree were *MAT1-2*; this leads to think that sexual reproduction is not occurring on these hosts.

Production of trichothecenes, fusarin C, fumonisin FB1 and enniatin A, was not detected on the *F. mexicanum* or *F. pseudocircinatum* isolates. Interestingly, of four *F. pseudocircinatum* isolates that produced moniliformin, three were recovered from the unidentified Meliaceae tree and one was isolated from big-leaf mahogany. A fifth isolate of *F. mexicanum* recovered from mango also produced significant levels of moniliformin. Moniliformin production by *F. pseudocircinatum* has been tested positively by Schütt et al. (1998) and Fotso et al. (2002), who found production of monoliformin on *F. pseudocircinatum* at levels of 100 µg/g while our isolates ranged from 3.07 to 92.22 µg/ml. The beauvericin levels produce by our isolates were low compared with those obtained by Moretti et al. (2007), where they found production of beauvericin by *F. pseudocircinatum* ranging from 90 to 200 µg/g. There is no mycotoxin analysis for *F. mexicanum* published to date. Only two isolates produced fusaric acid (one *F. mexicanum* and one *F. pseudocircinatum*), but in low quantities compared with previously reported analysis in the FFSC (Bacon et al. 1996).

The results in this study showed that *F. pseudocircinatum* was the only species associated with malformation symptoms in the neotropical trees cooperwood and rosy trumpet in the central western region of Mexico. Koch's postulates confirmed that *F. pseudocircinatum* is the causal agent of rosy trumpet malformation disease. A *F. pseudocircinatum* cooperwood isolate induced malformation symptoms on inoculated rosy trumpet seedlings; however, its pathogenicity on cooperwood is pending until seedlings of this host can be grown. Although, *F. pseudocircinatum* has been isolated from a variety of substrates and hosts, it has only been reported causing *Acacia koa* wilt (Shiraishi et al. 2012) and malformation diseases of mango and big- leaf mahogany (Freeman et al. 2014; Santillán-Mendoza et al. 2018). More studies are needed to understand the origin and host range of *F. pseudocircinatum* and the diseases it can cause. This is the first report of malformation disease on the neotropical tree rosy trumpet caused by *F. pseudocircinatum* in Mexico.

8.2.6. LITERATURE CITED

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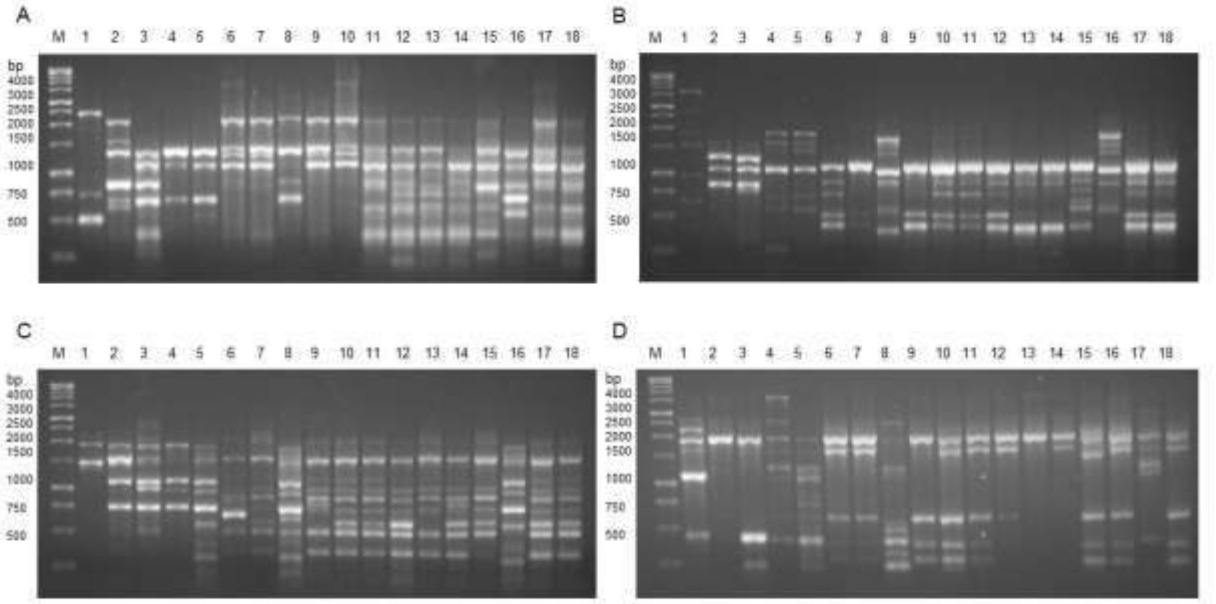

Supplementary Figure 1. ISSR fingerprint patterns of *Fusarium* isolates recovered from tropical trees with malformation disease. A, Oligonucleotide 809. B, Oligonucleotide 812. C, Oligonucleotide 835. D, Oligonucleotide 891. Panels A-C, lanes: M, 1 kb molecular weight marker. 1, *F. mangiferae* CML409. 2, *F. sterilihyphosum* CML280. 3, *F. tupiense* CML345. 4, *F. mexicanum* MICMW-32.13a. 5, *F. mexicanum* MXMIC-698. 6, *F. pseudocircinatum* MICMW 30.2. 7, *F. pseudocircinatum* MICMW-26.6. 8, *F. mexicanum* GRM-3. 9, *F. pseudocircinatum* GRW-7. 10, *F. pseudocircinatum* GRW-8. 11, *F. pseudocircinatum* GRW-12. 12, *F. pseudocircinatum* MIW-30. 13, *F. pseudocircinatum* MIW-36. 14, *F. pseudocircinatum* MIW-46. 15, *F. pseudocircinatum* MICMW-48.1. 16, *F. mexicanum* MICMW-1A. 17, *F. pseudocircinatum* MICMW-16B. 18, *F. pseudocircinatum* MICMW-18A. The isolates included in panel D are the same as in panels A-E except for lanes 16 and 17, which include *F. pseudocircinatum* MICMW-16B and *F. mexicanum* MICMW-1A, respectively.

Supplementary Table 3. *Fusarium* isolates obtained from malformed tissues form several tropical trees in Guerrero and Michoacán, Mexico.

Fusarium	**Isolate**	**Host[a]**	**Tree/ Sample[b]**	**Collection date**	**Location[c]**	**Coordinates[d]**	**Mating Type**
F. mexicanum	GRM-1	*M. indica* (v)	18/a	2016	Pungarabato, Gro.	18°19’59.1’’ N 100°41’20.3’’ W	1
F. mexicanum	GRM-3	*M. indica* (v)	18/a	2016	Pungarabato, Gro.	18°19’59.1’’ N 100°41’20.3’’ W	1
F. mexicanum	GRM-2	*M. indica* (f)	19/a	2016	Pungarabato, Gro.	18°19’59.1’’ N 100°41’20.3’’ W	1
F. mexicanum	GRM-4	*M. indica* (f)	19/a	2016	Pungarabato, Gro.	18°19’59.1’’ N 100°41’20.3’’ W	1
F. mexicanum	MICMM-4	*M. indica* (v)	26/a	2011	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	2
F. mexicanum	MICMM-2	*M. indica* (v)	23/a	2011	La Huacana, Mich.	18°57’18.6’’ N 101°48’21.6’’ W	2
F. mexicanum	MICMW-1A	*S. macrophylla* (v)	22/a	2011	La Huacana, Mich.	18°57’18.6’’ N 101°48’21.6’’ W	1
F. mexicanum	MICMW-1B	*S. macrophylla* (v)	22/a	2011	La Huacana, Mich.	18°57’18.6’’ N 101°48’21.6’’ W	1
F. mexicanum	MICMW-1C	*S. macrophylla* (v)	22/a	2011	La Huacana, Mich.	18°57’18.6’’ N 101°48’21.6’’ W	2
F. mexicanum	MICMW-3A	*S. macrophylla* (v)	24/a	2011	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	2
F. mexicanum	MICMW-3B	*S. macrophylla* (v)	24/a	2011	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	2
F. mexicanum	MICMW-3C	*S. macrophylla* (v)	24/a	2011	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	2
F. pseudocircinatum	GRW-1	*S. macrophylla* (v)	3/a	2016	Coyuca de Catalán, Gro.	18°19’07.2’’ N 100°41’52.2’’ W	1
F. pseudocircinatum	GRW-2	*S. macrophylla* (v)	4/a	2016	Coyuca de Catalán, Gro.	18°19’09.9’’ N 100°41’46.1’’ W	2
F. pseudocircinatum	GRW-3	*S. macrophylla* (v)	4/a	2016	Coyuca de Catalán, Gro.	18°19’09.9’’ N 100°41’46.1’’ W	2
F. pseudocircinatum	GRW-4	*S. macrophylla* (v)	5/a	2016	Coyuca de Catalán, Gro.	18°19’12.7’’ N 100°41’45.4’’ W	2
F. pseudocircinatum	GRW-5	*S. macrophylla* (v)	5/a	2016	Coyuca de Catalán, Gro.	18°19’12.7’’ N 100°41’45.4’’ W	2
F. pseudocircinatum	GRW-13	*S. macrophylla* (v)	17/a	2016	Coyuca de Catalán,	18°19’27.5’’ N	2

