**Diaporthe** species associated with symptomatic and asymptomatic infection of soybean stems in Minnesota: identity, virulence, and growth characteristics

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**Abstract:** Stem diseases of soybean with symptoms typical of infection by *Diaporthe* spp. are common in the Northern USA. Information is limited, however, on the characteristics of the associated pathogens. This study determined the identity and characteristics of *Diaporthe* spp. associated with symptomatic and asymptomatic soybean stems across diverse environments in Minnesota. Plants with symptoms of stem canker, pod and stem blight, and plant dieback, as well as asymptomatic plants, were sampled. Agar-based media and PCR assays were used to isolate and detect *Diaporthe* spp. from stems. The isolates were identified to species based on DNA sequencing and morphology. *Diaporthe* spp. were common in symptomatic and asymptomatic plants across all environments. Three species were detected: *D. longicolla*, *D. caulivora*, and *D. cucurbitae*. *Diaporthe longicolla* was the most widespread in distribution. *Diaporthe cucurbitae* was isolated from symptomatic soybean and confirmed to be a soybean pathogen for the first time. Only *D. longicolla* was detected in asymptomatic plants. While all isolates were pathogenic to soybean, virulence differed among isolates, and cultivars differed in susceptibility. Temperatures of 25°C and 32°C differentially influenced the growth of *D. longicolla* and *D. caulivora*. All isolates were sensitive to pyraclostrobin and tebuconazole. The results advance our understanding of the *Diaporthe* species associated with different soybean stem diseases, as well as their characteristics and management.

**Keywords:** blight, canker, *Diaporthe caulivora*, *Diaporthe cucurbitae*, *Diaporthe longicolla*, *Phomopsis*

**Résumé:** Les maladies de la tige du soya affichant des symptômes typiques de l’infection causée par *Diaporthe* spp. sont courantes dans le Nord-Est américain. Toutefois, il y a peu d’information sur les caractéristiques des agents pathogènes qui leur sont associés. Cette étude a établi l’identité et les caractéristiques des *Diaporthe* spp. associés aux tiges de soya symptomatiques et asymptomatiques dans divers environnements au Minnesota. Les plants présentant des symptômes de chancre de la tige, de brûlure phomospienne et de dépérissement ainsi que des plants asymptomatiques ont été échantillonnés. Des milieux gélosés et des épreuves par PCR ont été utilisés pour isoler et détecter *Diaporthe* spp. sur les tiges. Les isolats ont été identifiés à l’espèce en se basant sur le séquençage de l’ADN et la morphologie. *Diaporthe* spp. étaient courants chez les plants symptomatiques et asymptomatiques, et ce, dans tous les environnements. Trois espèces ont été détectées: *D. longicolla*, *D. caulivora* et *D. cucurbitae*. *Diaporthe longicolla* était le plus largement disséminé. *Diaporthe cucurbitae* a été isolé de plants de soya symptomatiques et confirmé en tant qu’agent pathogène du soya pour la première fois. Seul *D. longicolla* a été détecté chez des plants asymptomatiques. Tandis que tous les isolats étaient pathogènes pour le soya, la virulence variait chez ces derniers et les cultivars ont réagi diversement quant à leur susceptibilité à leur égard. Des températures de 25°C et de 32°C ont influencé différemment la croissance de *D. longicolla* et de *D. caulivora*. Tous les isolats étaient sensibles à la pyraclostrobine et au tébuconazole. Les résultats font progresser notre compréhension des espèces de *Diaporthe* associées aux différentes maladies de la tige du soya de même que de leurs caractéristiques et de leur gestion.

**Mots clés:** pourriture, chancre, *Diaporthe caulivora*, *Diaporthe longicolla*, *Diaporthe cucurbitae*, *Phomopsis*

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Introduction

Fungal stem diseases of soybean [Glycine max (L.) Merrill] are common in many regions of the world. These include stem canker, pod and stem blight, brown stem rot, and white mould (Malvick et al. 2003; Harrington et al. 2003; Malvick and Bussey 2008; Peltier et al. 2012). Stem canker and pod and stem blight diseases incited by Diaporthe Nitschke [= Phomopsis (Sacc.) Bubak] spp. are especially significant worldwide and cause large yield losses (Kulik 1984; Sinclair 1988, 1993; Koenning and Wrather 2010; Vidić et al. 2011; Udayanga et al. 2015; Vidić et al. 2015; Allen et al. 2017). Although stem diseases caused by Diaporthe spp. occur in the Northern USA, information is limited on their aetiology and the phenotypic and genotypic characteristics of the associated pathogens.

The identification of Diaporthe spp. in many studies has been based on morphology, host–pathogen interactions, and physiological characteristics, sometimes leaving uncertainty about the identity of pathogens involved (van Niekerk et al. 2005; Mengistu et al. 2007; Lu et al. 2010). Molecular and phylogenetic methods now facilitate the identification of Diaporthe spp.; and sequences of two or more genes have enabled resolving and re-assessment of Diaporthe spp. associated with soybean diseases (Santos et al. 2011; Gomes et al. 2013; Guillin et al. 2014; Udayanga et al. 2015; Dissanayake et al. 2017; Hosseini et al. 2020; Petrović et al. 2021). For example, a primary soybean pod and stem blight pathogen is D. sojae (syn D. phaseolorum var. sojae), the northern stem canker pathogen is D. caulivora (syn D. phaseolorum var. caulivora), and a common seed decay pathogen is D. longicolla (syn Phomopsis longicolla) (Santos et al. 2011; Gomes et al. 2013; Guillin et al. 2014; Udayanga et al. 2015). This, however, is likely an incomplete depiction of the Diaporthe spp. associated with different diseases on soybean. For example, D. longicolla also infects soybean stems, D. gulyae and other Diaporthe spp. can cause stem canker, and multiple species were recently identified as causal agents of seed decay (Mathew et al. 2017; Sinclair 1988; Rupe 1990; Li et al. 2010; Hartman et al. 2015; Petrović et al. 2021). Thus, the identity and characteristics of Diaporthe spp. associated with various soybean diseases in different geographic areas warrants further study.

In the North Central USA and Canada, several Diaporthe spp. have been associated with soybean stem diseases (Gravert et al. 2001; Li et al. 2004; Bradley and Li 2007; Xue et al. 2007; Lu et al. 2010; Gebreil et al. 2015; Abdelmagid et al. 2019). Diaporthe caulivora and D. sojae have been associated with northern stem canker and pod and stem blight, respectively, whereas D. longicolla appears more adaptable and has been associated with seed, pod, and stem diseases (Hartman et al. 2015). Much research on the Diaporthe spp. associated with soybean has focused on southern stem canker and seed decay in regions with hot and humid summers (Rupe 1990; Hartman et al. 2015). Less is known about stem canker, pod and stem blight, and the identity of Diaporthe spp. associated with these diseases in northern soybean production areas, which has implications for disease management and breeding for resistance.

