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SHORT COMMUNICATION

First record of the white root rot fungus *Dematophora necatrix* on indigenous South African trees

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Abstract

The soilborne fungus *Dematophora* (=*Rosellinia*) *necatrix* and causal agent of white root rot, has a wide host range that includes many tree species of economic importance. The pathogen has a worldwide distribution, including reports from commercial orchards in South Africa since the 1970s. During tree health surveys conducted as part of a sentinel plant project, we detected the pathogen on two symptomatic individuals from the indigenous South African tree species *Diospyros whyteana* and *Noronhia foveolata* subsp. *major*. Observed symptoms included wilting and root rot, with characteristic white mycelium present under the bark. *Dematophora necatrix* was isolated from both hosts and its identity confirmed by sequencing two gene regions (ITS and actin). Pathogenicity was confirmed through inoculation tests under semi-controlled conditions by inoculating the soil with bamboo sticks colonized with the fungus. Plants of both species developed similar symptoms to those observed naturally, and the fungus was successfully recovered from all symptomatic plants. This is the first record in South Africa of *D. necatrix* on indigenous species and outside of commercial orchards. Given the environmental threat posed we recommend the fungus is considered for regulation in South Africa.

Keywords *Noronhia foveolata* subsp. *major* · *Diospyros whyteana* · *Rosellinia necatrix* · White root rot

Dematophora (=*Rosellinia*) *necatrix* is the causal agent of white root rot disease on a wide range of mainly woody hosts globally. On susceptible hosts, the fungus infects the roots and root collars, with white mycelium developing both outside and under the bark as it rots the tissues causing the host to wilt and ultimately die. This cryptogenic pathogen has been reported from more than 340 hosts and more than 30 countries on all continents except Antarctica (GBIF 2024; USDA 2024). The most serious impacts reported to date are from economically important crop plants including apple, pear, avocado, olive and grapevine (Kulshrestha et al. 2014). This has led to it being regulated as a quarantine organism in countries including the USA, Canada and Egypt and as a non-quarantine pathogen in the European Union (EPPO 2024).

Dematophora necatrix was first recorded in South Africa in 1974 from pear and apple orchards in the Western Cape (WC) province (Van der Merwe and Matthee 1974). Since then, it has been recorded in vineyards (Marais 1980) as well as avocado and macadamia orchards (van den Berg et al. 2018; Botha et al. 2021), with its presence confirmed in three provinces (WC, Limpopo and Mpumalanga). Although the pathogen is known to have a wide host range, reports on South African indigenous species are limited to commercially cultivated Proteaceae on Madeira Island (Portugal; Moura and Rodrigues 2001). However, there is no mention of pathogenicity tests being carried out (Koch's postulates) and thus susceptibility of those plants is based only on field observations.

Symptoms characteristic of white root rot were observed on two indigenous South African tree species during tree health surveys conducted in 2022 and 2023. These surveys were undertaken as part of the project 'Monitoring tree health at sentinel sites: botanic gardens and arboreta' (https://www.fabinet.up.ac.za/index.php/sentinel-plant-network), a collaboration between the South African National

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Fig. 1 White root rot symptoms on *Diospyros whyteana*. **a** Sample collected at Arderne Gardens (Cape Town, Western Cape) with root and collar rot and small pockets of white mycelium under the bark. **b**–**g** Results of pathogenicity tests showing aerial organs, roots and root collar of plants used as controls (**b**–**d**) and those inoculated (**e**–**g**) with *Dematophora necatrix*, respectively