					Gro.	100°41’51.9’’ W	
F. pseudocircinatum	GRW-16	*S. macrophylla* (v)	17/a	2016	Coyuca de Catalán, Gro.	18°19’27.5’’ N 100°41’51.9’’ W	2
F. pseudocircinatum	GRW-6	*S. macrophylla* (v)	6/a	2016	Pungarabato, Gro.	18°19’59.1’’ N 100°41’20.3’’ O	1
F. pseudocircinatum	GRW-7	*S. macrophylla* (v)	6/a	2016	Pungarabato, Gro.	18°19’59.1’’ N 100°41’20.3’’ O	1
F. pseudocircinatum	MIW-30	*S. macrophylla* (v)	1/a	2016	San Lucas, Mich.	18°35'1.48’’ N 100°47'0.41’’ W	2
F. pseudocircinatum	MIW-31	*S. macrophylla* (v)	1/a	2016	San Lucas, Mich.	18°35'1.48’’ N 100°47'0.41’’ W	2
F. pseudocircinatum	MIW-32	*S. macrophylla* (v)	2/a	2016	San Lucas, Mich.	18°35'1.48’’ N 100°47'0.41’’ W	2
F. pseudocircinatum	MIW-33	*S. macrophylla* (v)	2/a	2016	San Lucas, Mich.	18°35'1.48’’ N 100°47'0.41’’ W	2
F. pseudocircinatum	MIW-34	*S. macrophylla* (v)	7/a	2016	San Lucas, Mich.	18°35’2.8’’ N 100°47’10.3’’ W	2
F. pseudocircinatum	MIW-35	*S. macrophylla* (v)	7/a	2016	San Lucas, Mich.	18°35’2.8’’ N 100°47’10.3’’ W	2
F. pseudocircinatum	MIW-36	*S. macrophylla* (v)	8/a	2016	Huetamo, Mich.	18°36’13.1’’ N 100°53’28.6’’ W	2
F. pseudocircinatum	MIW-37	*S. macrophylla* (v)	8/a	2016	Huetamo, Mich.	18°36’13.1’’ N 100°53’28.6’’ W	2
F. pseudocircinatum	MIW-47	*S. macrophylla* (v)	14/a	2016	Huetamo, Mich.	18°37'38.37’’ N 100°53'9.38’’ W	2
F. pseudocircinatum	MIW-52	*S. macrophylla* (v)	14/a	2016	Huetamo, Mich.	18°37'38.37’’ N 100°53'9.38’’ W	2
F. pseudocircinatum	MIW-50	*S. macrophylla* (v)	12/a	2016	Tiquicheo, Mich.	19°04’10.7’’ N 100°46’09.1’’ W	1
F. pseudocircinatum	GRW-8	*T. rosea* (f)	11/a	2016	Coyuca de Catalán, Gro.	18°19’28.7’’ N 100°41’46.0’’ W	1
F. pseudocircinatum	GRW-9	*T. rosea* (f)	11/b	2016	Coyuca de Catalán, Gro.	18°19’28.7’’ N 100°41’46.0’’ W	1
F. pseudocircinatum	GRW-10	*T. rosea* (f)	11/c	2016	Coyuca de Catalán, Gro.	18°19’28.7’’ N 100°41’46.0’’ W	1
F. pseudocircinatum	GRW-11	*T. rosea* (f)	15/a	2016	Coyuca de Catalán, Gro.	18°19’26.8’’ N 100°41’49.4’’ W	1
F. pseudocircinatum	GRW-14	*T. rosea* (f)	15/a	2016	Coyuca de Catalán, Gro.	18°19’26.8’’ N 100°41’49.4’’ W	1

F. pseudocircinatum	GRW-12	*T. rosea* (f)	16/a	2016	Coyuca de Catalán, Gro.	18°19’26.8’’ N 100°41’49.4’’ W	1
F. pseudocircinatum	GRW-15	*T. rosea* (f)	16/a	2016	Coyuca de Catalán, Gro.	18°19’26.8’’ N 100°41’49.4’’ W	1
F. pseudocircinatum	MICMW-16A	*T. rosea* (f)	28/a	2010	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-16B	*T. rosea* (f)	28/a	2010	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-16C	*T. rosea* (f)	28/a	2010	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-16D	*T. rosea* (f)	28/a	2010	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-17A	*T. rosea* (f)	28/b	2010	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-17B	*T. rosea* (f)	28/b	2010	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-17C	*T. rosea* (f)	28/b	2010	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-18A	*T. rosea* (f)	28/c	2011	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MICMW-18C	*T. rosea* (f)	28/c	2011	Apatzingán, Mich.	19°05'13.5" N 102°21’48.7’’ W	1
F. pseudocircinatum	MIW-118	*T. rosea* (f)	29/a	2018	Gabriel Zamora, Mich.	19°9' 24.282" N 102° 3' 12.2394 W	1
F. pseudocircinatum	MIW-119	*T. rosea* (f)	29/a	2018	Gabriel Zamora, Mich.	19°9' 24.282" N 102° 3' 12.2394 W	1
F. pseudocircinatum	MIW-120	*T. rosea* (f)	29/a	2018	Gabriel Zamora, Mich.	19°9' 24.282" N 102° 3' 12.2394 W	1
F. pseudocircinatum	MIW-121	*T. rosea* (f)	29/a	2018	Gabriel Zamora, Mich.	19°9' 24.282" N 102° 3' 12.2394 W	1
F. pseudocircinatum	MIW-38	*B. ovalifolia* (f)	9/a	2016	San Lucas, Mich.	18°31’19.9’’ N 100°47’53.4’’ W	2
F. pseudocircinatum	MIW-39	*B. ovalifolia* (v)	9/b	2016	San Lucas, Mich.	18°31’19.9’’ N 100°47’53.4’’ W	2
F. pseudocircinatum	MIW40	*B. ovalifolia* (v)	9/c	2016	San Lucas, Mich.	18°31’19.9’’ N 100°47’53.4’’ W	2
F. pseudocircinatum	MIW-41	*B. ovalifolia* (f)	9/d	2016	San Lucas, Mich.	18°31’19.9’’ N 100°47’53.4’’ W	2
F. pseudocircinatum	MIW-42	*B. ovalifolia* (v)	10/a	2016	San Lucas, Mich.	18°28’28.6’’ N	2

						100°41’22.4’’ W	
F. pseudocircinatum	MIW-43	*B. ovalifolia* (v)	10/b	2016	San Lucas, Mich.	18°28’28.6’’ N 100°41’22.4’’ W	2
F. pseudocircinatum	MIW-44	*B. ovalifolia* (f)	10/c	2016	San Lucas, Mich.	18°28’28.6’’ N 100°41’22.4’’ W	2
F. pseudocircinatum	MIW-45	*B. ovalifolia* (v)	10/d	2016	San Lucas, Mich.	18°28’28.6’’ N 100°41’22.4’’ W	2
F. pseudocircinatum	MICMW-15A	*B. ovalifolia* (f)	27/a	2011	Huetamo, Mich.	18°38’17.2’’ N 100°53’40.0’’ O	2
F. pseudocircinatum	MICMW-15B	*B. ovalifolia* (f)	27/a	2011	Huetamo. Mich.	18°38’17.2’’ N 100°53’40.0’’ O	2
F. pseudocircinatum	MICMW-15C	*B. ovalifolia* (f)	27/a	2011	Huetamo. Mich.	18°38’17.2’’ N 100°53’40.0’’ O	2
F. pseudocircinatum	MIW-48	*B. ovalifolia* (f)	20/a	2016	Huetamo. Mich.	18°35’00.8’’ N 100°53’10.0’’W	2
F. pseudocircinatum	MIW-53	*B. ovalifolia* (f)	20/a	2016	Huetamo. Mich.	18°35’00.8’’ N 100°53’10.0’’W	2
F. pseudocircinatum	MIW-49	*B. ovalifolia* (f)	21/a	2016	Huetamo. Mich.	18°35’24.4’’ N 100°53’17.5’’ W	2
F. pseudocircinatum	MIW-54	*B. ovalifolia* (f)	21/a	2016	Huetamo. Mich.	18°35’24.4’’ N 100°53’17.5’’ W	2
F. pseudocircinatum	MIW-46	*B. ovalifolia* (f)	13/a	2016	Huetamo. Mich.	18°38’17.2’’ N 100°53’40.0’’ W	2
F. pseudocircinatum	MIW-51	*B. ovalifolia* (f)	13/a	2016	Huetamo. Mich.	18°38’17.2’’ N 100°53’40.0’’ W	2
F. pseudocircinatum	MICMW-5A	Melicaceae (v)	25/a	2011	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	1
F. pseudocircinatum	MICMW-5B	Melicaceae (v)	25/a	2011	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	1
F. pseudocircinatum	MICMW-5C	Melicaceae (v)	25/a	2011	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	1
F. pseudocircinatum	MICMW-48.1	Melicaceae (v)	25/b	2015	Nuevo Urecho, Mich.	19°10’11.3’’ N 101°51’35.6’’ W	1
F. pseudocircinatum	MICMW-48.2	Melicaceae (v)	25/b	2015	Nuevo Urecho, Mich	19°10’11.3’’ N 101°51’35.6’’ W	1
F. pseudocircinatum	MICMW-48.3	Melicaceae (v)	25/b	2015	Nuevo Urecho, Mich	19°10’11.3’’ N 101°51’35.6’’ W	1
F. pseudocircinatum	MICMW-48.4	Meliaceae (v)	25/b	2015	Nuevo Urecho, Mich	19°10’11.3’’ N 101°51’35.6’’ W	1

[a] Simptomatology: (f), floral malformation; (v) vegetative malformation.

[b] Tree number with the same letter indicates that the isolates were obtained from the same malformed sample; while different letters indicates different malformed samples from the same tree.

[c] Gro.= Guerrero, Mich.= Michoacán

[d] no data.

8.3. CAPITULO III: Diversity of *Fusarium* species associated with weeds in mango orchards in Mexico.

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8.3.1. ABSTRACT

Mexico is one of the main producers of mango (*Mangifera indica*) and the first exporter worldwide. In some producing areas of the country, mango malformation disease (MMD) can severely reduce fruit yields in the affected orchards. Two *Fusarium* species, *F. mexicanum* and *F. pseudocircinatum*, have been reported causing MMD in Mexico. Most studies of the genus *Fusarium* have focused on isolates collected from commercial crops. However, reports on *Fusarium* isolates recovered from non-agricultural plants are scarce. Studies on pathogenic *Fusarium* species that infect asymptomatically or induce diseases on native plants are needed to fully understand the disease cycles of these pathogens. The objective of this study was to determine whether weeds commonly found in mango orchards in Michoacán, Mexico, are asymptomatically infected by *F. mexicanum* and *F. pseudocircinatum*. Dormant apical and lateral bud samples collected from four weeds species produced 59 *Fusarium* isolates from five species complexes: FFSC, FNSC, FSAMSC, FIESC and FSSC. The *Fusarium fujikuroi* species complex included the two species already reported as MMD causal agents in Mexico. Trichothecene production was detected in five isolates from the FIESC and FSAMSC. Both mating types were present although most isolates were *MAT1-2*. The information provided could prove valuable to study further the epidemiological role of weeds in MMD, which might help to improve management of the disease in mango growing areas.

8.3.2. INTRODUCTION

The genus *Fusarium* Link (Nectriaceae, Hypocreales) is estimated to comprise over 300 phylogenetically distinct species, representing one of the most important groups of plant pathogens and mycotoxigenic fungi. Species within this genus can be parasites, endophytes or pathogens of plants, depending on the ecological context

(Aoki et al. 2014). Diverse *Fusarium* species produce a wide array of secondary metabolites that are associated with plant diseases, as well as food contamination, posing a risk to human and animal health (O'Donnell et al. 2018). Some mycotoxins that had been detected and studied in this genus include fumonisins, monoliformin, beauvericin, bikaverin and trichothecenes (Busman et al. 2012; Desjardins 2006; Fotso et al. 2002; Leslie et al. 2004; O'Donnell et al. 2018; Proctor et al. 2004).

