Differential Reactions of Soybean Genotypes to Isolates of *Phialophora gregata*

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ABSTRACT

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Ten soybean (*Glycine max*) genotypes, three with the major genes for resistance (Rbs_1, Rbs_2, and Rbs_3) and five that expressed resistant and two that expressed susceptible phenotypes in field trials, reacted differentially when inoculated by a root-dip technique with six single-spore isolates of *Phialophora gregata* obtained from a single field. Disease severity was determined by the percentage of internal stem discoloration (PISD) and percentage of necrotic leaves (PNL). Each isolate, obtained from symptomatic, field-grown plants, caused internal stem discoloration and leaf necrosis in susceptible cultivars. An important conclusion from this this study is that isolates of *P. gregata* from the same field differed for virulence against genotypes of soybean.

Brown stem rot of soybean (*Glycine max* (L.) Merr.), caused by the soilborne fungus *Phialophora gregata* (Allington & D. W. Chamberlain) W. Gams (6), occurs commonly in the major soybean producing regions of North America, especially in the midwestern (1,3,12,13) and southeastern (18,21) United States. *P. gregata* invades young plants through the roots (1) and spreads systematically up the plant via mycelia and conidia (1,22). The disease is characterized by a reddish brown discoloration of vascular and pith tissues of the stem and petiole that often occurs in conjunction with interveinal leaf chlorosis and necrosis (1).

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Yield losses attributable to brown stem rot have been estimated to be up to 38% when plants were artificially inoculated (8) and up to 30% based on the yield of susceptible vs. resistant cultivars in soil infested by the pathogen (14).

High seasonal variation in incidence and severity of brown stem rot (2) has been attributed to variability in plant spacing (17), maturity (25), and soil moisture (14). Air temperature also influences disease development (1,2,13,19). Inoculum levels (4) and degrees of aggressiveness among isolates of *P. gregata* (7,13,20,27) also may contribute to seasonal variation of incidence and severity of brown stem rot.

All of the isolates of *P. gregata* collected in a 3-yr period after its discovery appeared to be identical and had stable morphology and pathogenicity (1). Hamilton and Boosalis (9) described sporulating and nonsporulating types of isolates on potato-dextrose agar. Gray (7) distinguished two pathotypes of *P. gregata* on susceptible soybean lines. Type I isolates cause both stem and leaf symptoms and type II isolates cause only internal stem discoloration. Phillips (20) compared 11 mass and 37 monoconidial isolates from several states. He distinguished 15 cultural types and reported sectoring in the fungus. Mengistu and Grau (13) found a wide range of pathogenicity on a single susceptible cultivar among 24 Wisconsin isolates of *P. gregata*. They also showed that classification of isolates as type I or type II can be influenced by air temperature.

Willmot et al (27) tested 27 type I isolates from Illinois, Iowa, Minnesota, and Wisconsin on four different soybean genotypes. Highly significant isolate × cultivar effects were noted for stem and leaf symptom severity, indicating physiologic specialization within *P. gregata*.

The objective of this research was to test for and interpret isolate × soybean genotype interactions for stem and leaf symptom severity with a set of type I isolates of *P. gregata* collected from a single Wisconsin field and 10 genotypes of soybean that expressed resistant or susceptible phenotypes in past experiments (16,26).

MATERIALS AND METHODS

Soybean plants that expressed characteristic symptoms of brown stem rot at growth stage R6–R7 (5) were collected from a field in its 10th consecutive year of soybeans at the University of Wisconsin Research Station at Hancock. Stems were rinsed in running tap water for 30 min. Pieces of discolored stem tissue (2 × 5 mm) were surface-disinfested in 0.5% NaOCl for 60 sec, blotted, and plated on acidified potato-dextrose agar

(APDA) to obtain field isolates of *P. gregata*.

Twenty-six single-spore isolates of *P. gregata*, obtained from six field isolates, were tested in the greenhouse for virulence on Ozzie, a susceptible soybean cultivar. After 7 wk of incubation, the single-spore isolates were ranked for aggressiveness based on severity of foliar symptoms. Each isolate was reisolated on APDA from infected stems. Six single-spore isolates that caused the most severe foliar symptoms were chosen for the isolate × soybean genotype interaction study.

Isolates were stored on silica gel at 4 C to maintain their genetic integrity. Inoculum was produced on soybean stem broth, which was prepared from 40 ml of distilled water and 1 g of dried soybean stem pieces autoclaved in 125-ml Erlenmeyer flasks for 30 min. The broth was adjusted to pH 4.0 with two drops of 50% lactic acid. Mycelial plugs (0.1 cm^2) from APDA were added to each flask and incubated on a rotary shaker at 100 rpm at 22 C. After 11 days, the colonized broth was filtered through four layers of cheesecloth to obtain a conidial suspension. The concentration of conidia was determined with a hemacytometer and then diluted to 1×10^7 ml^{-1} with distilled water. Methylcellulose (400 centipoise viscosity) was added at 0.75% (w/v) to the inoculum.

