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## Introduction

Diaporthe/Phomopsis species are pathogens on a wide range of hosts including soybeans (*Glycine max* L.), and responsible for several diseases, some of which are of economic importance. Under favorable environmental conditions, these diseases can result in in significant yield losses (Backman et al. 1985). For example, yield losses from Stem canker in the Midwest have ranged from minor to in excess of 50% (Hartman et al. 1999). Four *Diaporthe* species have been reported pathogenic on soybean – *Diaporthe sojae*, the causal agent of pod and stem blight; *Diaporthe caulivora* and *Diaporthe aspalathi*, causal agents of Northern and Southern stem cankers, respectively; and *Diaporthe longicolla*, causing seed decay (Hartman et al. 1999).

## *Diaporthe/Phomopsis* species on soybeans

The foliar symptoms are similar to brown stem rot and sudden death syndrome, which result in yellowing and browning of leaf tissues between the veins during pod-filling stages. However, the disease begins with the development of a small, reddish-brown lesion at the base of branches or petioles, which progress to browning and deterioration of the pith. During this time, the stem symptoms may be confused with those caused by white mold or Phytophthora stem rot.

Since there has been an increase in the prevalence of stem canker in the Midwest over the past few years, one objective of this study was to determine if the diseases caused by *Diaporthe/Phomopsis* species is present in North Dakota soybean fields. Another objective was to evaluate the cross-pathogenicity of *Diaporthe* species isolated from sunflowers (*Helianthus annuus* L.) and soybeans on the two hosts. Given expansion of soybeans to new production areas in North Dakota, soybean production can directly overlap with acreage of sunflowers. For example, the top soybean county in the United States (Cass county) planted 464,500 acres planted to soybeans and 2,800 acres to sunflowers in 2012 (Anonymous, 2013). Similarly, the top sunflower producing North Dakota counties in 2012 (Emmons county) planted 71,300 acres of sunflowers and 50,300 acres of soybeans (Anonymous, 2013). Overlapping production regions of soybeans with sunflowers in North Dakota has increased disease management concerns among growers. These questions have resulted in an increased awareness and monitoring for both documented and undocumented pathogens infecting soybeans and sunflowers.

## Materials and methods

### *Survey*

Enumerators employed by the National Agriculture Statistics Service (NASS) – North Dakota Field Office took 20 soybean stems from 100 fields in over 24 counties throughout North Dakota in 2011. Stem samples (n = 1,975) were cut longitudinally and visually assessed for the presence/absence of external and internal disease symptoms.

### *Isolation and molecular identification*

Stem pieces (~205) were surface sterilized and placed on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) amended with 0.02% streptomycin sulfate. Plates were incubated at room temperature for 7- to 14-d under 12-h of alternating light and dark conditions. Cultures were scored for presence or absence of different fungi, including Diaporthe, based on morphology (Barnett and Hunter 1972). DNA was extracted from lyophilized mycelium scraped from the surface of a 7-d Diaporthe culture growing on PDA using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI). The Diaporthe isolates were identified to species by amplifying and sequencing the internal transcribed spacer (ITS) regions using primers ITS5 and ITS4 (White et al. 1990).

### *Cross-pathogenicity*

The stem-wound method (Mathew et al. 2013) was adopted to evaluate cross-pathogenicity of *Diaporthe* isolates from soybeans and sunflowers. Isolates representing three *Diaporthe* species from soybeans were randomly selected from the isolations. *Diaporthe* isolates from sunflowers, previously characterized to *Diaporthe helianthi* and *Diaporthe gulyae*, were selected from collections made during a stem-disease survey in the North Great Plains (Mathew et al. 2011). The inoculum was prepared by culturing isolates on PDA and taking mycelial plugs (4 mm in diameter) from the margin of the growing colony. The stems of sunflower plants cv. 'HA 288' and soybean cv. 'RG200RR' were wounded on the 2nd internode and a Diaporthe-infested mycelial plug placed on the wound. Plants were assessed for lesion development on the stem 10-d after inoculation on a scale of 0 to 5 (Thompson et al. 2011). Analysis of data based on observed ranks was performed in SAS using the nonparametric procedure described by Shah and Madden (2004).

## Results

### *Survey*

Of the 2478 samples, 205 stems were positive for diseases (Brown stem rot, Stem canker, Charcoal rot) based on visual symptoms such as browning of the vascular and pith tissues of the stem.

### *Isolation and molecular identification*

Approximately 600 bp region of the ITS was amplified from 205 Diaporthe isolates and was used to query the GenBank database directly. A BLASTN search of GenBank identified the isolates as *D. caulivora* (50.2%), *D. longicolla* (48.8%), and *D. gulyae* (2.0%) based on comparison with the type isolates *D. caulivora* strain CBS 127268 (Accession # HM347712), *D. longicolla* strain CBS 659.78 (Accession # KC343201) and D. gulyae (*Phomopsis* sp. AJY-2011a strain T12505G, Accession # JF431299). These isolates differed at a single nucleotide site from the type isolate for the ITS gene. To the best of our knowledge, this is the first report of *D. gulyae* on soybeans in the United States.

### *Cross-pathogenicity*

The cross-pathogenicity study comparing *Diaporthe* species isolated from sunflowers and soybeans identified a significant interaction between species and hosts ($p<0.001$). Disease caused by the *Diaporthe* species was evaluated at 10-d after inoculations using relative effects (RE) and their 95% CI (Table 1). Among *Diaporthe* species infecting soybeans, significant differences in their RE was more evident; for example, the RE of the *D. gulyae* isolate from soybeans was significantly higher than that of *D. caulivora* isolate and *D. longicolla* isolate on soybeans (Table 1).

**Table 1.** Median, mean rank and relative treatment effects for stem canker severity rating caused by *Diaporthe* species on soybeans cv. 'RG200RR' and sunflowers cv. 'HA 288' at 10-d after inoculation.

| Host[a] | Species[a] | Median disease rating[b] | Mean rank | Estimated relative effect[c] |
|---|---|---|---|---|
| *Glycine max* (sb) | *D. gulyae* (sb) | 4.0 | 82.83 | 0.69 (0.61, 0.75) |
| | *D. longicolla* (sb) | 4.0 | 63.25 | 0.52* (0.45, 0.59) |
| | *D. caulivora* (sb) | 3.0 | 27.00 | 0.22 (0.19, 0.25) |
| | *D. helianthi* (sf) | 4.0 | 61.33 | 0.51* (0.41, 0.60) |
| | *D. gulyae* (sf) | 5.0 | 104.42 | 0.87 (0.81, 0.90) |
| *Helianthus annuus* (sf) | *D. gulyae* (sb) | 4.0 | 70.50 | 0.58* (0.55, 0.61) |
| | *D. longicolla* (sb) | 3.0 | 27.00 | 0.22* (0.19, 0.25) |
| | *D. caulivora* (sb) | 2.5 | 15.50 | 0.13* (0.09, 0.18) |
| | *D. helianthi* (sf) | 3.5 | 48.75 | 0.40* (0.31, 0.50) |
| | *D. gulyae* (sf) | 5.0 | 104.42 | 0.87* (0.81, 0.90) |

[a] sb=soybeans; sf=sunflowers
[b] Plants were assessed for lesion development at 10-d after inoculation on a scale of 0 to 5 (Thompson et al., 2011)
[c] The relative effect is significantly different based on confidence intervals ($p \leq 0.05$)

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