There is a considerable amount of studies of *Fusarium* species, diversity and toxin production among isolates collected from major grain and fruit crops, such as rice (Desjardins et al. 1997), maize, sorghum (Leslie et al. 2005, 1990), wheat (Laraba et al. 2017a, 2017b; O'Donnell et al. 2000), banana (Koenig et al. 1997; Moretti et al., 2004; O'Donnell et al. 1998b), date palm (Abdalla et al. 2000; Aoki et al. 2015) and mango (Britz et al. 2002; Haggag et al. 2011; Liu et al. 2014). Studies that focus on *Fusarium* isolates from non-agricultural plants as weeds and natural habitats, are needed to understand the disease cycles and to determine the range of alternative hosts of these pathogens, in order to achieve effective management of the diseases. In this sense, some studies on grasses (Fulcher et al. 2019; Leslie et al. 2004) and a diversity of non-agricultural plants (Laurence et al. 2016; Summerell et al. 2011) in natural areas, have shown the presence of *F. graminearum, F. verticillioides, F. kozum, F. subglutinans, F. thapsinum*. In other works that focused on weeds near agricultural crops (Hennessy et al. 2005; Mourelos et al. 2014; Pearson et al. 2016) or during crop rotation (Jenkinson and Parry 1994; Postic et al. 2012; Suproniene et al*.* 2019), diverse fusaria were recovered, including *F. oxysporum, F. subglutinans, F. graminearum, F. verticillioides, F. culmorum*.

Malformation disease is present in most mango producing areas worldwide (Kumar and Beniwal 1992). Due to its impact on floral organs, fruit yield can be severely reduced (Ploetz and Freeman 2009). This disease was observed initially in India (Watt 1985) and reported by Burns (1910) twenty years later. In more recent years, several *Fusarium* species members of the *F. fujikuroi* species complex (FFSC) (O'Donnell et al. 1998a), have been confirmed as causal agents of the disease. *Fusarium mangiferae* Britz, M. J. Wingf. & Marasas (Britz et al. 2002) has been reported from most mango producing areas worldwide, including Australia, China, Egypt, Florida (United States), India, Israel, Malaysia, Oman, South Africa, Spain, and Sri Lanka (Freeman et al. 2014b; Liew et al. 2016); *F. tupiense* C. S. Lima, Pfenning & J. F. Leslie has been reported in three countries, Brazil, Senegal and Spain (Crespo et al. 2016; Lima et al. 2012; Senghor et al. 2012); *F. sterilihyphosum* Britz, Marasas & M. J. Wingf. has been reported from two countries only, Brazil and South Africa (Britz et al. 2002; Lima et al. 2012); *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, has been reported in China only (Zhan et al. 2010); *F. pseudocircinatum* has been detected in two countries, Mexico and Dominican Republic (Freeman et al. 2014a; García-López et al. 2016); and *F. mexicanum* T. Aoki, S. Freeman, Otero-Colina, Rodríguez-Alvarado, Fernández-Pavía, Ploetz & O'Donnell has been detected in Mexico only (Otero-Colina et al. 2010).

Fusarium mexicanum and *F. pseudocircinatum* are the main causal agents of mango malformation disease (MMD) in Mexico; however, there is a lack of information on ecological and epidemiological studies on these pathogens. The role and importance of weeds in the MMD disease cycle and the establishment of these pathogens in agroecosystems is still not fully understood. The objective of this study was to identify the *Fusarium* species associated with weeds present on mango orchards with high MMD incidence, and examine the secondary metabolites produced by isolates recovered from these non-agricultural host.

8.3.3. MATERIALS AND METHODS

Sampling sites. Site 1, orchard located in Zimanca, Buenavista Tomatlán, Mich. (19°08'01.17" N, 102°32'48.68" W) (Fig. 7) with mango 'Haden' var., 15 to 20-year-old, and 10 to 15 m tall. This orchard was selected because presented 100% incidence of MMD and there were numerous dicotyledonous weed plants. Samples of mango malformed tissues and weed plants were collected in April 2016 and May 2017. Ten mango trees with floral and vegetative malformation symptoms were randomly selected. Two samples were obtained from each mango tree, one with floral malformation and one with vegetative malformation. Three different weed plant species were selected for sampling due to their abundance in the orchard and the results of a preliminary sampling that produced *Fusarium* isolates from dormant buds (data not shown). The selected plants were two species of *Senna* sp. Mill. (Fabaceae) that showed differences in the form and length of the pods and leaves, *Senna occidentalis* (L.) Link and *Senna uniflora* (Mill.) H.S. Irwin & Barneby, and *Sida rhombifolia* (L.). Three to five weed samples were obtained from under the canopy of each one of the selected mango trees. In the case of *Senna* plants, a sample was represented by the complete foliage of one plant, without the roots. In the case of *S. rhombifolia*, a sample was represented by several branches from one plant.

Site 2, orchard located in Nuevo Urecho, Mich. (19°10'15.6" N, 101°51'34.5" W) (Fig. 7) with mango ‘Haden’ var., 10 to 15-year-old, and 5 to 10 m tall. This orchard was also selected because presented a high incidence, 90-100%, of MMD and dicotyledonous weed plants were abundant. Samples of mango malformed tissues and weed plants were collected in September 2017. Seven mango trees with floral and vegetative malformation symptoms were randomly selected. Samples were collected as in Site 1. Two different weed plant species were selected for sampling due to their abundance in the orchard; *S. rhombifolia* and *Desmodium procumbens* (Mill.) Hitchc.

Site 3, orchard located in Nuevo Urecho, Mich. (19º 11' 32.2476" N, 101º 53' 47.3172" W) (Fig. 7) with mango ‘Haden’ var. 10 to 15-year-old, and 1 to 10 m tall. The incidence of MMD in this orchard was approximately 50%. The diversity and number of dicotyledonous weed plants was lower in this orchard, because most of the orchard was covered with grass weeds. Samples of *S. rhombifolia* were placed in labeled plastic bags in an ice chest, transported to the laboratory and stored at 4°C before being processed.

Figure 7. Map of Mexico showing the three mango orchard sites in the state of Michoacán where weeds and mango malformation samples were collected.

Fungal isolation from apical and lateral dormant buds of weed plants. Weed stems were cut into 2 to 3 cm segments (containing a dormant apical or lateral bud) and disinfested as described by Santillan-Mendoza et al. (2018) without the use of diluted sodium hypochlorite. Subsamples, 0.5 cm^2, containing one dormant bud were removed from the stem segments and plated on 60x15 mm Petri plates containing Peptone-PCNB-Agar (PPA) (Leslie and Summerell 2006) supplemented with antibiotics (Santillán-Mendoza et al. 2018). *Fusarium* isolates were purified and stored as indicated by Santillan-Mendoza et al. (2018).

In an additional isolation procedure, single apical or lateral dormant buds were ground in sterile mortars containing sterile, deionized water. Fifty µl of the ground tissue were transferred and dispersed with a glass rod on the surface of PPA in 100x15 mm Petri plates. Hyphal tips from individual colonies were transferred to SNA plates, incubated and stored as purified isolates as described previously (Santillán-

Mendoza et al. 2018). The protocol used to isolate fusaria from the mango malformed tissues was described by Santillán-Mendoza et al. (2018). The isolates were stored at -80°C in 15% glycerol in the laboratory of Kerry O'Donnell or deposited in the ARS Culture Collection (NRRL), Peoria, IL where they are available upon request (https://nrrl.ncaur.usda.gov/).

DNA extraction and phylogenetic analysis of *Fusarium* isolates. Genomic DNA was obtained from yeast-malt cultures using a hexadecyltrimethyl-ammonium bromide (CTAB, Sigma-Aldrich, St. Louis, MO) protocol (Gardes and Bruns 1993). Portions of translation elongation factor 1-α (*TEF1*) and RNA polymerase largest and second largest subunits (*RPB1* and *RPB2*) were PCR amplified and sequenced as previously described (O'Donnell et al. 2010; O'Donnell and Cigelnik 1997). PCR products were sequenced with BigDye 3.1 (Applied Biosystems, Foster City, CA), purified with BigDye Xterminator, and run on an ABI 3730 genetic analyzer. Sequence chromatograms were edited with Sequencher 5.2.4 (Gene Codes Corp., Ann Arbor, MI), exported as NEXUS files, and aligned with MUSCLE in SeaView ver. 4.3.0 (Edgar 2004; Gouy et al. 2010) prior to conducting partitioned maximum likelihood (ML) phylogenetic analyses with IQ-TREE 1.6.10 (Nguyen et al. 2015 http://www.iqtree.org/). ModelFinder (Kalyaanamoorthy et al. 2017) was used to identify the best-fit models of molecular evolution for each partition based on the Bayesian information criterion scores (Chernomor et al. 2016). DNA sequences were deposited in the National Center for Biotechnology Information GenBank under accession numbers MN242906-MN242944, MN724981-MN725022 and MN724932-MN724937.

Secondary metabolite production. Thirty-two selected *Fusarium* isolates were inoculated onto cracked corn kernels (50 g cracked corn + 22 mL water) (Aoki et al. 2015). The solid cultures were incubated for 28 d in the dark, at which time 10 g of culture material was extracted with 50 mL acetonitrile-water, 86:14 (v/v). The filtered extracts were analyzed by high-performance liquid chromatography–mass spectrometry (LC-MS), with a Dionex model U3000 liquid chromatography system

(Thermo Scientific, Waltham, Massachusetts) and a QTRAP 3200 mass spectrometer (AB SCIEX, Concord, Ontario, Canada). The LC-MS analysis was conducted with full scan in positive ionization mode to observe a wide range of metabolites and in negative-mode ionization specifically for the detection of the mycotoxin moniliformin. Positive-mode tandem mass spectrometry was used to assay for production of fumonisin FB1, beauvericin, bikaverin, aurofusarin, methylbostrycoidin and fusaric acid (Busman 2017; Busman et al. 2012; O'Donnell et al. 2018).

Two types of liquid culture media (agmatine medium and YEPD) and autoclaved rice were used to evaluate trichothecene production by strains comprising the *Fusarium incarnatum-equiseti* species complex (FIESC) and *F. sambucinum* species complex (FSAMSC), following the methodology described by O'Donnell et al. (2018). Trichothecenes were identified based on retention time and comparison of ion fragmentation patterns with a library prepared with purified standards, using GC-MS analyses with an Agilent 5873 chromatograph (Wilmington, Delaware) fitted with a HP-5MS column (Wilmington, Delaware) and products detected with a mass spectrometer with an electron impact source. Mycotoxins analyzed were 15-acetyldeoxy- nivalenol (15-ADON) and diacetoxyscirpenol (DAS), 4-acetylnivalenol (4-ANIV).