Greenhouse experiment. Each of the six isolates and a control broth without *P. gregata* were used to inoculate each of 10 soybean genotypes. Genotypes L78-4094, PI 437833, and PI 437970 each contain a single known resistance gene, Rbs_1 (10,23), Rbs_2 (10), and Rbs_3 (26), respectively. Genotypes PI 437366, PI 437475, PI 437497, PI 437685D, and PI 437934A each expressed a resistant phenotype in field trials at the Hancock Research Station (16). Two susceptible commercial cultivars, Sibley and Corsoy 79, were used for comparison.

Seeds were surface-disinfested in 0.5% NaOCl for 5 min and rinsed in sterile distilled water. Seeds were planted in steam-pasteurized sand in steam-pasteurized wooden flats and grown in the greenhouse. After 15–16 days, plants were removed and sand was rinsed from the roots. Roots were blotted with paper towels, the lower 1–2 cm was trimmed with scissors, and the remaining roots were dipped into the inoculum suspension in a manner similar to that used by Sebastian et al (24). Five inoculated seedlings were transplanted into a steam-pasteurized sand/soil (silty loam) mixture (1:1) in each steam-pasteurized 15-cm-diameter clay pot. Because only conidia were used and no additional inoculum was distributed around the roots in the pots, as in previous reports (10, 23,24,26,27), and preliminary tests had occasional plants that apparently escaped infection, host roots were trimmed and a higher concentration of inoculum was used to aid in infection. Uninoculated plants were dipped into a control broth solution lacking the fungus.

The factorial experiments were conducted in a randomized complete block design with 70 (7 × 10) treatments and three replications. The experiment was repeated in time and denoted as run 1 and run 2. Plants were grown in a greenhouse with a 14-hr photoperiod. Each pot was watered daily to field capacity

Table 1. Percentage of necrotic leaves (PNL) and percentage of internal stem discoloration (PISD) for 10 soybean genotypes inoculated with different isolates of *Phialophora gregata* (run 1)

	Isolate of *P. gregata*													
	1		2		3		4		5		6		Host mean	
Soybean genotype	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD
PI 437366	100	100	97	100	79	84	70	85	98	100	72	87	86	93
PI 437475	98	100	86	93	81	88	92	95	93	93	74	76	88	91
PI 437497	78	95	45	65	27	49	28	53	39	64	17	26	39	59
PI 437685D	32	23	35	38	7	6	4	1	28	37	8	8	19	19
PI 437934A	42	47	41	54	41	47	31	35	47	50	20	29	37	44
L78-4094	47	50	62	60	33	42	40	49	52	61	30	46	44	51
PI 437833	100	99	57	66	84	81	39	40	69	67	47	64	66	70
PI 437970	31	43	24	43	27	29	35	48	42	53	33	50	32	45
Sibley	86	83	80	79	40	48	64	77	84	85	86	86	73	77
Corsoy 79	100	99	89	90	42	57	50	74	83	93	75	81	73	82
Isolate mean	71	74	62	69	46	53	46	56	64	70	46	56		
Isolate × soybean genotype interaction:								PNL		PISD				
						LSD (0.01)		29		30				
						LSD (0.05)		22		23				

Table 2. Percentage of necrotic leaves (PNL) and percentage of internal stem discoloration (PISD) for 10 soybean genotypes inoculated with different isolates of *Phialophora gregata* (run 2)

	Isolate of *P. gregata*													
	1		2		3		4		5		6		Host mean	
Soybean genotype	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD	PNL	PISD
PI 437366	56	67	58	67	69	82	74	92	45	72	40	66	57	74
PI 437475	67	67	66	51	61	56	54	50	51	51	63	61	60	56
PI 437497	21	33	30	45	29	37	13	28	29	56	30	51	26	41
PI 437685D	1	2	1	2	6	7	2	4	6	5	3	7	3	4
PI 437934A	30	28	28	29	27	29	10	21	34	37	33	41	27	31
L78-4094	15	25	26	33	25	40	15	36	21	28	34	46	23	35
PI 437833	23	30	51	52	67	62	47	56	46	47	43	53	43	50
PI 437970	7	15	11	27	24	31	29	36	29	31	27	39	21	30
Sibley	88	92	75	80	82	86	80	75	71	79	76	77	79	82
Corsoy 79	79	88	47	55	66	79	76	81	74	86	78	84	70	79
Isolate mean	39	45	39	44	46	51	40	48	41	49	43	52		
Isolate × soybean genotype interaction:								PNL		PISD				
						LSD (0.01)		21		20				
						LSD (0.05)		16		15				

and fertilized weekly with 0.90 g of Peter's fertilizer (W. R. Grace & Co., Cambridge, MA) (20% N, 20% P_2O_5, 20% K_2O). Nighttime low temperatures were 13 ± 3 C in both runs. Daytime high temperatures varied from 19 to 33 C in run 1 and 19 to 30 C in run 2. The mean weekly high temperatures varied over weeks and runs.

