Use of Reciprocal Grafting to Study Brown Stem Rot Resistance in Soybean

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Accepted for publication 28 September 1998.

ABSTRACT

Bachman, M. S., and Nickell, C. D. 1999. Use of reciprocal grafting to study brown stem rot resistance in soybean. *Phytopathology* 89:59-63.

Resistance to brown stem rot of soybean has been identified and utilized in cultivar development and germ plasm enhancement. However, little is known about the nature of resistance to this disease. In an attempt to

better understand the resistance mechanism by locating the tissue or tissues responsible for resistance, reciprocal grafts were made between brown stem rot-resistant and -susceptible genotypes including near-isogenic lines for resistance gene *Rbs2*. Results of brown stem rot evaluation of grafted plants indicate that brown stem rot resistance is conditioned by the root in all resistance sources evaluated.

Brown stem rot (BSR) of soybean (*Glycine max* (L.) Merr.), caused by *Phialophora gregata* (Allington & D.W. Chamberlain) W. Gams, is an economically important disease in northern soybean production regions. Estimated seed yield losses up to 38% have been reported under conditions that favor disease development (10,17,25,31). Genetic resistance to *P. gregata* has been identified and shown to reduce yield losses by more than 10% when incidence of the disease is high (3).

Genetic analyses of resistant soybean lines have identified three independently segregating dominant genes that condition resistance to *P. gregata*. Two of these loci, *Rbs1* (13) and *Rbs3* (7), were identified in lines derived from plant introduction (PI) 84946-2, a resistant accession identified by Chamberlain and Bernard (5). *Rbs3* was also identified as the source of resistance in PI 437970 (34), an accession from northeastern China. Genetic analysis of another resistant accession from China, PI 437823 (later changed to PI 437833) (11), identified a third dominant BSR resistance allele, *Rbs2* (13). Analyses of additional resistant soybean lines have been conducted, but genes for resistance in these lines have not been conclusively identified (16,30). Other resistant accessions have been identified through germ plasm evaluation (2,19), but the relationship between the source of resistance in these lines and *Rbs* resistance alleles is unknown.

Early experiments conducted by Kunkel and Dunleavy (14,15) attempted to delimit morphological regions in which the resistance response occurred. To do this, Kunkel and Dunleavy (14,15) evaluated the response to *P. gregata* of reciprocal grafts made with resistant cultivar Midwest (24) and susceptible cultivar Ontario (18). They concluded that the resistance response was confined to the aerial portion of the plant based on observations that the roots (rootstock) of 'Midwest' and 'Ontario' (18) were equally susceptible to infection by *P. gregata* when grafted reciprocally. However, Phillips (22) found that PI 84946-2, which was usually resistant to BSR in field studies, had a susceptible reaction when inoculated in the hypocotyl with *P. gregata*. Those data presented evidence for a BSR resistance response in the root of PI 84946-2. Although results of the former and latter experiments conflict on the morphological location of the resistance response to *P. gregata*, no further studies have been published on this topic.

Studies on *P. gregata* and its soybean host have shown that variability exists in the pathogen (23,26,35) and that sources of resis-

tance are not immune to BSR (2,19,26). While resistant types have been identified including resistance derived from several distinct loci, there is little information on the morphological location or mechanism of resistance to *P. gregata*. If duality indeed exists for the morphological location of the resistance response, complementation of these resistance sources could lead to a greater level or higher stability of resistance, or both. This may be advantageous in future attempts to prevent yield loss to BSR. The objective of this study was to determine the morphological location of the resistance response to *P. gregata* in various characterized and uncharacterized sources of resistance using reciprocal grafting with susceptible cultivars followed by inoculation with *P. gregata*.

