Analyzing isolate variability of *Macrophomina phaseolina* from a regional perspective

Zachary F. Sexton, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907¹; Teresa J. Hughes, USDA-ARS, West Lafayette, IN 47909²; Kiersten A. Wise*, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

*corresponding author: Kiersten A. Wise

email: kawise@purdue.edu

Current addresses for authors: ¹Department of Plant Pathology, Washington State University, Pullman, WA 99164; ² Monsanto, 700 Chesterfield Parkway W., Chesterfield, MO. 63017

Abstract

Sexton, Z.S., Hughes, T.J., and Wise, K.A. 20XX. Analyzing isolate variability of *Macrophomina phaseolina* from a regional perspective. Crop Protection. XX:XXXX

Charcoal rot of soybean (*Glycine max* L.), caused by *Macrophomina phaseolina* (Tassi) Goid, has been reported more frequently in the north-central United States in recent years. Growth of isolates collected from the northern and southern U.S. was compared in vitro under various incubation temperatures, and isolate pathogenicity was assessed on cultivars adapted to each region. Results indicate that northern isolates had increased growth at 15°C compared to isolates from southern states, while southern isolates grew 23% more at 40°C compared to northern isolates. Isolates from both regions were

pathogenic on charcoal rot resistant and susceptible cultivars adapted for each region. This is the first research to suggest the importance of screening germplasm under regional environmental conditions and with local or regionally adapted isolates when evaluating cultivar resistance to charcoal rot.

Keywords: charcoal rot, *Macrophomina phaseolina*, isolate, variability

Introduction

The fungus *Macrophomina phaseolina* (Tassi) Goid, infects more than 500 plant species and is the causal agent of charcoal rot for many economically important crops including soybean (*Glycine max* (L.) Merr) (Grau et al., 2004). Although charcoal rot occurs throughout the world, it is most severe in warm, tropical to subtropical regions (Grau et al., 2004; Kaur et al., 2012). *Macrophomina phaseolina* survives in soil and host residue as microsclerotia. Under favorable conditions (28-35°C), microsclerotia will germinate and infect plant roots. Following infection, hyphae of *M. phaseolina* will progressively colonize the vascular tissues of the root, stem, and petioles, resulting in plant wilting (Kaur et al., 2012).

In the United States, charcoal rot of soybeans resulted in an average yield loss of approximately 850 thousand metric tons per year from 1996 through 2012 (Koenning and Wrather, 2010; United Soybean Board, 2012; Wrather et al, 2003; Wrather et al., 2001;

Wrather and Koenning, 2006). This makes it the 5th most important soybean disease in the U.S. in terms of yield impact. In terms of dollar amounts, these yield loss estimates equate to an average loss of over 250 million dollars annually (USDA National Agriculture Statistics Service, 2014). This disease, which is typically associated with periods of hot and dry weather, has impacted soybean production in the southern U.S. more consistently than the northern U.S. (Koenning and Wrather, 2010). However, yield losses caused by charcoal rot have been greater in the northern U.S. due to the higher amount of soybean production overall in this area (Koenning and Wrather, 2010). Furthermore, there has been a recent increase in the incidence and severity of charcoal rot in northern soybean producing states. This may be the result of changing weather patterns that favor charcoal rot, or the increased use of higher planting populations in soybean production, which can contribute to increase stress, making plants more susceptible to this disease (Bradley et al., 2003; Brown, 2007; Cummings and Bergstrom, 2013; Elaraby, 2003; Hughes, 2009; Yang and Navi, 2005).

Currently there are limited methods available to manage charcoal rot. Farmers are encouraged to plant less susceptible cultivars and prevent plant stress by planting at recommended plant populations, preventing crop injury, and reducing drought stress through irrigation during soybean reproductive growth stages (Smith et al., 2014).