Plants were assessed 6 wk after inoculation for plant height, total number of nodes, highest node with a necrotic or chlorotic leaf, and the height of internal stem discoloration. The unifoliate leaf node was considered the first node, and the top node at which a leaf had completely unrolled was considered the terminal node. Percentage of internal stem discoloration (PISD) was used to assess stem symptom severity as follows: PISD = 100 × (height internal stem discoloration/plant height) (15). The percentage of nodes with necrotic or chlorotic leaves in relation to the total number of nodes (PNL) was used as a measure of leaf symptom severity. For analyses of variance, runs were treated as random effects, and isolates and soybean genotypes were treated as fixed effects.

Field experiment. The same 10 soybean genotypes used in the greenhouse studies were evaluated for incidence and severity of brown stem rot in a field naturally infested with *P. gregata* at the Hancock Research Station. This field was the source of isolates of *P. gregata* used in the greenhouse experiments. Sixty seeds of each genotype were planted in 3-m rows in two-row plots on 22 May 1989. Rows were spaced 75 cm apart. The experimental design was a randomized complete block with four replications. Evapotranspiration rates were used as a criteria for irrigation, and plots were irrigated with 1–2 cm of water twice weekly throughout the growing season.

The severity of stem symptoms was assessed in terms of the mean PISD of six randomly chosen plants per plot. Leaf symptoms were rated visually on a whole plot basis with the Horsfall-Barratt scale (11) where 0 = no foliar symptoms, 1 = 0–3%, 2 = 3–6%, 3 = 6–12%, 4 = 12–25%, 5 = 25–50%, 6 = 50–75%, 7 = 75–87%, 8 = 87–94%, 9 = 94–97%, 10 = 97–100%, and 11 = 100% leaf necrosis. The severity of leaf and stem symptoms was assessed at growth stage R6 (5).

RESULTS

Greenhouse experiments. Leaf necrosis was first observed 19 and 27 days after inoculation in run 1 and run 2, respectively. After 6 wk of incubation, plants of the susceptible cultivars Sibley and Corsoy 79 expressed severe stem and leaf symptoms (type I reaction) in both replicates in time. However, overall symptom severity was greater in run 1 than in run 2. PISD and PNL data were distributed normally and a combined analysis of variance on the nontransformed data revealed a highly significant run × isolate × soybean genotype interaction for both traits. This indicates that isolate × soybean genotype interaction phenotypes were not consistent over the two runs. Within each run, isolate, soybean genotype, and isolate × soybean genotype effects for both PNL and PISD were highly significant, except in run 2 where a significant isolate effect on PNL was not detected. Based on treatment means combined over runs, PNL correlated strongly with PISD ($r = 0.96$; $P < 0.01$). Uninoculated plants did not develop stem and leaf symptoms and were not included in the analysis of variance.

All host genotype-isolate combinations resulted in leaf and stem symptom expression (Tables 1 and 2). PI 437366 and PI 437475 reacted similarly to Sibley and Corsoy 79 with high values for PNL and PISD. Severity of symptom expression in other soybean genotypes varied with the isolate. PI 437685D exhibited particularly low symptom expression with some isolates.

Field experiment. A killing frost on 24 September 1989 made disease assessment in PI 437685D at growth stage R6 impossible because of the later maturity of that genotype. Highly significant differences between soybean genotypes were found based on PISD and Horsfall-Barratt scores for leaf symptoms. Data for PI 437685D were not included in the analysis.

The susceptible cvs. Sibley and Corsoy 79 developed extensive stem and leaf symptoms of brown stem rot. Disease severity, based on leaf and stem ratings, in resistant and putatively resistant genotypes was significantly less than in the susceptible checks (Table 3).

DISCUSSION

Symptoms expressed by plants of resistant and putatively resistant genotypes were more severe than expected in greenhouse experiments. In previous greenhouse studies, minimal leaf symptoms were caused by single isolates of *P. gregata* on known resistant lines L78-4094 (10,23,27), PI 437833 (10,27), and PI 437970 (26). However, Willmot et al (27) found that each of 27 isolates caused leaf symptoms on PI 437833 with the root-dip inoculation technique. They rated the reaction of PI 437833 to four isolates as susceptible, intermediate, or intermediate to resistant based on leaf symptom severity. In our study, severe leaf symptoms were expressed by PI 437833 in each replicate in time. L78-4094 and PI 437970 also expressed an extensive development of leaf symptoms when inoculated with certain isolates.

