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First Report of Fusarium Wilt and Pink Rot of *Phoenix canariensis* in South Africa

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ABSTRACT

Canary Island date palm, *Phoenix canariensis*, is a popular ornamental species commonly planted in urban areas worldwide, including South Africa. In November 2023, symptoms typical of Fusarium wilt were detected on ornamental palms at the Waterfront in Cape Town, Western Cape Province, South Africa. Samples were collected from three wilting palms with one-sided frond death and pink sporulation on the bark. Isolations to culture media yielded two fungal species, which were confirmed based on DNA sequence data as *Fusarium oxysporum* f.sp. *canariensis* (Foc) and *Nalanthamala vermoesenii*. Fusarium wilt, caused by Foc, is among the most serious diseases of these palms. There is no effective treatment for this vascular wilt disease and infected palms inevitably die. Pink rot, caused by *N. vermoesenii,* is commonly found as a secondary infection associated with Fusarium wilt in *P. canariensis*. This is the first detection of the Fusarium wilt and pink rot pathogens in South Africa.

1 | Introduction

Canary Island date palm, *Phoenix canariensis* (Arecaceae), is endemic to the Canary Islands (Spain) but widely cultivated as an ornamental in warm temperate areas of the world. In South Africa, it is a popular species in ornamental plantings, especially in the Western Cape Province (WC), which features a Mediterranean climate.

One of the most serious diseases affecting *P. canariensis* is Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *canariensis* (Foc). The pathogen infects and blocks the vascular tissues of the fronds leading to wilting of aerial organs. Distinctive symptoms include one-sided frond wilting, vascular discolouration and eventually frond death. The pathogen *Nalanthamala vermoesenii* causes 'pink rot' disease on various palm species (Schroers et al. 2005); however, it is also commonly transmitted together with Foc where it acts as secondary pathogen (Downer et al. 2009). There is no known effective treatment for Fusarium wilt and infected palms inevitably die in a period that can span from two to several years (Laurence, Summerell, and Liew 2015). The disease has been detected in Morocco, United States, China, Japan, France, Greece, Italy, Spain (Canary Islands), Argentina and Australia (EPPO Global Database, https://gd.eppo.int, [Access date: 22 July 2024]).

In November of 2023, three ornamental palms displaying typical symptoms of wilt were identified at the waterfront area in Cape Town, Western Cape, South Africa (Figure 1a,b). Samples were collected and isolations were made in the laboratories of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

FIGURE 1 | Symptoms, signs and isolations from diseased *Phoenix canariensis* in Cape Town, South Africa. (a-b) Wilting ornamental palms at the V&A Waterfront. (c) Section of frond displaying 'one-sided' wilt. (d-f) Cross sections of rachis (d-e) and petiole (f) with vascular discolouration. (g) Pink sporulation of *Nalanthamala vermoesenii* on pieces of bark. (h) Isolates on culture media, *N. vermoesenii* on MEA (left) and *Fusarium oxysporum* f.sp. *canariensis* on ½ PDA (right). Photo credits: (a-b) Paul Barker.

2 | Materials and Methods

Samples were collected from three adult *P. canariensis* palms at the V&A Waterfront, Cape Town (GPS coordinates: −33.904405, 18.41816). These included segments of petioles, rachis and pinnae and pieces of bark. Isolations were made from the margins of vascular discolouration and from mycelium and spores present in the samples. For isolations from lesion margins, tissues were briefly surface disinfested with 70% ethanol before plating onto 2% malt extract agar (MEA: 20 g/L malt extract and 20 g/L agar; Biolab) and *Fusarium* selective medium (FSM; Nash and Snyder 1962). Isolations were incubated at room temperature (~23°C) for 4 days, after which emerging mycelium was transferred to fresh MEA and half strength potato dextrose agar (½PDA: 19.5 g/L PDA and 10 g/L agar; Merck).

The isolated fungal cultures were initially identified based on morphology, using a light microscope. They were then grouped into two distinct morphotypes and representative isolates were selected for DNA sequencing. DNA extractions were carried out using the Prepman Ultra Sample Preparation Reagent kit (Thermo Fisher Scientifc, Waltham, MA), following the manufacturer's protocols. The translation elongation factor 1 alpha (*tef1*) partial gene region was amplified for isolates putatively identified as *Fusarium*, using primers EF1 and EF2. For isolates of the second morphotype, putatively identified as a *Nalanthamala* sp., the ITS gene region was sequenced using primers ITS-1 and ITS-4. PCR products were cleaned using ExoSAP-IT PCR Product Cleanup Reagent (Applied Biosystems, Thermo Fisher). The BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific, USA)

was used for sequencing the PCR product in both directions. Sequencing was carried out at the DNA Sanger sequencing facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria.

The produced DNA sequences were submitted to GenBank's BLAST utility (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Based on the results, a sequence dataset was built using MEGA7 for phylogenetic analyses. Alignment was done using MAFFT v.7 online server (https://mafft.cbrc.jp/alignment/server/), then trimmed using MEGA7. A maximum likelihood phylogenetic tree was built using IQ-TREE online server (http://iqtree.cibiv.univie.ac.at/) using the best model selected by ModelFinder built into IQ-TREE. The resulting tree was visualised and modified using FigTree v.1.4.4 and Affinity Designer v. 1.10.5.1342.

