Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Eucalyptus Wilt Caused by *Chalaropsis thielavioides* in China

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Eucalyptus species are widely planted in the tropics and subtropics, and are an important cash crop in Southern China. A major disease on eucalyptus is Ceratocystis wilt, caused by the fungus *Ceratocystis fimbriata* Ellis & Halst., and the genus name *Chalaropsis* has been proposed for anamorphs of *Ceratocystis* species (de Beer et al. 2014). In April 2018, severely infected *Eucalyptus robusta* trees were observed in Kunming, Yunnan Province, China. Symptomatic trees initially exhibited yellowing and wilting of foliage on individual branches that spread to the whole canopy, sometimes followed by tree death. Reddish-brown to dark-brown discoloration in the woody xylem of affected trees was observed, and sometimes a grayish white layer of fungal growth was seen. The disease was observed on 16% of trees surveyed. The bases of trunks with typical symptoms were collected, and discolored xylem tissues were surface disinfected with 75% ethanol for 30 s and 0.1% HgCl₂ solution for 2 min, rinsed three times with sterile distilled water, plated onto potato dextrose agar medium, and incubated at 25°C. After 6 days, a fungus was consistently observed growing from the tissue. Three isolates were obtained. In culture, colonies reached a 54-mm diameter within 15 days. Mycelium was initially white and then became celadon. Endoconidia were unicellular, smooth, cylindrical, straight, biguttulate, and 11.21 to 32.26 × 4.12 to 5.25 μm. Lageniform phialides were produced on short, septate, aerial hyphae. A chain of phialoconidia (3.62 to 5.89 × 31.39 to 65.76 μm) was also observed. Chlamydospores (11.45 to 14.26 × 10.06 to 12.22 μm) were single, dark, and thick-walled. Morphological characteristics were consistent with the description of *Chalaropsis thielavioides* (Paulin-Mahady et al. 2002). Two of the isolates (EKY2-2-1 and EKY2-2-2) were used for molecular identification,

and their genomic DNA was extracted using the Chelex-100 method (Xu et al. 2020). The ITS region of rDNA was sequenced following Thorpe et al. (2005). BLAST analysis of ITS sequence data (GenBank accessions MW242701 and MW242702) showed that the isolates were 99 to 100% homologous to isolates of *C. thielavioides* from *Hevea brasiliensis*, *Monstera deliciosa* L., and ants in China and *Rosa* sp. in Australia (GenBank accessions KT963172, KJ511482, KT963173, and KX954598, respectively). Neighbor-joining phylogenetic analyses were performed using MEGA 6.06 based on ITS sequences, and the evolutionary distances were computed using the maximum composite likelihood method. Both isolates were in the same clade with all *C. thielavioides* and clustered with the *C. thielavioides* strains with high bootstrap support (97 to 100%). Thus, the fungus was identified as *C. thielavioides* based on morphology and molecular evidence. Pathogenicity of *C. thielavioides* was tested by inoculation of six 1-year-old, pot-grown *Eucalyptus citriodora* seedlings. The sterilized soil was inoculated by drenching it with 20 ml of spore suspension (2.0 × 10⁶ spores/ml). Control plants were inoculated with 20 ml of sterile distilled water. Seedlings were kept in a controlled greenhouse at 25°C and watered weekly. After 1 month, all the isolates produced wilt symptoms, and controls showed no symptoms. The original fungus was successfully reisolated from inoculated trees and identified as *C. thielavioides* according to the methods described above, and no fungal growth was observed in the controls, thus satisfying Koch's postulates. Wilt and canker caused by *C. fimbriata* on eucalyptus have been previously reported in Brazil, Uruguay, Uganda, China, and Pakistan (Alam et al. 2017; Ferreira et al. 1999; Li et al. 2014), but eucalyptus wilt caused by *C. thielavioides* has not been reported anywhere. Also, wilt of rubber tree and postharvest rot on carrot caused by *C. thielavioides* have been reported (Li et al. 2021; Xu et al. 2020). To our knowledge, this is the first report of eucalyptus wilt caused by *C. thielavioides* in China.

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