



## First report of citrus black spot disease caused by *Phyllosticta citricarpa* on *Citrus limon* and *C. sinensis* in Tunisia

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Citrus products are economically important in Tunisia and constitute a significant part of agricultural exports. In March and April 2019, symptoms resembling citrus black spot were observed on citrus fruit (*Citrus limon* and *C. sinensis*) in Nabeul Gouvernorat. From June to September 2019, an intensive survey was undertaken in 339 orchards located in this region. Symptoms were observed in 69 orchards of lemon and orange trees located in the areas of Bou Argoub, Beni Khalled, Menzel Bouzelfa, Soliman, Grombalia, Takelsa, Korba, Nabeul and Hammem Ghezaz. Diseased fruits had freckle spots or hard spots bearing pycnidia, whereas no symptom was observed on leaves.

For isolation of the pathogen, sections of fruit peel (5 × 5 mm) were cut aseptically and surface-sterilised in 10% sodium hypochlorite solution for 20 seconds, followed by 70% ethanol for 30 seconds, and rinsed three times in sterile distilled water. The dried sections were then plated on petri dishes containing potato dextrose agar (PDA) amended with 100 mg/l of sulphate streptomycin. The plates were incubated at 25°C for seven days. The resulting colonies developed slowly and were characterised by irregular margins with numerous pycnidia. The centre of the colony was dark-coloured, whereas the edge of the culture was colourless with a submerged mycelium (Fig. 1). Conidia (3.463-) 6.482 (-10.378) × (8.009-) 4.226 (-0.229) µm were ellipsoid-obovoid, and encased in a mucoid sheath (Fig. 2). The isolates were identified tentatively as *Phyllosticta* spp.

The species designation, and discrimination from the sister species *P. paracitricarpa*, was confirmed by sequencing two genes (*tef1* and LSU), according to Guarnaccia *et al.* (2017) followed by comparison with reference sequences. Fungal material was collected from two PDA cultures, one each from lesions on fruits of *Citrus limon* and *Citrus sinensis*, by scraping the mycelium with a sterile needle and transferring into 2-ml microtubes. Total fungal DNA was then extracted and *tef1* and LSU regions amplified by PCR using the EF1-728F/EF2 and LR0R/LR5 primer pairs, respectively (Guarnaccia *et al.*, 2017). Nucleotide sequences were deposited in GenBank (Accession Nos. MN823081-84). BLAST analysis

showed 100% identity with *P. citricarpa* (JN979767, KX280353 and FJ538375) for both isolates.

To verify the pathogenicity of 21 isolates, a bioassay was conducted on detached lemon fruits. Prior to inoculation, the fruits were surface-sterilised with 70% ethanol for 10 seconds. A toothpick was used to create 4 wound points per fruit which were then inoculated with a pellet of 14-day fungal culture. Six fruits were used for each isolate and a sterile PDA plug was used as negative control. The inoculation points were wrapped with paraffin film to prevent contamination and placed in a closed plastic container. After three weeks incubation at 25°C, citrus black spot symptoms were observed (Fig. 3) and the fungus could be isolated again from the lesion, thus fulfilling Koch's postulate.

To our knowledge, this is the first report of *Phyllosticta citricarpa* causing citrus black spot on citrus in Tunisia. The disease may have a severe impact on citrus production in Tunisia by reducing yield and affecting the quality of *Citrus limon* and *C. sinensis*. Surveys are ongoing and management strategies have been developed to control and limit the spread of the disease in Tunisia.

### Acknowledgements

Drs. Boughalleb-M'Hamdi and Fathallah contributed equally as first authors of this report.

### References

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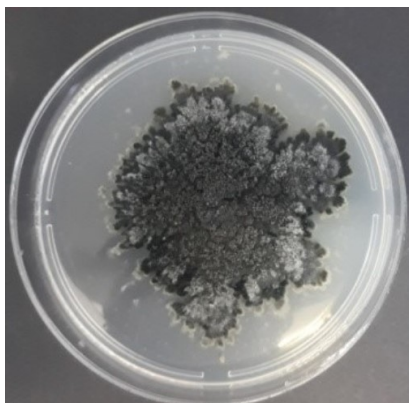


Figure 1

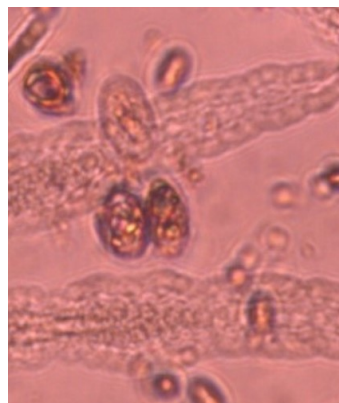


Figure 2



Figure 3

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