# Comparison of Pathogenic Variation among *Phakopsora pachyrhizi* Isolates Collected from the United States and International Locations, and Identification of Soybean Genotypes Resistant to the U.S. Isolates

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## Abstract

Paul, C., Frederick, R. D., Hill, C. B., Hartman, G. L., and Walker, D. R. 2015. Comparison of pathogenic variation among *Phakopsora pachyrhizi* isolates collected from the United States and international locations, and identification of soybean genotypes resistant to the U.S. isolates. Plant Dis. 99:1059-1069.

A major constraint in breeding for resistance to soybean rust has been the virulence diversity in *Phakopsora pachyrhizi* populations. In greenhouse experiments, reactions of 18 soybean genotypes to 24 U.S. isolates from 2007 and 2008 and 4 foreign isolates were compared. Reactions of four differentials (*Rpp1* to *Rpp4*) to these U.S. isolates were also compared with reactions to nine foreign isolates and three U.S. isolates from 2004. Principal component analysis (PCA) of the reaction types grouped the U.S. isolates into a single virulence group, whereas each of the foreign isolates had a unique virulence pattern. In another experiment, reactions of 11 differentials to the 24 U.S. isolates were compared and significant interactions ($P < 0.001$) were found between the isolates and host genotypes for rust severity and uredinia densities. PCA of these two measures of disease placed the 24 isolates into seven or six aggressiveness groups, respectively. In a third experiment, evaluation of 20 soybean genotypes for resistance to the previously established aggressive groups identified 10 genotypes resistant to isolates representing most of the groups. This study confirmed the pathogenic diversity in *P. pachyrhizi* populations and identified soybean germplasm with resistance to representative U.S. isolates that can be used in breeding.

Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd., is one of the most destructive foliar diseases of soybean. It originated in eastern Asia and has gradually spread to soybean production areas throughout the world, including the continental United States, where it was first observed in late 2004 (9,42). SBR was found in 19 states in 2007 and in 16 states in 2008 and 2009 (http://sbr.ipmpipe.org/cgi-bin/sbr/public.cgi), and has been observed on kudzu (*Pueraria montana*) as far north as Illinois (8). Heavy infections have caused seed yield losses of 30 to 80% in southern Africa (24,28) and in South America (57), and 43% in the southern United States (45). None of the soybean cultivars currently grown in the United States are reported to be resistant to SBR; therefore, destructive epidemics could result if inoculum spread and weather conditions were conducive to disease development (5,32,57). Although SBR can be managed with fungicides, it requires diligent scouting and timely chemical applications when weather conditions are favorable for spraying, and it increases production input costs (18,30). For example, the cost of controlling SBR in Brazil in 2003 was estimated to be U.S.$544 million (57). Widespread use of fungicides to manage SBR also increases the environmental impact of soybean production and puts selection pressure on pathogen populations to evolve tolerance to widely used chemistries (17,18). Therefore, SBR-resistant cultivars could be useful for reducing soybean yield losses and production costs in areas where rust occurs, but they must have genes that are likely to be effective in the specific geographic regions where the cultivars are to be grown.

Deployment of resistant soybean cultivars has been hampered by considerable geographical and temporal pathogenic variation among *Phakopsora pachyrhizi* populations (8,39,47,55). Although germplasm lines with SBR resistance have been released in the Unites States (3,15), resistant cultivars have yet to be marketed in North America. Although *P. pachyrhizi* has no known sexual stage (35), virulence diversity exists in and among field populations (47,48,52,54,55), and is likely to continue to evolve through mechanisms such as somatic hybridization resulting from germ tube and hyphal anastomosis during asexual reproduction (11,51). Yeh (56) found three races among 50 Taiwanese *P. pachyrhizi* uredinial isolates based upon rust reaction patterns on differentials, while Twizeyimana et al. (50) observed a high degree of genetic variation within *P. pachyrhizi* populations in Nigeria.

Dominant genes conditioning soybean resistance to *P. pachyrhizi* (*Rpp* genes) have been reported at six independent loci, with more than one

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Accepted for publication 8 January 2015.

http://dx.doi.org/10.1094/PDIS-09-14-0989-RE

alternative resistance allele at some loci (10,12,16,19–22,25,29,31,40,46). Although some soybean accessions exhibit an immune (IM) or type 0 reaction to specific *P. pachyrhizi* isolates, resistance is more often characterized by the development of reddish-brown (RB) lesions with few uredinia and little or no sporulation. Susceptible (TAN) reactions are typically tan in color, at least partly due to the abundance of beige-colored urediniospores on the abaxial side of infected leaves (10). The efficacy of each *Rpp* gene varies among locations, years, and continents (34,37,52,53). Miles et al. (33) identified 805 soybean plant introductions (PIs) from the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Soybean Germplasm Collection that were resistant to a mix of four foreign *P. pachyrhizi* isolates in greenhouse seedling assays. However, only 16 of 530 of these PIs that were screened in the field in Paraguay were resistant (34), and only 64 of the accessions were resistant to SBR in the southern United States between 2006 and 2008 (52). Kudzu accessions from northern Florida also exhibited different levels of resistance to a *P. pachyrhizi* isolate (23).

Greater knowledge about *P. pachyrhizi* virulence diversity is needed to guide the development of soybean cultivars with broad and durable resistance that is likely to be effective in the management of SBR in the United States. Such information would be useful in screening early-generation breeding lines for resistance against a range of pathogen genotypes reflecting the genetic diversity of the *P. pachyrhizi* field population in a given region. The objectives of the present study were to (i) assess the virulence and aggressiveness of 24 purified U.S. isolates collected in 2007 and 2008 on a set of differential soybean genotypes, (ii) compare the virulence of the U.S. isolates with that of a sample of foreign isolates, and (iii) evaluate 20 additional soybean genotypes for resistance to the U.S. isolates to identify genotypes with potentially novel resistance genes.

## Materials and Methods

**Soybean genotypes.** The 31 soybean genotypes in Table 1 or subsets of those were used in a series of assays. In the first experiment, the 31 lines were challenged with the 24 U.S. *P. pachyrhizi* isolates listed in the upper part of Table 2. Eleven soybean lines were selected to serve as a differential panel, including PI 200492 (*Rpp1*), PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), PI 459025B (*Rpp4*), PI 471904 (resistance allele at the *Rpp5* locus), PI 200526 (resistance allele at the *Rpp5* locus), PI 594538A (*Rpp1-b*), PI 587880A (resistance gene in the *Rpp1* region), and PI 567102B (*Rpp6*). The *Rpp5* alleles in PI 200526 and PI 471904 exhibit different degrees of dominance, and are likely to be different genes (16). Of the other two differentials, UG-5 is an SBR-resistant line from Uganda (24), which was also found to be resistant to some U.S. isolates (36). The SBR resistance genes in UG-5 have not yet been reported. 'Williams 82' (2) was included as a susceptible check because it has consistently produced TAN lesions to all U.S. and foreign *P. pachyrhizi* isolates. Of the 20 remaining genotypes, 12 were selected because they had SBR severities <0.2% and RB reactions with low sporulation in field trials conducted in Paraguay (34). 'DT 2000', a cultivar from Vietnam, and PI 423972 were included because they had developed RB reactions to U.S. isolates collected in 2004 and to several foreign isolates (39). PI 203398, PI 224268, PI 417089A, PI 567039A, PI 567041A, and PI 567046A, which were resistant to six purified domestic isolates in detached-leaf assays conducted by Paul and Hartman (36), were also included.

