Methods and Evaluation of Soybean Genotypes for Resistance to *Colletotrichum truncatum*

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Abstract

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Anthracnose of soybean (*Glycine max*) occurs throughout the soybean production areas of the world. There is little information on evaluating inoculation techniques or evaluating soybean germplasm for resistance. The objectives of this study were to develop a reliable inoculation technique for evaluating soybean for resistance to *Colletotrichum truncatum* and to evaluate soybean genotypes for resistance. Inoculated plants incubated in a dew chamber for 48 or 72 h had higher ($P = 0.05$) area under disease progress curve (AUDPC) values than when incubated for 24 h. Three experiments evaluated soybean genotypes for resistance to *C. truncatum*. In the first experiment using 15 soybean genotypes, 'Mandarin' had lower ($P = 0.05$) AUDPC values than all the other genotypes except for 'Mandarin 507', 'Mandarin (Ottawa)', and 'Boone'. In the second experiment using 28 soybean genotypes, Man-

darin 507 had lower ($P = 0.05$) AUDPC values compared with all other soybean genotypes except 'Early White Eyebrow', 'Mandarin Yowa', Boone, and 'Manchuria'. In the third experiment, Mandarin 507 had lower ($P = 0.05$) AUDPC values compared with 23 other soybean genotypes except 'Spry', Mandarin, and 'Iroquois'. Plants of Mandarin 507 and 'Williams 82' were inoculated at the vegetative and reproductive growth stages; Mandarin 507 had lower ($P = 0.05$) AUDPC values compared with Williams 82 for both growth stages evaluated, and Mandarin 507 had less ($P = 0.05$) pod area covered by lesions compared with the pods of Williams 82. This study provided a descriptive method to inoculate soybean plants with *C. truncatum* and identified soybean genotypes with resistance to anthracnose.

Anthracnose of soybean (*Glycine max* (L.) Merr.) occurs throughout the soybean production areas of the world (16). Soybean plants can be infected at any stage of development. Symptoms include irregularly shaped brown blotches and cankering on stems, petioles, and pods; necrosis of laminar veins; rolling leaves; and premature defoliation. Infected seed often become discolored and may die during germination (9). The disease is more prevalent under humid and warm conditions. Yield reduction from anthracnose has been reported in Asia, Europe, and South and North America (3,16,28,29). Anthracnose was reported to cause 16 to 26% seed yield loss in the southern area of the United States (3). From 2003 to 2005, anthracnose was among the top 10 most important soybean diseases in terms of monetary losses averaged over 15 southern states (28). Disease management includes fungicide application, crop rotation with nonhosts, and planting pathogen-free seed (16). Although no commercial cultivars are advertised to have resistance to anthracnose, sources of resistance have been reported (5,14,18–20).

Colletotrichum truncatum (Schwein.) Andrus & W.D. Moore, one species commonly reported to cause anthracnose, survives in

soybean debris and is seedborne (16,24). The disease cycle and infection process include primary infection on plants from over-wintered spores or mycelia on diseased plant tissues, germination of spores, development of appressoria and infection pegs, penetration and hyphal colonization of host tissue, and production of acervuli and spores. During the growing season, infection continues as acervuli produce spores that are rain splashed and land, germinate, and penetrate plant tissues (1,21). To study anthracnose of soybean, plants have been inoculated with conidial and mycelial suspensions with different *Colletotrichum* spp. (4,11,14,20,21,23). Conditions to promote anthracnose development on soybean seedlings showed that longer incubation times in a mist chamber produced more severe symptoms, with younger plants having more stem tip necrosis than older plants (20).

There are few studies that have evaluated anthracnose resistance in soybean. Several studies reported on the evaluation of 414 soybean germplasm accessions to *C. truncatum* (18–20). These studies found no immune genotypes; however, 10 genotypes in maturity groups (MGs) 00 to IV ('Bavendar Sp. A', 'Boone', 'Early White Eyebrow', 'Gibson', 'Manchuria', 'Mandarin', 'Mandarin [Ottawa]', 'Waseda', 'Wea', and 'Willomi'), plant introduction (PI) 96860 in MG VI, and 'Tarheel Black' in MG VII were classified as resistant. In another study (14), 'A. K. (Kansas)', Boone, 'Corsoy 79', and 'Williams 82' soybean were used to investigate the pathogenicity of sclerotia- and nonsclerotia-forming isolates of *C. truncatum* on seedling roots; all were reported susceptible to root infection by *C. truncatum*. In addition, 48 commercial soybean cultivars in Brazil were evaluated for the resistance to *C. truncatum* under greenhouse conditions. Although no cultivar was immune, 45 were classified as resistant or intermediate, and only 3 cultivars were susceptible (5). Breeding efforts to produce anthracnose-resistant soybean cultivars are lacking despite the potential for yield losses due to this disease. In addition, there is a lack of knowledge about soybean resistance in different tissues (from roots to seed) and whether any described resistance would be effective against the several species of *Colletotrichum* that cause anthracnose.

