Identification of *Diaporthe longicolla* on Dry Edible Pea, Dry Edible Bean, and Soybean in North Dakota

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Dry edible bean (*Phaseolus vulgaris* L.), dry edible pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.) are important crops in North Dakota, with the state ranking first in U.S. production of dry edible bean and dry edible pea and 10th in soybean production in 2013 (http://www.nass.usda.gov/Statistics_by_State/North_Dakota/). North Dakota soybean production has expanded well beyond its traditional growing region in recent years, resulting in direct overlap with dry edible bean and pea production areas. Possible short rotations among these crops raise concerns about the increase of soil and/or residue-borne pathogens known to infect the crops, and give rise to the possibility of other pathogens developing overlapping host ranges. One pathogen of concern is *Diaporthe longicolla* (Hobbs) J. M. Santos, Vrandečić & A. J. L. Phillips, the cause of Phomopsis seed decay and stem disease of soybean (Harrington et al. 2000, Li et al. 2010).

In July 2009, dry edible pea stems with red, elongated lesions were observed and collected from a commercial production field in northwest North Dakota. In September 2010, lower stems of soybean and dry edible bean showing reddish brown lesions and small, black pycnidia were collected from field research plots in Fargo, ND.

Isolations were made from the reddish-brown lesions by surface-sterilizing and plating small stem pieces (5 mm) on potato dextrose agar (PDA) medium amended with 0.02% streptomycin sulfate. The fungal isolates were labelled SB (from soybean), DEB (from dry edible bean), and DEP (from dry edible pea). Plates were incubated for 14 days at 25°C under 16 h of alternating light and dark conditions. Morphological characteristics of all cultures were consistent with *Diaporthe* spp. (Barnett and Hunter 1972). The mycelium of the isolates from the three hosts was white, dense, and floccose. Large, black pycnidia were formed in concentric patterns or were scattered. Alpha conidia exuded from pycnidia in creamy-to-yellowish drops and were ellipsoid and biguttulate with an average length of 6.6 µm and width of 2.1 µm. To molecularly identify isolates, DNA was extracted from the mycelium and the ITS region was amplified and sequenced using primers ITS4 and ITS5 (White et al. 1990). BLASTn analysis of approximately 600-bp fragment from the three isolates showed the best match was *Diaporthe longicolla* (Hobbs) J. M. Santos, Vrandečić & A. J. L. Phillips (Santos et al. 2011, GenBank Accession AY745021) from *G. max* with identities = 524/524 (100%) and gaps = 0/524 (0%). DNA sequences generated in this study have been deposited in GenBank under accession numbers KJ608553 (SB), KJ605682 (DEP) and KJ605683 (DEB).

Pathogenicity of the three *D. longicolla* isolates (one from each host) was tested on each of the three host crops (soybean cv. 'RG200RR', dry edible bean cv. 'Maverick': market class Pinto, and dry edible pea cv. 'Admiral': market class yellow). Each treatment consisted of one seed planted per 1.5 liter pot of Sunshine Mix # 1 and was replicated twelve times. The pots were placed on greenhouse benches under a 16-h photoperiod at 25 ± 2°C. At the V3 stage of plant growth, a 5 mm long vertical slit on the stem of the 2nd internode was made using a sterile scalpel, and a single mycelial plug cut (4 mm diameter) was placed on the wound with parafilm (Li et al. 2010). Correspondingly, plugs of noninoculated PDA were used for the negative control plants. Disease symptoms 14 days after inoculation (Figs. 1, 2, and 3) included brown lesions with black pycnidia and dry rot with discoloration at the inoculation point. Noninoculated (control) plants had no disease symptoms. All isolates caused disease on each host, with the exception of the DEP isolate on dry edible bean.

To complete Koch's postulates, *D. longicolla* was re-isolated from each host of origin and identity was confirmed via sequencing. The pathogen could not be re-isolated from the control plants. To the best of our knowledge, this is the first report of *D. longicolla* causing stem disease on dry edible beans and dry edible peas, and stem disease on soybean in North Dakota. Yield losses from *D. longicolla* have been reported in soybeans (Wrather and Koenning 2009), but its impact on dry edible beans and dry edible peas is uncertain.

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FIGURE 1
A dry edible bean plant (cv. Maverick) 14 days after greenhouse inoculation with a *Diaporthe longicolla* isolate from dry edible bean (DEB).

FIGURE 2
A soybean plant (cv. RG200RR) 14 days after greenhouse inoculation with a *Diaporthe longicolla* isolate from soybean (SB).

FIGURE 3
Dry edible pea plant (cv. Admiral) 14 days after greenhouse inoculation with a *Diaporthe longicolla* isolate from dry edible pea (DEP).

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