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NEW DISEASE REPORT

First report of *Botrytis cinerea* causing flower blight on macadamia in South Africa

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Abstract

Macadamia cultivation plays significant role in the economy of South Africa. Despite its importance, the industry grapples with disease-related challenges, notably flower blight, which threatens substantial economic losses by affecting yield and nut quality. In 2022, diagnostic services at the Agricultural Research Council and the Forestry and Agricultural Biotechnology Institute received macadamia flowers displaying blight symptoms. Employing two methods for fungal isolation, 25 isolates were obtained which were classified into one group based on morphological characteristics. DNA analysis identified the isolates as *Botrytis cinerea*. The pathogenicity testing was conducted on macadamia flowers to confirm Koch's postulates. This is the first report of *B. cinerea* affecting *Macadamia integrifolia* Maiden & Betche in South Africa, underlining its potential threat to the industry.

KEYWORDS
grey mould, nut disease, *Sclerotiniaceae*

The macadamia crop holds significant economic importance in South Africa, with approximately 65,516 hectares dedicated to macadamia cultivation across four provinces: Limpopo, Mpumalanga, KwaZulu-Natal and Western Cape. As the largest global producer, South Africa accounts for 22% of the total macadamia production worldwide (https://samac.org.za). However, the industry is facing challenges due to several diseases including flower diseases that can lead to significant economic losses.

During 2022, diagnostic services at the Agricultural Research Council (ARC) and Forestry and Agricultural Biotechnology Institute (FABI) at University of Pretoria received macadamia flowers showing blight symptoms. To isolate the fungi, two methods were applied: (1) inspection of the flowers under a stereo microscope, followed by the transfer of growing fungal mycelia from different parts of the flowers onto potato dextrose agar (PDA) (Biolab, Midrand, South Africa), (2) direct transfer of small pieces of the flowers onto PDA plates. After 2–3 days, all isolates showing typical fast-growing white aerial hyphae were transferred to half-strength PDA. In total, 25 isolates were obtained from samples. The isolates were classified as identical based on their morphological characters; therefore, four isolates were selected for DNA analyses.

The sequence data set for four representative isolates (PPRI32797, PPRI31534, PPRI32795 and PPRI32762) were generated from 4-day-old pure cultures. The internal transcribed spacer region of the ribosomal RNA (rRNA) operon was amplified with primers ITS-1 (Gardes & Bruns, 1993) and ITS-4 (White