3 | Results and Discussion

Cross sections of diseased tissues revealed vascular discolouration in all aerial organ samples from the three sampled palms (Figure 1c–f), with white mycelium emerging from some of the tissues. Bark samples from one of the palms were extensively covered with white/pink mycelium and sporulation (Figure 1g,h). Isolations from plant tissues and fungal structures yielded cultures having two distinct morphotypes, with both present in samples from all three palms sampled.

Based on morphological observations, one of the morphotypes was a *Fusarium* species (isolated from vascular discoloured tissues) and the other resembled *Nalanthamala* species (isolated from plant tissues and sporulation). All *Fusarium* isolates had identical morphological features and *tef1* sequences (Genbank accession numbers PP782538–PP782542). BLAST analyses resulted in 100% identity exclusively with isolates of *Fusarium oxysporum* f.sp. *canariensis* (highest score accession numbers: KM893865, HM591537 and JF826442). Phylogenetic analysis (Figure 2) resolved sequences of our isolates as identical to those of Foc isolates from various geographic locations, confirming their identity. The ITS sequences for the second morphotype (Genbank accession numbers: PP767248 and PP767249) had 100% identity exclusively with isolates of *Nalanthamala vermoesenii* (highest score accession numbers: NR_145023 and KC894849). It was not necessary to perform phylogenetic analysis for this fungus. Isolates of these fungi have been deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Based on the observed symptoms, consistency of isolations, robustness of sequence data-based identification and association of the two known fungal pathogens, this is considered as sufficient evidence to confirm the presence of Fusarium wilt and pink rot in South Africa. This is the first report of *F. oxysporum* f.sp. *canariensis* and *N. vermoesenii* in South Africa.

The emergence of Fusarium wilt and pink rot on palms in the Western Cape is likely the result of a recent introduction. The V&A Waterfront, which has approximately 150 adult *P. canariensis* palms, is a popular international tourist destination. In addition, this area is in close proximity to the highly active Port of Cape Town. Fusarium wilt is most commonly spread through the movement of contaminated plant material, which may remain asymptomatic for long periods of time. Additionally, suboptimal hygiene practices in plant management, such as the use of contaminated pruning tools, can also contribute to its spread. The pink rot pathogen was likely moved together with the same material.

Fusarium wilt has caused drastic changes in urban *P. canariensis* plantings in countries where it has established, particularly in the United States and Australia (Elliott 2018). Although *P. canariensis* is the main and most susceptible host, other palm species including *P. reclinata*, *P. sylvestris* and *P. dactylifera* and *Washingtonia* sp. are also known to be susceptible (Elliott 2018). These palm species are found in South Africa as ornamentals, cultivated for agricultural purposes (*P. dactylifera*), and in the case of *P. reclinata*, as part of its native range. The detection of *F. oxysporum* f.sp. *canariensis* on palms in South Africa deserves careful consideration. In the light of reported pathogenicity towards *P. reclinata* (Elliott 2018), a risk analysis should be carried out to support regulatory listing of Foc under NEM:BA

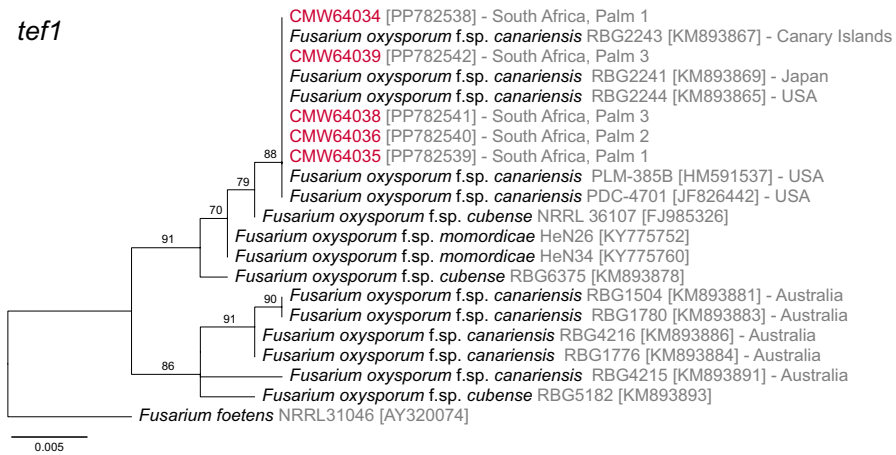

FIGURE 2 | Maximum likelihood phylogenetic tree with sequences of the translation elongation factor 1 alpha (*tef1*). Isolates obtained in the present study are highlighted in colour. GenBank accession numbers are shown in between brackets. Numbers on branches indicate bootstrap values ($n = 1000$).

A&IS Regulations (South African National Environmental Management: Biodiversity Act [NEM:BA, Act 10 of 2004] Alien and Invasive Species Regulations; Department of Environment Forestry and Fisheries 2020). Based on current observations of the extent of the incursion, we regard Foc as 'reproducing' in South Africa under the guidelines of Paap et al. (2022), albeit with low confidence (Foc might have spread further that the current site or alternatively all infected individuals might have been infected from the same initial introduction without any requirement for spread post-introduction). Further surveys and engagement with those cultivating palms is recommended to establish the current status of Foc in South Africa and to limit future spread.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

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