RESEARCH

Evaluation of USDA Soybean Germplasm Accessions for Resistance to Soybean Rust in the Southern United States

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] resistance to soybean rust (SBR) caused by *Phakopsora pachyrhizi* could reduce reliance on fungicides to manage this disease. The objective of this study was to identify soybean germplasm with resistance to field populations of *P. pachyrhizi* in the United States. Field evaluations of 576 accessions from the USDA Soybean Germplasm Collection for resistance to SBR were conducted at seven locations in the southern United States between 2006 and 2008. Accessions from maturity groups (MG) 000 to X and North American susceptible check cultivars from each MG except X were rated for disease severity in all year–location environments, and for disease incidence, fungal sporulation, lesion type, and/or uredinia density in certain environments. While none of the accessions was immune in all environments, 64 were resistant in two or more locations each year that they were tested. Some accessions appeared to be more resistant in certain environments than in others. Of the original four *Rpp* genes described in the literature, *Rpp1* provided the highest level of resistance, and among the accessions with uncharacterized *Rpp* genes, PI 567104B had the highest overall resistance across environments. The plant introductions confirmed to be resistant in these evaluations should be useful sources of genes for resistance to North American populations of *P. pachyrhizi*.

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Abbreviations: MG, maturity group; PI, plant introduction; SBR, soybean rust.

Soybean rust caused by *Phakopsora pachyrhizi* Syd. & P. Syd. is an economically important fungal disease of soybean. *Phakopsora pachyrhizi* is able to infect more than 150 legume species, including kudzu [*Pueraria lobata* (Willd.) Ohwi], which can serve as an overwintering host and inoculum reservoir in the southeastern United States (Slaminko et al., 2008). Soybean rust can cause soybean seed yield losses as high as 80% (Bromfield, 1984; Yorinori et al., 2005),

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primarily as a result of premature defoliation, reductions in photosynthetic area and dry matter accumulation, and a lower harvest index (Hartman et al., 1991; Kumudini et al., 2008). *Phakopsora pachyrhizi* is an obligate biotroph, and is thus unable to survive winter temperatures in the major soybean production areas of North America. After its hosts emerge or regrow, however, the pathogen can move long distances rapidly via windborne urediniospores. Due to this capability and the ability of the pathogen to evolve quickly, race shifts in regional *P. pachyrhizi* populations may occur rapidly (Hartman et al., 2005; Ribeiro et al., 2007). For example, the *Rpp1* and *Rpp3* genes provided resistance to SBR when it first appeared in South America in 2001, but within 2 yr both had been defeated by a Brazilian isolate (Silva et al., 2008).

Soybean rust was first reported in the continental United States in November 2004 (Schneider et al., 2005), after having been confirmed in South America in 2001 (Yorinori et al., 2005). Soybean rust can be managed effectively through applications of strobilurin and triazole fungicides (Mueller et al., 2009), but this increases production costs, and proper timing relative to the onset of infection is critical to obtaining optimal control. Furthermore, by the 2008–2009 growing season some South American *P. pachyrhizi* populations were already showing increasing tolerance to certain fungicides (Godoy, 2009). Agronomically competitive soybean cultivars with resistance to SBR would therefore be useful components of a disease management program.

Genes conditioning soybean resistance to *P. pachyrhizi* (*Rpp* genes) have been identified at five independent loci. Plant introduction (PI) 200492 with *Rpp1* (McLean and Byth, 1980); PI 230970 with *Rpp2* (Bromfield and Hartwig, 1980); PI 462312 with *Rpp3* (Bromfield and Melching, 1982; Hartwig and Bromfield, 1983); and PI 459025B with *Rpp4* (Hartwig, 1986) were reported in the 1980s. *Rpp1* provides immunity to certain isolates, whereas the accessions carrying the other three *Rpp* genes develop an "RB" infection type characterized by reddish-brown lesions with few uredinia and meager sporulation when challenged with incompatible fungal isolates (Bromfield and Hartwig, 1980). In contrast to RB-type reactions, susceptible soybean genotypes typically develop a "TAN" infection type characterized by tan-colored lesions with multiple uredinia and abundant sporulation (Bromfield and Hartwig, 1980). *Rpp1* and *Rpp4* have been mapped to two different loci on chromosome 18 (LG G; Hyten et al., 2007; Silva et al., 2008), *Rpp2* to chromosome 16 (LG J; Silva et al., 2008), and *Rpp3* to chromosome 6 (LG C2; Hyten et al., 2009). Monteros et al. (2007) mapped the *Rpp?*(Hyuuga) resistance allele from the Japanese cultivar Hyuuga (PI 506764) to the vicinity of the *Rpp3* locus. Chakraborty et al. (2009) mapped a gene from PI 594538A (*Rpp1-b)* that was distinct from *Rpp1* to the *Rpp1* locus, and Ray et al. (2009) also mapped resistance genes from PI 587880A and PI 587886 that have different specificities from *Rpp1* to the same region of the genome. Garcia et al. (2008) recently discovered a novel independent locus (*Rpp5*) on chromosome 3 (LG N). In their mapping populations, resistance at this locus was dominant in PI 200487 and PI 200526, incompletely dominant in PI 471904, and recessive in PI 200456. Garcia et al. (2008) also reported a recessive resistance allele at or near the *Rpp2* locus in PI 224270. Since none of the known *Rpp* genes provides resistance against all isolates of *P. pachyrhizi* (Hartman et al., 2005), mapping of *Rpp* genes offers breeders the opportunity to pyramid two or more *Rpp* genes to obtain broader and/or more durable SBR resistance (Pedersen and Leath, 1988). Partial or rate-reducing resistance that delays and/or reduces SBR growth and sporulation has also been reported in soybean (Wang and Hartman, 1992).

To identify additional sources of SBR resistance genes, Miles et al. (2006) screened seedlings of 16,595 *G. max* accessions from the U.S. Department of Agriculture Soybean Germplasm Collection for SBR resistance in a Biosafety Level 3 containment greenhouse at the USDA-ARS Foreign Disease-Weed Science Research Unit at Ft. Detrick, Maryland (subsequently referred to here as "Ft. Detrick"). In two rounds of screening for resistance to a mixture of four *P. pachyrhizi* isolates originating from Brazil, Paraguay, Thailand, and Zimbabwe, 805 accessions with a mean severity of 2.7 or less, and/or with RB lesions on at least two of the three plants assayed were selected. Miles et al. (2008) subsequently evaluated 530 of the selected PIs from MGs III to IX for adult plant resistance in the field in Itapúa, Paraguay. During the 2005–2006 growing season, approximately 25% of the PIs tested there appeared to be resistant, and the resistance of 10 of these was confirmed in greenhouse assays.

Following the discovery of SBR in the continental United States in late 2004, researchers at the University of Georgia screened 778 of the 805 Ft. Detrick PI selections from MGs 000 to X in field tests at the Attapulgus Research and Education Center in southwestern Georgia (D.R. Walker et al., unpublished data, 2006). To compress maturity dates, promote canopy growth, and delay flowering until cooler weather favored a disease epidemic (Christiano and Scherm, 2007), the nursery was not planted until 3 Sept. 2005, and the natural photoperiod was extended for 1 mo using portable lighting units. Almost half of the Ft. Detrick selections tested in the field were susceptible to SBR in the field at Attapulgus, GA.

The objective of the present research was to evaluate soybean germplasm accessions selected from the Ft. Detrick greenhouse assays and the 2005 University of Georgia field screening for resistance to SBR over multiple years and locations in the southern United States.

MATERIALS AND METHODS

Plant Material

Subsets of the 805 accessions that Miles et al. (2006) considered putatively resistant to four foreign isolates were tested for

Table 1. Experimental details for 2006–2008 evaluations of USDA soybean accessions for resistance to soybean rust.

Year	Location	MG range†	Accessions‡	Replications	Row length/spacing	Planting date	Suppl. lighting§
					m		
2006	Bossier City, LA	III–VIII	137	2	3.0/1.0	13 June	No
	Alexandria, LA	000–X	295	1	4.6/1.0	14 July	No
	Baton Rouge, LA	000–X	295	1	2.7/0.8	~25 July	No
	Fairhope, AL	VI–VIII	105	4	2.4/0.9	21 May	No
	Attapulgus, GA	000–X	347	3	1.8/0.9	15 Aug.	Yes
	Quincy, FL	III-VIII	229	2	1.2/0.9	21 June	Yes
	Blackville, SC	V–X	198	2	3.0/1.0	21 June	No
2007	Bossier City, LA	III–X	305	2	3.0/1.0	9 Aug.	No
	Alexandria, LA	000–X	422	2	4.6/1.0	6 Aug.	No
	Baton Rouge, LA	000–X	422	2	2.7/0.8	10 Oct.	No
	Fairhope, AL	IV–X	265	3	2.4/0.9	11 July	No
	Attapulgus, GA	00–X	422	2	1.8/0.9	22 Aug.	Yes
	Quincy, FL	000–X	463	2	1.2/0.9	16 Aug.	Yes
	Blackville, SC	000–X	422	2	3.0/1.0	4 July	Yes
2008	Bossier City, LA	V–X	79	2	3.0/1.0	25 Aug.	No
	Alexandria, LA	I–X	91	2	4.6/1.0	~6 Aug.	Yes
	Baton Rouge, LA	I–X	96	2	2.7/0.8	23 Sept.	Yes
	Fairhope, AL	V–X	84	2	2.4/0.9	8 Aug.	No
	Attapulgus, GA	I–X	96	2	1.8/0.9	20 Aug.	Yes
	Quincy, FL	I–X	96	3	1.2/0.9	18 Aug.	Yes
	Blackville, SC	0–X	91	2	3.0/1.0	24 July	Yes

†Maturity group (MG) range represented by the accessions tested.

‡Number of germplasm accessions planted.

§Use of supplementary lighting to extend natural photoperiod and delay flowering.

resistance to field populations of *P. pachyrhizi* in the southern United States between 2006 and 2008 (Table 1). In 2006 and 2007, accessions from MGs 000 through X were tested each year, and disease ratings from each of these years were used to select which PIs to re-evaluate the following year. In 2008 no accessions from MGs 000 or 00 were planted because none of the ones that had been previously tested were resistant, and only one MG X accession was retested. North American cultivars representing each MG except X were planted in the SBR nurseries as susceptible checks, and germplasm accessions with known *Rpp* genes were included whenever seed were available. Due to limited seed, PI 200492 (*Rpp1*; MG VII) was only planted in 2008, but L85–2378 (PI 547875), a Williams 82 isoline with *Rpp1*, was planted all 3 yr (Bernard, 1995). PI 230970 (*Rpp2*; MG VII) was tested in 2007 and 2008, and PI 462312 (*Rpp3*; MG VIII) and PI 459025B (*Rpp4*; MG VIII) were planted every year. The MG VII Japanese cultivar Hyuuga (PI 506764), which has the *Rpp?*(Hyuuga) resistance gene at or near the *Rpp3* locus (Monteros et al., 2007), was planted in Attapulgus in 2006, and at all locations in 2007 and 2008.