MATERIALS AND METHODS

Seedlings of resistant and susceptible cultivars and germ plasm lines were grafted in various combinations including self-grafts, reciprocal grafts using rootstocks and scions of the same BSR reaction types, and reciprocal grafts using rootstocks and scions of opposite BSR reaction types. Two separate experiments were conducted. The first experiment was designed to study the morphological location of resistance response in various published sources of BSR resistance. The second experiment was conducted to confirm results of an experiment by Kunkel and Dunleavy (14,15) that indicated that resistance was limited to the aerial portion of 'Midwest'. In the first experiment, sources of BSR resistance and susceptibility were evaluated in grafted combinations for reaction to *P. gregata* (Table 1). Soybean lines L78-4094 (*Rbs1*), PI 437833 (*Rbs2*), and PI 437970 (*Rbs3*) were included to allow identification of the morphological location of resistance in at least three unique sources of resistance. Line PI 84946-2, which has indirectly been shown to carry *Rbs1* and *Rbs3* (7,13), was included to observe any effect caused by the presence of two independently segregating resistance alleles. Line PI 86150 contains a single resistance gene that is not allelic to *Rbs1* or *Rbs3* (33) and was included to investigate this unidentified gene. A total of 13 additional BSR-resistant soybean accessions from China also were included. These accessions were identified as BSR-resistant in a germ plasm evaluation by Bachman et al. (2), but genetic analyses have not been conducted to characterize these sources of resistance. Cultivar Century 84 (29) was included as a susceptible type because it had a highly susceptible reaction to *P. gregata* in previous studies (2,7,30). Clark 63 (32), a BSR-susceptible cultivar (27), was also included to determine if reactions of grafted plants depended on the source of susceptible donor tissue. Finally, five pairs of *Rbs2* near-isogenic lines (20) were included. The responses of these isolines to *P. gregata* were verified in a separate experiment (3). These lines

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Publication no. P-1998-1112-01R

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theoretically differ by only the *Rbs2* resistance allele, and when grafted reciprocally and evaluated for BSR resistance, should provide an indication of the location of the resistance response.

In the second experiment, 'Midwest' and 'Ontario' were evaluated in grafted combinations in an effort to confirm the results of a study by Kunkel and Dunleavy (14,15). These cultivars were evaluated as reciprocal grafts with one another and as reciprocal grafts with BSR-resistant accession PI 437970 and susceptible 'Century 84'.

In both experiment 1 and experiment 2, grafted and ungrafted controls were included to determine if grafting affected disease development in the plant.

Grafting of soybean seedlings was performed as described by Cho and Harper (6), modified from Bezdicsek et. al. (4), with the following additional modifications. Seedlings were planted in torpedo sand in 10-cm-diameter plastic pots. After grafts were made, pots containing grafted soybeans were covered with plastic and misted with water at 4- to 8-h intervals in the greenhouse. Grafted soybeans received no supplemental light for 24 h after grafting. After 5 days, the chamber was opened slightly to allow reduction in relative humidity. After 6 days, the plastic cover was removed, and 7 days after grafting, seedlings were inoculated with *P. gregata*.

Liquid cultures of PgOh2, a monoconidial isolate of *P. gregata* (obtained from L. Gray, USDA-ARS, University of Illinois, Urbana-Champaign), were initiated by transferring three agar plugs (approximately 2.7 cm³) containing hyphal tips of an active culture from soybean stem agar minimal medium (1) to 100 ml of soybean seed broth (100 g of soybean seed per liter of water steamed, strained, and autoclaved). Both stem agar and seed broth were made from a susceptible standard, 'Century 84'. Stationary liquid cultures were incubated at 24°C in the dark. After 4 to 5 weeks, seed broth cultures of *P. gregata* were ground for 75 s in a blender at high speed. The concentration of mycelial fragments and conidia was determined using a hemacytometer; mycelial fragments of all sizes were included in counts. Blended cultures were then diluted with distilled water to a concentration of 1.2×10^6 propagules per ml. Carboxymethyl cellulose was blended for 15 s with the diluted, blended culture at a rate of 7.5 g/liter to serve as a sticking agent.

