

First Report of *Lasiodiplodia brasiliense* Causing Disease in Apple Trees in Brazil

M. V. V. Martins; J. S. Lima; F. J. Hawerth; M. A. Ootani; F. S. A. Araujo; J. E. Cardoso; L. A. L. Serrano; F. M. P. Viana. Embrapa Agroindústria Tropical, Caixa Postal 3761, CEP 6060511-110, Fortaleza, CE Brazil

In the last years, several apple varieties have been well-adapted to grow under the tropical conditions of northeastern Brazil. However, fungal diseases can be a limiting growing factor under these conditions. Cankers, dieback and eventual plant death were observed in two-year-old apple trees, cv. Eva in Paraipaba county, State of Ceará, Brazil in 2015. In an attempt to identify the causal agent, symptomatic tissue from trunk was collected and disinfected in 70% alcohol and 1.5% sodium hypochlorite. Tissue was plated onto potato-carrot agar (PCA) and incubated at $\pm 25^{\circ}\text{C}$ for seven days. Fungal colonies developed from infected tissue were dark or greyish. Hyphal tips were plated again in PCA medium with sterilized pine needles and incubated during ten to fifteen days to produce pycnidia and sporulation. Conidia were ellipsoid to ovoid and measured $22.83\ \mu\text{m}$ length and $11.58\ \mu\text{m}$ wide ($n = 140$). Conidia were hyaline when immature and brown with a central transverse septum and longitudinal striations when mature. These characteristics indicated that this specie is morphologically similar to those in the genus *Lasiodiplodia* (Netto et al. 2014). A monosporic culture grown in PCA medium was used for DNA extraction. To confirm identification, three genes were amplified and sequenced, including ITS (internal transcribed spacer region), and part of the TUB2 (β -tubulin) and elongation factor (EF1- α) genes. ITS, TUB2 and EF1- α sequences showed 99% homology with *Lasiodiplodia brasiliense* (KX278010.1, KP308524.1 and JX464049.1) from Genbank. Based on sequence analysis, isolate BOT458 was confirmed to be *L. brasiliense* and sequences were deposited in GenBank with accession numbers KY696839 (ITS), KY711350 (TUB2) and KY711351 (EF1- α). Pathogenicity test was confirmed on 2-year-old healthy apple trees under field conditions. Colonized mycelium plugs of a 10 day-old colony of *L. brasiliense* were inserted into a 6.5 mm hole in ten regions of the stems and branches (ten replicates). Sterile PCA was inserted for negative control in seven replicates. Twenty days after inoculation, disease symptom on stem was recorded. No symptoms were observed in the controls. The pathogen was re-isolated from the lesions of inoculated plants. Although other Botryosphaeriaceae species have been reported on apple trees in South America (Delgado-Cerrone et al. 2016; Sessa et al. 2016), this is the first report of *L. brasiliense* causing stem canker and dieback in apple trees in Brazil. Further studies to identify apple genetic resistance and management strategies, e.g., pruning and the effectiveness of fungicides are desirable.



Apple plant killed by *Lasiodiplodia brasiliense*.

431x575mm (72 x 72 DPI)



External symptom caused by *Lasiodiplodia brasiliense*.

431x575mm (72 x 72 DPI)