In addition to their roles as stem pathogens, Diaporthe spp. have been detected in asymptomatic soybean stems as endophytes or latent pathogens (Miller and Roy 1982; Sinclair 1988; Impullitti and Malvick 2013; Leite et al. 2013; Kontz et al. 2016; Batzer and Mueller 2020). The relationships between endophytic or latent Diaporthe spp. that colonize soybean early in the growing season and the development of stem canker or stem blight later in the season when plants are maturing or stressed is incompletely understood. Kulik (1984) reported that Diaporthe (Phomopsis) spp. inoculated on soybean did not cause symptoms or produce pycnidia until after plant senescence, and Hill (1981) reported that D. sojae could be dormant in soybean near the inoculation site until plant senescence.

Management of Diaporthe stem diseases of soybean may include the use of resistant soybean varieties and fungicides (Hartman et al. 2015; Tormen and Blum 2020). For example, varieties have been identified with resistance to southern stem canker caused by D. aspalathia and northern stem canker caused by D. caulivora (Benavidez et al. 2010; Chang et al. 2016; Peruzzo et al. 2019). There is limited understanding of resistance to stem blight caused by Diaporthe spp., and more research is needed to clarify the role of pathogen variability on resistance (Kontz et al. 2016; Ghimire et al. 2019). In addition, fungicides may play a role in the management of Diaporthe soybean stem diseases (Lu et al. 2010; Soto-Arias and Munkvold 2011). Information is limited, however, on the sensitivity of Diaporthe spp. from soybean stems to fungicides. Moreover, the effects of fungicide sprays on infection of soybean stems by Diaporthe spp. have been inconsistent, where fungicides have decreased and increased infection in different studies (Soto-Arias and Munkvold 2011; Batzer and Mueller 2020).

This study was undertaken to address knowledge gaps in the identity, pathogenicity, and other characteristics of Diaporthe spp. associated with soybean stem diseases across diverse environments in Minnesota. The specific objectives were to: (i) determine the Diaporthe species associated with symptomatic and asymptomatic infection
of soybean stems, (ii) determine if isolates and species vary in virulence, sensitivity to fungicides, and growth at different temperatures, and (iii) determine if selected northern soybean cultivars differ in susceptibility to prevalent Diaporthe spp. and isolates. This work aims to expand our knowledge of the identity, characteristics, and management of Diaporthe species associated with soybean stem disease.

**Materials and methods**

**Sampling and identification of Diaporthe spp. in symptomatic plants**

Soybean plants were sampled that had symptoms of stem disease and grouped into three categories: stem canker [discrete brown to reddish brown lesion at node(s)], pod and stem blight (dark pycnidia on stem arranged in rows, darkened stem without discrete lesions), and plant dieback (yellowing and/or death of upper stem with no other apparent root or stem symptoms) (Yang 2001; Hartman et al. 2015; Mueller et al. 2016). The plants (n = 87) were collected at R3 – R6 growth stages (beginning pod to full seed) from 50 fields in 19 counties across Minnesota (Fig. 1).

Sections of symptomatic stems, 2 by 8 mm, were surface disinfected with 0.26% NaOCl for 2 min, rinsed in sterile distilled water for 1 min, and blotted dry on sterile paper towels. The pieces were then placed on 0.5x potato dextrose agar (PDA, BD Difco Lab., Sparks, MD) in Petri dishes and incubated at 25°C for 7 to 10 d with a 10 h photoperiod.

**Fig. 1** Location (counties) in Minnesota where soybean stems were collected and tested for the presence of Diaporthe spp. (shaded). Symbols indicate where Diaporthe caulivora (●), D. longicolla (X), and D. cucurbitae (▲) were detected. Includes plants with symptoms of pod and stem blight, stem canker, and top dieback, as well as asymptomatic plants.
Fungal isolates with colony morphology characteristic of *Diaporthe*, i.e. white floccose growth on PDA, large stromata, and perithecia (Hartman et al. 2015; Vidić et al. 2015) were selected from 37 plants collected over a large geographical area. All putative *Diaporthe* isolates were subcultured by hyphal-tipping two to three times to purify the cultures. The genus and species of the isolates were subsequently identified based on DNA sequences and morphological characteristics. The morphology of isolates from this study, as well as isolates *D. caulivora* De IA41 and *D. longicolla* DI IA17 from Iowa, was studied on 0.5x PDA following incubation at 24°C under 14 h fluorescent lights for 14 d. For sequencing analysis, the isolates were grown in PD broth (BD Difco Laboratories, Franklin Lakes, NJ) for 7 d, and total DNA was extracted from all 37 isolates with a FastDNA® kit (MP Biomedical, Solon, OH). A standard PCR (sPCR) assay using primers Phom I and Phom II was used for presumptive confirmation that the isolates were *Diaporthe* spp. (Zhang et al. 1997). The species identity for all 37 isolates was then determined by sequencing the nuclear ribosomal internal transcribed spacer (ITS) region, amplified with ITS4 and ITS5 primers, and the beta-tubulin (TUB) gene amplified using the primers BT2A and BT2B (White et al. 1990; Glass and Donaldson 1995; Zhang et al. 1997; Lu et al. 2010; Udayanga et al. 2012) (Table 1). DNA sequencing was performed at the University of Minnesota BioMedical Genomics Center (St. Paul, MN) using an Applied Biosystems Prism 3730xl DNA analyzer. Amplification conditions used for all sequences were as follows: 1 cycle of 96°C for 60 seconds followed by 25 cycles of the following: 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 75 seconds. Consensus sequences were created using BioEdit 7.0.5.3 (Hall 1999) and compared to known sequences using BLASTN (www.ncbi.nlm.nih.gov/).

For further confirmation of the identity and to determine relationships among isolates, ITS region and the TUB gene sequences for 13 *D. longicolla* and 11 *D. caulivora* isolates were analyzed phylogenetically (Table 1). The ITS and TUB sequences were concatenated (~1400 bp) and aligned using Muscle in MEGA7.0.21 (Kumar et al. 2016). Sequences from GenBank and *Diaporthe* spp. isolates from Iowa were included for comparison (Santos et al. 2011; Gomes et al. 2013; Udayanga et al. 2015) (Table 1). Analysis was also conducted in MEGA and a tree was constructed with concatenated ITS and TUB sequences (34 nucleotide sequences) and inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura 1992) using MEGA7. The tree was rooted to *D. sojae*. Initial tree(s) for the heuristic search were obtained by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach and selecting the topology with superior log likelihood (~2834.2204). A discrete Gamma distribution was used to model evolutionary rate differences [5 categories (+G, parameter = 1.5788)]. The tree was drawn to scale with branch lengths measured in the number of substitutions per site. Positions with < 95% site coverage were eliminated. Confidence levels were determined with bootstrap analysis (1000 reps).

