

Fungal pathogens of mango (*Mangifera indica* L.) inflorescences^{1,2}

Lydia I. Rivera-Vargas³, Manuel Pérez-Cuevas⁴, Irma Cabrera-Asencio⁵, María R. Suárez-Rozo⁶ and Luz M. Serrato-Díaz⁷

J. Agric. Univ. P.R. 104(2):139-164 (2020)

ABSTRACT

This is the first comprehensive study to identify fungal pathogens of mango (*Mangifera indica* L.) inflorescences in Puerto Rico. A total of 452 mango inflorescences were collected from four cultivars at seven developmental stages during two blooming seasons. Samples were gathered from the germplasm collection at the Agricultural Experiment Station of the University of Puerto Rico in Juana Díaz, Puerto Rico. Eight different symptoms were observed: cankers, flower abortion, powdery mildew, rachis necrotic lesions, rachis soft rot, tip blight, vascular wilt, and insect perforations with necrotic borders. Necrosis was the most prevalent symptom (47%), followed by powdery mildew (19%) and tip blight (6%). Symptoms of malformation were never observed in the field. Using a modified Horsfall and Barratt scale, data on all mango cultivars pooled from two blooming seasons showed that the full bloom stage, the last inflorescence developmental stage (G), displayed the highest mean disease severity (42.67%). This severity value was significantly higher than those of the other developmental stages evaluated ($P < 0.05$). Early inflorescence developmental stages were asymptomatic or showed the lowest percentage of disease severity. An ANOVA was performed to compare disease severity among all mango cultivars regardless of developmental stage. Results showed that there were significant differences ($P < 0.05$) between mean disease severity of cultivars 'Parvin' and 'Haden'. Mean disease severity was higher in 'Haden' (20%) when compared to 'Parvin' (10.7%). There were no statistical differences in mean disease severity between cultivars 'Irwin', 'Keitt' and 'Parvin', or between 'Irwin', 'Haden' and 'Keitt'. In addition to the powdery mildew caused by *Pseudoidium anacardii*, 26 genera of fungi, mainly of Ascomycetes,

¹Manuscript submitted to Editorial Board 18 June 2019.

²This work was supported by the USDA National Institute of Food and Agriculture, Hatch project H-425. The authors thank agronomists Víctor M. González, Luis E. Collazo and Lorena L. Simbaña for their assistance in the development of this research.

³Professor, Department of Agro-environmental Sciences, University of Puerto Rico-Mayagüez, Mayagüez, P.R. 00680; e-mail: lydiai.rivera@upr.edu.

⁴Ex-graduate student, Department of Agro-environmental Sciences, University of Puerto Rico-Mayagüez.

⁵Professor, Department of Agro-environmental Sciences, University of Puerto Rico, Agricultural Experiment Station, Juana Díaz, P.R. 0079.

⁶Research Associate, Department of Agro-environmental Sciences, University of Puerto Rico-Mayagüez.

⁷Research Plant Pathologist, USDA-ARS-Tropical Agriculture Research Station, 2200 P.A. Campos Avenue Ste. 201, Mayagüez, Puerto Rico 00680-5470.

were identified from a total of 569 fungal isolates, from symptomatic and asymptomatic inflorescences. The most common fungi were: *Diaporthe* spp. (29%), followed by members of the Botryosphaeriaceae (16%), *Curvularia* spp. (11%) and *Fusarium* spp. (11%). Many fungal pathogens identified in this study were isolated from asymptomatic tissue, occurring as endophytes or latent pathogens: *A. alternata*, various members of the Botryosphaeriaceae, *C. gloeosporioides* complex, *Cladosporium* spp. and *F. decemcellulare*. Thus, the use of protectant fungicides will not be as effective as systemics in their control. Correct identification of fungal pathogens affecting mango inflorescences is important when quarantine regulations are applied. In addition, this information will facilitate the development of better management strategies in mango orchards.

Key words: mango, inflorescences, powdery mildew, tip blight, necrosis, fungi

RESUMEN

Hongos fitopatógenos de las inflorescencias de mangó (*Mangifera indica* L.)

Este es el primer estudio comprensivo para identificar los hongos patógenos de la inflorescencia del mangó (*Mangifera indica* L.) en Puerto Rico. Se recolectaron 452 inflorescencias de cuatro cultivares de mangó en siete etapas de desarrollo, durante dos periodos de inflorescencia. Las muestras se recolectaron en la colección de germoplasma de mangó localizada en la Estación Experimental Agrícola de la Universidad de Puerto Rico, Juana Díaz, Puerto Rico. Se observaron ocho síntomas diferentes: añublo polvoriento; aborto de las flores; canchales; lesiones necróticas en el raquis; pudrición blanda del raquis; quemazón del ápice; marchitez vascular; y perforaciones, causadas por insectos, con bordes necróticos. La necrosis fue el síntoma prevalente (47%), seguido de añublo polvoriento (19%) y quemazón (6%). No se observaron síntomas de malformación en los huertos. El análisis estadístico de todos los datos en conjunto, que incluyó todos los cultivares y ambos periodos de floración, mostró que la última etapa de floración o flor completamente madura (etapa G) mostró la más alta severidad de enfermedad (42.67%), utilizando la escala modificada de Horsfall and Barratt. Esta severidad fue significativamente mayor a la observada en las otras etapas de desarrollo evaluadas. Las etapas tempranas de la inflorescencia fueron asintomáticas o mostraron bajos porcentajes de severidad de enfermedad. Se realizó una ANOVA para comparar la severidad de la enfermedad entre todos los cultivares de mangó, independientemente de la etapa de desarrollo de la inflorescencia. Los resultados mostraron diferencias significativas ($P < 0.05$) entre el promedio de la severidad de la enfermedad entre los cultivares 'Parvin' y 'Haden'. El promedio de la severidad de la enfermedad fue mayor en 'Haden' (20%) comparado con 'Parvin' (10.7%). No hubo diferencias significativas estadísticas en el promedio de la severidad de la enfermedad entre los cultivares 'Irwin', 'Keitt' y 'Parvin', o entre 'Irwin', 'Haden' y 'Keitt'. Además del añublo polvoriento causado por *Pseudoidium anacardii*, se aislaron 26 géneros de hongos, principalmente de Ascomycetes, de un total de 569 aislados a partir de inflorescencias sintomáticas y asintomáticas. Los hongos más comúnmente identificados fueron: *Diaporthe* spp. (29%), seguido de miembros de Botryosphaeriaceae (16%), *Curvularia* spp. (11%) y *Fusarium* spp. (11%). Muchos hongos fitopatógenos identificados en este estudio fueron aislados de inflorescencias asintomáticas, ocurriendo como endófitos o patógenos latentes: *A. alternata*, varios miembros de Botryosphaeriaceae, el complejo de *C. gloeosporioides*, *Cladosporium*

spp. y *F. decemcellulare*. De modo que el uso de fungicidas de protección no será tan efectivo como el uso de fungicidas sistémicos en su control. La identificación correcta de los hongos patógenos de las inflorescencias de mangó es importante en la aplicación de regulaciones cuarentenarias. Además, esta información facilitará el desarrollo de mejores estrategias de manejo en los huertos de mangó.

Palabras clave: mangó, inflorescencias, añublo polvoriento, quemazón del ápice, necrosis, hongos

INTRODUCTION

Mango (*Mangifera indica* L.) ranks as one of the most important traded tropical fruits (FAO, 2020). Worldwide, approximately 2 billion ton were exported during 2019, where India, China and Thailand dominated the market, producing up to 35 million ton of fruits (FAO, 2020). Even though mangoes have been grown in Puerto Rico since about 1750, it was not until 1948 that the Agricultural Experiment Station of the University of Puerto Rico (UPR-AES) started a program of mango improvement with the introduction and testing of over 150 cultivars (Morton, 1987). The most important commercial cultivar produced is 'Keitt' (80%). The other 20% includes cultivars such as 'Palmer', 'Parvin', 'Tommy Atkins' and 'Haden' (Alvarado et al., 2004). Ninety percent of mango production in Puerto Rico is exported, mostly to Europe (80%) and the USA (10%). Only 10% is available for local consumption (Morton, 1987; USDA-NAAS, 2014).

During 2018, among the Caribbean Greater Antilles, Haiti was a major producer of mangoes, mangosteens and guavas with 642,880 ton, followed by Cuba (391,111 ton) (FAO, 2020). In Puerto Rico, mango is an important fruit with a market value of \$25 million (Puerto Rico Department of Agriculture, 2015) (Figure 1). According to the most recent data from the Census of Agriculture for Puerto Rico, 1,263 ha (3,120 acres) were used for mango production in 2012 (USDA-NASS, 2014). On the island, mango fruit enterprises face major constraints. Besides the impact of devastating atmospheric disturbances such as Hurricane María, which caused \$11.2 million in losses (Gómez, 2018), disease and insect pests can decimate production and consequently, exports (Alfaro, 2010). Another limitation, not related to production, is increased competition from other mango exporting countries that forces farmers to seek ways to improve fruit appearance and quality, and to reduce production costs.