The soybean genotype Mandarin was among the soybean genotypes reported to have resistance to anthracnose (18,19), although

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the PI number was not provided in these publications. In the soybean germplasm collection (26), along with Mandarin (listed as PI 548378) there also are 'Mandarin Yowa' (PI 189890), 'Mandarin A' (PI 438363), and 'Mandarin 507' (PI 548380) that, to our knowledge, have not been evaluated for resistance to anthracnose. The *C. truncatum* isolates used in previous studies to evaluate soybean accessions for anthracnose resistance have not been curated and are not available for use today. However, the soybean genotypes that were previously reported can be evaluated with newly collected isolates.

The genetic base of North American soybean cultivars is from a narrow range of ancestral lines. Fourteen soybean introductions were reported to constitute about 70% of the genes in North American public cultivars released from 1971 to 1981 (6,9). A set of 35 soybean ancestors that contributed to >95% of the genes found in North American cultivars were released between 1947 and 1988 (10). These ancestral lines have been evaluated for resistance to soybean sudden death syndrome (22) and for four soybean viruses (27) but not for resistance to anthracnose.

The objectives of this study were to (i) develop a reliable inoculation technique to evaluate soybean genotypes for resistance to anthracnose and (ii) use this technique to evaluate soybean genotypes previously reported to be resistant to anthracnose, a set of soybean ancestral lines, and a set of public soybean cultivars.

Materials and Methods

Fungal isolate. An isolate of *C. truncatum* (IL15B) was obtained from soybean petioles and stems collected in Urbana, IL in 2009. This isolate was selected as the most aggressive isolate in a preliminary detached-leaf assay for screening the pathogenicity of 84 *Colletotrichum* isolates collected in the United States in 2009 (30). The isolate was identified as *C. truncatum* according to its morphological characteristics and DNA sequence analysis (30). The isolate was maintained on potato dextrose agar (PDA; BD Difco, Sparks, MD) at the National Soybean Research Center in the Laboratory for Soybean Diseases Research at the University of Illinois (www.soydiseases.illinois.edu). The isolate was transferred periodically as needed and incubated under 12 h of alternating cool-white fluorescent light (45 μmol m^{-2} s^{-1}) and darkness at 24 $\pm$ 1°C.

Inoculum preparation. Six 4-mm-diameter mycelial disks from an actively growing culture of isolate IL15B on PDA were added to 50 ml of autoclaved and cooled potato dextrose broth (PDB; BD Difco) in 250-ml flasks. The flasks were secured to a shaker (Lab-Line Instrument, Melrose Park, IL) at 100 rpm and incubated for 5 days at room temperature (24 $\pm$ 1°C). The mycelial suspension was transferred to 50-ml tubes (Corning Inc., Corning, NY) and centrifuged at 2,500 rpm for 20 min (Centrifuge 5810; Eppendorf, Hauppauge, NY). The pellet was collected, squeezed to minimize liquid content, then weighed. The pellet was resuspended in distilled water, fragmented with a blender (Model 31BL92; Waring Laboratory, Torrington, CT) at low speed for 30 s, stopped for 30 s, and ground for another 30 s. In preliminary tests, final concentrations of mycelial suspensions of 20 to 100 mg/ml in distilled water caused severe symptoms on Williams 82 plants, with no significant differences in anthracnose severity among the suspensions (*data not shown*).

Incubation chambers. To keep plants moist after inoculation, space limitations necessitated the use of two kinds of chambers (commercial dew chambers and homemade moist chambers). Two dew chambers (Percival Scientific Inc., Perry, IA) with the lights switched off were set at a wall temperature of 19°C and a water temperature of 32°C. The temperature recorded was 24 $\pm$ 2°C, with a relative humidity of over 80%. The moist chambers were constructed using plastic crates (interior size: 57 by 37 by 41 cm), covered and sealed with insulated foil bubble wrap. A stainless steel test-tube rack (34 by 18 by 10 cm) was placed inside each crate to support one flat (52 by 26 by 6 cm) with plants in multiple pot inserts. Water (5 liters) was added to the bottom of each crate and the inside walls were sprayed with water using a hand-held

sprayer (Do it Best Corp., Fort Wayne, IN) before setting plants inside. The moist chambers were kept in a growth chamber room at 28 $\pm$ 2°C with a relative humidity greater than 90%.