Seedlings were inoculated with *P. gregata* using the root-dip inoculation method described by Willmot and Nickell (34). At 7 days after grafting, seedlings (VC-V1 growth stage [4]) were removed from pots and the sand was thoroughly rinsed with water from the roots of all plants. Two to five uniform, healthy-appearing seedlings

TABLE 1. Experimental treatments (grouped by rootstock) and respective numbers of replications evaluated for response to *Phialophora gregata*

Treatment ^a	Reps ^b	Treatment ^a	Reps ^b
Experiment 1		R = PI 567609, S = 'Century 84'	2
R = PI 437970, S = PI 437970	4	R = PI 567333A, S = 'Century 84'	2
R = PI 437970, S = PI 437833	4	R = PI 567544, S = 'Century 84'	2
R = PI 437970, S = L78-4094	4	R = PI 567552, S = 'Century 84'	2
R = PI 437970, S = 'Century 84'	4	R = PI 567542, S = 'Century 84'	2
R = PI 437970, S = 'Clark 63'	2	R = PI 567535, S = 'Century 84'	2
R = PI 437833, S = PI 437833	4	R = PI 567523, S = 'Century 84'	2
R = PI 437833, S = PI 437970	4	R = PI 567479, S = 'Century 84'	2
R = PI 437833, S = L78-4094	4	R = PI 567615, S = 'Century 84'	2
R = PI 437833, S = 'Century 84'	4	R = PI 567323A, S = 'Century 84'	2
R = PI 437833, S = 'Clark 63'	2	R = PI 567381A, S = 'Century 84'	2
R = L78-4094, S = L78-4094	4	R = PI 567296B, S = 'Century 84'	2
R = L78-4094, S = PI 437970	4	R = PI 567546, S = 'Century 84'	2
R = L78-4094, S = PI 437833	4	R = LN92-12077, S = LN92-12070	2
R = L78-4094, S = 'Century 84'	4	R = LN92-12070, S = LN92-12077	2
R = L78-4094, S = 'Clark 63'	2	R = LN92-11976, S = LN92-11995	2
R = 'Century 84', S = PI 437970	4	R = LN92-11995, S = LN92-11976	2
R = 'Century 84', S = PI 437833	4	R = LN92-12117, S = LN92-12137	2
R = 'Century 84', S = L78-4094	4	R = LN92-12137, S = LN92-12117	2
R = 'Century 84', S = PI 84946-2	2	R = LN92-12014, S = LN92-12022	2
R = 'Century 84', S = PI 86150	2	R = LN92-12022, S = LN92-12014	2
R = 'Century 84', S = 'Century 84'	4	R = LN92-12033, S = LN92-12054	2
R = 'Century 84', S = PI 567609	2	R = LN92-12054, S = LN92-12033	2
R = 'Century 84', S = PI 567333A	2	R = LN92-12033, S = LN92-12033	2
R = 'Century 84', S = PI 567544	2	R = LN92-12054, S = LN92-12054	2
R = 'Century 84', S = PI 567552	2	'Century 84' (ungrafted)	2
R = 'Century 84', S = PI 567542	2	PI 437970 (ungrafted)	2
R = 'Century 84', S = PI 567535	2		
R = 'Century 84', S = PI 567523	2	Experiment 2	
R = 'Century 84', S = PI 567479	2	R = 'Ontario', S = 'Century 84'	2
R = 'Century 84', S = PI 567615	2	R = 'Ontario', S = 'Midwest'	4
R = 'Century 84', S = PI 567323A	2	R = 'Ontario', S = PI 437970	2
R = 'Century 84', S = PI 567381A	2	R = 'Ontario', S = 'Ontario'	4
R = 'Century 84', S = PI 567296B	2	R = 'Midwest', S = PI 437970	2
R = 'Century 84', S = PI 567546	2	R = 'Midwest', S = 'Century 84'	2
R = 'Clark 63', S = 'Clark 63'	4	R = 'Midwest', S = 'Ontario'	4
R = 'Clark 63', S = PI 437970	2	R = 'Midwest', S = 'Midwest'	4
R = 'Clark 63', S = PI 437833	2	R = PI 437970, S = 'Midwest'	2
R = 'Clark 63', S = L78-4094	2	R = PI 437970, S = 'Ontario'	2
R = 'Clark 63', S = PI 84946-2	2	R = PI 437970, S = PI 437970	2
R = 'Clark 63', S = PI 86150	2	R = 'Century 84', S = 'Ontario'	2
R = PI 84946-2, S = PI 84946-2	2	R = 'Century 84', S = 'Midwest'	2
R = PI 84946-2, S = 'Clark 63'	2	R = 'Century 84', S = 'Century 84'	2
R = PI 84946-2, S = 'Century 84'	2	'Midwest' (ungrafted)	4
R = PI 86150, S = 'Century 84'	2	'Ontario' (ungrafted)	4
R = PI 86150, S = PI 86150	2	'Century 84' (ungrafted)	2
R = PI 86150, S = 'Clark 63'	2	PI 437970 (ungrafted)	2

