Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia theobromae* Causing Decline of Blueberry (*Vaccinium corymbosum*) in the Czech Republic

J. Pečenka, D. Tekielska, M. Kocanová, E. Peňázová, A. Berraf-Tebbal, and A. Eichmeier[†]

Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics, 69144, Lednice, Czech Republic

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The popularity of blueberries (*Vaccinium corymbosum* L.) has been rising worldwide, due to their nutritional bioactive compounds such as flavonoids, anthocyanins, and polyphenols (Sinelli et al. 2008). These factors led to an increase of the demand for fresh blueberries in the Czech market, which resulted in increased blueberry planted in the Czech Republic. In spring 2018, about 200,000 1-year-old blueberry plants originally from the Netherlands were planted on 47 ha in the South Moravian region. In September 2019, wilting of leaves and shoots, bark necrosis, and dieback of the plants were observed. The occurrence of blueberry decline symptoms in the field was near 2% of 5,000 observed plants, which resulted in significant yield losses at the beginning of the production. Twenty shoots of symptomatic plants were collected (one shoot from each plant, 20 plants in total) and surface disinfected using 75% ethanol. Necrotic tissues of xylem were aseptically placed on potato dextrose agar (PDA) (HiMedia, India) amended with streptomycin and incubated at 25°C in the dark for 1 week. Nine isolates of Botryosphaeriaceae-like fungi were obtained from 20 plants and were transferred on PDA. Aerial mycelium grew rapidly, initially white, and then became gray to black. To enhance the conidia production, the isolates were transferred onto water agar (HiMedia) with autoclaved poplar twigs, placed near day light, and incubated at room temperature. Three weeks later, black pycnidia were observed on the poplar twigs. Mature pycnidia exuded white or gray colored masses of conidiospores. Conidia were granular, subcylindrical to elliptical. Immature conidia were single-celled and hyaline, measuring $9.73 \pm 1.39 \times 16.86 \pm 1.81 \mu\text{m}$ ($n = 60$). Mature conidia were thick-walled with a septum, $11.30 \pm 1.17 \times 16 \pm 1.58 \mu\text{m}$ ($n = 60$). Based on conidiogenous cells, paraphyses, and longitudinal striations

on mature conidia characteristics, the isolates were morphologically assigned to the *Lasiodiplodia* genus (Phillips et al. 2013). Isolates were single spored, and total DNA was extracted with a NucleoSpin Tissue kit (Macherey-Nagel, Germany) following manufacturer's protocol. The internal transcribed spacer (ITS) region, including the 5.8S, and part of the translation elongation factor 1 alpha (TEF-1α) were amplified with ITS1/ITS4 and EF1-728F/EF1-986R primers, respectively (Carbone and Kohn 1999; White et al. 1990). Consensus sequences were blasted using the NCBI nucleotide BLASTn search. The sequences of the ITS region were 468/469 nt (MEND-F-00167) and 486/490 nt (MEND-F-00168) similar to *Lasiodiplodia theobromae* isolate CBS 287.47 and were 286/289 nt (MEND-F-00167) and 287/290 nt (MEND-F-00168) in the case of TEF-1α. Sequences of two representative isolates (MEND-F-00167 and MEND-F-00168) were deposited in GenBank under accession numbers MN983133 and MN983134 for ITS and MN989914 and MN989915 for TEF-1α. For pathogenicity tests, 6-mm agar plugs with mycelium obtained from 10-day-old mycelia of both fungal isolates were inoculated on ten 1-year-old blueberry shoots (cv. Top Shelf) collected from one plant, using a cork borer. Five shoots and pure agar plugs were used as a control. Plugs were covered with wet cotton wool to keep moisture and fixed with Parafilm. After 3 weeks, characteristic discoloration and lesions appeared on the inoculated shoots. Control shoots remained symptomless. Necrotic tissue was aseptically cut, and *L. theobromae* was reisolated on PDA. Identity of the fungus was confirmed by morphological characterization and sequencing, fulfilling Koch's postulates. Blueberry decline caused by Botryosphaeriaceae spp. has already been described around the world (Hilário et al. 2020; Tennakoon et al. 2018; Wiseman et al. 2017; Xu et al. 2015). To our knowledge, this is the first report of *L. theobromae* on blueberry in the Czech Republic.

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[†]Indicates the corresponding author.

E-mail: ales.eichmeier@mendelu.cz (A.E.)