Effect of inoculum type and incubation period on anthracnose severity. Six treatments, including two inoculum types (mycelia and conidia) and three dew periods (24, 48, and 72 h), were arranged as a factorial in a completely randomized design with three replications. Each experimental unit consisted of three plants.

Seed of Williams 82 were planted in three pots (11 cm in diameter) and thinned to three plants per pot. The plants were grown in a growth chamber (Percival Scientific Inc.) with 12 h of alternating light (350 μmol m^{-2} s^{-1}) and darkness at 25°C and, when 12 days old or at growth stage V1 (8), they were moved to a dew chamber for inoculation.

Mycelial inoculum at a concentration of 40 mg/ml was prepared as previously described. Conidial inoculum was prepared according to Manandhar et al. (20), with slight modifications. Briefly, IL15B cultures were grown on sodium chloride-yeast extract agar (SYA) plates (17) with alternating 12 h of light (45 μmol m^{-2} s^{-1}) and dark condition at 24 $\pm$ 1°C for 3 to 4 weeks to facilitate spore production. Conidia were harvested from the plates by adding 20 ml of sterile distilled water per plate. The surface was brushed using a plastic disposable loop (Nunc, Thermo Fisher Scientific, Rochester, NY). The conidia in suspension were calculated by a hemacytometer (Reichert Bright Line, Buffalo, NY) and diluted to a final concentration of 3 $\times$ 10^6 conidia/ml in distilled water.

Plants were atomized with the mycelial or conidial suspension until run-off. Plants were incubated in a dew chamber at 25°C in the dark for 24, 48, and 72 h. Plants were removed and placed back in the growth chamber. Disease severity was rated on a whole plant basis daily from 4 to 10 days postinoculation (DPI).

Disease severity was based on visual assessment of inoculated plants using a pretransformed rating scale (15) from 0 to 5, where 0 = no visible symptoms, 1 = 1 to 10% of the plant affected (primarily leaf vein necrosis), 2 = 11 to 35% of the plant affected (primarily leaf and some petiole necrosis), 3 = 36 to 65% of the plant affected (primarily leaf, petiole, and some stem necrosis), 4 = 66 to 90% of the plant affected (extensive leaf, petiole, and stem necrosis), and 5 = 91 to 100% of the plant affected (severe necrosis on all plant parts to plant death).

Effect of plant age on disease severity. Plants of five different growth stages (VC, V1, V2 to V3, R3 to R4, and R4 to R5) were arranged in a completely randomized design with four replications. Each experimental unit consisted of three plants per pot.

Seed of Williams 82 were planted weekly for 5 weeks in pots (11 cm in diameter) and thinned to three plants per pot. Plants were grown in a growth chamber (Percival Scientific Inc.) with 12 h of alternating light (350 μmol m^{-2} s^{-1}) and darkness at 25°C. When plants from the first sowing date developed to growth stages R4 to R5 (43-day-old plants), plants for all the treatments were inoculated with a mycelial suspension of isolate IL15B, as previously described, by atomizing plants until run-off. Inoculated plants were placed in moist chambers in the dark at 28 $\pm$ 2°C and, after 72 h, transferred back to the growth chamber. Disease severity, based on the previously described visual assessment, was rated at 4, 5, 6, and 9 DPI. The experiment was repeated.

Evaluation of three sets of soybean genotypes for anthracnose resistance. The first set included 15 soybean genotypes consisting of 10 genotypes reported to be resistant (18,19), plus Mandarin Yowa, Mandarin A, Mandarin 507, 'T269H' (random selection from the collection with an unknown reaction to anthracnose), and the susceptible check Williams 82. The second set included 28 soybean genotypes consisting of all the genotypes in the first set (excluding T269H), plus 12 soybean ancestral lines (9), Hartwig, and Kingwa. The third set included 25 public soybean cultivars and Williams 82 as the susceptible check, and Mandarin 507 and Mandarin as the resistant checks.