**Table 1.** Soybean genotypes inoculated with *Phakopsora pachyrhizi* isolates used in this study

| Genotype[a] | Geographic origin[b] | MG[c] | Resistance genes | Reference |
|---|---|---|---|---|
| PI 200492 [D][d] | Japan | VII | *Rpp1* | 10,21 |
| PI 594538A [D][d] | China | VIII | *Rpp1-b* | 12 |
| PI 587880A | China | VI | Unique allele at *Rpp1* | 40 |
| PI 230970 [D][d] | Japan | VII | *Rpp2* | 31,46 |
| PI 462312 [D][d] | India | VIII | *Rpp3* | 20,22 |
| PI 459025B [D][d] | China | VIII | *Rpp4* | 19,46 |
| PI 200526 | Japan | VIII | *Rpp5* allele | 16 |
| PI 471904 | Indonesia | IX | *Rpp5* allele | 16 |
| PI 567102B [D][d] | Indonesia | IX | *Rpp6* | 29 |
| UG-5 [D][d] | Uganda | VI | *Rpp1*, *Rpp3*[e] | Unpublished |
| DT 2000 | AVRDC, Taiwan | VI | Unknown | 39 |
| PI 203398[d] | Brazil | VIII | Unknown | 36 |
| PI 224268[d] | Japan | VIII | Unknown | 36 |
| PI 398288 | Korea | V | Unknown | 33 |
| PI 417089A[d] | Indonesia | VIII | Unknown | 36 |
| PI 423972[d] | Japan | IX | Unknown | 39 |
| PI 506863 | Japan | IV | Unknown | 33 |
| PI 507305 | Japan | V | Unknown | 33 |
| PI 567039A | Japan | IX | Unknown | 36 |
| PI 567041A | Indonesia | VIII | Unknown | 36 |
| PI 567046A[d] | Indonesia | VIII | Unknown | 36 |
| PI 567104B[d] | Indonesia | IX | Unknown | 33 |
| PI 567341[d] | China | IV | Unknown | 33 |
| PI 567351B[d] | China | III | Unknown | 33 |
| PI 587880B | China | VI | Unknown | 33 |
| PI 587886 | China | VI | Unknown | 33 |
| PI 587905[d] | China | VII | Unknown | 33 |
| PI 605779E | Vietnam | VIII | Unknown | 33 |
| PI 605833 | Vietnam | IX | Unknown | 33 |
| PI 605891A[d] | Vietnam | V | Unknown | 33 |
| Williams 82 [D][d] | Illinois, United States | III | Susceptible | 33 |

[a] All genotypes were used in the U.S. isolate assays conducted in Urbana, IL. The first 10 genotypes and the susceptible check Williams 82 comprised a differential set.
[b] AVRDC = Asian Vegetable Research Development Center, Taiwan.
[c] MG = maturity group, based on information from the Germplasm Resources Information Network.
[d] In all, 18 genotypes, including eight differentials (indicated with a "D" in brackets), were used in the foreign isolate assays conducted in Fort Detrick, MD.
[e] Unpublished data indicate that UG-5 has resistance genes at the *Rpp1* and *Rpp3* loci.

**U.S. isolate assay.** The first 24 U.S. isolates listed in Table 2 originated from single pustules on soybean or kudzu leaves collected from fields in the southern United States in 2007 and 2008, as previously described (36). These isolates had been purified through three cycles of uredinia transfers from a single spore isolated from uredinia, after which each isolate was considered to be clonal and genetically homogeneous. Isolates were increased on detached leaves of Williams 82 cultured in sealed petri dishes to obtain sufficient quantities of inoculum.

Three seeds of each of the 31 soybean genotypes were planted per pot in 32-pot trays (52 by 27 cm) filled with soil-less Sunshine LC1 Mix (Sun Grow Horticulture Inc., Belleview, WA). Each pot was fertilized at planting with 10 to 20 Osmocote 14-14-14 pellets (Scotts-Sierra Horticultural Products Co., Marysville, OH), and the seedlings were grown in a greenhouse with maximum daytime temperatures of 24 to 30°C and a 12-h photoperiod. After 3 weeks, the seedlings were thinned to two plants per pot. Assays were arranged in a randomized complete block design with three blocks, and the experimental units were individual pots containing two plants. Each flat was a complete block containing one experimental unit of each of the 31 soybean genotypes.

Approximately 3 weeks after planting, when most seedlings were at the first fully expanded trifoliolate leaf stage (V2), plants in each flat were inoculated with a single *P. pachyrhizi* isolate. Inoculum of each isolate was prepared by suspending urediniospores in a solution of 0.01% Tween 20 (vol/vol) in sterile distilled water, mixing the suspension vigorously, and filtering it through a 53-μm sieve. Urediniospore concentrations were adjusted using a hemacytometer to a final concentration of $4 \times 10^4$ urediniospores/ml for inoculation (39,50). The inoculation experiments were conducted in a Plant

**Table 2.** Reactions produced by *Phakopsora pachyrhizi* isolates on 11 soybean differential genotypes with rust resistance (*Rpp*) genes following inoculation in greenhouse assays

| | Differential genes[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Isolates** | ***Rpp1*** | **Allele at *Rpp1* locus** | ***Rpp1-b*** | ***Rpp2*** | ***Rpp3*** | ***Rpp4*** | ***Rpp5* (a)** | ***Rpp5* (b)** | ***Rpp6*** | **UG-5** | **Williams 82** |
| U.S. 2007–2008[b] | | | | | | | | | | | |
| AL07-1 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | RB | RB | TAN |
| AL07-2 | IM | TAN | TAN | RB | RB | RB | RB | TAN | HR | IM | TAN |
| AL08-3 | RB | TAN | TAN | RB | RB | TAN | TAN | TAN | RB | RB | TAN |
| AR08-1 | IM | TAN | TAN | RB | RB | TAN | RB | TAN | RB | IM | TAN |
| AR08-3 | RB | RB | TAN | RB | RB | RB | RB | TAN | RB | RB | TAN |
| AR08-14 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | RB | RB | TAN |
| FL07-1 | IM | TAN | TAN | RB | RB | TAN | TAN | TAN | HR | IM | TAN |
| FL07-3 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | RB | RB | TAN |
| FL07-6 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | HR | IM | TAN |
| FL07-8 | IM | TAN | TAN | RB | RB | TAN | RB | TAN | HR | IM | TAN |
| FL07-10 | IM | TAN | TAN | RB | RB | TAN | RB | TAN | HR | IM | TAN |
| FL07-13 | IM | TAN | TAN | RB | RB | RB | RB | TAN | IM | IM | TAN |
| GA08-1 | RB | RB | TAN | RB | RB | RB | RB | TAN | RB | RB | TAN |
| GA08-2 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | RB | RB | TAN |
| IL07-1 | RB | TAN | TAN | RB | RB | RB | TAN | TAN | RB | RB | TAN |
| IL08-2 | RB | TAN | TAN | RB | RB | RB | RB | TAN | IM | IM | TAN |
| IL08-4 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | HR | RB | TAN |
| LA08-1 | IM | TAN | TAN | RB | RB | TAN | RB | TAN | HR | IM | TAN |
| MS07-3 | IM | TAN | TAN | RB | RB | RB | RB | TAN | IM | IM | TAN |
| MS07-4 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | HR | IM | TAN |
| OK07-1 | IM | TAN | TAN | RB | RB | TAN | RB | TAN | HR | IM | TAN |
| TX07-1 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | HR | RB | TAN |
| TX07-6 | IM | TAN | TAN | RB | RB | TAN | RB | TAN | HR | IM | TAN |
| TX08-1 | RB | TAN | TAN | RB | RB | TAN | RB | TAN | RB | IM | TAN |
| U.S. 2004 | | | | | | | | | | | |
| AL04-1[c] | TAN | … | … | RB | TAN | RB | … | … | … | … | TAN |
| AL04-3[c] | TAN | … | … | RB | TAN | RB | … | … | … | … | TAN |
| LA04-1[c] | RB | … | … | RB | RB | RB | … | … | … | … | TAN |
| Foreign[d] | | | | | | | | | | | |
| BZ01-1[c] | TAN | … | … | RB | RB | RB | … | … | … | … | TAN |
| IN73-1 | IM | … | RB | RB | RB | RB | … | … | RB | IM | TAN |
| PG05-1[c] | TAN | … | RB | RB | TAN | RB | … | … | RB | … | TAN |
| SA01-1 | TAN | … | RB | RB | RB | RB | … | … | RB | RB | TAN |
| TH01-1[c] | TAN | … | … | RB | TAN | RB | … | … | … | … | TAN |
| TW72-1[c] | TAN | … | … | RB | TAN | RB | … | … | … | … | TAN |
| TW80-2 | TAN | … | RB | T | TAN | RB | … | … | RB | T | TAN |
| ZM01-1 | TAN | … | RB | RB | TAN | RB | … | … | RB | T | TAN |
| NIG-05-06[e] | RB/Mixed | … | HR | RB/Mixed | RB | RB/Mixed | … | … | … | RB/HR | … |

[a] *Rpp1* = PI 200492, *Rpp1* locus = PI 587880A, *Rpp1-b* = PI 594538A, *Rpp2* = PI 230970, *Rpp3* = PI 462312, *Rpp4* = PI 459025B, *Rpp5* (a) = PI 471904, *Rpp5* (b) = PI 200526, and *Rpp6* = PI 567102B. *Rpp1* locus = an allele that is different from *Rpp1* gene, *Rpp1b* = an allele of *Rpp1* gene or linked gene. HR = hypersensitive response characterized by brown discoloration with no uredinia development, RB = reddish-brown reaction type, T = TAN indicates tan-colored reaction type, IM = an immune response with no visible symptoms, MIXED = both RB and TAN reaction types present on the same leaf.