Symptomatic plants (n = 56) that did not yield isolates of *Diaporthe* spp. were tested for the presence and identity of *Diaporthe* spp. using standard and quantitative PCR (sPCR and qPCR) assays. Individual stem pieces were ground and DNA was extracted using a modified FastDNA® protocol (Malvick and Grunden 2005). The presence of *Diaporthe* spp. was confirmed in 10 of the plants using the sPCR assay with Phom I and Phom II primers as noted above. The identity of *Diaporthe* spp. in the 10 plants was then determined with species-specific quantitative PCR (qPCR) assays using primer/probe sets PL-3 and DPC-3; which were specific for *D. longicolla* and *D. caulivora*, respectively (Zhang et al. 1999; Kontz et al. 2016). The assays were performed on an Applied Biosystems 7500 Real-Time PCR System (Foster City, CA).

Asymptomatic soybean plants (n = 154) at the R6 growth stage were also arbitrarily collected from 111 fields in 62 counties representing diverse soybean production areas across Minnesota (Fig. 1). The fields were selected using distance-based transects in each county and plants were sampled arbitrarily 4 m from the edge of the fields. DNA was extracted from the stems and the presence of *Diaporthe* spp. was determined with sPCR as described above. The 73 samples that were positive with the sPCR assay were then tested with the previously described qPCR assay to determine species.

Pathogenicity of *Diaporthe* isolates and resistance of soybean cultivars

Six soybean cultivars (cvs.) were randomly chosen and inoculated to determine their reaction to 24 isolates of *Diaporthe* species (Table 1). The cvs. were: ‘AG2107’ (Monsanto Co., St Louis, MO), ‘MN1606SP’ (Univ. of Minnesota), ‘BSR101’, ‘Corsoy 79’, ‘IA2008R’ (Albert Lea Seed Co, Albert Lea, MN), and ‘S19-A6’ (Syngenta AG, Basel, Switzerland). Three seeds of each cv. were sown in pots containing Sunshine LC8 growing mix (Sun Gro Horticulture, Bellevue, WA).
Isolates of *D. longicolla* (n = 13) and *D. caulivora* (n = 11) were selected for virulence studies to represent different geographic areas of origin. The isolates were grown on 0.5x PDA (BD Difco Lab.) for 7 d at 25°C, and then used to inoculate the plants growing in pots using a cut-stem inoculation method. Stems were cut just below the fourth node at growth stage V3, and 200 μL sterile micropipette tips containing two PDA plugs (4-mm diameter) colonized with individual isolates were placed over the cut stems (Bradley and Li 2007; Li et al. 2010). Sterile PDA plugs were used for controls. Plants in four replicate pots per cv. were inoculated with each isolate. Pots were arranged in a randomized complete block design and maintained in a greenhouse at 24°C day and 18°C night with a 14 h photoperiod. Pipette tips were removed from plants 10–12 d post inoculation. Disease severity for each inoculated plant was determined 6 weeks post inoculation (wpi) based on percentage necrotic length of the inoculated internode and on percentage branch dieback (number of dead branches/total number of branches on each plant x 100). The average disease severity and branch dieback were calculated per pot. Symptomatic stem pieces for each variety and isolate combination were collected, surface disinfected with bleach, rinsed in sterile water, and placed on 0.5x PDA. Petri dishes were incubated at 25°C with a 10 h photoperiod for 7 to 10 d. The morphology of each isolate from inoculated plants was compared to

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**Table 1.** Isolate code, species, geographical origin, associated symptoms, source, and virulence group for isolates of *Diaporthe* spp. used in these studies.

| Isolate code<sup>a</sup> | Species | Geographical origin (MN County) or state/country | Symptoms on soybean or identity of other original host | Source or Reference | Virulence group<sup>b</sup> |
|-------------------------|---------|-----------------------------------------------|-----------------------------------------------|-------------------|---------------------|
| DC DA                   | *D. caulivora* | Dakota | Stem canker | This study | Moderate |
| DC DB                   | *D. caulivora* | Sherburne | Stem canker | This study | High |
| DC M4*                  | *D. caulivora* | Chisago | Pod & stem blight | This study | Low |
| DC M6*                  | *D. caulivora* | Le Sueur | Pod & stem blight | This study | High |
| DC M7*                  | *D. caulivora* | Kandiyohi | Early dying | This study | High |
| DC OL                   | *D. caulivora* | Olmsted | Pod & stem blight | This study | Low |
| DC R6                   | *D. caulivora* | Redwood | Stem canker | This study | Moderate |
| DC R10                  | *D. caulivora* | Redwood | Pod & stem blight | This study | Low |
| DC R11*                 | *D. caulivora* | Redwood | Stem canker | This study | Moderate |
| DC SW                   | *D. caulivora* | Swift | Pod & stem blight | This study | Moderate |
| DI B3*                  | *D. longicolla* | Sherburne | Stem canker | This study | High |
| DI B4                   | *D. longicolla* | Sherburne | Stem canker | This study | High |
| DI GO*                  | *D. longicolla* | Goodhue | Stem canker | This study | High |
| DI M1                   | *D. longicolla* | Blue Earth | Pod & stem blight | This study | High |
| DI M2*                  | *D. longicolla* | Mower | Pod & stem blight | This study | Moderate |
| DI M5                   | *D. longicolla* | Jackson | Pod & stem blight | This study | High |
| DI M8*                  | *D. longicolla* | Jackson | Pod & stem blight | This study | High |
| DI M9                   | *D. longicolla* | Nicollet | Pod & stem blight | This study | High |
| DI M10                  | *D. longicolla* | Le Sueur | Pod & stem blight | This study | High |
| DI R1                   | *D. longicolla* | Redwood | Stem canker | This study | High |
| DI R2                   | *D. longicolla* | Redwood | Stem canker | This study | High |
| DI W1                   | *D. longicolla* | Waseca | Pod & stem blight | This study | High |
| DI W2                   | *D. longicolla* | Waseca | Stem canker | This study | High |
| DI W3*                  | *D. longicolla* | Waseca | Stem canker | This study | High |
| CBS117168               | *D. aspalathi* | South Africa | *Aspalathus linearis* | Gomes et al. 2013 |
| CBS127268               | *D. caulivora* | Croatia | Glycine max | Santos et al. 2011 |
| DC IA41*                | *D. caulivora* | USA, IA | *G. max* | Alison Robertson, IA State U. |
| Deu*                    | *D. cucurbitae* | USA, MN | *G. max* | This study |
| CBS127267               | *D. longicolla* | Croatia | *G. max* | Santos et al. 2011 |
| DI FAU644               | *D. longicolla* | USA, MD | *G. max* | Udayanga et al. 2015 |
| DI IA17*                | *D. longicolla* | USA, IA | *G. max* | Alison Robertson, IA State U. |
| DI IA62                 | *D. longicolla* | USA, IA | *G. max* | Alison Robertson, IA State U. |
| Dp CBS                  | *D. phaseolorum* | USA, DE | Phaseolus vulgaris | Udayanga et al. 2015 |
| 139281                  | *D. sojae* | USA, MS | *G. max* | Udayanga et al. 2015 |

<sup>a</sup>Denotes use for detailed morphological studies.