Locations

Germplasm accessions were evaluated in SBR screening nurseries at seven locations in five states (Table 1). From west to east, these locations were: (i) The Louisiana State University Agricultural Center (LSU AgCenter) Red River Research station at Bossier City (near Shreveport) in northwestern Louisiana (32°25′ N, 93°38′ W); (ii) The LSU AgCenter Dean Lee Research Station at Alexandria, in central Louisiana (31°10′ N, 92°24′ W); (iii) the LSU AgCenter Central Research Station in Baton Rouge, east-central Louisiana (30°22′ N, 91°10′ W); (iv) Auburn University's Gulf Coast Substation in Fairhope, in southern Alabama (30°32′ N, 87°52′ W); (v) the University of Florida's North Florida Research and Education Center in Quincy, Florida (30°32′ N, 84°35′ W); (vi) the University of Georgia's Attapulgus Research and Education Center in southwestern Georgia (30°45′ N, 84°29′ W); and (vii) Clemson University's Edisto Research and Education Center in Blackville, SC (33°21′ N, 81°19′ W).

Experimental Designs and Plot Details

Experimental and plot details for each year–location environment are shown in Table 1. Accessions tested at most locations were divided into two or more separate tests to group PIs from the same or similar MGs, and each test had one to three replications, depending on the location and year. Entries from each test were planted as single rows in a randomized complete block design, and plots were laid out in such a way as to minimize the effects of possible disease pressure gradients resulting from differences in proximity to neighboring fields infected with SBR. Row lengths and distances between rows varied among locations in accordance with the standards used at each research station (Table 1). In locations where supplementary lighting was used, lights were typically set up within a week after planting, and were used to extend the natural photoperiod for 1 mo. The total photoperiod length during the period of extended lighting was at least 16 h, except in Quincy in 2008, when lights were used between 2200 and 2300 h to interrupt the dark period. The use of supplementary lighting was determined simply by whether someone at a specific location was willing and able to turn the lights on and off every night.

In Attapulgus (2006–2008) and Quincy (2007 only) plots were artificially inoculated with *P. pachyrhizi* urediniospore suspensions containing the local isolate, but most evaluations relied exclusively on passive natural infection (Table 2). The germplasm screening nurseries were often located close to soybean fields that were already infected with SBR, and these were most likely the primary source of inoculum at most locations. Artificial

Table 2. Soybean rust inoculation and rating methods and scales used in the 2006–2008 USDA soybean germplasm evaluations.

Year	Location	Inoculation†	Rating method‡	Scale range	Rating date(s)	Comments
2006	Bossier City, LA	Natural only	-	-	-	Frost damaged plants before they could be evaluated
	Alexandria, LA	Natural only	Bayer Scale	1–9	11 Oct.	
	Baton Rouge, LA	Natural only	Bayer Scale	1–9	9 Nov.	
	Fairhope, AL	Natural only	Miles Scale	1–9	15 Sept. and 4 Oct.	Data not reported
	Attapulgus, GA	Natural + manual	Attapulgus	1–5	16 Nov.	Severity
	Quincy, FL	Natural only	Quincy 2006	0–5	Oct.	Severity
	Blackville, SC	Natural only	-	-	-	Frost damaged plants before they could be evaluated
2007	Bossier City, LA	Natural only	Bayer Scale	1–9	19 Nov.	Severity and lesion type
	Alexandria, LA	Natural only	Bayer Scale	1–9	18 and 29 Oct.	Data not reported
	Baton Rouge, LA	Natural only	-	-	-	Frost damaged plants before they could be evaluated
	Fairhope, AL	Natural only	Miles Scale	1–9	28 Oct.	Severity, lesion type, and uredinia density
	Attapulgus, GA	Natural + manual	-	-	-	Frost damaged plants before they could be evaluated
	Quincy, FL	Natural + manual	Lesion density	Lesions cm^{-2}	3 Oct.	Lesion density determined on collected leaf samples
	Blackville, SC	Natural only	Blackville 2007	-	-	Data not reported due to low disease pressure
2008	Bossier City, LA	Natural only	-	-	-	Frost damaged plants before they could be evaluated
	Alexandria, LA	Natural only	-	-	-	Frost damaged plants before they could be evaluated
	Baton Rouge, LA	Natural only	Bayer Scale	1–9	18 Nov.	
	Fairhope, AL	Natural only	Bayer Scale	0–8	28 Oct.	Severity rated on two samples of five leaves each
			Quincy 2008	1–5	13 Oct. and 15 Nov.	Severity rated on whole plants in field
	Attapulgus, GA	Natural + manual	Attapulgus	1–5	18 Nov.	
	Quincy, FL	Natural only	Quincy 2008	1–5	6 Nov.	
	Blackville, SC	Natural only	Blackville 2008	0–10	25 or 31 Oct.	

†All tests were subject to passive inoculation from infected plants in adjacent fields, but artificial inoculation with a urediniospore suspension was also used in Attapulgus (2006–2008) and in Quincy in 2007.

‡Criteria and other details of rating methods used are described in detail in the text. In all scales, lower rating scores indicated less disease, the lowest rating indicated no lesions, the highest rating indicated profuse lesion development (i.e., severity typically >65%, and intermediate ratings indicated disease severity levels between those of the most resistant accessions and the susceptible check cultivars.

inoculation was used at Attapulgus because it had become a standard practice there following the 2005 germplasm evaluations at that station, and it was used in Quincy in 2007 because the natural SBR epidemic on the research station had been delayed by drought. Inoculum suspensions were prepared by rinsing locally collected infected leaves or whole plants in basins of water, and then straining the suspension through cheesecloth to remove debris. The concentrations of *P. pachyrhizi* urediniospores in these suspensions were not determined, but the inoculum was applied uniformly to all plots. In Attapulgus, plots were inoculated several times at weekly intervals, whereas the 2007 plots in Quincy were inoculated only once. Also, in Attapulgus Bac-Master agricultural streptomycin (AMVAC Chemical Corp., Los Angeles, CA) was added to the urediniospore suspensions and applied at a rate of 236.6 mL 378.6 L^{-1} to reduce the incidence of bacterial foliar diseases in the tests. Susceptible border rows were planted around the perimeters of all the nurseries, and at Quincy in 2006 spreader rows were planted throughout the nursery at least 2 wk in advance of the plot planting dates. Overhead irrigation of both the test plots and nearby SBR-infected fields was sometimes used to promote the spread of disease during dry periods.

Evaluations of Soybean Rust Resistance

Information on rating methods and scales used in different year–location environments is summarized in Table 2. Depending on the year and location, data were collected for disease severity (percentage of leaf area affected with lesions or density of lesions per unit area), disease incidence (percent of plants in a plot with SBR symptoms), lesion type (RB, tan, or mixed), sporulation relative to susceptible controls, number of uredinia per cm^2, percentage of sampled leaves with a certain disease severity level, and stage of maturation of the plants at the time of evaluation. Plants were typically in the R3 to R6 developmental stages at the time of rating (Fehr et al., 1971), and ratings were generally made on leaves from the middle level of the canopy. Evaluations were made during these reproductive stages of development because spore production and pustule development generally increase after plants begin to flower (Bromfield, 1984), and because variation in disease severity was typically high at these stages, while the most susceptible genotypes were not yet heavily defoliated. From 5 to 10 leaves per plot were examined. Less mature plants with apparent resistance were re-examined 1 to 2 wk after the initial rating date whenever possible to confirm resistance. Disease severity was

assessed in all year–location environments where disease pressure was sufficiently high to result in moderate to high disease severity on susceptible cultivars, and severity was used as the primary criterion for assessing resistance to SBR.

Due to the number and personal preferences of researchers involved in rating plots in different locations and years, a variety of rating criteria and scales were used in different years and locations, but for all scales, a lower rating indicated less disease. Two general types of severity rating scales were used: a longer scale with either 9 or 11 points, and a shorter scale with either five or six points. One of the nine-point scales was based on a series of photographs in a booklet entitled the "Asian Soybean Rust Disease Severity Evaluation Scale" that was published in 2006 by Bayer CropScience (Research Triangle Park, NC). Median severities for the ratings in the "Bayer Scale" were as follows: 1 = 0%, 2 = 1.2%, 3 = 3.8%, 4 = 7.5%, 5 = 12.5%, 6 = 20%, 7 = 30%, 8 = 51%, and 9 = ≥ 68% severity. Ratings of 0 to 8 instead of 1 to 9 were used in association with the Bayer Scale to rate leaves from Fairhope, AL in 2008. The other nine-point scale, which was used in Fairhope in 2006 and 2007, corresponded to a series of diagrams adapted from Miles et al. (2005), and was more conservative than the Bayer Scale in that plots with moderate amounts of disease were given a higher numerical rating. In the "Miles Scale", the average severity for each rating score was as follows: 1 = 0%, 2 = 0.25%, 3 = 0.5%, 4 = 1.0%, 5 = 2.5%, 6 = 5%, 7 = 10%, 8 = 20%, and 9 = > 20% of the leaf surface covered with SBR lesions. In Blackville in 2008, an 11-point scale was used in which 0 = no SBR lesions, 1 = 1 to 10 pustules per leaf, 2 = 11 to 25 pustules, 3 = 26 to 50 pustules, 4 = 51 to 100 pustules, 5 = 1% of leaf area affected by SBR, 6 = 3% of leaf area affected, 7 = 10% of leaf area affected, 8 = 25% of leaf area affected, 9 = 50% of leaf area affected, and 10 = 75 to 100% of leaf area affected by SBR. In contrast to the longer scales, the shorter severity scales were not associated with sets of photos or diagrams, but were "calibrated" to the level of disease in specific assays. In these scales a rating of either "0" (in the six-point scale used at Quincy in 2006) or "1" (in the five-point scales) indicated an absence of SBR lesions, a "5" indicated a level of disease similar to that on the most susceptible check cultivars, and a "3" indicated approximately half as much disease as on the susceptible checks from the same MG.

In some evaluations, lesion color was noted as being either RB, tan, a mixture of these two types, or indistinct. Entries with tan-colored lesions are referred to here as "tan" rather than "TAN", because some accessions developed tan-colored lesions that lacked the "many uredinia and abundant sporulation" criteria for Bromfield and Hartwig's (1980) TAN infection type. The terms "resistant" and "moderately resistant" as used here refer to relative levels of disease severity and/or sporulation in comparison with susceptible checks and the most resistant entries from the same experiment and MG. "Moderate resistance" implies that disease symptoms were intermediate between the susceptible and resistant extremes, whereas "resistant" accessions were characterized by (i) low severity ratings relative to check cultivars from the same MG and/or (ii) little or no sporulation in two or more year–location environments.