[b] Isolate names indicate state of origin (AL = Alabama, AR = Arkansas, FL = Florida, IL = Illinois, LA = Louisiana, MS = Mississippi, GA = Georgia, OK = Oklahoma, and TX = Texas), year of collection (2007 or 2008), and isolate number for location of collection.

[c] Reaction type data for differentials originally reported by Pham et al. (39).

[d] Foreign isolate names indicate the country of origin, followed by year and location of collection. BZ = Brazil, IN = India, PG = Paraguay, SA = South Africa, TH = Thailand, TW = Taiwan, ZM = Zimbabwe, and NIG = Nigeria.

[e] Reaction type data for differentials originally reported in Twizeyimana et al. (50) or Twizeyimana and Hartman (47).

Biosafety Level 2 greenhouse at the University of Illinois in Urbana in the winter months of 2009 and 2010, under a permit from the USDA Animal and Plant Health Inspection Service (APHIS). The abaxial (lower) leaf surface of the first trifoliolate leaf on each plant was sprayed with a urediniospore suspension at a rate of 50 ml/flat using an atomizer attached to an air compressor at a pressure of $1.4 \times 10^5$ Pa (Paasche Airbrush Co., Taiwan). Inoculated plants were incubated in a mist chamber overnight at 20 to 22°C. Plants were removed from the mist chamber and placed on greenhouse benches in a room maintained at 24 to 25°C and with a 12-h photoperiod, where they were watered daily. Two isolates were tested at a time at 2-day intervals due to limited space in the mist chamber. Urediniospore viability was determined by spraying inoculum of each isolate immediately after inoculation onto the surface of 2% water agar medium in petri dishes and then observing the percentage of germinated urediniospores after 24 h of incubation at room temperature in the dark using a stereoscope at ×100 magnification (5,25).

At 15 to 16 days after inoculation, SBR reactions and disease severity were assessed on the abaxial surface of the first trifoliolate leaf of each plant. The reaction types were recorded as (i) IM, if there were no visible lesions; (ii) RB, if there were reddish-brown lesions with either no uredinia or only a few sparsely sporulating uredinia, indicating a resistance reaction; or (iii) TAN, if there was a tan-colored reaction with abundantly sporulating uredinia, indicating a susceptible reaction (10). Leaflets that developed light-brown lesions with no uredinia were recorded as having a hypersensitive response (HR), as previously described by Jordan et al. (23) on kudzu leaves. When no lesions were observed on a trifoliolate, additional leaflets were examined to confirm the absence of lesion development. Disease severity was assessed in the greenhouse using a visual scale of 1 to 5 devised by Miles et al. (32), in which 1 = no visible lesions, 2 = a few scattered lesions, 3 = a moderate number of lesions on part of the leaflet, 4 = an abundant number of lesions on part of the leaflet, and 5 = prolific lesion development over most of the leaflet. After reaction type and rust severity were rated, two leaflets of each soybean genotype per replication were excised, placed in an envelope, and baked in an oven overnight at 50°C to kill the urediniospores and other fungal tissue. This was necessary to comply with the APHIS rust permit regulations for working with *P. pachyrhizi* in Illinois. The numbers of lesions and sporulating uredinia per square centimeter of leaf tissue were determined by counting the numbers within two circles, each 1 $cm^2$ in area, on either side of the midrib on the abaxial side of each leaflet. This was done using a dissecting microscope at ×100, as described by Paul et al. (38). The mean of the four circles was then calculated for each experimental unit. The average number of uredinia per lesion on each leaflet was calculated by dividing the number of sporulating uredinia per circle by the number of lesions. Genotypes with mean severity ratings <2.5 or with fewer than 2.0 uredinia/lesion were considered to be resistant. Inoculations with all of the isolates were repeated once, with three replications (blocks).

**Foreign isolate assay.** Assays were conducted with four foreign *P. pachyrhizi* isolates from India (IN73-1), South Africa (SA01-1), Taiwan (TW80-2), and Zimbabwe (ZM01-1) (Table 2) on a subset of 18 soybean genotypes, including eight differentials (Table 1). The differentials included accessions with the genes *Rpp1* through *Rpp4*, *Rpp1-b,* and *Rpp6*, as well as UG-5 and the susceptible check Williams 82. Another 10 soybean genotypes (Table 1) were also included to evaluate the phenotypic reactions to the foreign isolates. These assays were arranged in a randomized complete block design with two blocks (replications). An experimental unit consisted of 1 pot with three plants of each genotype and 32 pots/tray, as previously described for the assay with the U.S. isolates. Inoculations with four isolates on 18 genotypes were made once, with two replications.

All four foreign isolates had been established from urediniospores on soybean leaves collected from the countries and years indicated by the isolate name (6). All increases were made on the susceptible soybean genotype Williams 82 in the greenhouse at the USDA-ARS Foreign Disease-Weed Science Research Unit Biological Safety Level 3 Containment Facility at Fort Detrick, MD (26). Urediniospores were collected and stored in liquid nitrogen, as previously described (14,33). Prior to inoculation, frozen urediniospores were heat shocked at 40°C for 5 min and then rehydrated with sterile, distilled water by overnight incubation at 100% relative humidity in a covered petri plate at room temperature. Inoculum was prepared as previously described (6). Urediniospores were quantified using a hemacytometer and were suspended in 0.01% (vol/vol) Tween 20 (sodium monolaurate) in distilled water. The suspension was adjusted to a final concentration of $25 \times 10^3$ urediniospores/ml (7), and the inoculations were performed as previously described (39). Two weeks after inoculation, reaction types were recorded using the criteria of Bromfield and Hartwig (10) as TAN, RB, or IM by inspecting the abaxial side of the leaflets.

**Comparison of the U.S. isolates with nine foreign isolates.** Variation in virulence was also assessed by comparing the reactions that the 24 U.S. isolates from 2007 and 2008 induced on a set of four differentials (*Rpp1* to *Rpp4*) with the reactions induced by nine foreign isolates and three U.S. isolates from 2004. Four of the foreign isolates and the 2004 isolates from Alabama and Louisiana had been used in one previous study (39), and the Nigerian isolate NIG-05-06, that was previously reported (49), was also included to investigate isolate grouping patterns using principal component analysis (PCA) (Table 2).

**Data analysis.** Analysis of disease severity, number of lesions, number of uredinia, and uredinia per lesion from the assays with the 24 U.S. isolates was performed using PROC MIXED, with the analysis of variance (ANOVA) option restricted maximum likelihood (REML) (SAS version 9.3; SAS Institute Inc., Cary, NC). Soybean genotype and isolate were fixed effects and block was treated as a random effect. Least square means within each genotype–isolate combination were generated with LSMEANS statements. Data were combined from the two trials prior to analysis because error variance heterogeneity was found to be nonsignificant using ANOVA in PROC GLM with an option Hovtest = levene. Least square means for severity, number of lesions, number of uredinia, and number of uredinia per lesion were estimated from these analyses for PCA. Principal component (PC) scores were calculated from the resulting data matrix of 24 observations (isolates) and 11 variates (differential genotypes), and the first two PCs were plotted to visualize isolate clustering. To assess virulence diversity between the 4 foreign and 24 U.S. isolates, reaction types on 18 genotypes were recorded for each isolate, and were then coded for the PCA as follows: IM and RB were both considered resistance reactions, and were coded as 0, while the TAN susceptible reactions were coded as 1. Pathotype clusters were determined for foreign and U.S. isolates using the average linkage method and clustering criteria in PROC CLUSTER and PROC TREE.

**Determination of *P. pachyrhizi* isolate aggressiveness groups.** Least square means for rust severity for each isolate from the REML analysis of the U.S. isolate assays were used for multivariate analysis. Prior to multivariate analysis, rust severity data for the U.S. isolates were used to delineate three severity classes using PROC UNIVARIATE in SAS (version 9.3; SAS Institute Inc.). The first, second, and third categories consisted of values that were greater than one standard deviation above the grand mean, within one standard deviation of the mean, or lower than one standard deviation below the grand mean, respectively. PCA was used to examine the multivariate structure of the *P. pachyrhizi* isolates, as described by Chakraborty et al. (13), using three severity classes (i.e., classes 1 to 3) for each isolate and each genotype as separate variates. PCA was conducted using the SAS procedure PRINCOMP, and the first two PCs were plotted to visualize isolate groupings. Aggressiveness groups were determined using the average linkage method and clustering criteria in PROC CLUSTER and PROC TREE. The number of isolate clusters was optimized by plotting the proportional reduction in residual sum of squares against the number of clusters (44). A similar procedure was used to examine the distribution of the uredinia density data and uredinia per lesion on leaflets, with low, medium, and high classes based on departure from the mean. Twenty soybean genotypes were evaluated for resistance to each isolate within each of the respective aggressiveness groups to identify genotypes resistant to *P. pachyrhizi*.