^a R = rootstock, S = scion.

^b Reps = number of treatment replications.

were selected, and their roots were blotted dry using paper towels and then dipped into a beaker containing 10 ml plant⁻¹ of the suspension of mycelial fragments and conidia. Special care was taken to exclude the graft union from the liquid inoculum. The seedlings were removed from the inoculum and placed into a 6- to 8-cm depression in steam-treated 1:1 sand/topsoil mixture filling a 15-cm-diameter steam-sterilized clay pot. The remaining inoculum was then poured over the roots of the seedlings, and the roots were covered with the 1:1 sand/soil mixture to a level approximately 1 cm below the cotyledons.

Plants were maintained under a 14-h photoperiod at an average nighttime temperature of 18°C and an average daytime temperature of 24°C. All entries received approximately 150 ml of water daily for 7 days after grafting and approximately 300 ml of water daily for the remainder of the experiment. Pots were fertilized weekly with 150 ml of a nutrient solution containing 0.121 g of N, 0.112 g of P, 0.107 g of K, 0.000044 g of B, 0.00022 g of chelated Fe, and 0.00011 g of chelated Cu, Mn, and Zn.

Each pot contained between two and five plants of the same treatment and was considered one replication. Resistant and susceptible self-grafts and reciprocal grafts made with L78-4094 (*Rbs1*), PI 437833 (*Rbs2*), PI 437970 (*Rbs3*), and 'Century 84' (*rbs1*, *rbs2*, *rbs3*) were evaluated sequentially in two sets of two replications to identify variability and repeatability of experimental procedures. All other

treatments in experiment 1 were evaluated sequentially in two replications because of space limitations. Self-grafts, reciprocal grafts, and ungrafted controls of 'Midwest' and 'Ontario' were replicated four times. All other self-grafts and reciprocal grafts in experiment 2 were replicated twice because of labor and space limitations (Table 1). Treatments were arranged in a completely randomized design on greenhouse benches lined with 3 cm of torpedo sand.

After approximately 6 weeks (R1 to R2 growth stage [8], 8 to 11 aboveground nodes), plants were evaluated for BSR reaction. Individual plants in a pot were rated for foliar and stem symptoms. Foliar symptoms of each plant were recorded as the height of leaf necrosis, measured in nodes with a leaf showing BSR-induced necrosis, as a fraction of the total height, measured in nodes with an expanded leaf. Stem symptoms were measured by splitting the stem of each plant lengthwise and recording the height of stem browning, measured in nodes, as a fraction of the total height, measured in nodes with an expanded leaf. Foliar and stem ratings were averaged for all plants in a pot and recorded as a single observation. To insure that foliar symptoms were the result of BSR, only those plants showing stem browning were assigned the corresponding observed foliar symptom rating (13). Foliar and stem symptom data were subjected to separate analyses of variance for treatments (individual graft combinations) and rootstock-scion groups

TABLE 2. Brown stem rot (BSR; causal agent, *Phialophora gregata*) foliar and stem symptoms for experimental treatments

