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DISEASE NOTES

First Report of *Colletotrichum theobromicola* Causing Leaf Spot in Sapote (*Manilkara zapota*) Seedlings in Brazil

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Citation |

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Sapote (*Manilkara zapota* L., family Sapotaceae) is a tropical perennial plant native to Asia largely cultivated on the coastal and semiarid areas of northeastern Brazil, mainly for fresh fruit consumption. Sapote is a succulent and sweet fruit, esteemed as a nutritious food for its content of vitamins A, B1, B2, B5, and C, as well as carbohydrates, calcium, phosphorus, and iron. In 2014, during the April to May rainy season, necrotic leaf spots were observed on 70% of the seedlings in a nursery at Embrapa Agroindústria Tropical, in Pacajus, Ceará State. The causal agent was isolated from small pieces of the symptomatic disinfested leaf (70% ethanol, 0.5% sodium hypochlorite, and washed with sterilized distilled water) and plated on potato carrot agar (PCA). After 5 days of incubation at 25°C, the isolated colony (CMM-4726) was grayish-white in color, and conidia were straight, hyaline, aseptate, and subcylindrical with rounded ends, measuring 12.24 to 20.34 μm long and 2.70 to 5.48 μm wide ($n = 110$). The colony from this isolate was similar in color and shape and was within the range of conidia sizes for *Colletotrichum theobromicola* described by Santos et al. (2017). A monosporic culture grown in PCA medium was used for DNA extraction according to the method of Zhang et al. (2010). To confirm identification, six genes were amplified and sequenced: internal transcribed spacer region (ITS), actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), calmodulin (CAL), chitin synthase (CHS-1), and β-tubulin (TUB2) (Weir et al. 2012). The ITS, ACT, and TUB2 sequences had 100% sequence similarity with *C. theobromicola* (KX721068.1,

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KP642639.1, and KJ883593.1), CAL and CHS-1 sequences had 99% (JX009588.1 and KP642641.1), and GAPDH sequences had 98% (KX721064.1). Based on sequence analysis and morphological characteristics, the isolate CMM-4726 was confirmed to be *C. theobromicola*, and sequences were deposited in GenBank with accession numbers KY206758 (ITS), KY206759 (ACT), MF685342 (CAL), MF685343 (CHS-1), MF681690 (TUB2), and KY206760 (GAPDH). Pathogenicity was confirmed on 2-month-old sapote seedlings sprayed with a conidial suspension (2×10^6 conidia/ml). Ten inoculated and 10 noninoculated seedlings (only sprayed with sterile water, to serve as controls) were incubated in a growth chamber at 28°C. Leaf spot symptoms were observed 7 days after inoculation, and a fungus with the same morphological characteristics was reisolated from infected tissues. Control seedlings remained asymptomatic. This *Colletotrichum* species has been reported in other fruit plants in Brazil (Bragança et al. 2014; Santos et al. 2017) and in eucalyptus (Rodrigues et al. 2014). This is the first report of *C. theobromicola* causing leaf spot on sapote seedlings in a nursery in Brazil.

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