Plant Disease • 2020 • 104:1087-1095 • https://doi.org/10.1094/PDIS-09-18-1704-RE

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Reactions of Soybean Germplasm Accessions to Six *Phakopsora pachyrhizi* Isolates from the United States

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Abstract

Soybean rust, caused by *Phakopsora pachyrhizi* Syd. & P. Syd., is one of the most economically important foliar diseases of soybean. Resistant cultivars could reduce yield losses and management costs but considerable pathogenic diversity exists among populations of the fungus; thus, resistance to a range of pathotypes is essential. Seedling and detached-leaf assays were conducted to characterize the resistance of 55 soybean plant introductions (PIs) to six purified isolates of *P. pachyrhizi* originating from the southern United States. In the greenhouse resistance assays, the differentials Hyuuga (PI 506764) and PI 471904 and accessions PI 224268, PI 567025A, PI 567039, PI 567046A, and DT 2000 (PI 635999) were resistant to all six isolates, including Florida isolates from 2011 and 2012 that were able to defeat resistance conditioned by the *Rpp1* through *Rpp4* genes. Twenty-six other PIs were resistant to four or five of the six isolates. In the detached-leaf assays, eight accessions developed reddish-brown reactions to all six isolates, with an average of only 0.23 to 0.55 uredinia/lesion. These included Hyuuga, DT 2000, two differentials with a resistance allele at the *Rpp5* locus, and accessions PI 224268, PI 423960B, PI 567025A, and PI 567046A. Many of the resistant accessions have subsequently been reported to have a resistance allele at the *Rpp3* locus, and two others have resistance genes at the *Rpp4* or *Rpp6* locus. This study provided new information about resistance reaction phenotypes that can be useful for understanding mechanisms of resistance, which *Rpp* genes and alleles could be combined to obtain broader and more durable rust resistance in soybean cultivars, and pathotype diversity among the six isolates used.

Keywords: cultivar/resistance < disease, *Phakopsora pachyrhizi*, soybean, soybean rust

Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd., is one of the most economically important foliar diseases of soybean (*Glycine max* (L.) Merr.) worldwide. It is most often a threat to soybean in production regions with mild winter temperatures. The pathogen is endemic to East Asia but it has gradually spread from there to Africa and the Americas. In South America, it was first found in Paraguay and Brazil in 2001 (Yorinori et al. 2005), and the first positive identification in the United States was made in November 2004 (Schneider et al. 2005). Although the disease can be managed effectively with mixtures of strobilurin and triazole fungicides, this adds to production costs, and the overuse of some fungicide chemistries has resulted in resistance to demethylation inhibitors and quinone-outside-inhibitors in some *P. pachyrhizi* populations and isolates from South America (Godoy 2012; Schmitz et al. 2014).

Resistant evaluation studies have identified more than 120 SBR-resistant germplasm accessions (plant introductions [PIs]) in the United States Department of Agriculture (USDA) Soybean Germplasm Collection (Miles et al. 2008; Walker et al. 2011, 2014a,b) but pathogenic variation within and among *P. pachyrhizi* is high, and none of the resistant soybean accessions has been resistant to all of the isolates it has been challenged with. Susceptible soybean plants develop a "TAN" infection type, characterized by the extrusion of clumps of urediniospores from uredinia (Bromfield 1984). The tan or beige color can be from host tissue discoloration or clumps of urediniospores that have been extruded from lesions. Highly resistant, immune hosts have a type 0 infection type with no macroscopically visible lesions; however, most resistant hosts develop reddish-brown (RB) lesions, which often have some uredinia but limited sporulation compared with susceptible hosts. The level of sporulation can vary within both the TAN and RB reaction types, however, indicating that there is variability in how well different host genotypes can recognize and react to *P. pachyrhizi* infections (Bromfield 1984).

Thus far, seven independent loci with SBR resistance (*Rpp*) genes have been reported, and several of those are known to have multiple resistance alleles based on differential reactions to fungal isolates (Childs et al. 2018a). Although most of the resistance genes are dominant, recessive and partially dominant alleles have also been reported (Garcia et al. 2008). Walker et al. (2011, 2014a) reported the resistance of more than 100 PIs to *P. pachyrhizi* field populations at various locations in the southern United States. However, the

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Funding: This study was funded by the United States Department of Agriculture–Agricultural Research Service and H. Z. Motter was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) program of the Brazilian Ministry of Education.

*The *e*-Xtra logo stands for "electronic extra" and indicates that four supplementary tables are published online.

The author(s) declare no conflict of interest.

Accepted for publication 3 November 2019.

pathotype composition of those field populations of the fungus was unknown. Twizeyimana et al. (2011) reported that >90% of the total genetic diversity of 116 Nigerian isolates existed within individual fields that were in the same geographical zone. In an investigation of pathogenic variation among 72 purified isolates from the United States, Twizeyimana and Hartman (2012) identified three pathotypes based on virulence and six aggressiveness groups based on the level of sporulation from uredinia. The germplasm screening studies by Walker et al. (2011, 2014a) also demonstrated that *P. pachyrhizi* pathotype diversity between growing seasons and among locations within a growing season can vary considerably in the southern United States.

To develop soybean cultivars with broad and durable resistance to SBR, it is critical for breeders to know which known *Rpp* genes provide the most effective resistance in the geographical target area, and which soybean germplasm accessions have resistance to a majority of the fungal field populations and isolates from the targeted region (Childs et al. 2018a). Screening soybean germplasm accessions in the field has been effective for identifying those with moderate to high levels of SBR resistance in most year–location environments where disease pressure is sufficiently high and uniform in a field (Walker et al. 2011, 2014a). The reactions of highly susceptible and highly resistant hosts are generally relatively simple to assess unless there is a high density of lesions caused by other pathogens such as *Xanthomonas axonopodis* pv. *glycines*, which causes bacterial pustule, or *Peronospora manshurica*, an oomycete that causes downy mildew. Although lesions caused by other pathogens can usually be distinguished from SBR lesions, an abundance of non-SBR lesions slows or severely interferes with the process of rating SBR severity, particularly if the host has the RB infection type. Field ratings can also be more complicated, however, on plants with only moderate levels of resistance, or if there are differential interactions between the resistance genes in a host plant and multiple pathotypes present in the local *Phakopsora pachyrhizi* population that result in TAN and RB infection types on the same leaf.

The objectives of this study were to confirm and more thoroughly characterize the SBR reactions of some of the soybean PIs that had previously appeared to be resistant to field populations of *P. pachyrhizi* in the southern United States or in Paraguay. The PIs were challenged with six different isolates collected between 2008 and 2012.

Materials and Methods

Greenhouse inoculation experiment. In the first experiment, 45 *G. max* lines were inoculated with the six U.S. *P. pachyrhizi* isolates shown in Table 1. The experimental design was a randomized complete block with three replications. Of primary interest were 30 accessions from the USDA Soybean Germplasm Collection, many of which had been resistant to SBR in field evaluations in the southeastern United States (Walker et al. 2011, 2014a,b) or in detached-leaf assays with U.S. isolates (Paul and Hartman 2009; Paul et al. 2011). Eleven of the entries were included as differentials with known resistance genes, including the original sources of *Rpp1* through *Rpp6* and several accessions with alternative alleles at the *Rpp1* and *Rpp5* loci. Hyuuga (PI 506764), which has resistance genes at the *Rpp3* and *Rpp5* loci (Kendrick et al. 2011), was also included. Although some resistance genes such as the *Rpp1-b* allele in PI 594538A and the *Rpp5* allele from PI 200526 were already known to be ineffective against U.S. field populations of *P. pachyrhizi*, PIs with those genes were also included. Williams 82 (PI 518671) (Bernard and Cremeens 1988), 5601T (PI 630984) (Pantalone et al. 2003), and two maturity group IV breeding lines (LD00-2817P from the University of Illinois and R00-1194F from the University of Arkansas) were used as susceptible checks.

