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Gummosis of citrus in Ghana caused by *Phytophthora citrophthora*

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Abstract Citrus in Ghana are seriously affected by gummosis, which causes trunk cankers and tree death. The disease was originally related to *Phytophthora parasitica* but more recently to *Lasiodiplodia theobromae*. The aetiology of citrus gummosis in Ghana was reassessed in the same locations surveyed by previous studies. *Phytophthora citrophthora* was confirmed as the causal agent of the disease.

Keywords Oomycota · *Citrus sinensis* · West Africa · Aetiology

The citrus-growing area in Ghana has expanded significantly in the last decades with 671,000 tonnes of citrus fruit produced in 2012 (FAOSTAT 2012). Surveys conducted in 1959 indicated that about 40 % of the citrus orchards in Ghana were affected by a severe trunk rot disease (Leather 1959) and it is currently considered of major economic importance (Offei et al. 2002; Ofosu-Budu et al. 2007). The *Citrus* species affected included sweet orange (*C. sinensis*), mandarin (*C. reticulata*), lime (*C. aurantifolia*), lemon (*C. limon*) and grapefruit (*C. paradisi*) (Assuah et al. 1999).

The typical symptom associated with the disease was the exudation of gum in the affected area which later dried on the bark surfaces (Fig. 1a). The affected bark became darker than the surrounding healthy tissues. When the affected bark tissues were removed, the diseased wood surface appeared dark brown with decreasing intensity to light brown. The infected bark often cracked and peeled off with disease progression. Affected trees showed leaf chlorosis and twig die-back with reduced fruit production. When lesions girdled the trunk, trees eventually died, especially after an exceptional heavy crop load.

In Ghana, the disease was originally associated with *Phytophythora parasitica*, but details of the aetiological methods used were not provided (Leather 1959; Clerk 1974). A more recent study identified *Lasiodiplodia theobromae* (syn. *Diplodia natalensis*) as the causal agent of the disease in Kade (Assuah et al. 1999). This study pointed out that several attempts were made to isolate *Phytophthora* but without success. The objective of the current study was to reassess the aetiology of citrus gummosis in Ghana.

One sample from each of 50 sweet orange trees (cv. Valencia Late) grafted onto rough lemon rootstock (*C. jambhiri*) and severely affected by gummosis were collected from the experimental orchards at five locations of the University of Ghana, Forest and Horticultural Crops Research Centre located at Kade (06° 08′ 54.76″ N, 0° 54′ 53.35″ W). This site is one of the main citrus-growing areas in Ghana and was surveyed by Assuah et al. (1999). Affected tissues of the scion were removed from the margins of gummosis lesions. Samples were washed under running tap water, surface sterilised with 70 % ethanol and air dried in the laboratory. Tissues were cut into small pieces and immersed in 10 % sodium hypochlorite for 3 min followed by 70 % ethanol for 1 min and rinsed in sterile distilled water. Tissue fragments were dried on filter paper and placed on modified PARBPH selective agar (Jeffers and Martin 1986). Growing colonies

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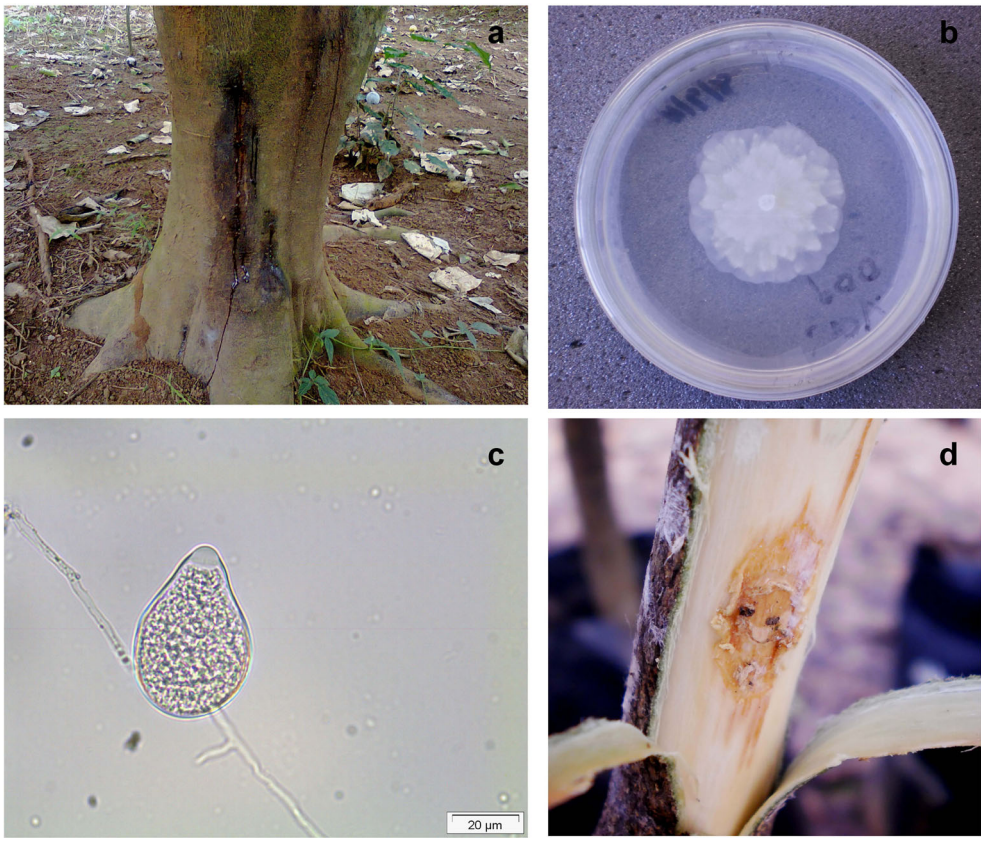