For all three experiments, seed were sown in a soil-less medium, Sunshine Mix LC1 (Sun Gro Horticulture Inc.), in 18 or 36 multi-celled flats (52 by 26 by 6 cm). The design for each experiment

was a randomized complete block, with soybean genotypes randomized within each block. Each flat was a block (replication), with three blocks per each experiment. In each cell, two to three seeds were sown and thinned, if needed, to two plants per cell (experimental unit) for the first experiment and one plant per cell for experiments two and three. Plants were grown in a growth chamber (Percival Scientific Inc.) at 25°C under 14 h of light (350 µmol m^{-2} s^{-1}) per day for 21 ± 3 days, corresponding to a growth stage of V2 to V3 before inoculation. Plants were inoculated with a mycelial suspension of isolate IL15B as previously described. After inoculation, plants were placed in moist chambers at 28 ± 2°C in the dark. After 48 h of incubation, plants were moved back to the growth chamber. Anthracnose severity, based on the previously described visual assessment, was rated daily from 3 to 8 DPI and 10 DPI for the first experiment, daily from 3 to 7 DPI for the second experiment, and daily from 3 to 9 DPI for the third experiment. All three experiments were repeated with different randomizations within blocks.

Effect of plant age on a resistant and a susceptible genotype. Seed of two soybean genotypes, Mandarin 507 (resistant) and Williams 82 (susceptible), were sown in plastic pots (13 cm in diameter) and thinned to three plants per pot in a growth chamber (Percival Scientific Inc.) with 12 h of alternating light (350 µmol m^{-2} s^{-1}) and darkness at 25°C. To obtain plants at two different growth stages, seed of both genotypes were planted 35 and 40 days after the initial planting for trial 1 and trial 2, respectively. For each trial, 36 plants (3 plants per pot, three replications, two genotypes, and two growth stages) were inoculated with isolate IL15B when plants were in the vegetative growth stage (V1 to V2, or 13 and 19 days after sowing for trial 1 and trial 2, respectively) and in the reproductive growth stage (R4 up to R6, or 47 and 50 days after sowing for trial 1 and trial 2, respectively). Disease severity, based on the previously described visual assessment, was rated daily from 3 to 8 DPI. In trial 2, plants that were inoculated in the reproductive growth stage were also rated for anthracnose severity on pods. In total, 57 and 68 pods of Mandarin 507 and Williams 82, respectively, were evaluated at 20 DPI.

Data analysis. Midpoint percentages of the ratings were used to calculate area under the disease progress curve (AUDPC) (25), which was used as the variable in all experiments. In addition, pods were evaluated in the experiment where one resistant and one susceptible genotype were compared at their vegetative and reproductive growth stages. The pod evaluation was rated once, and the variable used for analysis to compare the soybean genotypes was

the pretransformed rating scale that was detransformed to percent for presentation. The homogeneity of variance for the AUDPC values among repeated experiments was tested using Bartlett's test (JMP, version 11; SAS Institute Inc., Cary, NC). Restricted or residual maximum likelihood (REML) analysis of the AUDPC values was performed with JMP with replications as random effects and treatments as fixed effects. When significant differences were indicated in the REML analysis, least square means were compared using least significant difference at $P = 0.05$.

Results

Effect of inoculum type and incubation period on anthracnose severity. There were significant ($P < 0.0001$) differences among the treatments for AUDPC values (Table 1), with the highest AUDPC value for plants inoculated with a mycelial suspension and incubated for 48 or 72 h and for plants inoculated with a conidial suspension and incubated for 72 h (Table 2). The AUDPC values between the 24-h incubation period with the mycelial inoculum and the 48-h incubation period with the conidial inoculum were not significantly different. The treatment with the lowest AUDPC value was conidial inoculum with an incubation period of 24 h.

Effect of plant age on disease severity. The homogeneity of variance for the AUDPC values between the two trials was not significant ($P < 0.3859$) based on the Bartlett's test and the data were combined for analysis and presentation. There were significant ($P < 0.0001$) differences among the growth stages for AUDPC values (Table 1). The AUDPC values for plants inoculated at vegetative growth stages were greater than values of plants inoculated at reproductive stages (Table 3). Plants inoculated at early vegetative growth stages had leaf necrosis and lesions on petioles and stems, and were defoliated by 4 DPI. Plants inoculated at the growth stage VC died 6 DPI. The majority of plants inoculated at V1 or V2 to V3 died at 9 DPI. Plants inoculated at R3 to R4 or R4 to R5 did not develop stem lesions until 6 DPI, although pod lesions were observed as brown, irregular-shaped blotches beginning at 4 DPI.

