Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fruticola* Causing Anthracnose on *Pouteria campechiana* in China

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Pouteria campechiana (Kunth) Baehni (= *Lucuma nervosa* A. DC.) is a fruit crop originally from Central America grown in southern China (Awang-Kanak and Abu Bakar 2018; Gao et al. 2019). In March 2019, a leaf spot disease was found on *P. campechiana* in Baoshan, Yunnan, China. Leaf spots were found on 90% of 6-year-old plants in a 0.06-ha orchard and were observed in other planting areas. Symptoms initially appeared as small, round, brown spots. Lesion centers became sunken with a dark brown border. Under severe conditions, spots joined into larger irregular spots, and whole leaves died. Disease severity varied; some leaves showed only a few brown spots, and others showed many spots. Fragments of diseased tissues (3 × 3 mm) were disinfected in 75% ethanol for 10 s, 1% NaClO for 1 min, and rinsed thrice in sterilized water. Tissues were placed on PDA and incubated at 25°C in the dark for 5 days. Isolates with similar morphology were consistently recovered. The 25 colonies were initially cottony, pale white to pale gray on the upper side and greyish-green with black zonation on the underside of plates. Conidia were single-celled and hyaline, aseptate, straight, and cylindrical, with rounded ends. The length and width of 200 conidia were measured for representative isolates DHG-1 and DHG-2, and these averaged 14.48 × 5.59 and 14.92 × 5.57 μm. Appressoria were ovoid, sometimes clavate, brown, and averaged 7.47 × 5.86 and 7.25 × 5.85 μm (*n* = 30). Brown and globose ascocarp were observed on the leaves of *P. campechiana*. Asci were unitunicate, thin-walled, six- to eight-spored, clavate, and averaged 51.53 × 13.01 and 50.21 × 13.32 μm (*n* = 30). Ascospores were hyaline, one-celled, slightly curved to curved with obtuse to slightly rounded ends, and averaged 14.64 × 5.97 and 15.19 × 6.23 μm

(*n* = 30). These isolates were selected for molecular identification. DNA was extracted from mycelia with the DNA secure Plant Kit (Tiangen). For further molecular identification, the ITS, partial ACT, CAL, CHS-1, GAPDH, TUB2, and ApMat genes of DHG-1 and DHG-2 were amplified using the primer pairs ITS1/ITS4, ACT-512F/ACT-783R, CL1C/CL2C, CHS-79F/CHS-345R, GDF1/GDR1, T1/Bt-2b, and AM-F/AM-R (Silva et al. 2012; Weir et al. 2012), respectively. The sequences were obtained and compared with GenBank, and they all showed over 99% identity to the type strain of *Colletotrichum fruticola* ICMP 18581 (JX010165, JX010033, JQ807838, FJ907426, JX010405, JX009866, and FJ917508) (Weir et al. 2012). A phylogenetic tree based on the combined ITS, ACT, CAL, CHS-1, TUB2, GAPDH, and ApMat sequences using the neighbor-joining algorithm revealed that the isolates were *C. fruticola*. The sequences were deposited into GenBank as MN955541, MN955542, and MN966581 to MN966592. Pathogenicity tests were conducted on 18 healthy and tender leaves of six 1-year-old *P. campechiana* plants in a greenhouse. The experiment was repeated twice. Inoculated leaves were 8 to 13 × 2.5 to 3.6 cm. The epidermis of each tested leaf was lightly scratched in six separate areas with a sterilized needle. Each isolate was inoculated onto at least three wounded leaves by placing 20 μl of 10⁶ conidia/ml suspension on the wound sites. Control leaves were wounded and inoculated with distilled water. All plants were sprayed with distilled water and covered with plastic bags. After 10 days, initial symptoms appeared as circular and deep yellow spots. After a few more days, the spots became brown and grew to up to 4.0 mm, similar to symptoms in the field; controls remained symptomless. Koch's postulates were fulfilled by reisolation of *C. fruticola* from diseased leaves and identification confirmed by sequencing. *C. fruticola* has been associated with anthracnose on mango, apple, pear, and cassava (Oliveira et al. 2018). This is the first report of *C. fruticola* associated with anthracnose of *P. campechiana* worldwide. These results will provide crucial information for future epidemiological studies and for management of this disease.

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