Sources of Soybean Rust Resistance Challenged with Single-Spored Isolates of *Phakopsora pachyrhizi*

C. Paul and G. L. Hartman*

ABSTRACT

Soybean rust, caused by the fungus *Phakopsora pachyrhizi* Syd., is a potentially devastating disease that can cause significant yield losses. Resistance in soybean [*Glycine max* (L.) Merr.] germplasm, both qualitative and quantitative, may be effective in providing at least partial control of soybean rust. A number of soybean genotypes have resistance to soybean rust, but few of these have been challenged with the recently recovered U.S. isolates. The objective of this study was to evaluate known sources of soybean rust resistance against U.S. isolates of *P. pachyrhizi*. Twenty-eight soybean genotypes that either contained known major-genes for resistance or had been reported as new sources of soybean rust resistance, along with two susceptible checks, were challenged with six *P. pachyrhizi* isolates collected in the U.S. All six isolates produced similar phenotypic reactions within each of the genotypes. Five genotypes, including the *Rpp1* source and the isoline of 'Williams 82' with *Rpp1*, had no visible lesions. Eleven genotypes produced red-brown lesions with few uredinia, including the sources of *Rpp2* and *Rpp3*, and the remainder had susceptible tan lesions, including the source of resistance for *Rpp4*. Uredinial counts from genotypes producing red-brown lesions on live and fixed leaflets showed significant variation in the number of uredinia with a genotype × isolate interaction. Uredinial counts from genotypes producing tan lesions on live and fixed leaflets showed significant variation in the number of uredinia among genotypes, but there was no genotype × isolate interaction. There were significant correlations ($r = 0.8$, $P < 0.0001$; and $r = 0.4$, $P < 0.0001$) between uredinial counts based on live and fixed leaflets within genotypes producing red-brown lesions and those producing tan lesions, respectively.

C. Paul, Dep. of Crop Sciences, Univ. of Illinois, 1101 West Peabody Dr., Urbana, IL 61801; G.L. Hartman, USDA-ARS and Dep. of Crop Sciences, Univ. of Illinois, 1101 West Peabody Dr., Urbana, IL 61801. 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 the USDA implies no approval of the product to the exclusion of others that may also be suitable. Received 17 Dec. 2008. *Corresponding author (ghartman@illinois.edu).

Abbreviations: AVRDC, Asian Vegetable Research and Development Center; IITA, International Institute of Tropical Agriculture.

Soybean rust, caused by *Phakopsora pachyrhizi* Syd., has been widely distributed throughout the tropics and subtropics of Asia for many decades (Hartman et al., 1999) and more recently has been reported in Africa (Levy, 2005), South America (Yorinori et al., 2005), and the United States (Killgore and Heu, 1994). Since the first report in the continental United States (Schneider et al., 2005), the occurrence of soybean rust has been intensely monitored (USDA, http://sbr.ipmpipe.org/cgi-bin/sbr/public.cgi).

The sources of resistance and the genetics of resistance of the four single dominant genes to specific soybean rust isolates were reviewed (Hartman et al., 2005). In addition, *Rpp5* was recently identified (Calvo et al., 2008), along with a new allele of *Rpp1* (Chakraborty et al., 2009). Resistance to *P. pachyrhizi* in soybean [*Glycine max* (L.) Merr.] has been described as producing a resistant response when no visual lesions are observed or when red-brown lesions develop, while a susceptible response occurs when tan lesions develop (Bromfield, 1984; Bromfield and Hartwig, 1980). Many reports indicated that these four single dominant genes (*Rpp1–4*) are not effective when challenged with different

Published in Crop Sci. 49:1781–1785 (2009).
doi: 10.2135/cropsci2008.12.0710

677 S. Segoe Rd., Madison, WI 53711 USA

isolates of *P. pachyrhizi* (Bonde et al., 2006; Miles et al., 2008; Pham et al., 2009). For example, when each of the known resistance sources (*Rpp1–4*) were challenged with three U.S. *P. pachyrhizi* isolates, PI 200492 (*Rpp1*) and PI 462312 (*Rpp3*) produced a differential response whereas PI 230970 (*Rpp2*) and PI 459025B (*Rpp4*) produced red-brown lesions (Pham et al., 2009).

