First Report of Charcoal Rot, caused by *Macrophomina phaseolina,* on Blueberry in Southwestern Spain

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In Europe, Huelva province in Southwestern Spain is the main berry production area. Blueberry (*Vaccinium* spp.) was introduced during the early 90's as an addition to strawberry cultivation. From 2011 to 2018, blueberry acreage increased from 777 ha to 3,000 ha. In May 2015 and September 2017, wilted southern highbush blueberry plants (cvs. 'Star', 'Ventura', and 'Legacy') were collected from three orchards located in the Huelva production area (Gibraleón and Moguer). The diseased plants showed drying of foliage and brown discoloration of stems and roots. Root and stem of symptomatic plants were surface sterilized (2 min,1% sodium hypochlorite), rinsed, dried, and plated on potato dextrose agar (PDA). After 7 days at 30º C in the dark, fungal isolates produced numerous black, round to ovoid shaped sclerotia with an average diameter of 98 µm (range: 61 to 128 µm). Genomic DNA from a single sclerotium isolate (TOR-872) was extracted following the technique described by Bekesiova et al. (1999). Four DNA regions were amplified and sequenced: the exon region of translation elongation factor 1- α (TEF-1 α), β-tubulin (β-TUB), calmodulin (CAL), and the ITS region. TEF-1 α was amplified with the EF1-728F and EF1-986R primers (Carbone and Kohn, 1999), the β-TUB with the T1 and T22 primers (O'Donnell and Cigelnik, 1997), the CAL with the CAL-228F and CAL-737R primers (Carbone and Kohn, 1999) and the ITS with the ITS5 and ITS4 primers (White et al., 1990). After BLASTing the 4 sequences against the GenBank database, the top hits corresponded to *Macrophomina phaseolina* with a 99-100% of sequence identity for all cases. Our sequences were submitted to GenBank under Accession numbers: MK447854 (TEF-1 α), MK447918 (β-TUB), MK447823 (CAL) and MK447886 (ITS). Morphological and molecular results confirmed this isolate as *M. phaseolina* (Holliday and Punithalingam, 1970). In Gibraleón, in 7.87% of nearly dead plants (cv. Ventura) only *M. phaseolina* was isolated, whereas in Moguer disease incidence was 30, 7, and 2.27% in cvs. 'Star', 'Ventura', and 'Legacy', respectively. Inoculum for pathogenicity testing was produced by growing isolates TOR-872 and TOR-862 (both from diseased blueberry plants) on PDA. In addition, pathogenicity of a *M. phaseolina* isolate (TOR-102), from a strawberry soil and confirmed as pathogenic to strawberry, was tested because blueberry is usually cultivated in soils where strawberry had grown. Six potted blueberry plants (cv. 'Star') per isolate were inoculated by substrate irrigation with 50 ml of a sclerotia suspension (10^4 sclerotia/ml) of each isolate. Six control plants were irrigated with water. Plants were held at 28°C and 40/70% relative humidity (day/night) in a growth chamber with a 16-h photoperiod. Four months after inoculation, the mortality of the inoculated plants was 33, 50 and 50% for isolates TOR-862, TOR-872, and TOR-102, respectively. *M. phaseolina* was reisolated from all dead plants. No symptoms were observed in control plants. *Macrophomina phaseolina* has been associated with a blight disease on blueberry in Serbia (Popović et al., 2018) but this is the first report of charcoal rot on blueberry in Spain. The aggressiveness of the strawberry soil isolate was also confirmed on blueberry. In Spain, chemical fumigation in soil is banned in blueberry production. Thus, blueberry may be grown on fields with a

previous history of strawberry production and carry-over of *M. phaseolina* causing charcoal rot may significantly impact crop production.

References:

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