Evaluation of three sets of soybean genotypes for anthracnose resistance. The test of equal variances for the AUDPC values

Table 1. Restricted or maximum likelihood analysis of experiments to evaluate inoculation techniques and to evaluate soybean genotypes for resistance to *Colletotrichum truncatum* (anthracnose) based on the area under disease progress curve values

Experiment	Source	df	$P > F$
Inoculum type and incubation[u]	Type × time	5	<0.0001
Plant age at inoculation[v]	Growth stages	4	<0.0001
Previously reported sources[w]	Soybean genotype	14	<0.0001
Ancestral set, previous sources[x]	Soybean genotype	27	<0.0001
Public cultivars[y]	Soybean genotype	27	<0.0001
Resistant versus susceptible[z]	Growth stages	3	<0.0001

[u] Based on six treatments, two inoculum types (mycelia and conidia), three dew periods (24, 48, and 72 h), and three replications using three plants per experimental unit.

[v] Based on five growth stage treatments (VC, V1, V2 to V3, R3 to R4, and R4 to R5) and eight replications (combined two trials of four replications each) using three plants per experimental unit.

[w] Based on 15 soybean genotypes and six replications (combined two trials of three replications each) using two plants per experimental unit.

[x] Based on 28 soybean genotypes and six replications (combined two trials of three replications each) using one plant per experimental unit.

[y] Based on 28 soybean genotypes and six replications (combined two trials of three replications each) using two plants per experimental unit.

[z] Based on four treatments, two plant growth stages, two soybean genotypes, and six replications (combined two trials of three replications each) using three plants per experimental unit.

Table 2. Area under disease progress curve (AUDPC) values based on daily visual severity rating from 4 to 10 days after inoculation for soybean Williams 82 inoculated with a mycelial or a conidial suspension of *Colletotrichum truncatum* and incubated in the dark at three different incubation periods

Inoculum type[y]	Incubation period (h)	AUDPC[z]
Mycelial	72	353.4 A
Mycelial	48	303.0 AB
Conidial	72	266.3 B
Mycelial	24	151.8 C
Conidial	48	138.0 C
Conidial	24	11.0 D

[y] Mycelial suspension = 40 mg/ml and conidial suspension = 3×10^6 conidia per ml.

[z] Means not connected by the same letter are significantly ($P = 0.05$) different by least significant difference.

Table 3. Area under disease progress curve (AUDPC) values based on daily visual severity rating at 4, 5, 6, and 9 days after inoculation of soybean Williams 82 inoculated at five different growth stages with a mycelial suspension of *Colletotrichum truncatum*

Growth stage[y]	Plant age at inoculation (days)	AUDPC[z]
VC	8	372.8 A
V2 to V3	23	335.0 AB
V1	14	309.2 B
R3 to R4	36	148.6 C
R4 to R5	43	144.8 C

[y] V = vegetative growth stages and R = reproductive growth stages (8).

[z] Means not connected by the same letter are significantly ($P = 0.05$) different by least significant difference.

among the three experiments was not significant ($P < 0.861$, 0.2729, and 0.244). Therefore, the data were combined for analysis and presentation. In all three experiments, AUDPC values were significantly different for soybean genotypes (Table 1).

In the first experiment, Mandarin had lower AUDPC values than all the other genotypes except for Mandarin 507, Mandarin (Ottawa), and Boone (Table 4). Early White Eyebrow, Mandarin A, and Mandarin Yowa had AUDPC values higher than Mandarin but lower than Williams 82.

In the second experiment, Mandarin 507 had lower AUDPC values compared with all other genotypes except Early White Eyebrow, Mandarin Yowa, Boone, and Manchuria (Table 5). Two genotypes, 'A. K. Harrow' and Kingwa, had higher AUDPC values than Williams 82. Of the genotypes classified as "ancestors", no genotypes differed in AUDPC from Williams 82 except A. K. Harrow, which had a higher value.

In the third experiment, Mandarin 507, 'Spry', Mandarin, and 'Iroquois' had lower AUDPC values than the 24 other genotypes

tested (Table 6). Only seven genotypes had lower AUDPC values than Williams 82. This included two breeding lines (LN92-7369 and LN97-15076), three named cultivars ('Macon', Iroquois, and Spry), and the two resistant checks (Mandarin and Mandarin 507).