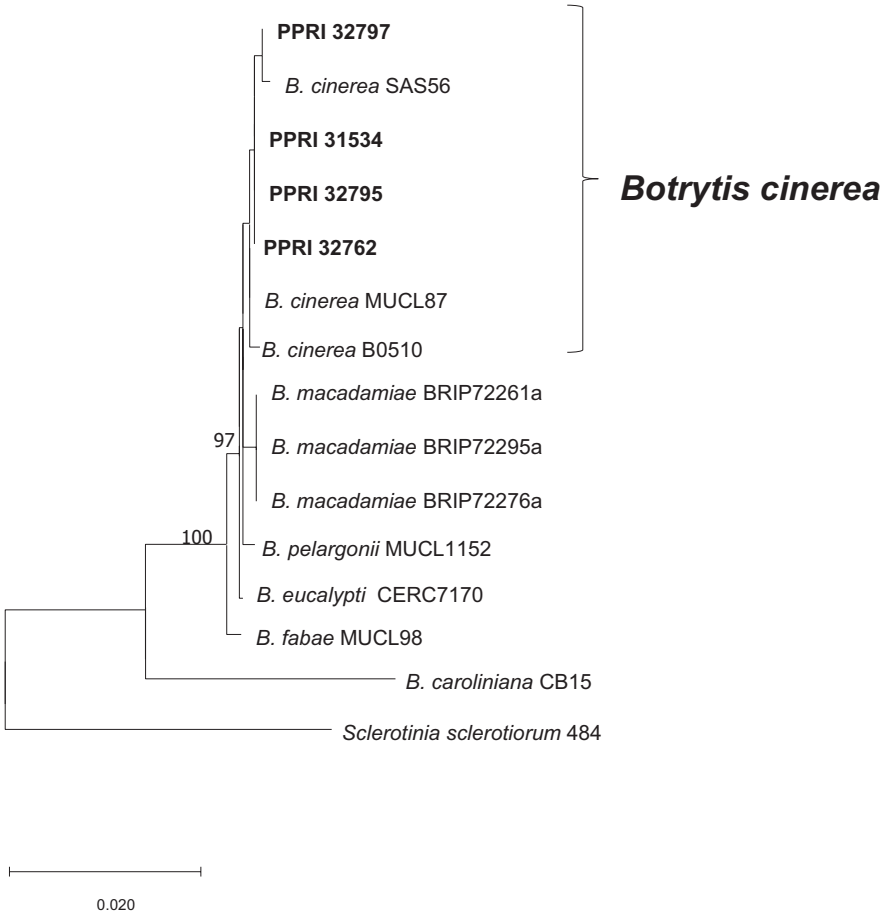

FIGURE 1 Maximum likelihood tree of the combined data set of *ITS*, *G3PDH*, *HSP60* and *rpb2* loci sequences. Bootstrap values above 75% are given at the nodes. Isolates of this study are indicated in bold.

Species	Collection number	GenBank accession numbers			
		ITS	*G3PDH*	*HSP60*	*rpb2*
Botrytis cinerea	PPRI32797	OR758637	PP155422	PP155426	PP155430
Botrytis cinerea	PPRI32795	OR758638	PP155421	PP155424	PP155429
Botrytis cinerea	PPRI32762	OR758639	PP155420	PP155425	PP155428
Botrytis cinerea	PPRI31534	OR758640	PP155419	PP155423	PP155427

TABLE 1 *Botrytis cinerea* isolates and sequences of this study including GenBank accession numbers for the four loci *ITS*, *G3PDH*, *HSP60* and *rpb2*.

et al., 1990), glyceraldehyde 3-phosphate dehydrogenase (G3PDH) gene with primers G3PDHfor and G3PDHrev, and the RNA polymerase subunit II (RPB2) gene with primers RPB2for and RPB2rev and heat shock protein 60 (HSP60) gene with primers HSP60for and HSP60rev (Staats et al., 2005). Sequences of the isolates were edited using CLC-Bio Workbench V9.0 (Qiagen, Hilden, Germany). DNA sequences for relevant *Botrytis* species previously published (Prasannath et al., 2021) were retrieved from GenBank (http://www.ncbi.nlm.gov). The resulting data matrices were rooted to *Sclerotinia sclerotiorum* AJ745716 and were aligned online using MAFFT (http://align.bmr.kyushuu.ac.jp/mafft/online/server/) version 6 (Katoh et al., 2019) and checked manually for alignment errors. Phylogenetic analyses for all the data sets were performed using maximum likelihood (ML) using PhyML 3.0 online (http://www.atgc-montpellier.fr/phyml). The confidence levels for ML analyses were determined with 1000 bootstrap replications. The isolates were identified as *Botrytis cinerea* (Figure 1). All the sequences of this study were deposited in GenBank (Table 1).

To fulfil Koch's postulate, three isolates were inoculated on flowers of 12-year-old macadamia trees in August 2023 at a macadamia farm in White River, Mpumalanga, South Africa. The methods described by Prasannath et al. (2022) were employed. Stage two flowers of the macadamia cultivar HAES '788' were selected for inoculation (Figure 2a). Each isolate was inoculated on three trees and four racemes per tree. Conidial suspensions were derived from 2-week-old pure PDA-grown cultures, and the final concentration of the conidial suspension for each isolate was adjusted to 10^5 conidia per millilitre. To facilitate inoculation, a drop of Tween 80 (Sigma-Aldrich) was added to the conidial suspension, and each raceme was gently sprayed until run-off. For controls, racemes were sprayed

