First Report of *Lasiodiplodia theobromae* Causing Dieback Symptoms on Plantain (*Musa* AAB subgroup) in Nigeria

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Bananas (banana and plantains) rank sixth among staple food crops (FAO 2018), with production challenged by biotic factors, mainly fungal diseases that may cause a total loss in some orchards (Jones 2018).

In April 2017, dieback symptoms (progressive blackening and necrotic aerial plant parts, leaves, fruits and peduncles) were observed on plantain (*Musa* AAB subgroup), in Onne, Rivers State, Nigeria (4°42′55.4012″N, 7°10′35.92128″E). Diseased plants (n=112) were either wilted with blackened necrotic areas, or dead (Fig. S1). Nearly 10% of the plants had blackened pseudostems and fruits with slate gray to black internal tissues when sliced (Fig. S1) and black, erumpent pycnidia were observed on diseased fruits.

A fungal species was consistently isolated when surface disinfected pieces of diseased samples were cultured on PDA plates. Plates were incubated at 25±2°C for 4 to 15 d to observe conidia. Isolates had colonies and conidia consistent with members of the Botryosphaeriaceae family (Phillips et al. 2013). Immature conidia were single-celled, ellipsoidal and hyaline while mature conidia were two-celled, had a thick wall, a central septum, longitudinal striations, and a dark brown, cinnamon-like color. Size of mature conidia ($n = 20$) ranged 22.9 to 30.0 × 14.2 to 18.4 µm ($x = 27.0 \times 15.6$ µm; Fig. S1). DNA templates of three isolates (23688-2_R16; 19144-18_R15 and PITA_22-1) were amplified using primers ITS1 and ITS4 for the ITS locus, EF1-688F and EF1-1251R for the translation elongation factor 1-α (TEF-1α) locus (Phillips et al. 2013) and sequenced (GenBank accession Nos. MZ413346, MZ413347, and MZ413348 for ITS; and MZ420177, MZ420178, and MZ420179 for TEF-1α). BLASTn query showed 100% identity with reference sequences of various isolates of *Lasiodiplodia theobromae*. Based on morphological characters and nucleotide homology, the isolates were identified as *L. theobromae* (Fig. S1 & S2). To fulfil Koch's postulates, 4-month-old plants of plantain hybrid PITA 24, and mature fruits from three genotypes (PITA 24, plantain cultivar Obino L'ewai) were inoculated with mycelial plugs from the margins of 5-d-old cultures of the three *L.*

theobromae isolates. Pseudostems were drilled with a sterile 5 -mm cork borer, a mycelial plug placed down into the wound, covered with sterilized cotton, and sealed with parafilm. Sterile water was injected every third day to maintain moisture at the inoculated area. Toothpicks containing mycelia were used to inoculate fruits, placed in plastic Crisper boxes. Sterile PDA plugs or toothpicks were used for the controls. Inoculated plants and fruits were kept in a screenhouse at room temperature (~26°C) for 14 d. All inoculated materials developed symptoms similar to the diseased plants in the field. Control plants and fruits remained asymptomatic. *L. theobromae* was re-isolated from the artificially inoculated plant parts and its identity was confirmed. The fungus *L. theobromae* is distributed in tropical and subtropical regions and has a wide host range (Phillips et al. 2013; Mehl et al. 2017). This fungus was previously reported in grey literature as the causal agent of *Musa* spp. basal rot at Onne, Nigeria (Mwangi et al. 2005) but its molecular identification was not conducted; it was unknown whether the isolates were indeed *L. theobromae* or other cryptic species (*L. pseudotheobromae* or *L. parva*) (Alves et al. 2008). Over 15 years later, the present study confirms *L. theobromae* as the causal agent of basal rot of bananas based on nucleotide homology, and to our knowledge, this is the first report of *L. theobromae* causing dieback disease on plantain in Nigeria and in Africa. There is need to conduct a more comprehensive distribution surveys and develop appropriate control strategies in Nigeria.