## Results

**Infection types induced by 24 U.S. *P. pachyrhizi* isolates from 2007 and 2008.** All of the U.S. isolates produced an RB reaction on PI 230970 (*Rpp2*) and PI 462312 (*Rpp3*) and a TAN reaction on PI 200526 (allele at *Rpp5*), PI 594538A (*Rpp1-b*), and Williams 82 (Table 2). PI 200492 (*Rpp1*) and UG-5 plants had an IM reaction to 45 and 60% of the isolates, respectively, and an RB reaction to the remaining isolates. PI 459025B (*Rpp4*) developed RB lesions with profusely sporulating uredinia following infection with 30% of the isolates, while other isolates induced a TAN reaction. RB lesions were induced on PI 471904 (resistance allele at the *Rpp5* locus) by 88% of the isolates. PI 567102B (*Rpp6*) developed RB lesions when challenged by 42% of the isolates and the previously described HR type of response or IM when challenged with the rest of the isolates. Although 8% of the U.S. isolates produced RB lesions on PI 587880A, which has a unique allele at the *Rpp1* locus (36), the rest of the isolates produced a TAN reaction.

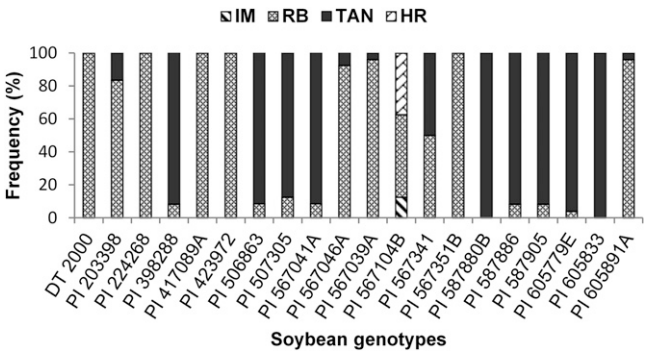


**Fig. 1.** Frequencies of infection types on soybean genotypes with unidentified resistance genes on plants challenged with 24 *Phakopsora pachyrhizi* isolates collected in the United States in 2007 or 2008. Reaction type abbreviations: IM = immune, RB = reddish-brown lesions (incomplete resistance), TAN = tan-colored reaction (susceptible), and HR = hypersensitive reaction with brown lesions.

The soybean genotypes with uncharacterized resistance genes differed in their reactions to the 24 U.S. isolates (Fig. 1). For example, all 24 isolates produced RB reactions on five soybean genotypes, but a TAN reaction on PI 587880B and PI 605833. More than 90% of the isolates induced TAN reactions on seven other host genotypes, while four genotypes developed RB lesions. RB lesions or brown lesions resembling the HR described (23) were induced on PI 567104B plants by 50 and 38% of the isolates, respectively. The remaining isolates produced an immune reaction. AL07-2, FL07-1, FL07-6, and LA08-1 were the most virulent isolates, producing a TAN reaction on 52 to 60% of the soybean genotypes (data not shown). The numbers of lesions with sporulating uredinia per square centimeter varied, however, depending on the virulence of the isolate on specific host genotypes.

**Comparison of virulence between U.S. and foreign isolates.** The virulence of the nine foreign isolates and the three 2004 U.S. isolates differed from that of the 2007 and 2008 U.S. isolates on some of the soybean differentials (Table 2). For instance, all of the 2007 and 2008 U.S. isolates produced either RB or IM reactions on PI 200492 (*Rpp1*) and PI 462312 (*Rpp3*), whereas the two 2004 Alabama isolates and most of the foreign isolates produced TAN reactions. The one exception was an isolate from India which induced an IM reaction on the PI 200492 (*Rpp1*) genotype. In addition, most of the U.S. isolates produced a TAN reaction on PI 459025B (*Rpp4*), whereas all of the foreign isolates produced an RB reaction, or an RB/mixed reaction in the case of the NIG-05-06 isolate. On PI 594538A (*Rpp1-b*), all of the U.S. isolates produced a TAN reaction, while five of the foreign isolates produced an RB reaction and the NIG-05-06 isolate induced an HR reaction (Table 2). All U.S. isolates, including three isolates from 2004, and seven of the nine foreign isolates produced an RB reaction on PI 230970 (*Rpp2*). The isolate from Taiwan produced a TAN reaction and the NIG-05-06 isolate produced an RB or mixed reaction. The 24 U.S. isolates produced RB, HR, or IM reactions on PI 567102B (*Rpp6*), and the five foreign isolates produced an RB reaction.

PCA performed on the reactions produced by the 24 U.S. isolates and the foreign isolates IN73-1, SA01-1, ZM01-1, and TW80-2 on 18 soybean genotypes revealed one primary isolate group containing all of the U.S. isolates, whereas none of the 4 foreign isolates were

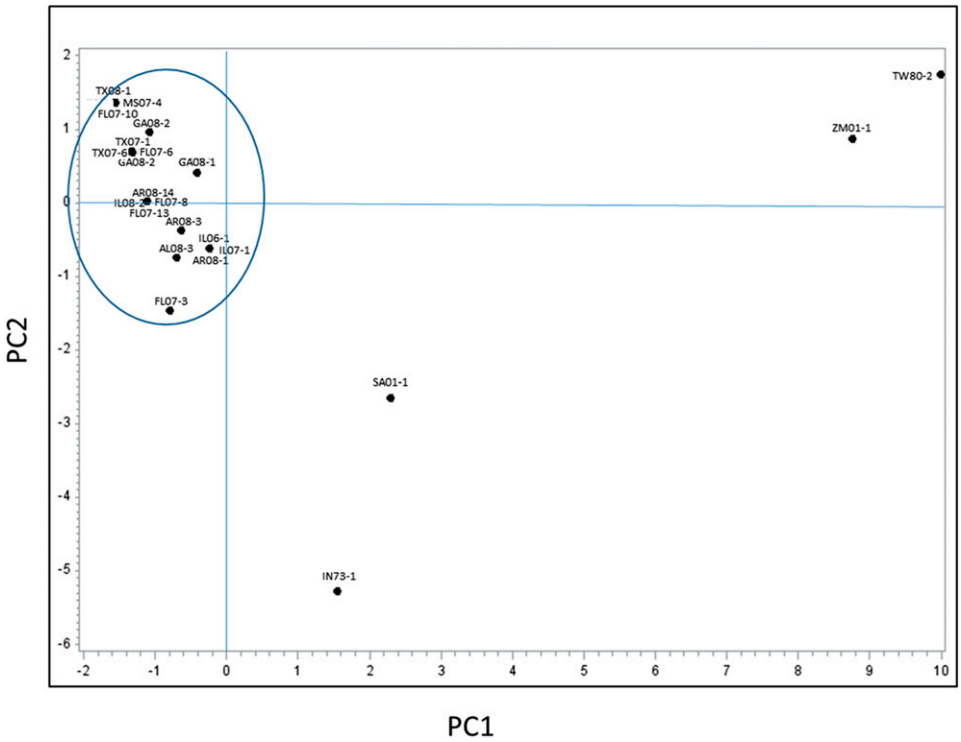


**Fig. 2.** Principal component analysis of reaction types on 18 soybean genotypes, including seven differential lines, after inoculation with 24 *Phakopsora pachyrhizi* isolates from the United States and four foreign isolates, plotted in a plane defined by the first two principal components (PC1 × PC2). The U.S. isolates are circled.

grouped with any other isolate (Fig. 2). In a comparative analysis of the reactions induced on the *Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4* soybean genotypes by the 9 foreign isolates, 3 U.S. isolates from 2004, and 24 U.S. isolates from 2007 and 2008, PCA grouped all 24 of the more recent U.S. isolates into a single group while 4 of the foreign isolates (BZ01-1, TH01-1, PG05-1, and ZM01-1) formed another group which also included U.S. isolate AL04-1 (Fig. 3). The remaining four foreign isolates (IN73-1, SA01-1, TW80-2, and

