

## NEW DISEASE REPORT

# First report of citrus black spot disease caused by *Phyllosticta citricarpa* in Benin

H. G. Toessi<sup>1,2,3</sup>  | G. E. Ler-N'Ogn Dadé Amari<sup>1,2</sup> | R. Sikirou<sup>3</sup> | D. Kone<sup>1,2</sup>

<sup>1</sup>Centre d'Excellence Africain sur le Changement climatique, la Biodiversité et l'Agriculture Durable (CEA-CCBAD), Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire

<sup>2</sup>Laboratoire de Biotechnologie, Agriculture et Valorisation des Ressources Biologiques, UFR Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire

<sup>3</sup>Laboratoire de Défense des Cultures (LDC), Institut National des Recherches Agricoles du Bénin (INRAB), Cotonou, Benin

## Correspondence

H.G. Toessi, Centre d'Excellence Africain sur le Changement climatique, la Biodiversité et l'Agriculture Durable (CEA-CCBAD), Université Félix Houphouët-Boigny, 22 BP 582 Abidjan, Côte d'Ivoire  
Email: [habib.toessi@yahoo.com](mailto:habib.toessi@yahoo.com)

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## KEYWORDS

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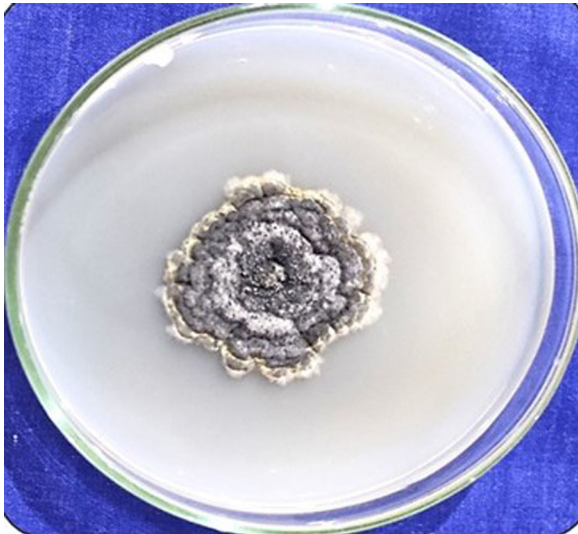
In Benin, citrus production represents an important activity in the national economy and source of income for farmers. For a decade, the majority of orange orchards in Benin have been affected by a disease that is expressed by the appearance of spots on the fruits (Lokossou *et al.*, 2009). A preliminary study conducted on farmers' perception on the presence of this disease revealed positive answers from all 417 interviewed orchardists. In July 2021, 15 orchards were randomly selected for inspection in four orange production districts in Benin: Djidja (7°20'N, 1°56'E), Zakpota (7°15'N, 2°11'E), Lalo (6°56'N, 1°56'E) and Bopa (6°35'N, 1°57'E). Between 50 to 100% of the trees in the inspected orchards were affected by the disease. Disease symptoms consisted of numerous tiny to middle-sized dark brown to black spots, ranging in size from 3 to 10 mm in diameter. The tiny spots had a grey centre with a dark brown to black border surrounded by a green or yellow halo, depending on the maturity of the fruit. In cases of severe infection, the fruits were completely covered with black spots, and rot (Fig. 1). The leaves also had numerous black spots. In cases of less severe infection, symptoms were limited to the fruits. Four fruits per orchard, in total sixty fruits, showing black spots were randomly selected each of the inspected orchards.



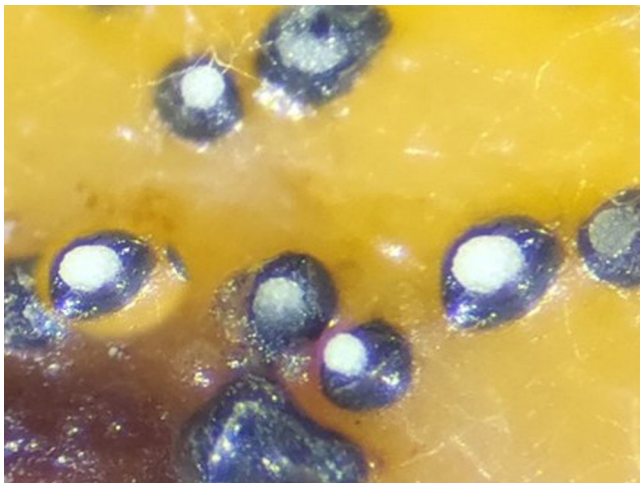
**FIGURE 1** Symptoms of black spot disease in orange orchards in Benin