Fig. 1 **a** Gummosis lesion in a sweet orange tree grafted onto rough lemon rootstock at Kade, Ghana; **b** Petaloid colony of *Phytophthora citrophthora* growing in potato dextrose agar; **c** Papillate sporangium of *P. citrophthora*; and **d** Lesion in a sweet orange tree inoculated with *P. citrophthora*

were transferred to potato dextrose agar (PDA) and maintained at 24 °C in the dark for characterisation of colony morphology. Growth at cardinal temperatures, 5 and 35 °C, was evaluated on V8 juice agar after 30 days in the dark as indicated by Erwin and Ribeiro (1996). Sporangia were produced by cutting 5-mm-wide strips from colonies growing on V8 and floating them on 10 ml of 1.5 % sterile soil extract for 4–5 days at 24 °C under fluorescent light. Mycelial characteristics, morphology and dimensions of 100 sporangia were evaluated microscopically at 400X.

Oomycete strains with coenocytic hyphae and petaloid colony pattern were consistently isolated from affected tissues in all 50 samples analysed (Fig. 1b). One isolate from each sample was selected for morphological and cultural analysis. Typical *Phytophthora*-like noncaducous, papillate sporangia were observed when incubated in sterile soil extract (Fig. 1c). Shape of sporangia was obpyriform or ovoid with the following lengths and widths: 50.6 (36.5–64.4) × 31.1 (23.6–38.6) µm and with a L:W ratio of 1.6 (1.3–2.3) based on 100 measurements. No chlamydospores were observed. Isolates grew at 5 °C but not at 35 °C. These morphological and cultural characteristics were similar to those described for *Phytophthora citrophthora* (Erwin and Ribeiro 1996).

The internal transcribed spacer regions (ITS1, ITS2), the 5.8S and 28S rRNA genes were amplified using the primers ITS5 and ITS4 (White et al. 1990) from DNA extracted from a representative isolate designated as FHCRC-PHY1, obtained from *C. sinensis* at Kade, Ghana. The sequence was submitted to the GenBank database with accession No. KP676165 and the isolate deposited in the Spanish Type Culture Collection (http://www.uv.es/cect). PCR products were purified with the High Pure PCR Product Purification Kit (Roche Diagnostics GmbH) and directly sequenced using the Tag DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). Sequences were compared with those available in the *Phytophthora* database 2.0 (Park et al. 2013) and the *Phytophthora*-ID database 2.0 (Grunwald et al. 2011). The sequence obtained from isolate FHCRC-PHY1 had 99.87 % identity with those of *P. citrophthora* isolates PD_01697, PD_01696 and PD_01705 from the *Phytophthora* database 2.0 (Park et al. 2013) and was 99 % homologous to the *P. citrophthora* sequence HQ643205 of the isolate CBS 950.87 (Robideau et al. 2011) in the *Phytophthora*-ID database 2.0 (Grunwald et al. 2011).

Pathogenicity tests were carried out on 2-year-old sweet orange trees cv. Valencia-Late grafted on rough lemon grown in plastic pots (40 cm diameter × 60 cm deep) containing potting mix (sterilised soil and rice husk Boucher). Ten isolates, including FHCRC-PHY1, were grown on PDA and used in the pathogenicity tests. Inoculation sites in the plants were previously disinfested with 70 % ethanol. Plants were

stem-inoculated by removing a 5-mm-diameter disc of the bark of the scion on each plant using a cork borer to expose the cambium and placing a mycelial plug obtained from 5-day-old colonies (Álvarez et al. 2008). Control plants were treated with PDA plugs. All inoculation points were immediately covered with moistened sterile cotton wool and parafilm. The experiment was repeated once using five plants for each of the 10 isolates studied and five other plants as controls. Four weeks after inoculation all plants inoculated with *P. citrophthora* showed symptoms of the disease, consisting of gum exudation and bark discoloration (Fig. 1d). Depending on the isolate, lesion lengths ranged from 22 to 76 mm and *P. citrophthora* was re-isolated. No symptoms were observed on control plants.

According to Dade (1940), the presence of *Phytophthora* in Ghana was first reported between 1928 and 1930, but not on *Citrus* species. Leather (1959) indicated that citrus gummosis was caused by *P. parasitica*. However, the pathogenicity tests conducted by Assuah et al. (1999) indicated that the causal agent of citrus gummosis in Ghana was *L. theobromae*. These results were later supported by Offei et al. (2002), who also induced lesions in different citrus rootstocks inoculated with *L. theobromae*. Species of the family Botryosphaeriaceae were associated with twig and branch dieback of citrus (Fawcett and Burger 1911; Adesemoye et al. 2014). Benzimidazole fungicides were recommended for the control of *L. theobromae* in citrus (Hearn and Fenton 1970; Assuah et al. 1999). However, in Ghana, the anti-oomycete fungicide metalaxyl (Syngenta Crop Protection AG, Switzerland) is recommended for the control of citrus gummosis (Ofosu-Budu et al. 2007). Moreover, before the spread of *citrus tristeza virus* (CTV) in Ghana (~1938–1948), control of gummosis was obtained by budding onto sour orange (*C. aurantium*), which is known to be highly resistant to *Phytophthora* (Leather 1959). In conclusion, the present study confirmed earlier reports indicating that *Phytophthora* is the causal agent of citrus gummosis in Ghana. Further country-wide surveys will elucidate the role of *P. citrophthora* and other *Phytophthora* species in a gummosis of *Citrus* species in Ghana.

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