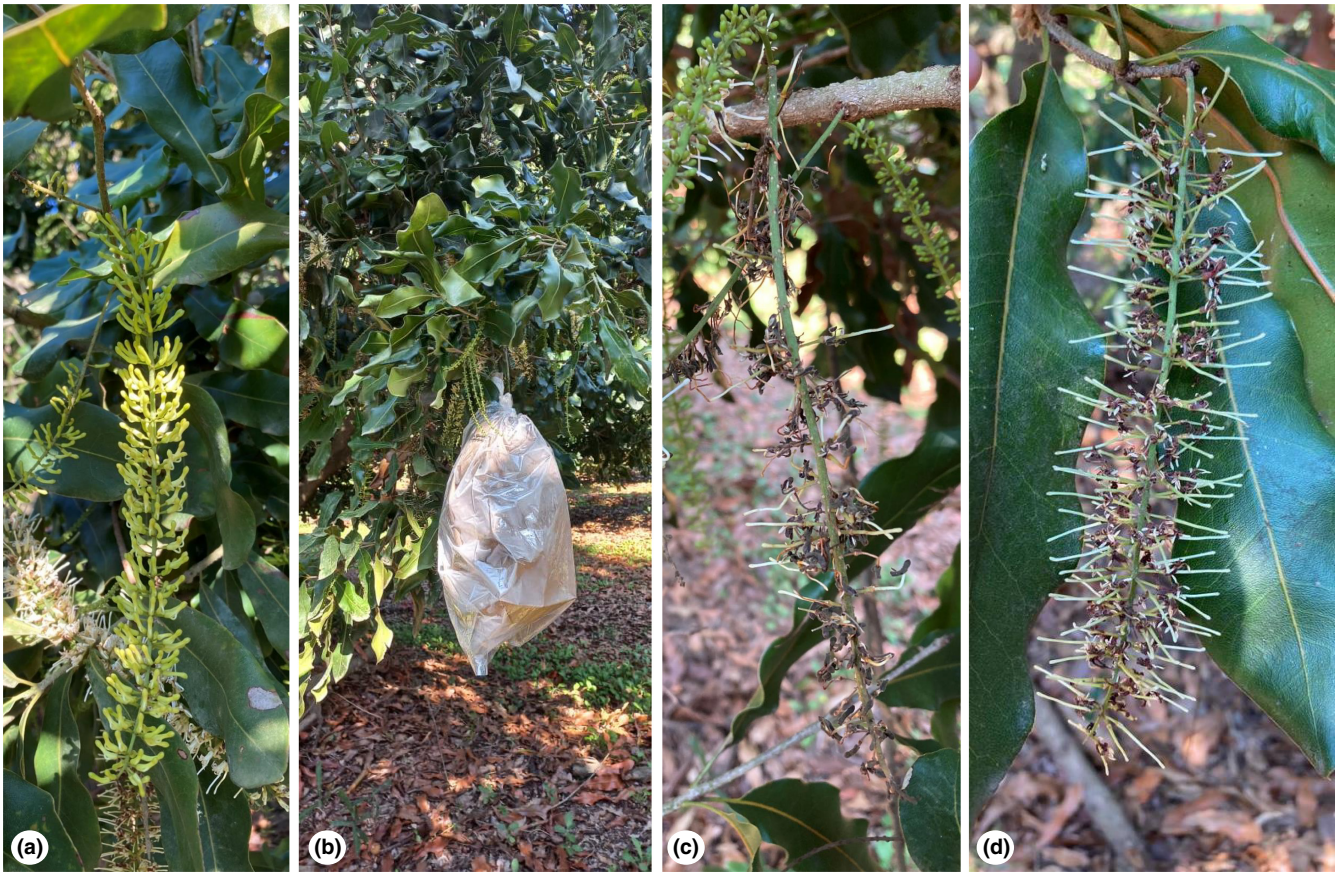

FIGURE 2 Pathogenicity trial: (a) stage two flower before inoculation. (b) Covered inoculated flowers. (c) Blight symptoms after 14 days. (d) Control.

with sterile distilled water. The inoculated racemes were enclosed for 24 h using paper bags and polythene bags to contain the moisture (Figure 2b). After this 24-h period, the polythene bags were removed, but the paper bags were left in place to cover the racemes for an additional 7 days. Symptoms of flower blight were observed after 14 days on 85% of the inoculated racemes (Figure 2c), and no symptoms developed on control racemes (Figure 2d). *Botrytis cinerea* was re-isolated and confirmed based on morphological characteristics.

Botrytis cinerea causes substantial losses in more than 200 crop species globally, primarily through the development of grey mould (Williamson et al., 2007). This species has been reported from Protea, Citrus, Begonia, amaryllis, apple, guava, strawberry, grape and figs in South Africa (Crous et al., 2004); however, this is the first report on *Macadamia integrifolia* for the country.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare that are relevant to this article.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jph.13325.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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