<sup>b</sup>Virulence groups based on studies in a greenhouse: Low <46% severity; Moderate 46–71% severity; High >71% severity
the isolate used for inoculations. The experiment was repeated once.

Detection and characterization of Diaporthe cucurbitae

Soybean plants exhibiting stem cankers and severe dieback of leaves and upper branches were harvested from a field near Rosemount, Dakota County, MN in September 2017 (Fig. 1). Stems of five diseased plants were split and vascular browning was also present. Isolations were made from the stems as previously described, resulting in a culture with characteristics of Diaporthe spp. (Hartman et al. 2015; Udayanga et al. 2015). The fungus was grown in potato dextrose broth, DNA was extracted, and partial ITS and TUB regions were sequenced as described above, and the translation elongation factor-1 alpha (EF1-α) was sequenced using primers EF1-728 F/EF1-986 R (Glass and Donaldson 1995; Carbone and Kohn 1999; Udayanga et al. 2012). The isolate was also tested using the sPCR and specific qPCR assays described above. Koch’s postulates were completed on soybean cv. ‘MN1606SP’ using the cut-stem inoculation method noted above. Diaporthe caulivora isolate (De M6) was used as a positive control and sterile PDA was the negative control. Four pots with three seeds each were used per treatment. Stems were rated for symptom development 6 weeks post inoculation (wpi). Fungi were isolated from symptomatic stems 6 wpi as described above, and the morphology and DNA sequences of recovered isolates were compared to the isolate used for inoculation.

Effects of temperature and fungicides on growth of Diaporthe isolates

Radial growth of D. longicolla (n = 13) and D. caulivora (n = 11) isolates (Table 1) was determined at 15, 25 and 32°C. An agar plug (5-mm-diam.) from each isolate grown 2 weeks on 0.5x PDA at 25°C was transferred to the centre of three replicate Petri dishes containing 0.5x PDA. Cultures were incubated in darkness for 14 d at each temperature in a completely random design and colony diameters were measured twice across each colony at right angles. The experiment was repeated once.

Sensitivity to pyraclostrobin (Headline®, BASF Corp.) and tebuconazole (Folicur® 3.6 F, Bayer CropScience) was determined with 13 isolates of D. longicolla and 11 isolates of D. caulivora. Each fungicide was added to 0.5x PDA at 0.01, 0.05, 0.1, 0.5, 1 and 5 µg a.i. mL⁻¹. Salicylhydroxamic acid (SHAM) was dissolved in methanol and added at 100 µg mL⁻¹ to media with pyraclostrobin. Non-amended media was used as a control for tebuconazole, and media with 100 µg mL⁻¹ SHAM was the control for pyraclostrobin. Agar disks (5 mm) from 2-week-old cultures were placed on media in three replicate Petri dishes per treatment and incubated in darkness for 7 d at 25°C in a completely random design. Colony diameters were measured and EC₅₀ values were calculated by regression against the log fungicide concentration using the two concentrations bracketing 50% growth (Lonergan et al. 2015). This study was repeated once.

Data analysis

Data were analyzed using JMP version 14.3.0 (SAS Institute Inc., Cary, NC). For the greenhouse studies, virulence data were arcsine transformed and analysis of variance (ANOVA) was calculated. When significant treatment effects were detected, means were separated by Tukey’s test (P < 0.05). There was an isolate by cultivar interaction and factors were analyzed separately. Average lesion severity (%) was calculated across all cvs., and virulence groups were generated using the ‘Interactive Binning’ add-in (low virulence <46%; moderate virulence 46–71%; high virulence >71%). Due to non-normality of data for percentage branch dieback, a non-parametric analysis was conducted using Fit Y by X function, and a Wilcoxon test was used for mean separation. For the temperature study on fungal growth, a Kruskal-Wallis test was used for mean separation, and means were separated using Dunn’s test (Bonferroni adjustment). EC₅₀ data from the two fungicide sensitivity experiments could not be combined and were analyzed separately; then the data were log transformed and ANOVA was conducted. When significant treatment effects were detected, means were separated by Tukey’s test for isolates and Student’s T-Test for fungicides and species (P < 0.05). Fungicide by isolate interactions occurred and fungicides were analyzed separately.

Results

Identification of isolates of D. caulivora and D. longicolla

For all 37 isolates sequenced, except one as described below, the ITS and TUB sequences had 99–100% sequence identity matches to either D. longicolla or D. caulivora in BLASTN. The sequences were deposited in the NCBI GenBank database (Table S1). The sequencing and specific qPCR results agreed for the isolates. Phylogenetic analysis of the Diaporthe isolates based on
concatenated ITS and TUB sequences separated the isolates into two main clusters representing *Diaporthe longicolla* and *D. caulivora* (Fig. 2). The *D. longicolla* isolates from Minnesota, along with two *D. longicolla* isolates from Iowa and two reference isolates of *D. longicolla* are in one main cluster. *Diaporthe sojae* is related and a more distant but related branch contains *D. cucurbitae* and *D. phaseolorum*. The other main cluster contains all of the *D. caulivora* isolates included in this study. This analysis resulted in the same clustering of isolates regardless of whether ITS or TUB sequences were used alone or concatenated for analysis (Fig. 2).

The morphology of the isolates identified as either *D. caulivora* or *D. longicolla* was the same as described previously (Hobbs et al. 1985; Santos et al. 2011; Hartman et al. 2015; Udayanga et al. 2015; Hosseini et al. 2020; Mena et al. 2020; Petrović et al. 2021). Growth of the isolates of *D. caulivora* was white with tan to brown areas and the reverse side of the dish developed tan pigmentation. Growth of the isolates of *D. longicolla* was dense, white, with occasional greenish-coloured patches, and the underside of colonies developed large, black stromata (Fig S1). Growth on 0.5x PDA was fast for all isolates of *D. caulivora* and *D. longicolla* (average 8.3 mm d⁻¹).

