First report of Fusarium anthophilum causing drying on Butia odorata in Rio Grande do Sul, Brazil

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Short Report

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Abstract

Butia spp. is a palm tree native to southern Brazil and Uruguay. The fruits are consumed fresh and used to make many food products, while the leaves are commonly used to make crafts. In January 2018, severe symptoms of wilt and drying were identified in clusters of *Butia odorata* occurring on the leaf and the rachis (in a proportion of 50% of leaf area). The pathogen was isolated from a stump extracted from a symptomatic tree. The morphologic characters showed a presence of macroconidia and microconidia with a classic salmon color of some species of the *Fusarium* genus. The molecular identification of the locus of actin (*ACT*), calmodulin (*CAL*), factor elongation 1-alpha (*TEF1*) and histone (*HIS3*), confirmed the identity of *Fusarium anthophilum*. This finding will help growers and agronomists prevent and manage this disease. To the best of our knowledge, this is the first report of *Fusarium antophilum* causing disease in *Butia odorata*.

Introduction

The genus *Butia* (Arecaceae) originated in South America and is widely distributed in the southern and southeastern regions of Brazil (Lorenzi et al., 2010). This genus comprises five species of which *Butia odorata*, grown in Rio Grande do Sul, is widely consumed as fresh fruit as well as in processed food products (ice cream, juice, liqueur, and candy) (Schwartz et al., 2010).

Several problems threaten the survival of natural populations of palm trees such as *Butia* spp., including the lack of regeneration due to seed dormancy, limited availability of studies focused on management, loss of natural habitat to urbanization, and attack of phytopathogens and pests (Buttow et al., 2010; Hoffmann et al., 2014). According to the U.S. National Fungus Collections Fungus-Host Distributions Database, the genus *Butia* has 33 records of pathogens associated with diseases, and 14 of these are described only in Florida (USA). Brazil has 6 records, of which, *Periconia* sp. and *Trabutia* sp. have been associated with *Butia leiospatha*, *Colletotrichum theobromicola* on *Butia odorata*, and *Coccostroma palmicola* and *Mycosphaerella advena* on *Butia* sp. (Farr and Rossman, 2022).

In the summer of 2018, several clusters of *Butia odorata* (called “butiazais”) with symptoms of wilt and drying in the basal and middle leaves were detected in Rio Grande do Sul, Brazil (Fig. 1). Drying was observed in the middle of the leaflet, sometimes accompanied by necrosis, and was limited by the midrib. Some trees with severe decay symptoms were dead or with most of the basal leaves showing one-side desiccated, and another side with a streak from reddish brown to dark brown at the base of the rachis and petiole. The diseased trees were found in an area of approximately one hectare, in which there was an incidence of 10% of a total of 100 adult trees (varying from 10 to 25 years old). In addition, 5% showed a severity of 100% and ended in the death of the palm tree. To preserve the conservation of the species and contribute to the study of the pathogenic factors that threaten the survival of this taxonomic group, this study aimed to determine the identity of the pathogen associated with drying and necrosis symptoms in *Butia odorata* trees.

Material and Methods

Isolation, identification, and pathogenicity

The pathogen was isolated from the stump of a cut tree with symptoms described previously. Portions of infected tissue (about 0.5 cm in diameter) were placed on the surface of petri plates with potato dextrose agar (PDA; KASVI) and incubated at 26°C under white fluorescent light with a 12 h photoperiod for 48 h. Subsequently, the hyphal tips were isolated from emerging colonies and transferred to a new plate with PDA under the conditions previously described.

For morphological identification, slides were prepared from fungal structures from a seven-day old colony using cotton blue and clear lactophenol. The observations and measurements were made with a light microscope (Leica DM1000) coupled to a camera (Leica DFC295) and the Leica Application Suite Core software (LAS V3.7). The intervals were generated from 30 measurements.

Molecular identification was performed from mycelium grown on PDA plates for nine days at 25°C. Genomic DNA was extracted using a DNA isolation kit (Norgen, Ontario, Canada) according to manufacturer's instructions. Five regions were targeted for PCR amplification and sequencing, including actin (*ACT*), calmodulin (*CAL*), histone (*HIS3*), translation elongation factor 1-α (*TEF1*), and 5.8S nrDNA of the internal transcribed spacer region (ITS). Amplifications were performed with an automated thermal cycler (MJ Research PTC–200) using a PCR master mix (Thermo Fisher Scientific, MA, USA). The reaction was carried out at final volume of 25 µL, composed of 12.5 µL of PCR master mix, 1 µL (10 pmol) of each primer pair, 1 µL of DNA (30 ng/µL), and 9.5 µL of sterile distilled water. Primers and PCR programs used for the amplification are described in Victoria Arellano *et al*., (2021). Amplicons were purified with a PCR cleaning product (Mebep Bioscience, Shenzhen, China) and the PCR fragments were sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems, USA) following the manufacturer's protocol. The products were analyzed on an ABI Prism 3730 XL DNA sequencer (Applied Biosystems). Contigs were generated by comparing (forward and reverse sequences) and manually editing in MEGA (Molecular Evolutionary Genetics Analysis, version 11) when necessary (Tamura et al., 2021). Consensus sequences were compared in megablast (NCBI), to determine the percentage of similarity of the pathogen for each analyzed locus (Altschul et al., 1990). The sequences derived in this study were deposited in GenBank.