Despite the relative economic importance of mangoes worldwide, and to Puerto Rico in particular, knowledge of pathogens in inflorescence tissue is practically non-existent. Very few studies have been conducted to understand fungal pathogen species and insect pest pop-

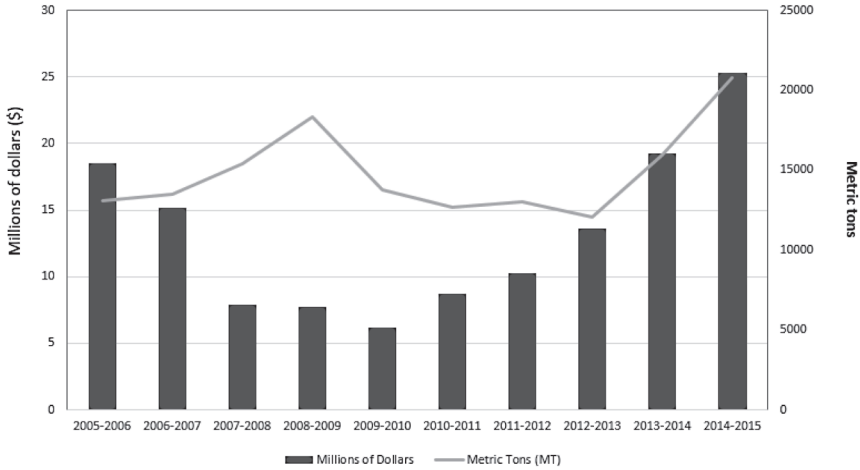


FIGURE 1. Puerto Rico's mango production in metric tons (MT) and market value in millions of dollars (\$) from 2005 to 2015.

ulations affecting mango inflorescences (Lonsdale and Kotzé, 1993; Prakash, 2003; Ramos et al., 1991; Ploetz, 2003; Slippers et al., 2004). More important, little is known about the mycobiota and their interactions with other pathogens and insect pests.

Fungal diseases associated with mango inflorescence

Lonsdale and Kotzé (1993) recognized different diseases that affect mango inflorescence in South Africa. These are blossom blight and spots, malformation and powdery mildew. Curved and necrotized peduncles with tip die-back characterize blossom blight disease (Ploetz, 2003). A complex of fungal pathogens has been implicated in the disease, including a group of species belonging to the Botryosphaeriaceae (Lonsdale and Kotzé, 1993; Ploetz, 2003; Slippers et al., 2005). Another important mango inflorescence disease is blossom spot caused by *Alternaria* spp. In India, *Alternaria tenuissima* and *A. alternata* caused significant decreases in fruit set (Prakash, 2003). In Africa, *A. alternata* was reported infecting panicles besides reducing fruit set (Cronje et al., 1990 cited by Ploetz, 2003). In Australia, *Colletotrichum gloeosporioides* and *C. gloeosporioides* var. *minor* were reported as causal agents of blossom blight (Ploetz, 2003). In Homestead, Florida, USA, besides *C. gloeosporioides*, *C. acutatum* was reported infecting mango flowers and panicles (Rivera et al., 2006). Currently, *C. acutatum* and *C. gloeosporioides* are considered species complexes (Damm et al., 2012; Weir et al., 2012).

Powdery mildew, caused by *Pseudoidium anacardii* (syn. *Oidium mangiferae* and teleomorph: *Erysiphe quercicola*), is another major disease of mango inflorescence (Johnson, 1991; Ploetz, 2003; Prakash, 2003). In Sinaloa, Mexico, *Pseudoidium anacardii* has been reported affecting mango inflorescence (Félix-Gastélum et al., 2013). No teleomorph of this fungus has been described. Necrosis is observed on affected panicles with few or no fruit (Ploetz, 2003). In South Africa, the first symptoms of the disease appeared two to three weeks after 20% of the inflorescence attained the red-colored to red-open stage of development in mango cv. 'Tommy Atkins'. These authors reported 80 to 90% crop losses caused by this disease (Schoeman et al., 1995). In Puerto Rico and Florida, complete losses occur, especially when cooler temperatures and drier conditions prevail, and no management practices are used (Toro, 1988; Ploetz, 2003). Resistance to powdery mildew appears to vary among mango cultivars, but no methodological studies have been conducted on the island.

Mango malformation as a biotic disorder has been disputed. Its etiology remained unclear for a century; physiological conditions, in addition to a diverse group of organisms, have been implicated in the disease (Kumar et al., 1993). Symptoms associated with malformation are shortening, thickening and branching of the inflorescences, increases in flower number and size, increases in the number of male flowers, sterility or abortion of the flowers and the development of leaves within the inflorescence (Marasas et al., 2006; Ploetz, 2003; Prakash, 2003; Freeman et al., 2014). It has been demonstrated using Koch's postulates that *Fusarium mangiferae* (= *F. subglutinans*, formerly *F. moniliforme* var. *subglutinans*), isolated from vegetative shoots and floral tissue, is the causal agent of malformation in South Africa, Egypt, Israel and Florida, USA (Marasas et al., 2006). An interaction between mango bud mite, *Aceria mangiferae*, and *F. mangiferae* has been established. The researchers suggested that the mites can act as a conidial vector and assist in fungal penetration (Gamliel-Atinsky et al., 2009). In South Africa, two new *Fusarium* species belonging to section *Liseola* have been associated with mango malformation (Britz et al., 2002). Worldwide, other *Fusarium* species have been implicated in the disease: *F. sterilihyphosum* was isolated from malformed tissues in South Africa and Brazil; *Fusarium* sp. nov. and *F. proliferatum*, in Malaysia; *F. oxysporum*, in Egypt, Mexico and India, but their pathogenicity has not been demonstrated (Haggag et al., 2010). In Mexico, a novel species, *Fusarium mexicanum*, was reported as the etiological agent of mango malformation (Otero-Colina, 2010). The disease has not been reported in Puerto Rico, but the mite, *A. mangiferae*, has been detected in mango seedlings at nurseries (Nieves-Méndez, 2005).

In Puerto Rico, a disease described as mango wither-tip caused by *C. gloeosporioides* was reported by Nolla (1926). These symptoms are

currently described as tip blight. In 1967, Álvarez-García reported a dieback disease of mango caused by *Botryodiplodia theobroma* and later in 1968, as *Physalospora rhodina*; both are synonymous of *Lasiodiplodia theobromae*. He also reported this fungus as the causal agent of gummosis, die-back and fruit rot of mango (Álvarez-García and López-García, 1971). Our group has reported tip blight of mango caused by different fungal species such as *Diaporthe pseudomangiferae*, *L. theobromae*, *Neofusicoccum mangiferae* and *N. parvum* (Serrato-Díaz et al., 2013a, 2013b and 2014b).

Correct identification of fungi is critical to assure effective orchard disease management and is key to enforce phytosanitary regulations. Even so, robust knowledge of key inflorescence diseases is also needed to understand the dynamics of different endophytic, pathogenic and saprophytic species present in mango orchards. Thus, the goal of this research is to provide breeders, plant pathologists, farmers and integrated pest managers with the basic knowledge needed to devise sustainable management practices, adapted to our horticultural conditions, that will reduce flower losses, and, consequently, improve mango yield.

MATERIALS AND METHODS

Collection of plant material

Field surveys were conducted during two mango blooming seasons to collect symptomatic and asymptomatic inflorescences of cultivars 'Keitt', 'Haden', 'Irwin' and 'Parvin'. Inflorescences were collected at seven flowering stages of development as described by Schoeman et al. (1995) from the UPR-AES Mango Germplasm Collection located in Juana Díaz, Puerto Rico. Samples were placed in plastic bags, labeled, refrigerated and processed at the Plant Pathology Laboratory of the Department of Agroenvironmental Sciences, UPR-Mayagüez. Disease symptoms were described and disease severity (%) was estimated based on the scale developed by Lonsdale and Kontzé (1993). Inflorescences were rated from 0 to 4, based on a visual scale, where 0 is an asymptomatic inflorescence, 1 equals to 1 to 25% of the diseased area, 2 equals to 26 to 50%, 3 equals to 51 to 75%, and 4 is greater than 76% of diseased area (Lonsdale and Kotzé, 1993).

Statistical analyses

Data from two surveys was consolidated into one data set and analyzed based on the percentage of diseased tissue (symptomatic) or mycelium observed at each inflorescence stage per mango cultivar. Data

conversion was adjusted as suggested by Horsfall and Barratt (H-B) (1945). For this conversion the percentage range midpoint was taken directly for each estimated interval of the visual scale of Lonsdale and Kotzé (1993) described above, in which a zero value (asymptomatic) was replaced by the value of 0.001 (Table 1). Once the data was converted, means comparisons between mango cultivars and inflorescence development stages were performed through analysis of variance and the Tukey test ($\alpha = 0.05$) using Infostat Statistical Program (InfoStat/Professional, v 2017p).

Isolation of fungi from mango inflorescences

Symptomatic and asymptomatic inflorescence tissues (1 mm²) were surface sterilized with 70% ethanol, 0.7% sodium hypochlorite and rinsed with de-ionized-sterile-distilled water for one minute for each treatment. Tissue sections were transferred to potato dextrose agar acidified with 25% lactic acid (APDA). For fungal identification, pure colonies were transferred to APDA. Different culture media such as carnation leaf agar (CLA), water agar (WA), oatmeal agar (OA) or cornmeal agar (CMA) were used to induce sporulation. Isolates were incubated at room temperature (approx. 26° C) for a week.