Treatment ^a	BSR reaction ^b		BSR symptoms		Treatment ^a	BSR reaction ^b		BSR symptoms	
	Rootstock	Scion	Foliar ^c	Stem ^d		Rootstock	Scion	Foliar ^c	Stem ^d
Characterized sources ^e					R = 'Century 84', S = PI 567523	— ^f	+ ^f	0.60	0.74
R = PI 437970, S = PI 437833	+ ^f	+ ^f	0.00	0.04	R = PI 567523, S = 'Century 84'	+	—	0.07	0.11
R = PI 437833, S = PI 437970	+	+	0.03	0.01	R = 'Century 84', S = PI 567479	—	+	0.59	0.64
R = PI 437970, S = L78-4094	+	+	0.00	0.00	R = PI 567479, S = 'Century 84'	+	—	0.14	0.14
R = L78-4094, S = PI 437970	+	+	0.02	0.04	R = 'Century 84', S = PI 567615	—	+	0.58	0.65
R = PI 437970, S = 'Century 84'	+	—	0.00	0.00	R = PI 567615, S = 'Century 84'	+	—	0.24	0.29
R = 'Century 84', S = PI 437970	—	+	0.96	0.96	R = 'Century 84', S = PI 567323A	—	+	0.73	0.81
R = PI 437970, S = 'Clark 63'	+	—	0.00	0.00	R = PI 567323A, S = 'Century 84'	+	—	0.00	0.00
R = 'Clark 63', S = PI 437970	—	+	0.76	0.80	R = 'Century 84', S = PI 567381A	—	+	0.86	0.84
R = PI 437833, S = L78-4094	+	+	0.00	0.00	R = PI 567381A, S = 'Century 84'	+	—	0.09	0.10
R = L78-4094, S = PI 437833	+	+	0.10	0.11	R = 'Century 84', S = PI 567296B	—	+	0.85	0.82
R = PI 437833, S = 'Century 84'	+	—	0.07	0.07	R = PI 567296B, S = 'Century 84'	+	—	0.07	0.07
R = 'Century 84', S = PI 437833	—	+	0.86	0.81	R = 'Century 84', S = PI 567546	—	+	0.57	0.72
R = PI 437833, S = 'Clark 63'	+	—	0.00	0.00	R = PI 567546, S = 'Century 84'	+	—	0.07	0.11
R = 'Clark 63', S = PI 437833	—	+	0.96	0.93	<i>Rbs2</i> near-isogenic lines				
R = L78-4094, S = 'Century 84'	+	—	0.06	0.07	R = LN92-12077, S = LN92-12070	+	—	0.06	0.06
R = 'Century 84', S = L78-4094	—	+	0.73	0.71	R = LN92-12070, S = LN92-12077	—	+	0.73	0.76
R = L78-4094, S = 'Clark 63'	+	—	0.21	0.23	R = LN92-11976, S = LN92-11995	+	—	0.03	0.02
R = 'Clark 63', L78-4094	—	+	0.55	0.70	R = LN92-11995, S = LN92-11976	—	+	0.93	0.78
R = PI 84946-2, S = 'Clark 63'	+	—	0.09	0.10	R = LN92-12117, S = LN92-12137	+	—	0.10	0.10
R = 'Clark 63', S = PI 84946-2	—	+	0.49	0.54	R = LN92-12137, S = LN92-12117	—	+	0.88	0.76
R = PI 84946-2, S = 'Century 84'	+	—	0.00	0.04	R = LN92-12014, S = LN92-12022	+	—	0.12	0.10
R = 'Century 84', S = PI 84946-2	—	+	0.57	0.68	R = LN92-12022, S = LN92-12014	—	+	0.46	0.53
Uncharacterized sources ^e					R = LN92-12033, S = LN92-12054	+	—	0.08	0.08
R = PI 86150, S = 'Century 84'	+	—	0.10	0.11	R = LN92-12054, S = LN92-12033	—	+	0.85	0.87
R = 'Century 84', S = PI 86150	—	+	0.59	0.58	Grafted controls				
R = PI 86150, S = 'Clark 63'	+	—	0.08	0.01	R = PI 437970, S = PI 437970	+	+	0.00	0.00
R = 'Clark 63', S = PI 86150	—	+	0.57	0.61	R = PI 437833, S = PI 437833	+	+	0.00	0.00
R = 'Century 84', S = PI 567609	—	+	0.80	0.80	R = L78-4094, S = L78-4094	+	+	0.02	0.03
R = PI 567609, S = 'Century 84'	+	—	0.08	0.08	R = 'Clark 63', S = 'Clark 63'	—	—	0.64	0.52
R = 'Century 84', S = PI 567333A	—	+	0.93	0.93	R = 'Century 84', S = 'Century 84'	—	—	0.67	0.73
R = PI 567333A, S = 'Century 84'	+	—	0.00	0.05	R = PI 84946-2, S = PI 84946-2	+	+	0.00	0.00
R = 'Century 84', S = PI 567544	—	+	0.75	0.76	R = PI 86150, S = PI 86150	+	+	0.00	0.00
R = PI 567544, S = 'Century 84'	+	—	0.06	0.04	R = LN92-12033, S = LN92-12033	+	+	0.00	0.00
R = 'Century 84', S = PI 567552	—	+	0.85	0.84	R = LN92-12054, S = LN92-12054	—	—	0.62	0.66
R = PI 567552, S = 'Century 84'	+	—	0.06	0.06	Ungrafted controls				
R = 'Century 84', S = PI 567542	—	+	0.67	0.82	'Century 84'	—		0.65	0.71
R = PI 567542, S = 'Century 84'	+	—	0.18	0.19	PI 437970	+		0.04	0.07
R = 'Century 84', S = PI 567535	—	+	0.61	0.73	LSD (<i>P</i> = 0.05)				0.34 0.36
R = PI 567535, S = 'Century 84'	+	—	0.00	0.00					