Additional details about rating methods and scales used in various locations and years that are not adequately explained in Table 2 are described here: **Bossier City**–*2007*: Independent ratings were made for the upper and lower sectors of the canopy when the majority of the plants were at the R6 growth stage (Fehr et al., 1971). Since ratings were very similar for the two canopy levels, they were averaged for each replication and the overall means are reported in Table 3. **Alexandria**–*2006*: SBR severity was rated in both the upper canopy and the lower canopy. **Fairhope**–*2007*: Two samples of five leaves each were evaluated for disease incidence (percent of plants infected), disease severity, lesion type, and the number of uredinia in an area of approximately 1 cm^2. Average severity was rated using the Miles Scale, and was based on evaluation of up to four replications. *2008*: Independent ratings were made on incubated leaf samples and on leaflets examined in the field (Table 2). On 28 October five leaves were collected from each plot in the first replication and incubated for 7 d in a moist chamber. The leaves were then inspected with a microscope and rated 0 to 8 using the Bayer Scale. Middle canopy leaflets from the first replication were evaluated for disease severity on 13 October and on 15 November, and sporulation was also rated using a 1 to 5 scale on the second date. **Quincy**–*2006*: The 244 accessions were planted on four different dates and were rated when most lines were at the R6 growth stage using a 0 to 5 scale in which 0 = no SBR, 1 = light severity, 2 = light to moderate, 3 = moderate, 4 = heavy, and 5 = very heavy SBR severity. *2007*: Due to severe and rapid wilting of numerous accessions that were susceptible to a suite of soil-borne pathogens (*Phytophthora* spp., *Fusarium* spp., *Pythium* spp., and/or *Rhizoctonia* spp.), it was not possible to rate plants in the field. Five leaflets collected from each plot between 2 and 4 October were incubated overnight in sealed plastic bags. A dissecting microscope at 40× magnification was then used to count the number of SBR lesions in a 1.0 cm^2 area on the abaxial (lower) side of each leaf to determine lesion density. If there were more than 50 lesions per cm^2, the lesion density was recorded as " >50", and means calculated from these are preceded by a " > " sign to indicate that the actual mean would have been higher than the number reported. Lesions were deliberately counted near the tips of leaflets because lesion densities were typically highest there, and mean lesion density for each plot was calculated from the four leaflets with the highest lesion densities. The five-point sporulation rating scale used was: 1 = no leaflets with sporulating lesions, 2 = one leaflet with sporulating lesions, 3 = two leaflets with sporulating lesions; 4 = three or four leaflets with sporulating lesions, and 5 = sporulating lesions on all five leaflets. *2008*: Disease severity on both replications and sporulation on leaflets from the second replication were rated on 6 November using scales of 1 to 5. Soybean rust severity and sporulation in the first replication were rated a second time on 13 November. **Attapulgus**–*2006*: Plots were rated 16 November using a 1 to 5 rating scale in which 1 = no lesions, 2 = one to three leaves per plant with a few lesions, 3 = four or more leaves per plant with a few lesions, 4 = one or two leaves per plant with many lesions, and 5 = four or more leaves per plant with many lesions. *2008*: Plants were rated 18 November using a 1 to 5 scale that reflected disease severity and progression up through the canopy. Entries with no macroscopically visible lesions were rated "1", and plots with SBR lesions in the upper canopy were rated "5". **Blackville**–*2007*: For the "Blackville 2007" evaluations, 10 leaves were sampled from each row on either 25 October (MGs 0 through VII) or 31 October (MGs VIII through X). After being incubated at room temperature for 48 to 72 h, leaves

Table 3. Soybean rust severity ratings for resistant accessions, accessions with described *Rpp* genes, and susceptible cultivars in the southern United States (2006–2008). Disease severity or lesion density was rated using various scales in different locations, as indicated, but in all scales a low score represented resistance. Accessions with PI numbers in bold font are also listed in Table 2.

			Quincy (FL)			Attapulgus (GA)		Fairhope (AL)			Blackville (SC)	Alexandria (LA)	Bossier City (LA)	Bat. Rg. (LA)
			Oct. 2006	3 Oct. 2007	6 Nov 2008	16 Nov. 2006	18 Nov. 2008	28 Oct. 2007	28 Oct. 2008	15 Nov 2008	25 or 31 Oct. 2008	11 Oct. 2006	19 Nov. 2007	18 Nov. 2008
Accession	Name [resistance gene]	MG	Severity[†] (0–5)	Les. dens.[‡] (les. cm$^{2\ -1}$)	Severity (1–5)	Severity[§] (1–5)	Severity[§] (1–5)	Severity (1–9)	Lf. Sev.[¶] (0–8)	Severity[#] (1–5)	Severity (0–10)	Severity (1–9)	Severity (1–9)	Severity (1–9)
PI 547875	L85–2378 [Rpp1]	III	3.6 ± 0.4	0.1	2.0 ± 0.6	2.3 ± 0.3	1.0 ± 0.0	-	-	-	0.00	6.0	1.0 ± 0.0	5.5 ± 1.5
PI 567189A	Ekhabac	IV	4.0 ± 0.0	2.1	2.8 ± 0.6	2.3 ± 0.3	1.0 ± 0.0	-	-	-	0.10	3.5	3.0 ± 0.0	5.0 ± 1.0
PI 606440A	VX 92	IV	2.8 ± 0.3	6.2	3.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	-	-	-	0.20	6.0	4.0 ± 0.0	2.0 ± 0.0
PI 398399		V	4.8 ± 0.3	>30.0	4.7 ± 0.3	3.7 ± 0.7	2.0 ± 1.0	2.5	0.0 ± 0.0	2.0	1.35	-	4.0 ± 0.0	2.0 ± 1.0
PI 476905A	Nguu mao hong	V	2.8 ± 0.5	4.6	3.3 ± 0.9	2.7 ± 0.7	2.0 ± 1.0	-	0.0 ± 0.0	2.0	0.00	3.0	2.5 ± 0.5	7.0 ± 0.0
PI 567059		V	5.0 ± 0.0	1.2	4.3 ± 0.7	3.0 ± 0.6	1.0 ± 0.0	1.5	1.2 ± 0.8	3.0	0.05	3.0	-	2.5 ± 0.5
PI 605773		V	2.8 ± 0.3	0.8	3.7 ± 0.7	2.7 ± 0.3	1.0 ± 0.0	1.0	0.0 ± 0.0	3.0	0.10	3.0	4.0 ± 0.0	2.0 ± 1.0
PI 605829		V	-	1.4	2.3 ± 0.3	-	2.0 ± 0.0	1.0	0.4 ± 0.2	3.0	0.00	-	4.0 ± 0.0	3.5 ± 0.5
PI 605838	Xanh si man	V	3.5 ± 0.3	3.8	2.7 ± 0.3	2.7 ± 0.3	1.0 ± 0.0	-	0.0 ± 0.0	2.0	0.00	1.5	[4.0]	3.5 ± 0.5
PI 605854B		V	3.8 ± 0.5	1.5	3.3 ± 0.3	2.7 ± 0.3	1.0 ± 0.0	2.0	0.0 ± 0.0	2.0	0.15	1.5	-	1.5 ± 1.5
PI 605865B		V	3.5 ± 0.3	-	3.0 ± 1.0	2.7 ± 0.7	1.0 ± 0.0	2.0	0.6 ± 0.4	2.0	0.25	2.5	3.0 ± 0.0	7.0 ± 0.0
PI 605885B		V	-	3.0	3.3 ± 0.3	-	1.0 ± 0.0	1.0	0.0 ± 0.0	2.0	0.10	-	5.5 ± 0.5	6.0 ± 1.0
PI 605891A		V	3.0 ± 0.0	0.5	4.0 ± 0.3	2.3 ± 0.3	-	2.0	0.4 ± 0.2	2.0	-	4.0	7.5 ± 0.5	2.0 ± 0.0
PI 606397B	(Hat nho duc trong)	V	3.5 ± 0.5	2.8	3.0 ± 0.6	2.7 ± 0.3	1.0 ± 0.0	2.5	0.4 ± 0.2	3.0	0.05	1.0	6.0 ± 0.0	4.0 ± 0.0
PI 606405	Madrak	V	4.0 ± 0.4	3.2	3.0 ± 0.0	2.3 ± 0.7	1.0 ± 0.0	-	1.6 ± 0.2	3.0	1.80	2.5	5.5 ± 0.5	5.5 ± 1.5
PI 615445	Hi long 3	V	3.3 ± 0.3	4.1	4.0 ± 0.6	2.0 ± 0.0	-	1.0	0.6 ± 0.2	3.0	-	4.5	7.0 ± 0.0	7.0 ± 0.0
PI 417503	Pioneira	VI	3.5 ± 0.5	0.0	3.3 ± 0.7	2.7 ± 0.3	1.0 ± 0.0	1.0	1.4 ± 0.2	3.0	0.00	1.5	8.5 ± 0.5	7.0 ± 0.0
PI 506695	Gogaku	VI	4.3 ± 0.3	10.4	3.7 ± 0.3	3.0 ± 0.6	1.5 ± 0.5	1.5	2.0 ± 0.0	4.0	0.00	3.5	4.0 ± 0.0	2.5 ± 1.5
PI 507009	Kyuushuu 43	VI	3.0 ± 0.0	8.8	4.0 ± 0.6	4.0 ± 0.6	1.0 ± 0.0	2.0	1.0 ± 0.3	3.0	0.00	5.0	8.0 ± 0.0	4.0 ± 0.0
PI 567190	Halang 4 thang	VI	3.0 ± 0.0	4.1	3.7 ± 0.3	3.0 ± 0.6	1.0 ± 0.0	-	0.8 ± 0.2	3.0	0.00	4.5	5.0 ± 0.0	3.5 ± 0.5
PI 605791A		VI	3.5 ± 0.3	-	[3.5] ± 0.5	3.0 ± 0.0	1.0 ± 0.0	1.0	1.2 ± 0.2	3.0	0.10	7.0	7.5 ± 0.5	3.5 ± 0.5
PI 605891B		VI	2.8 ± 0.3	-	3.0 ± 0.3	4.3 ± 0.7	1.0 ± 0.0	-	0.2 ± 0.2	3.0	0.00	3.5	8.5 ± 0.5	3.5 ± 0.5
PI 615437	A 9	VI	3.0 ± 0.7	18.5	3.3 ± 0.7	3.7 ± 0.7	1.0 ± 0.0	1.0	0.6 ± 0.2	3.0	0.00	3.0	6.0 ± 0.0	5.5 ± 1.5
PI 635999	DT2000	VI	-	11.0	4.0 ± 0.6	-	1.0 ± 0.0	1.0	0.0 ± 0.0	2.0	0.00	-	5.5 ± 0.5	7.0 ± 0.0
PI 200492	Komata [Rpp1]	VII	2.3 ± 0.3	-	1.7 ± 0.7	2.0 ± 0.3	-	-	0.0 ± 0.0	1.0	0.00	1.5	-	3.0 ± 1.0
PI 230970	[Rpp2]	VII	3.7 ± 0.3	>13.4	3.7 ± 0.7	2.0 ± 0.0	5.0 ± 0.0	2.5	1.8 ± 1.1	4.0	0.40	3.0	8.0 ± 0.0	1.0 ± 0.0
PI 417116	Kyushu 19	VII	-	>13.4	3.3 ± 0.7	-	1.0 ± 0.0	1.5	0.6 ± 0.2	4.0	0.00	-	9.0 ± 0.0	2.5 ± 1.5
PI 417128	Kyushu 37	VII	3.5 ± 0.3	[>19.0]	3.8 ± 0.7	2.3 ± 0.3	1.5 ± 0.5	1.0	0.6 ± 0.2	2.0	0.05	2.5	-	7.0 ± 0.0
PI 417132	Kyushu 56	VII	4.3 ± 0.3	14.2	3.7 ± 0.3	2.3 ± 0.3	-	2.0	7.2 ± 0.4	4.0	-	1.0	[3.0]	2.5 ± 0.5
PI 506764	Hyuuga [Rpp?(Hyuuga)]	VII	-	>26.0	3.7 ± 0.3	3.3 ± 0.9	1.0 ± 0.0	2.0	2.2 ± 0.4	3.0	0.00	-	[4.0]	4.0 ± 0.0
PI 594172A	Gogaku	VII	3.3 ± 0.3	>31.1	3.0 ± 0.0	2.7 ± 0.3	1.5 ± 0.5	1.0	0.6 ± 0.2	3.0	0.05	3.0	4.0 ± 0.0	4.0 ± 0.0
PI 628932	FT-2	VII	2.3 ± 0.3	16.8	4.3 ± 0.3	2.3 ± 0.3	1.0 ± 0.0	7.5	1.8 ± 0.6	3.0	0.00	8.0	7.0 ± 0.0	5.5 ± 1.5
PI 197182	Raub 16.1422	VIII	-	>19.0	3.0 ± 0.6	-	1.0 ± 0.0	2.0	1.2 ± 0.5	4.0	0.05	-	9.0 ± 0.0	1.5 ± 0.5
PI 200487	Kinoshita [Rpp5]	VIII	-	10.4	4.3 ± 0.7	-	3.0 ± 0.0	2.0	1.0 ± 0.3	3.0	0.05	-	9.0 ± 0.0	1.0 ± 0.0
PI 200488	Kiro Aki Daizu	VIII	-	7.4	3.3 ± 0.3	-	1.0 ± 0.0	1.0	2.2 ± 0.4	3.0	0.10	-	[6.0]	2.5 ± 0.5
PI 203398	Abura	VIII	3.5 ± 0.5	13.0	4.3 ± 0.3	2.0 ± 0.0	2.0 ± 1.0	1.0	0.6 ± 0.2	3.0	0.15	1.0	[6.0]	1.5 ± 0.5
PI 416778	Aki sengoku (Kyushu 11)	VIII	4.3 ± 0.3	>30.0	4.7 ± 0.3	2.0 ± 0.6	3.5 ± 0.5	2.0	3.2 ± 0.4	2.0	-	5.0	[3.0]	3.0 ± 1.0
PI 416826A	Cha sengoku 81	VIII	3.0 ± 0.0	1.0	[1.0] ± 0.0	2.3 ± 0.3	1.0 ± 0.0	1.0	-	-	0.05	3.5	5.0 ± 0.0	5.5 ± 1.5

