Research Note

FIRST REPORT OF *COLLETOTRICHUM ALATAE* ON WATER YAM (*DIOSCOREA ALATA*) CAUSING LEAF ANTHRACNOSE IN PUERTO RICO[1,2]

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Anthracnose caused by *Colletotrichum* spp. is the most destructive yam disease of *Dioscorea alata* cv. Florido in Puerto Rico. Under high disease levels, yield losses can be 50 to 90% (González-Vélez, 2006). From 2013 to 2015, yam plants (cv. Florido) with typical anthracnose-like symptoms were sampled at farms in four municipalities (Moca, Aguada, Barranquitas and Utuado). Observed symptoms ranged from small necrotic spots that enlarged to form reddish dry lesions to irregular brown lesions with necrotic borders and chlorotic halos. Pieces (1 to 2 mm) of symptomatic leaves and stem cut from lesions' margins were surface sterilized with 70% ethanol (1 min), 0.5% sodium hypochlorite (1 min), and rinsed three times with sterile water. Surface sterilized pieces were placed on Potato Dextrose Agar (PDA), amended with ten drops of 85% lactic acid to prevent bacterial contamination, and incubated at 27° C for seven days. Three single-spore isolates, F52251A, F52253A and F133251A, were selected for molecular, morphological and pathogenic characterization. Monosporic cultures grown on PDA ranged from 35 to 60 mm diameter after ten days at 25° C. Isolate F133251A showed the slowest growth rate (35.5 mm), followed by F52253A (49.0 mm) and F52251A (60.5 mm). Phenotypically fungal colony grown on PDA varied widely between isolates (Figures 1a, d and g). Isolate F52251A showed white sparse aerial mycelium, while F52253A showed aerial greyish white mycelium with dark acervuli and orange conidial masses near the center. Isolate F133251A showed a cottony white colony without visible acervuli and with abundant orange conidial masses. Conidia were one-celled, hyaline, straight with rounded to ovoid ends, and 14.5 µm x 4.7 µm in size, matching previous descriptions of *C. alatae* (Weir et al., 2012) (Figures 1c, f and i). Simple melanized appressoria varied in shape between isolates from elliptic to ovate (Figures 1b, e and h). Perithecia was absent on all isolates.

For molecular identification to species level, genomic DNA was extracted, and partial β-tubulin (TUB2), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and rDNA internal transcribed spacer region (ITS) genes were amplified and sequenced (Weir et al., 2012). Nucleotide sequences were deposited in the GenBank (Table 1) and compared with similar reference sequences of *Colletotrichum* spp. (Lin et al., 2018; Weir et al., 2012). Although it was established that rDNA ITS sequences distinguish *C. alatae* from all other taxa (Weir et al., 2012), a blast nucleotide search showed 100% homology with