Along with single gene resistance, partial resistance to soybean rust has been described (Hartman et al., 2005). This kind of resistance may be controlled by minor genes and may be expressed as reduced uredinial number and size, a longer latent period, and other components related to fungal reproduction. Latent period was identified as an important component of partial resistance in wheat (*Triticum aestivum* L.) that reduced the rate of wheat leaf rust (caused by *Puccinia triticina*) epidemics (Parlevliet, 1979). Recently, the average number of uredinia per lesion and average uredinial diameter were reported to be components of partial resistance in soybean rust and were a reflection of the growth of the fungus in the host tissue (Bonde et al., 2006).

Over the last decade, there has been a concerted effort to find additional sources of resistance to soybean rust. Nearly the entire USDA germplasm collection was evaluated for resistance to *P. pachyrhizi* under controlled conditions (Miles et al., 2006). In addition, many of these sources were evaluated under field conditions (Miles et al., 2008; Twizeyimana et al., 2008). Some of these sources were evaluated under controlled conditions to three U.S. isolates collected in 2004 (Pham et al., 2009). Since then, a collection of U.S. isolates has been assembled (Smith et al., 2007) and initial results reported (Paul and Hartman, 2008). In this study, six isolates of *P. pachyrhizi* were used to challenge 30 soybean genotypes. Both qualitative assessments, based on the reaction type, and quantitative assessment, based on the number of uredinia per unit of inoculum in both live and fixed soybean leaflets, were used to assess resistance.

MATERIALS AND METHODS

Plant Materials

Thirty soybean genotypes were used in this study, including 'Williams 82' and 'Dwight,' which were susceptible checks (Miles et al., 2006). The remaining 28 genotypes had reported resistance to *P. pachyrhizi*. These included PI 200492 (*Rpp1*), PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), PI 459025B (*Rpp4*), PI 547875 (an isoline of Williams 82 carrying *Rpp1*), TGx1740-2F, TGx1903-3F, and TGx1835-10E (breeding lines from International Institute of Tropical Agriculture [IITA], Ibadan, Nigeria) (Twizeyimana et al., 2008), a first-generation genotype, MT-1, from PI 483218 × *G. tomentella* (Patzoldt et al., 2007), SRE-B-15A and SRE-G-56F (Asian Vegetable Research and Development Center [AVRDC]) (Hartman, 1995), 'UG-5' a source from Uganda (Twizeyimana et al., 2008), PI 224268 and PI 227687 reported from Australia (McLean, 1979), and other Plant Introductions reported as potential sources of resistance (Miles et al., 2006). Seeds of these genotypes were collected from AVRDC, IITA, and the USDA soybean germplasm collection center at Urbana, IL.

Table 1. Origin of single-spored *Phakopsora pachyrhizi* isolates used to inoculate 30 soybean genotypes under controlled conditions. These isolates are maintained at the National Soybean Research Center, University of Illinois, Urbana, IL.

Code	State	County	Date received	Host
FL06-1	Florida	Gadsden	3 Nov. 2006	soybean
FL07-1	Florida	Gadsden	26 Jan. 2007	soybean
IL06-1	Illinois	Jefferson	23 Oct. 2006	soybean
MS06-1	Mississippi	Warren	7 Nov. 2006	soybean
SC06-1	South Carolina	Calhoun	30 Oct. 2006	kudzu
TX07-1	Texas	Hidalgo	21 Feb. 2007	soybean

Isolates and Inoculations

Urediniospores from rust-infected leaves obtained from Florida, Illinois, Mississippi, South Carolina, and Texas (Table 1) in double-sealed bags in 2006 and 2007 (APHIS Permit No. 73149) were collected using a vacuum-type spore collector (Barnant, Barrington, IL). Isolates were purified and cultured as previously reported (Smith et al., 2007). Urediniospores for each isolate were increased using detached leaves of Williams 82 (Twizeyimana et al., 2007) in tissue culture chambers (Conviron, Winnipeg, Canada) maintained at 20 to 23°C with 12/12 h light/dark.