Although host genetic resistance is the cornerstone of charcoal rot management, there are limited resistant cultivars available. In addition, resistance has primarily been studied in cultivars adapted to southern U.S. growing conditions (maturity group (MG) IV-V) where the disease has been more frequently observed. However, the growing presence of

69
70 charcoal rot in the north central U.S. has prompted researchers to evaluate cultivars in
71 northern maturity groups for resistance to this disease. To date, these screenings have
72 occurred primarily in greenhouse locations in northern climates, or in field trials in more
73 southern locations, such as Tennessee, as a part of larger germplasm screening projects
74 (Mengistu et al., 2007; Mengistu et al., 2011; Mengistu et al., 2014; Smith and Carvil,
75 1997).

76 Pathogen diversity and screening conditions across regions have previously been
77 observed to affect cultivar resistance. For instance, geographically diverse isolates of
78 another soil-borne fungal pathogen, *Fusarium virguliforme*, which causes sudden death
79 syndrome in soybean, were noted to have varying levels of virulence on susceptible and
80 moderately resistant cultivars (Li et al., 2009; Malvick and Bussey, 2008). However,
81 there is little information on *M. phaseolina* diversity in regards to regional differences in
82 temperature and growth requirements of isolates, and how this may impact screening for
83 disease resistance. The objectives of this study were 1) to determine if temperature affects
84 in vitro growth of *M. phaseolina* isolates collected from northern and southern regions of
85 the U.S., and 2) assess pathogenicity and virulence of isolates on resistant and susceptible
86 cultivars adapted to each region.

87 88 **Materials and Methods**

89 **Impact of temperature on *M. phaseolina* growth**

91 Isolates of *M. phaseolina* were collected from both the northern and southern U.S.
92 Isolate collection locations are listed in Table 1. A total of 42 isolates, 21 from the
93 northern U.S. and 21 from the southern U.S. were selected for the experiments. Because
94 some of the isolates had been stored in long-term culture collections, all isolates were
95 tested to ensure cultures had maintained virulence in storage by inoculating the charcoal
96 rot-susceptible soybean cultivar Pharaoh in a greenhouse assay using the cut-stem
97 method (Twizeyimana et al., 2012). Approximately nine days after plant inoculation,
98 plants were observed for symptoms of charcoal rot, and plants with charcoal rot lesions
99 were sampled and isolates were collected for storage. Samples were confirmed to be *M.*
100 *phaseolina* by plating portions of colonized stem tissue on potato dextrose agar (PDA;
101 Becton Dickinson BBL Media, Franklin Lakes, NJ) incubating at 30°C for 4 days and
102 observing the resulting fungal colony. After confirmation, 5 to 6 cm portions of
103 colonized stem tissue which were then lyophilized, and stored at -20°C.

104 The experiment was designed as a randomized complete block with two replicates
105 per isolate, and an experimental unit was considered a single 100 x 15 mm petri dish.
106 Growth (colony diameter) of each isolate was assessed at 15, 30, and 40°C. These three
107 temperatures were selected based on the results of preliminary studies that suggested that
108 isolates demonstrated the most variation in growth rate at these temperatures and
109 represent soil temperatures observed during the planting and growing seasons in these
110 areas. Approximately six days prior to the start of the experiment, a section of colonized
111 stem tissue from each isolate was retrieved from storage and placed on PDA and
112 incubated at 32°C in the dark for two to three days. The resulting colony was then sub-

cultured to a new plate of PDA to obtain a pure culture, and incubated at 32°C in the dark for an additional three days.

A single agar plug, 6 mm, was taken from the leading edge of a 3 day old pure culture of each isolate and placed in the center of a 100 x 15 mm petri dish containing PDA. Three replicate plates per isolate were established and plates were sealed with parafilm. Plates were arranged in an incubator (Percival Scientific, Perry, IA Model # CU22LC8) according to the experimental design and incubated for seven days. All 42 isolates were tested at the same time at each incubation temperature, and then the experiment was repeated twice at each temperature. Colony diameter measurements were taken at 3, 5, and 7 days after plating for a total of three measurements per replicate per isolate. Colony diameter was calculated by averaging the length in mm, of the widest and narrowest portions of each colony for a single value. Diameter measurements were used to calculate area under the growth progress curve (AUGPC) for each isolate using the following formula,

$$AUGPC = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

where t = days accumulated before colony size measurement and y = colony diameter averaged over two measurements. (Jeger and Viljanen-Rollinson, 2001). Area under the growth progress curve was used to represent colony growth because at optimal temperatures *M. phaseolina* grows very quickly, reaching the edge of a 90mm petri dish in three to four days. The AUGPC statistic incorporates time, allowing us to differentiate between an isolate that reaches the edge of the petri dish in four days from one that takes seven days.