**Distribution of Diaporthe spp. associated with symptomatic and asymptomatic infection of soybean**

*Diaporthe* spp. were common inhabitants of symptomatic and asymptomatic soybean stems across the diverse environments and soybean maturity group zones that were sampled. *Diaporthe longicolla* was widespread in symptomatic (29%) and asymptomatic (47%) soybean plants across Minnesota from the Iowa border to near the Manitoba border (Fig. 1, Table 2). *Diaporthe caulivora* was detected only in plants in the southern half of Minnesota and was not detected in asymptomatic plants (Fig. 1, Table 2). *Diaporthe cucurbitae* was isolated and confirmed from soybean in one field in southern Minnesota. Other *Diaporthe* spp. were
Diaporthe spp. associated with soybean stems in MN

Table 2. Diaporthe spp. detected in soybean stems with different types of symptoms.

| Stem symptom     | Number of plants tested | Number from which Diaporthe spp. was isolated | Number positive for Diaporthe spp. with qPCR | Diaporthe longicolla | Diaporthe caulivora | Diaporthe cucurbitae |
|------------------|-------------------------|-----------------------------------------------|---------------------------------------------|----------------------|---------------------|---------------------|
| Pod & stem blight| 25                      | 14                                            | 4                                           | 8                    | 10                  | 0                   |
| Stem canker      | 46                      | 22                                            | 5                                           | 17                   | 9                   | 1                   |
| Top dieback      | 22                      | 1                                             | 2                                           | 2                    | 1                   | 0                   |
| Asymptomatic     | 154                     | N/A                                           | 73                                          | 73                   | 0                   | 0                   |

*a*Number of stems from which Diaporthe spp. were isolated and later sequenced to determine species.

*b*Number of stems from which isolates were not obtained but were positive for Diaporthe spp. with sPCR. These were later tested with specific quantitative PCR (qPCR) assays to determine species identification.

*c*Identification based on sequencing and qPCR results.

not detected in symptomatic or asymptomatic plants via isolations or PCR assays.

Diaporthe spp. were detected differentially via isolation on media or with qPCR from soybean stems with different types of symptoms. Diaporthe longicolla was detected in 32%, 37%, and 9% of plants with symptoms of pod and stem blight, stem canker, and top dieback, respectively. Whereas D. caulivora was isolated or detected in 40%, 20%, and 5% of plants with these symptoms, respectively (Table 2). Isolation of Diaporthe was most successful from plants with stem canker and pod and stem blight symptoms (47% and 54%, respectively) and less successful from plants with top dieback symptoms (4.5%). Of the symptomatic plants that did not yield Diaporthe in isolations, 20% were positive for Diaporthe with the sPCR assay and all of those were determined to be either D. longicolla or D. caulivora based on the species-specific qPCR positive assays (Table 2).

**Detection and characterization of Diaporthe cucurbitae**

One isolate from soybean was confirmed to be D. cucurbitae based on ITS, TUB, and EF1 sequences and morphology, and its pathogenicity to soybean was confirmed with Koch’s postulates. The sequences were deposited in the NCBI GenBank database as accession numbers OL843922, OL860962, and OL999089 for ITS, EF1-α, and TUB, respectively. The ITS, TUB, and EF1-α sequences were 98–100% identical to D. cucurbitae sequences in GenBank (accessions KP118848, NR_147563, KM453211, DAOM 42078). The D. cucurbitae culture produced white, fluffy mycelia with dark green to brown colouration in the centre. Growth rate of the isolate was moderate (4.5 mm d⁻¹) on 0.5x PDA. Alpha conidia were 8.3–10 µm by 4.3 µm and aseptate, hyaline, and biguttulate. Beta conidia were abundant and curved, hooked, aseptate, and 25 µm by 1.25 µm (Fig S2). In the greenhouse pathogenicity assays, 100% of the plants inoculated with D. cucurbitae developed dark brown stem cankers and 17% developed top dieback (Figs S3 and S4). For comparison, 67% of the check plants inoculated with D. caulivora developed stem cankers and >50% developed dieback. Diaporthe cucurbitae and D. caulivora (used as a control) were recovered from symptomatic tissue in inoculated plants and their identities confirmed with sequencing and qPCR assays. The negative control plants did not develop symptoms of infection and Diaporthe was not isolated from them.

**Virulence of Diaporthe isolates on different soybean cultivars**

Inoculation of soybean with D. longicolla and D. caulivora resulted in sunken brown cankers, wilted leaves, and branch dieback starting at inoculated nodes. Virulence differed between D. caulivora and D. longicolla and among isolates within each species (Tables 1 and 3). Diaporthe longicolla produced significantly greater lesion severity than D. caulivora on all six soybean cvs., and D. caulivora produced significantly greater branch dieback on four of the cvs. (Table 3, Figs 3, 4). All isolates of D. longicolla were moderately to highly virulent (64–91% severity), whereas the D. caulivora isolates ranged from low to high (15–84% severity).

The isolates within each species of Diaporthe also varied in virulence on each soybean cv. (Tables 1 and
3). For each of the six cvs., at least one isolate was significantly different from others based on lesion severity (Table 3). The two isolates that caused the most severe branch dieback (De DB and De M6) were also highly virulent based on stem lesion severity.

The six soybean cvs. differed significantly in reaction to D. caulivora and D. longicolla (Figs 3 and 4). Cultivars ‘S19-A6’, ‘Corsoy 79’, and ‘IA2008R’ were least susceptible to D. caulivora based on lesion severity, but all cvs. were similarly susceptible to D. longicolla (Fig. 3, Table 3). Branch dieback was greater following inoculation with D. caulivora than D. longicolla for all cvs. except for ‘Corsoy 79’ and ‘BSR 101’ (Fig. 4). The cvs. ‘IA 2008 R’ and ‘S19-A6’ had the highest levels of branch dieback following inoculation with D. caulivora, and ‘Corsoy 79’ and ‘BSR 101’ had the lowest (Fig. 4). In all virulence studies, Diaporthe isolates were recovered from the inoculated stem tissue and Diaporthe was not isolated from control plants.

Influence of temperature and fungicides on growth of Diaporthe isolates

Average colony diameters at 14 d for all D. caulivora isolates combined at 15°C, 25°C, and 32°C were 8.5, 8.3, and 0.8 cm, respectively (Fig. 5). Average diameters for all D. longicolla isolates combined at 15°C, 25°C, and 32°C were 8.4, 7.5, and 5.4 cm, respectively. Growth of the two species was significantly different at 25 and 32°C (p < 0.05). Diaporthe caulivora isolates grew significantly more than D. longicolla isolates at 25°C and significantly less than D. longicolla at 32°C (Fig. 5). Both species of Diaporthe grew significantly slower at 32°C than at 15 and 25°C.