The *P. pachyrhizi* isolates had all been purified through three sequential cycles of transferring a few urediniospores from a single, isolated uredinium to a fresh detached leaf of the SBR-susceptible cultivar Williams 82. It was surmised that at least one of the uredinia was likely to have originated from infection by a single urediniospore in the previous inoculation. The isolates were originally collected in Alabama (in 2008 and 2009), Louisiana (2009), or Florida (2009, 2011, and 2012), and all of them readily reproduced on susceptible soybean leaves (Table 1). The Alabama isolates originated from soybean plants at Auburn University's Gulf Coast Substation in Fairhope, AL, and the LA09-BC isolate came from soybean plants growing in northwestern Louisiana at the Louisiana State University AgCenter's Red River Research Station in Bossier City. The three Florida isolates were from soybean plants growing at the University of Florida's North Florida Research and Education Center in Quincy, FL. The source soybean plants for the FL12-Q isolate had previously been inoculated with urediniospores collected from infected kudzu (*Pueraria* spp.) plants growing on the same station. The unpurified isolate was found to be highly aggressive on soybean and able to reproduce on plants with the *Rpp1* and *Rpp6* resistance genes, and the FL12-Q isolate used in this study was subsequently purified from it. The Alabama and Louisiana isolates were maintained and increased on leaves of susceptible hosts, primarily Williams 82. The 2011 and 2012 Florida isolates, which were known to be virulent on hosts with the *Rpp1* and *Rpp6* genes (Paul et al. 2013), were maintained on PI 200492, the source of the original *Rpp1* gene. The purpose of this was to prevent loss of virulence, a phenomenon that we had previously observed in another highly virulent isolate maintained for several months on a susceptible host.

Inoculation methods were the same as those described by Paul et al. (2013). Urediniospores were suspended in a solution of 0.01% Tween 20 in autoclaved distilled water and filtered through a 53-μm sieve to remove clumped spores. The suspensions were then diluted to a concentration of 4×10^4 urediniospores/ml prior to inoculation. The abaxial sides of the first trifoliate leaf on each plant were inoculated by spraying them until runoff using a Paasche H1208 single-action, siphon-fed airbrush (Paasche Airbrush Co., Taiwan) at a pressure of 1.4×10^5 Pa. Inoculated plants were incubated for 24 h in a mist chamber to promote infection. The greenhouse room was maintained at temperatures ranging between 22°C during the day and 20°C at night, with a 14-h photoperiod.

Reactions to the six *P. pachyrhizi* isolates were evaluated 14 days postinoculation using several criteria. Infection type was noted as being either TAN (susceptible), RB (incomplete resistance), or IM (immune or type 0) if no symptoms or signs of disease were visible. SBR severity on the first trifoliate leaf of each plant was rated on a scale of 1 to 5, in which 1 indicated that there were no visible symptoms or signs of infection, 2 corresponded to severity ranging from 1 to 10%, 3 corresponded to approximately 11 to 25%, 4 to 26 to 50%, and 5 indicated that at least 50% of the abaxial surface of a sample leaflet was covered with SBR lesions. Two 1.0-cm^2 circles were marked on either side of the midrib of a sample leaflet, and the numbers of lesions and uredinia inside each circle were counted. Mean lesion and uredinia densities were then calculated from those data and were used to calculate the average number of uredinia per lesion. Plants with

Table 1. Purified isolates of *Phakopsora pachyrhizi* used in soybean rust greenhouse seedling assays and detached-leaf assays

Isolate name	Geographical origin	Coordinates	Year collected
AL08-FH	Fairhope, AL (southern Alabama)	30°32′ N, 87°52′ W	2008
AL09-FH	Fairhope, AL (southern Alabama)	30°32′ N, 87°52′ W	2009
LA09-BC	Bossier City, LA (northwestern Louisiana)	32°25′ N, 93°38′ W	2009
FL09-Q	Quincy, FL (north-central Florida)	30°32′ N, 84°35′ W	2009
FL11-Q	Quincy, FL (north-central Florida)	30°32′ N, 84°35′ W	2011
FL12-Q	Quincy, FL (north-central Florida)	30°32′ N, 84°35′ W	2012

either no visible reaction or with RB lesions, or with significantly fewer uredinia per lesion than the susceptible checks were considered to be resistant.

Detached-leaf inoculation experiment. Detached-leaf inoculation assays were conducted to investigate the reactions of 27 accessions from the USDA Soybean Germplasm Collection, two susceptible checks, and the *Rpp1* backcross line L85-2378 to the six isolates used in the greenhouse assays (Supplementary Tables S3 and S4). A randomized complete block design with three replications was used. Nineteen PIs with unknown resistance genes were assayed, including 14 accessions that were not in the greenhouse experiment. One of these, PI 567046C, was a different subline from PI 567046A, which was assayed in both the greenhouse and detached-leaf experiments. The other accessions not previously tested in the greenhouse assay were PI 200487, PI 416778, PI 417126, PI 417129B, PI 423959, PI 423960B, PI 506938, PI 518295, PI 567024, PI 567054C, PI 567104B, and PI 594796. Nine accessions were included as differential genotypes representing the *Rpp1* through *Rpp6* resistance genes alone, and the combination of resistance genes at the *Rpp3* and *Rpp5* loci in Hyuuga (PI 506764). L85-2378 (PI 547875) is an isoline of Williams 82 into which the *Rpp1* gene from PI 200492 was backcrossed by R. L. Bernard. Data from some other studies suggest that PI 200487 and PI 471904 might have different alleles of the *Rpp5* gene (Garcia et al. 2008). PI 605823 could also be regarded as a differential because it is now known to have the *Rpp7* gene (Childs et al. 2018b). The lines used as susceptible controls were Williams 82 and LD00-3309 (PI 739740), a maturity group IV breeding line from the University of Illinois (Diers et al. 2006).

Seedlings from each soybean accession were grown in a growth chamber, as described by Paul and Hartman (2009). At the V3 to V4 stage of development, fully expanded leaves were collected and rinsed four to five times in autoclaved distilled water. These were briefly air dried and then transferred to sealable, clear plastic "clamshell" containers (21 by 13 by 3 cm) of the type designed to display items for retail sale. Each container had a moistened, autoclaved paper towel in the bottom to maintain a high humidity level. Inoculum used for this experiment was prepared using the same method that we used for the greenhouse study. Individual leaflets were placed adaxial side down on the moistened towels and the abaxial sides were inoculated until runoff occurred. Individual leaflets were labeled with 9-mm Tough-Spots round, adhesive colored labels (Diversified Biotech, Dedham, MA, U.S.A.) so that multiple genotypes could be assayed in the same container. The detached leaflets were then inoculated using a Paasche airbrush as described for the greenhouse seedling assay. The containers were sealed and placed in a growth chamber, where they were maintained for 2 weeks at 20 to 23°C and under a photoperiod of 12 h of light and 12 h of darkness. For 24 h after inoculation, the culture containers were covered with aluminum foil to shade the urediniospores in order to promote infection of the plant tissue.

Infection type was determined and disease severity was rated using the same 1-to-5 scale used in the greenhouse assays. Four 1.0-cm^2 circles were marked and the number of lesions in each was counted as had been done in the greenhouse assays. The circles were then excised from each leaflet using a cork borer and the discs were transferred to one well in a 24-well plate with a flat bottom and a low evaporation lid. The discs were bathed for 24 h in a 3:1 (vol/vol) solution of absolute ethanol and acetic acid to fix and clear the tissue of chlorophyll and other pigments (Bonde et al. 2006; Paul and Hartman 2009). The discs were then transferred to lactophenol for 24 h to further clear the tissue, and urediniospores were subsequently stained with 0.1% cotton blue in lactophenol for 24 h. After the leaf tissue was rinsed twice with distilled water, the stained uredinia were counted at ×100 magnification. The number of uredinia per lesion was then determined by dividing the number of uredinia on a leaf disc by the number of SBR lesions. Plant reactions were considered to be resistant if inoculated leaves had no visible lesions or had RB lesions with an average number of uredinia per lesion that was significantly lower than the numbers on the susceptible checks.

