Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Boeremia exigua* var. *exigua* Causing Black Spot-Like Symptoms on Commercially Grown Soybean in Germany

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Soybean (*Glycine max* [L.] Merr.) is economically the most important protein crop grown worldwide. However, Europe largely depends on soybean imported from the Americas (European Commission 2019; Haupt and Schmid 2020). In Germany, soybean production was not formally recorded before 2016, but since then a steady increase along with an expansion of the growing area from the south of Germany to northern states occurred. In 2019 an area of 29,000 ha was under soybean cultivation (Federal Ministry of Food and Agriculture [Germany] 2019). In the state of North Rhine-Westphalia (NRW, western part of Germany), farmers have started in recent years to cultivate soybean, making it increasingly important to monitor pathogens associated with this new crop. At the beginning of October 2019, shortly before harvest, rows of black spots on pods and stems of soybean plants of cultivar Viola throughout a field site near Jülich (NRW) were observed. Close observation identified them as pycnidia with similarity to symptoms reported from soybean in Austria in 2015 (Hisek and Bedlan 2016). The collected samples were thoroughly surface sterilized (two washes with 70% EtOH, a rinse in 0.5% sodium hypochlorite solution, and a final wash in sterile double-distilled water) and placed on plates containing potato dextrose agar at 22°C in the dark. Fungal colonies were transferred to malt extract agar plates (MEA) and examined by microscopy. Thus, 34 of 41 isolates looked morphologically similar, producing colonies that appeared dark gray with white aerial mycelium and round to irregular margins. A single-spore isolate was generated and designated IPP1903. Spores derived from IPP1903 were unicellular and mostly oblong to cylindrical with a mean width of $2.6 \pm 0.3 \mu\text{m}$ and a mean length of $5.9 \pm 0.8 \mu\text{m}$ ($n = 50$, mean value $\pm$ standard deviation). Colonies on MEA were 5.4 to 5.8 cm in diameter after growth for 7 days at 20 to 25°C with a photoperiod of 12 h and 3.3 to 3.7 cm in diameter after growth for 7 days in the dark at 22°C. These morphological observations led to the

conclusion that the isolate may belong to the genus *Phoma*. To test this hypothesis, we performed a drop test with 5 M NaOH, which is used routinely to check for the presence of a genus-specific metabolite. We observed a change in color, indicating a positive test result. The color change was even more pronounced on the plates incubated in the light, further confirming the presence of “metabolite E” (Boerema et al. 2004; Kövics et al. 2014). Next, DNA was extracted, and PCR was performed with primers specific for ITS regions (GenBank MT397284), LSU (MT397285), *rbb2* (MT414713), or *tub2* (MT414712). Sequencing results of PCR products were used to create a combined phylogenetic tree, including sequences published previously (Chen et al. 2015). Our sequencing results together with the morphological observations clearly identified the fungal isolate to be *Boeremia exigua* var. *exigua*. The isolate is publicly available in the CBS collection of the Westerdijk Fungal Biodiversity Institute with the accession number CBS 146730. Koch’s postulates were fulfilled by inoculating a spore suspension of the isolate IPP1903 ($5 \times 10^5/\text{ml}$ in 0.05% Tween 20 solution in distilled water) onto healthy primary leaves of twenty soybean plants (14 days old) of the cultivar Abelina. Whereas the mock-inoculated plants (inoculated with 0.05% Tween 20 solution in distilled water) stayed healthy, the inoculated plants developed lesions on the leaves after 7 days. Six weeks after inoculation the fungus could be reisolated from cuttings of the infected leaves after surface sterilization. Fungal colonies were confirmed to be *B. exigua* var. *exigua* by morphological examination and via NaOH drop test. To our knowledge, this is the first report of *B. exigua* var. *exigua* causing disease on commercially grown soybean in Germany.

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