^a R = rootstock, S = scion.

^b BSR reaction = brown stem rot reactions of stock and scion sources, obtained from prior published work.

^c Proportion of total nodes that exhibited BSR-induced foliar necrosis.

^d Proportion of total nodes that exhibited internal stem browning.

^e Characterized sources = BSR resistance that has been genotypically identified; uncharacterized sources = BSR resistance that has not been genotypically identified.

^f + = resistant, — = susceptible.

(composed of BSR reaction types). A protected least significant difference test at the 0.05 probability level was used to identify differences in BSR foliar and stem symptom ratings between treatments and rootstock-scion groups.

RESULTS

The number of aboveground nodes (cotyledonary node and above) was not significantly different between ungrafted and grafted control plants, indicating that grafting did not affect this component of plant growth.

Experiment 1. BSR foliar and stem symptoms were not significantly different for ungrafted and grafted controls, indicating that the grafting procedure did not interfere with BSR resistance or BSR symptom development above the graft union in these lines (Table 2). Isolation of *P. gregata* from stem tissue above the graft union in plants with a rootstock donated by a susceptible plant and a scion derived from a resistant plant (data not shown) demonstrated the ability of the fungus to colonize scion tissue from a resistant donor. This provided evidence that symptoms above the graft union were not solely the result of a diffusible toxin (12) produced in the colonized susceptible rootstock.

Results of BSR foliar and stem symptom evaluation indicated that, in all treatments inoculated with *P. gregata*, the response of the scion, regardless of source, was governed by the phenotype of the rootstock. Of the individual graft combinations tested including grafts with characterized resistance sources, uncharacterized resistance sources, and *Rbs2* near-isogenic lines, those with a rootstock derived from a resistant type had foliar and stem symptom means that were not significantly different from the corresponding means of the grafted and ungrafted resistant controls (Table 2). In addition, the group of treatments with a resistant rootstock and susceptible scion was not significantly different from either the grafted or ungrafted resistant controls (Table 3). In contrast, both individual graft combinations and the rootstock-scion group with a rootstock derived from a susceptible type and a scion derived from a resistant type had a susceptible reaction to *P. gregata*, evidenced by foliar and stem symptom means that were not significantly different from susceptible grafted control 'Century 84' (Tables 2 and 3). This indicates that the scion tissue was not providing the necessary response to confer resistance to *P. gregata*. This conclusion was supported by data from an additional experiment in which stem sections of resistant types were grafted between a susceptible rootstock and susceptible scion. Results of that experiment indicated that the section of resistant stem did not impair BSR symptom development in the scion of the grafted plant (data not shown).