Plants were grown in growth chambers (Percival Scientific Inc., Perry, IA) maintained at 20 to 24°C and 60 to 70% relative humidity with 12 h of light (300 μmol m^{-2} s^{-1} photosynthetically active radiation). Four plants per genotype per cell (8 by 8 cm) were planted in trays (52 by 26 cm) containing potting mixture mixed with 14–14–14 N–P–K osmocote fertilizer. Plants were watered daily as needed. Leaflets were removed from 2- to 3-wk-old plants that had one to two trifoliolates, rinsed four to five times in sterile distilled water, air-dried in a biological safety cabinet, and transferred with the lower leaf surface facing up to water agar medium amended with 6-benzylaminopurine (1.5% v/w) in plates (100 by 15 mm).

Urediniospores, collected separately for each isolate from previously infected detached leaflets of Williams 82 using a vacuum spore collector, were suspended in sterile distilled water containing 0.01% (v/v) Tween20 in 15-mL tubes, counted using a hemocytometer, and adjusted as needed to a final solution of 50 urediniospores per microliter. Each leaflet was inoculated with 20-μL (approximately 1000 urediniospores) of each isolate using different pipette tips for each isolate. Three 20-μL inoculation drops were placed on each side of the leaflet midrib approximately equal distance from each other making a total of six inoculation sites per leaflet. Petri plates containing inoculated leaflets on amended water agar were incubated for 12 h in a tissue chamber at 20 to 22°C in the dark. After dark incubation, plates were sealed with parafilm and incubated at 20 to 22°C in a 12-hour light (380 μmol m^{-2} s^{-1})–dark cycle.

Experimental Design and Evaluation

The experiment was a completely randomized design with three replications. There were 540 total experimental units based on a single leaflet from each of 30 genotypes randomly inoculated with six isolates in three replications on separate leaflets. The entire experiment was repeated with only reaction type recorded in the repeat.

Sixteen days after inoculation, leaflets were examined visually at each inoculation point with a stereoscope (Olympus Corp., Tokyo, Japan) at ×10 magnification and assigned a reaction type of either immune (no uredinia formation), red-brown with uredinia, or tan. The number of sporulating uredinia per inoculation point was counted at ×100 magnification.

After the live visual evaluation, leaflets were transferred to petri plates containing a solution of absolute ethanol:acetic acid (3:1 vol:vol) for 24 h to fix and remove the chlorophyll and other pigments from the tissue (Bonde et al., 2006). The leaflets were then transferred to lactophenol for 24 h and stained in 0.1% cotton blue in lactophenol for 24 h. Leaves were washed with distilled water twice and stained uredinia were counted at ×100 magnification.

Statistical Analyses

The data for those genotypes producing red-brown lesions and those producing tan lesions were analyzed separately. The data of uredinial counts per unit of inoculum from live and fixed tissue were log-transformed after adding 100 to each number before running the analysis of variance in JMP 7 (SAS Institute, Cary, NC). Mean separation was done by calculating the LSD at $P = 0.05$ when treatment means were significantly different ($P < 0.05$) in the ANOVA. For the tables, nontransformed uredinial counts from live and fixed tissue were presented. Pearson coefficient of correlation was calculated in JMP 7 between the uredinial counts in live and fixed tissue.

Table 2. Analysis of variance for live and fixed uredinial counts within soybean genotypes producing red-brown and tan lesions after inoculation with *Phakopsora pachyrhizi*.

Variable	Source of variation	df	Mean square	*F* value	Pr > *F*
	Red-brown lesions				
Live uredinia	Genotype	15	0.5668376	29.7855	<0.0001
	Isolate	5	0.0190117	2.9970	0.0128
	Genotype × isolate	75	0.2296062	2.4130	<0.0001
Fixed uredinia	Genotype	15	1.9833967	40.8522	<0.0001
	Isolate	5	0.0256746	1.5865	0.1664
	Genotype × isolate	75	0.3974867	1.6374	0.0046
	Tan lesions				
Live uredinia	Genotype	13	1.4210091	10.1509	<0.0001
	Isolate	5	0.0533354	0.9906	0.4258
	Genotype × isolate	65	0.4517040	0.6453	0.9759
Fixed uredinia	Genotype	13	3.2911522	9.2615	<0.0001
	Isolate	5	0.0425157	0.3111	0.9057
	Genotype × isolate	65	1.2668174	0.7130	0.9371