Statistical analyses. Quantitative data were analyzed for statistically significant differences using PROC MIXED in SAS (SAS Institute, Cary, NC, U.S.A.). Data were first tested for heterogeneity of error variances and transformed prior to analysis of variance and means separation if necessary. In the case of the severity scores, a square-root transformation was used and, for the lesion and uredinia density data, the log to the base 10 was used after the addition of 1 to each data point. After confirmation that there were significant differences among the treatment means based on Fisher's protected least significant difference (LSD) ($P \leq 0.05$), the transformed means were separated based on the LSD. Transformed data means were later back-transformed to obtain the values presented in the tables. For the data from the detached-leaf study, a Pearson correlation coefficient analysis was conducted on the disease severity ratings, the counts of uredinia per square centimeter made on the live tissue, and the uredinia counts made on the cleared tissue.

Results

Greenhouse experiment. Infection type (i.e., IM, RB, or TAN) reactions and mean numbers of uredinia per lesion from the greenhouse experiment are shown in Table 2, where they are listed in order of increasing uredinia numbers, averaged across the six isolates. *Rpp* genes and resistance alleles are included in Table 2 if known, though the *Rpp* genes in many of the PIs were not yet known when the experiments were begun. Although disease severity ratings and numbers of lesions per square centimeter were often higher for lines with TAN reactions (Supplementary Tables S1 and S2), the number of uredinia per lesion was a more informative indicator of resistance and the reproduction potential of each isolate on a specific host genotype. For example, the numbers of lesions per square centimeter in RB and TAN lesions induced by the FL11-Q isolate were often similar (Supplementary Table S2), whereas all but one of the soybean lines that developed RB infection types averaged 1.1 or fewer uredinia per lesion (Table 2). The mean number of uredinia per RB lesion in this experiment was 0.17 ± 0.28 (standard error [SE]), compared with an average of 2.15 ± 0.42 (SE) uredinia in TAN lesions. The exception, PI 417089A, averaged 1.3 uredinia per RB lesion caused by the LA09-BC isolate. PI 417089B leaves infected with the FL12-Q isolate also averaged 1.3 uredinia per lesion but the infection type was considered to be TAN due to a greater abundance of urediniospores. The average number of uredinia per lesion in TAN lesions for specific soybean-isolate combinations ranged from 1.3 to 3.6.

Because soybean PI–SBR isolate interactions had a significant ($P \leq 0.05$) effect on the quantitative disease ratings, the quantitative disease reactions to each isolate were analyzed separately. The 2011 and 2012 isolates from Quincy, FL were more virulent than the 2009 isolate from Quincy or the 2008 and 2009 isolates from Alabama and Louisiana (Table 2; Supplementary Tables S1 and S2). The FL12-Q isolate was the most virulent and aggressive isolate, causing a TAN reaction on 33 of 41 PIs (80%), and on nine accessions that had RB reactions to the FL11-Q isolate, which induced a TAN reaction on 24 of the PIs (Table 2).

All of the cultivars and elite lines used as susceptible checks (Williams 82, LD00-2817P, R00-1194F, and 5601T) developed TAN reactions to all of the six *P. pachyrhizi* isolates (Table 2). Across isolates, the checks had 2.30 to 2.85 uredinia/SBR lesion. In contrast, 32 of the other 41 entries averaged less than 1.0 uredinium/lesion. The reactions of the lines that were included as differentials (i.e., accessions whose *Rpp* gene locations were known at the time the assays were conducted) were highly variable. PI 471904 and PI 506764 (Hyuuga) were the only accessions from the differential set that had an RB reaction to all six isolates. The former has a resistance allele at the *Rpp5* locus and Hyuuga has *Rpp3* and a second resistance allele at the *Rpp5* locus. The *Rpp6* gene in PI 567102B provided a high level of resistance to all of the isolates except FL12-Q, while *Rpp1* (PI 200492), *Rpp2* (PI 230970), *Rpp3* (PI 462312), and *Rpp7* (PI 605823) provided resistance to all of the isolates except the 2011 and 2012 isolates from Florida (Table 2). At the time the plants were inspected (2 weeks postinoculation), no SBR

symptoms were visible on PI 200492 plants challenged with the FL09-Q isolate. In contrast, the *Rpp1-b* gene in PI 594538A and the allele at the *Rpp1* locus in PI 587880A did not confer resistance to any of the isolates, and the *Rpp4* gene from PI 459025B also provided little or no resistance to any of the isolates. Like PI 200492, PI 416826A did not develop any macroscopically visible lesions when challenged with the FL09-Q isolate.

Three differentials known to have a resistance allele at the *Rpp5* locus had different reaction patterns to the six isolates (Table 2). Although PI 471904 had RB reactions to all of the isolates, the recessive *rpp5* allele from PI 200456 conferred resistance to only the Alabama and Louisiana isolates. PI 200526, which has a dominant allele at the *Rpp5* locus, was susceptible to all six isolates. PI 567102B (*Rpp6*) had RB reactions to all of the isolates except FL12-Q. PI 605823, which carries the *Rpp7* gene recently discovered by Childs et al. (2018b), was resistant to all of the 2008 and 2009 isolates but had TAN reactions to the FL11-Q and FL12-Q isolates.

PI 224268, PI 567025A, PI 567039, PI 567046A, and the Vietnamese cultivar DT 2000 (PI 635999) developed RB reactions to all six isolates, whereas PI 203398, PI 417208, PI 567129, PI 567189A, PI 605773, PI 605791A, PI 605854B, PI 605891A, and PI 606440A had resistance to all of the isolates except for FL12-Q

Table 2. Infection type and average number of uredinia per lesion on 41 soybean germplasm accessions (plant introductions [PIs]) and four susceptible checks challenged with six *Phakopsora pachyrhizi* (soybean rust) isolates from locations in the southern United States in greenhouse seedling assays[a]