(cont'd)

Table 3. Continued.

			Quincy (FL)			Attapulgus (GA)		Fairhope (AL)			Blackville (SC)	Alexandria (LA)	Bossier City (LA)	Bat. Rg. (LA)
			Oct. 2006	3 Oct. 2007	6 Nov 2008	16 Nov. 2006	18 Nov. 2008	28 Oct. 2007	28 Oct. 2008	15 Nov 2008	25 or 31 Oct. 2008	11 Oct. 2006	19 Nov. 2007	18 Nov. 2008
Accession	Name [resistance gene]	MG	Severity[†] (0–5)	Les. dens.[‡] (les. cm$^{2\ -1}$)	Severity (1–5)	Severity[§] (1–5)	Severity[§] (1–5)	Severity (1–9)	Lf. Sev.[¶] (0–8)	Severity[#] (1–5)	Severity (0–10)	Severity (1–9)	Severity (1–9)	Severity (1–9)
PI 416873B	(Fusanari daizu)	VIII	3.5 ± 0.5	4.4	4.3 ± 0.3	2.0 ± 0.0	1.0 ± 0.0	-	1.0 ± 0.0	4	0.15	-	4.0 ± 0.0	5.5 ± 1.5
PI 416886	Ginsui Zairai	VIII	-	>19.8	4.7 ± 0.3	-	1.0 ± 0.0	2.0	1.8 ± 0.6	4	0.65	-	7.0 ± 0.0	3.0 ± 1.0
PI 417120	Kyushu 25	VIII	-	5.0	[1.0] ± 0.0	-	1.5 ± 0.5	1.0	0.0 ± 0.0	3.0	0.00	-	5.0 ± 0.0	7.0 ± 0.0
PI 417125	Kyushu 31	VIII	5.0 ± 0.0	>29.5	4.3 ± 0.3	3.0 ± 0.0	-	2.5	0.6 ± 0.2	2 or 3	-	5.0	4.0 ± 0.0	5.5 ± 1.5
PI 417126	Kyushu 32	VIII	4.7 ± 0.3	>35.6	4.7 ± 0.3	2.3 ± 0.3	1.0 ± 0.0	4.5	0.0 ± 0.0	2.0	0.05	2.5	3.0 ± 0.0	3.0 ± 1.0
PI 417134	Magarikawa Zairai	VIII	4.3 ± 0.3	>28.4	4.3 ± 0.3	2.3 ± 0.3	1.0 ± 0.0	-	1.4 ± 0.2	2.0	0.15	7.0	7.5 ± 0.5	1.0 ± 0.0
PI 417208	Oka Kaizu	VIII	3.3 ± 0.3	>18.9	4.0 ± 0.6	2.3 ± 0.3	1.5 ± 0.5	1.0	1.6 ± 0.7	2.0	0.70	3.0	3.0 ± 0.0	6.0 ± 1.0
PI 459025B	(Bing nan) [Rpp4]	VIII	-	-	5.0 ± 0.0	-	-	-	4.2 ± 0.8	4.0	-	-	-	5.5 ± 1.5
PI 462312	Ankur [Rpp3]	VIII	-	14.1	4.0 ± 0.6	-	1.0 ± 0.0	1.0	0.4 ± 0.2	3.0	0.00	-	-	1.0 ± 0.0
PI 506491	Akanida	VIII	-	>23.0	4.3 ± 0.7	-	1.0 ± 0.0	1.5	0.0 ± 0.0	4.0	0.25	-	4.0 ± 0.0	4.0 ± 0.0
PI 506947	Kumaji 2	VIII	2.7 ± 0.3	16.4	3.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0	1.0	1.8 ± 0.2	2.0	0.05	2.0	5.5 ± 0.5	5.5 ± 1.5
PI 567024		VIII	3.0 ± 1.0	>29.8	3.3 ± 0.7	2.3 ± 0.3	1.5 ± 0.5	1.0	1.0 ± 0.0	3.0	0.00	4.0	5.5 ± 0.5	7.0 ± 0.0
PI 567025A		VIII	3.7 ± 0.7	10.8	3.3 ± 0.7	1.7 ± 0.3	1.0 ± 0.0	2.0	3.4 ± 1.9	2.0	0.05	1.5	[4.0]	5.5 ± 1.5
PI 567031B		VIII	2.8 ± 0.5	4.6	3.7 ± 1.3	2.3 ± 0.3	2.5 ± 0.5	1.0	0.0 ± 0.0	4.0	0.50	1.0	[4.0]	6.0 ± 1.0
PI 567034		VIII	-	9.5	1.0 ± 0.0	-	2.0 ± 1.0	1.0	0.0 ± 0.0	2.0	0.05	-	6.0 ± 0.0	7.0 ± 0.0
PI 567046A		VIII	2.8 ± 0.5	16.0	3.0 ± 0.0	1.7 ± 0.3	2.0 ± 1.0	1.0	3.0 ± 1.1	3.0	0.00	2.0	5.5 ± 0.5	6.0 ± 1.0
PI 567056A		VIII	3.5 ± 0.6	>19.9	4.3 ± 0.3	2.0 ± 0.0	1.0 ± 0.0	2.0	0.8 ± 0.4	3.0	0.25	2.0	5.5 ± 0.5	1.5 ± 0.5
PI 567123A		VIII	-	>29.8	[1.0] ± 0.0	1.3 ± 0.3	1.0 ± 0.0	1.0	0.0 ± 0.0	3.0	0.00	1.0	7.5 ± 0.5	7.0 ± 0.0
PI 578457A	May den	VIII	4.0 ± 0.0	12.0	3.3 ± 0.3	2.3 ± 0.3	1.0 ± 0.0	1.0	6.0 ± 0.8	3.0	0.20	1.0	6.0 ± 0.0	3.5 ± 0.5
PI 416810	Ban kuro daizu	IX	-	8.8	3.0 ± 0.6	-	1.0 ± 0.0	1.0	0.0 ± 0.0	3.0	0.05	-	8.5 ± 0.5	4.0 ± 0.0
PI 417089A	Kuro daizu	IX	-	7.6	3.0 ± 0.6	3.0 ± 0.6	1.0 ± 0.0	1.0	1.8 ± 0.2	2 or 4	0.00	1.0	7.0 ± 0.0	6.0 ± 1.0
PI 423972	Takema	IX	-	21.6	3.3 ± 0.3	-	1.5 ± 0.5	3.5	1.0 ± 0.3	3.0	0.25	-	-	4.0 ± 0.0
PI 567053		IX	-	>21.0	3.7 ± 0.3	2.0 ± 0.0	1.0 ± 0.0	1.0	2.0 ± 0.0	3.0	0.00	1.5	[4.0]	2.0 ± 0.0
PI 567058D		IX	-	>17.9	3.3 ± 0.7	1.3 ± 0.3	5.0 ± 0.0	3.5	0.8 ± 0.2	2.0	0.30	1.5	[8.0]	4.5 ± 0.5
PI 567102B		IX	-	>20.9	2.0 ± 1.0	-	4.0 ± 0.0	2.0	0.0 ± 0.0	2.0	0.05	-	[1.0]	2.0 ± 1.0
PI 567104B		IX	-	13.8	1.7 ± 0.3	1.3 ± 0.3	1.0 ± 0.0	4.5	0.0 ± 0.0	2.0	0.00	1.0	1.0 ± 0.0	1.0 ± 0.0
PI 567129		IX	-	>36.4	3.7 ± 0.9	1.7 ± 0.3	1.5 ± 0.5	3.5	0.0 ± 0.0	3.0	0.00	7.0	6.0 ± 0.0	1.5 ± 0.5
PI 605823		IX	-	13.0	3.0 ± 0.0	1.7 ± 0.3	1.0 ± 0.0	1.5	1.0 ± 0.8	2.0	0.00	-	-	[1]
Susceptible checks														
PI 518671	Williams 82	III	4.9 ± 0.1	>45.9	5.0 ± 0.0	5.0 ± 0.0	4.0 ± 0.0	-	-	-	3.90	8.5	7.5 ± 0.5	5.5 ± 1.5
PI 534646	Flyer	IV	5.0 ± 0.0	15.9	-	4.0 ± 0.6	-	-	-	-	-	-	4.0 ± 0.0	-
PI 586981	KS4694	IV	4.9 ± 0.1	-	5.0 ± 0.0	3.7 ± 0.9	1.0 ± 0.0	-	-	-	3.10	-	-	4.0 ± 0.0
PI 518664	Hutcheson	V	4.8 ± 0.3	-	5.0 ± 0.0	4.0 ± 0.0	5.0 ± 0.0	-	-	-	2.85	9.0	-	5.5 ± 1.5
PI 630984	5601T	V	5.0 ± 0.0	>35.6	-	4.7 ± 0.3	-	-	-	-	-	6.0	9.0 ± 0.0	-
PI 548986	Brim	VI	-	-	5.0 ± 0.0	-	3.5 ± 1.5	-	8.0 ± 0.0	3.0	1.35	-	-	4.0 ± 0.0
PI 599333	Musen	VI	5.0 ± 0.0	>50.0	-	4.7 ± 0.3	-	7.5	-	-	-	-	7.0 ± 0.0	-
PI 595645	Benning	VII	5.0 ± 0.0	>41.8	-	4.7 ± 0.3	-	5.0	-	-	-	6.0	5.0 ± 0.0	-