Fungal characterization

Fungal isolates were identified using taxonomic keys (Barnett and Hunter, 1998; Boerema et al., 2004; Hanlin, 1997; Leslie and Summerell, 2006; Simmons, 2007; Úrbez-Torres et al., 2011). Semi-permanent

TABLE 1.—*Number of inflorescences evaluated using a disease severity visual scale and converted to a midpoint based on H-B scale during two surveys.*

Disease Severity Scale ¹	Midpoint for Conversion based on H-B Scale ²	Number of inflorescences rated
0	0.001	230
1	13	142
2	38	23
3	63	22
4	88	35
Total		452

¹Disease severity was rated from 0 to 4, based on a visual scale developed by Lonsdale and Kontzé (1993), where 0 is an asymptomatic inflorescence, 1 equals to 1 to 25% of diseased area, 2 equals to 26 to 50% of diseased area, 3 equals to 51 to 75% of diseased area, and 4 is greater than 76% of diseased area.

²Data was converted as first suggested by Horsfall and Barratt (H-B) (1945). During this conversion the percent range midpoint was taken directly for each estimated interval of the visual scale, a zero disease severity (asymptomatic) was replaced by the value of 0.001.

microscopic slides were prepared from pure fungal colonies and their morphological characteristics such as mycelium, production of sexual and asexual reproductive structures including conidial size and shape, ascocarps, asci and ascospores, among others structures, were examined. Fifty conidia per isolate were measured at random (length and width) for each isolate using a compound microscope (400X, Olympus, Model 40BX, Melville, NY)⁸.

Morphological characterization of powdery mildew of mango was performed using a scanning electron microscope (SEM), in addition to light microscopy (Braun et al., 2002). In brief, for SEM, inflorescence tissue sections (7 to 8 mm) were fixed using 3% glutaraldehyde in 0.1M phosphate buffer pH 7.2 (Electron Microscopy Sciences, Washington, PA). After 24 h, tissues were rinsed twice for 15 min with phosphate buffer pH 7.2. Then, samples were dehydrated by a series of ascending ethyl alcohol concentrations that ranged from 10% up to 99.9%; concentrations were increased at intervals of 10% every 15 min. Tissues were rinsed with ethyl alcohol for 15 min. A critical point drying was performed for two hours using an EMS 850 (Electron Microscopy Sciences, Washington, PA). Later, samples were mounted on an aluminum stub with a 10 mm diameter carbon cover. After that, samples were concealed with gold film for 10 min using an EMS 550X (Electron Microscopy Sciences, Washington, PA). Micrographs were taken with a Scanning Electron Microscope (JSM-5410 LV Jeol Ltd. Model, Tokio, Japan) at the center of Microscopy, Department of Biology of the UPR-Mayagüez.

Genomic DNA of fungal isolates was extracted using a commercial extraction kit (DNeasy Plant Mini Kit, Qiagen, California, USA). Analysis of the ITS1-5.8-ITS2 rDNA operon was used to complement morphological characterization (White et al., 1990). Polymerase chain reaction (PCR) was used to amplify the ITS region in a reaction containing 25 μ L Amplitaq Gold® PCR Master Mix (Roche, New Jersey USA), 12 pmol of each primer, 17 μ L of ultrapure water (Sigma) and 20 to 30 ng of the DNA template to reach a reaction volume of 50 μ L. Polymerase chain reaction products were separated by electrophoresis (Fisher Scientific, NJ) at 100V for 45 min in a 1% (w/v) agarose prepared with 1X sodium borate buffer and 4 μ L ethidium bromide (1 μ g/1 μ L, Sigma®, St. Louis, MO) and visualized under UV light (Quantity One® 4.5 2003, BioRad Laboratory, Inc., Japan). Amplification products were purified with QIA quick Gel Extraction Kit (QIAGEN, CA).

⁸Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of Puerto Rico.

Purified products were sequenced in both directions using commercial facilities. Once sequenced, sequences were edited and aligned with the program Sequencher® 4.9 (Gene Codes Corporation, Minnesota, USA) and compared to GenBank database. DNA sequences were deposited in GenBank (Table 2).

TABLE 2.—*Fungi isolated from mango inflorescences and their molecular identification based on ITS region of rDNA and GenBank accession number.*

Fungal isolate number	BLAST id.	Accession no.
K-283 E (04/04) (20)	<i>Alternaria</i> sp.	HM060597
K-282 (4) (38Q)	<i>Alternaria alternata</i>	GU968430
I-142 D (12/01) (19)	<i>Cochliobolus</i> sp.	HM060596
K-283 G (04/04) (48)	<i>Cochliobolus lunatus</i>	HM060602
I-141 C (09/02) (3)	<i>C. lunatus</i>	HM060592
I-140 B1 (12Q)	<i>Cochliobolus specifer</i>	GU968419
I-142 G (04/04) (17)	<i>C. specifer</i>	GU968422
I-141 G (20/12) (13)	<i>Colletotrichum gloeosporioides</i>	GU968420
I-142 D (02/03) (22)	<i>C. gloeosporioides</i>	HM060599
VII	<i>C. gloeosporioides</i>	HM060607
K-284 G (04/04) (2)	<i>Curvularia</i> sp.	HM060591
I-141 G (02/03) (40)	<i>Curvularia</i> sp.	HM060600
H-172 G (17/02) (23)	<i>Curvularia</i> sp.	GU968426
K-284 (1) 1 (4Q)	<i>Diaporthe</i> sp.	GU968413
K-282 D (04/04) (7)	<i>Diaporthe</i> sp.	GU968416
I-141 (1) (30Q)	<i>Diaporthe pseudomangiferae</i>	GU968429
H-172 A (26Q)	<i>Fusarium</i> sp.	GU968427
K-283 F (04/04) (6)	<i>Fusarium</i> sp.	GU968414
K-283 G (04/04) (9)	<i>Fusarium decemcellulare</i>	GU968418
K-284 F (20/04) (18)	<i>F. decemcellulare</i>	GU968423
K-282 F (04/04) (29)	<i>Fusarium equiseti</i>	GU968428
K-283 F (04/04) (6)	<i>F. equiseti</i>	HM060593
H-174 (1) (13Q)	<i>Neofusicoccum parvum</i>	GU968421
I-140 G (17/02) (4)	<i>N. parvum</i>	GU968412
I-142 G (18/03) (52)	<i>N. parvum</i>	HM060603
P-47 (2) (21Q)	<i>N. parvum</i>	GU968425
45Q	<i>N. parvum</i>	GU968431
50Q	<i>N. parvum</i>	GU968432
73Q	<i>N. parvum</i>	HM060604
75Q	<i>N. parvum</i>	HM060605
76Q	<i>N. parvum</i>	HM060606
86Q	<i>N. parvum</i>	GU968434
87Q	<i>N. parvum</i>	GU968435
52Q	<i>Pestalotiopsis</i> sp.	GU968433
H-133 A (05/02) (16)	<i>Pyrenochaeta romeroi</i>	HM060595
K-284 (1) 4 (8Q)	<i>Hypoxylon</i> sp. (O. Xylariales)	GU968417

Pathogenicity tests

Pathogenicity tests were conducted on healthy mango inflorescences at orchards of cultivars 'Haden' and 'Irwin' located in the UPR-AES Mango Germplasm Collection, Juana Díaz, Puerto Rico. Under field conditions, inflorescences were superficially sterilized with a solution of sodium hypochlorite 0.07% and rinsed with de-ionized-sterile-distilled water for one minute. Three inflorescence rachises (wounded or unwounded) were inoculated with a conidial suspension or mycelial disks, depending on the fungal isolate evaluated. Conidial suspensions were prepared by washing the surface of the colony with 50 ml de-ionized-sterile-distilled water with three drops of Tween 20. Conidia concentration was adjusted to 10^4 conidia/ml using a hemacytometer. Mycelial disks (5 mm) were removed from the edge of a fungal colony grown on APDA for a week. A total of 24 fungal isolates were evaluated (Table 3). Untreated controls were inoculated with APDA disks or de-ionized-sterile-distilled water. Inflorescences were covered with plastic bags containing a wet cotton ball to retain humidity and reduce contamination. Data of disease severity was evaluated five and eight days after inoculation (DAI).

RESULTS

A total of 452 inflorescences were evaluated during two mango blooming seasons for four mango cultivars (i.e., 'Irwin', 'Haden', 'Keitt' and 'Parvin'). Sample sizes for the first and second survey consisted of 188 and 264 inflorescences, respectively. Of these, 50% were asymptomatic ($n=230$) and over 31% of the inflorescences showed low disease severity or category 1 on the Lonsdale and Kotzé scale (Table 1). Only 8% of the inflorescences showed the highest disease severity belonging to category 4 (Table 1). One-fourth of the inflorescences analyzed belongs to the earlier developmental stages (stages A to C). More than 75% of the inflorescence evaluated fell into mature developmental stages (stages D to G). The majority of the inflorescence evaluated were from mango cv. 'Irwin' ($n=168$; 37%) followed by 'Keitt' ($n=126$; 28%), 'Haden' ($n=103$; 23%) and 'Parvin' ($n=55$; 12%).