Line	Resistance genes[b]	Comments	AL08-FH	AL09-FH	LA09-BC	FL09-Q	FL11-Q	FL12-Q	Mean[c]	SE[d]
PI 567025A	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.1*	*0.4*	0.08	0.07
PI 506764	*Rpp3* + *Rpp5* allele	Hyuuga	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.7*	0.12	0.12
PI 567046A	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.2*	*0.6*	0.13	0.10
PI 567039	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.1*	*0.8*	0.15	0.13
PI 635999	*Rpp3* + *Rpp4* allele	DT 2000	*0.2*	*0.0*	*0.0*	*0.0*	*0.2*	*0.5*	0.15	0.08
PI 471904	*Rpp5* allele	Differential	*0.0*	*0.0*	*0.0*	*0.1*	*0.1*	*1.1*	0.22	0.18
PI 224268	Unknown	…	*0.0*	*0.1*	*0.1*	*0.0*	*0.5*	*1.1*	0.30	0.18
PI 203398	Unknown	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.2*	**1.9**	0.35	0.31
PI 605854B	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.3*	**1.9**	0.37	0.31
PI 567102B	*Rpp6*	Differential	*0.0*	*0.1*	*0.0*	*0.0*	*0.1*	**2.0**	0.37	0.33
PI 417208	Unknown	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.3*	**2.0**	0.38	0.33
PI 605773	Unknown	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.3*	**2.0**	0.38	0.33
PI 567189A	Unknown	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.3*	**2.1**	0.40	0.34
PI 605891A	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	*0.3*	**2.1**	0.40	0.34
PI 605865B	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	**1.6**	**1.9**	0.58	0.37
PI 417503	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	**1.7**	**2.0**	0.62	0.39
PI 567129	(*Rpp6*)	…	*0.3*	*0.0*	*0.0*	*0.4*	*0.5*	**2.5**	0.62	0.39
PI 416826A	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	0.0	**1.9**	**2.0**	0.65	0.41
PI 567104B	(*Rpp6*)	…	*0.0*	*0.2*	*0.0*	*0.0*	**1.7**	**2.0**	0.65	0.38
PI 417132	(*Rpp3*)	…	*0.1*	*0.0*	*0.0*	*0.0*	**1.7**	**2.4**	0.70	0.44
PI 605885B	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	**1.7**	**2.7**	0.73	0.48
PI 606405	(*Rpp3*)	…	*0.0*	*0.0*	*0.1*	*0.0*	**1.7**	**2.6**	0.73	0.46
PI 417125	Unknown	…	*0.1*	*0.6*	*0.5*	*0.1*	**1.4**	**1.8**	0.75	0.29
PI 605838	(*Rpp3*)	…	*0.0*	*0.0*	*0.0*	*0.0*	**1.8**	**2.7**	0.75	0.49
PI 606440A	Unknown	…	*0.0*	*0.6*	*0.5*	*0.6*	*0.4*	**2.4**	0.75	0.34
PI 615437	(*Rpp3*)	…	*0.0*	*0.4*	*0.0*	*0.0*	**1.7**	**2.4**	0.75	0.43
PI 230970	*Rpp2*	Differential	*0.3*	*0.1*	*0.5*	*0.9*	**1.3**	**1.5**	0.77	0.23
PI 605791A	(*Rpp4*)	…	*0.6*	*0.5*	*1.0*	*0.3*	*0.4*	**1.8**	0.77	0.23
PI 417089B	(*Rpp3*)?	…	*0.2*	*0.4*	*0.0*	*0.0*	**2.7**	**1.3**	0.77	0.43
PI 417089A	(*Rpp3*)	…	*0.2*	*0.0*	*1.3*	*0.0*	**2.3**	*0.9*	0.78	0.37
PI 462312	*Rpp3*	Differential	*0.1*	*0.1*	*0.0*	*0.3*	**2.0**	**2.7**	0.87	0.48
PI 200492	*Rpp1*	Differential	*0.7*	*0.2*	*0.1*	0.0	**1.8**	**2.5**	0.88	0.42
PI 605823	*Rpp7*	Differential	*1.1*	*0.6*	*1.0*	*0.6*	**1.7**	**2.5**	1.25	0.30
PI 200456	*rpp5*	Differential	*0.4*	*0.4*	*0.5*	**2.2**	**2.1**	**2.4**	1.33	0.40
PI 459025B	*Rpp4*	Differential	**1.5**	**1.6**	**2.1**	**1.5**	**2.0**	**2.3**	1.83	0.14
PI 567351B	Unknown	…	**1.5**	**2.0**	**2.0**	**1.9**	**2.0**	**2.3**	1.95	0.11
PI 200526	*Rpp5* allele	Differential	**1.9**	**1.9**	**1.9**	**1.9**	**2.2**	**2.5**	2.05	0.10
PI 594538B	*Rpp1-b*	Differential	**1.9**	**1.7**	**2.2**	**1.9**	**2.3**	**2.5**	2.08	0.12
PI 567056B	(*Rpp3*)	…	**2.2**	**2.0**	**2.3**	**1.9**	**2.1**	**2.3**	2.13	0.07
PI 470227B	Unknown	…	**2.3**	**2.0**	**2.7**	**1.9**	**1.7**	**2.3**	2.15	0.15
LD00-2817P	None	Check	**2.1**	**2.3**	**2.5**	**1.6**	**2.5**	**2.8**	2.30	0.17
PI 587880A	*Rpp1* allele	Differential	**2.7**	**3.1**	**2.0**	**2.0**	**1.9**	**2.1**	2.30	0.20
R00-1194F	None	Check	**2.3**	**2.1**	**2.5**	**2.2**	**2.4**	**2.6**	2.35	0.08
5601T	None	Check	**3.4**	**2.0**	**2.3**	**2.2**	**2.4**	**2.7**	2.50	0.20
Williams 82	None	Check	**2.6**	**2.4**	**2.5**	**2.8**	**3.2**	**3.6**	2.85	0.19
LSD (0.05)[e]	…	…	0.88	0.64	0.96	0.55	0.86	0.85	0.79	…

[a] Infection type is indicated by bold, italics, or roman type and numerical values represent the mean number of uredinia per lesion that developed on seedlings of a soybean line inoculated with a specific fungal isolate. Host–inoculum combinations that resulted in a reddish-brown resistance infection type are in italics and those resulting in a TAN susceptibility infection type are in bold. Combinations that produced no macroscopically visible disease symptoms are in roman. *Rpp* resistance genes are listed if known, and lines used as differentials are indicated.

[b] Gene names in parentheses indicate genomic locations of resistance alleles that may or may not be the same allele as the original gene given that name.

[c] Means are the number of uredinia per lesion averaged across the six isolates.

[d] Standard error.

[e] Least significant difference.

(Table 2). Ten other accessions were resistant to the 2008 and 2009 isolates but were susceptible to the Florida isolates from 2011 and 2012. PI 417089A, which is now known to have a resistance allele at the *Rpp3* locus, was unique among the lines tested in having a TAN reaction to the FL11-Q isolate but an RB reaction to FL12-Q. The closely related accession PI 417089B was rated as having a TAN reaction to the 2011 and 2012 Florida isolates but the number of uredinia per lesion from infection with FL12-Q was not significantly higher than the number in the RB lesions of PI 417089A. Although PI 417089A had an RB reaction to LA09-BC, the lesions had an unusually high number of uredinia compared with other lines with RB lesions. Among the 10 PIs that were resistant to all of the isolates except FL11-Q and FL12-Q, PI 416826A was unique in having no visible reaction to the FL09-Q isolate at the time ratings were done (Table 2).

Fifteen accessions had RB reactions to the Alabama and Louisiana isolates and no visible uredinia resulting from infection by those three isolates (Table 2). PI 567025A, PI 567039, PI 567046A, PI 203398, PI 417208, PI 567189A, PI 605773, PI 605854B, PI 605891A, PI 417503, PI 605838, PI 605865B, PI 605885B, PI 416826A, and PI 635999 had similarly high levels of resistance to the 2009 isolate from Florida. Of these 15 PIs, 9 also had RB reactions to the FL11-Q isolate, though some of the lesions developed uredinia (Table 2). Accessions that averaged 0.2 or fewer uredinia per lesion from infection by only one or two of the 2008 and 2009 isolates included the differentials PI 471904 (allele of *Rpp5*) and PI 567102B (*Rpp6*) and the lines PI 224268, PI 417132, PI 567104B, and PI 606405. In contrast, PI 470227B, PI 567056B, and PI 567351B were susceptible to all six isolates, despite an allele at the *Rpp3* locus in PI 567056A that provided resistance to some foreign *P. pachyrhizi* isolates (Harris et al. 2015). Averaged across the six isolates, these susceptible PIs had 1.95 to 2.15 uredinia per lesion.

PI 605854B and PI 605891A have a resistance gene at the *Rpp3* locus, PI 605791A has a gene at the *Rpp4* locus, and PI 567129 has a gene at the *Rpp6* locus (Harris et al. 2015). The locations of the resistance genes in PI 203398, PI 417208 567189A, PI 605773, and PI 606440A have not yet been reported. Although these nine accessions had TAN reactions to the FL12-Q isolate, seven averaged 2.1 or fewer uredinia per lesion compared with 2.6 to 3.6 for the four susceptible checks.

Overall, the average number of uredinia per lesion was 2.0 or higher on most leaves that developed TAN lesions, and was usually less than 1.0 on most of those with RB lesions (Table 2). Many RB lesions had no visible uredinia 2 weeks after inoculation. Only four host–isolate combinations resulted in RB lesions that averaged more than 1.0 uredinium/lesion.