RESULTS

All six isolates produced similar phenotypic reactions within each of the accessions. Five genotypes produced no visible lesions (PI 200492, PI 547875, PI 081765, PI 417089A, and UG-5), 11 produced red-brown lesions (PI 230970, PI 462312, PI 506764, PI 203398, PI 224268, PI 567039A, PI 605891A, PI 567046A, PI 467323A, PI 561377, and PI 227687), and 14, including the two susceptible checks and PI 459025B (the source of *Rpp4*), produced tan lesions.

Within the genotypes producing red-brown lesions, there was an interaction between isolate and genotype for the two variables, live and fixed counts of uredinia (Table 2). Four genotypes (PI 227687, PI 230970, PI 467323A, and PI 561377) produced more uredinia than none for one or more isolates (Table 3). The other 12 genotypes did not have uredinial counts for both variables greater than none for all the isolates.

Within the genotypes producing tan lesions, there was no interaction between isolate and genotype for the two variables, and only genotypes produced a significant source of variation (Table 2). Six genotypes that produced tan lesions had counts of live uredinia that differed from the susceptible check Williams 82 (Table 4). One of these, PI 459025B, was higher than Willaims 82; the other five had lower counts than Williams 82. All genotypes, except PI 651356 and TGx17402-F, had lower fixed uredinial counts than Williams 82.

There were significant correlations ($r = 0.8$, $P < 0.0001$; $r = 0.4$, $P < 0.0001$) between uredinial counts based on live

Table 3. Mean number of uredinia per inoculation unit with red-brown or no lesions in live and fixed tissue on soybean genotypes 16 d after inoculation with U.S.-collected isolates of *Phakopsora pachyrhizi*.

Genotypes†	Isolates‡											
	FL06–1		FL07–1		IL06–1		MS06–2		SC06–1		TX07–1	
	Live	Fixed	Live	Fixed	Live	Fixed	Live	Fixed	Live	Fixed	Live	Fixed
PI 200492 (*Rpp1*)	0	0	0	0	0	0	0	0	0	0	0	0
PI 462312 (*Rpp3*)	0	0	1	2	2	6	0	1	2	3	3	4
PI 506764	0	0	0	2	2	8	0	0	0	0	0	0
PI 567046A	0	0	0	0	0	0	0	0	8	10	0	0
PI 203398	0	1	0	1	1	1	0	0	0	0	0	0
PI 567039A	0	0	0	0	0	0	2	2	0	1	2	3
PI 547875 (*Rpp1* iso)	0	1	0	0	0	0	0	1	0	0	0	0
PI 081765	0	0	0	1	0	1	0	0	0	0	0	0
PI 224268	2	3	7	9	4	7	8	13	2	3	3	3
PI 227687	7	9	4	9	16*	20*	6	13	7	13	2	8
PI 230970 (*Rpp2*)	17*	20*	4	5	35*	41*	16*	17*	7	9	2	2
PI 467323A	20*	32*	9*	15	20*	23*	15*	22*	13*	35*	16*	55*
PI 561377	6	39*	10*	33*	3	38*	13*	28*	5	32*	3	43*
'UG-5'	0	0	0	0	0	0	0	0	0	0	0	0
PI 605891A	0	0	0	0	0	1	0	0	0	0	0	0
PI 417089A	0	0	0	1	0	0	0	0	0	0	0	0

*Significantly different from 0 at $P = 0.05$.

†All genotypes are from the USDA–ARS Soybean Germplasm Collection, Urbana, IL except for UG-5, from the National Agricultural Research Organization, Kampala, Uganda.

‡Designated by state abbreviation and year collected.