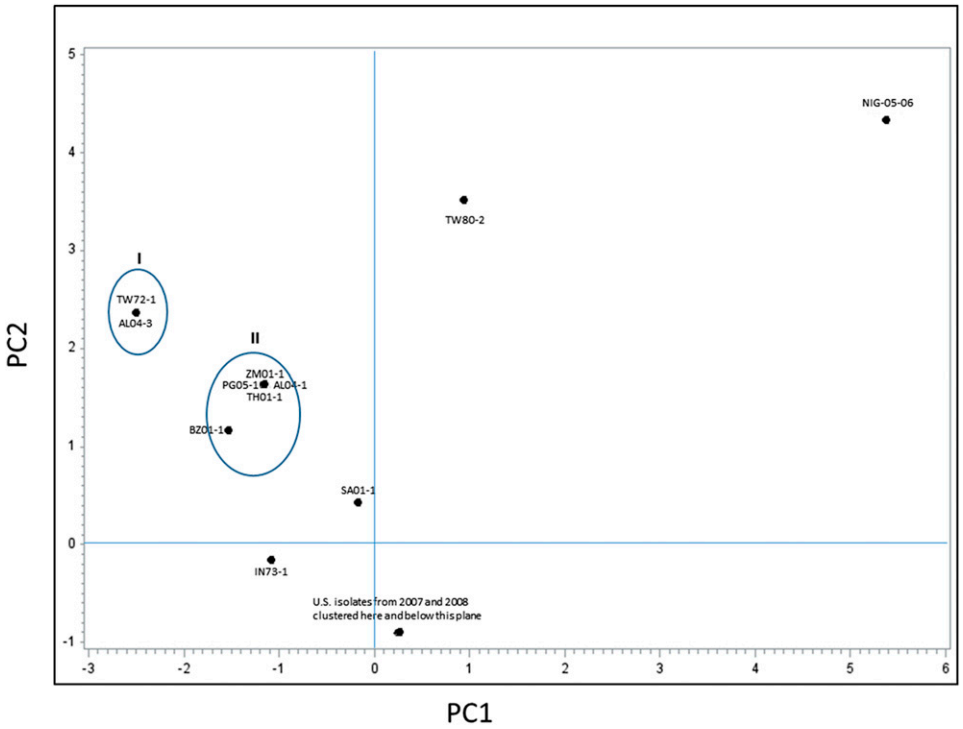


**Fig. 3.** Principal component analysis of reaction types on soybean *Rpp1* to *Rpp4* differentials after inoculation with 24 U.S. *Phakopsora pachyrhizi* isolates from 2007 and 2008, 3 U.S. isolates from 2004, and 9 foreign isolates plotted in a plane defined by the first two principal components (PC1 × PC2). Group I consisted of the U.S. isolate AL04-3 and Taiwanese isolate TW72-1, and group II consisted of four foreign isolates and the U.S. isolate AL04-1.

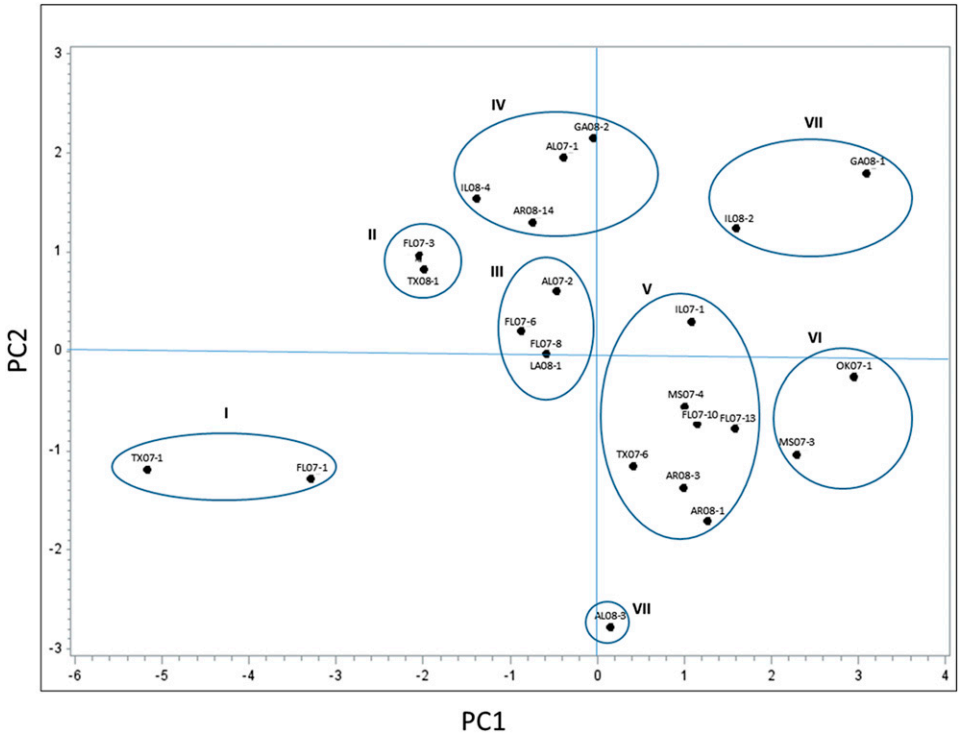


**Fig. 4.** Principal component and cluster analyses of disease severity after inoculation of 11 soybean differential genotypes with 24 *Phakopsora pachyrhizi* isolates collected in the United States in 2007 or 2008. Sample size represents the number of isolates within each cluster (circle), plotted in a plane defined by the first two principal components (PC1 × PC2). Roman numerals adjacent to each cluster indicate aggressiveness aggressive groups referred to in the text.

NG-05-06) did not group with any other isolates but TW72-1 grouped with U.S. isolate AL04-3.

**Aggressiveness diversity among the U.S. *P. pachyrhizi* isolates From 2007 and 2008.** Isolate–soybean genotype interactions for rust severity and uredinia density were highly significant ($P < 0.001$). PCA of rust severity ratings from 11 differential soybean genotypes grouped the 24 U.S. *P. pachyrhizi* isolates into seven aggressiveness groups (Fig. 4). The cumulative proportion of total variation for the first three PCs was 65%. Large positive coefficients were revealed for PI 471904, PI 567102B, PI 462312, and UG-5, and negative coefficients for PI 594538A and Williams 82. PCA based on the densities of uredinia revealed six aggressiveness groups (Fig. 5). In the cluster analysis of the 24 U.S. isolates, the $R^2$ value increased steadily by 5 and 10% in the first six clusters. After six clusters, the subsequent incremental rise in the $R^2$ value with each additional cluster was less than 3%. Thus, the optimal number of representative groups was determined using $R^2 > 5\%$ as the cut-off point to represent the six groups of isolates. The densities of uredinia produced by the six isolate groups on the 11 differentials are summarized in Table 3. Isolates in groups I, II, and III were the most aggressive, producing significantly higher uredinial densities on most soybean genotypes than isolates in the other groups. Sporulation of isolates from all of the groups was absent or very low on the *Rpp1* and *Rpp6* genotypes and on UG-5. Based on an independent gene mapping study, UG-5 appears to have resistance genes at the *Rpp1* and *Rpp3* loci (unpublished data). Isolates in groups I and II were highly aggressive on PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), and PI 459025B (*Rpp4*), despite the association of these resistance genes with the RB reaction type. Isolates in group VI were the least aggressive isolates.

**Identification of soybean lines resistant to the 2007 and 2008 U.S. *P. pachyrhizi* isolates.** A significant ($P < 0.001$) soybean genotype–rust isolate interaction was found for both rust severity and uredinia density. Isolates in aggressiveness groups established from severity data on differential soybean genotypes were used to evaluate the resistance of soybean lines (Fig. 4). Soybean lines with severity ratings ≤2.5 were considered to be resistant. Groups I, II, and III contained the most aggressive isolates, and PI 567104B was the only genotype with a disease severity score ≤2.5 (Table 4). When the average severities were summed across the seven aggressiveness groups, PI 567104B had the lowest sum, followed by PI 417089A, PI 567089A, DT2000, and PI 567046A. Seven genotypes were found to be resistant to isolates in aggressiveness group IV, while two genotypes were resistant to isolates in aggressiveness group V.