Detached-leaf experiment. Infection type and average number of uredinia per lesion data from the detached-leaf assays are presented in Table 3 in order of increasing numbers of uredinia per lesion, averaged across the six isolates, and disease severity rating and lesion density data are provided in Supplementary Tables S3 and S4. Twelve PIs screened in this experiment were not included in the greenhouse experiment but the reactions of all of the soybean

Table 3. Infection type and average number of uredinia per lesion on 27 germplasm accessions (plant introductions [PIs]), two susceptible checks, and one *Rpp1* isoline (L85-2378) challenged with six *Phakopsora pachyrhizi* (soybean rust) isolates from locations in the southern United States in detached-leaf assays[a]

Line	*Rpp* genes[b]	Comments	AL08-FH	AL09-FH	LA09-BC	FL09-Q	FL11-Q	FL12-Q	Mean	SE[c]
PI 471904	*Rpp5*	Differential	*0.2*	*0.0*	*0.1*	*0.1*	*0.0*	*1.0*	0.23	0.16
PI 567046A	(*Rpp3*)	…	*0.1*	*0.0*	*0.0*	*0.0*	*0.4*	*1.0*	0.25	0.16
PI 200487	*Rpp5*	Differential	*0.2*	*0.0*	*0.0*	*0.0*	*0.2*	*1.2*	0.27	0.19
PI 567025A	(*Rpp3*)	…	*0.3*	*0.0*	*0.0*	*0.3*	*0.2*	*1.2*	0.33	0.18
PI 635999	*Rpp3* + *Rpp4*	DT 2000	*0.1*	*0.0*	*0.1*	*0.3*	*0.2*	*1.3*	0.33	0.20
PI 567054C	(*Rpp3*)	…	*0.3*	*0.0*	*0.0*	*0.0*	*0.2*	**1.7**	0.36	0.27
PI 567024	(*Rpp4*)	…	*0.0*	*0.1*	*0.1*	*0.0*	*0.5*	**1.8**	0.40	0.28
PI 506764	*Rpp3* + *Rpp5*	Hyuuga	*0.0*	*0.1*	*0.0*	*0.3*	*0.7*	*1.5*	0.44	0.24
PI 224268	Unknown	…	*0.0*	*0.1*	*0.1*	*0.0*	*1.3*	*1.6*	0.52	0.30
PI 567102B	*Rpp6*	Differential	*0.0*	*0.0*	*0.1*	*0.0*	*0.6*	**2.5**	0.54	0.40
PI 423960B	Unknown	…	*0.2*	*0.0*	*0.0*	*0.7*	*0.9*	*1.5*	0.55	0.25
PI 417125	Unknown	…	*0.0*	*0.2*	*0.0*	*0.3*	**1.6**	**1.7**	0.63	0.33
PI 594796	Unknown	…	**1.5**	*0.2*	*0.6*	*0.3*	*0.3*	**1.3**	0.69	0.23
PI 423959	Unknown	…	*0.6*	*0.1*	*0.0*	*0.0*	**1.2**	**2.3**	0.70	0.38
PI 567056A	(*Rpp3*)	…	*0.0*	*0.0*	*0.1*	*1.0*	**1.3**	**1.9**	0.72	0.33
PI 518295	Unknown	…	*0.4*	*0.2*	*0.4*	0.0	*0.9*	**3.5**	0.91	0.53
PI 567104B	(*Rpp6*)	…	*0.0*	*0.1*	*0.4*	*0.0*	**1.9**	**3.5**	0.98	0.59
PI 200492	*Rpp1*	Differential	*0.4*	*0.1*	*0.2*	0.0	**2.4**	**2.9**	1.00	0.53
L85-2378	*Rpp1* isoline	Differential	*0.0*	*0.3*	*0.3*	0.0	**2.0**	**3.4**	1.01	0.57
PI 462312	*Rpp3*	Differential	*0.2*	*0.1*	*0.1*	*0.5*	**1.8**	**3.9**	1.10	0.62
PI 417126	Unknown	…	*0.6*	*0.5*	**1.4**	**1.4**	**1.6**	**1.9**	1.21	0.22
PI 230970	*Rpp2*	Differential	*1.1*	*1.4*	*0.7*	*1.0*	**1.5**	**2.0**	1.28	0.19
PI 605823	*Rpp7*	Differential	*0.2*	*0.8*	*0.9*	*0.3*	**2.1**	**3.4**	1.28	0.51
PI 416778	Unknown	…	*0.5*	*1.2*	**1.5**	**1.8**	**2.0**	*1.6*	1.43	0.21
PI 417129B	Unknown	…	**1.5**	*0.0*	*0.5*	**2.2**	**2.7**	**2.9**	1.64	0.48
PI 506938	Unknown	…	*1.1*	**2.3**	**2.3**	*1.1*	**2.0**	**2.7**	1.91	0.28
PI 567046C	Unknown	…	**3.5**	**4.6**	**2.7**	*0.5*	**1.6**	*0.6*	2.25	0.66
PI 459025B	*Rpp4*	Differential	**2.6**	**1.2**	**3.7**	**2.2**	**2.4**	**2.6**	2.44	0.33
LD00-3309	None	Check	**2.2**	**2.0**	**3.2**	**2.9**	**3.6**	**4.1**	3.00	0.33
Williams 82	None	Check	**3.3**	**5.1**	**3.3**	**3.3**	**3.9**	**4.4**	3.89	0.30
LSD (0.05)[d]	…	…	0.13	0.10	0.10	0.07	0.08	0.11	0.10	…

[a] Infection types are indicated by bold, italics, and roman type, and the numerical values represent mean numbers of uredinia per lesion that developed on seedlings of a soybean line challenged with a specific fungal isolate. Host-inoculum combinations that resulted in a reddish-brown resistance infection type are in italics and those resulting in a TAN susceptibility infection type are in bold. Combinations that produced no macroscopically visible disease symptoms are in roman. *Rpp* resistance genes are listed if known, and the lines used as differentials are indicated.

[b] Gene names in parentheses indicate genomic locations of resistance alleles that may or may not be the same allele as the original gene given that name.

[c] Standard error.

[d] Least significant difference.

accessions that were in both experiments were very similar. As in the greenhouse assays, differential reactions to the isolates were observed, often involving reactions to the 2011 and 2012 isolates from Florida. Averaged across the six isolates, the mean number of uredinia per lesion ranged from 0.23 on PI 471904 to 3.89 on Williams 82 (Table 3). The mean number of uredinia per RB lesion in this experiment was 0.36 ± 0.04 (SE), and TAN lesions averaged 2.38 ± 0.09 (SE) uredinia/lesion.

The two susceptible checks, LD00-3309 and Williams 82, developed a TAN infection type to all six isolates (Table 3). Based on the number of uredinia per lesion, Williams 82 appeared to be more susceptible to the AL09-FH isolate than to the other isolates; however, because this was not observed in the greenhouse assays, it would need to be confirmed. Although LD00-3309 was susceptible to all of the isolates, it appeared to be somewhat less susceptible than Williams 82 to all of the isolates except LA09-BC based on uredinia counts. It even had fewer uredinia per lesion than PI 459025B (source of the *Rpp4* gene) when challenged with the Alabama and Louisiana isolates, and fewer than PI 567046 when both were challenged with the two Alabama isolates. Most of these differences were small but they were statistically significant.

Eight lines, including the differentials PI 200487, PI 471904, and Hyuuga (PI 506764), had an RB reaction to all of the isolates (Table 3). PI 200487 and PI 471904 both averaged 0.33 or fewer uredinia per lesion. Other highly resistant accessions included two lines with resistance alleles at the *Rpp3* locus (PI 567046A and PI 567025A) and two whose resistance genes have not yet been reported (PI 224268 and PI 423960B). Three lines were resistant to all of the isolates except FL12-Q.