In the current study, PI 437366 and PI 437475 were susceptible to all isolates in both runs in the greenhouse but showed significantly less leaf symptoms than susceptible cultivars in the infested field at Hancock. PI 437497 and PI 437934A developed moderate leaf symptoms when averaged across all isolates in the greenhouse; however, certain isolates were less virulent on these genotypes. PI 437685D was the most resistant genotype to all isolates in both runs. In a previous greenhouse study, PI 437366, PI 437497, PI 437685D, and PI 437934A expressed no leaf symptoms when inoculated with a single isolate of *P. gregata* (16). PI 437475 was classified as resistant 1 yr and as having intermediate resistance the following year, based on a rating of leaf symptoms (16).

Willmot et al (27) reported physiologic specialization among isolates of *P. gregata* obtained from diverse geographic regions. Differential reactions of soybean genotypes in this study suggested that variation in virulence exists within a set of six isolates of *P. gregata* obtained from a single field.

Leaf symptoms associated with brown stem rot in the PI genotypes and L78-4094 were less severe than those on Sibley and Corsoy 79 in the naturally infested field at Hancock. None of these lines expressed more than 12% leaf necrosis, whereas Corsoy 79 and Sibley averaged no less than 75% necrotic leaves. Nelson et al (16) reported similar results with the putatively resistant soybean genotypes and Corsoy 79 over two seasons at the same location. Willmot and Nickell (26) reported that in field-grown plants inoculated with a single isolate of *P. gregata*, leaf symptoms progressed through an average of 0.9, 2.3, and 0.6 nodes in L78-4094, PI 437833, and PI 437970, respectively. These genotypes were classified as resistant. PI 437970 expressed the second highest level of resistance to all isolates in the greenhouse study but

Table 3. Leaf symptom severity ratings (LSS)[x] and percentage of internal stem discoloration (PISD)[y] for nine soybean genotypes grown in a field naturally infested with *Phialophora gregata* at the Hancock Research Station, University of Wisconsin

Soybean genotype	LSS	PISD
L78-4094	1.0 a[z]	5 a
PI 437366	1.0 a	47 c
PI 437934A	1.5 a	19 ab
PI 437970	1.5 a	48 c
PI 437833	2.0 ab	27 b
PI 437497	2.8 b	19 ab
PI 437475	3.0 b	28 b
Corsoy 79	7.3 c	65 d
Sibley	8.3 c	79 d

[x] Based on Horsfall-Barratt leaf symptom severity scores (11): 0 = no symptoms to 11 = 100% necrotic.

[y] Based on percentage of total main stem height with internal discoloration.

[z] Means with the same letter in a column are not significantly different ($P = 0.05$) as determined by Fisher's least significant difference.

expressed an intermediate stem reaction in the field.

Resistance observed in the field was not always expressed in the greenhouse. Several unique inoculum and inoculation factors may have contributed to the increased brown stem rot symptoms observed in this greenhouse study. First, the six most virulent isolates (from among 26) were used in this study. Second, inoculum concentration in this study was based solely on the number of conidia/ml^{-1} whereas the concentration of fungal propagules, including conidia and mycelial fragments, has been used in previous greenhouse tests. The higher number of conidia may have been important because sporulating mycelia induced symptoms sooner than nonsporulating mycelia in stem inoculated plants (1). Third, inoculum concentration was adjusted to 10^7 conidia/ml^{-1} vs. 1.2×10^6 propagules/ml^{-1} in the previous studies. Seedling roots were cut before inoculation, aiding penetration of the pathogen into the plant host. Finally, plants were evaluated after 6 wk rather than 5–5.5 wk, allowing more time for symptom development.

The significant run × isolate × soybean genotype interaction observed in this study indicated that some combinations of host and fungal isolates reacted differently in the two replicates in time. During the first week after inoculation, high temperatures averaged 21.7 C in run 1 and 26.1 C in run 2. Phillips (19) reported a rapid drop in symptom ratings at temperatures above 27 C. If temperature differences contributed to the significant run effect, temperatures may affect the expression of host resistance to certain isolates. Studies on temperature effects on symptom development in susceptible cultivars are numerous (1,2,13,19), although results disagree. The effects of temperature on the interaction of resistant lines with isolates of *P. gregata* have not been researched.

Statistically significant interactions between isolates and soybean genotypes in each run of this study supports the conclusion (27) that physiologic specialization exists within *P. gregata*. Willmot et al (27) obtained fungal isolates from four states and found that there was variability for virulence among isolates from a given region. This study shows that isolates of *P. gregata* differing in virulence were obtained from a single field that was heavily infested with the fungus.

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