Data were analyzed in STATISTICA software version 10.0 (Statsoft, Tulsa, OK). Colony diameter and AUGPC values were checked for normality and did not require transformation. Individual isolate values by replicate were averaged prior to analysis and analyzed using a two-way analysis of variance (ANOVA). Temperature and isolate region of origin were considered fixed effects and replicate was treated as a random effect. Homogeneity of variance and interactions between experiments and fixed effects were examined to determine if data from replicated experiments could be combined within a given temperature, and across temperatures. Means were separated using Fisher's least significant difference (LSD) at $P = 0.05$.

Effect of isolate region on pathogenicity and virulence of *M. phaseolina*

The effect of isolate region of origin was tested on cultivars adapted to both northern and southern conditions, including the charcoal rot-moderately resistant DT97-4290 (Paris et al., 2006) and susceptible Pharaoh (Mengistu et al., 2007). Both cultivars are maturity group (MG) IV and adapted to the southern U.S. Northern-adapted cultivars included Asgrow AG2632 and Wyckoff W2990CRR, which are MG II-III cultivars. AG2632 was classified as moderately resistant and W2990CRR as susceptible based on previous greenhouse studies (Sexton and Hughes, 2012).

Experiments were conducted in the United States Department of Agriculture Agricultural Research Service (USDA-ARS) greenhouse located on the Purdue University campus in West Lafayette, IN. The experiment was designed as a randomized complete block design with five replicates, and an experimental unit consisted of two soybean plants per cultivar per replicate.

Plants were planted into MetroMix 510 (SunGro Horticulture, Agawam MA) growth medium in 48-cell plastic flats (T.O. Plastics, Clearwater, MN), with 3.8 x 6 x 5.8 cm cells. Plants were watered two to three times a week to maintain constant moisture on the soil surface. The experiment was conducted under a programmed 14-h photoperiod, supplemented by 1000w sodium lamps (Xtrasun, Grand Prairie, TX), and temperatures were maintained between 24°C and 28°C for the duration of the experiment. Due to space constraints, assays on the northern and southern isolates were conducted separately.

Inoculum for all 42 previously described isolates was prepared four to six days prior to inoculation as described in the previous experiment. The cut-stem method was used to evaluate pathogenicity and virulence of individual isolates on soybean (Twizeyimana et al., 2012). Stems of plants at growth stage V2 to V3 (second or third trifoliate leaf; approximately two weeks old), were cut 38 mm above the unifoliate node. Immediately following excision, the reverse end of a 200 µL pipette tip containing a plug of mycelium (6 mm in diameter) from the margin of an actively growing *M. phaseolina* colony on PDA was placed firmly over the cut stem of the soybean plant. Three days after inoculation (DAI), pipette tips were removed and the length of the resulting stem lesion was measured at 3, 6, and 9 DAI. The three measurements were then used to calculate an area under the disease progress curve (AUDPC) value. The experiments were repeated twice. AUDPC values were averaged across each experimental unit (two plants) and data were analyzed using STATISTICA software version 10.0. Colony diameter and AUDPC values were checked for normality and did not require transformation. Individual isolate values by replicate were averaged prior to analysis and analyzed using ANOVA. Temperature and isolate region of origin were

considered fixed effects and replicate was treated as a random effect. Means were separated using Fisher's least significant difference (LSD) at $P = 0.05$. Spearman's rank correlation (Rho) was also used to gauge the homogeneity of ranks between experiments.