The pathogenicity test followed Koch's postulates. Four sets of three healthy one year old *Butia odorata* plants were inoculated by needle injection. Three of these sets were inoculated using three different concentrations of spores (10^6, 10^7, and 10^8 conidia mL^{-1}), and the remaining set was inoculated with sterile distilled water. All the concentrations were applied in the same volume (2 mL) in the bulb of the plant. Plants were transferred to a greenhouse maintained at 24 to 28°C, and 85 ± 5% relative humidity, and checked for symptoms every 8 h. Fifteen days after inoculation, fungal reisolation was performed from infected necrotic tissue. The original fungi isolated were compared morphologically with those previously inoculated.

Results

According to morphologic, biometric, and cultural characteristics, the isolated fungi (named ACRE) were identified as *Fusarium fijikuroi* species complex sensu lato and with high similarity to *Fusarium anthophilum* (A. Braun) Wollenweber 1916. The fungus was characterized by showing white farinose colonies with abundant white floccose mycelia. Colony on PDA initially presented white aerial mycelium, becoming pink-pale to pale-salmon in color on the undersurface. Two morphologic types (mainly) of microconidia were observed: oval to clavate microconidia, (1.32–2.76 × 3.33–7.02 µm), 0-or 1-septate, and the cylindrical or allantoid microconidia (1.82–3.42 × 7.02–11.7 µm) produced on false heads on mono- and polyphialidic conidiogenous cells. Macroconidia were few, very slightly sickle shaped to nearly straight, 2–7 septate, measuring 33–62 × 2.5–3.4 µm. Chlamydospores and the teleomorph were not observed.

Molecular identification based on target genes *ACT*, *CAL*, *HIS3, TEF1*, and ITS revealed that the ACRE fungal isolate showed 100% identity with *Fusarium anthophilum* sensu stricto in the *Fusarium fijikuroi* complex for the accession numbers MT010879 (ACT) and MT010898 (CAL), 99% for KF466359 (HIS3), and 99.6% for MT010997 (TEF1). On the other hand, the ITS region had 100% identity for the accession EU567316 of *Gibberella moniliformis*, which is a synonym of *F. anthophilum* according to the Index Fungorum database. The sequences derived from this study were saved in the GenBank database under the accession numbers OQ184876 (ACT), OQ184877 (CAL), OQ184878 (HIS3), OQ184875 (TEF1), and OQ176228 (ITS).

At least one complete leaf of one-year-old plants (containing two to three leaves) showed necrosis symptoms on one side of the plant. The symptom first developed twelve days after inoculation (dai) with $2x10^{8}$ conidia mL^{-1} on the youngest leaf starting at the apical part of the leaf. The necrosis progressed downwards towards the bulb, which presented symptoms of necrosis through day 36 (dai), yet the plant survived with only one leaf. It should be noted that with the remaining lower inoculum concentrations, no alteration was observed during the experiment. The pathogen reisolated after 15 days had the same morphology and coloration of the colony as the fungus initially inoculated.

Discussion

In this study, clusters of *Butia odorata* were found in southern Brazil with symptoms of necrosis in adult palm leaves, mainly in the basal and middle part of the plant. The ACRE isolate was found associated with a rot in the center of the stem in xylem vessels. The ACRE isolate was pathogenic to one-year-old plants. The morphological and molecular identification revealed the identity of the associated fungus, which corresponds to *Fusarium athophilum*. This pathogen is pathogenic to plants of *Allium cepa*, *Acacia koa*, *Austrostipa aristiglumis, Dasylirion longissimum*, *Eucalyptus grandis*, *Euphorbia pulcherrima*, *Gossypium* sp., *Hippeastrum* sp., *Lotus corniculatus, Lythrum salicaria, Mangifera indica, Pinus elliottii, Plantago* sp., *Protea burchellii, Protea longifolia, Protea magnifica, Protea neriifolia, Protea nitida, Protea repens, Triticum aestivum*, and *Zizania palustris* (Farr and Rossman, 2022). In Brazil, *Fusarium*

anthophilum has been previously reported in *Vitis* sp. and *Tabebuia impetiginosa* (Farr and Rossman, 2022). *Fusarium anthophilum* has not previously been described to cause disease in *Butia odorata*.