Mating type determination. For those isolates within the *F. fujikuroi* (FFSC) *and F. nisikadoi* species complexes (FNSC), screening for mating type idiomorph was accomplished by using two diagnostic primers pairs as described by Montoya-Martínez et al. (2019), in multiplex PCR reactions performed in 30 µl volumes. For isolates within the FIESC, FSSC (*F. solani* species complex) and FSAMSC, mating type determination was performed as described by Steenkamp et al. (2000) and Kerenyi et al., (2004). Amplicons were sized in a 2% agarose gel in 1X TAE buffer pH 8.0 (40 mM Tris, 20 mM acetic acid, 1 mM EDTA), using the EZ load 100 bp molecular weight marker (Bio-Rad, Hercules, CA), followed by staining with ethidium bromide and visualization over a UV trans-illuminator (Fotodyne Inc.; Hartland, WI).

8.3.4. RESULTS AND DISCUSSION

A total of 59 *Fusarium* isolates were recovered from apical and lateral dormant buds from *S. occidentalis* (2), *S. uniflora* (10), *S. rhombifolia* (37) and *D. procumbens* (10) (Table 6). Additionally, 33 *Fusarium* isolates were obtained from mango trees with malformation to compare with those present on weeds in the same orchards. The incidence of MMD on the three orchards surveyed was 90% to 100%. None of the weeds sampled showed malformation symptoms.

Table 6. *Fusarium* isolates obtained from malformed tissues form weeds and mango trees in Michoacán, Mexico.

***Fusarium* species complex[a]**	**Specie**	**Strain**	**Host [b]**	**Sample[c]**	**Collection date**	**Site[d]**
FFSC	*F. mexicanum*	MIM-1	*M. indica* (f)	1/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-2	*M. indica* (v)	1/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-3	*M. indica* (f)	2/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-4	*M. indica* (v)	2/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-5	*M. indica* (f)	3/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-6	*M. indica* (v)	3/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-7	*M. indica* (f)	4/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-8	*M. indica* (v)	4/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-9	*M. indica* (f)	5/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-10	*M. indica* (v)	5/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-11	*M. indica* (f)	6/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-12	*M. indica* (v)	6/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-13	*M. indica* (f)	7/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-14	*M. indica* (v)	7/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-15	*M. indica* (f)	8/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-16	*M. indica* (v)	8/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-17	*M. indica* (f)	9/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-18	*M. indica* (v)	9/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-19	*M. indica* (f)	10/1	2017	Site 1
FFSC	*F. mexicanum*	MIM-20	*M. indica* (v)	10/2	2017	Site 1
FFSC	*F. mexicanum*	MIM-21	*M. indica* (f)	11/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-22	*M. indica* (f)	12/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-23	*M. indica* (f)	12/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-24	*M. indica* (f)	13/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-25	*M. indica* (f)	13/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-26	*M. indica* (f)	14/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-27	*M. indica* (f)	14/1	2017	Site 2
FSSC	*F. striatum* (FSSC 21-d)	MIM-28	*M. indica* (f)	15/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-29	*M. indica* (f)	16/1	2017	Site 2
FFSC	*F. mexicanum*	MIM-30	*M. indica* (f)	16/1	2017	Site 2
FIESC	*Fusarium sp.* (FIESC 39-a)	MIM-31	*M. indica* (v)	17/1	2017	Site 2
FSSC	*Fusarium* sp. (FSSC)	MIM-32	*M. indica* (v)	17/1	2017	Site 2
FFSC	*F. mexicanum*	MICMM-21	*M. indica* (f)	18/1	2011	Site 1
FFSC	*F. mexicanum*	MIW-55	*Senna occidentalis* (a)	19	2017	Site 1
FFSC	*F. mexicanum*	MIW-56	*Senna occidentalis* (a)	19	2017	Site 1

FFSC	*F. mexicanum*	MICMW-19A	*Senna uniflora* (a)	50	2011	Site 1
FFSC	*F. mexicanum*	MICMW-19B	*Senna uniflora* (a)	50	2011	Site 1
FFSC	*F. mexicanum*	MICMW-20A	*Senna uniflora* (a)	51	2011	Site 1
FFSC	*F. mexicanum*	MICMW-20B	*Senna uniflora* (a)	51	2011	Site 1
FFSC	*F. mexicanum*	MICMW-20C	*Senna uniflora* (a)	51	2011	Site 1
FIESC	*F. irregulare* (FIESC)	MICMW-49.1	*Senna uniflora* (a)	52	2015	Site 1
FIESC	*F. irregulare* (FIESC 15-g)	MICMW-49.2	*Senna uniflora* (a)	52	2015	Site 1
FIESC	*F. irregulare* (FIESC)	MICMW-49.3	*Senna uniflora* (a)	52	2015	Site 1
FFSC	*F. mexicanum*	MICMW-50.1	*Senna uniflora* (a)	53	2015	Site 1
FIESC	*F. irregulare* (FIESC 15)	MICMW-50.2	*Senna uniflora* (a)	53	2015	Site 1
FSSC	*F. falciforme*	MIW-57	*Sida rhombifolia* (a)	23	2017	Site 1
FSSC	*F. falciforme* (FSSC 3+4-iiii)	MIW-58	*Sida rhombifolia* (a)	23	2017	Site 1
FFSC	*F. mexicanum*	MIW-65	*Sida rhombifolia* (a)	24	2017	Site 2
FIESC	*Fusarium* sp. (FIESC)	MIW-66	*Sida rhombifolia* (a)	24	2017	Site 2
FIESC	*F. sulawense* (FIESC 16-h)	MIW-67	*Sida rhombifolia* (a)	24	2017	Site 2
FFSC	*F. mexicanum*	MIW-74	*Sida rhombifolia* (a)	27	2017	Site 2
FSAMSC	*F. boothii*	MIW-75	*Sida rhombifolia* (a)	27	2017	Site 2
FFSC	*F. pseudocircinatum*	MIW-76	*Sida rhombifolia* (a)	27	2017	Site 2
FFSC	*F. mexicanum*	MIW-80	*Sida rhombifolia* (a)	29	2017	Site 2
FSSC	*F. striatum* (FSSC 21-d)	MIW-81	*Sida rhombifolia* (a)	29	2017	Site 2
FFSC	*F. mexicanum*	MIW-82	*Sida rhombifolia* (a)	30	2017	Site 2
FFSC	*F. mexicanum*	MIW-83	*Sida rhombifolia* (a)	30	2017	Site 2
FIESC	*F. sulawense* (FIESC)	MIW-84	*Sida rhombifolia* (a)	31	2017	Site 2
FIESC	*F. caatingaense.* (FIESC 20-e)	MIW-85	*Sida rhombifolia* (a)	32	2017	Site 2
FIESC	*F. caatingaense.* (FIESC)	MIW-86	*Sida rhombifolia* (a)	32	2017	Site 2
FSAMSC	*F. boothii*	MIW-87	*Sida rhombifolia* (a)	33	2017	Site 2
FFSC	*F. pseudocircinatum*	MIW-89	*Sida rhombifolia* (a)	35	2017	Site 2
FFSC	*F. mexicanum*	MIW-90	*Sida rhombifolia* (a)	35	2017	Site 2
FFSC	*F. pseudocircinatum*	MIW-93	*Sida rhombifolia* (a)	37	2017	Site 3
FIESC	*F. irregulare* (FIESC)	MIW-94	*Sida rhombifolia* (a)	38	2017	Site 3
FIESC	*F. sulawense* (FIESC)	MIW-95	*Sida rhombifolia* (a)	39	2017	Site 3
FIESC	*F. sulawense* (FIESC 16-g)	MIW-96	*Sida rhombifolia* (a)	39	2017	Site 3
FIESC	*F. irregulare* (FIESC)	MIW-97	*Sida rhombifolia* (a)	40	2017	Site 3
FIESC	*F. sulawense* (FIESC)	MIW-98	*Sida rhombifolia* (a)	40	2017	Site 3
FFSC	*F. pseudocircinatum*	MIW-99	*Sida rhombifolia* (a)	41	2017	Site 3
FIESC	*F. sulawense* (FIESC 16-i)	MIW-100	*Sida rhombifolia* (a)	41	2017	Site 3
FIESC	*Fusarium* sp. (FIESC)	MIW-101	*Sida rhombifolia* (a)	42	2017	Site 3
FNSC	*F. foetens*	MIW-102	*Sida rhombifolia* (a)	43	2017	Site 3
FIESC	*F. sulawense* (FIESC)	MIW-103	*Sida rhombifolia* (a)	44	2017	Site 3

FIESC	*Fusarium* sp. (FIESC)	MIW-104	*Sida rhombifolia* (a)	45	2017	Site 3
FFSC	*F. cf. sudanense*	MIW-105	*Sida rhombifolia* (a)	45	2017	Site 3
FIESC	*F. caatingaense.* (FIESC)	MIW-106	*Sida rhombifolia* (a)	46	2017	Site 3
FFSC	*F. pseudocircinatum*	MIW-107	*Sida rhombifolia* (a)	47	2017	Site 3
FIESC	*Fusarium* sp. (FIESC)	MIW-108	*Sida rhombifolia* (a)	48	2017	Site 3
FSSC	*Fusarium sp.* (FSSC 25-p)	MIW-109	*Sida rhombifolia* (a)	48	2017	Site 3
FFSC	*F. mexicanum*	MIW-110	*Sida rhombifolia* (a)	49	2017	Site 3
FIESC	*Fusarium* sp. (FIESC)	MIW-111	*Sida rhombifolia* (a)	49	2017	Site 3
FFSC	*F. mexicanum*	MIW-68	*Desmodium procumbens* (a)	25	2017	Site 2
FFSC	*F. mexicanum*	MIW-69	*Desmodium procumbens* (a)	25	2017	Site 2
FFSC	*F. verticilioides*	MIW-71	*Desmodium procumbens* (a)	26	2017	Site 2
FFSC	*F. verticilioides*	MIW-72	*Desmodium procumbens* (a)	26	2017	Site 2
FFSC	*F. verticilioides*	MIW-73	*Desmodium procumbens* (a)	26	2017	Site 2
FIESC	*Fusarium sp.* (FIESC 40-a)	MIW-77	*Desmodium procumbens* (a)	28	2017	Site 2
FFSC	*F. mexicanum*	MIW-78	*Desmodium procumbens* (a)	28	2017	Site 2
FIESC	*Fusarium* sp. (FIESC)	MIW-79	*Desmodium procumbens* (a)	28	2017	Site 2
FFSC	*F. pseudocircinatum*	MIW-88	*Desmodium procumbens* (a)	34	2017	Site 2
FFSC	*F. mexicanum*	MIW-91	*Desmodium procumbens* (a)	36	2017	Site 2

[a] FFSC, *F. fujikuroi* species complex; FIESC, *F. incarnatum-equiseti* species complex; FSAMC, *F. sambucinum* species complex; FSSC, *F. solani* species complex.