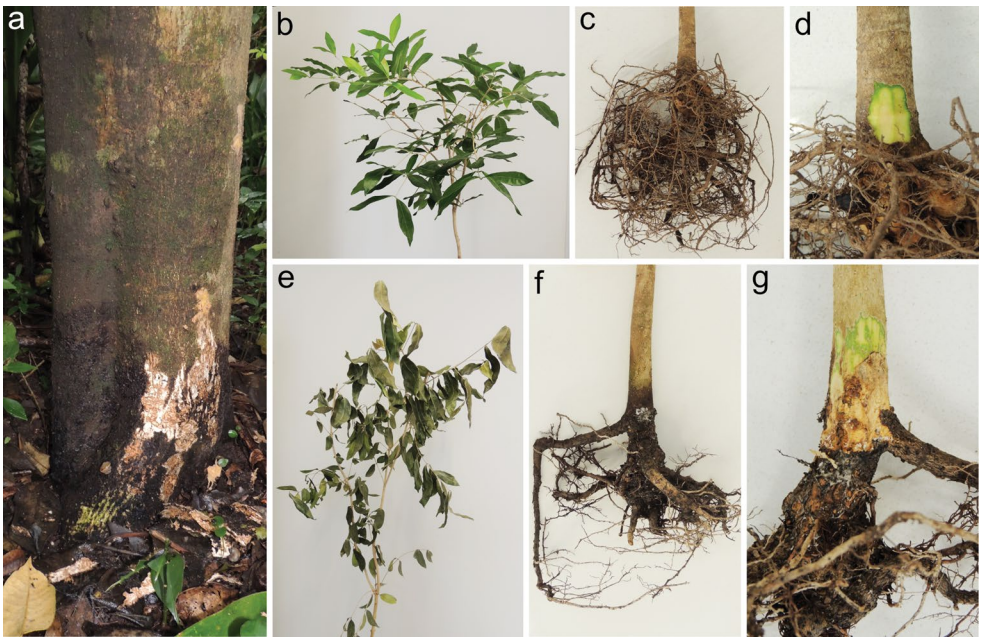

Fig. 2 White root rot symptoms on *Noronhia foveolata* subsp. *major* (**a**) Sampled tree at Lowveld NBG (Mbombela/Nelspruit, Mpumalanga) with collar rot and white mycelium under the bark. **b**–**g** Results of pathogenicity tests showing aerial organs, roots and root collar of plants used as controls (**b**–**d**) and those inoculated (**e**–**g**) with *Dematophora necatrix*, respectively

Biodiversity Institute (SANBI) and the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. A wilting *Diospyros whyteana* (bladdernut, Ebenaceae) plant was found at the Arderne Gardens in Cape Town (Fig. 1a; August 2022, GPS coordinates: −33.987579, 18.463875) and a wilting *Noronhia fovoleata* subsp. *major* (=*Chionanthus foveolatus* subsp. *major*; forest pock ironwood; Oleaceae) tree was detected at the Lowveld National Botanical Garden (NBG) in Mbombela (=Nelspruit; Fig. 2a; January 2023, GPS coordinates: −25.44488, 30.96839). Samples were collected from both species and isolations were made on the day of collection. These included isolations from lesion margins (for *Di. whyteana*) and from white mycelium present under the bark (both species). Isolations were made to Petri dishes containing 2% malt extract agar (MEA; 20 g/L malt extract, 20 g/L agar; Biolab), and then allowed to grow at room temperature for up to 14 days before fungal colonies were transferred into fresh MEA plates. In both cases, a predominant fungal morphotype was obtained, which consisted of fluffy white colonies with abundant aerial mycelium mostly at the edges. Fungal isolates obtained from both hosts were deposited in the culture collection (CMW) of FABI.

One fungal isolate from each host (CMW61830 from *Di. whyteana* and CMW61831 from *N. foveolata* subsp. *major*) was selected for identification by DNA sequencing. DNA extractions from growing colonies were made using the Prepman® Ultra Sample Preparation Reagent kit (Thermo