Results of BSR evaluation of reciprocally grafted *Rbs2* near-isogenic lines suggest that the presence of *Rbs2* differentially affects the BSR reaction of the rootstock and scion of the plant. Results indicated that the *Rbs2* gene conferred resistance to the rootstock but not the scion of the plant, suggesting root-limited resistance conditioned by *Rbs2*. The 13 resistant accessions from China identified by Bachman et al. (2) also were observed to have resistant

rootstocks and susceptible scions when inoculated with *P. gregata*. Although little is known about the pedigree of these accessions, these results, coupled with similar results for other graft combinations (Table 2), suggest that resistance in the rootstock may be common among BSR-resistant types.

Experiment 2. 'Midwest' was not highly resistant to the isolate of *P. gregata* used in this study. Although it had BSR foliar and stem symptoms significantly lower than the ungrafted susceptible control 'Century 84', 'Midwest' had BSR symptoms significantly higher than the resistant control PI 437970 (Table 4). This may indicate that 'Midwest' lacks a major gene for BSR resistance. Brown stem rot foliar symptom development in 'Midwest' and 'Ontario' appeared to be inhibited by grafting, because grafted and ungrafted controls were significantly different for this character. However, there were no significant differences in BSR stem symptoms between grafted and ungrafted controls of these cultivars, nor were there significant differences in foliar or stem symptoms between grafted and ungrafted controls of 'Century 84' and PI 437970. The significant differences between foliar symptoms of 'Midwest' and 'Ontario' grafted and ungrafted controls may be attributable to the high frequency of disease escapes in the grafted control treatments. Over 14 and 47%, respectively, of 'Ontario' and 'Midwest' grafted control plants escaped disease, while 0 and 10% of the corresponding ungrafted controls escaped disease. We have no explanation for this discrepancy, except that there may be differences between cultivars for BSR reaction after grafting. However, Kunkel and Dunleavy (14,15) did not observe this phenomenon in their study, indicating that it may have been specific to the current experiment. Regardless of the cause, it appears that if grafting does affect symptom development, it has an inhibitory effect. If disease development was indeed inhibited, it was not inhibited to the degree that the majority of experimental treatments could not be declared significantly different from the appropriate controls.

In this study, 'Midwest' appeared to have some level of BSR resistance in the root and little or no resistance in the stem. Graft combinations of 'Midwest' rootstocks and 'Century 84' scions had significantly lower BSR foliar and stem symptoms compared with

TABLE 4. Brown stem rot (BSR; causal agent, *Phialophora gregata*) foliar and stem symptoms for experimental treatments

Treatment ^a	BSR reaction ^b		BSR symptoms	
	Rootstock	Scion	Foliar ^c	Stem ^d
R = 'Midwest', S = 'Ontario'	+	–	0.46	0.51
R = 'Ontario', S = 'Midwest'	–	+	0.56	0.56
R = 'Midwest', S = PI 437970	+	+	0.30	0.36
R = PI 437970, S = 'Midwest'	+	+	0.00	0.00
R = 'Midwest', S = 'Century 84'	+	–	0.39	0.33
R = 'Century 84', S = 'Midwest'	–	+	0.67	0.60
R = 'Ontario', S = PI 437970	–	+	0.71	0.73
R = PI 437970, S = 'Ontario'	+	–	0.02	0.03
R = 'Ontario', S = 'Century 84'	–	–	0.53	0.55
R = 'Century 84', S = 'Ontario'	–	–	0.71	0.64
Grafted controls				
R = 'Midwest', S = 'Midwest'	+	+	0.26	0.30
R = 'Ontario', S = 'Ontario'	–	–	0.39	0.53
R = 'Century 84', S = 'Century 84'	–	–	0.71	0.66
R = PI 437970, S = PI 437970	+	+	0.00	0.00
Ungrafted controls				
'Midwest'	+		0.47	0.49
'Ontario'	–		0.72	0.71
'Century 84'	–		0.76	0.83
PI 437970	+		0.00	0.00
LSD (<i>P</i> = 0.05)			0.19	0.21

^a R = rootstock, S = scion.

^b BSR reaction = brown stem rot reactions of stock and scion sources, obtained from prior published work.