[b] Symptomatology: (f), floral malformation; (v), vegetative malformation; (a), asymptomatic

[c] Number of sample /number of malformation in mango trees

[d] Site 1, Buenavista Tomatlán, Michoacán 19°08'01.17" N, 102°32'48.68" W; Site 2, Nuevo Urecho, Michoacán 19°10’15.6” N, 101°51’34.5” W; Site 3, Nuevo Urecho, Michoacán 19º 11' 32.2 N, 101º 53' 47.3" W

Phylogenetic analysis of *Fusarium* isolates*.* BLASTn searches of *Fusarium* MLST (http://www.westerdijkinstitute.nl/fusarium/), using the partial *TEF1* sequences as the queries, identified the isolates from mango and weed plants within five *Fusarium* species complexes FSAMSC (2), FIESC (24), FFSC (59), FNSC (1) and FSSC (6). For precise identification, partitioned ML analyses of a 2-locus dataset conducted with IQ-tree (Nguyen et al., 2015) that included portions of *RPB1* and *RPB2* (for 32 selected (based on host and sampling site) isolates collected in the present study together with 18 previously characterized isolates, including the ex-type strains *F. pseudocircinatum* NRRL 22946 and *F. mexicanum* NRRL 53147 (Fig. 8). The isolates identities ranged across five *Fusarium* species complexes, most of them belonging to the FFSC, including MMD pathogens *F. mexicanum* and *F. pseudocircinatum*.

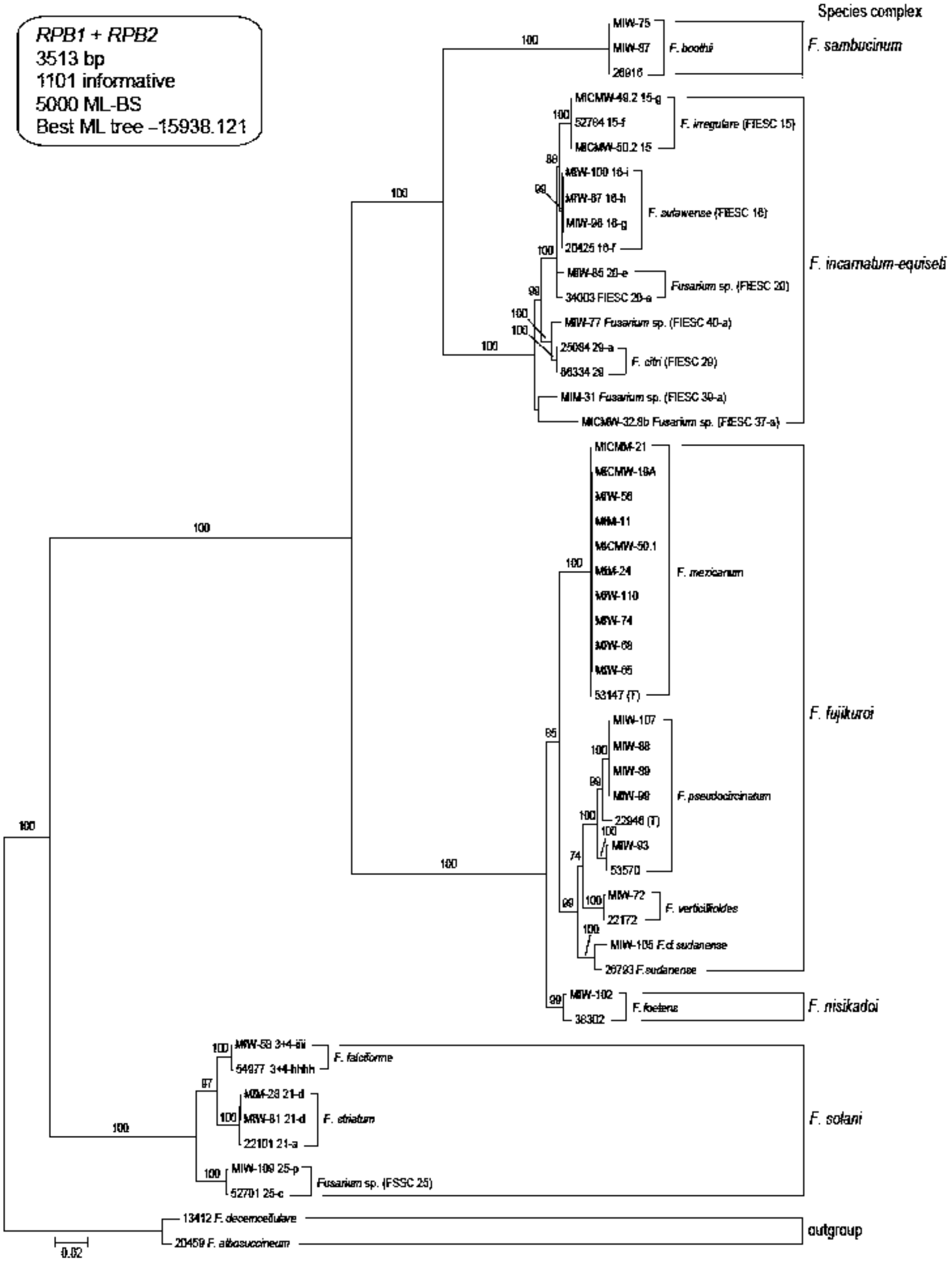

Figure 8. Maximum likelihood tree (ML) inferred from a two-locus dataset. The ML bootstrap value (ML-BS) of 100% is based on 5,000 pseudoreplicates of the data; MICMW, MIW and MIM = Fungal Collection, Laboratorio de Patología Vegetal, UMSNH; NRRL = Agricultural Research Service Culture Collection, Peoria, IL; T = ex-type strain.

Secondary metabolite production and mating type determination. The 32 *Fusarium* isolates collected in the surveys that were included in the phylogenetic analysis, were tested for their ability to produce secondary metabolites and mating type characterized by a PCR assay (Table 7). Trichothecene production was detected on five isolates from the FIESC and FSAMSC species complexes. Both *MAT* idiomorphs were identified; 9 isolates were *MAT1-1*, while 23 isolates were *MAT1-2.*

Table 7. Secondary metabolites production and mating type of *Fusarium* isolates from weeds and mango malformation.

Fusarium	**Strain**	**FB1**[a]	**BEA**[a]	**AUR**[a]	**BIK**[a]	**MBO**[a]	**FA**[b]	**MON**[b]	**Trichothecene**[c]	**Mating type**
F. mexicanum	MICMM-21	0	0	0	0.03	0.02	0	0	NA	2
F. mexicanum	MICMW-19A	0	0	0	0.28	33.98	0.1	0	NA	2
F. mexicanum	MICMW-50.1	0	12.98	0	0	14.03	0.12	0	NA	2
F. mexicanum	MIM-11	0	32.43	0	0	4.02	0.13	0	NA	2
F. mexicanum	MIM-24	0.01	43.23	0	0	24.54	0.02	0	NA	2
F. mexicanum	MIW-110	0	0	0.75	0.23	46.28	0.23	0	NA	2
F. mexicanum	MIW-56	0	0	0	0.2	21.04	0.03	0	NA	2
F. mexicanum	MIW-65	0	0	0	0.23	0.99	0.12	0	NA	2
F. mexicanum	MIW-68	0	0	0	0.51	33.78	0.36	0	NA	2
F. mexicanum	MIW-74	0	0	0	1.8	0.06	0.48	0	NA	2
F. pseudocircinatum	MIW-107	0	0	0	1.96	2.07	0.11	75.78	NA	2
F. pseudocircinatum	MIW-88	0	0	0	3.27	5.62	0.1	0	NA	1
F. pseudocircinatum	MIW-89	0	0	0	0.57	0.01	1.81	0.45	NA	1
F. pseudocircinatum	MIW-93	0	84.83	0	2.41	3.31	0.21	0.01	NA	2
F. pseudocircinatum	MIW-99	0	23.13	0	4.02	0.22	0.11	35.27	NA	1
F. verticilioides	MIW-72	17.39	0	0	0.94	0.02	0.37	0	NA	1
F. cf. sudanense	MIW-105	0.53	4.59	0	0.78	2.11	0.23	0	NA	2
F. foetens	MIW-102	0	1713.47	0	0.49	0.07	0.62	0.02	NA	1
F. boothii	MIW-75	0	0	3.31	0.04	0.12	0.18	0.01	15-ADON	1
F. boothii	MIW-87	0	0	2.77	0	0.02	0.38	0	15-ADON	1
F. irregulare (FIESC 15-g)	MICMW-49.2	0	0	0	0	0	0.22	0.01	4-ANIV	2
F. irregulare (FIESC 15)	MICMW-50.2	0	0	0	0	0.02	0.25	0	4 ANIV	2
F. sulawense (FIESC 16-i)	MIW-100	0	0	0	0.09	0	0.31	0.01	4-ANIV and DAS	2
F. sulawense (FIESC 16-h)	MIW-67	0	0	0	0	0.01	0.34	0	None detected	2
F. sulawense (FIESC 16-g)	MIW-96	0	0	0	0.04	0	0.51	0	NA	1
Fusarium sp. (FIESC 20-e)	MIW-85	0	0	0	0	0	0.5	0	None detected	2
Fusarium sp. (FIESC 40-a)	MIW-77	0	0	0.13	0.01	0	0.65	0	None detected	1
Fusarium sp. (FIESC 39-a)	MIM-31	0	0	0	0.01	0	0.36	0	None detected	2
F. falciforme (3+4-iiii)	MIW-58	0	0	0	0.01	0.03	0.23	0	NA	2
F. striatum (FSSC 21-d)	MIM-28	0	0.26	0	0	0.03	0.33	0	NA	2
F. striatum (FSSC 21-d)	MIW-81	0	0	0	0.01	0	0.36	0	NA	2
Fusarium sp. (FSSC 25-p)	MIW-109	0	0	0	0.01	0.01	0.26	0	NA	2

[a] ng/ml; FB1, Fumonisin FB1; BEA, beauvericin; AUR, aurofusarin; BIK, bikaverin; MBO, 8-O-methylbostrycoidin

[b] µg/ml; FA, Fusaric acid; MON, moniliformin.