Symptomatology

Eight different symptoms were observed in mango orchards: cankers, flower abortion, powdery mildew, rachis necrotic lesions, rachis soft rot, tip blight, vascular wilt, and insect perforations with necrotic borders (Figure 2). Symptoms of inflorescence malformation were never observed in the field during the surveys.

TABLE 3.—Selected fungi evaluated during pathogenicity tests conducted in mango inflorescences under field conditions at UPR Agricultural Experiment Station, Juana Diaz, Puerto Rico.

Fungal species	Isolate no.	Mango cultivar	Inflorescence Stage	Symptoms in the field	Symptoms observed during pathogenicity tests ¹
<i>Alternaria</i> sp.	K- 283 G	'Keitt'	G	Necrotic spots	Ellipsoidal necrotic lesions, 5 DAI in 'Haden'.
<i>A. alternata</i>	I56F1	'Haden'	G	Ellipsoidal necrotic lesions	Necrotic lesions. Low levels of infection (<20% in lesions with wounds).
<i>A. alternata</i>	K - 282- 4	'Keitt'	ND ²	Tip blight	Ellipsoidal necrotic lesions with wounds in 'Haden'.
<i>A. infectoria</i>	I120F3	'Haden'	C	Tip blight	Low levels of infection (<25% with wounds), 5 DAI* in 'Irwin'.
<i>Botryosphaeria dothidea</i>	I21F2	'Irwin'	A	Asymptomatic	Tip blight; Rachis necrosis with >35% of mycelium coverage in 'Haden' and 'Irwin', 8 DAI.
<i>Colletotrichum gloeosporioides</i> ³	H - 172 G	'Haden'	G	Petal necrosis and necrotic spots in rachis	Necrotic spots on rachis; Petal necrosis in 'Irwin' and 'Haden'.
<i>C. gloeosporioides</i> ³	I - 141 G	'Irwin'	G	Petal necrosis	Ellipsoidal necrotic lesion on rachis and flower abortion in 'Haden'.
<i>Diaporthe</i> sp.	K - 284 D	'Keitt'	D	Asymptomatic	Necrotic spots on rachis; Petal necrosis in 'Irwin' and 'Haden'.
<i>Diaporthe</i> sp.	P - 49 A2	'Parvin'	A	Asymptomatic	Irregular necrotic lesions; Rachis soft rot observed 8 DAI in 'Haden'.
<i>Diaporthe pseudomangiferae</i>	I69F2	'Keitt'	C	Necrosis, associated with insect abrasions	Irregular necrotic lesions in rachis. Necrotic spots, rachis soft rot, flower abortion and cankers in 5 and 8 DAI in 'Irwin'.

¹DAI = Days after inoculation of inflorescences at stages E (*), F (**), or G (no asterisk) in 'Haden' or 'Irwin'.

²ND = Stage not determined

³Currently, *Colletotrichum gloeosporioides* is considered a fungal complex (Weir et al., 2012).

TABLE 3.—(Continued) Selected fungi evaluated during pathogenicity tests conducted in mango inflorescences under field conditions at UPR Agricultural Experiment Station, Juana Diaz, Puerto Rico.

Fungal species	Isolate no.	Mango cultivar	Inflorescence Stage	Symptoms in the field	Symptoms observed during pathogenicity tests ¹
<i>Fusarium</i> sp.	ISR1	'Parvin'	G	Necrosis, associated with insect abrasions	Inflorescence covered by mycelium 66 and 100% at 5 and 8 DAI, respectively, in 'Irwin'.
<i>F. decemcellulare</i>	H - 172 -1	'Haden'	E	Asymptomatic	Asymptomatic
<i>F. decemcellulare</i>	IFS3	'Parvin'	Fruit set	Asymptomatic	Vascular necrosis (wilt), flower death with wounds, 8 DAI in 'Haden'.
<i>Lasiodiplodia theobromae</i>	K -283 -1	'Keitt'	ND ²	Tip Blight	Mycelial threads covered 100% of inflorescence, 5 DAI in 'Haden'; Rachis necrosis in 'Haden' 8 DAI; Tip blight in 'Irwin'.
<i>L. theobromae</i>	K -283 -2	'Keitt'	ND	Tip Blight	Rachis necrosis in 'Haden' 8 DAI; Tip blight in 'Irwin'.
<i>Leptosphaerulina charitarum</i>	I4R6	'Parvin'	F	Asymptomatic	Asymptomatic
<i>Neofusicoccum mangiferae</i>	I23F2	'Irwin'	C	Rachis necrosis	Rachis necrosis in 'Haden' 8 DAI; Tip blight in 'Irwin'.
<i>Neofusicoccum parvum</i>	H - 172 -3	'Haden'	ND	Tip Blight	A 60 to 85% of inflorescences covered by mycelium. Rachis necrosis

¹DAI = Days after inoculation of inflorescences at stages E (*), F (***) or G (no asterisk) in 'Haden' or 'Irwin'.

²ND = Stage not determined

³Currently, *Colletotrichum gloeosporioides* is considered a fungal complex (Weir et al., 2012).

TABLE 3.—(Continued) Selected fungi evaluated during pathogenicity tests conducted in mango inflorescences under field conditions at UPR Agricultural Experiment Station, Juana Diaz, Puerto Rico.

Fungal species	Isolate no.	Mango cultivar	Inflorescence Stage	Symptoms in the field	Symptoms observed during pathogenicity tests ¹
<i>N. parvum</i>	H-174-1	'Haden'	ND ²	Tip blight	Necrotic lesions (2.5 cm) in 'Haden' at 8 DAI
<i>N. parvum</i>	I23F3	'Irwin'	C	Flower necrosis	Rachis necrosis in 'Haden' 8 DAI; Tip blight in 'Irwin'.
<i>N. parvum</i>	K-284-2	'Keitt'	ND	Tip Blight	Irregular necrotic lesions in rachis in 'Haden'
<i>N. ribis</i>	IFS3	'Parvin'	Fruit set	Asymptomatic	Rachis necrosis with 100% mycelium coverage 8 DAI cv. 'Haden'. Tip blight in 'Irwin'.
<i>Phoma exigua</i>	H-172 G	'Haden'	G	Rachis necrosis	Asymptomatic
<i>Phoma sorghina</i>	I52F1	'Keitt'	A	Flower necrosis	Cankers and irregular necrotic lesions, 8 DAI** in 'Irwin'.

¹DAI = Days after inoculation of inflorescences at stages E^(*), F^(**) or G (no asterisk) in 'Haden' or 'Irwin'.

²ND = Stage not determined

³Currently, *Colletotrichum gloeosporoides* is considered a fungal complex (Weir et al., 2012).

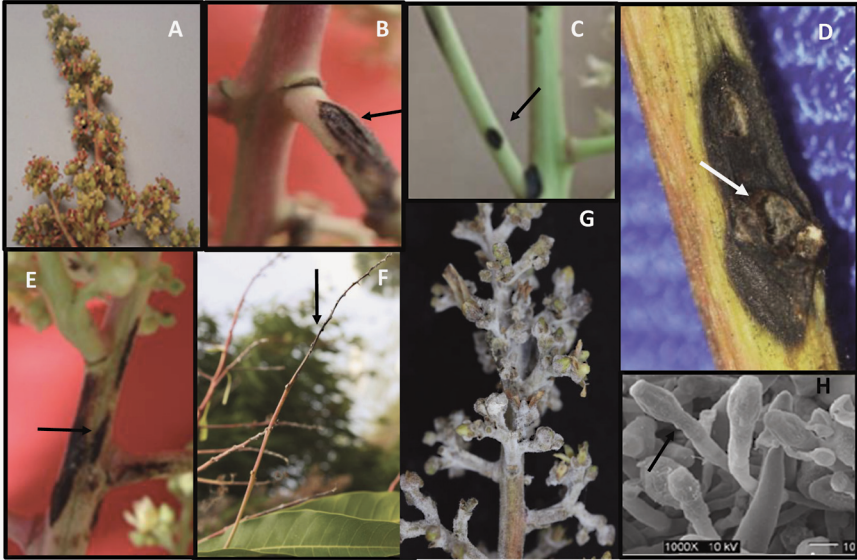


FIGURE 2. Symptoms observed in mango inflorescences at the orchards: A) Healthy mango inflorescence at full bloom (stage G); B) Raquis' cankers; C) Necrotic spots; D) Insect perforations with necrotic borders; E) Ellipsoidal necrotic lesions (arrow); F) Tip blight (arrow); G) Powdery mildew, *Pseudoidium anacardii*; and H) Detail of a powdery mildew conidiophore (arrow) with conidia attached.

Over all, necrosis was the most prevalent symptom (47%), followed by powdery mildew (19%) and tip blight (6%). Fifteen percent of the inflorescences showed a combination of symptoms, especially necrosis and powdery mildew. Less than 13% of the inflorescences showed symptoms of cankers, flower abortion, insect perforations, rachis soft rot or vascular wilts. The predominant symptom was necrosis, expressed as round, irregular or ellipsoidal lesions on inflorescence rachises and flower petals (Figure 2C to E). Powdery mildew, caused by *Pseudoidium anacardii* (syn. *Oidium mangiferae* Berthet), was first observed on the inflorescence rachises and flowers of 'Irwin' at the green-colored stage (stage D). The disease affected all four mango cultivars evaluated. The fungus completely covered the inflorescence, causing flower and fruit abortion (Figure 2G and H). Tip blight of mango is described as a die-back caused by an array of fungal species (Figure 2F).