Sensitivity to the fungicides pyraclostrobin and tebuconazole varied among isolates of D. longicolla and D. caulivora and between the two fungicides tested, although all isolates of both species were sensitive to the fungicides. Isolates were generally less sensitive to
tebuconazole. The mean EC$_{50}$ for all isolates in the experiments were 0.05 µg mL$^{-1}$ a.i. for pyraclostrobin and 0.38 µg mL$^{-1}$ a.i. for tebuconazole (Table 4). The mean EC$_{50}$ for both species were similar for
pyraclostrobin, but for tebuconazole the mean EC$_{50}$ for _D. caulivora_ was 0.34 µg mL$^{-1}$ a.i. and for _D. longicolla_ it was 0.42 µg mL$^{-1}$ a.i. The _Diaporthe_ isolates responded similarly to both fungicides in both experiments.

**Discussion**

Soybean stem diseases caused by _Diaporthe_ spp. are significant problems worldwide. The associated pathogen species and their characteristics are complex, creating challenges for understanding and managing them. In this study, the _Diaporthe_ spp. associated with symptomatic and asymptomatic soybean stems were characterized across an ecologically and climatically diverse geographic region in Minnesota. The _Diaporthe_ diversity and species composition was narrow. _Diaporthe caulivora_ and _D. longicolla_ were the only species detected in 98% of plants sampled. _Diaporthe longicolla_ was the predominant species associated with stem blight and stem canker symptoms and asymptomatic stems. Isolates in both species varied in virulence to soybean, response to temperature, and fungicide sensitivity. _Diaporthe cucurbitae_, the only other _Diaporthe_ species detected, was confirmed to be a pathogen of soybean stems for the first time. The results expand understanding of the identity and characteristics of _Diaporthe_ spp. associated with stem infection of soybean.

_Diaporthe caulivora_ is reported as the primary cause of northern stem canker in soybean, although _D. longicolla_ has also occasionally been reported as a causal agent (Cui et al. 2009; Lu et al. 2010; Mathew et al. 2015, 2017, 2017, 2017; Mena et al. 2020). In this study of plants with stem canker symptoms, _D. caulivora_ was detected in only 20% of plants and _D. longicolla_ was detected at nearly twice that frequency. _Diaporthe caulivora_ was also detected in 40% of plants with pod and stem blight symptoms (Table 2). There were no clear differences in appearance of symptoms produced by _D. caulivora_ or _D. longicolla_ on inoculated plants, but virulence was greater for _D. longicolla_ in greenhouse trials. The results from this study support previous studies that suggest that _D. caulivora_ and _D. longicolla_ are both associated with stem canker.

_Diaporthe sojae_ (syn _D. phaseolorum_ var. _sojae_) has been considered the primary cause of pod and stem blight disease of soybean (Udayanga et al. 2012; Hartman et al. 2015; Mueller et al. 2016). This species, however, was not detected in any of the plants in this study with stem blight symptoms. Rather, _D. longicolla_ and _D. caulivora_ were detected at similar levels from stems with stem blight symptoms. _Diaporthe longicolla_ has often been described in association with seed decay, although it has also been associated with stem infection (Harrington et al. 2000; Xue et al. 2007; Lu et al. 2010; Ghimire et al. 2019; Mena et al. 2020). The results from

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**Fig. 5** Average colony diameter (cm) of _Diaporthe caulivora_ (cross hatch bars) and _D. longicolla_ (black bars) isolates after 14 d of growth on 0.5x PDA at three temperatures. Each temperature was analysed separately. Treatments designated by different letters are significantly different ($\alpha = 0.05$).
Table 4. Sensitivity of 11 isolates of Diaporthe caulivora (De) and 13 isolates of D. longicolla (Di) to the fungicides pyraclostrobin and tebuconazole. The EC₅₀ (µg mL⁻¹ a.i.) was determined based on radial growth on agar plates containing these fungicides. Fungicides were analysed separately. Treatments in the same column not designated by the same letter are significantly different (α = 0.05).

| Isolate  | Pyraclostrobin | Tebuconazole |
|----------|----------------|--------------|
| De DA    | 0.03 abcd      | 0.35 abcde   |
| De DB    | 0.04 abcde     | 0.37 abcd    |
| De M4    | 0.11 a         | 0.16 bedf    |
| De M6    | 0.03 abcde     | 0.34 abcd    |
| De M7    | 0.03 bc        | 0.37 abcd    |
| De OL    | 0.03 bc        | 0.35 abcd    |
| De R6    | 0.05 abcde     | 0.70 ab      |
| De R10   | 0.08 ab        | 0.26 bcdef   |
| De R11   | 0.00 c         | 0.32 bcde    |
| De SW    | 0.01 c         | 0.26 bcdef   |
| De W1    | 0.06 ab        | 0.27 abdef   |
| De Avg.  | 0.04 a         | 0.34 a       |
| Di B3    | 0.04 abcde     | 0.22 bcdef   |
| Di B4    | 0.05 abcde     | 0.71 ab      |
| Di GO    | 0.04 abcde     | 0.68 abcde   |
| Di M1    | 0.05 abcde     | 0.03 g       |
| Di M2    | 0.03 abcde     | 0.18 bcdef   |
| Di M5    | 0.03 abcde     | 0.69 abcde   |
| Di M8    | 0.03 bc        | 1.70 a       |
| Di M9    | 0.03 abcde     | 0.36 bcde    |
| Di M10   | 0.03 abcde     | 0.07 efgh    |
| Di R1    | 0.06 ab        | 0.45 abcde   |
| Di R2    | 0.06 abcde     | 0.16 cdef    |
| Di W2    | 0.14 ab        | 0.09 defg    |
| Di W3    | 0.04 abcde     | 0.06 fg      |
| Di Avg.  | 0.05 a         | 0.42 a       |
| Grand Avg.| 0.05          | 0.38         |

this and previous studies suggest that D. sojae is not the only and primary cause of pod and stem blight disease; D. longicolla is and D. caulivora may be more important causal agents in at least some areas, perhaps depending on environmental differences.

Two species of Diaporthe that have been widely reported to be associated with soybean exhibiting stem blight or stem canker symptoms are Diaporthe sojae and D. aspalathii. These species were not detected from any plants in this study. The absence of D. sojae may be due to the relatively greater importance of D. longicolla as a stem pathogen in the geographic region sampled. In addition, the historical challenges with identification of Diaporthe spp. and the recent taxonomic reassessment of species in this genus may also be relevant. The absence of D. aspalathii (syn D. phaseolorum var. meridionalis), causal agent of southern stem canker, was not surprising given that it appears to be uncommon in the northern US (Smith and Backman 1988; Hartman et al. 2015).