Results

Impact of temperature on *M. phaseolina* growth

Analysis of variance indicated that experimental variances were homogeneous within experiments, and across temperatures, and so colony growth values were combined for further analysis. ($P=0.498$). There was a significant interaction of region of isolate origin by temperature on colony growth ($P < 0.001$), and both the region of origin ($P=0.022$) and temperature ($P < 0.001$) significantly affected colony growth. Northern isolates had greater AUGPC than southern isolates at both 15 and 30°C, while southern isolates had greater AUGPC than northern isolates at 40°C (Fig. 1). Incubator space limited the experimental size and number of replications possible, so individual isolates or smaller groupings of isolates were not compared.

Effect of isolate region on pathogenicity and virulence of *M. phaseolina*

There was a significant experiment by isolate interaction for the replicated experiments, ($P < 0.001$ for all three experiments), and therefore, data are presented by individual experiments. A significant cultivar by isolate interaction was observed in all three experiments ($P=0.037, 0.0497, 0.024$), but there was no significant cultivar by isolate region interactions in any experiment. AUDPC was highly variable across experiments, likely due to environmental variability within the greenhouse.

In all experiments, the moderately resistant southern adapted cultivar DT97-4290, had significantly less charcoal rot compared to the similarly adapted susceptible cultivar Pharaoh. ($P<0.001$; Fig. 2), regardless of the region of origin of the isolate of *M. phaseolina*. The effect of isolate region was highly significant ($P<0.001$) in two of the three experiments with northern isolates causing significantly more charcoal rot on both southern-adapted resistant and susceptible cultivars (Fig. 2).

Across all experiments, the northern-adapted susceptible cultivar (W2990CRR) consistently averaged a higher AUDPC than the northern-adapted resistant cultivar (AG2632) (Fig. 3). The effect of the region of isolate origin was only significant in experiment 2 ($P<0.001$), although the isolates from the northern U.S. resulted in increased disease on the northern adapted susceptible cultivar compared to southern isolates in all three experiments (Fig. 3).

Discussion

This research demonstrates that there are distinct differences among regional populations of *M. phaseolina* in relation to temperature effects on growth and also virulence on regionally adapted cultivars. Our results indicate northern isolates grow more quickly at 15 and 30°C, when compared to southern isolates, and that isolates from both regions have significantly greater growth at 30°C compared to other temperatures examined in this study. These results support research that demonstrated the optimum temperature for *M. phaseolina* mycelial growth on agar to be 30-35°C (Collins et al., 1991).

The temperatures examined in this study represent typical soil temperatures at planting (15°C) in the northern U.S. and average soil temperatures during soybean

reproductive growth stages in each region (30°C in northern regions; 40°C in southern regions) (De Bruin and Pederson, 2008; Mulekar et al. 2008). *Macrophomina phaseolina* infects early in the growing season, and is dormant in the plant until stress occurs, usually in the reproductive growth stages (Pearson et al., 1984). Severe charcoal rot is associated with heat and drought stress in the reproductive stages, and the higher range of temperatures in our study reflects temperatures that would be common during the reproductive stages when stress triggers increased pathogen colonization of soybean tissue (Mengistu et al., 2011). Therefore, our results indicate that in northern adapted climates, northern isolates may colonize soybean more quickly in the early growth stages and during reproductive stages when compared to southern isolates. The implication of this finding is that northern adapted germplasm, such as MG I-III, should be screened in typical northern climates using region-specific isolates to ensure that a representative level of disease is obtained in any greenhouse or field experiments.

Isolate origin for northern isolates consisted primarily of isolates collected in Indiana, Michigan and Wisconsin. Indiana isolates were selected from areas that represent a range of growing conditions from the southernmost counties in Indiana where the climate is representative of Mid-South conditions, to the northern tier of the state where earlier maturity group soybeans are grown in a cooler climate. Isolates collected across the southern states represent a wider geographic area, including those from the Mid-South (Tennessee) down to Mississippi and Georgia. Due to the range of environmental conditions within Indiana, it would be interesting to compare the Indiana isolates with those collected in Wisconsin and Michigan and those in the Mid-South to further determine if temperature gradients such as latitude influence isolate growth or

virulence. However, due to space and incubator limitations, we could not test all isolates simultaneously at multiple temperatures. Despite being unable to compare growth of individual isolates we were still able to see differences in growth by region, and subsequent experiments should examine *M. phaseolina* diversity within and among regions of the United States.