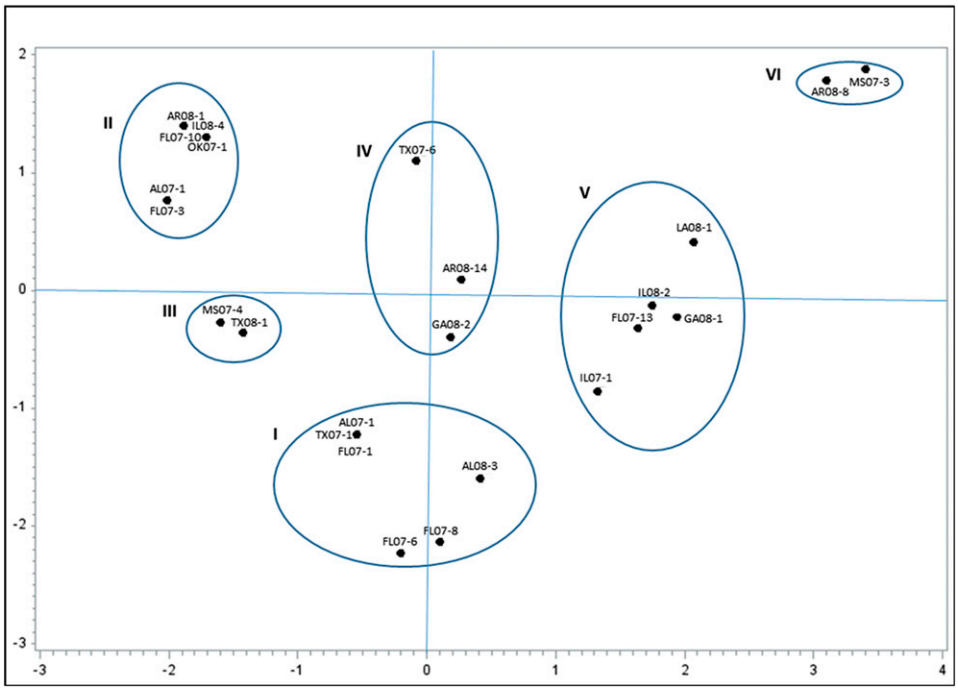


**Fig. 5.** Principal component and cluster analyses of number of uredinia per square centimeter after inoculation of 11 soybean differential genotypes with 24 *Phakopsora pachyrhizi* isolates collected in the United States in 2007 or 2008. Sample size represents the number of isolates within each group (circle), plotted in a plane defined by the first two principal components (PC1 × PC2). Roman numerals adjacent to each group indicate aggressiveness groups referred to in the text.

**Table 3.** Average numbers of uredinia per square centimeter on 11 soybean differentials challenged with 24 isolates of *Phakopsora pachyrhizi* from the United States representing six aggressiveness groups identified using cluster analysis[a]

| Group | Isolate | PI 200492 (*Rpp1*) | PI 594538A (*Rpp1-b*) | PI 587880A (*Rpp1* locus) | PI 230970 (*Rpp2*) | PI 462312 (*Rpp3*) | PI 459025B *Rpp4* | PI 200526 *Rpp5* | PI 471904 *Rpp5* | PI 567102B *Rpp6* | UG-5[b] | Williams 82 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 6 | 0 | 42.5 (7.5) | 38.7 (7.0) | 6.7 (2.3) | 5.4 (1.7) | 25.7 (4.8) | 51.0 (9.5) | 1.4 (1.0) | 0.1 (0.1) | 0 | 45.7 (8.7) |
| II | 6 | 0 | 42.9 (0.5) | 39.0 (6.0) | 17.8 (4.0) | 8.8 (3.2) | 25.7 (4.1) | 36.3 (5.7) | 1.9 (0.5) | 0.8 (0.7) | 0 | 39.2 (7.0) |
| III | 2 | 0.6 (0.6) | 55.3 (10.0) | 33.4 (7.5) | 13.0 (2.7) | 2.4 (0.8) | 37.7 (4.5) | 48.0 (7.7) | 6.3 (2.0) | 0.8 (0.8) | 0 | 35.3 (13.5) |
| IV | 3 | 0.9 (0.6) | 31.3 (0.1) | 28.9 (5.5) | 5.7 (1.5) | 0.7 (0.6) | 16.8 (3.4) | 19.4 (3.2) | 0.1 (0.1) | 0.4 (0.3) | 0 | 19.5 (3.0) |
| V | 5 | 0.4 (0.2) | 13.7 (1.7) | 11.2 (2.0) | 1.5 (0.6) | 0.4 (0.2) | 6.6 (1.6) | 11.4 (1.9) | 3.0 (1.5) | 0 | 0.6 (0.4) | 16.9 (3.1) |
| VI | 2 | 0 | 11.1 (3.9) | 1.8 (1.1) | 0.2 (0.2) | 0.2 (0.2) | 4.6 (1.4) | 16.3 (3.8) | 0.7 (0.2) | 0 | 0 | 4.9 (0.9) |

[a] Numbers in parentheses are standard errors.
[b] UG-5 carries resistance alleles at the *Rpp1* and *Rpp3* loci (unpublished data).

Using isolates in aggressiveness groups identified in PCA from the uredinia densities (Fig. 5), the soybean genotypes were further evaluated. PI 417089A, PI 567089A, PI 203398, and PI 567104B were resistant to the isolates in aggressiveness groups I, II, III, and IV, with uredinia densities of 0.1 to 2.5 uredinia/cm$^2$ (Table 5). Soybean genotypes with a uredinia density ≤2.5 were considered to be resistant. Cluster V isolates were highly aggressive on PI 203398, which was resistant in Paraguay (34) and in Attapulgus, GA in 2012 (53), and which had much lower uredinia densities when challenged by isolates in the other five aggressiveness groups. PI 605891A and PI 567046A were similar in being highly resistant to isolates in every group except for those in group II. Eleven other genotypes were highly susceptible, with densities of 19.5 to 60.4 uredinia/cm$^2$. The sums of the numbers of uredinia per square centimeter across the six aggressiveness groups reveal a 10-fold difference between the PI with the lowest density (PI 567104B) and the PIs with the highest densities (PI 506863 and PI 605833) (Table 5). Eight of the PIs had sums <25.0, two had sum counts between 40 and 50, and the rest of the entries, including Williams 82, had sum counts of more than 150.

**Table 4.** Disease severity on 21 selected soybean genotypes inoculated with 24 *Phakopsora pachyrhizi* isolates from the United States representing seven aggressiveness groups determined by principal component analysis

| Genotypes | Aggressiveness group[a] | | | | | | | Sum across groups |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | |
| PI 567089A | 3.3 | 3.3 | 2.8 | 2.4 | 2.9 | 2.0 | 1.7 | 18.4 |
| PI 567046A | 3.5 | 3.9 | 3.3 | 1.8 | 3.0 | 2.2 | 2.5 | 20.2 |
| PI 567104B | 3.5 | 2.3 | 2.2 | 1.1 | 1.7 | 0.2 | 0.7 | 11.7 |
| PI 417089A | 3.7 | 3.0 | 3.0 | 1.6 | 2.4 | 2.3 | 1.2 | 17.2 |
| DT 2000 | 3.8 | 3.8 | 2.7 | 2.3 | 3.0 | 2.8 | 1.7 | 20.1 |
| PI 423972 | 3.8 | 4.4 | 4.0 | 3.0 | 3.3 | 2.5 | 2.3 | 23.3 |
| PI 506863 | 3.8 | 4.4 | 4.3 | 4.2 | 3.9 | 2.8 | 3.3 | 26.7 |
| PI 605891A | 3.8 | 4.2 | 3.8 | 3.1 | 3.1 | 3.0 | 2.5 | 23.5 |
| PI 203398 | 4.0 | 4.0 | 3.8 | 2.4 | 3.0 | 2.5 | 3.0 | 22.7 |
| PI 398288 | 4.0 | 4.4 | 3.9 | 4.1 | 3.7 | 2.5 | 3.7 | 26.3 |
| PI 224268 | 4.2 | 3.7 | 3.8 | 2.1 | 3.0 | 2.3 | 1.8 | 20.9 |
| PI 587880B | 4.3 | 4.2 | 4.2 | 4.2 | 3.7 | 2.8 | 2.8 | 26.2 |
| PI 587886 | 4.3 | 4.2 | 3.8 | 4.1 | 3.3 | 2.8 | 2.3 | 24.8 |
| PI 605779E | 4.3 | 3.9 | 4.4 | 4.1 | 3.4 | 2.8 | 2.8 | 25.7 |
| PI 507305 | 4.5 | 3.6 | 4.1 | 4.0 | 3.9 | 2.7 | 4.2 | 27.0 |
| PI 587905 | 4.5 | 4.4 | 3.8 | 4.6 | 3.7 | 2.8 | 3.3 | 27.1 |
| PI 567351B | 4.7 | 4.9 | 4.2 | 3.6 | 3.5 | 2.7 | 2.2 | 25.8 |
| PI 567041A | 4.8 | 4.9 | 4.3 | 4.6 | 3.9 | 2.3 | 3.2 | 28.0 |
| PI 567341 | 4.8 | 4.9 | 4.3 | 3.2 | 3.5 | 3.2 | 2.8 | 26.7 |
| PI 605833 | 4.8 | 4.9 | 4.1 | 4.9 | 3.9 | 2.3 | 3.2 | 28.1 |
| Williams 82 | 5.0 | 5.0 | 4.7 | 4.4 | 4.0 | 2.5 | 4.2 | 29.8 |
| Isolates/cluster (*n*) | 2 | 3 | 4 | 3 | 8 | 2 | 2 | … |
| Trial mean | 4.2 | 4.1 | 3.8 | 3.3 | 3.3 | 2.5 | 2.6 | … |

[a] Scored on a scale of 1 to 5, where 1 = no visible lesions, 2 = a few scattered lesions, 3 = a moderate number of lesions on part of the leaflet, 4 = an abundance of lesions on part of the leaflet, and 5 = prolific lesion development over most of the leaflet (33). Means are from three replications. Classification categories: resistant = score ≤2.5, moderately resistant = score 2.6 to 3.0, and susceptible = score 3.1 to 5.0.