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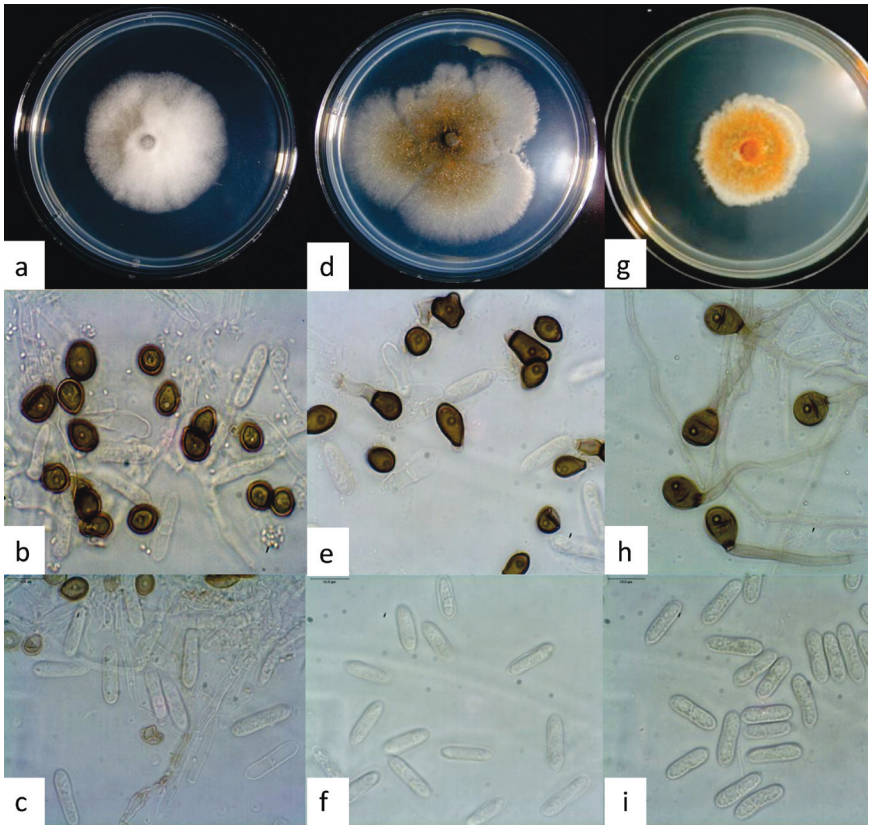

FIGURE 1. Isolates of *Colletotrichum alatae*. Isolate F52251A (a-c); F52253A (d-f); and F133251A (g-i). Appresorium (b, e and h) and conidium (c, f and i).

TABLE 1.—*GenBank accession numbers for Colletotrichum spp.* sequences of ITS, TUB2 and GAPDH gene.

		GenBank accession numbers		
	Isolate code	ITS[2]	TUB2	GAPDH
C. alatae	F52253A[1]	KR445669	–[3]	KU743260
	F52251A	KR445667	KU743279	KU743258
	F133251A	KR445671	KU743262	KU743262

[1]F= Isolates obtained from the survey of yam farms in four municipalities (Moca, Aguada, Barranquitas and Utuado).

[2]Abbreviations; ITS (Internal transcribe spacer), TUB2 (beta tubulin 2) and GAPDH (Glyceraldehyde-3-Phosphate Deshydrogenase).

[3]No accession number due to the low quality percentage in sequencing analysis.

TUB2 and GADPH genes of *Colletotrichum alatae* with reference sequences in GenBank (Accession No. TUB2: JX010449, GADPH: JX009990 and ITS: JX0101911).

Pathogenicity tests were conducted on detached healthy yam leaves artificially wounded with a sterile needle and unwounded, under controlled conditions. A 10-microliter drop of conidial suspension of 1×10^6 conidia/ml for each isolate was placed onto wounded and unwounded leaves and incubated in a moist chamber at 25° C. Control leaves were inoculated with sterilized water after wound inoculation with a sterile needle. After seven days of incubation at room temperature + 24° C. Isolates F133251A and F52253A did not cause lesions on unwounded leaves, while F52251A caused lesions on wounded and unwounded plant tissue. All isolates caused brown lesions with necrotic borders and chlorotic halos. All isolates were successfully re-isolated from symptomatic tissue, fulfilling Koch's postulates.

The pathogen was hence identified as *Colletotrichum alatae* based on its morphological and molecular characteristics. *Colletotrichum alatae* was a newly recognized species as a yam-specialized pathogen within the *C. gloesporioides* species complex (Weir et al., 2012), even though several names have been applied to *Colletotrichum*'s specimens causing anthracnose symptoms on yam worldwide. In 1966, it was named *C. gloeosporioides* "f. *alatae*" related to *Dioscorea alata* (Singh et al., 1966). In Puerto Rico, in 1982, based on morphological descriptions, the causal agent of foliar anthracnose on cv. Florido was described as *C. gloesporioides* (Mignucci et al., 1988). To our knowledge, this is the first record of *C. alatae* causing anthracnose on *D. alata* cv. Florido in Puerto Rico and the Caribbean Region.

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