[c] NA Not tested; 15-ADON, 15-acetyldeoxy- nivalenol; 4-ANIV, 4,15-diacetylnivalenol; DAS, diacetoxyscirpenol.

***Fusarium* species associated to weeds.** A description of the 32 characterized isolates by an ML analyses of a 2-locus dataset is shown below.

Fusarium mexicanum. Ten isolates from weeds (7) and mango (3) were identified as *F. mexicanum*. All were *MAT1-2*. The ten isolates produced non-detectable levels of fumonisin FB1, aurofusarin, fusaric acid or moniliformin; three isolates produce detectable levels of beauvericin (from 12.98 to 43.23 ng/ml); one isolate produced the pigment bikaverin (1.8 ng/ml); seven isolates produced methylbostrycoidin (from 4.02 to 46.28 ng/ml). *Fusarium mexicanum* has only been reported from Mexico causing malformation disease of mango and big-leaf mahogany (*Swietenia macrophylla* King) (Otero-Colina et al. 2010; Santillán-Mendoza et al. 2018).

***Fusarium pseudocircinatum*.** Five isolates were identified as *F. pseudocircinatum*. Both mating types were identified; three isolates corresponded to *MAT 1-1* and two to *MAT1-2*. None of the isolates produced fumonisin FB1 or aurofusarin. Two isolates produced detectable levels of beauvericin (from 23.13 to 84.83 ng/ml); four produced bikaverin (from 1.96 to 4.02 ng/ml); three produced methylbostrycoidin (from 2.07 to 5.62 ng/ml); one isolate produced fusaric acid (1.81 µg/ml) and two produced moniliformin (35.27 to 75.78 µg/ml). Previous studies have reported production of moniliformin, fusaproliferin, beauvericin and fumonisins FB1, and FB2 (Fotso et al. 2002; Moretti et al. 2007). *Fusarium pseudocircinatum* has been isolated from diverse substrates and hosts from tropical regions, such as dead leaves and textiles in Panama, the insect *Heteropsylla incisa* Šulc in Papua New Guinea, *Pinus kesiya* Royle ex Gordon in Philippines, *Solanum* sp. L. in Ghana (Nirenberg and O'Donnell 1998), and causing wilt on *Acacia koa* A. Gary in Hawaii, USA (Shiraishi et al. 2012). This fungus has been reported causing MMD in Mexico (Freeman et al. 2014a) and Dominican Republic (García-López et al. 2016). It has also been identified as causal agent of malformation in big-leaf mahogany (Santillán-Mendoza et al. 2018).

Fusarium verticillioides. One isolate was identified as *F. verticillioides*. Mating type was identified as *MAT1-1*. Produced detectable levels of fumonisin FB1 (17.39

ng/ml). Fumonisins are one of the most important toxins produced by *F. verticillioides*, as they have serious implications in human and animal health (Desjardins et al. 1994; Gelderblom et al. 1991, 1988; Miller et al. 1995). It did not produce any of the other mycotoxins tested. It is a pathogen of maize and is found widely distributed throughout the world wherever maize is cultivated (Bacon and Nelson 1994; Gai et al. 2018). In Mexico, it has been isolated from maize (Desjardins et al. 1994; Leyva-Madrigal et al. 2017, 2015; Morales-Rodríguez et al. 2007). It has also been recovered post-harvest from banana fruits (Moretti et al. 2004), as an endophyte from a variety of native grasses in natural ecosystems in northern Australia (Summerell et al. 2011), from native grasses on the Konza prairie tallgrass prairie reserve in the Great Plains of the United States (Leslie et al. 2004), and from finger millet in Uganda (Saleh et al. 2012).

***Fusarium cf. sudanense*.** One recovered isolate was identified as *F. cf. Sudanense*. Mating typing corresponded to *MAT1-2*. Full-scan LC-MS analysis indicated that it produced beauvericin (4.59 ng/ml) and the AZ anthraquinone pigment methylbostrycoidin (2.11 ng/ml). *Fusarium sudanense* was recently described as a species by Moussa et al. (2017). It was isolated from the weed *Striga hermonthica* (Delile) Benth. from Australia (Summerell et al. 2011).

***Fusarium foetens*.** One isolate was identified as *F. foetens*. It was characterized as *MAT1-1*. It produced only beauvericin (1713.47 ng/ml). González-Jartín et al. (2019) found similar results, detecting production of beauvericin and fusaric acid from *F. foetens* isolates in different culture media; the isolates from that study were obtained from forage maize in Galicia (NW Spain). *Fusarium foetens* was initially described as a species in 2004 by Schroers et al. (2004). It was isolated from begonia elatior hybrid (*Begonia X hiemalis*) causing basal rot, vein yellowing and wilting. Additionally, two isolates of this species were recovered from soil in southern Western Australia (Summerell et al. 2011), but they were not associated to any plant tissue.

Fusarium boothii. Two isolates were identified as *F. boothii*. Both isolates were identified as *MAT1-1*. Production of aurofusarin (2.77 to 3.31 ng/ml) and trichothecene 15-ADON was detected in both isolates. It was described as a species from the *F. graminearum* clade (former lineage 3) (O'Donnell et al. 2004). Isolates have been recovered from wheat in Mexico (Cerón-Bustamante et al. 2018; Malihipour et al. 2012); these isolates were 15-ADON chemotypes (Malihipour et al. 2012). It has also been recovered from wheat and maize in USA and China (Duan et al. 2016; Okello et al. 2019; Wegulo et al. 2018), from pecan and camel thorn in South Africa (Gryzenhout et al. 2016), and causing post-harvest fruit rot in tomato in Brazil (Gomes et al. 2015).

***Fusarium irregulare* FIESC 15.** Two isolates were identified as *F. irregulare*. Both isolates were *MAT1-2*. Production of trichothecene 4-ANIV was detected in both isolates. It has been isolated from bamboo (Bambusoideae) in China (Wang et al. 2019), bell pepper in Trinidad and Tobago (Ramdial et al. 2017) and from human tissue (O'Donnell et al. 2009). It has also been isolated from insects from Colombia and Benin (O'Donnell et al. 2012).

***Fusarium sulawense* FIESC 16.** Three isolates were identified as *F. sulawense*. Both mating types were identified [*MAT1-1* (1) and *MAT1-2* (2)]. One isolate produced trichothecenes 4-ANIV and DAS. *Fusarium sulawense* has been isolated from several hosts worldwide: banana in China and Indonesia (Maryani et al. 2019; Wang et al. 2019), *Luffa aegyptiaca* Mill, *Ipomea batatas* (L.) Lam., *Oryza sativa* L. and bamboo in China (Wang et al. 2019), bell pepper in Trinidad and Tobago (Ramdial et al. 2017), rice in Brazil (Avila et al. 2019), insects in Papua New Guinea (O'Donnell et al. 2012) and from human tissues (O'Donnell et al. 2009). In a recent study, Avila et al. (2019) found FIESC 16 isolates capable of producing deoxynivalenol (DON) and nivalenol (NIV), although they did not produce 4-ANIV, as the ones reported in this study.

***Fusarium caatingaense* FIESC 20.** One isolate was identified as *F. caatingaense*. This isolate was identified as *MAT1-2*. It did not produce mycotoxins. The haplotype FIESC 20 was recently named *F. caatingaense*. It has been was isolated from rice in Brazil (Santos et al. 2019), also from *Juniperus chinensis* L., human sputum (O'Donnell et al. 2009) and insects from California, USA (O'Donnell et al. 2012). Recently, it was recovered from big-leaf mahogany malformation in Mexico, but did not caused the disease in pathogenicity tests (Santillán-Mendoza et al. 2018). Avila et al. (2019) found that isolates of this haplotype produced NIV and 4-ANIV.

***Fusarium* sp. FIESC.** Two isolates from the FIESC were identified as haplotypes FIESC 40-a (*MAT1-1*; isolated from *D. procumbens*) and FIESC 39-a (*MAT1-2*, isolated from mango malformation). Previous reports have shown that the majority of the FIESC strains produce trichothecenes (O'Donnell et al. 2018); however, these isolates did not produce detectable levels of those mycotoxins. The mechanism involved in the regulation of secondary metabolite production is not yet completely understood; it seems to be related to the phenological state of the host (Bluhm and Woloshuk 2005) or nutrients available for inducing mycotoxin production (Gardiner et al. 2009; O'Donnell et al. 2018).

***Fusarium falciforme* FSSC 3+4.** One isolate was identified as *F. falciforme*. It was identified as *MAT1-2*. Mycotoxin production was not detected. *Fusarium falciforme* has been isolated from *Pinus maximinoi* H. E. Moore in Colombia (Herron et al. 2015), causing root rot in lima bean in Brazil (Sousa et al. 2017), from maize, papaya, tomato and onion in Mexico (Douriet-Angulo et al. 2019; Tirado-Ramírez et al. 2018; Vega-Gutiérrez et al. 2019b, 2019a), soybean (Chitrampalam and Nelson 2016) and from human eye in USA (O'Donnell et al. 2008).

***Fusarium striatum* FSSC 21.** Two isolates were identified as *F. striatum* [*S. rhombifolia* (1), mango (1)]. Both isolates were identified as *MAT1-2*. Mycotoxin production was not detected. Isolates of this species have been recovered from tomato causing root rot symptoms, similar to those caused by *F. oxysporum* (Moine et al. 2014).

***Fusarium* sp. FFSC 25-p.** One isolate was identified as haplotype FFSC 25-p. it was identified as *MAT1-2*. Mycotoxin production was not detected. This haplotype was found associated with thousand cankers disease of *Juglans* spp. in Italy (Montecchio et al. 2015) and with big-leaf mahogany malformation in Mexico (Santillán-Mendoza et al. 2018). O'Donnell et al. (2018) tested six FSSC strains for production of mycotoxins and the only toxin detected was beauvericin.