(cont'd)

Table 3. Continued.

Accession	Name [resistance gene]	MG	Quincy (FL) Oct. 2006 Severity† (0–5)	Quincy (FL) 3 Oct. 2007 Les. dens.‡ (les. cm^{2} $^{-1}$)	Quincy (FL) 6 Nov 2008 Severity (1–5)	Attapulgus (GA) 16 Nov. 2006 Severity§ (1–5)	Attapulgus (GA) 18 Nov. 2008 Severity§ (1–5)	Fairhope (AL) 28 Oct. 2007 Severity (1–9)	Fairhope (AL) 28 Oct. 2008 Lf. Sev.¶ (0–8)	Fairhope (AL) 15 Nov 2008 Severity# (1–5)	Blackville (SC) 25 or 31 Oct. 2008 Severity (0–10)	Alexandria (LA) 11 Oct. 2006 Severity (1–9)	Bossier City (LA) 19 Nov. 2007 Severity (1–9)	Bat. Rg. (LA) 18 Nov. 2008 Severity (1–9)
PI 641156	NC-Raleigh	VII	-	-	4.3 ± 0.7	-	4.5 ± 0.5	-	7.6 ± 0.4	5.0	2.50	-	-	4.0 ± 0.0
PI 608033	Kuell	VIII	5.0 ± 0.0	-	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	8.0	-	3.0	3.75	-	-	5.5 ± 1.5
PI 612157	Prichard	VIII	2.5 ± 0.3	>50.0	-	2.3 ± 0.3	-	-	-	-	-	3.5	9.0 ± 0.0	-
PI 548969	Alamo	IX	-	>49.4	4.7 ± 0.3	-	5.0 ± 0.0	-	7.0 ± 0.8	4.0	2.15	-	-	3.0 ± 1.0
PI 556805	H9190	IX	-	>45.0	5.0 ± 0.0	-	4.5 ± 0.5	7.5	8.0 ± 0.0	4.0	2.60	-	9.0 ± 0.0	1.0 ± 0.0
	LSD (0.05)	I-IV			0.7	1.4								
		V–VII			1.1	1.3								
		VIII-X			1.4	1.4								
		Overall			1.3	1.4	1.4							2.1

† Disease severity (mean ± standard error) was rated on scales with the indicated ranges in each year-location environment. Higher ratings indicate more disease. (See Materials and Methods for detailed descriptions of rating scales.) Means in brackets were calculated from incomplete data sets; those with no standard error are based on a single replication, whereas those with a standard error are from multiple replications, but with incomplete sample sizes within one or more replications.

‡ Lesion density (Les. dens.) refers to the number of soybean rust lesions per cm^{2} on sample leaflets. A " > " sign indicates that one or more leaflets had > 50 lesions per cm^{2}, and that these were not counted precisely.

§ Natural inoculum in the environment was supplemented by one or more applications of urediniospore suspensions.

¶ Five leaves per plot were collected from the field, incubated in a moist chamber for 7 d, and rated 0 to 8 for disease severity (Sev.) corresponding to the series of nine photos in the Bayer CropScience Asian Soybean Rust Disease Severity Disease.

Whole plots from a single replication were rated from 1 to 5 for soybean rust symptoms in the field.

were examined to determine lesion type (R,B, tan, or mixed) and to estimate percent of leaf surface affected by SBR. The numbers of leaves from each plot with 0, 1, 3, 5 10, 15, or 25% of the leaf area affected were recorded. *2008*: Ten leaflets per plot were collected from the two replications and rated for disease incidence (percentage of leaflets showing any pustules) and severity. In the "Blackville 2008" severity scale, 0 = no pustules, 1 = 1 to 10 pustules, 2 = 11 to 25 pustules, 3 = 26 to 50 pustules, 4 = 51 to 100 pustules, 5 = 1% of leaf area affected by SBR, 6 = 3% affected, 7 = 10% affected; 8 = 25% affected, 9 = 50% affected, and 10 = 75 to 100% of leaf area affected by SBR. Accessions in MGs I through V were rated on 22 October and again on 6 November. Those in MG VI were rated 22 and 30 October, and the accessions in MGs VII through X were rated one time on 30 October.

Statistical Analyses

PROC UNIVARIATE in SAS Version 9.1 was used to calculate entry means and standard deviations (SAS Institute, Cary, NC). Significant differences among entries within a test or in the combined tests were determined for some data sets by calculating Fisher's protected LSD using PROC GLM in SAS. In year–location environments where the germplasm accessions had been planted in independent tests according to MG, LSD values were initially determined for each test, and if these values were the same or nearly identical, a mean LSD value was calculated for the entire set of germplasm accessions screened in a particular environment. A Spearman rank correlation coefficient (ρ or r_s) was calculated using disease severity ratings from each pair of year–location environments listed in Table 3 using a worksheet available at http://udel.edu/~mcdonald/statspearman.html.

RESULTS AND DISCUSSION

A total of 576 soybean germplasm accessions were evaluated for SBR resistance in one or more years between 2006 and 2008. Informative data for variation in SBR disease severity were obtained from the 12 year–location environments listed in Table 3. Due to low disease pressure before the occurrence of the first killing frost or other reasons, informative data sets were not collected in the following environments: Bossier City, LA in 2006 and 2008; Baton Rouge, LA in 2006 and 2007; Fairhope, AL in 2006; Blackville, SC in 2006 and 2007; and Attapulgus, GA in 2007. Data from these environments are therefore not presented here. Although differences in experimental details and rating methods limit direct comparisons of severity ratings among the accessions from different locations and years, each of the rating methods used was designed to identify the accessions with resistance to SBR in a particular year–location environment. Lower means for disease incidence, disease severity, lesion density, and sporulation relative to susceptible North American check cultivars from the same MG were considered indicative of resistance. Spearman rank correlation coefficients (ρ) for pairs of year–location

environments indicated that the 2008 rust severity ratings from Quincy were significantly correlated with relative rankings for disease severity on accessions in all other environments except Bossier City, LA in 2007 and Baton Rouge, LA in 2008 (Supplementary Table 1). The two different rating methods used in Fairhope in 2008 (i.e., a nine-point rating of laboratory-incubated leaves vs. a five-point rating of leaflets in the field at a later date) produced similar rankings for resistance (Supplementary Table 1).

Table 3 shows disease severity data for 61 MG IV through IX accessions that exhibited at least moderate resistance to SBR in two or more of 12 different year–location environments from which informative data were obtained. Data for 13 susceptible check cultivars, the accessions with the *Rpp1* through *Rpp4* genes, and PI 547875, a Williams 82 isoline with the *Rpp1* gene, are also included in Table 3. Data for SBR lesion densities (average number of SBR lesions per cm^2 on the undersides of middle canopy leaves) are presented in lieu of disease severity ratings for Quincy in 2007 because infections from soil pathogens made it impossible to rate SBR severity at the whole plant level on many germplasm accessions that year. All of the accessions in Table 3 were screened at two or more locations in 2008, and most had exhibited some level of resistance in the field during the previous 2 yr. Additional disease evaluation data for 46 of the most resistant accessions from Table 3 are presented in Table 4. These include sporulation ratings (on a 1–5 scale) from three year–location environments, lesion type classifications from two environments, average numbers of uredinia per cm^2 in Fairhope in 2007, and SBR incidence in Blackville in 2008. Data from susceptible check cultivars are also included in Table 4.

None of the germplasm accessions evaluated was immune to SBR in every year–location environment, and only a few PIs were highly resistant in most environments (Tables 3 and 4). This may indicate differences in virulence among *P. pachyrhizi* populations among testing environments. On the basis of the Spearman correlation coefficients shown in Supplementary Table 1, the rankings of the accessions from Table 3 for resistance in Bossier City (2007) and Baton Rouge (2008) were not positively correlated with each other or with rankings from any of the other locations, with the sole exception of the correlation between rankings at Bossier City in 2007 and Fairhope in 2008.