Disease severity

Direct estimates of disease severity in the field ranged from 0 to >75% of the inflorescences affected. It varies among mango cultivars and inflorescence developmental stages. After data conversion of dis-

ease severity to H-B values, disease severity ranged from 0.003 to 52.30% (Table 4).

Earlier inflorescence developmental stages (A and B) did not show signs of disease severity in ‘Haden’ and ‘Parvin’ (Table 4). At these early stages, disease severity varied from 5 to 21% using a modified H-B scale in ‘Irwin’ and ‘Keitt’. Disease severity was less than 21% at developmental stages A to E. For all mango cultivars, as inflorescences mature (stages F to G) disease severity increased, with ‘Haden’ showing the highest numbers at red-opened (F) and full bloom (G) stages (Table 4). Cultivar ‘Parvin’ in comparison, showed no significant differences between developmental stages ($P < 0.05$).

When we considered the data of all mango cultivars pooled together, the final inflorescence developmental stage (G) showed the highest mean disease severity (42.67%) using the modified HB scale (Table 5). This was significantly different from the other developmental stages. No significant differences were observed between inflorescence stages A to E, nor between stages A, C, E and F ($P > 0.05$) (Table 5). Developmental stages B and D showed the lowest percentage of disease severity. In addition, an ANOVA was performed to compare disease severity between all mango cultivars regardless of inflorescence developmental stage. Results showed that there were significant differences ($P < 0.05$) between ‘Parvin’ and ‘Haden’. Mean disease severity was higher in ‘Haden’ (20%) when compared to ‘Parvin’ (10.7%). There were no statistical differences in mean disease severity between ‘Irwin’, ‘Keitt’ and ‘Parvin’, or between ‘Irwin’, ‘Haden’ and ‘Keitt’ (Table 6).

TABLE 4.—Disease severity (%) per developmental stages of mango inflorescences for four mango cultivars.

Inflorescence Developmental Stage ³	Disease severity (%) per mango cultivar ^{1,2}			
	‘Haden’	‘Irwin’	‘Keitt’	‘Parvin’
Bud-swoll to bud-break (A)	0.003 a	12.64 a	20.63 ab	0.003 a
Mouse ear (B)	0.003 a	7.36 a	4.88 a	0.003 a
Protected (C)	14.25 ab	7.89 a	7.92 a	0.003 a
Green-colored (D)	2.71 a	4.18 a	3.76 a	4.73 a
Red-colored (E)	12.24 ab	9.27 a	6.50 a	16.00 a
Red-opened (F)	41.20 bc	19.63 a	10.00 a	8.24 a
Full bloom (G)	52.30 c	48.11 b	31.00 b	26.60 a

¹Disease severity was rated from 0 to 4, based on a visual scale developed by Lonsdale and Kontzé (1993), and converted to a midpoint as first suggested by Horsfall and Barratt (H-B) (1945).

²Different letters mean statistical differences using Tukey tests $\alpha = 0.05$.

³Inflorescences were collected at the seven flowering developmental stages as described by Schoeman et al. (1995).

TABLE 5.—ANOVA of disease severity (%) using an H-B scale by mango developmental stages regardless of mango cultivar.

Inflorescence Developmental Stages	Mean ¹	n	E.E.
Bud-swell to bud-break (A)	10.69 ab ²	32	3.87
Mouse ear (B)	4.44 a	32	3.87
Protected (C)	8.35 ab	43	3.34
Green-colored (D)	3.73 a	101	2.18
Red-colored (E)	10.19 ab	78	2.48
Red-opened (F)	18.47 b	79	2.46
Full bloom (G)	42.67 c	87	2.35

¹Disease severity was rated from 0 to 4, based on a visual scale developed by Lonsdale and Kontzé (1993), and converted to a midpoint as first suggested by Horsfall and Barratt (H-B) (1945). Data was pooled together from four mango cultivars 'Haden', 'Irwin', 'Keitt' and 'Parvin'.

²Different letters mean statistical differences using Tukey tests $\alpha = 0.05$.

Fungal isolation and identification

A total of 569 fungal isolates from mango inflorescences were examined during the two surveys, which included 26 genera, primarily of Ascomycetes. DNA sequence analysis using ITS region of rDNA confirmed fungal morphological characterization of 36 specimens (Table 2). Specimens identified as *Bipolaris* sp. or *Dreschlera* sp. using morphology were placed within the genera *Cochliobolus* spp. using the ITS region of rDNA. In addition, specimens that we placed in the Order Xylariales were grouped within the genus *Hypoxylon* spp.

The most common fungal genus identified was *Diaporthe* spp. (29%), followed by members of the Botryosphaeriaceae (16%), *Fusarium* spp. (11%), *Curvularia* spp. (11%) and *Cladosporium* spp. (9%) (Figure 3). *Diaporthe* spp. were isolated from necrotic tissues of rachises and flowers, as well as from asymptomatic tissues of all mango cultivars examined. Members of Botryosphaeriaceae, which are important plant pathogens of mango, were isolated from asymptomatic as well as

TABLE 6.—ANOVA of disease severity (%) using a H-B scale by mango cultivar regardless of developmental stage.

Mango cultivar	Mean ¹	n	E.E.
'Parvin'	10.66 a ²	55	3.45
'Keitt'	11.14 ab	126	2.28
'Irwin'	18.43 ab	168	1.98
'Haden'	20.39 b	103	2.52

¹Disease severity was rated from 0 to 4, based on a visual scale developed by Lonsdale and Kontzé (1993), and converted to a midpoint as first suggested by Horsfall and Barratt (H-B) (1945). Data was pooled together from all inflorescence developmental stages evaluated.

²Different letters mean statistical differences using Tukey tests $\alpha = 0.05$.

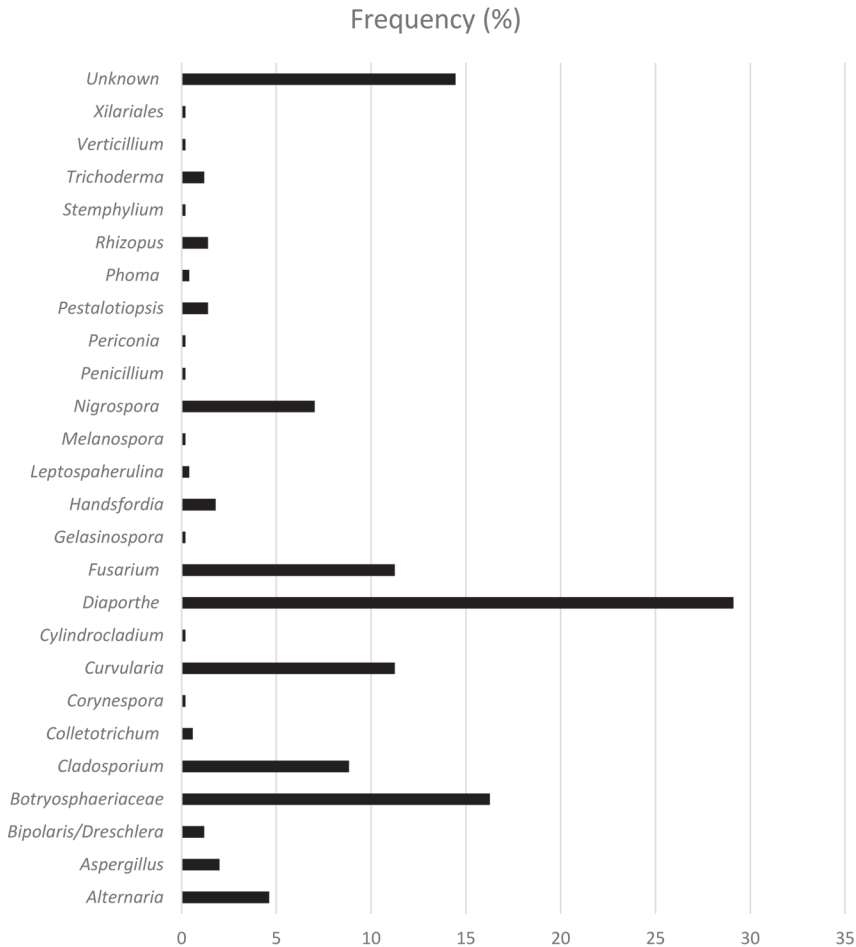


FIGURE 3. Frequency (%) of fungal genera isolated from mango inflorescences.

symptomatic tissue showing necrotic round spots and ellipsoidal lesions of rachis, pedicels and tip blight (Figure 2). In addition, they were also isolated from rachis cankers and insect perforations with necrotic borders (Figure 2D). Profuse black, dark to light grey mycelial growth was often associated with inflorescences harboring *Botryosphaeriaceae*. Mycelial threads were often confused with spider’s webs in the field and observation recreated after pathogenicity tests (Figure 4A). Among the species belonging to the Botryosphaeriaceae we identified: *B. dothidea*, *Lasidiopliidia theobromae* (syn. *B. rhodina*), *Neofusicoccum parvum* (syn. *B. parva*), *N. ribis* (syn. *B. ribis*), and *N. mangiferae*.