The primary objective of this study was to determine whether weeds commonly found on mango orchards serve as reservoirs of the MMD pathogens, *F. mexicanum* and *F. pseudocircinatum*. *Fusarium mexicanum* was recovered from the four weeds species sampled; *S. occidentalis, S. uniflora, Sida rhombifolia*, and *D. procumbens*, as well from the mango malformation samples. *Fusarium pseudocircinatum* was isolated from *S. rhombifolia* and *D. procumbens*; however, it was not isolated from mango malformations. We also found other *Fusarium* species of agricultural importance as *F. verticillioides* and *F. boothii*, known pathogens of maize. Weeds, plant debris and non-agricultural plants have been demonstrated to serve as an alternative host for *Fusarium* species. Jenkinson and Parry (1994) recovered two hundred and twenty-six *Fusarium* isolates from 15 broad-leaved weeds species and found them to be *F. avenaceum, F. culmorum, F. poae, F. sambucinum* and *F. graminearum*; 77 isolates were pathogenic when inoculated on wheat seedlings. Leslie et al. (2004) isolated *Fusarium* species of the FFSC from a non-agricultural ecosystem and found similarities on terms of species present with those found in the agricultural fields that surround it, but also found species unique to the tall-grass prairie. Our results showed similarities with these previous studies, since we also found pathogens of the agricultural crop (mango) on the weeds. It is worth mentioning that the weeds sampled on mango orchards did not showed any symptoms and appear to be healthy. Mourelos et al. (2014) and Postic et al. (2012) found *Fusarium* species, commonly associated with diseases in agricultural crops, on healthy, asymptomatic weeds.

We have shown that asymptomatic weeds within mango orchards may serve as hosts for *F. mexicanum* and *F. pseudocircinatum*, causal agents of MMD in Mexico. The impact of alternative hosts on the survival MMD pathogens *F. mexicanum* and *F. pseudocircinatum* in mango orchards needs to be determined, but even a small amount of inoculum could be important in the establishment of this disease. With this information, producers should be recommended to clear mango orchards from weeds and develop management practices aimed at reducing the amount of inoculum available; this is because alternative hosts may serve as reservoirs for potential MMD outbreaks. Additional studies are needed to evaluate pathogenicity and population genetic diversity of recovered *Fusarium* isolates.

8.3.5. LITERATURE CITED

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9. DISCUSIÓN GENERAL

Se diseñaron oligonucleótidos para la determinación del tipo de apareamiento dentro del complejo *Fusarium fujikuroi*, herramienta que es de gran importancia para el estudio de las poblaciones de este grupo de especies con gran impacto económico en el sector agrícola. Determinar si un patógeno posee la habilidad de reproducirse sexualmente, provee información importante para el control de la enfermedad, ya que los patógenos recombinantes suponen un mayor riesgo para superar los métodos de control basados en fungicidas y hospedantes resistentes. (McDonald y Linde 2002).

El objetivo principal de este trabajo fue demostrar que las especies de *Fusarium* que causan malformación en mango y las que causan malformación en árboles neotropicales, en México, son las mismas. Además, que estas especies de *Fusarium* se encuentran en las yemas de malezas comunes en huertas de mango de

Michoacán. Los resultados obtenidos confirmaron este supuesto, ya que se logró aislar con éxito a *F. pseudocircinatum* de malformaciones de *Bursera ovalifolia*, *Tabebuia rosea* y *Swietenia macrophylla*, además de que mediante los postulados de Koch se demostró en plántulas de *T. rosea*, que este patógeno es el agente causal de la malformación en estos árboles. Un estudio previo mostró que *F. pseudocircinatum* es el agente causal de malformación en árboles de *S. macrophylla* (Santillán-Mendoza et al. 2018).

A pesar de que *F. mexicanum* es el patógeno prevalente en huertas de mango con malformación en México (Otero-Colina et al. 2010, Betancourt-Resendes et al. 2012), esta especie no fue recuperada de los árboles neotropicales muestreados en este trabajo; sin embargo, si fue encontrado en las yemas ápicales y laterales de malezas en huertas comerciales de mango en Michoacán. En las malezas analizadas, se encontró una diversidad de especies de *Fusarium* pertenecientes a cinco complejos de especies (FFSC, FIESC, FSSC, FSAMSC y FNSC), dentro de los cuales se encuentran importantes patógenos de plantas (p.ej. *F. verticillioides, F. boothi, F. falciforme*) incluyendo a los patógenos reportados para la malformación de mango, *F. mexicanum* y *F. pseudocircinatum*. Resultados similares han sido reportados con anterioridad, donde se obtuvieron aislados de especies de *Fusarium* que causan enfermedades en cultivos de importancia económica (p.ej. plátano, trigo, maíz), de malezas dentro y/o cercanas a los campos de cultivo de éstos (Hennessy et al. 2005; Mourelos et al. 2014; Leslie et al. 2004; Postic et al. 2012).

La asociación de varias especies de *Fusarium* con tejidos malformados de mango y árboles neotropicales, y a tejido asintomático de malezas silvestres, sugiere que podría estar ocurriendo un flujo de aislados de *Fusarium* entre estos hospedantes. Además, las malezas presentes en las huertas de mango o en áreas adyacentes, podrían estar actuando como reservorios de especies de *Fusarium* causantes de malformación. Al senescer los tejidos de las plantas de malezas con infecciones quiescentes, el hongo podría ser activado, colonizar saprofiticamente el tejido senescente y producir propágulos (microconidios), que serían diseminados por insectos (hormigas), viento y/o lluvia, a las yemas apicales de otras plantas de malezas y/o árboles de mango, donde infectarían exclusivamente yemas en dormancia. En un experimento preliminar, Santillán-Mendoza (datos no publicados), incubó en cámara húmeda, ramas de *Senna* sp. infectadas con *F. mexicanum* y cuando estas ramas senecieron, estructuras del hongo crecieron sobre el tejido, mostrando que este patógeno puede actuar como saprófito. Existen diversos trabajos que apoyan la hipótesis de que ocurrió un salto de hospedero cuando el mango fue introducido a México, encontrándose en malezas y especies silvestres nativas de la región. Esta hipótesis ha sido planteada anteriormente (O'Donnell et al. 1998) para explicar la infección en África de dos plantas neotropicales, *Zea mays* L. e *Ipomoea batatas* (L.) Lam., por los patógenos paleotropicales *F. verticillioides* (Sacc.) Nirenberg y *F. denticulatum* Nirenberg & O'Donnell, respectivamente. Algunos investigadores consideran que esta hipótesis podría ser mejor resuelta si se analizaran aislados de *Fusarium* obtenidos de plantas silvestres, no de plantas cultivadas (Summerell et al. 2010).

10. CONCLUSIONES GENERALES

En este estudio se demostró que *F. pseudocircinatum*, agente causal de malformación en mango y caoba en México, también causa malformación en árboles de *Tabebuia rosea*. Este es el primer reporte de malformación en árboles de *T. rosea* causada por *F. pseudocircinatum* en México. Además, se encontró que *F. pseudocircinatum* se encuentra asociado al tejido malformado de *Bursera ovalifolia*; aún se necesita comprobar su patogenicidad en este hospedante. A pesar de no obtener aislados de *F. mexicanum* de los árboles neotropicales muestreados, su patogenicidad fue demostrada en plántulas de *T. rosea* y en *S. macrophylla*.

Otro de los objetivos principales de este estudio fue identificar a las especies de *Fusarium* asociadas a malezas presentes en huertas comerciales de mango en Michoacán, con alta incidencia de MMD. Se encontraron diversas especies de *Fusarium*, incluyendo a *F. mexicanum* y *F. pseudocircinatum*. Esto demuestra que las malezas asintomáticas dentro de las huertas, están infectadas latentemente por los patógenos de MMD y pueden servir como un reservorio. Son necesarios más estudios para determinar si las cepas obtenidas de malezas son patogénicas.

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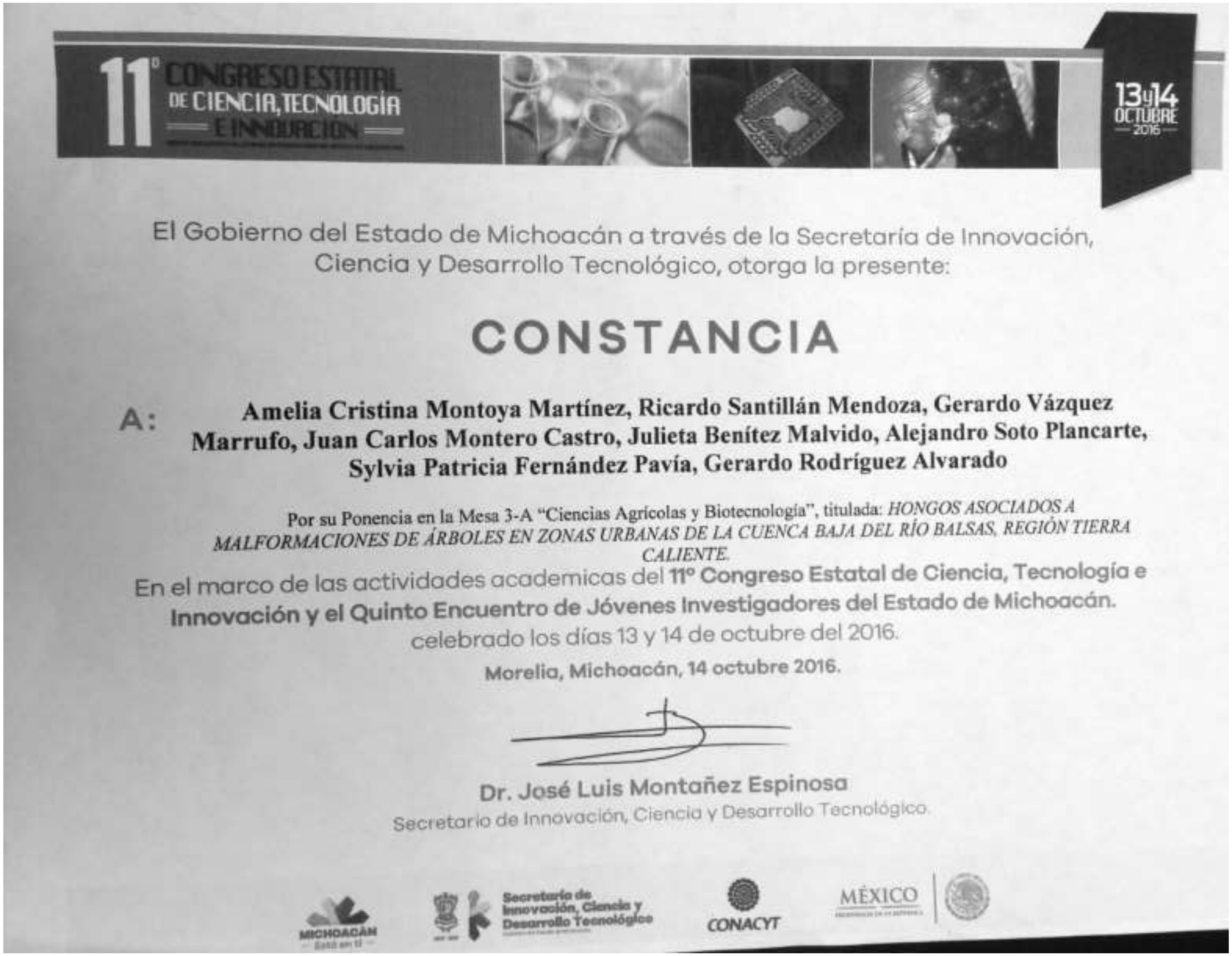
12. ANEXOS: Constancias de participación en congresos



11° CONGRESO ESTATAL DE CIENCIA, TECNOLOGÍA E INNOVACIÓN

13 y 14 OCTUBRE 2016

El Gobierno del Estado de Michoacán a través de la Secretaría de Innovación, Ciencia y Desarrollo Tecnológico, otorga la presente:

CONSTANCIA

A: **Amelia Cristina Montoya Martínez, Ricardo Santillán Mendoza, Gerardo Vázquez Marrufo, Juan Carlos Montero Castro, Julieta Benítez Malvido, Alejandro Soto Plancarte, Sylvia Patricia Fernández Pavía, Gerardo Rodríguez Alvarado**

Por su Ponencia en la Mesa 3-A "Ciencias Agrícolas y Biotecnología", titulada: *HONGOS ASOCIADOS A MALFORMACIONES DE ÁRBOLES EN ZONAS URBANAS DE LA CUENCA BAJA DEL RÍO BALSAS, REGIÓN TIERRA CALIENTE.*

En el marco de las actividades academicas del **11° Congreso Estatal de Ciencia, Tecnología e Innovación y el Quinto Encuentro de Jóvenes Investigadores del Estado de Michoacán.** celebrado los días 13 y 14 de octubre del 2016.