Based on isolates in six aggressiveness groups identified from the uredinia density, nine soybean genotypes were resistant to isolates from five of the six groups as determined by the number of uredinia per lesion, with an average of 0.1 to 2.0 uredinia/lesion (Table 6). The sums of the average number of uredinia per lesion across the six aggressiveness groups indicated that PI 567104B (0.4), PI 567089A (0.5), and PI 417089A (0.5) had very high levels of resistance, though at least five other PIs had much fewer sporulating uredinia per lesion than Williams 82 (sum of 24.5) and several other genotypes.

## Discussion

Knowledge of the pathogenic variation of pathogens is critical in plant breeding programs for guiding decisions about development and deployment of cultivars with genes controlling resistance to pathotypes prevalent in the target region. In this study, differences in the reaction patterns on the differential sets were considered indicative of differences in the virulence of the *P. pachyrhizi* isolates. Variation in disease severity and uredinia densities on the same host genotype was considered to reflect differences in aggressiveness, whereas different reaction types reflected the ability of an isolate to defeat resistance conditioned by a specific *Rpp* gene (43). Although SBR reaction type and disease severity are often correlated, there are occasional exceptions (6,7).

Differences in measurements of rust traits induced on the differential set by the 24 U.S. *P. pachyrhizi* isolates from 2007 and 2008

**Table 5.** Average numbers of uredinia per square centimeter on 20 soybean genotypes and a susceptible check (Williams 82) challenged with 24 *Phakopsora pachyrhizi* isolates from the United States representing six aggressiveness groups determined by principal component and cluster analyses

| Genotypes | Aggressiveness group[a] | | | | | | Sum across groups |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | |
| DT 2000 | 0.1 (0.1) | 1.0 (0.4) | 5.5 (2.6) | 0.4 (0.2) | 0.0 (0.0) | 0.0 (0.0) | 7.0 |
| PI 417089A | 0.2 (0.2) | 1.1 (0.3) | 0.2 (0.2) | 0.3 (0.1) | 0.2 (0.2) | 0.0 (0.0) | 2.0 |
| PI 605891A | 0.6 (0.2) | 10.8 (5.3) | 2.5 (1.2) | 0.8 (0.2) | 0.2 (0.1) | 1.0 (0.5) | 15.9 |
| PI 567089A | 0.8 (0.4) | 0.9 (0.5) | 0.4 (0.4) | 0.0 (0.0) | 0.0 (0.0) | 1.7 (0.8) | 3.8 |
| PI 203398 | 0.9 (0.4) | 2.1 (0.9) | 2.5 (1.7) | 0.4 (0.2) | 15.5 (4.2) | 0.0 (0.0) | 21.4 |
| PI 567104B | 1.2 (0.7) | 0.3 (0.3) | 0.8 (0.6) | 0.1 (0.1) | 0.0 (0.0) | 0.1 (0.1) | 2.5 |
| PI 567046A | 1.6 (1.2) | 10.1 (6.0) | 0.4 (0.3) | 0.4 (0.1) | 0.0 (0.0) | 2.1 (2.0) | 14.6 |
| PI 224268 | 2.8 (1.0) | 4.4 (1.2) | 2.7 (1.0) | 2.2 (0.6) | 0.8 (0.3) | 0.3 (0.1) | 13.2 |
| PI 423972 | 9.5 (2.0) | 16.2 (4.0) | 11.0 (2.5) | 8.3 (2.7) | 2.7 (0.7) | 1.6 (0.8) | 49.3 |
| PI 567351B | 16.7 (3.7) | 8.1 (1.9) | 11.7 (2.5) | 4.0 (1.6) | 1.7 (0.9) | 0.1 (0.1) | 42.3 |
| PI 567341 | 34.6 (5.6) | 42.8 (4.6) | 57.0 (0.7) | 5.9 (1.3) | 16.8 (5.4) | 0.7 (0.3) | 157.8 |
| PI 605779E | 35.3 (7.2) | 32.7 (5.0) | 51.8 (7.4) | 22.8 (0.1) | 14.3 (3.2) | 2.2 (1.4) | 159.1 |
| PI 587886 | 39.1 (10.1) | 49.4 (5.5) | 45.5 (3.1) | 22.3 (2.9) | 10.6 (2.3) | 9.6 (2.1) | 176.5 |
| PI 567041A | 40.5 (8.6) | 48.7 (8.6) | 49.7 (14.0) | 34.8 (9.3) | 15.3 (3.7) | 0.8 (0.4) | 189.8 |
| PI 587880B | 42.0 (10.0) | 37.0 (4.5) | 46.1 (10.6) | 24.7 (5.4) | 10.0 (2.2) | 18.3 (4.5) | 178.1 |
| PI 398288 | 44.1 (12.6) | 47.5 (5.1) | 43.1 (8.9) | 19.7 (3.7) | 14.1 (3.1) | 6.6 (4.8) | 175.1 |
| Williams 82 | 45.7 (8.6) | 39.2 (7.0) | 35.4 (13.5) | 19.5 (8.7) | 16.9 (3.1) | 5.0 (0.9) | 161.7 |
| PI 506863 | 47.1 (8.5) | 51.6 (3.8) | 60.4 (12.8) | 35.2 (5.0) | 13.0 (2.3) | 10.4 (3.4) | 217.7 |
| PI 605833 | 48.5 (7.8) | 46.8 (6.6) | 42.8 (11.0) | 42.3 (8.0) | 12.3 (4.2) | 22.3 (6.2) | 215.0 |
| PI 507305 | 51.1 (8.5) | 21.4 (4.2) | 53.8 (6.1) | 20.2 (3.5) | 20.0 (6.1) | 2.1 (0.9) | 168.6 |
| PI 587905 | 53.3 (7.0) | 38.9 (7.1) | 41.7 (4.7) | 27.8 (5.7) | 17.2 (3.2) | 2.1 (0.1) | 181.0 |

[a] Numbers in parentheses indicate standard errors.

indicated considerable variation for pathogenicity and aggressiveness. Despite this, PCA of reaction types revealed that the 24 isolates had a similar virulence, which differed from that of the 4 foreign SBR isolates that were tested. In contrast, the two 2004 Alabama isolates from the Pham et al. study (39) were grouped with foreign isolates, suggesting the possibility that some pathotypes that existed in the southern United States in 2004, the first growing season that SBR was found in the region, disappeared or were suppressed due to subsequent winter damage to kudzu.

Qualitative assessment of soybean reactions to SBR by classification into IM (immune), RB (resistant), or TAN (susceptible) infection types has been used in previous studies to describe virulence diversity in *P. pachyrhizi* (39,47) but, within RB and TAN infection types, there are frequently quantitative differences in the number of uredinia per lesion and in the intensity of sporulation, as well as in the color of the lesions (6,9,32,52). In the present study, the *Rpp3* genotype PI 462312 developed nonsporulating RB lesions when challenged by all of the 2007 and 2008 U.S. isolates. PI 462312 has shown less resistance to some U.S. field populations of *P. pachyrhizi* (47,48). The *Rpp4* genotype PI 459025B developed an RB reaction with abundant sporulation to 25% of the 24 U.S. *P. pachyrhizi* isolates, and similar variation among RB or TAN infection types has been previously reported (47). PI 200492 (*Rpp1*) developed an immune or RB infection type against all 24 of the U.S. isolates, whereas the two Alabama isolates collected in 2004 were reported to produce TAN infection types on PI 200492 and PI 462312 (*Rpp3*), indicating susceptibility (6,39). Avirulence genes interacting with *Rpp1* and *Rpp3* in the 2004 population may have been lost through genetic drift resulting from restricted survival of overwintering rust populations.