Several Diaporthe species may cause similar symptoms on soybean, and the challenges and changes with taxonomy and identification may have led to inconsistencies in ID of species associated with different symptoms in different studies. In this study, identification was based primarily on ITS region and TUB gene sequences as well as morphological features (Mathew et al. 2015; Udayanga et al. 2015; Dissanayake et al. 2017; Petrović et al. 2021). Gomes et al. (2013), Dissanayake et al. (2017), and Hosseini et al. (2020) suggested that molecular data, at least sequences of ITS and TUB or HIS, are necessary for reliable identification of Diaporthe species. Additionally, if isolates are not available, PCR approaches are also useful. In this study, Diaporthe spp. in asymptomatic plants and in symptomatic plants that did not yield Diaporthe isolates were identified using qPCR assays (Zhang et al. 1999; Lu et al. 2010; Kontz et al. 2016). Kontz et al. (2016) also applied the qPCR assay to symptomatic and asymptomatic soybean samples and detected Diaporthe spp. in plants that failed to produce cultures with isolation techniques, similar to what was found in this study and reported by Impullitti and Malvick (2013). In this study, the identity of a set of isolates was cross-validated using ITS and TUB sequences and qPCR assays, and the sequencing and the specific qPCR assay results agreed.

The unexpected species of Diaporthe detected in this study was D. cucurbitae. To our knowledge, D. cucurbitae has not been previously reported to be a pathogen of soybean. Diaporthe cucurbitae was isolated from a soybean plant expressing stem canker symptoms and confirmed to be pathogenic and cause stem cankers on soybean using Koch’s postulates. Diaporthe cucurbitae has been reported on Cucumis and Arctium spp. causing fruit rot (Udayanga et al. 2015). A synonym for D. cucurbitae based on the host was previously considered to be D. melonis, which has been reported on soybean, but they are clearly different species (Udayanga et al. 2015; Dissanayake et al. 2017; Zaw et al. 2020). While this study suggests the distribution of this pathogen may be limited on soybean, the results also suggest it has the potential to be an important soybean pathogen. The detection and confirmation of D. cucurbitae as a soybean pathogen for the first time also supports two broader issues with this genus. First, the species associated with and potentially important as pathogens of soybean are not well understood. Second, more species are found associated with soybean as more
studies are conducted. For example, recent studies of soybean seed decay revealed several *Diaporthe* spp. not previously associated with soybean seed infection (Petrović et al. 2021).

The phylogenetic comparison shows that *D. caulivora* and *D. longicolla* from this study are clearly delineated based on ITS and TUB sequences, and *D. caulivora* and *D. aspalathi* are separated from *D. sojae*, as reported in other studies (Udayanga et al. 2015; Hosseini et al. 2020). *Diaporthe cucurbitae* was clearly delineated as a separate species and related to *D. phaseolorum*, as seen in other studies (Zaw et al. 2020). The sequencing and phylogenetic studies detected minimal sequence differences among isolates within each species, indicating there is little genetic diversity among isolates in this diverse geographic region that could be measured with those two sequences. The low levels of diversity detected within each species were not related to virulence (data not shown). Lu et al. (2010) also reported minimal nucleotide differences among *D. longicolla* isolates or *D. caulivora* isolates from soybean stems.

*Diaporthe longicolla* was the only *Diaporthe* species detected in asymptomatic plants, where it was detected at high frequency in plants collected over a large area with different soil types, climate patterns, and growing zones. We do not know the aetiology or potential outcomes of these endophytic or latent infections. However, the *D. longicolla* isolates from symptomatic stems were aggressive stem pathogens in this study and this species also might be a significant latent pathogen in the asymptomatic plants that cause disease damage when plants mature or are stressed (Hartman et al. 2015). Numerous other studies have also found *D. longicolla* and undescribed *Diaporthe* spp. in association with asymptomatic soybean stems (Harrington et al. 2000; Lu et al. 2010; Impullitti and Malvick 2013; Batzer and Mueller 2020) and have speculated on their potential importance as pathogens.

Differential growth at various temperatures in vitro clearly separated *D. caulivora* and *D. longicolla*. For example, all isolates of *D. longicolla* isolates tested grew significantly more than isolates of *D. caulivora* at 32°C. The differential effects of temperature suggests that these two pathogens may have different temperature optima for infection and disease development. Further studies are needed to determine the influence of temperature on infection and disease development by these *Diaporthe* spp.

Fungicides are often applied to soybeans in mid-growing season to manage foliar diseases and to enhance yields; however, their potential to manage stem diseases caused by *Diaporthe* spp. is unclear. In this study, a common QoI fungicide (pyraclostrobin) and triazole fungicide (tebuconazole) were used to determine their potential to suppress growth of the *Diaporthe* spp. These fungicides inhibited mycelial growth of *D. longicolla* and *D. caulivora* isolates at low levels, suggesting that the isolates were sensitive to these fungicides and that these fungicides have potential for managing stem diseases caused by these *Diaporthe* spp. Soto-Arias and Munkvold (2011) reported that incidence of stem infection by *Phomopsis* spp. (*syn* *Diaporthe* spp.) in Iowa was reduced by pyraclostrobin but not tebuconazole applications at R3 and R5 growth stages. However, in a more recent field study in Iowa, the fungicides fluxapyroxad and pyraclostrobin appeared to increase the frequency of *Diaporthe* spp. detected in soybean stem (Batzer and Mueller 2020). Thus, there is a need for more studies to determine if fungicides can influence infection, colonization, and stem disease development in soybean caused by *Diaporthe* spp.

The pathogenicity/virulence studies illustrate two key findings. One is that virulence differed between the species *D. caulivora* and *D. longicolla*; and another is that isolates within each species vary in virulence on and among individual soybean cultivars. The isolates of *D. longicolla* were more virulent than the isolates of *D. caulivora*. Greater virulence may partially explain why *D. longicolla* was more common than *D. caulivora* in the stems in this study. Other studies have also found differences in virulence among isolates of *D. longicolla* and *D. caulivora* (Keeling 1985; Pioli et al. 2003; Lu et al. 2010; Mena et al. 2020). In this study, the original symptoms of the plant from which isolates were obtained had no association with how aggressive the isolate was as a stem pathogen.