Effect of plant age on a resistant and a susceptible genotype. There were significant ($P < 0.0001$) differences between the soybean genotypes Mandarin 507 and Williams 82 in AUDPC at each of the growth stages and the severity of anthracnose on pods (Table 1). The highest AUDPC value occurred on Williams 82 plants at V1 to V2, which differed ($P = 0.05$) from all other treatments. The lowest AUDPC value occurred on Mandarin 507 at R4 to R6, which differed ($P = 0.05$) from all other treatments (Table 7). In trial 2, percentage of pods covered with lesions was lower in Mandarin 507 (3.8%) than in Williams 82 (39.5%) (Fig. 1).

Discussion

The objectives of this study were fulfilled because a reliable inoculation technique was developed to evaluate soybean genotypes

Table 4. Area under disease progress curve (AUDPC) for soybean genotypes inoculated with *Colletotrichum truncatum* with their origin, plant introduction (PI) number, and maturity group (MG)

Genotypes	Origin	PI	MG	AUDPC[z]
Williams 82	United States	518671	III	335.6 A
Willomi	Japan	548425	III	312.5 AB
Waseda	Japan	548423	II	299.3 AB
T269H	United States	548246	III	282.9 A–C
Wea	China	548424	II	277.1 A–D
Manchuria	China	548375	I	269.9 A–D
Gibson	United States	548330	IV	249.2 A–D
Bavendar Sp. A	United States	548304	III	239.0 A–D
Mandarin Yowa	France	189890	I	234.4 B–D
Mandarin A	Canada	438363	I	216.5 B–E
Early White Eyebrow	China	548320	0	213.7 B–E
Boone	China	548309	IV	185.5 C–F
Mandarin (Ottawa)	China	548379	0	179.2 D–F
Mandarin 507	China	548380	I	116.1 EF
Mandarin	China	548378	I	88.1 F

[z] Means not connected by the same letter are significantly ($P = 0.05$) different by least significant difference.

Table 5. Area under disease progress curve (AUDPC) for soybean genotypes inoculated with *Colletotrichum truncatum* with their reported use as ancestors to modern day public soybean cultivars released between 1947 to 1988, their origins, plant introductions number (PI), and maturity group (MG)

Genotype	Ancestor[y]	Origin	PI	MG	AUDPC[z]
A. K. Harrow	Yes	China	548298	III	268.5 A
Kingwa	No	China	548359	IV	250.1 AB
Arksoy	Yes	Korea	548438	VI	243.3 A–C
S-100	Yes	China	548488	V	243.3 A–C
Peking	Yes	China	548402	IV	241.0 A–D
Wea	No	China	548424	II	227.2 A–E
Gibson	No	United States	548330	IV	224.9 A–E
Willomi	No	Japan	548425	III	215.8 A–E
Dunfield	Yes	China	548318	III	215.8 A–E
Palmetto	Yes	China	548480	VII	215.8 A–E
Manchu	Yes	China	548365	III	206.6 B–F
Tokyo	Yes	Japan	548493	VII	202.0 B–G
Mukden	Yes	China	548391	II	197.4 B–G
Hartwig	No	United States	543795	V	188.3 C–G
Williams 82	No	United States	518671	III	186.0 C–G
Waseda	No	Japan	548423	II	183.7 D–G
Mandarin A	No	Canada	438363	I	179.9 E–G
Richland	Yes	China	548406	II	179.9 E–G
Roanoke	Yes	China	548485	VII	172.2 E–G
CNS	Yes	China	548445	VII	169.9 E–H
Bavendar Sp. A	No	United States	548304	III	154.1 F–I
Mandarin	Yes	China	548378	I	148.7 F–I
Mandarin (Ottawa)	Yes	China	548379	0	147.0 G–I
Manchuria	No	China	548375	I	112.6 H–J
Boone	No	China	548309	IV	109.9 I–J
Mandarin Yowa	No	France	189890	I	80.3 J
Early White Eyebrow	No	China	548320	0	76.8 J
Mandarin 507	No	China	548380	I	62.8 J

[y] Reported as ancestral to modern day public soybean cultivars released between 1947 to 1988 (10).
[z] Means not connected by the same letter are significantly ($P = 0.05$) different by least significant difference.

for resistance to *C. truncatum*. The method to inoculate soybean plants may be adapted to anthracnose on other crops or to other foliar diseases on soybean. The homemade moist incubation chambers alleviated the need for commercialized dew chambers. The

Table 6. Area under disease progress curve (AUDPC) for selected public soybean cultivars inoculated with *Colletotrichum truncatum* with their plant introduction (PI) number, maturity group (MG), and year of release