This family of fungi was isolated from all mango inflorescence stages of all cultivars evaluated including asymptomatic tissues.

Fusarium spp. were isolated from all inflorescence developmental stages and mango cultivars evaluated, from symptomatic and asymptomatic tissue. *Fusarium decemcellulare* was isolated from inflorescences with vascular wilt, necrotic margins of flowers, rachises and pedicels; and insect perforations with necrotic borders. It was also isolated from asymptomatic tissue. *Fusarium solani* was isolated from long ellipsoidal lesions of flowers and small necrotic spots of rachises, as well as from asymptomatic flowers. *Fusarium equiseti* and *Fusarium oxysporum* were isolated from irregular or elliptic necrotic lesions and tip blight.

All *Curvularia* spp. isolates were obtained from asymptomatic tissues of flowers and rachises from all mango cultivars examined. By molecular identification, two *Curvularia* sp. isolates were classified as *Cochliobolus lunatus* (Accession No. HM060592 and HM060602). *Cladosporium* spp. were isolated from symptomatic tissues of all mango cultivars examined and all flowering stages except for bud swell to bud break (stage A). *Cladosporium* spp. were associated with necrosis of rachis and flower sepals, and necrotic ellipsoidal lesions. *Cladosporium* spp. were often associated with other phytopathogenic fungi such as *Alternaria* spp., *B. rhodina*, *Diaporthe* spp. and *Fusarium solani*.

Alternaria spp. were isolated from flower, rachis and sepals associated with round and ellipsoidal necrotic lesions from all cultivars examined including asymptomatic tissues. *Alternaria alternata* was isolated from tip blight symptoms (Accession No. GU968430). In addition, *A. alternata* and *A. tenuissima* were isolated from asymptomatic tissues of flowers, rachises and sepals, often associated with *Fusarium decemcellulare* and *Bipolaris* spp.

To our surprise *Colletotrichum gloeosporioides* species complex (Weir et al., 2012) occurred at a very low frequency during the surveys (0.6%), even though necrotic symptoms in mango inflorescence are often attributed to this pathogen. It was associated with flower necrosis of bud-swell to bud-break stage (stage A), the first stage of development in cultivar 'Irwin'. Fungal complexes were often detected, for example, between *Alternaria* spp., the Botryosphaeriaceae, *Curvularia* sp., *Diaporthe* spp. and *Fusarium* spp.

Certain genera of plant pathogens identified occurred at very low frequencies, ranging from 0.2 to 3%, among them *Bipolaris/Dreschlera* spp., *Cylindrocladium* sp., *Pestalotiopsis* spp., *Phoma* spp., *Stemphylium* spp. and *Verticillium* sp. (Figure 3). Fourteen percent of the fungal specimens did not produce reproductive structures on culture media nor were they identified using molecular tools. These were categorized as unknown (Figure 3).

Fifty-nine percent of the fungi were isolated from asymptomatic inflorescences. Of these, 74% are important fungal pathogens. Among them, *Alternaria* spp., *Diaporthe* spp., various members of the Botryosphaeriaceae, and *Fusarium* spp.

Meteorological variables

Meteorological variables measured during the first survey of mango blooming season were: precipitation which averaged 1143 mm, relative humidity that ranged from 60 to 85 percent and temperatures that fluctuated from 21 to 28 °C. During the second survey, precipitation averaged 2108 mm and temperatures fluctuated from 29 to 33 °C, both variable measurements were higher than the previous year. Data of relative humidity was not available for the second survey.

Pathogenicity tests

Twenty-four fungal isolates were selected and evaluated during pathogenicity tests. Of these, the majority were pathogenic to mango inflorescences: *Alternaria* sp., *A. alternata*, *A. infectoria*, *B. dothidea*, *C. gloeosporioides* (complex), *Diaporthe* spp., *D. pseudomangiferae*, *Fusarium* sp., *F. decemcellulare*, *Lasiodiplodia theobromae*, *Neofusicoccum mangiferae*, *N. parvum*, *N. ribis* and *Phoma sorghina* (Table 3).

The most virulent fungi, affecting from 60% to 100% of the inflorescences, were *Diaporthe* spp., *D. pseudomangiferae*, *Fusarium* sp., *F. decemcellulare*, *L. theobromae*, *N. ribis*, *N. mangiferae* and *N. parvum*. Inflorescences of 'Haden' were entirely covered with grey mycelium of Botryosphaeriaceae: *L. theobromae*, *N. mangiferae*, *N. parvum* and *N. ribis*, eight days after inoculation (Figures 4A and G). All of them caused inflorescence tip blight in 'Haden' and 'Irwin' (Figures 4A, F and G; Table 3).

Some inflorescences exhibited severe necrosis, rachis soft rots or wilting, five or eight days after inoculation on 'Haden' or 'Irwin' (Figure 4). For example, *Diaporthe* spp. and *D. pseudomangiferae* caused extensive necrotic irregular lesions, cankers, and rachis soft rot. *Fusarium decemcellulare* caused vascular wilt and flower abortion along the rachises.

Various fungal species such as: *Alternaria* sp., *A. alternata*, *A. infectoria*, *B. dothidea*, *C. gloeosporioides* and *P. sorghina* were moderately pathogenic, affecting from 16 to 30% of the inflorescences. *Alternaria alternata* caused ellipsoidal necrotic lesions (Figure 4E). *Colletotrichum gloeosporioides* complex caused ellipsoidal necrotic lesion on rachis and flower abortion (Figure 4D). *Phoma sorghina* caused cankers in rachises of 'Irwin' (Figure 4B). Isolates identified as *Leptosphaerulina* spp. and *Phoma exigua* were not pathogenic to mango inflorescences.



FIGURE 4. Symptoms observed in mango inflorescences during pathogenicity tests: A) Mycelial threads of *L. theobromae* covering 100% of inflorescence, five days after inoculation (DAI); B) Cankers observed on rachis, 8 DAI with *Phoma sorghina*; C) Rachis rot observed 8 DAI with *Diaporthe* sp.; D.) Ellipsoidal necrotic lesion on rachis and flower abortion caused by *C. gloeosporioides*; E) Ellipsoidal lesions caused by *A. alternata* 5 DAI; F) Tip blight (arrow) and G) Mycelial threads of Botryosphaeriaceae, 8 DAI; H) Vascular wilt caused by *F. decemcellulare*, 8 DAI. Photos A to H of cv. 'Haden', with the exception of B, cv. 'Irwin'.

Fourteen percent of the fungal species isolated from inflorescences were not evaluated in pathogenicity tests. These were: *Aspergillus* spp., *Bipolaris* / *Dreschlera* spp. (syn. *Cochliobolus* spp.), *Cladosporium* spp., *Curvularia* spp., *Cylindrocladium* sp., *Gelasinospora* sp., *Handsfordia* spp., *Melanospora* sp., *Nigrospora* spp., *Penicillium* spp., *Periconia* sp., *Pestalotiosis* spp., *Rhizopus* spp., *Stemphylium* sp., *Trichoderma* spp., *Verticillium* sp. and specimens belonging to the Order Xylariales.

DISCUSSION

This is the first comprehensive study to identify fungal pathogens of inflorescences at different developmental stages of four mango cultivars in Puerto Rico. Necrosis, powdery mildew and tip blight were the most common symptoms observed in mango inflorescences. The most

affected inflorescence stage was full bloom (stage G) whereas early season stages such as bud swell to bud break (stage A) and mouse ear (stage B) were either asymptomatic or showed moderate symptoms ranging from 0.003 to 20.63% on the H-B scale. Antifungal compounds such as resorcinols [5-(12-cis-heptadecenyl)-resorcinol], present in the mango peel of immature fruit, could be responsible for the resistance against fungal diseases in inflorescences at early stages of development (Cojocarú et al., 1986). Another aspect to consider is that inflorescences at the full bloom stage (stage G) have been in the field longer, exposed to fungal spores, insect and scald damage, thus rendering them susceptible to pathogens.

Mango powdery mildew, *P. anacardii*, was observed starting at the green-colored stage (stage D) in 'Irwin', with full bloom (stage G), the most affected stage. Our findings are similar to those reported by Schoeman et al. (1995) in an epidemiological study conducted in powdery mildew of mango in South Africa. They observed powdery mildew symptoms from two to three weeks after inflorescences reached the red-colored stage (stage E) to full bloom (stage G); this last stage showed the most severe symptoms. Thus, mango inflorescences are susceptible to *P. anacardii* from the protected stage (stage C) to full bloom (stage G). Climatological conditions, especially cooler temperatures, are conducive to recurrent powdery mildew outbreaks in the southern part of the island.