Excluding the Williams 82 *Rpp1* isoline and the PIs carrying *Rpp1* through *Rpp4*, the resistant accessions in Table 4 are from MGs IV (2; 3%), V (13; 21%), VI (8; 13%), VII (6; 10%), VIII (23; 38%), and IX (9; 15%). The failure to identify resistant PIs from MGs 000 through III may reflect a real lack of resistance among those accessions, or simply limitations in our ability to effectively screen early MG material in the field at the latitudes where the seven nurseries were located. Although artificial extension of the natural photoperiod seemed to have been effective in delaying flowering on most early MG entries, those accessions still tended to reach the R1 stage 1 to 2 wk earlier than entries from later MGs. Susceptible checks from early MGs might be expected to have higher disease severities on a given rating date than checks from later MGs, but this pattern was not observed. In 2006, for example, disease severity ratings on the MG I checks 'BSR101' (3.0) and 'MN1302' (3.7) were lower in Attapulgus than ratings on the MG VII checks 'Haskell' (5.0) and 'Benning' (4.7) (Table 3). Possible explanations for this are that the more limited canopy development on accessions from early MGs may have exposed urediniospores on the adaxial (upper) sides of the leaves to damaging levels of solar irradiation (see Isard et al., 2006), and/or that the more rapid evaporation of moisture from leaf surfaces in sparser canopies might have reduced the percentage of successful infection events. Nevertheless, L85–2378, the Williams 82 MG III isoline with *Rpp1* (PI 547875), was consistently more resistant than Williams 82 itself (Tables 3 and 4).

Of the most resistant accessions, which are listed in Table 4, 17 originated from Japan (36%), 16 from Vietnam (34%), and 11 from Indonesia (23%). China and Malaysia were each the source of a single resistant accession, and two accessions (Pioneira and FT-2) originated from Brazil. Nearly all of the Japanese accessions were from the southern islands of Kyūshū and Shikoku (latitudes 31–34° N), and were in MGs VII and VIII (Table 4). The Vietnamese accessions, which were predominantly in MGs V and VI, were nearly all from northern provinces located close to the border with China (latitudes 21–23° N), and the Indonesian accessions, most of which were from the island of Java (latitudes 6–9° S), were in MGs VIII and IX (Table 4). There is no obvious relationship between geographical origins of accessions and regional variation in resistance in the southern United States. For example, PIs 417120 and 506947 from Kyūshū, Japan; PIs 567034, 567046A, and 567123A from Java, Indonesia; and PI 605865B from Lao Cai Province in Vietnam were all more resistant at the southeastern sites than in the Louisiana locations, whereas PIs 417126, 506491, and 506947 from Kyūshū, and PIs 567102B and 567104B from East Java were more resistant in the Louisiana locations (Table 3). The limited number of source locales increases the chance that some of the resistant PIs identified in these evaluations may have *Rpp* genes that are identical by descent or allelic.

Reactions of Accessions Carrying Known *Rpp* Genes

Rpp1

The two entries with the *Rpp1* gene, PI 200492 ('Komata') in MG VII, and PI 547875 (L85–2378) in MG III (Bernard 1995), were among the most resistant entries across environments (Tables 3 and 4). L85–2378 had higher disease severity than PI 200492 in Quincy and Alexandria in 2006, however, and neither was as resistant as some other accessions in Baton Rouge in 2008 (Table 3). For example,

Table 4. Reactions of 48 resistant soybean accessions and 13 check cultivars to soybean rust at four locations in the southern United States (2006–2008). Data include relative sporulation, lesion type, uredinia density, and disease incidence. The place of origin of each accession is also indicated.

			Quincy (FL)			Fairhope (AL)			Blackville (SC)	Boss. City (LA)	Place of origin	
			3 Oct. 2007	6 Nov. 2008	13 Nov. 2008	28 Oct. 2007		15 Nov. 2008	25 or 31 Oct. 2008	19 Nov. 2007		
Accession	Name [resistance gene]	MG	Spor.† (1–5)	Spor. (1–5)	Spor.‡ (1–5)	Lesion type§	Uredinia¶ (ured. $cm^{2\ -1}$)	Spor. (1–5)	Dis. Incidence# (%)	Lesion type	Province	Country
PI 547875	L85–2378 [*Rpp1*]	III	1.0	1	-	-	-	-	0	-	Illinois	United States
PI 567189A	Ekhabac	IV	1.5	1	2	-	-	-	10	Mix	unknown	Vietnam
PI 606440A	VX 92	IV	3.5	2	3	-	-	-	15	Mix	(north)	Vietnam
PI 476905A	Nguu mao hong	V	1.0	1	5 or 1	-	-	2	0	RB	unknown	China
PI 567059		V	1.0	2	2	HR/RB	0.5	3	5	-	unknown	Indonesia
PI 605773		V	1.0	1	1	HR	0.0	3	10	Mix	Cao bang	Vietnam
PI 605829		V	1.5	2	2	HR	0.0	3	0	Mix	Ha Giang	Vietnam
PI 605838	Xanh si man	V	2.5	1	4	-	-	2	0	Mix	Ha Giang	Vietnam
PI 605854B		V	1.0	1	2	RB	3.5	2	15	-	Tuyen quang	Vietnam
PI 605865B		V	1.0	1	3	RB	3.5	2	25	RB	Lao Cai	Vietnam
PI 605885B		V	1.0	1	3	-	0.0	3	10	Tan	Lao Cai	Vietnam
PI 605891A		V	1.0	2	3	RB	3.5	2	-	Tan	Son La	Vietnam
PI 606397B	(Hat nho duc trong)	V	1.0	1	1 or 4	Tan	7.0	3	5	Tan	(north)	Vietnam
PI 606405	Madrak	V	1.0	3	3	-	-	3	55	Tan	(north)	Vietnam
PI 417503	Pioneira	VI	1.0	1	2	-	0.0	3	0	Tan	unknown	Brazil
PI 506695	Gogaku	VI	1.5	1	4	RB/HR	0.5	5	0	Mix	Kyūshū	Japan
PI 567190	Halang 4 thang	VI	1.0	2	3	-	-	3	0	Tan	unknown	Vietnam
PI 615437	A 9	VI	1.0	2	4	-	0.0	3	0	Tan	(north)	Vietnam
PI 635999	DT2000	VI	1.5	1	3	-	0.0	2	0	Tan		Vietnam
PI 200492	Komata [*Rpp1*]	VII	-	1	3	-	-	1	0	-	Shikoku	Japan
PI 230970	[*Rpp2*]	VII	3.0	3	4	RB	4.5	3	35	Tan	unknown	Japan
PI 417116	Kyushu 19	VII	1.5	1	3	-	0.0	2	0	Tan	Kyūshū	Japan
PI 417132	Kyushu 56	VII	1.0	3	5	Tan?	4.5	4	-	-	Kyūshū	Japan
PI 506764	Hyuuga [*Rpp?*(Hyuuga)]	VII	2.0	2	3	RB	4.5	5	0	Tan	Kyūshū	Japan
PI 594172A	Gogaku	VII	3.0	2	3	-	0.0	4	5	Tan	Kumamoto	Japan
PI 200488	Kiro Aki Daizu	VIII	1.0	2	3	-	0.0	2	10	Tan	Shikoku	Japan
PI 203398	Abura	VIII	1.0	1	3	-	0.0	4	15	Tan	unknown	Brazil
PI 416826A	Cha sengoku 81	VIII	1.0	-	1	-	0.0	1	5	Mix	unknown	Japan
PI 417120	Kyushu 25	VIII	1.0	1	5	-	0.0	2.5	0	Tan	Kyūshū	Japan
PI 417126	Kyushu 32	VIII	2.5	3	4	RB	9.0	1	10	RB	Kyūshū	Japan
PI 417208	Oka Kaizu	VIII	1.5	2	3	-	0.0	1	30	RB	Kyūshū	Japan
PI 459025B	(Bing nan) [*Rpp4*]	VIII	-	5	4	-	-	5	-	-	Fujian	China

(cont'd)

disease severity on PI 200492 in Baton Rouge in 2008 was higher than that observed on PI 230970, which carries the *Rpp2* gene, though the difference may not have been statistically significant (Table 3). Due to limited seed availability, PI 200492 was tested for resistance in only seven year–location environments. Although PI 200492 was not planted in 2007, line L85–2378 was highly resistant to SBR in Quincy and Bossier City that year, and none of the leaflets collected from L85–2378 in Quincy had sporulating lesions (Tables 3 and 4). This level of resistance was higher than that observed in Quincy and Alexandria the previous year, and in 2008 L85–2378 was substantially more resistant than Williams 82 in Quincy and Attapulgus (Table 3). Li and Young (2009) reported that PI 200492 seedlings were immune to a 2006 *P. pachyrhizi* isolate from southwestern Mississippi. In a related study, PI 200492 was also immune to two 2007 isolates from west-central Mississippi, and out of the *Rpp1* through *Rpp4* genes, *Rpp1* conditioned the highest level of resistance to the three isolates (Li, 2009). Although we did not observe complete immunity in every year–location environment, we also found *Rpp1* to provide the highest level of resistance overall. Similarly, Paul and Hartman (2009) found both PI 200492 and L85–2378 to be nearly immune to a panel of six 2006 and 2007 *P. pachyrhizi* isolates from five states. In contrast, Pham et al. (2009) reported that PI 200492 developed an RB reaction type against a 2004 isolate from near Baton Rouge, LA, but TAN lesions when challenged with 2004 isolates from Fairhope, AL and a site in a neighboring county. A race shift that occurred in Brazil between 2002 and 2003 resulted in the defeat of *Rpp1*-mediated resistance by some populations of the pathogen (Yorinori 2008).

Table 4. Continued.

