Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neofusicoccum parvum* Causing Leaf Spot on *Geodorum eulophioides* in China

C. J. Du,¹ D. Yang,¹ Y. F. Ye,² Z. B. Zhang,³ L. F. Pan,¹ J. Zhang,¹ and G. Fu^{1,†}

¹ Guangxi Key Laboratory of Biology for Crop Diseases and Insect Pests, Plant Protection Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, P.R. China

² Horticultural Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, P.R. China

³ Flower Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, P.R. China

Funding: This research was financially supported by Guangxi Key Research and Development Plan Project (GK AB16380061) and Guangxi Natural Science Foundation Program (2017GXNSFDA198002). Plant Dis. 105:486, 2021; published online as <https://doi.org/10.1094/PDIS-03-20-0517-PDN>. Accepted for publication 25 September 2020.

Geodorum eulophioides Schltr. is a critically endangered orchid listed in the International Union for Conservation of Nature Red List of threatened species. At present, only two natural populations were found in China. It has important scientific and ornamental value because of its uniqueness. During the summer of 2019, a black leaf spot disease occurred on *G. eulophioides*, in Yachang Orchid National Nature Reserve (E106°13'32", N24°44'19") in Guangxi province, China. More than 60% of leaves of these plants were infected. The disease symptoms initially appeared as small yellow circular spots, which enlarged into irregular brown spots (6 to 9 cm length and 3 to 5 cm width). In later stages of the disease development, the center of the spots became dark brown with a clear edge and surrounded by a yellow halo. In severe infections, the spots coalesced, covering the entire leaf. Six symptomatic leaves were collected from three infected plants, surface sterilized in 75% ethanol for 15 s and 0.1% HgCl₂ for 4 min, washed three times with sterile water, plated onto potato dextrose agar (PDA), and incubated at 28°C for 3 days. Eighteen fungal cultures with similar morphological characteristics were obtained from the infected tissues. Colonies were initially white, then turned dark gray after 9 days. To induce sporulation, isolates were grown on 2% water agar and incubated under UVA light at 28°C for 9 days. Three isolates were selected for morphological characterization. Conidia were hyaline, unicellular, non-septate, ellipsoidal to fusiform, externally smooth, thin-walled, and ranged from 10.7 to 16.6 (avg. 13.8) × 4.1 to 6.7 (avg. 5.1) μm (*n* = 50). The isolate DBL-1 was selected as a representative for molecular identification.

Genomic DNA was extracted and used for PCR to amplify the rDNA internal transcribed spacer region (ITS), translation elongation factor 1- α gene (EF1- α), and beta-tubulin gene (TUB2), using the primer pairs ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Alves et al. 2008; Carbone and Kohn 1999), and T1/T2 (O'Donnell et al. 1997), respectively. The obtained ITS sequence (GenBank accession no. MN918440), EF1- α sequence (MN963815), and TUB2 sequence (MN963816) showed >99% homology with several GenBank sequences of *Neofusicoccum parvum* (JX513636, KU997497 for ITS; KU997261, MH252401 for EF1- α ; and KJ841779, MK412882 for TUB2, respectively). Based on morphological characteristics of the asexual morph and maximum-likelihood analyses of combined rDNA-ITS, EF1- α , and TUB2 gene sequences, it was identified as *N. parvum*. A pathogenicity test was performed using isolate DBL-1 by inoculating three leaves of *G. eulophioides* plants. The test was repeated three times. Each leaf was wounded using a sterile needle, and a mycelial plug (6-mm diameter) harvested from the periphery of a 3-day-old colony grown on PDA was placed on each wound. Plants were then covered with plastic bags to maintain high relative humidity of 90% and kept at 28°C in a greenhouse under natural daylight conditions. An equal number of leaves on the same plant were inoculated using sterile PDA plugs and served as mock-inoculated controls. After 3 days, all the inoculated leaves showed black spot symptoms resembling those observed in the field, whereas controls remained symptomless. The fungus was reisolated from the symptomatic leaves, thus completing Koch's postulates. *N. parvum* has been reported to cause leaf spot disease on *Myristica fragrans* (Jayakumar et al. 2011), *Ginkgo biloba* (Mirhosseini et al. 2014), *Vitis heyneana* (Wu et al. 2015), and *Hevea brasiliensis* (Liu et al. 2017), respectively. To the best of our knowledge, this is the first report of *N. parvum* causing leaf spot disease on *G. eulophioides* in China. The disease control measures and in-situ conservation method need to be strengthened to protect this rare species.

References:

- Alves, A., et al. 2008. Fungal Divers. 28:1.
- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Jayakumar, V., et al. 2011. New Dis. Rep. 23:19.
- Liu, Y. X., et al. 2017. Plant Dis. 101:1545.
- Mirhosseini, H. A., et al. 2014. New Dis. Rep. 30:12.
- O'Donnell, K., et al. 1997. Mol. Phylogenet. Evol. 7:103.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Wu, D. D., et al. 2015. Plant Dis. 99:417.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Neofusicoccum parvum*, *Geodorum eulophioides*, leaf spot, EF1- α , TUB2

[†]Indicates the corresponding author.
E-mail: G. Fu; fug110@gxaas.net