Butia palms in Brazil and Uruguay, where this species is native, are threatened by this pathogen which has been seen to cause plant death. The identification of this organism provides a starting point for growers and agronomists to develop management strategies to counteract this disease. To the best of our knowledge, this is the first report of *Fusarium anthophilum* causing necrosis in *Butia odorata*.

Declarations

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Statements and Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

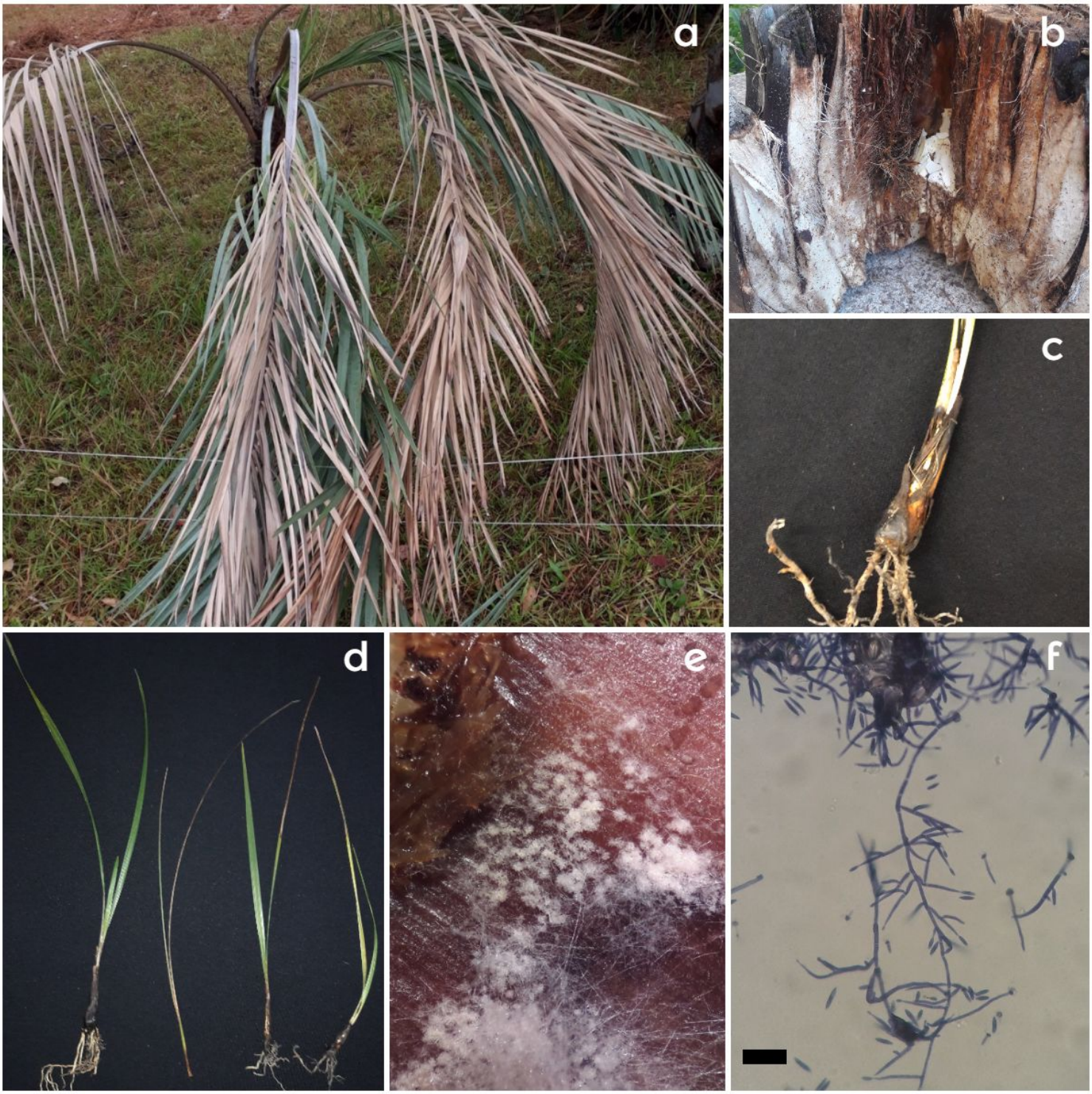

Figure 1

a. *Fusarium anthophilum* symptoms on *Butia odorata.* **b.** infected stump of an adult *Butia odorata* plant. **c.** Infected bulb of a one-year-old plant, 15 days after inoculation. **d.** Plants inoculated using three different concentrations of spores (10^6, 10^7 and 10^8 conidia mL^{-1} and the control) starting from right to left. **e.** *Fusarium anthophilum* colony on the surface of xylem tissue. **f.** clavate or allantoid microconidia, scale bar (10 µm).