Cultivar	PI	MG	Year of release	AUDPC[x]
Dwight	597386	II	1997	304.1 A
Hack	548569	II	1984	284.7 AB
Hamilton	540555	IV	1989	276.3 A–C
Williams 82	518671	III	1983	263.7 A–D
Omaha	597382	IV	1996	263.1 A–D
LD00–3309	639740	IV	2006	251.3 A–D
Savoy	597381	II	1996	240.8 A–E
Cisne	593256	IV	1995	231.0 A–F
Jack	540556	II	1989	230.7 A–F
Maverick	598124	III	1996	229.5 A–F
Bell	540554	I	1989	226.1 A–F
LD00-2817P	658519	IV	2010	222.6 A–F
Saline	578057	III	1993	213.0 A–G
Ina	606749	IV	1998	211.2 B–G
Chamberlain	548635	III	1986	205.5 B–H
Pana	597387	III	1997	204.9 B–H
Burlison	533655	II	1988	199.7 B–H
Loda	614088	II	2000	197.5 B–H
Rend	606748	IV	1998	193.0 B–I
Yale	584441	III	1994	189.0 C–I
Piatt	574534	III	1993	178.1 D–I
LN92-7369	607385	II	1999	154.5 E–I
LN97-15076	633983	IV	2003	153.1 F–I
Macon	593258	III	1995	142.2 F–I
Iroquois	593259	III	1995	124.2 G–J
Mandarin[y]	548378	I	1920	116.2 H–J
Spry	553051	IV	1991	104.6 I–J
Mandarin 507[z]	548380	I	1943	49.3 J

[x] Means not connected by the same letter are significantly (*P* = 0.05) different by least significant difference.

[y] PI 548378 origin is China; historical origin is the United States, United States Department of Agriculture Arlington Farm, Arlington, VA; selected from PI 36653 from Sui Hua, Heilongjiang, China, in 1913; and released in 1920 (26).

[z] PI 548380 origin is China; historical origin is the United States, Wisconsin AES, Wisconsin, 507; selected from Mandarin; and released in 1943 (26).

soybean genotypes that exhibited lower AUDPC values did not have an immune reaction to the pathogen; instead, the reaction was quantitative. Additional research will be needed to determine the inheritance of this type of resistance and to determine the nature of the resistance mechanisms producing this quantitative resistance.

In this study, a mycelial suspension for inoculation was used to test soybean for resistance to anthracnose. Results showed that this technique provided several advantages over conidial suspensions. The mycelial suspensions were highly infectious and easy to prepare in large quantities in liquid cultures. The preparation time for mycelial suspension was less than that for conidial suspension and could be used to compare isolates that produce no or few conidia in culture plates (*unpublished*). Other studies have also shown benefits of using mycelial fragments, including for *Mycosphaerella fijiensis* infection of banana plants (7).

Incubation period and humidity are important components for many foliar pathogens, including *Colletotrichum* spp. (12,13,20). We found that longer incubation periods after inoculation increased the severity of anthracnose. Results were similar to those of a previous study, which showed that a longer period of wetness caused more severe anthracnose symptoms on inoculated soybean plants (20). Although it was not part of this study, it is presumed that the increased duration of moisture benefitted the fungus either directly, by allowing a longer period of optimal conditions for fungal growth, or indirectly, by increasing the susceptibility of plants to the pathogen with the increase of succulence of plant tissues (2).

Our results corroborated a previous study (20) that showed that younger plants were more susceptible to infection by *C. truncatum*. Soybean genotypes that had extreme differences in AUDPC values at the vegetative growth stage had similar differences at the reproductive growth stage. This indicates that the seedling stage may be

Table 7. Area under disease progress curve (AUDPC) for soybean genotypes Mandarin 507 and Williams 82 inoculated with *Colletotrichum truncatum* at two different growth stages

Soybean genotype	Growth stage[y]	AUDPC[z]
Williams 82	V2–V3	12.4 A
Mandarin 507	V2–V3	7.2 B
Williams 82	R4–R6	4.9 C
Mandarin 507	R4–R6	1.7 D

[y] V = vegetative growth stages and R = reproductive growth stages (8).