The virulence studies also suggest that soybean cultivars differ in susceptibility to *D. caulivora* and *D. longicolla*, as determined with a set of arbitrarily selected cultivars. The different levels of susceptibility among cultivars, which presumably were not deliberately selected for resistance to these *Diaporthe* spp., is encouraging and suggests that genetic traits for partial resistance may be common. The fact that isolates of *Diaporthe* varied in virulence across soybean cultivars and individual cultivars differed in susceptibility to different isolates, however, also suggests a need for developing resistance against a set of different isolates. These results were obtained using one inoculation method and one set of environmental conditions, which may influence the interactions between isolate and host (Ghimire et al. 2019). Resistance to *D. caulivora* has been reported (Peruzzo et al. 2019), but there appears to be limited knowledge on resistance to *D. longicolla* stem infection. The widespread occurrence of soybean stem diseases attributed
Diaporthe spp. associated with soybean stems in MN

Bradley CA, Li S. 2007. First report of northern stem canker of soybean caused by Diaporthe phaseolorum var. caulivora in North Dakota. Plant Disease. 90(5):687. doi: 10.1094/PDIS-90-0687-A

Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 91(3):553–556. doi: 10.1080/00275519.1999.12601051

Chang H-X, Lipka AE, Domier LL, Hartman GL. 2016. Characterization of disease resistance loci in the USDA soybean germplasm collection using genome-wide association studies. Phytopathology®. 106(10):1139–1151. doi: 10.1094/PHYTO-01-16-0042-FI

Cui Y-L, Duan C-X, Wang X-M, Li H-J, Zhu Z-D. 2009. First report of Phomopsis longicolla causing soybean stem blight in China. Plant Pathol. 58(4):799. doi: 10.1111/j.1365-3059.2009.02057.x

Dissanyake AJ, Phillips AJL, Hyde KD, Yan JY, Li XH. 2017. The current status of species in Diaporthe. Mycosphere. 8(5):1106–1156. doi: 10.5943/mycosphere/8/5/5

Gebreil A, Micijevic A, Weber A, Hyronimus L, Mathew F. 2015. Characterization of Diaporthe species infecting soybeans (Glycine max L.) in South Dakota. Proc 100th Anniv South Dakota Acad Sci. 94:362.

Ghimiře K, Petrović K, Kontz BJ, Bradley CA, Chilvers MI, Mueller DS, Smith DL, Wise KA, Mathew FM. 2019. Inoculation method impacts symptom development associated with Diaporthe aspalothi, D. caulivora, and D. longicolla on soybean (glycine max). Plant Dis. 103(4):677–684. doi: 10.1094/PDIS-06-18-1078-RE

Glass NL, Donaldson G. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol. 61(4):1323–1330. doi: 10.1128/aem.61.4.1323-1330.1995.

Gomes RR, Gliemke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW. 2013. Diaporthe: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia - Molecular Phylogeny and Evolution of Fungi. 31(1):1–41. doi: 10.3767/0301358513X66844

Gravert CE, Li S, Hartman GL. 2001. Occurrence of Diaporthe phaseolorum var. meridionalis on Soybean in Illinois Plant Dis. 85:1211.

Guillin EA, Grijalba PE, Oliveira LO, Gottlieb AM. 2014. Specific boundaries between the causal agents of the soybean stem canker. Crop Plant Pathol. 39(4):316–325. doi: 10.1590/S1982-56762014000400006

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. 41:95–98.

Harrington TC, Steimel J, Workneh F, Yang XB. 2000. Molecular identification of fungi associated with vascular discoloration of soybean in the North Central United States. Plant Dis. 84(1):83–89. doi: 10.1094/PDIS.2000.84.1.83.

Harrington TC, Steimel J, Workneh F, Yang XB. 2003. Characterization and distribution of two races of Phialophora gregata in the North-Central United States. Phytopathology®. 93(7):901–912. doi: 10.1094/PHYTO.2003.93.7.901.

Hartman GL, Rupe JC, Sikora EJ, Domier LL, Davis JA, Steffey KL, editors. 2015. Compendium of Soybean Diseases and Pests. 5th ed. ed. St Paul (MN): APS Press.

Hill HC, Horn NL, Steffens WL. 1981. Mycelial development and control of Phomopsis sojae in artificially inoculated soybean stems. Plant Dis. 65:132–134. doi: 10.1094/PD-65-132.

Hobbs TW, Schmitthenner AF, Kuter GA. 1985. A New Phomopsis species from soybean. Mycologia. 77(4):535. doi: 10.1080/00275519.1985.12025139.

Hosseini B, El-Hasan A, Link T, Vogele RT. 2020. Analysis of the species spectrum of the Diaporthe/Phomopsis complex in European soybean seeds. Mycological Progress. 19(5):455–469. doi: 10.1007/s11557-020-01570-y.

Impulliti AE, Malvick DK. 2013. Fungal endophyte diversity in soybean. J Appl Microbiol. 114(5):1500–1506. doi: 10.1111/jam.12164.

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Supplementary material

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References

Abdelmagid A, Hafez M, Lawley Y, Adam LR, Daayf F. 2019. First report of northern stem canker caused by Diaporthe caulivora on soybean in Western Canada. Plant Dis. 103(2):372. doi: 10.1094/PDIS-07-18-1185-PDN.

Allen TW, Bradley CA, Sisson AJ, Byamukama E, Chilvers MI, Coker CM, Collins A, Damicone JP, Dorrance AE, Dufault NS, et al. 2017. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Health Progress. 18(1):19–27. doi: 10.1094/PHP-PS-16-0066.

Batzer JC, Mueller DS. 2020. Soybean fungal endophytes Alternaria and Diaporthe spp. are differentially impacted by fungicide application. Plant Dis. 104(1):52–59. doi: 10.1094/PDIS-05-19-1001-RE.

Benavides R, Pioli RN, Morandi EN. 2010. Response of the edamame edible soybean germplasm to Diaporthe phaseolorum, causal agent of soybean stem canker, in Argentina. Trop Plant Pathol. 35(1):48–51. doi:10.1590/S1982-56762010000100008.
White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols, A Guide to Methods and Applications. New York (NY): Academic Press; p. 315–322.

Xue AG, Morrison MJ, Cober E, Anderson TR, Rioux S, Ablett GR, Rajcan I, Hall R, Zhang JX. 2007. Frequency of isolation of species of Diaporthe and Phomopsis from soybean plants in Ontario and benefits of seed treatments. Can J Plant Pathol. 29(4):354–364. doi:10.1080/07060660709507481.

Yang XB. 2001. Biology and management of premature yellowing and death of soybean caused by Phomopsis idiaporthe fungi. Proc Int Crop Mgmt Conf. 23:181–185.

Zaw M, Aye SS, Matsumoto M. 2020. Colletotrichum and Diaporthe species associated with soybean stem diseases in Myanmar. Journal of General Plant Pathology. 86(2):114–123. doi:10.1007/s10327-019-00902-5.

Zhang AW, Hartman GL, Riccioni L, Chen WD, Ma RZ, Pedersen WL. 1997. Using PCR to Distinguish Diaporthe phaseolorum and Phomopsis longicolla from Other Soybean Fungal Pathogens and to Detect Them in Soybean Tissues. Plant Dis. 81(10):1143–1149. doi:10.1094/PDIS.1997.81.10.1143.

Zhang AW, Hartman GL, Curio-Penny B, Pedersen WL, Becker KB. 1999. Molecular Detection of Diaporthe phaseolorum and Phomopsis longicolla from Soybean Seeds. Phytopathology®. 89(9):796–804. doi:10.1094/PHYTO.1999.89.9.796.