In our study, northern isolates were more virulent than southern isolates on southern-adapted cultivars in two of three experiments. This response may be due to the experimental conditions in the greenhouse favoring the growth of northern isolates. Temperatures within the greenhouse averaged 26° C, which favors northern isolate growth, as indicated in our growth chamber study. This indicates that regional differences between *M. phaseolina* populations are important to consider in cultivar screening programs. However, it is reassuring that the previously established resistant and susceptible cultivars used in this experiment remained consistent across both regional groups of isolates. Isolate variability among experiments prevents us from drawing any conclusions about the virulence of individual isolates, but the significant isolate by cultivar interaction observed in several experiments suggests that individual isolates vary in virulence and how individual isolates impact resistance ratings warrants further investigation.

We advocate that based on this research local isolates should be used when screening both germplasm and commercial varieties for charcoal rot resistance. Adapted maturity groups should be tested in the region of origin, or under greenhouse conditions that mimic the soil conditions expected in the area specific to the maturity group of interest. Additional research into the genetic basis of isolate differences observed in these

experiments is a logical next step in the investigation of *M. phaseolina* isolate variability in the U.S.

Acknowledgements

We gratefully acknowledge the United Soybean Board and the United States Department of Agriculture: Agricultural Research Service for funding this research. We thank Judy Santini for statistical consulting and Derrick McFall for help with maintenance of experiments.

References:

Bradley C.A. and del Rio, L.E. 2003. First report of charcoal rot on soybean caused by *Macrophomina phaseolina* in North Dakota. Plant Dis. 87:601.

Brown, D. 2007. Charcoal rot in soybeans. Michigan State University Extension Crop Advisory Team Alert for August 23, 2007. Online.

http://msue.anr.msu.edu/news/charcoal_rot_in_soybeans. Access date: 10 Sept. 2015.

Collins, D.J., Wyllie, T.D., and Anderson, S.H. 1991. Biological activity of *Macrophomina phaseolina* in soil. Soil Biol. Biochem. 23:495-496.

Cummings, J.A., and Bergstrom, G.C. 2013. First report of charcoal rot caused by *Macrophomina phaseolina* in soybean in New York. Plant Dis. 97:1506.

De Bruin, J.L., and Pedersen, P. 2008. Soybean seed yield response to planting date and seeding rate in the upper Midwest. Agron J. 100:696-703

295 Elaraby, M.E. 2003. First report of charcoal rot (*Macrophomina phaseolina*) on soybeans
 296 in Minnesota. Plant Dis. 87:202.

297 Grau, C.R., Dorrance, A.E., Bond, J., Russin, J.S. 2004. Charcoal Rot. Pages 684-687 in:
 298 H.R. Boerma and J.E. Specht, eds. Soybeans: Improvement, Production, and
 299 Uses. 3rd Ed. ASA-CSSA-SSSA, Inc., Madison, WI.

300 Hughes, T.J. 2009. Taking advantage of a stressful situation: Stem canker & charcoal rot
 301 in soybeans. Wisc. Crop Man. 16:9-11.

302 Jeger, M.J., and Viljanen-Rollinson, S.L.H. 2001. The use of the area under the disease-
 303 progress curve (AUDPC) to assess quantitative disease resistance in crop
 304 cultivars. Theor. Appl. Genet. 102:32-40.

305 Kaur, S., Dhillon, G.S., Brar, S.K., Vallad, G.E., Chand, R., and Chauhan, V.B. 2012.
 306 Emerging phytopathogen *Macrophomina phaseolina*: biology, economic
 307 importance and current diagnostic trends. Critical Rev. Micro. 38:136-151.