In addition to powdery mildew, 26 fungal genera, mainly Ascomycetes, were identified as associated with these symptoms. *Diaporthe* (29%) and Botryosphaeriaceae (16%) were the most common fungi of mango inflorescences. Future studies should focus on the characterization of other *Diaporthe* spp., the most abundant genera (145 isolates) and members of the Botryosphaeriaceae (81 isolates). Botryosphaeriaceae are considered to be stress associated pathogens; for example, *B. dothidea* is one of the most widespread and important endophytes or latent pathogens, occurring on trees of agriculture, forestry and natural ecosystems of importance (Marsberg et al., 2017). In our study, this species was isolated from asymptomatic tissue and caused tip blight and rachis necrosis with >35% of mycelium coverage of inflorescences in 'Haden' and 'Irwin', 8 DAI. Other Botryosphaeriaceae species identified were: *L. theobromae*, *N. mangiferae*, *N. parvum* and *N. ribis*, common mango pathogens causing tip blight and extensive rachis necrosis, worldwide. We have previously reported *L. theobromae*, *N. mangiferae* and *N. parvum* as important fungal pathogens of mango inflorescences in Puerto Rico (Serrato-Díaz et al., 2013a, 2013b and 2014a). *Neofusicoccum parvum* has been reported as causing mango tip blight in Australia (Slippers et al., 2005), Brazil (de Oliveira Costa et al., 2010), Italy (Ismail et al., 2013), Perú (Javier-Alva et al., 2009), South Africa

(Jacobs et al., 2002) and New Zealand (Slippers et al., 2005). More recently, *Lasiodiplodia iraniensis* and *Neofusicoccum batangarum* isolated from mango tip blight were shown to be pathogenic, causing die-back to rambutan seedlings in Puerto Rico (Serrato-Díaz et al., 2020).

Sixty-four isolates belonging to different *Fusarium* species were identified as associated with mango inflorescences. Among those were *F. decemcellulare*, *F. equiseti*, *F. oxysporum* and *F. solani*. In 2015, we first reported that *F. decemcellulare* caused wilt and vascular flower necrosis in Puerto Rico (Serrato-Díaz et al., 2015). *Fusarium equiseti* is a cosmopolitan soil inhabitant and a common colonizer of senescent and damaged plant tissue; thus, its role as a plant pathogen should be treated cautiously (Leslie and Summerell, 2006). *Fusarium oxysporum*, a widely dispersed fungus, contains non-pathogenic and many pathogenic forms usually associated with vascular wilts (Leslie and Summerell, 2006). This heterogeneous species includes many *forma specialis* or host specific forms. *Fusarium oxysporum* has been reported as the predominant species associated with root rot and wilt of plantings in mango nurseries in Pakistan (Salam-Mengal et al., 2016). *Fusarium solani* species complex is cosmopolitan and has been recorded as a pathogen in diverse plant species. Detailed studies on the implications of *Fusarium* species in mango inflorescences need to be clarified. In mango, *Fusarium* spp. are often implicated in malformation of inflorescences and vegetative portions of the plant (Freeman et al., 2014). This symptom was not observed in the orchards and has not been reported in Puerto Rico.

Certain fungal species were not evaluated during pathogenicity tests because of their low frequencies (0.2 to 1.4 %) during the surveys or their recognized ecological habit as saprophytes (i.e., *Gelasinospora* sp., *Nigrospora* spp., *Periconia* sp., *Rhizopus* spp.), fungal parasites (i.e., *Handsfordia* sp.) or biological control agents (i.e., *Trichoderma* sp.). Some fungal species such as *Cladosporium* spp., *Cochliobolus* spp., *Curvularia* spp., *Cylindrocladium* sp., *Stemphylium* sp., and *Verticillium* sp. might have pathogenic potential on mango inflorescences and need to be further evaluated.

Various studies have shown the importance of endophytes or latent pathogens colonizing mango tissue as a key route for disease development during fruit maturity (Slippers et al., 2005; Morales-Rondón and Rodríguez-González, 2006). Many fungal pathogens identified in this study were isolated from asymptomatic tissues, occurring as endophytes or latent pathogens: *A. alternata*, various members of the Botryosphaeriaceae including *L. theobromae*, *C. gloeosporioides*, *Cladosporium* spp. and *F. decemcellulare*. Thus, the use of protectant fungicides will not be as effective as systemics in their control. The majority of these fungal species are known worldwide as necrotrophs of man-

go inflorescences (Ploetz, 2003). Endophytes such as *Curvularia* sp., which is the third most common genus isolated in this study, have been shown to provide thermal protection when growing inside plant tissues (Redman et al., 2002). According to Jumpponen (2001), under certain scenarios, dark septate endophytes are capable of forming mutualistic associations similar to those produced by mycorrhizas in roots. The ubiquitous presence of dark septate endophytes in plant tissues, besides roots, may imply a potential mutualistic nature that will provide benefits to the tree, an aspect that needs to be explored.

LITERATURE CITED

- Alfaro, A.N., 2010. Fincas de mangó pierden millones. El Nuevo Día. Retrieved from <https://www.elnuevodia.com/negocios/finanzas/nota/fincasdemangopierdenmillones-759504/>
- Alvarado, A.N., N. Acín and J. Zamora, 2004. Crop profile for mangos in Puerto Rico. <http://www.ipmcenters.org/cropprofiles/docs/PRmango.pdf>
- Álvarez-García, L., 1967. A dieback disease of mangos (*Mangifera indica* L.). *J. Agric. Univ. P.R.* 51(2): 191. <https://doi.org/10.46429/jaupr.v51i2.11467>
- Álvarez-García, L., 1968. *Physalospora rhodina* (Berk. Curt.) Cooke, the perfect stage of a *Diplodia* responsible for a dieback of mango in Puerto Rico. *J. Agric. Univ. P.R.* 52(3): 260. <https://doi.org/10.46429/jaupr.v52i3.11513>
- Álvarez-García, L.A. and J. López-García, 1971. Gummosis, die-back and fruit rot diseases of mango (*Mangifera indica* L.) caused by *Physalospora rhodina* (B&C.) Cke. in Puerto Rico. *J. Agric. Univ. P.R.* 55(4): 435-450. <https://doi.org/10.46429/jaupr.v55i4.11007>
- Barnett, H.L. and B.B. Hunter, 1998. Illustrated Genera of Imperfect Fungi. 4th ed. APS Press, St. Paul, Minnesota, USA. 218 pp.
- Boerema, G.H., J. de Gruyter, M.E. Noordeloos and M.E.C. Hamers, 2004. *Phoma* Identification Manual: Differentiation of Specific and Infra-specific Taxa in Culture. CABI Publishing, Wallingford, UK.
- Britz, H., E.T. Steenkamp, T.A. Coutinho, B.D. Wingfield, W.F.O. Marasas and M.J. Wingfield. 2002. Two new species of *Fusarium* section *Liseola* associated with mango malformation. *Mycologia* 94(4): 722-730.
- Braun U., R.T.A. Cook, A.J. Inman and H.-D Shin, 2002. The taxonomy of powdery mildew fungi: pp 13-55, In: R.R. Bélanger, W.R. Bushnell, A.J. Dik and T.L.W. Carver (eds). The Powdery Mildew: A Comprehensive Treatise. APS Press, St. Paul, MN, USA.
- Cojocaru, M., S. Drobny, E. Glotter, A. Goldman, H.E. Gottlieb, B. Jacoby and D. Prusky, 1986. 5-(12-heptadecenyl)-resorcinol, the major component of the antifungal activity in the peel of mango fruit. *Phytochemistry* 25(5): 1093-1095.
- Damm, U., P.F. Cannon, J.H.C. Woudenberg and P.W. Crous, 2012. The *Colletotrichum acutatum* species complex. *Studies in Mycology* 73: 37-113.
- de Oliveira Costa, V.S., S.J. Michereff, R. Brainer Martins, C.A. Tuão Gava, E. S. Gomide Mizubuti, M.P. Saraiva Câmara, 2010. Species of Botryosphaeriaceae associated on mango in Brazil. *Eur. J. Plant Pathol.* 127: 509-519.
- FAO, 2020. Major tropical fruits - Preliminary market results, 2019. Rome.
- FAO Statistic Database, 2018. <http://www.fao.org/faostat>.
- Félix-Gastélum, R., G. Herrera-Rodríguez, C. Martínez-Valenzuela, R.M. Longoria-Espinoza, I. E. Maldonado-Mendoza, F.R. Quiroz-Figueroa, J.C. Martínez-Álvarez, L.M. García-Pérez and S. Espinosa-Matías, 2013. First report of powdery mildew (*Pseudoidium anacardii*) of mango trees in Sinaloa, Mexico. *Plant Dis.* 97(7): 994.