^c Proportion of total nodes that exhibited BSR-induced foliar necrosis.

^d Proportion of total nodes that exhibited internal stem browning.

^e + = resistant, – = susceptible.

TABLE 3. Brown stem rot (BSR; causal agent, *Phialophora gregata*) foliar and stem symptoms for rootstock-scion groups

Rootstock-scion group ^a	BSR symptoms	
	Foliar ^b	Stem ^c
Rootstock = –, Scion = +	0.74	0.76
Rootstock = –, Scion = –	0.66	0.67
'Century 84' (ungrafted)	0.65	0.71
Rootstock = +, Scion = –	0.07	0.08
Rootstock = +, Scion = +	0.02	0.02
PI 437970 (ungrafted)	0.04	0.07
LSD (<i>P</i> = 0.05)	0.15	0.13

^a + = group composed of resistant types, – = group composed of susceptible types.

^b Proportion of total nodes that exhibited BSR-induced foliar necrosis.

^c Proportion of total nodes that exhibited internal stem browning.

grafted control 'Century 84', indicating root-specific resistance (Table 4). However, when scions of 'Midwest' were grafted onto rootstocks of 'Century 84' and 'Ontario', respectively, the foliar and stem symptoms means were not significantly different from the corresponding 'Century 84' and 'Ontario' grafted controls. This indicates that 'Midwest' did not have a resistance response in the aerial portion of the plant. The intermediate BSR reaction of 'Midwest' also made it impossible to declare statistical differences between reciprocal grafts of 'Midwest' and 'Ontario'. In summary, resistance to the PgOh2 isolate of *P. gregata* in 'Midwest' appears to be root specific and of intermediate level.

DISCUSSION

Results of previous studies have conflicted on the morphological location of the resistance response to *P. gregata* in soybean. Experiments conducted by Kunkel and Dunleavy (14,15) indicated that resistance to *P. gregata* in the soybean cultivar Midwest was confined to the aerial portion of the plant. However, Phillips (22) suggested the possibility of a resistance factor in the roots of PI 84946-2, because it had a BSR-susceptible reaction following hypocotyl inoculation with *P. gregata*. In addition, Sills et al. (26) speculated that an observed increase in BSR symptoms on normally resistant lines may have been caused by root trimming prior to root-dip inoculation. If this were indeed the case, root trimming may have removed a resistance factor or disabled a resistance response, thus allowing the fungus to more easily colonize the aerial portions of the plant. The current study indicates that a resistance response to *P. gregata* occurs in the root in at least three unique sources of monogenic BSR resistance. There was no conclusive evidence of stem-specific resistance to *P. gregata* in any of the lines tested.

A possible explanation for the discrepancies between the results of the current study and those of Kunkel and Dunleavy (14,15) arises from differences in experimental procedure. First, Kunkel and Dunleavy (14,15) evaluated grafted seedlings in the field, relying on *P. gregata* inoculum present in field soil. Because differences in pathogenicity have been reported for different isolates of *P. gregata* (9,23,26,35), it is possible that Kunkel and Dunleavy and the current authors observed different responses of 'Midwest' and 'Ontario' to different isolates of *P. gregata*. It is also possible that environmental conditions or other organisms interacted with the fungus and plant to produce variations in disease development. However, it is clear from the results of both studies that 'Midwest' is only moderately resistant to BSR.

This paper has presented evidence for root-limited resistance to BSR in several unique sources that have been used in the development of cultivars and germ plasm lines (20,21,28). It has also provided evidence that the *Rbs2* resistance gene is responsible for the observed BSR resistance in the rootstock of near-isogenic lines that carry it. Because root-limited resistance sources currently in use have shown susceptibility in some situations, whole-plant resistance to *P. gregata* may be a desirable goal of soybean breeders. However, the data presented in this paper indicate that many of the known sources of BSR resistance appear to be of the root-limited type.

ACKNOWLEDGMENTS

Contribution of the Illinois Agriculture Experimental Station. Research supported, in part, by Illinois Soybean Program Operating Board. We thank T. D. Vuong for instruction on soybean grafting procedures.

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