Morelia, Michoacán, 14 octubre 2016.

Dr. José Luis Montañez Espinosa

Secretario de Innovación, Ciencia y Desarrollo Tecnológico.

MICHOACÁN Está en ti

Secretaría de Innovación, Ciencia y Desarrollo Tecnológico

CONACYT

MÉXICO

XIX Congreso Internacional &

XLIV Congreso Nacional de la Sociedad Mexicana de Fitopatología A.C.

Otorga la siguiente

CONSTANCIA

a

Sylvia Patricia Fernández-Pavía

Amelia Cristina Montoya-Martinez, Ricardo Santillán-Mendoza, Alejandro Soto-Plancarte, Sylvia Patricia Fernández-Pavia, Gerardo Vázquez-Marrufo, Gerardo Rodriguez-Alvarado.

Por su participación con la presentación del poster:

USO DE SECUENCIAS REPETIDAS SIMPLES INTERNAS (ISSR) PARA DIFERENCIAR ESPECIES DE *Fusarium* CAUSANTES DE MALFORMACIÓN EN ÁRBOLES TROPICALES. [Use of Inter Simple Sequence Reapets to differentiate *Fusarium* species causing malformation in tropical trees].

Tuxtla Gutiérrez, Chiapas, México
del 17 al 20 de julio del 2017

Dr. Eduardo R. Garrido Ramírez
Presidente de la Mesa Directiva 2016-2018

inifap
BAYER
syngenta

12° Congreso Estatal de Ciencia
Tecnología e Innovación
6° Encuentro de Jóvenes Investigadores del Estado de Michoacán

5 y 6 octubre 2017
Centro de Convenciones y Exposiciones de Morelia

El Gobierno del Estado de Michoacán de Ocampo, a través de la Secretaría de Innovación, Ciencia y Desarrollo Tecnológico otorgan la presente:

CONSTANCIA

A: Amelia Cristina Montoya Martínez

Por su ponencia en la "Mesa 3.- Ciencias Agrícolas y Biotecnología", titulada: **DIVERSIDAD GENÉTICA DE Fusarium AISLADO DE MALFORMACIONES DE ÁRBOLES EN ZONAS URBANAS DE LA CUENCA BAJA DEL RÍO BALSAS, REGIÓN TIERRA CALIENTE**

En el marco de las actividades académicas del 12° Congreso Estatal de Ciencia, Tecnología e Innovación y el Sexto Encuentro de Jóvenes Investigadores del Estado de Michoacán.

Morelia, Michoacán a 6 de octubre de 2017.

Dr. José Luis Montañez Espinosa
Secretario de Innovación, Ciencia y Desarrollo Tecnológico.

CENTENARIO Constitución Política de los Estados Unidos Mexicanos 1917 - 2017
MÉXICO
CONACYT
Secretaría de Innovación, Ciencia y Desarrollo Tecnológico
MICHOACÁN — Está en ti —

El Gobierno del Estado de Michoacán de Ocampo, a través del Instituto de Ciencia, Tecnología e Innovación del Estado,

otorgan la presente

CONSTANCIA

A: Amelia Cristina Montoya Martínez

Por su ponencia en la "Mesa 3.- Ciencias Agrícolas y Biotecnología", titulada: **ANÁLISIS DE DIVERSIDAD GENÉTICA DE AISLADOS DE Fusarium spp. OBTENIDOS DE MALEZAS EN HUERTAS COMERCIALES DE MANGO EN MICHOACÁN**

En el marco de las actividades académicas del **13° Congreso Estatal de Ciencia, Tecnología e Innovación y el Séptimo Encuentro de Jóvenes Investigadores** del Estado de Michoacán.

Morelia, Michoacán; a 19 de octubre de 2018.

Dr. José Luis Montañez Espinosa

Director General del Instituto de Ciencia,
Tecnología e Innovación del Estado de Michoacán

PIDCB

La Universidad Michoacana de San Nicolás de Hidalgo a través del Programa Institucional de Doctorado en Ciencias Biológicas otorga la presente

CONSTANCIA A:

Amelia C. Montoya-Martínez, Gerardo Vázquez-Marrufo, Juan Carlos Montero-Castro, Julieta Benítez-Malvido, Sylvia P. Fernández-Pavía, Gerardo Rodríguez-Alvarado

CON LA PONENCIA:

COMPARACIÓN GENÉTICA Y PATOGÉNESIS DE *Fusarium* spp. ASOCIADOS A LA MALFORMACIÓN DE MANGO Y A PLANTAS SILVESTRES

En la Reunión Académica 2017
del Programa Institucional de Doctorado en Ciencias Biológicas
27 y 28 de noviembre del 2017. Morelia, Michoacán.

Dr. Carlos Cortés Penagos
Coordinador del PIDCB

Dra. Rosa Elva Norma de Río Torres
Coordinadora General de Estudios de Posgrado

SOCIEDAD MEXICANA
DE FITOPATOLOGÍA A.C.

Otorga la presente

CONSTANCIA

a:

Amelia Cristina Montoya-Martínez, Ricardo Santillán-Mendoza, Sylvia Patricia Fernández-Pavía, Nuria Gómez-Dorantes, Kerry O'Donnell, Gerardo Rodríguez-Alvarado

Por su participación con la presentación oral:

***Fusarium mexicanum* y *F. pseudocircinatum* CAUSANTES DE MALFORMACIÓN EN ÁRBOLES TROPICALES EN ZONAS URBANAS DE LA CUENCA BAJA DEL RÍO BALSAS**

XXI Congreso Internacional
& XLVI Congreso Nacional

del 24 al 28 de agosto 2019
Morelia, Michoacán

Sylvia Fernández

Dra. Sylvia Fernández Pavía
Presidente
Mesa Directiva 2018 - 2020

SOCIEDAD MEXICANA
DE FITOPATOLOGÍA A.C.

Otorga la presente

CONSTANCIA

a:

Amelia Cristina Montoya-Martínez, Gerardo Rodríguez-Alvarado,
Sylvia Patricia Fernández-Pavía, Robert H. Proctor, Hye Seon Kim,
Kerry O'Donnell

Por su participación con la presentación del cartel:

DISEÑO Y VALIDACIÓN DE OLIGONUCLEÓTIDOS PARA LA DETERMINACIÓN DEL TIPO DE APAREAMIENTO DENTRO DEL COMPLEJO *Fusarium fujikuroi*

XXI Congreso Internacional
& XLVI Congreso Nacional

del 24 al 28 de agosto 2019
Morelia, Michoacán

Sylvia Fernández
Dra. Sylvia Fernández Pavía
Presidente
Mesa Directiva 2018 - 2020

ASOCIACIÓN MEXICANA DE MICROBIOLOGÍA
1949-2019 - 70 ANIVERSARIO

BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE BACTERIAS
SOCIEDAD MEXICANA DE BIOQUÍMICA

Se otorga la presente

CONSTANCIA a:

Amelia Cristina Montoya Martínez

Quien asistió y presentó el trabajo:

Design and validation of a multiplex PCR assay for MAT idiomorph determination within *Fusarium fujikuroi* species complex

por:
Amelia C. Montoya-Martínez, Gerardo Rodríguez-Alvarado, Sylvia P. Fernández-Pavía, Robert H. Proctor, Hye-Seon Kimb, Kerry O'Donnell

En la modalidad de cartel durante el
VI Congreso de Bioquímica y Biología Molecular de Bacterias y XLI Congreso Nacional de Microbiología
27 – 31 de octubre de 2019 en la ciudad de Oaxaca, Oax.

Atentamente
Por el Comité Organizador

Dr. José Luis Puente García

Dra. María de Lourdes Girard Cuesy

10º Foro Académico del Posgrado en Ciencias Biológicas y Agropecuarias

La Universidad Michoacana de San Nicolás de Hidalgo y la Coordinación General de Estudios de Posgrado a través de los Programas Institucionales de Maestría y Doctorado en Ciencias Biológicas otorga la presente:

CONSTANCIA A:

Amelia Cristina Montoya Martínez, Sylvia Patricia Fernández Pavía, Juan Carlos Montero Castro, Julieta Benítez Malvido, Gerardo Vázquez Marrufo, Gerardo Rodríguez Alvarado

POR SU PARTICIPACIÓN

En 10º Foro Académico del Posgrado en Ciencias Biológicas y Agropecuarias, los días 27 y 28 de noviembre del 2019, en la ciudad de Morelia, Michoacán.

CON LA PONENCIA TITULADA

IDENTIFICACIÓN Y PATOGÉNESIS DE FUSARIUM SPP. ASOCIADOS A MALFORMACIÓN DE ÁRBOLES NEOTROPICALES

DR. LUIS FELIPE MENDOZA CUENCA
COORDINADOR GENERAL DEL PROGRAMA
INSTITUCIONAL DE MAESTRÍA EN CIENCIAS
BIOLÓGICAS

DRA. ESPERANZA MELENDEZ HERRERA
COORDINADORA GENERAL DEL PROGRAMA
INSTITUCIONAL DE DOCTORADO EN CIENCIAS
BIOLÓGICAS

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