References

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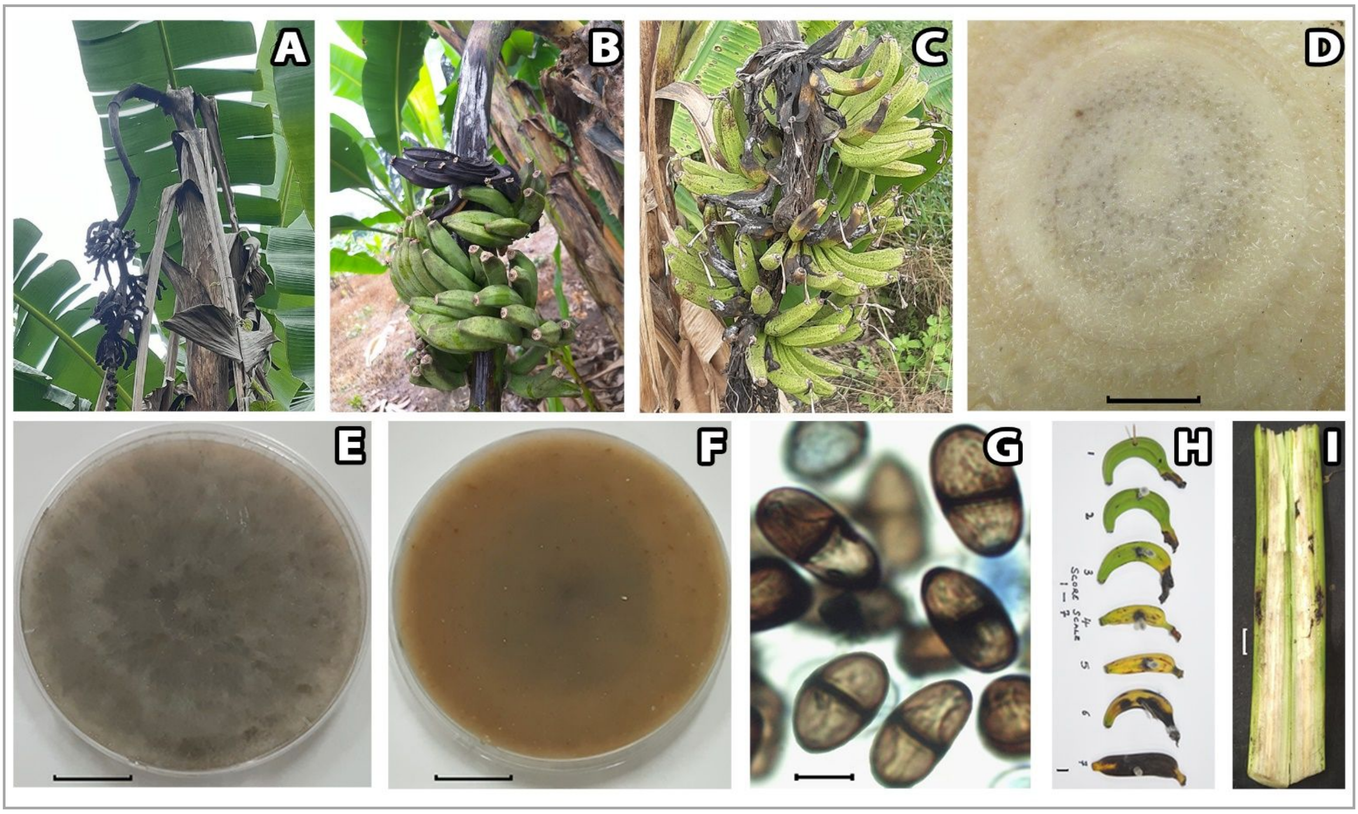