308 Koenning, S.R., and Wrather, J.A. 2010. Suppression of soybean yield potential in the
 309 continental United States by plant diseases from 2006-2010. Online. Plant Health
 310 Prog. doi:10.1094/PHP-2010-1122-01-RS.

311 Li, S., Hatman, G.L., and Chen, Y. 2009. Evaluation of aggressiveness of *Fusarium*
 312 *virguliforme* isolates that cause soybean sudden death syndrome. J. Plant Path.
 313 91:77-86.

314 Malvick, D., and Bussey, K. 2008. Comparative analysis of characterization of the
 315 soybean sudden death syndrome pathogen *Fusarium virguliforme* in the northern
 316 United States. Can.J. Plant Pathol. 30:467-476.

317 Mengistu, A., Ray, J.D., Smith, G.D., and Paris, R.L. 2007. Charcoal rot disease
 318 assessment of soybean genotypes using colony-forming units index. Crop Sci.
 319 47:2453-2461.

320 Mengistu, A., Smith, J.R., Ray, J.D., and Bellaloui, N. 2011. Seasonal progress of
 321 charcoal rot and its impact on soybean productivity. Plant Dis. 95: 1159-1166.

322 Mengistu, A., Ray, J.D., Smith, J.R., and Boykin, D.L. 2014. Maturity effects on colony-
 323 forming units of *Macrophomina phaseolina* infection as measured using near-
 324 isogenic lines of soybeans. J. Crop Improv. 28:38-56.

325 Mulekar, M.S., Kimball, S., and Sowell, V.J. 2008. Comparison of soil temperatures at
 326 Pascagoula and Agricola, MS. Section on Statistics and the Environment. JSM.

327 Paris, R.L., Mengistu, A., Tyler, J.M. and Smith, J.R. 2006. Registration of soybean
 328 germplasm line DT97-4290 with moderate resistance to charcoal rot. Crop Sci.
 329 46:2324-2325.

330 Pearson, C.A.S., Schwenk, F.W., Crowe, F.J., and Kelley, K. 1984. Colonization of
 331 soybean roots by *Macrophomina phaseolina*. Plant Dis. 68:1086-1088.

332 Sexton, Z.F., and Hughes, T.J. 2012. Impact of temperature on growth and development
 333 of northern and southern isolates of *Macrophomina phaseolina*. Phytopathology
 334 102:S5.9.

335 Smith, G.S., and Carvil, O.N. 1997. Field screening of commercial and experimental
 336 soybean cultivars for their reaction to *Macrophomina phaseolina*. Plant Dis. 81:
 337 363-368.

338 Smith, D., Chilvers, M., Dorrance, A., Hughes, T., Mueller, D., Niblack, T., and Wise, K.
 339 2014. Charcoal rot management in the North Central region. University of
 340 Wisconsin Extension Bulletin A4037.

341 Twizeyimana, M., Hill, C.B., Pawlowski, M., Paul, C., and Hartman, G.L. 2012. A cut-
 342 stem inoculation technique to evaluate soybean for resistance to *Macrophomina*
 343 *phaseolina*. Plant Dis. 96:1210-1215.

344 United Soybean Board 2012. 2010-2012 Soybean yield loss estimates. Online.
 345 www.unitedsoybean.org. Accessed 10 Sept. 2015.

346 United States Department of Agriculture National Agricultural Statistics Service 2014.
 347 Soybeans price received. Online. quickstats.nass.usda.gov. Accessed 10 Sept.
 348 2015.

349 Wrather, J.A., Koenning S.R., and Anderson T.R. 2003. Effect of diseases on soybean
 350 yields in the United States and Ontario (1999–2002). Online. Plant Health Prog.
 351 doi10.1094/PHP-2003-0325-01-RV.

352 Wrather, J.A., Stienstra W.C., and Koenning S.R. 2001. Soybean disease loss estimates
 353 for the United States from 1996 to 1998. Can. J. of Plant Path. 23:122-131.

354 Wrather, J.A., and Koenning S.R. 2006. Estimates of disease effects on soybean yields in
 355 the United States 2003 to 2005. J.of Nem. 38:173.

