Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia theobromae* and *L. pseudotheobromae* Causing Canker Disease of Sacha Inchi (*Plukenetia volubilis*) in Hainan, China

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Sacha inchi (*Plukenetia volubilis* L.; Euphorbiaceae) is a perennial wooden oilseed crop and is a good source of polyunsaturated fatty acids, protein, and other bioactive compounds, such as tocopherols, carotenes, and phytosterols (Chirinos et al. 2013). During a 2017 to 2018 survey, canker disease showing greyish-brown sunken lesions was observed on the branches of sacha inchi in Danzhou campus, Hainan University, China. Disease incidence was <5%; however, it can lead to leaf yellowing, wilt, and eventually plant death. In November 2017, 12 branches showing typical canker symptoms were collected and covered with parafilm at both ends to prevent desiccation and placed in black plastic bags at 4°C until isolations were made. Samples were rinsed with tap water and dried with paper towels. Fragments, 5 mm in length and cut from the junction of diseased and healthy parts of branches, were surface sterilized with 2% NaOCl for 2 min, rinsed with sterilized distilled water five times, dried with sterilized filter paper, plated on PDA medium amended with 100 µg/ml of streptomycin, and incubated in the dark for 4 days at 28°C. Pure cultures of fungal isolates were obtained by transferring mycelial fragments from colony margins onto fresh PDA plates and incubated as described before. The colonies of cultures were initially white and turned black after 4 days on PDA. The morphology characterization of conidia was initially hyaline and aseptate, and a single median septum formed in mature conidia. The average size of 50 conidia was $16.39 \pm 1.46 \times 8.52 \pm 0.92$ µm for J6, and $15.64 \pm 1.73 \times 8.94 \pm 0.86$ µm for J3. Three genes were used for phylogenetic analysis (Alves et al. 2006). *ITS* regions and the partial β -tubulin gene (*TUB*) were amplified using primer pairs ITS1/ITS4 (White et al. 1990) and Bt2a/Bt2b

(Glass and Donaldson 1995), respectively, and EF1-688F/EF1-1251R for J3 and EF1-728F/EF1-986R for J6 were used to amplify translation elongation factor 1-alpha (*TEF*) (Alves et al. 2008). The sequences of *ITS*, *TUB*, and *TEF* from J3 and J6 were deposited in GenBank. BLAST searches in GenBank with *ITS*, *TUB*, and *TEF* amplified from isolates J3, respectively, revealed 100, 99, and 100% identities with *L. pseudotheobromae*, and isolate J6 showed 100, 100, and 99% of identity with *L. theobromae*. The phylogenetic analysis of the combined *ITS*, *TUB*, and *TEF* sequences of J3, J6, and 28 reference strains was performed using MEGA 6.0 with 1,000 bootstrap replications, and the result was consistent with the conclusion above. With the phylogenetic studies supported by morphological characters, J3 was identified as *L. pseudotheobromae* and J6 as *L. theobromae*. For the pathogenicity test, J3 and J6 were used to inoculate 4-week-old healthy sacha inchi potted seedlings. One wound about 5 mm in depth per seedling stem was made using a sterile blade. A 5-mm-diameter mycelium plug of each isolate taken from the edge of a 4-day-old culture growing on PDA was placed on the fresh wound of each plant stem and wrapped with parafilm. Sterile PDA plugs were used for controls. Nine healthy seedlings were inoculated with each isolate or PDA plugs in a completely randomized design. After inoculation, plants were placed in a greenhouse at 26 to 30°C (80% RH) and irrigated when needed. The experiment was conducted twice. Five days later, black or dark-brown canker lesions formed on the stems of inoculated plants and expanded upward and downward from the inoculation points. Pycnidia were produced on the necrotic regions and were used to observe the morphology of conidia. *L. pseudotheobromae* or *L. theobromae* could be reisolated from the inoculated plants but not from the controls. *L. pseudotheobromae* was recorded to be collected from dead leaves of *P. volubilis* in Yunnan Province, China, but did not prove this fungus to be pathogenic (Tennakoon et al. 2016). This is the first report of *L. theobromae* and *L. pseudotheobromae* causing stem canker in sacha inchi in Hainan, China. The results pave the way for the development of management strategies.

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