[z] Means not connected by the same letter are significantly (*P* = 0.05) different by least significant difference.
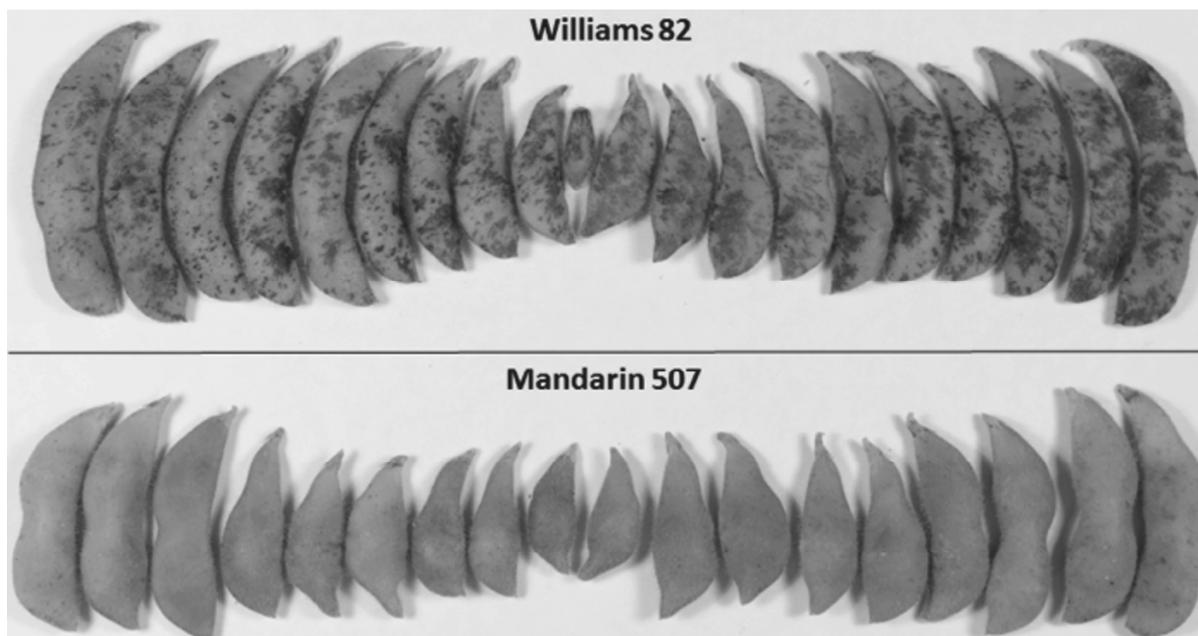
Fig. 1. Anthracnose on pods removed from plants of soybean genotypes Williams 82 and Mandarin 507 that were inoculated 20 days previously with *Colletotrichum truncatum*.

an acceptable time to evaluate soybean genotypes for resistance to *C. truncatum*. Using seedlings allows the researcher to maximize space and time when evaluating many genotypes or populations to determine the inheritance of resistance.

The resistance in soybean to anthracnose discovered in our study was not complete because even the genotypes with the lowest severity ratings had some leaf and petiole necrosis. One of the most resistant genotypes in a previous study was Mandarin (18). We tested an additional four PI accessions with the name Mandarin from the soybean germplasm collection (26). Of these five, Mandarin 507 was also in the most resistant group and showed good consistency in all of the experiments. This cultivar, as well as Mandarin (Ottawa), was derived and selected from Mandarin (26), which originated from China. However, Mandarin, Mandarin (Ottawa), and Mandarin Yowa (from France) performed differently, suggesting that they are different genotypes.

Among the 14 ancestral lines examined in the present study, 8 were tested by Manandhar et al. (18), including A. K. Harrow, 'Dunfield', 'Manchu', Mandarin, Mandarin (Ottawa), 'Mukden', 'Peking', and 'Richland'. All had ratings similar to Williams 82, with the exception of Mandarin and Mandarin (Ottawa). Mandarin and Mandarin (Ottawa) contributed less than 1 and 12%, respectively, of genes for the North American cultivars released from 1947 to 1988 (10).

Of the public soybean cultivars screened, only Iroquois and Spry had a level of resistance comparable with Mandarin 507. Both Iroquois and Spry have 'Harosoy' in their background (26), which has Mandarin (Ottawa) as one of its parents. In addition to showing a good level of foliar, petiole, and stem resistance, Mandarin 507 had few pod symptoms. Mandarin 507 and, possibly, Iroquois and Spry may be useful in further studies on inheritance and understanding the mechanisms of resistance which, to date, remain unknown. Further research needs to determine whether soybean resistance in the aboveground green tissues relates to resistance in seed and roots, and if the resistance is effective to multiple species of *Colletotrichum* that cause anthracnose.

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