356 Yang, X.B.,and Navi, S.S. 2005. First report of charcoal rot epidemics caused by
 357 *Macrophomina phaseolina* in soybean in Iowa. Plant Dis. 89:526.

358
 359

Table 1. Isolates of *Macrophomina phaseolina* tested in growth chamber and greenhouse experiments examining the impact of temperature on isolate growth based on region of origin. County specific data not available for all isolates.

Isolate	Origin	Source	Region
TN262	Tennessee	A. Mengistu	South
TN550	Texas	A. Mengistu	South
TN501	Louisiana	A. Mengistu	South
TN270	Mississippi	A. Mengistu	South
MP203	Louisiana	R. Baird	South
MP220	Tennessee	R. Baird	South
TN551	Mississippi	A. Mengistu	South
TN5	Tennessee	A. Mengistu	South
TN272	Mississippi	A. Mengistu	South
MP223	Texas	R. Baird	South
TN279	Tennessee	A. Mengistu	South
TN4	Tennessee	A. Mengistu	South
MP144	Kentucky	R. Baird	South
MP249	Georgia	R. Baird	South
TN380	Mississippi	A. Mengistu	South
MP250	Georgia	R. Baird	South
TN280	Kentucky	A. Mengistu	South
TN264	Tennessee	A. Mengistu	South
MP258	South Carolina	R. Baird	South
TN271	Mississippi	A. Mengistu	South
IN12-PO-3	Posey, IN	PPDL ^a	North
IN12-9-4	Vermillion, IN	PPDL	North
IN12-8-3	Lagrange, IN	PPDL	North
W23	Hamilton, MI	M. Chilvers	North
Et18	E. Troy, WI	T. Hughes	North
Md7	Muscoda, WI	T. Hughes	North
IN12-4	Benton, IN	PPDL	North
W3-5	Hamilton, MI	M. Chilvers	North
W25	Hamilton, MI	M. Chilvers	North
W12-6	Hamilton, MI	M. Chilvers	North
Md9	Muscoda, WI	T. Hughes	North
Md10	Muscoda, WI	T. Hughes	North
Et14	E. Troy, WI	T. Hughes	North
Et17	E. Troy, WI	T. Hughes	North
Md3	Muscoda, WI	T. Hughes	North
Md6	Muscoda, WI	T. Hughes	North

IN12-8-1	Lagrange, IN	PPDL	North
Et12	E. Troy, WI	T. Hughes	North
Et8	E. Troy, WI	T. Hughes	North
Dm13	Markesan, WI	T. Hughes	North
Md5	Muscoda, WI	T. Hughes	North
IN12-9-6	Vermillion, IN	PPDL	North

^a. Purdue Plant and Pest Diagnostic Laboratory

Fig. 1: Mean area under the growth progress curve (AUGPC) values for colony growth of 42 isolates of *Macrophomina phaseolina* separated by region of origin (North, South) and temperature. Letters represent means that are significantly different based of Fisher's least significant difference (LSD) test at $P=0.05$ across all experiments. Bars indicate standard error of the mean.

Fig. 2: Comparison of mean area under the disease progress curve (AUDPC) values, from three experiments testing pathogenicity of 42 *Macrophomina phaseolina* isolates obtained from the northern (North) or southern (South) U.S. on southern-adapted soybean cultivars DT97-4290 (Resistant) and Pharaoh (Susceptible). Different letters signify statistically different means based on Fishers least significant difference test at a threshold of $P=0.05$ within each experiment. Bars within a column signify standard error of the mean.

Fig. 3: Comparison of mean area under the disease progress curve (AUDPC) values, from three experiments, testing pathogenicity of 42 *Macrophomina phaseolina* obtained from the northern (North) or southern (South) U.S. on northern soybean cultivars, AG2632 (Resistant) and W2990CRR (Susceptible). Different letters signify statistically different means based on Fishers least significant difference test at a threshold of $P = 0.05$ within each experiment. Bars within a column signify standard error of the mean.