Diseases Caused by Oomycetes

The First Report of *Phytophthora multivesiculata* Causing Black Rot of *Cymbidium* and *Ansellia africana* from South Africa

T. Bose[1,2,†] and A. Hammerbacher[2]

[1] Department of Biochemistry, Genetics and Microbiology, Faculty of Natural and Agricultural Sciences, University of Pretoria Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, South Africa
[2] Department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of Pretoria Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, South Africa

Funding: Funding was provided by the National Research Foundation (Grant number 137971). Plant Dis. 107:588, 2023; published online as https://doi.org/10.1094/PDIS-03-22-0623-PDN. Accepted for publication 13 June 2022.

Globally, various species and hybrids of *Cymbidium* are of horticultural importance. In January 2022, we visited a private orchidarium near Pretoria (25°54′30″S, 28°24′34″E). During this visit, the owner reported mortality among various pure and hybrid *Cymbidium* and *Ansellia africana*, an indigenous South African ornamental orchid. *Phytophthora* was identified as a possible causative agent based on an initial examination of the affected orchids. The infected orchids exhibited vascular wilting. Brown, water-soaked lesions covered the roots. The pseudobulbs showed black rot symptoms. Necrotic lesions were also visible towards the basal part of the leaves. For isolation of the causal agent, pieces of infected tissues from roots, pseudobulbs, and leaves were surface sterilized using 70% ethanol and plated into *Phytophthora* selective medium, NARPH. All the plates were incubated at 21°C in darkness. After 3 days, *Phytophthora*-like mycelia emerged from all three tissue types. These colonies were transferred onto PDA medium. For molecular identification, genomic DNA was extracted from four representative isolates (CMW58027-30) using a Zymo Research Fungal/Bacterial DNA MiniPrep kit. The complete ITS and partial beta-tubulin (*BT*) and cytochrome oxidase 1 (*COX1*) gene regions were amplified using the primers DC6/ITS4 (Cooke et al. 2000; White et al. 1990), Btub_F1A/Btub_R1 (Blair et al. 2008; Kroon et al. 2004), and FM84/FM83 (Martin and Tooley 2003), respectively. BLAST searches in NCBI showed that the four isolates were from *Phytophthora* ITS Clades 2. ITS, *BT*, and *COX1* datasets from Bose et al. (2021) were used for the phylogenetic identification of our isolates. Single gene and concatenated datasets were analyzed using both maximum likelihood and Bayesian approaches, which confirmed the identity of the isolates as *Phytophthora multivesiculata*. All the sequences were submitted to GenBank: ITS (OM967212 to OM967215), *BT* (OM966588 to OM966591), and *COX1* (OM966592 to OM966595). Measurements of sporangia and gametangia overlapped with those from Ilieva et al. (1998): sporangia (28.3 to 56.3) 41.6 × 31.3 (21.5 to 39.6) µm; L:B 1.42 (1.08 to 1.69); exit pore 11.2 (7.1 to 14.6) µm; oogonia 44.2 (24.5 to 56.3) µm; oospore 34.2 (21.6 to 53.2) µm; antheridia (5.8 to 14.6) 11.8 × 15.3 (6.2 to 14.9) µm. The pathogenicity trial was conducted following the protocol suggested by Ilieva et al. (1998). Five *A. africana* roots and the cut ends of seven *Cymbidium* leaves were immersed in separate beakers containing 100 ml of sterile distilled water and 10 5-mm agar discs excised from a 7-day-old culture of *P. multivesiculata* (CMW58027) grown on PDA. Sterile distilled water was used as the control, with an equal number of plants and leaves. All of the sets were incubated at 21°C. After 7 days, the plants and leaves developed lesions similar to those observed on the symptomatic plants at the orchidarium. Trials were repeated once, and the pathogen was reisolated from both trials and the identity was confirmed by amplifying the complete ITS gene region. *P. multivesiculata* has been previously reported from the Netherlands (Ilieva et al. 1998), Taiwan (Chern et al. 2011), Australia (Cunnington et al. 2009), New Zealand (Hill 2004), and elsewhere causing black rot of *Cymbidium*. However, this is the first report of *P. multivesiculata* causing black rot of *Cymbidium* and *A. africana* from Africa. We are now conducting follow-up surveys to determine the distribution range of this pathogen in South Africa.

References:

Blair, J. E., et al. 2008. Fungal Genet. Biol. 45:266.
Bose, T., et al. 2021. Mycol. Prog. 20:755.
Chern, L.-L., et al. 2011. Plant Pathol. Bull. 20:1.
Cooke, D. E. L., et al. 2000. Fungal Genet. Biol. 30:17.
Cunnington, J. H., et al. 2009. Australas. Plant Dis. Notes 4:42.
Hill, C. 2004. Australas. Plant Pathol. 33:603.
Ilieva, E., et al. 1998. Eur. J. Plant Pathol. 104:677.
Kroon, L. P. N. M., et al. 2004. Fungal Genet. Biol. 41:766.
Martin, F. N., and Tooley, P. W. 2003. Mycologia 95:269.
White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: herbaceous/flowering plants, oomycetes, ornamentals, pathogen detection

†Indicates the corresponding author.
T. Bose; Tanay.Bose@fabi.up.ac.za