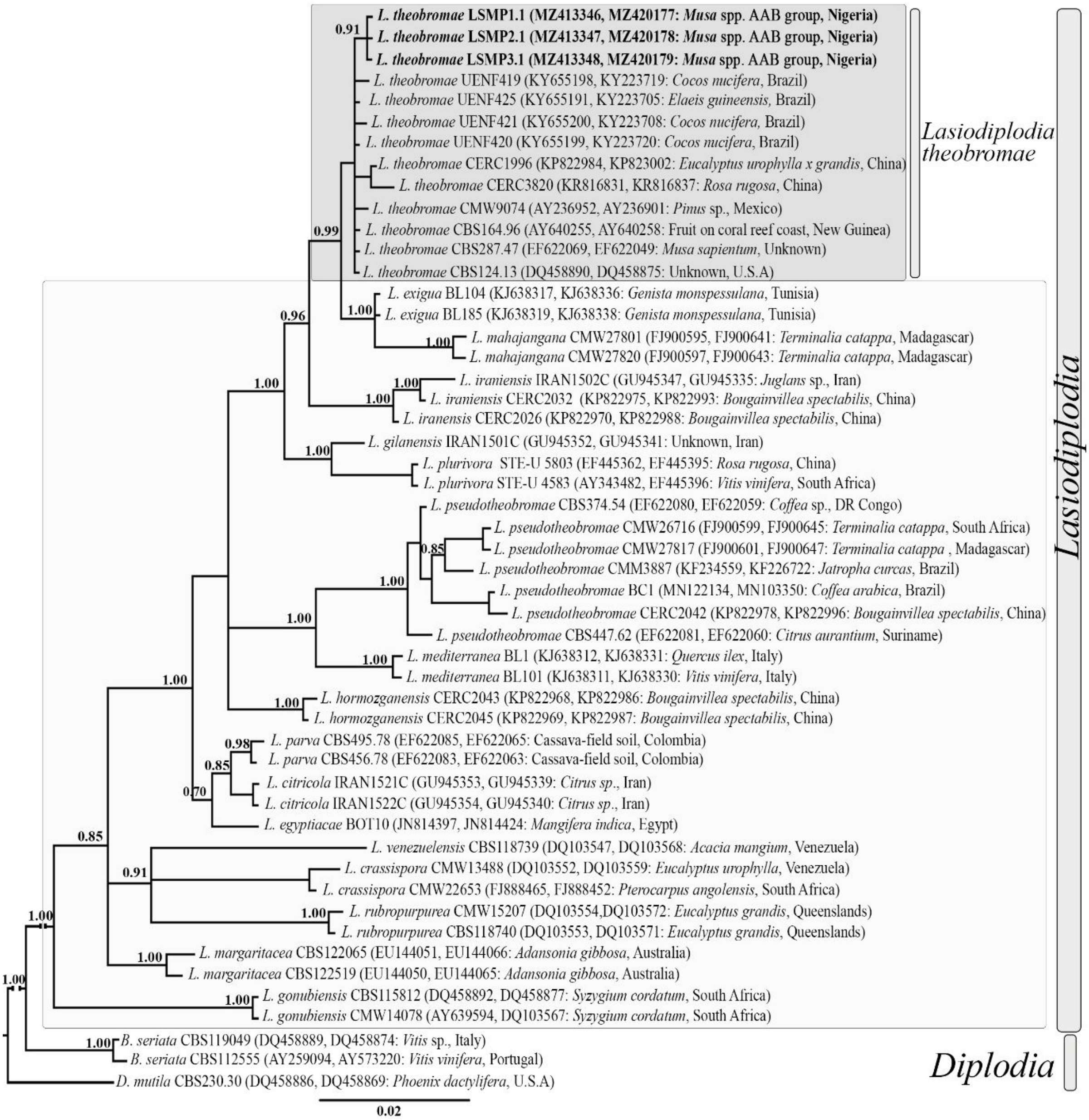
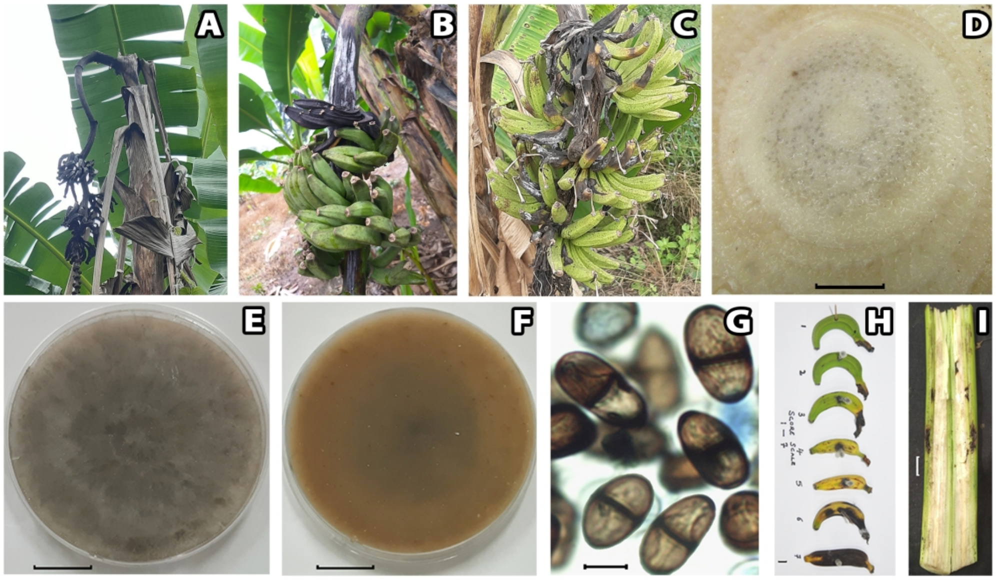