PI 200492 (*Rpp1*), PI 230970 (*Rpp2*), and PI 462312 (*Rpp3*) showed resistance to all of the isolates except FL11-Q and FL12-Q (Table 3). L85-2378, the Williams 82 isoline into which the *Rpp1* gene had been introgressed from PI 200492, had the same reaction pattern as PI 200492, and had similar numbers of uredinia per lesion for each isolate. PI 200492 and L85-2378, along with PI 518295, did not develop any visible lesions when infected with the FL09-Q isolate, confirming the immunity or near-immunity of PI 200492 against this isolate in the greenhouse assays (Table 2). As in the greenhouse experiment, the *Rpp4* gene of PI 459025B did not provide much resistance to any of the isolates, though it may have suppressed the number of uredinia per lesion somewhat on leaves infected with AL09-FH and the isolates from Florida. The *Rpp5* resistance alleles of PI 200487 and PI 471904 conditioned RB reactions to all six isolates, as did the resistance alleles at the *Rpp3* and *Rpp5* loci in Hyuuga (PI 506764). The *Rpp6* gene in PI 567102B provided resistance to all of the isolates except FL12-Q, and PI 567102B plants challenged with that isolate averaged only 2.5 uredinia/lesion compared with 4.4 on Williams 82 and 4.1 on LD00-3309, indicating that resistance mediated by the *Rpp6* gene had not been entirely overcome (Table 3). The *Rpp7* gene in PI 605823 conditioned an RB reaction to all of the isolates except FL12-Q and FL11-Q. The reactions of differential lines observed in the detached-leaf assay thus corroborate those seen in the greenhouse assay.

Fourteen of the germplasm accessions evaluated using the detached-leaf assays had also been in the greenhouse resistance evaluations, including Hyuuga and six other differentials with known *Rpp* genes (Table 3). These included PI 567046A, PI 567025A, DT 2000 (PI 635999), PI 224268, PI 417125, PI 567104B, and PI 605823, all of which had resistance to the 2008 and 2009 isolates in both types of assays (Tables 2 and 3). PI 567046A, PI 567025A, and DT 2000, which were among the most resistant accessions tested in the greenhouse assays (Table 2), were also resistant to all of the isolates and had some of the lowest uredinia counts in the detached-leaf assays (Table 3). PI 567025A and PI 567046A are now known to carry a resistance allele at the *Rpp3* locus, and there has been speculation that PI 423960B might have more than one resistance gene (Harris et al. 2015). PI 417125, PI 567104B, and PI 605823 (*Rpp7*) were resistant to all of the isolates except FL11-Q and FL12-Q, and had the same infection type reaction patterns as they had in the greenhouse experiment. PI 423959 and PI 567056A were also resistant to all of the isolates except FL11-Q and FL12-Q but had not been tested in the greenhouse experiment. Of the accessions in this experiment that were not included in the greenhouse assays, PI 518295 and PI 567024 (*Rpp4* allele) and PI 567054C (*Rpp3* allele) were resistant to all isolates except FL12-Q (Supplementary Tables S3 and S4).

Several of the accessions assayed had unusual or unexpected patterns of reaction to the six isolates. PI 416778 had an RB reaction to the two Alabama isolates and, surprisingly, to the FL12-Q isolate but was susceptible to the other isolates (Table 3). PI 417126, PI 594796, PI 417129B, and PI 506938 each had unique reaction patterns to the six isolates. All had a TAN reaction to the FL12-Q isolate but variable reactions to the other isolates. The reactions of PI 567046C were particularly unusual and were very different from the reactions of the PI 567046A subline, which was highly resistant in the greenhouse and detached-leaf experiments. The PI 567046C subline developed a TAN reaction to all of the isolates except the FL12-Q and FL09-Q isolates, even though FL12-Q was the most virulent and aggressive of the six isolates. In contrast, the "A" subline developed an RB response to all six isolates in the greenhouse experiment and in the detached-leaf assays (Tables 2 and 3). It is possible that 567046A has an *Rpp* gene that is absent in PI 567046C. Because of the level of sporulation observed on it, PI 594796 was classified as having a TAN reaction to 12FL-Q, even though it had fewer uredinia per lesion than some other accessions that had RB reactions (Table 3).

Correlations between severity and uredinia counts based on live tissue ($r = 0.45$, $P < 0.0001$) and fixed tissue ($r = 0.46$, $P < 0.0001$) were significant for the accessions in the detached-leaf assay. There was a much stronger correlation ($r = 0.98$, $P < 0.0001$) between the uredinia counts made on live tissue and fixed tissue, indicating that the tissue clearing and staining procedure should not be necessary for evaluating SBR resistance and selecting SBR-resistant plants and lines. The uredinia per lesion counts in the detached-leaf assays were higher. A higher inoculum dosage or more favorable germination conditions for urediniospores may have contributed to this.

***Phakopsora pachyrhizi* isolate pathotypes.** Variation in the reactions of the soybean PIs in this study show that the six *P. pachyrhizi* isolates represented different pathotypes. The differential reactions of the soybean lines to different isolates suggest temporal and more subtle geographical differences in pathogenicity. Although the reactions of the PIs assayed in the greenhouse suggested that the 2008 and 2009 isolates shared similarities in their pathotypes, the differential reactions of lines such as PI 416778, PI 417129B, and PI 506938 in the detached-leaf study suggested some pathotype differences among those isolates (Table 3). In the greenhouse assays, PI 200456 (*rpp5*) had RB reactions to the Alabama and Louisiana isolates but developed a TAN reaction and more uredinia per lesion from the FL09-Q isolate (Table 2). The unusual and unexpected reaction patterns of PI 416778, PI 417126, PI 417129B, PI 506938, PI 567046C, and PI 594796 to the 2008 and 2009 isolates in the detached-leaf experiment, however, revealed differences in pathogenicity. None of these six informative accessions had been evaluated in the greenhouse experiment.

The FL12-Q isolate was more virulent than the 2009 and 2011 isolates from Florida, inducing TAN reactions on 80% of the accessions tested compared with 59% for FL11-Q and 30% for FL09-Q (Tables 2 and 3). The virulence of the FL11-Q isolate was intermediate between those of the FL09-Q and FL12-Q isolates. It was similar to FL12-Q in being able to produce a TAN reaction on the PIs with the *Rpp2* and *Rpp3* genes and 11 other accessions in the greenhouse experiment but was less virulent than FL12-Q on 9 accessions (Table 2). As had been the case in the reactions to the Alabama and Louisiana isolates, the reactions of PI 416778, PI 417129B, PI 506938, and PI 594796 indicated differences in pathogenicity among the Florida isolates in the detached-leaf assays (Table 3).

In the greenhouse experiment, the uredinia per lesion counts on the four susceptible checks indicated that there were no large differences

in the aggressiveness of the six isolates, though the FL12-Q isolate induced the highest or second highest number of uredinia per lesion on each of the checks (Table 2). This was also true in the detached-leaf experiment, though the AL09-FH isolate induced the highest number of uredinia per lesion on Williams 82 (Table 3). Differences in the reactions of susceptible cultivars within each experiment and in the numbers of lesions counted on Williams 82 in the two experiments indicated that additional data would be needed to confirm any possible differences in aggressiveness.