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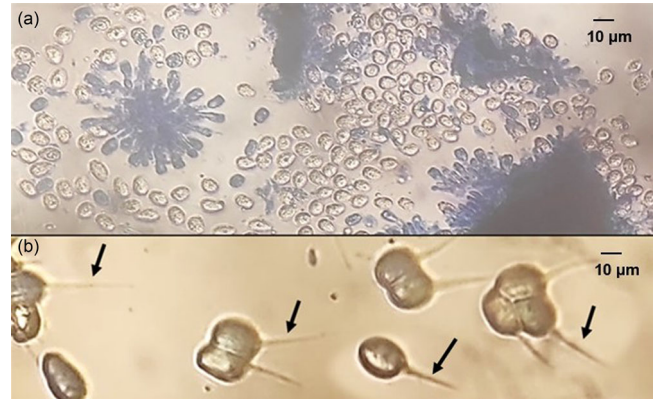


**FIGURE 2** Colonies of *Phyllosticta citricarpa* on potato dextrose agar medium

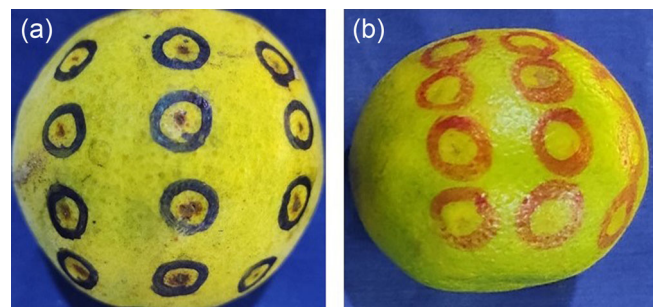


**FIGURE 3** Binocular observation of *Phyllosticta citricarpa* pycnidia at  $\times 40$  magnification

A fungus was isolated from the diseased orange fruits on potato dextrose agar medium. The fungus was identified as *Phyllosticta citricarpa* based on morphological and cultural characteristics. The fungus produced a slow mycelial growth with irregular edged colonies surrounded by a larger translucent zone of immersed clear mycelium (Fig. 2). The centre of the colonies was composed of grey aerial mycelium forming a plectenchymatous rind. The reverse had a very dark centre surrounded by grey and beige areas. Hard, black mass stromas appeared after seven to eight days and ripe pycnidia containing conidia after 10 to 14 days (Fig. 3). Conidia were  $9.4 - 12.7 \mu\text{m}$  long and  $5.0 - 8.5 \mu\text{m}$  wide, ellipsoid to obovoid with a truncate base, hyaline, guttulate, aseptate with a tiny apical mucoid and translucent layer appendage,  $3 - 10 \mu\text{m}$  long and  $1.5 \mu\text{m}$  wide (Fig. 4). These morphological features confirm the identity of the fungus as *Phyllosticta citricarpa* (Baayen *et al.*, 2002).



**FIGURE 4** Microscopic observation: (a) conidia surrounded by a thin mucoid layer, and (b) conidia with apical appendage (arrow)



**FIGURE 5** Pathogenicity test results of *Phyllosticta citricarpa* on orange fruits: (a) fruit inoculated by *Phyllosticta citricarpa*, and (b) control fruit inoculated with distilled water

Koch's postulates were performed in the laboratory on 30 healthy ripe orange fruits, with two replicates, by injecting  $5 \mu\text{l}$  of a suspension of  $10^6$  conidia/ml with a sterile medical needle approximately 2 mm deep in the albedo of fruit. On each fruit, 12 points were inoculated. Control fruits were inoculated with sterile needle and distilled water. Inoculated fruits were kept at room temperature ( $25 \pm 2^\circ\text{C}$ ). After 15 days, the inoculated fruits developed similar symptoms to those observed in the orange orchards while no symptoms were observed on the untreated fruits (Fig. 5). *Phyllosticta citricarpa*, was re-isolated from lesions developed on inoculated fruit.

*Phyllosticta citricarpa* (syn. *Guignardia citricarpa*) has been found previously in Australia, in tropical and subtropical citrus-growing areas of Africa (Angola, Ghana, Kenya, Mozambique, Tunisia, Uganda, Namibia, South Africa, Zambia, Zimbabwe), the Americas (Brazil, Florida), and in Asia (China, India) (EFSA *et al.*, 2020). This is the first report of *Phyllosticta citricarpa* causing citrus black spot in Benin. Management methods and strategies are being developed to control and limit the spread of the disease in the country.

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#### ORCID

H. G. Toessi  <https://orcid.org/0000-0001-9061-5110>

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