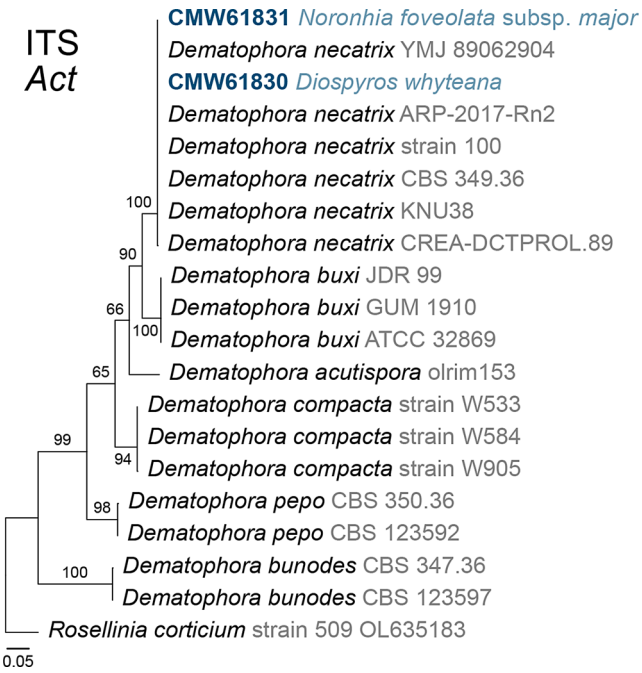

Fig. 3 Maximum likelihood phylogenetic tree for the concatenated ITS and *Act* gene regions for *Dematophora* species. Isolates obtained in this study are highlighted in blue. Numbers on branches represent Ultrafast Bootstrap values (n = 1000)

Fisher Scientifc, Waltham, MA) following the manufacturer's instructions. The ITS and partial actin gene (*Act*) regions were amplified using the ITS1/ITS 4 (White et al. 1990) and ACT-512F/ACT-783R (Carbone and Kohn 1999) primers, respectively. PCR products were cleaned using ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™, Thermo Fisher) and the BigDye™ Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific, USA) was used to sequence these in both directions. Sanger sequencing was carried out at the DNA Sanger sequencing facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria.

Resulting DNA sequences were assembled using CLC Main Workbench v. 21.0.3 and the generated consensus sequences (ITS: PQ498867, PQ498868; *Act*: PQ511219, PQ511220) were submitted to GenBank's BLAST utility (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST analyses with sequences from both hosts resulted in 100% identity with sequences of *D. necatrix* both for the ITS (MK888684, MH855818) and for the *Act* (EF025588, MG273315) gene regions. Based on these results, two datasets (ITS and *Act*) with the sequences from GenBank for all available *Dematophora* species were built. Datasets were aligned using MAFFT v.7 online server (https://mafft.cbrc.jp/alignment/server/) and then edited and trimmed using MEGA v7.0.26. This resulted in a dataset of 20 taxa and 567 bp long for the ITS gene region, and 9 taxa and 279 bp long for *Act.*

Phylogenetic analyses were carried using IQ-TREE online server (http://iqtree.cibiv.univie.ac.at/) for each datas et and both concatenated (using partitioned data). Maximum likelihood trees were calculated using the best model based on ModelFinder built into IQ-TREE. The resulting tree was visualized and modified using FigTree v.1.4.4 and Affinity Designer v. 1.10.5.1342. The calculated trees (individual and concatenated datasets; Fig. 3) resolved sequences from isolates obtained in this study together with those of *D. necatrix* with support values above 95%. These results confirmed the identity of our isolates as the white root rot pathogen *D. necatrix.*

To confirm pathogenicity, plants of both hosts were obtained and maintained in phytotrons with semi-controlled conditions at FABI. *Diospyros whyteana* plants were purchased at an indigenous nursery and *N. foveolata* subsp. *major* plants were provided by the Lowveld NBG. The same isolates used in phylogenetic analyses were used for pathogenicity tests on their respective isolation host. Inoculations were performed using the method described by Negishi et al. (2011) and van den Berg et al. (2018). Three copies of each isolate were made on MEA and allowed to grow at 25 ± 1 °C for seven days. Autoclaved pieces of bamboo sticks (2 cm; 8–10/plate) were then placed on top of the mycelium and incubated for an additional seven days. Once the mycelium had covered the sticks, these were inserted perpendicularly into the soil 2–3 cm from the collars of the plants and covered with soil. Four pieces of fungus-colonised bamboo sticks were used per plant and non-colonized sterile pieces were used for the controls. Six *Di. whyteana* plants (4 inoculated, 2 control) and 10 *N. foveolata* subsp. *major* (8 inoculated, 2 control) were used in the trial. Plants were kept in the phytotrons at FABI with watering every second day and continuous monitoring for the appearance of disease symptoms.

