Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease of Pineapple Caused by *Penicillium oxalicum*

J.-B. Wu,¹ Y.-B. He,^{2,†} and R.-S. Chen³

¹ South Subtropical Crops Research Institute, CATAS, Zhanjiang, Guangdong 524091, China; Key Laboratory of Hainan Province for Post-harvest Physiology and Technology of Tropical Horticultural Products, Zhanjiang, Guangdong 524091, China; Key Laboratory of Tropical Fruit Biology, Ministry of Agriculture, P.R., China; Hainan Tropical Crops Nutrition Key Laboratory, Zhanjiang, Guangdong 524091, China

² College of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology, Loudi, Hunan 417000, China

³ College of Life Science and Technology, Lingnan Normal University, Zhanjiang, Guangdong 524048, China

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Pineapple (*Ananas comosus* Merr.) is an economically important tropical fruit crop. In China, it is primarily distributed in tropical and subtropical southern regions, including Leizhou Peninsula (Guangdong province) and Hainan province. Other pineapple culturing areas also include Fujian, Guangxi, Yunnan, and Taiwan provinces. A pineapple leaf spot disease was observed in Leizhou Peninsula (20°47′52″ N, 110°5′7″ E) from July to August in 2019 to 2020, with a natural incidence of 10 to 15%. In the initial infection stage, grayish or yellowish white spots emerged on the leaf surfaces with dimensions of 1.25 to 1.75 × 0.8 to 1.0 cm. The leaf spots also had distinctive light brown-to-reddish brown banding pattern on the edges. At the late stage of infection, the leaves with spots withered and died, seriously affecting the plant growth. To isolate the pathogen, leaf pieces 5 mm in diameter were cut from the decaying edges. They were surface-sterilized with 75% ethanol solution for 30 s, washed with 0.1% sodium hypochlorite solution for 30 s, rinsed five times with sterile water, and placed on potato dextrose agar (PDA) medium. After incubation at 28°C for 6 days, the pathogen was purified using single conidial isolation for morphological and molecular

characterization. All 25 isolates showed similar phenotypes. The colonies on PDA were green, circular, flat, and velutinous. Conidiophores were broom-shaped (16.35 ± 1.30 μm). Conidia were colorless, unicellular, and kidney shaped (3.50 to 4.00 × 2.50 to 3.00 μm). Based on the morphological characteristics, the fungal isolates were tentatively identified as *Penicillium* species. From each culture, the internal transcribed spacer (ITS) region of rDNA and the partial β-tubulin genes of a representative isolate (ZN2019211) were amplified using ITS1/ITS4 and Bt2a/Bt2b primer pairs, respectively. The gene sequences were deposited in GenBank (accessions MT678576 for the ITS region and MT720907 for the Bt2 region) and were 100% identical to *Penicillium oxalicum* Currie & Thom isolates (MN592913 for the ITS region; KX961250 for the Bt2 region). Based on their morphological and molecular characteristics, the isolates were determined to be *P. oxalicum*. Pathogenicity tests were conducted in three replicates by inoculating surface-sterilized leaves of pineapple. The leaves were wounded and inoculated with mycelium PDA plugs (10 × 10 mm) from 6-day-old cultures. Control leaves wounded in the same way were inoculated with sterilized PDA plugs (10 × 10 mm). Each of the three replicates comprised two whole plants and two leaves (including a control) per plant (Wu et al. 2017). The inoculated leaves were placed in a greenhouse (25 to 30°C). Six days post inoculation, symptoms similar to those observed in the field were observed on the inoculated leaves, but not on the controls; the same fungus was isolated both times from the infected leaves, confirming Koch’s postulates. We also inoculated pineapple inflorescence and fruit, with or without injury, and observed that the pathogen could not cause pineapple fruit disease, but caused early senescence of the inflorescence after being dropped with 200 μl of a 10⁴ conidia/ml solution, although it did not affect subsequent flowering. *P. oxalicum* has been reported to cause blue mold disease in different plants (Liu et al. 2019; Paul et al. 2018; Picos-Munoz et al. 2011; Tang et al. 2020). However, to the best of our knowledge, this is the first report of pineapple leaf spot disease caused by *P. oxalicum* globally, and the disease has become a potential threat to the growth and production of pineapple in China.

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[†]Indicates the corresponding author.
Y.-B. He; heyianbiao2013@163.com