Supplementary Figure S1. Plants with fingers and peduncles rot, dieback symptoms caused by *Lasiodiplodia theobromae* (A-C), Pseudostem cross-section of infected plant (D), cultures of isolates on PDA (E-F), spores' morphology (G), and the rot caused by *L. theobromae* following the pathogenicity test on fruits (H) and pseudostems of plants (I). Scale bars: D-F, H-I = 2 cm; G = 10 μm



Supplementary Figure S2. Phylogenetic tree of *Lasiodiplodia* isolates from plantains (*Musa* spp. AAB group) plants in Nigeria and related species based on the Bayesian Inference of a combined internal transcribed spacer (ITS) and translation elongation factor 1-alpha (TEF 1-α). The posterior probability is indicated near the branch nodes. *Diplodia mutila* CBS 230.30 is used as outgroup. Isolates marked in bold represent those obtained from plantains (*Musa* spp. AAB group).



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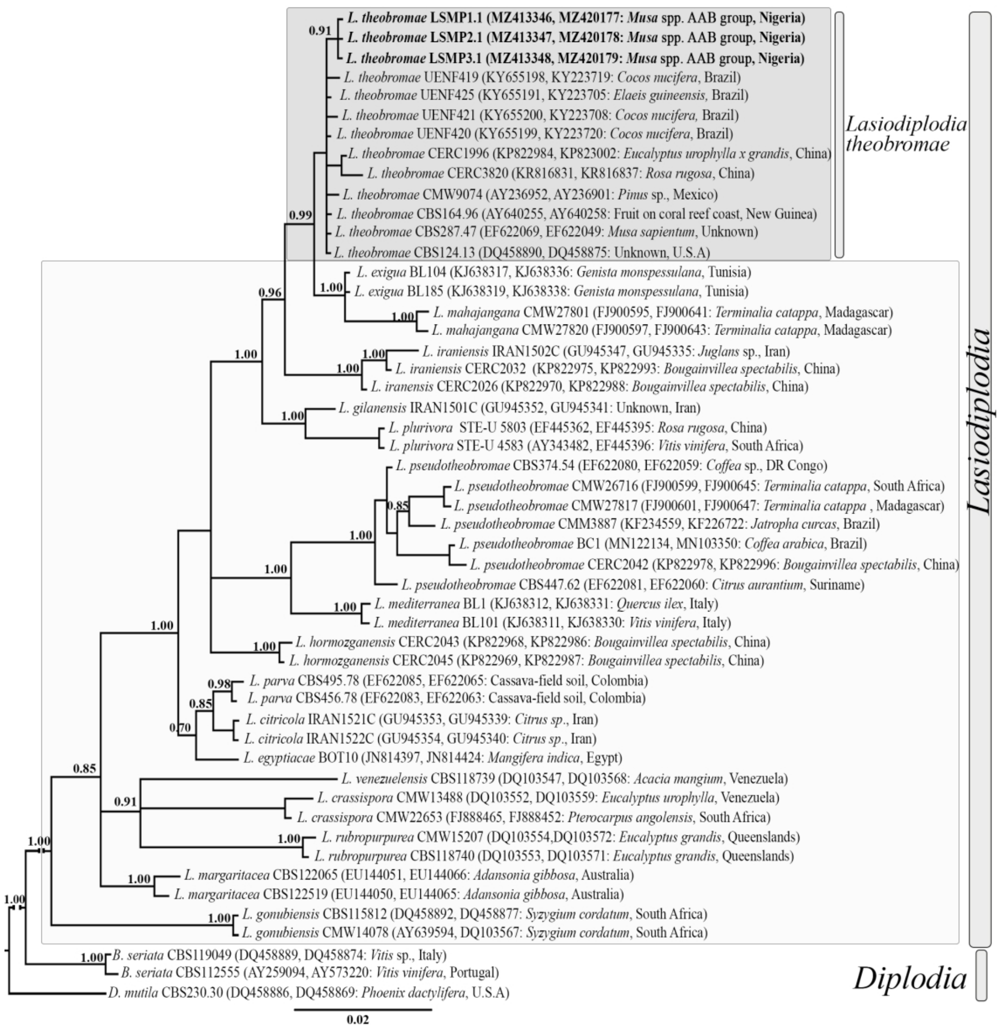

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