Disease symptoms (wilting and white mycelium on the collar) were first visible on *Di. whyteana* three weeks after inoculation. By the fourth week, all inoculated plants were wilting (Fig. 1e), while controls were asymptomatic (Fig. 1b). Inoculated plants had abundant white mycelium covering roots and collars. Inspection of these organs revealed severe root rot and a widespread black discolouration under the bark of both roots and root collars (Fig. 1f, g), similar to the symptoms originally observed in the field. Disease symptoms were slower to develop on *N. foveolata* subsp. *major*. After four weeks, water-soaked lesions had developed on the collars. Wilting of aerial organs (Fig. 2e) and appearance of white mycelium around the collars (Fig. 2f) were observed on two plants 12 weeks after inoculation. Examination of roots and root collars after 15 weeks revealed that all inoculated *N. foveolata* subsp. *major* plants had discoloured tissues under the bark. The infection in

most plants spread predominantly in the rootcollars and main roots, with discoloured tissues and pockets of white mycelium present (Fig. 2g). None of the control plants had signs of wilting or discoloured tissues under the bark, and roots were all apparently healthy. *Dematophora necatrix* was recovered from all inoculated plants of both species but not from any of the controls.

The results from this study confirm the presence and pathogenicity of *D. necatrix* on two South African indigenous tree species (*Di. whyteana* and *N. foveolata* subsp. *major*) and suggest that this disease represents a threat to these and potentially other indigenous trees. Our observations of the naturally infected trees and the inoculation trials suggest that the disease is facilitated by moist soils and high temperatures, a view also noted by Anselmi and Giorcelli (1990). Nevertheless, only a single tree of each species was found displaying symptoms in each of the sampled gardens, and the rate of spread and impact of the disease has not been estimated.

Both gardens visited are located in urban areas close to the native ranges of the respective host plants. Therefore, the disease could spread to natural populations, and so a management response should be considered. The pathway of introduction of *D. necatrix* into these gardens is unknown, as the inoculum could originate from and be transported via numerous sources, although it is most commonly in contaminated soil and/or plants. Nevertheless, both gardens are in provinces with extensive areas of agricultural production, where the presence of *D. necatrix* has been confirmed in numerous orchards (Hartley et al. 2022).

Although this is the first record of white root rot caused by *D. necatrix* on *Di. whyteana*, susceptibility of other *Diospyros* species has been demonstrated. This includes the commercially produced *Di. kaki* (Asian persimmon) and *Di. virginiana* (American persimmon; Sztejnberg and Jabareen 1986); the former being commercially cultivated in the WC province of South Africa (Nunez 2013). This the first record of *D. necatrix* on a plant in the genus *Noronhia*, but there is a single record on the sister genus *Chionanthus* (*C. retusus* in Japan; Takemoto et al. 2011) and numerous records for species in the Oleaceae, including in *Forsythia, Fraxinus, Jasminum, Ligustrum, Olea, Osmanthus* and *Syringa* (USDA 2024). Most of these records, however, are either inaccessible or lack details regarding impact on these hosts.

There are 18 indigenous *Diospyros* species (43 taxa if including sub-species; 11 endemic) and three *Noronhia* species (6 taxa including sub-species; 2 endemic) in South Africa. Results of this study showing pathogenicity on *Di. whyteana* and *N. foveolata* subsp. *major,* combined with reports of *D. necatrix* affecting cultivated South African Proteaceae in Madeira (Moura and Rodrigues 2001) as well as members of the Oleaceae in numerous countries, highlight the potential threat this invasive pathogen poses to indigenous South African flora. As such, a risk analysis should be conducted to consider the regulatory listing of *D. necatrix* under the NEM:BA 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).

The present study represents only an early indication of a potential problem and the pathogen has not yet been found in natural areas. Yet it is the first record of an aggressive pathogen on indigenous South African species and the first report from an environment outside of a commercial orchard. For this reason, we contend that *D. necatrix* should be regarded as 'colonising' (or invasive; D1) in South Africa following the guidelines of Paap et al. (2022). Further research on the extent and susceptibility of South African indigenous plants to *D. necatrix* should be undertaken to better understand the risks associated with this disease, and to further support the process of regulation and the development of a management strategy.

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Declarations

Conflict of interest The authors declare no competing interests.

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