Accession	Name [resistance gene]	MG	Quincy (FL) 3 Oct. 2007 Spor.† (1–5)	Quincy (FL) 6 Nov. 2008 Spor. (1–5)	Quincy (FL) 13 Nov. 2008 Spor.‡ (1–5)	Fairhope (AL) 28 Oct. 2007 Lesion type§	Fairhope (AL) 28 Oct. 2007 Uredinia¶ (ured. cm$^{2-1}$)	Fairhope (AL) 15 Nov. 2008 Spor. (1–5)	Blackville (SC) 25 or 31 Oct. 2008 Dis. Incidence# (%)	Boss. City (LA) 19 Nov. 2007 Lesion type	Place of origin Province	Place of origin Country
PI 462312	Ankur [*Rpp3*]	VIII	1.5	2	3	-	0.0	5	0	-	Uttar Pradesh	India
PI 506491	Akanida	VIII	1.0	2	5	RB/HR	1.5	3.5	25	Mix	Kyūshū	Japan
PI 506947	Kumaji 2	VIII	1.0	2	4	-	0.0	2	5	Tan	Kyūshū	Japan
PI 567024		VIII	3.0	1	2	-	0.0	2	0	Tan	unknown	Indonesia
PI 567025A		VIII	1.5	1	3	RB	4.0	2	5	Tan	unknown	Indonesia
PI 567034		VIII	1.0	1	2	-	0.0	2	5	Tan	Central Java	Indonesia
PI 567046A		VIII	1.5	1	2	-	0.0	2	0	Tan	Central Java	Indonesia
PI 567056A		VIII	1.5	1	4	RB	4.0	3	20	Tan	unknown	Indonesia
PI 567123A		VIII	2.0	-	1	-	0.0	2	0	Tan	East Java	Indonesia
PI 578457A	May den	VIII	1.0	2	5	-	0.0	3	20	Tan	An Giang	Vietnam
PI 416810	Ban kuro daizu	IX	1.5	2	2	-	0.0	3	5	Tan	Kyūshū	Japan
PI 417089A	Kuro daizu	IX	1.5	2	3	-	0.0	2 or 5	0	-	Kyūshū	Japan
PI 567053		IX	2.5	2	4	-	0.0	3	0	Tan	East Java	Indonesia
PI 567102B		IX	1.0	4	1	RB	4.5	1	5	-	East Java	Indonesia
PI 567104B		IX	1.0	1	1	Tan	34.5	2	0	-	East Java	Indonesia
PI 605823		IX	2.0	2	2	RB/HR	3.5	1	0	-	Ha Giang	Vietnam
Susceptible checks												
PI 518671	Williams 82	III	5.0	5	-	-	-	-	100	Tan	Illinois	Check
PI 534646	Flyer	IV	4.5	-	-	-	-	-	-	Tan	Ohio	Check
PI 586981	KS4694	IV	-	5	-	-	-	-	100	-	Kansas	Check
PI 518664	Hutcheson	V	-	5	5	-	-	-	100	-	Virginia	Check
PI 630984	5601T	V	4.5	-	-	-	-	-	-	Tan	Tennessee	Check
PI 548986	Brim	VI	-	5	-	Tan	97.5	5	75	-	North Carolina	Check
PI 599333	Musen	VI	5.0	-	-	-	-	-	-	Tan	South Carolina	Check
PI 595645	Benning	VII	5.0	-	-	Tan	43.0	-	-	Tan	Georgia	Check
PI 641156	NC-Raleigh	VII	-	2	5	-	-	5	100	-	North Carolina	Check
PI 608033	Kuell	VIII	-	5	5	-	-	3	100	-	Alabama	Check
PI 612157	Prichard	VIII	5.0	-	-	Tan	85.5	-	-	Tan	Georgia	Check
PI 548969	Alamo	IX	5.0	4	5	-	-	5	100	-	Florida	Check
PI 556805	H9190	IX	4.5	5	5	Tan	194.0	5	100	Tan	'-	Check

†Sporulation (spor.) was rated from 1 (no sporulation) to 5 (profuse sporulation equivalent to that observed on susceptible cultivar checks).

‡Accessions with two different rating values exhibited possible (unconfirmed) heterogeneity for sporulation.

§Lesion type abbreviations: HR = hypersensitive reaction (i.e., immune); RB = reddish brown; Mix = mixture of RB and Tan lesions.

¶Mean number of uredinia per cm^2 on the undersides of sample leaflets.

#Disease incidence refers to percentage of plants with soybean rust symptoms.

Rpp2

In 2006, PI 230970 (*Rpp2*) was immune in Fairhope and resistant in Attapulgus and Alexandria, but seemed less resistant in Quincy (Tables 3 and 4). In 2007, PI 230970 had a relatively high lesion density (>13.4 lesions per cm^2) and a moderate level of sporulation in Quincy, but appeared to be more resistant in Fairhope, where it had RB lesions and almost 10-fold fewer uredinia per cm^2 than the susceptible cultivar Benning (Tables 3 and 4). PI 230970 had moderate disease severity in Bossier City in 2007, while in 2008 it was susceptible or only moderately resistant in Quincy, Attapulgus, and Fairhope, but appeared highly resistant in Baton Rouge (Tables 3 and 4). Altogether these data suggest that the level of *Rpp2*-mediated resistance depended more on the virulence of local *P. pachyrhizi* populations than *Rpp1* resistance. Paul and Hartman (2009) also observed differential reactions of PI 230970 to 2006 and 2007 isolates from five different states, and Li and Young (2009) reported that PI 230970 seedlings challenged with a 2006 isolate from southwestern Mississippi exhibited similar or slightly lower rust severity and moderate sporulation compared to two susceptible cultivars. In greenhouse seedling assays conducted by Pham et al. (2009), however, PI 230970 actually appeared more resistant than PI 200492, developing RB reactions to 2004 isolates from Louisiana and southern Alabama. *Rpp2*-mediated resistance was defeated by some 2003 *P. pachyrhizi* populations in Brazil (Yorinori, 2008).

Rpp3

PI 462312 (*Rpp3*) was not tested in 2006 due to a shortage of seed, but in 2007 it averaged only 14.1 lesions per cm^2 compared to >50 lesions per cm^2 on 'Prichard' in Quincy, and developed no SBR symptoms in Fairhope (Table 3). Disease severity on PI 462312 was high in Quincy in 2008 (Table

3), but sporulation levels were low (Table 4). No SBR was observed on PI 462312 in Attapulgus in 2008, and leaflets collected from the Fairhope plots on 28 October had very low levels of disease (Table 3). By 2 wk later, however, PI 462312 leaflets in Fairhope had developed moderately high SBR severity and some heavily sporulating lesions (Tables 3 and 4). PI 462312 seemed immune to SBR at Baton Rouge in 2008. Li and Young (2009) reported that PI 462312 seedlings challenged with a Mississippi isolate had moderate SBR severity and low sporulation relative to susceptible checks. *Rpp3* also seemed to condition high to moderate resistance to several U.S. isolates in assays conducted by Paul and Hartman (2009), but PI 462312 developed TAN lesions when infected by two out of three domestic isolates from 2004 (Pham et al., 2009). Resistance conditioned by *Rpp3* has also been overcome by Brazilian *P. pachyrhizi* populations (Yorinori, 2008).

PI 506764, carrying the *Rpp?*(Hyuuga) gene, which may be allelic to *Rpp3* (Monteros et al., 2007), had a relatively high disease severity at Attapulgus in 2006 (Table 3). It also had a higher average SBR lesion density than PI 462312 in Quincy in 2008 (Table 3), though the two accessions had identical sporulation ratings (Table 4). In Fairhope, both PIs had low severity ratings and much fewer uredinia per cm^2 than the cultivar checks in 2007 (Tables 3 and 4). In Quincy, PI 506764 had a relatively high lesion density in 2007 and disease severity in 2008 (Table 3), but only low to moderate sporulation (Table 4). It appeared to be highly resistant in Attapulgus in 2008 and seemed similarly resistant at the time of the first rating in Fairhope, but by 15 November it had developed a moderate density of lesions, most of which were sporulating (Tables 3 and 4).

Rpp4

Due to limited seed availability, PI 459025B (*Rpp4*) was not planted in 2006 or 2007, but in 2008 it was highly susceptible in both Quincy and Fairhope, where it exhibited high disease severity and profuse sporulation (Tables 3 and 4). Overall, *Rpp4* may be the least effective of the original four *Rpp* genes against North American *P. pachyrhizi* populations. Li and Young (2009) reported that disease severity of PI 459025B seedlings inoculated with a 2006 Mississippi isolate was significantly lower than that of PI 230970 (*Rpp2*), but the sporulation rating for PI 459025B was almost twice as high. Pham et al. (2009) found that PI 459025B was resistant to three 2004 isolates from southern Alabama and Louisiana. It is interesting that in their assays, *Rpp2* and *Rpp4* conferred more resistance to the 2004 isolates than *Rpp1* or *Rpp3*, whereas in our field evaluations, *Rpp1* and *Rpp3* provided more consistent levels of resistance in most environments (Tables 3 and 4).

Rpp5

During the course of our germplasm evaluations, Pierozzi et al. (2008) and Garcia et al. (2008) independently identified PI 200487 as one of several accessions with an SBR resistance gene at the previously unreported *Rpp5* locus. Although recessive and partially dominant resistance alleles at this locus have been identified in other accessions, resistance in PI 200487 is dominant. This PI had relatively high disease severity ratings in 2008 at Quincy, Attapulgus, and on 28 October in Fairhope, but showed some resistance in Quincy and Fairhope in 2007 and in Blackville in 2008 (Table 3). The *Rpp5* allele in PI 200487 thus appeared to provide resistance to some, but not all *P. pachyrhizi* populations in the southeastern United States.

Most Resistant Accessions with Uncharacterized Soybean Rust Resistance

In addition to PI 200492 (*Rpp1*), accessions PI 567104B, PI 605823, and PI 605838 consistently had disease severity scores ≤3.5 on the five-point rating scales and ≤6.0 on the nine-point scales (Table 3), as well as sporulation ratings ≤2.5 in environments where sporulation was examined (Table 4). PI 567104B, an MG IX accession from East Java in Indonesia, appeared to be resistant in all environments except Fairhope in 2007, where it had moderate disease severity (as rated using the conservative Miles et al. rating scale), tan lesions, and an average of 34.4 uredinia per cm^2 (Tables 3 and 4). At the same location the following year, however, it had low severity and low sporulation. PI 605823 (MG IX) and PI 605838 (MG V), both from north-central Vietnam, had low to moderate disease severity across locations, and little or no sporulation, though severity data for PI 605823 from Louisiana locations were limited.

Variability in relative disease severity ratings across year–location environments would have made it difficult to identify accessions with broad resistance on the basis of data from only one or two random environments (Table 3 and Supplementary Table 1). For example, PI 417132 had relatively high SBR severity at Quincy in 2006 and at Quincy and Fairhope in 2008, but was resistant at Attapulgus and Alexandria in 2006 and at Baton Rouge in 2008, whereas PI 567102B seemed highly resistant at Quincy, Fairhope, and Baton Rouge in 2008, but relatively susceptible in Attapulgus (Table 3). While these discrepancies could be at least partly due to differences in environmental factors and/or differences in rating methods (e.g., different raters, different rating dates), the data indicate possible diversity in virulence among *P. pachyrhizi* populations among years and locations. The variable levels of resistance of some accessions at different locations in the same year suggests regional pathogen diversity between populations in the more eastern locations (i.e., Attapulgus, Quincy, and Blackville) and those at the two Louisiana sites (Table 3 and Supplementary Table 1). Although some accessions like PI 417134, PI 462312 (with *Rpp3*), PI 567053 and PI 567129 showed moderate SBR resistance in most environments, many accessions tended to be resistant in either southeastern locations or Louisiana locations, but not in both regions (Table 3). The general trend was

for certain accessions that were resistant in most eastern locations to be more susceptible in one or more of the Louisiana locations, though disease severity on certain PIs also differed considerably sometimes among locations within Louisiana or the Southeast (Table 3).