Discussion

This study provided new information about (i) the resistance of 44 germplasm accessions to a panel of diverse *P. pachyrhizi* (SBR) isolates representing four growing seasons and three geographical locations, (ii) allelic diversity among accessions that have resistance gene alleles at the same *Rpp* locus, (iii) the efficacy of natural *Rpp* gene pyramids in Hyuuga (PI 506764) and DT 2000 (PI 635999), and (iv) pathotype diversity among the six *P. pachyrhizi* isolates used. The soybean lines tested were either PIs with known *Rpp* genes, which were included as differentials, or PIs that had resistance in field tests in the southern United States (Walker et al. 2011, 2014a,b). Pathogenic variation among the six *P. pachyrhizi* isolates was manifested as differences in infection type (i.e., TAN, RB, or IM) and as quantitative variation in the numbers of uredinia per SBR lesion. The germplasm accessions that were assayed in both the greenhouse and detached-leaf experiments had similar reactions in both. The higher uredinia per lesion counts in the detached-leaf assays were due, in part, to the fact that clearing and staining the leaves made uredinia on both sides of a leaf visible. Developing uredinia that might not have been visible on a live leaf might have also been distinguishable. Although we have focused on infection type and differences in the number of uredinia per lesion in the text, severity ratings were almost always higher for soybean PIs with TAN reactions and higher lesion densities (Supplementary Tables S1 through S4).

Resistance to *P. pachyrhizi* has often been assessed primarily on the basis of infection type but, as Shaner et al. (1992) pointed out, virulence can be complex. Bromfield (1984) suggested that the RB and TAN infection types should be subdivided on the basis of the number of uredinia per lesion and the amount of sporulation, and other researchers have developed more precise reaction classification criteria for SBR lesions (Miles et al. 2011; Yamanaka et al. 2010). In the present study, we regarded uredinia per lesion to be an important measurement of resistance based on the conclusions of Yamanaka et al. (2010) and Miles et al. (2011). However, infection type (i.e., lesion color) was generally a reliable indicator of the average number of uredinia per lesion, and could be determined much more rapidly than uredinia per lesion counts.

The results of the experiments confirmed that PIs with resistance genes at the same *Rpp* locus or genomic region can have very different reactions to certain isolates of *P. pachyrhizi*. The more resistant lines most likely have either a different allele at the same *Rpp* locus or an additional undetected *Rpp* gene that contributes to their resistance. The results also confirmed that there was pathotype diversity among the six isolates used in this study, particularly between the more virulent FL11-Q and FL12-Q isolates and the four less virulent isolates from 2008 and 2009.

At the time this study was conducted, the SBR resistance genes in many of the PIs assayed in these experiments had not yet been mapped but, since then, the identities and locations of *Rpp* genes in many of the genotypes have been reported. PI 605823 is of particular interest, because it is now known to carry the *Rpp7* at a previously unreported locus on chromosome (Chr) 19 (Childs et al. 2018b). Many of the accessions genotyped by Harris et al. (2015) have a resistance gene at the *Rpp3* locus based on bulked segregant analysis, and resistance genes in other lines that were tested have been mapped to the *Rpp4, Rpp6*, or *Rpp3* and *Rpp4* loci (Harris et al. 2015).

Resistance genes at the *Rpp1* locus on Chr 18 were represented in the greenhouse experiment by PI 200492 (*Rpp1*), PI 594538B (*Rpp1-b*), and PI 587880A. Although the *Rpp1* gene of PI 200492 was initially effective against South American populations of *P. pachyrhizi*, the resistance was overcome by some fungal populations within two growing seasons after the appearance of SBR in Brazil (Ribeiro et al. 2007). PI 200492 and PI 547875 (L85-2378), a Williams 82 isoline with the same *Rpp1* allele, were resistant to *P. pachyrhizi* field populations in the southern United States in multiple locations and growing seasons but they were susceptible to the 2012 population in Quincy, FL (Walker et al. 2011, 2014a). At least one pathotype in that field population, from which the FL12-Q isolate was purified, had the ability to reproduce vigorously on both PI 200492 (*Rpp1*) and PI 567102B (*Rpp6*), lines which have historically had high levels of resistance to many U.S. fungal populations and isolates (Paul et al. 2013; Walker et al. 2011, 2014a,b). Although resistance conditioned by the *Rpp1* gene from PI 200492 was overcome by the 2011 and 2012 isolates from Quincy, FL, in the present study, it was effective against field populations and isolates from the same location in subsequent growing seasons (unpublished data). In contrast to the *Rpp1* gene from PI 200492, the *Rpp1-b* gene of PI 594538 and the *Rpp1* allele in PI 587880A have not provided any resistance to *P. pachyrhizi* populations in the United States, despite being effective against *P. pachyrhizi* isolates and populations in South America (Ray et al. 2009).

Harris et al. (2015) found that 52 of 75 PIs with SBR resistance in the United States have a resistance gene in the *Rpp3* region on Chr 6, and at least 19 of the lines evaluated in the present study are thought to have a resistance gene at the *Rpp3* locus. Moreover, Hyuuga (PI 506764) and DT 2000 (PI 635999) have a resistance gene at the *Rpp3* locus in addition to a second *Rpp* gene at a different locus. The lines from this study that carry an *Rpp3* resistance allele can be classified into four groups: (i) those that were resistant to all six isolates (based on having RB lesions with few or no uredinia), (ii) those that were resistant to all of the isolates except FL12-Q, (iii) those resistant to all of the 2008 and 2009 isolates but not to FL11-Q or FL12-Q, and (iv) those that were susceptible to all of the isolates. Five of the most resistant lines in the greenhouse assays, including Hyuuga and DT 2000, have a resistance allele at the *Rpp3* locus, and five of the eight most resistant lines in the detached-leaf assays also have an *Rpp3* resistance allele. It remains unclear whether PI 567025A, PI 567039, PI 567046A, and PI 567054C possess an allele of the *Rpp3* gene that is more effective in recognizing U.S. isolates of *P. pachyrhizi* than the "original" *Rpp3* gene of PI 462312, or if they carry an additional unreported *Rpp* gene at another locus. PI 567025A, PI 567039, and PI 567046A all originate from Indonesia; therefore, it is possible that they have the same allele of *Rpp3*. The second group of lines from the greenhouse experiment included PI 605854B and PI 605891A, and the third group, which was the largest, included PI 462312 (source of the original *Rpp3* gene) and at least eight other PIs. PI 567056A was only in the detached-leaf experiment but its reaction pattern was like that of PI 462312. This was particularly interesting because, in the greenhouse experiment, the closely related accession PI 567056B was the only line with a resistance gene at the *Rpp3* locus that was susceptible to all six isolates.

The extent to which the second *Rpp* gene in Hyuuga and DT 2000 contributes to their resistance to U.S. isolates of *P. pachyrhizi* is also unclear. In some previous investigations, resistance segregation ratios and genetic mapping analyses of both lines suggested that the *Rpp3* allele was responsible for most of their resistance (Monteros et al. 2007; Vuong et al. 2016). For both lines, phenotypic data for the reactions to *P. pachyrhizi* isolates from other countries were key to revealing the presence of a second *Rpp* gene. DT 2000 (PI 635999) is a cultivar from Vietnam with SBR resistance genes at the *Rpp3* and *Rpp4* loci (Vuong et al. 2016). The effect of the *Rpp4* gene was detected using SBR disease data from Hanoi, Vietnam, but was not significantly associated with resistance in Quincy, FL. In the present study, the allele of the *Rpp4* gene in PI 459025B may have slowed uredinium development from some isolates but it did not prevent the development of a TAN reaction to any of the isolates in either experiment. PI 459025B has also shown little or no resistance to U.S. populations of *P. pachyrhizi* in the field (Walker et al.

2011, 2014a). In contrast, the *Rpp4* allele from PI 605791A conditioned RB reactions to every isolate except FL12-Q in the greenhouse study. The availability of this effective allele from PI 605791A thus adds another locus to the possible combinations of *Rpp* genes that could be pyramided together to enhance the durability of SBR-resistant soybean cultivars in the southern United States (Yamanaka et al. 2013).