The *Rpp1* and *Rpp6* genes have historically been effective against *P. pachyrhizi* field populations in the southern United States (52,53) and they provided high levels of resistance to the 24 domestic isolates in this study (Table 2). In 2012, however, both of these resistance genes were ineffective against a field population in north-central Florida (53) and a purified single pustule isolate established from that population (37). Interestingly, the field population at the same location was unable to defeat these two genes in 2013 (unpublished data), providing further evidence of a winter survival bottleneck. With the exception of IN73-1, the foreign isolates in the present study were virulent on *Rpp1* but not on *Rpp6,* similar to the responses to the 2012 *P. pachyrhizi* population at Quincy, in northern Florida (53). Based on the limited number of samples, the *Rpp6* gene could be expected to provide broader resistance domestically and internationally than *Rpp1*.

Based on a quantitative assessment of uredinia densities, the U.S. isolates included six aggressiveness groups, which was the same number that Twizeyimana and Hartman (47) identified among 72 U.S. isolates based on uredinia density in detached leaf assays. The 24 U.S. isolates evaluated in this greenhouse study were a subset of those 72 isolates, and our results show that pathogenic variation among the 24 U.S. isolates from 2007 and 2008 was similar to that found in the 72 isolates collected between 2006 and 2009, but different from that of the three U.S. isolates from 2004 and the foreign isolates included.

Gene-for-gene interactions in some rust fungus pathosystems have been studied using molecular and genetic analysis (4). The six aggressiveness groups described in this study represent at least part of the pathogenic diversity that existed within and among U.S. *P. pachyrhizi* field populations in 2007 and 2008, and the variation found was similar to that reported by others (47,52). Altogether, the results demonstrate considerable variation in aggressiveness and virulence. Because single purified uredinial isolates from each U.S. location were used in this study, the amount of virulence diversity that existed within the *P. pachyrhizi* source populations is unknown; however, Twizeyimana et al. (48) found that, in Nigerian populations, >90% of the total genetic diversity was represented within fields. The reasons why U.S. isolates in some PCA-determined groups were less virulent than isolates in others are not known, but it is possible that factors other than pathogen–soybean interactions alone were involved. For example, penetration frequencies of urediniospores have been shown to be reduced as a result of parasitism by *Verticillium psalliotae* and *Trichothecium roseum* (27,41). It is also possible that natural selection on kudzu might affect virulence on soybean.

Categorization of the isolates into different aggressiveness groups on the basis of disease severity or uredinia densities revealed differential reactions between some of the 20 PIs tested in the third experiment and isolates in certain groups. Summing the data across the six or seven aggressiveness groups produced values that were useful for quick assessments of the overall resistance of each PI. Differences were particularly evident among the sums of the numbers of uredinia per square centimeter, with a 100-fold range from 2.0 to 217.7 (Table 5). PI 567104B, PI 567089A, PI 417089A, PI 567046A,

**Table 6.** Average numbers of uredinia per lesion on a set of 21 selected soybean genotypes produced by 24 *Phakopsora pachyrhizi* isolates from the United States representing six aggressiveness groups determined using principal component analysis

| | Aggressiveness group[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| **Genotypes** | **I** | **II** | **III** | **IV** | **V** | **VI** | **Sum across groups** |
| DT 2000 | 0.1 (0.1) | 0.2 (0.1) | 0.6 (0.2) | 0.1 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 1.0 |
| PI 567104B | 0.1 (0.1) | 0.2 (0.2) | 0.1 (0.1) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 0.4 |
| PI 567046A | 0.1 (0.1) | 0.7 (0.3) | 0.1 (0.1) | 0.1 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 1.0 |
| PI 567089A | 0.1 (0.1) | 0.1 (0.1) | 0.1 (0.1) | 0.0 (0.0) | 0.0 (0.0) | 0.2 (0.1) | 0.5 |
| PI 417089A | 0.2 (0.1) | 0.1 (0.0) | 0.0 (0.0) | 0.1 (0.0) | 0.1 (0.1) | 0.0 (0.0) | 0.5 |
| PI 605891A | 0.2 (0.1) | 0.7 (0.3) | 0.4 (0.2) | 0.2 (0.0) | 0.1 (0.1) | 0.5 (0.2) | 2.1 |
| PI 203398 | 0.2 (0.3) | 0.2 (0.1) | 0.3 (0.1) | 0.1 (0.1) | 2.7 (0.7) | 0.0 (0.0) | 3.5 |
| PI 224268 | 0.5 (0.1) | 0.6 (0.2) | 0.5 (0.2) | 0.7 (0.2) | 0.5 (0.2) | 0.0 (0.0) | 2.8 |
| PI 423972 | 1.5 (0.2) | 2.0 (0.3) | 1.6 (0.2) | 1.5 (0.3) | 1.4 (0.3) | 1.0 (0.5) | 9.0 |
| PI 567351B | 1.5 (0.3) | 0.6 (0.1) | 1.2 (0.1) | 0.7 (0.2) | 0.5 (0.1) | 0.0 (0.0) | 4.5 |
| Williams 82 | 4.5 (0.6) | 4.1 (0.7) | 5.6 (1.7) | 3.6 (0.5) | 4.5 (0.6) | 2.2 (0.7) | 24.5 |
| PI 587886 | 4.6 (0.4) | 4.8 (0.6) | 5.4 (0.3) | 4.0 (0.6) | 3.6 (0.7) | 1.5 (0.2) | 19.3 |
| PI 605779E | 4.8 (0.5) | 3.1 (2.7) | 8.1 (0.8) | 3.9 (0.3) | 4.9 (0.6) | 0.6 (0.4) | 25.4 |
| PI 587905 | 5.1 (0.3) | 5.1 (0.7) | 5.7 (1.0) | 3.7 (0.6) | 4.5 (0.7) | 1.1 (0.6) | 25.2 |
| PI 587880B | 5.1 (0.6) | 3.8 (0.4) | 5.7 (1.2) | 3.5 (0.5) | 3.6 (1.0) | 2.1 (0.4) | 16.2 |
| PI 398288 | 5.2 (2.6) | 4.5 (0.6) | 5.3 (0.9) | 4.0 (1.0) | 3.3 (0.7) | 1.3 (0.9) | 23.6 |
| PI 567341 | 5.4 (0.8) | 3.8 (0.5) | 5.4 (0.6) | 1.8 (0.5) | 4.3 (0.4) | 0.0 (0.0) | 20.7 |
| PI 567041A | 5.4 (4.1) | 4.2 (0.5) | 5.2 (1.4) | 3.4 (0.5) | 2.5 (0.4) | 0.6 (0.4) | 21.3 |
| PI 605833 | 5.5 (0.6) | 5.2 (0.7) | 4.2 (0.1) | 5.6 (0.8) | 2.8 (0.4) | 4.2 (0.9) | 27.5 |
| PI 506863 | 5.6 (0.5) | 4.9 (0.7) | 7.3 (1.0) | 4.7 (0.5) | 5.0 (1.2) | 3.4 (1.4) | 30.9 |
| PI 507305 | 6.1 (3.7) | 2.8 (0.6) | 9.3 (2.4) | 3.3 (0.4) | 3.0 (0.6) | 0.3 (0.1) | 24.8 |

[a] Numbers in parentheses are standard errors.

and DT 2000 all had high levels of resistance to the U.S. isolates used in this study and, therefore, would be good sources of *Rpp* genes in a breeding program targeting the southern United States. PI 605891A, PI 224268, PI 203398, PI 567351B, and PI 423972 developed more disease symptoms in our assays but, nevertheless, appear to have resistance genes that should also be useful in breeding for SBR resistance. Because counting uredinia per unit area using a microscope is far more time consuming than making visual assessments of disease severity in the field, it is an impractical criterion for selecting SBR-resistant plants in a breeding program. However, as can be seen in Table 4, disease severity can sometimes be quite high on resistant plants with RB reactions, indicating that severity data alone are not always reliable indicators of SBR resistance. Therefore, large-scale phenotypic selection of inoculated seedlings for SBR resistance should be based on a combination of disease severity and reaction type, which may sometimes be easier to rate on the basis of sporulation intensity rather than lesion color, which can change over time.

## Acknowledgments

We thank the United Soybean Board for partial financial support of this research through a project entitled "Identification and Utilization of Resistance to Soybean Rust". Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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