Comparison of Germplasm Accession Reactions with Their Responses in other Studies Involving North American Isolates of *Phakopsora pachyrhizi*

PI 567102B was one of the most resistant accessions in our evaluations (Tables 3 and 4). With the exception of its puzzling severity rating in Attapulgus in 2008, this accession was highly resistant in Quincy, Fairhope, and Baton Rouge. Out of 10 accessions resistant to SBR in southern Paraguay that were challenged with 2006 and 2007 *P. pachyrhizi* isolates from Mississippi, Li (2009) found PI 567102B to be the most resistant. While inoculated leaves on PI 567102B seedlings had few SBR lesions and no sporulation, the other nine accessions either lacked resistance or had a lower level of resistance than PI 567102B and PI 200492 (with the *Rpp1* gene). We screened these same nine PIs in 2006 and/or 2007, and found them all to be susceptible to SBR at two or more nurseries (data not shown). Although PI 567099A (MG IX) appeared to have some resistance in Attapulgus and Alexandria in 2006, it had a high density of heavily sporulating lesions in Quincy the following year. PI 594767A had low disease severity at Attapulgus in 2006 (no data were available from other locations that year), but had a high lesion density and sporulation level at Quincy in 2007. Li and Young (2009) evaluated seedling resistance of another set of 17 soybean accessions to the same 2006 Mississippi *P. pachyrhizi* isolate that was used in the Li (2009) assays. Several of the accessions that they reported to be susceptible were also susceptible in our evaluations (data not shown), but PI 594172A had a moderate level of resistance in both studies (Tables 3 and 4). Although PI 398399 and PI 605854B appeared to be resistant in certain environments in our evaluations, PI 398399 and the "A" subline of PI 605854 had high disease severity and sporulation in the Li and Young (2009) assays. In contrast, while PI 417560 showed some resistance in their evaluations, it was not resistant when we tested it in 2006 (data not shown). Unfortunately, we did not test the field resistance of PI 407730, one of the PIs that appeared to have seedling resistance against the 2006 Mississippi isolate.

Pham et al. (2009) tested four of the accessions that we screened (other than the sources of known *Rpp* genes) for resistance to two 2004 *P. pachyrhizi* isolates from southern Alabama and to another 2004 isolate collected near Baton Rouge. PI 594794 (MG IX) developed a TAN reaction to the Louisiana isolate and an RB reaction to both of the Alabama isolates (Pham et al., 2009). In 2006 this PI was susceptible in Attapulgus, GA and Alexandria, LA, but it appeared less susceptible in Baton Rouge (data not shown). The 2007 evaluations confirmed that it was susceptible in most locations, and we did not test it in 2008. PIs 423972 and 635999 ('DT 2000') also exhibited isolate-specific reactions in the Pham et al. (2009) assays and in our assays (Tables 3 and 4), but overall we consider them resistant, whereas PI 437323 was highly susceptible in Quincy in 2007 (data not shown). In assays with six domestic isolates from 2006 and 2007, Paul and Hartman (2009) also tested some of the accessions evaluated in our assays. In both studies, PI 467323A was found to be susceptible and PIs 203398 and PI 567046A were resistant. Reactions of accessions with known *Rpp* genes were similar in both of our assays, as mentioned previously.

Comparison of Soybean Rust Reactions in the United States (2006–2008) and Paraguay (2006) or Nigeria (2005–2006)

Of the 530 accessions that Miles et al. (2008) screened for SBR reactions in Capitán Miranda, Itapúa, Paraguay between March and July 2006, 345 were evaluated in the present study. Only about 15% of the accessions that were moderately to highly resistant in southern Paraguay were also resistant in Quincy, FL in the fall of 2008. Among these were PI 605829 and PI 605838 in MG V; PI 417503 in MG VI; and PI 567102B, PI 567104B, and PI 605823 in MG IX. About 16% of the 345 accessions were resistant in Quincy, but not in Paraguay; 23% were resistant in Paraguay, but not in Quincy; and 46% were susceptible in both locations. Ray et al. (2009) recently mapped resistance genes in populations derived from PI 587880A and PI 587886, but both had very high SBR severity in Attapulgus, Fairhope, and Alexandria in 2006 and were not tested further (data not shown).

Twizeyimana et al. (2008) screened 101 accessions from the USDA Soybean Germplasm Collection for SBR resistance in screenhouse assays at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, in 2005 and reported that PI 594538A (MG IX) was the only accession in a second round of evaluation that had a hypersensitive reaction and no sporulation. In our 2008 evaluation this PI was highly susceptible to the *P. pachyrhizi* populations in Quincy and Attapulgus (data not shown). In Nigeria, PI 417089A (MG IX) developed RB lesions and showed ≤10% sporulation (Twizeyimana et al., 2008). This accession also showed at least moderate resistance in Quincy and Attapulgus in 2008, but appeared to be more susceptible in Baton Rouge (Tables 3 and 4). The rating data from the IITA and Paraguay resistance evaluations would therefore have been of limited value for predicting which accessions would be resistant to North American *P. pachyrhizi* populations.

Soybean Rust Resistance Evaluation Criteria

Disease severity (or lesion density) and relative sporulation were among the most informative SBR resistance criteria

in our evaluations, particularly when considered together (Tables 3 and 4). Infection type or lesion color may have been of some value, but was probably less reliable for rating the resistance of adult plants in the field than it would be for rating the reactions of seedlings 2 wk after a controlled inoculation in a greenhouse or growth chamber. Miles et al. (2006) used infection type (Bromfield and Hartwig, 1980) and disease severity to rate seedlings from more than 16,500 germplasm accessions for SBR resistance in a greenhouse. In subsequent evaluations of a much smaller set of PIs for field resistance in southern Paraguay, Miles et al. (2008) also examined sporulation on leaves that developed RB-type lesions. Twizeyimana et al. (2008) likewise considered low relative sporulation in addition to lesion type and low disease severity as an indication of SBR resistance in Nigeria. In our evaluations, sporulation levels on some accessions were independent of lesion color/infection type, so sporulation was rated in Quincy beginning in 2007 and in Fairhope in 2008 (Table 4). For example, PI 230970 (*Rpp2*) and PI 567056A had RB lesions in Fairhope in 2007, but moderate levels of sporulation in 2008. In contrast, PI 467104B had tan-colored lesions and a high density of uredinia per cm^2 at Fairhope in 2007, but exhibited a low level of sporulation there in 2008 (Table 4). Kato and Yorinori (2008) reported that RB lesions sometimes produced as many urediniospores as tan lesions on a set of lines evaluated in Brazil, and Morel et al. (2008) addressed variation in sporulation levels on RB lesions by classifying RB lesions into six different categories, ranging from no sporulation (0) to profuse sporulation (5). Li and Young (2009) found sporulating RB lesions on PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), and PI 459025B (*Rpp4*) plants challenged with a Mississippi isolate. Moreover, Bonde et al. (2006) noted that the color of some "tan" lesions described in the literature was probably due to the abundance of tan-colored urediniospores rather than to the color of the underlying leaf tissue. These observations should be taken into consideration when interpreting lesion type data from field plots.

High levels of sporulation were generally associated with high disease severity or lesion density in our assays, but high disease severity was not always accompanied by heavy sporulation. For example, PI 567059 (MG V) had disease severity ratings similar to susceptible checks in Quincy in 2006 and 2008 (Table 3), but had low sporulation on both of the 2008 evaluation dates (Table 4). PI 462312 (with *Rpp3*) also had relatively high disease severity at Quincy in 2008, but comparatively light sporulation (Tables 3 and 4).

Without considering low sporulation in addition to disease severity and infection type/lesion color, some resistant accessions might be overlooked. Soares (2008) and Yorinori (2008) also suggested that germplasm lines with high severity but subdued sporulation could be useful sources of resistance, but stressed the importance of recognizing that both lesion color (RB vs. tan) and sporulation rate can vary with environmental conditions and leaf age. Cooler conditions can induce higher sporulation. During the week between 6 and 13 Nov. 2008, sporulation increased on almost all of the accessions growing in Quincy, and on accessions like PI 417120 and PI 567056A (both MG VIII), the percentage of sporulating lesions increased dramatically (Table 4). It is thus important to confirm that low sporulation levels remain steady over time.

In summary, evaluation of 576 *G. max* accessions from the USDA Soybean Germplasm Collection for resistance to *P. pachyrhizi* populations under field conditions in the southern United States identified about 65 that were resistant to *P. pachyrhizi* populations in several year–location environments. The 48 most resistant accessions, which are included in Table 4, therefore represent fewer than 10% of the accessions evaluated in these field assays. Although PI 200492, which has the *Rpp1* gene, was resistant in most locations and was among the most resistant accessions, none of the accessions tested in our evaluations was immune to all of the *P. pachyrhizi* populations encountered. Few of the accessions with uncharacterized resistance genes exhibited as much resistance overall as PI 200492, which was the most resistant of the accessions with the *Rpp1* through *Rpp5* genes. In retrospect, it would have been difficult to identify many of the resistant accessions in Table 4 on the basis of their reactions in just one or two random year–location environments, especially if disease severity had been used as the sole selection criterion. Due to the substantial influence that precipitation and temperature can have on soybean rust epidemiology (Melching et al., 1989; Christiano and Scherm, 2007), the extent to which location-to-location and year-to-year differences in ratings reflect temporal and geographic variation in virulence among southern U.S. *P. pachyrhizi* populations is unclear, but regional genetic variability among pathogen populations, particularly between Louisiana and southeastern populations, does appear to have been a factor. Furthermore, the fact that major resistance genes like *Rpp2* and *Rpp3* conditioned moderate resistance to many SBR populations will make it difficult to determine whether resistance in accessions with similar levels of resistance is qualitatively or quantitatively inherited until genetic and/or mapping studies are done on them. Breeders may need to pyramid two or more *Rpp* genes from different sources to develop soybean cultivars with broad and durable resistance to North American *P. pachyrhizi* populations. Additional molecular mapping and phenotypic evaluation studies are therefore needed to provide breeders with more information regarding which combinations of resistance genes are likely to be suitable candidates for pyramiding. Annual testing of the best accessions from this study will also be necessary to confirm that these accessions remain resistant to *P. pachyrhizi* populations currently in North America. Although the likelihood that SBR will

cause economically significant yield losses in the United States is not yet known, data from the evaluations described here are already being used by soybean breeders to develop high-yielding, agronomically acceptable lines with effective resistance to SBR in North America.

Supplemental Information Available

Supplemental Table 1 is available at http://www.crops.org/publications/cs.

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