The different patterns of reaction of PI 200456, PI 200526, and PI 471904 to the isolates in the greenhouse assays support the conclusion of Garcia et al. (2008) that these accessions have three different alleles at the *Rpp5* locus. The allele in PI 471904 provided a high level of resistance to all six isolates in both experiments, as did the allele from PI 200487 in the detached-leaf experiment. In contrast, the recessive *rpp5* allele in PI 200456 provided resistance against the isolates from Alabama and Louisiana but not against any of the Florida isolates. It was the only one of the PIs tested that had that particular reaction pattern. Although the allele from PI 200526 was not effective against any of the isolates in this study, it conditioned resistance to some South American isolates (Akamatsu et al. 2013). The *Rpp5* alleles from PI 471904 and PI 200487 would be of value for breeding SBR-resistant cultivars.

The *Rpp6* gene from PI 567102B discovered by Li et al. (2012) provided a high level of resistance to all of the isolates except FL12-Q in both experiments, and this PI has also shown consistent resistance to SBR in field evaluations (Walker et al. 2011, 2014a). Although PI 567102B and PI 567104B originate from the same research station in Indonesia, resemble one another morphologically, and have had very similar levels of resistance to field populations of *P. pachyrhizi* (Walker et al. 2014a), it is still not known whether they have the same allele at the *Rpp6* locus. Liu et al. (2016) mapped an SBR resistance gene in PI 567104B to the *Rpp6* locus, and Harris et al. (2015) found evidence for a second resistance gene at the *Rpp4* locus in PI 567104B, though that was never confirmed. If PI 567104b has another *Rpp* gene and the same *Rpp6* allele as PI 567102B, it would be expected to be at least as resistant as PI 567102B; however, in the present study, PI 567102B was resistant to the FL11-Q isolate in both the greenhouse and detached-leaf assays, whereas PI 567104B was not. Song et al. (2015) reported some genetic dissimilarity between PI 567102B and PI 567104 in the *Rpp6* region of Chr 18; thus, it is possible that the alleles in the two accessions are similar but not identical. PI 567129, another accession from East Java in Indonesia, also has a resistance gene at the *Rpp6* locus (Harris et al. 2015). In the greenhouse assays, it had a reaction pattern identical to that of PI 567102B, suggesting that it may have the same *Rpp6* allele. In both experiments, the *Rpp7* gene that Childs et al. (2018b) mapped on Chr 19 conferred resistance to all of the isolates except the 2011 and 2012 isolates from Florida. Because this gene is located at a unique locus, it could be useful for developing unique *Rpp* gene pyramids to obtain more durable resistance to *P. pachyrhizi*.

The genes responsible for the resistance of two accessions with resistance to all six isolates are not yet known. PI 224268 (Asomasari), a maturity group VIII accession from the southern Japanese island of Kyūshū (Konno 1970), had an RB reaction to all of the isolates and had low uredinia counts in both experiments. PI 423960B (Gokudaizu), also from Kyūshū, was not tested in the greenhouse experiment but it had good resistance in the detached-leaf assays. The *Rpp* genes responsible for the resistance of PI 203308, PI 417125, PI 417208, PI 423959, PI 423960B, PI 594796, and PI 605773 are also still unknown. Therefore, mapping of the resistance genes in the most resistant of the PIs from the present study should be a research priority. Six of the nine PIs that were only susceptible to FL12-Q in the greenhouse assays originate from northern Vietnam, while PI 417208 is another accession from Kyūshū, Japan. PI 203398 is a 1952 cultivar from Brazil named Abura. Most of these nine PIs had been screened in a 2011 field test planted in Quincy, FL and in 2012 field tests in Quincy and in Attapulgus, GA. In both years, the *P. pachyrhizi* populations in Florida caused considerably more disease on PIs than the 2012 population in Georgia (Walker et al. 2014a). PI 203398, PI 417208, and PI 567129 were susceptible to the Florida populations in both years, whereas PI 605773, PI605791A, PI 605854B, and PI 605891A, all of which originate from Vietnam, had less disease in 2011 than in 2012.

The identities of the *Rpp* genes in six accessions with unique or unusual reaction type patterns have also not yet been verified. PI 416778, PI 417126, PI 417129B, PI 506938, PI 567046C, and PI 594796 were only tested in the detached-leaf experiment; thus, we were unable to confirm their unusual reaction patterns using data from the greenhouse assays. Harris et al. (2015) found evidence for more than one *Rpp* gene in PI 417129B (Kyūshū 40) but the number and identify of the genes have not been confirmed.

The pathogenic variability among the six isolates used in this study reflects some portion of the *P. pachyrhizi* population pathogenic diversity that existed in the field in the years that the isolates were collected. The reactions of the germplasm accessions in the experiments clearly showed that the 2012 isolate from Quincy, FL was the most virulent of the six isolates, followed by the 2011 isolate from the same location. The 2012 isolate also appeared to be more aggressive than the 2011 isolate based on the average numbers of uredinia per lesion that each induced on the PIs that developed the same infection type to both isolates. Both isolates were more virulent than the 2009 isolate from Quincy, which was more similar in virulence to the 2008 and 2009 isolates from Alabama and Louisiana. This pattern is very similar to the pattern of virulence that Walker et al. (2014a) observed in the field in Quincy, FL during the period between 2009 and 2012.

Although pathogenic diversity was lower among the 2008 and 2009 isolates in this study, differences in infection types, particularly in the detached-leaf assays, and in the numbers of uredinia per lesion in both experiments indicated that the four isolates are not pathogenically identical. In the detached-leaf assays, the reactions of PI 417126 to these four isolates suggested that the 2008 and 2009 isolates from Fairhope, AL were similar, and that the 2009 isolates from Louisiana and Florida were similar to one another. In contrast, the reactions of PI 417129B and PI 506938 to the same four isolates suggested similarities between the 2009 isolates from Alabama and Louisiana, and that the 2008 isolate from Alabama and the 2009 isolate from Quincy, FL both had a different pathotype from them. Because the *Rpp* genes have not yet been identified in any of these accessions, they would not have been included in a standard differential set and the pathotype differences that they revealed in this study would have therefore been missed.

If the numbers of uredinia per lesions on the susceptible checks in the two experiments can be considered indications of isolate aggressiveness, the differences observed in the two experiments were either minor or inconsistent (in the case of the Williams 82 reaction to the AL09-FH isolate in the detached-leaf assays). On most of the susceptible control lines, the FL12-Q isolate induced the highest or second highest uredinia per lesion counts, suggesting that it might be somewhat more aggressive than most of the other isolates; however, this still needs to be confirmed with additional investigations.

In summary, this study confirmed moderate to high levels of resistance in at least 45 soybean germplasm accessions to six *P. pachyrhizi* isolates in seedling or detached-leaf assays. Differential reactions were observed in specific PI–isolate combinations that provided information about both the resistance of the plants and the comparative virulence of the isolates. Considerable differences were seen in the reactions of some accessions with a gene at the *Rpp1*, *Rpp3*, *Rpp4*, *Rpp5*, or *Rpp6* locus, confirming the likelihood of different alleles at the same *Rpp* locus or undetected resistance genes at other loci. Further molecular and phenotypic investigations will be necessary to resolve which genes are responsible for the resistance of these accessions and PIs with unmapped *Rpp* genes. The results of these experiments also confirmed that the 2012 isolate from Quincy, FL is unusually virulent against PIs with a variety of *Rpp* genes, including *Rpp6*, which is historically one of the most effective genes in providing resistance to U.S. populations of *P. pachyrhizi*. The highly resistant soybean PIs in the experiments described here should be of value to soybean breeders. The unusual reaction patterns of several of the accessions indicate that those could be informative if they are included as differentials in future pathotype studies.

Acknowledgments

We thank M. K. Pauls (formerly USDA-ARS), J. B. Buckley (Louisiana State University AgCenter, Bossier City, LA), E. Sikora (Auburn University, Auburn, AL), and M. Pegues (Auburn University, Fairhope, AL) for their assistance.

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