- Freeman, S., D. Shtienberg, M. Maymon, A.G. Levin and R.C. Ploetz, 2014. New insights into mango malformation disease epidemiology lead to a new integrated management strategy for subtropical environments. *Plant Dis.* 98(11): 1456-1466.
- Gamliel-Atinsky, E., S. Freeman, M. Maymon, E. Belausov, R. Ochoa, G. R. Bauchan, A. Skoracka, J. E. Peña and E. Palevsky, 2009. The role of eriophyoids in fungal pathogen epidemiology, mere association or true interaction? *Experimental and Applied Acarology* 51(1-3): 191-204. Doi: 10.1007/s10493-009-9302-y.
- Gómez, A.R., 2018 (September 16). La agricultura está en franca mejoría. *El Nuevo Día*. Retrieved from <https://www.elnuevodia.com/negocios/empresas/nota/laagricultura-aestaen-franca-mejoria-2447379/>.
- Haggag, M.W., M. Hazza, A. Sehab and M. Abd El-Wahab, 2010. Epidemiology and the association of the *Fusarium* species with the mango malformation disease in Egypt. *Nature and Science* 8(4): 128-135.
- Hanlin, R.T., 1997. Illustrated genera of Ascomycetes Vol 1. APS Press. St Paul, USA. 263 pp.
- Horsfall, J.G. and R.W. Barratt, 1945. An improved grading system for measuring plant diseases. *Phytopathology* 35: 655.
- Ismail, A.M., G. Cirvilleri, L. Lombard, P.W. Crous, J.Z. Groenewald and G. Polizzi, 2013. Characterisation of *Neofusicoccum* species causing mango dieback in Italy. *J. Plant Path.* 95(3): 549-557.
- Jacobs, R., 2002. Characterisation of *Botryosphaeria* species from mango in South Africa. M.S. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa. 162 pp.
- Javier-Alva, J., D. Gramaje, L.A. Alvarez and J. Armengol, 2009. First report of *Neofusicoccum parvum* associated with dieback of mango trees in Peru. *Plant Dis.* 93(4): 426.
- Johnson, G.I., A.W. Cooke, A.J. Mead and I.A. Wells, 1991. Stem rot of mango in Australia: causes and control. *Acta Horticulturae* 291 (Abstract).
- Jumpponen, A., 2001. Dark septate endophytes - are they mycorrhizal? *Mycorrhiza* 11: 207-211.
- Kumar, J., U.S. Singh and S.P.S. Beniwal, 1993. Mango malformation: One hundred years of research. *Annu. Rev. Phytopathol.* (31): 217-232.
- Leslie, J.F. and B.A. Summerell, 2006. The *Fusarium* Laboratory Manual. *Blackwell Publishing*, Oxford, UK. 388 pp.
- Lonsdale, J.H. and J.M. Kotzé, 1993. Chemical control of mango blossom diseases and the effect on fruit set and yield. *Plant Dis.* 77: 558-562.
- Marasas, W.F.O., R.C. Ploetz, M.J. Wingfield, B.D. Wingfield and E.T. Steenkamp, 2006. Mango malformation disease and the associated *Fusarium* species. *Phytopathology* 96: 667-672.
- Marsberg A., M. Kemler, F. Jami, J.H. Nagel, A. Postma-Smidt, S. Naidoo, M.J. Wingfield, P.W. Crous, J.W. Spatafora, C.N. Hesse, B. Robbertse and B. Slippers, 2017. *Botryosphaeria dothidea*: A latent pathogen of global importance to woody plant health. *Molecular Plant Pathology* 18(4): 477-488.
- Morales-Rondón, V. and M. Rodríguez-González, 2006. Hongos endófitos en plantaciones de mango 'Haden' de la planicie de Maracaibo, Venezuela. *Rev. Fac. Agron.* 23(3): 273-284.
- Morton, J., 1987. Mango: pp 221-239, *In*: J. F. Morton (ed) Fruits of warm climates. Echo Point Books & Media, Brattleboro, VT. 505 pp.
- Nieves-Méndez, N., 2005. Etiología de la malformación del tejido vegetativo del mango (*Mangifera indica* L.) en Puerto Rico. M.S. Thesis, Department of Crop Protection, University of Puerto Rico, Mayagüez, PR. 98 pp.
- Nolla, J.A.B., 1926. Mango wither-tip. *J. Agric. Univ. P.R.* 10(3-4): 257-258. <https://doi.org/10.46429/jaupr.v10i3-4.15071>
- Otero-Colina, G., G. Rodríguez-Alvarado, S. Fernández-Pavía, M. Maymon, R.C. Ploetz, T. Aoki, K. O'Donnell and S. Freeman, 2010. Identification and characterization of a novel etiological agent of mango malformation disease in Mexico, *Fusarium mexicanum* sp. nov. *Phytopathology* 100: 1176-1184.

- Ploetz, R.C., 2003. Diseases of mango: pp 327-363, *In*: R.C. Ploetz (ed) Diseases of tropical fruit crops. CABI Publishing, UK. 527 pp.
- Prakash, O., 2003. Compendium of mango diseases and disorders. Capital Publishing Company, New Delhi. 84 pp.
- Puerto Rico Department of Agriculture, 2020. Agricultural Annual Gross Income, revised data and preliminary data. Commonwealth of Puerto Rico, San Juan, PR. <http://www.estadisticas.gobierno.pr>
- Ramos, L., J.S.P. Lara, R.T. Jr. MacMillan and K.R. Narayanan, 1991. Tip dieback of mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. *Plant Disease* 75: 315-318.
- Redman, R.S., K.B. Sheehan, R.G. Stout, R.J. Rodríguez and J.M. Henson, 2002. Thermotolerance generated by plant/fungal symbiosis. *Science* 298 (5598): 1581. <http://www.sciencemag.org/>
- Rivera, L., Y. Lugo, R. McGovern, T. Seijo and M. Davis, 2006. Occurrence and distribution of *Colletotrichum* spp. on mango (*Mangifera indica* L.) in Puerto Rico and Florida, USA. *Plant Pathology Journal* 5: 191-198.
- Salam-Mengal, A., S. Hussain, M. A. Abro, T. Nisa, M. R. Khetran, G. Yaseen Dahar, U. Zaib, S. Najeebullah Mushwani, A. Keerio and S. Ahmed Maari, 2016. Investigations on *Fusarium* wilt disease of mango nursery and Its *In-Vitro* control by applying different fungicides on the linear colony growth of *Fusarium oxysporum*. *International Journal of Fauna and Biological Studies* 3(3): 107-112.
- Schoeman, M.H., B.Q. Manicom and M.J. Wingfield, 1995. Epidemiology of powdery mildew on mango blossoms. *Plant Dis.* 79: 524-528.
- Serrato-Díaz, L.M., A. Avilés-Noriega, A. Soto-Bauzó, L.I. Rivera-Vargas, R. Goenaga and P. Bayman, 2020. Botryosphaeriaceae as causal agents of dieback and corky bark in rambutan and longan. *Plant Dis.* 104(1): 105-115.
- Serrato-Díaz, L.M., L.I. Rivera-Vargas and R.D. French-Monar, 2014a. First report of *Diaporthe pseudomangiferae* causing inflorescence rot, rachis canker and flower abortion of mango. *Plant Dis.* 98(7): 1004.
- Serrato-Díaz, L.M., L.I. Rivera-Vargas and R.D. French-Monar, 2014b. First report of *Neofusicoccum mangiferae* causing necrosis and inflorescences blight of mango (*Mangifera indica*) in Puerto Rico. *Plant Dis.* 98(4): 570.
- Serrato-Díaz, L.M., M. Pérez-Cuevas, L.I. Rivera-Vargas and R.D. French-Monar, 2013a. First report of *Lasiodiplodia theobromae* causing inflorescence blight of mango. *Plant Dis.* 97(10): 1380.
- Serrato-Díaz, L. M., M. Pérez-Cuevas, L.I. Rivera-Vargas and R.D. French-Monar, 2013b. First report of *Neofusicoccum parvum* causing rachis necrosis of mango (*Mangifera indica*) in Puerto Rico. *Plant Dis.* 97(10): 1381.
- Serrato-Díaz, L.M., M. Pérez-Cuevas, L.I. Rivera-Vargas, R. Goenaga and R.D. French-Monar, 2015. First report of *Fusarium decemcellulare* causing inflorescences wilt and vascular and flower necrosis of rambutan (*Nephelium lappaceum*), longan (*Dioscarpus longan*), and mango (*Mangifera indica*). *Plant Dis.* 99(8): 1187.
- Simmons, E.G., 2007. *Alternaria*. An Identification Manual. CBS Fungal Biodiversity Centre, Utrecht, Netherlands. 775 pp.
- Slippers, B., P.W. Crous, S. Denman, T.A. Coutinho, B.D. Wingfield and M.J. Wingfield, 2004. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* 96(1): 83-101.
- Slippers, B., G.I. Johnson, P.W. Crous, T.A. Coutinho, B.D. Wingfield and M.J. Wingfield, 2005. Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica*. *Mycologia* 97(1): 99-110.
- Toro, E.E., 1988. El cultivo de mangos en Puerto Rico. Servicio de Extensión Agrícola. Universidad de Puerto Rico, Recinto de Mayagüez. Colegio de Ciencias Agrícolas. 95pp.
- Úrbez-Torres, J.R., S. Rooney-Latham and C. Blomquist, 2011. *Botryosphaeria* Identification Workshop. National Plant Diagnostic Network. 3rd National Meeting. Berkeley, CA, 112 pp.

- USDA-NASS, 2014. 2012 Census of Agriculture. Puerto Rico Island and Municipal Data. Volume 1. Geographic Area Series. Part 52. 350 pp.
- Weir, B.S., P.R. Johnston and U. Dam, 2012. The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology* 73: 115-180.
- White, T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics: pp 315-322, *In*: M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White (eds). PCR Protocols-A Guide to Methods and Applications. Academic Press, London. 482 pp.