Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Pod and Stem Blight and Seed Decay Caused by *Diaporthe longicolla* on Soybean in Western Canada

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In October 2019, soybean plants (*Glycine max*) (cv. 24-10RY, R7 growth stage) with dry rot, necrosis, reddish-brown lesions, and small black fruiting bodies in linear rows were collected from fields in Manitoba (Carman, St. Adolph, Dauphin), Canada. The pods and seeds were shriveled, small, and some seeds were covered with whitish mycelium. Symptoms began as brown lesions, which darkened, elongated, and caused wilt of the above stems and then plant death. Microscopy showed that the fruiting bodies were pycnidia. Symptomatic stems were cut into 1 to 2 cm pieces and seeds surface-sterilized in 0.5% NaOCl, rinsed twice in sterilized H₂O, air-dried on sterilized filter paper, and plated on potato dextrose agar (PDA) medium amended with 100 mg/liter of streptomycin sulfate at room temperature with 12-h fluorescent light/12-h dark for 3 days. The emerging hyphae were transferred using the hyphal tip method to new PDA Petri dishes and incubated for 21 days at room temperature. Mycelia of 20 isolates were dense, white, and floccose with occasional green-yellow areas. Black stromata in concentric patterns or scattered as large masses were visible on the cultures' back. Pycnidia formed solely or aggregated after 4 to 5 weeks of incubation on PDA. Alpha conidia emanated from pycnidia in creamy-to-yellowish drops and were hyaline, nonseptate, ellipsoid to fusiform, and biguttulate. The average length and width of alpha conidia were 5.5 µm and 1.5 µm, respectively ($n = 30$). No perithecia were seen. The cultures' morphology was consistent

with *Phomopsis longicolla*'s description (Hobbs et al. 1985). Seven isolates were selected for molecular characterization to confirm their identity by amplifying the ITS region with universal primers ITS4/ITS5 (White et al. 1990). All PCR amplicons were analyzed by electrophoresis through 1.5% agarose gels and the size of PCR amplicons estimated using 1-kb plus DNA ladder (Thermo Fisher, Canada). PCR amplicons (~650 bp) were purified and sequenced in two directions by Psomagen Inc. (Rockville, MD, U.S.A.). ITS sequences were identical for all isolates, and GenBank searches (BLASTn: Altschul et al. 1990) confirmed species identity. ITS sequences (accessions MW466183–89) were deposited in GenBank and matched the type sequence of *Diaporthe longicolla* strain ATCC 60325 (accession NR_144924) from *G. max* in the U.S.A. with identities = 473/475 (99.6%) and gaps = 0/475 (0%). To confirm the pathogenicity of the seven isolates, the stems of V4-stage (four open trifolilates) soybean plants (cv. 24-10RY) were excised using a sterile scalpel. Mycelial plugs (9 mm in diameter) from 1-week-old culture of each isolate were placed over the wounded stems (Abdelmagid et al. 2019). Sterile PDA plugs were used on control plants. Six plants were used per isolate and control. Plugs of both treatments were wrapped with Parafilm to avoid drying. The plants were incubated in a humidity chamber for 4 days and then in a greenhouse at 24:16°C day/night, 13:11-h light/dark cycle, and 70 to 80% relative humidity, and irrigated as needed. Symptoms similar to those observed in the field were seen on the stems and seeds of all artificially infected plants ~8 weeks after inoculation. Pods and seeds of inoculated plants were shriveled and small. No symptoms were observed on control plants. *D. longicolla* was reisolated only from diseased plants and seeds. To our knowledge, this is the first report following Koch's postulates to identify the causal pathogen of soybean pod and stem blight and seed decay in western Canada. This will be instrumental in determining the causes of stem and seed decay and contribute to properly dealing with soybean seed issues in western Canada in the future.

References:

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