Table 4. Mean number of uredinia per inoculation unit with tan lesions counted in live and fixed tissue on soybean genotypes 16 d after inoculation with six domestic isolates of *Phakopsora pachyrhizi.*

Genotypes†	Uredial counts	
	Live	Fixed
'Dwight'	33	34*
MT-1	34	47*
PI 417012	40	53*
PI 437241	24*	47*
PI 459025B (*Rpp4*)	60*	71*
PI 561356	22*	75
PI 567041A	19*	38*
PI 594538A	33	36*
SRE-B-15A	23*	35*
SRE-G-56F	17*	25*
TGx1740-2F	37	78
TGx1835-10E	38	62*
TGx1903-3F	31	66*
'Williams 82'	39	96

*Significantly different from Williams 82 at $P = 0.05$.

†All genotypes are from the USDA–ARS Soybean Germplasm Collection, Urbana, IL, except for TGx1835-10E, TGx1740-2F, and TGx1903-3F, from the International Institute of Tropical Agriculture, Ibadan, Nigeria; MT-1, from Dr. Hymowitz, University of Illinois, Urbana, IL; and SRE-B-15A and SRE-G-56F, from the Asian Vegetable Research and Development Center, Taiwan.

and fixed leaflets within genotypes producing red-brown lesions and those producing tan lesions, respectively.

DISCUSSION

For soybean rust resistance to be effective, knowledge of how resistance sources and known genes react to a wide range of isolates of *P. pachyrhizi* is important. In our study, there was no differential response among the genotypes when challenged with these six domestically collected isolates. Differential reactions were observed, however, when sources of resistance were challenged with 10 isolates, including three U.S.-collected isolates (Pham et al., 2009). That study utilized isolates collected in the U.S. in 2004, the first year that soybean rust was reported in the continental U.S. The isolates used in our study, although from a broad geographic area in the U.S., did not show this kind of variation and were not compared to the isolates collected in 2004 due to restrictions in transporting pathogens from a higher to a lower containment facility. The reason for this difference could be due to a small sampling size, a restriction in survival of some virulence types over others when the population is reduced to its overwintering subtropical locations, or selection of reduced variation in the process of purifying the isolates. The virulence pattern based on the four known genes in our study was not the same as that found in the 2004 isolates, indicating that these six isolates do not have a similar virulence profile to the isolates collected in 2004. None of our isolates caused a susceptible reaction on PI 200492 (*Rpp1*), PI 230970 (*Rpp2*), and PI 462312 (*Rpp3*), while all isolates caused a susceptible reaction on PI 459025B (*Rpp4*). This differed from the differential response caused by the three 2004 U.S. *P. pachyrhizi* isolates on PI 200492 (*Rpp1*) and PI 462312 (*Rpp3*) and the resistant response produced by PI 459025B (*Rpp4*) (Pham et al., 2009).

Our results indicate that the *Rpp1* source of resistance in PI 200492 and its isoline was resistant to the six isolates of *P. pachyrhizi*. However, PI 200492 has been reported to produce an immune response, red-brown lesions, or tan lesions depending on the isolates used to challenge it (Bonde et al., 2006; Pham et al., 2009). Differential responses of the sources of resistance for *Rpp2*, *Rpp3*, and *Rpp4*, were previously reported (Bonde et al., 2006; Pham et al., 2009) but were not found in our study. In the past, the immune reaction only was reported with *Rpp1* with certain isolates of *P. pachyrhizi*, such as one from India (Bonde et al., 2006; Pham et al., 2009). In our experiment, this reaction was produced in those genotypes with *Rpp1* (PI 200492 and PI 547875) and in other genotypes that may or may not have *Rpp1*.

In addition to the sources of resistance with known genes, a number of genotypes also had no or low uredinial counts. These included UG-5 and PI 417089A, which were reported to be useful sources of resistance in Nigeria (Twizeyimana et al., 2008), and PI 224268 and PI 227687, which were reported immune in Australia (McLean, 1979). The latter two genotypes were not immune in our test, although the counts of uredinia were relatively low.

Some of the genotypes we tested, such as PI 594538A, produced tan lesions despite being considered resistant in other tests using isolates collected from outside the United States (Chakraborty et al., 2009; Twizeyimana et al., 2008). Some genotypes, like PI 567041A and SRE-G-56F that produced tan lesions, had lower live uredinial counts than the susceptible checks and counts not different than some genotypes with red-brown lesions. This may be an indication that these genotypes have partial resistance. Partial resistance to soybean rust, one aspect of which is reduced sporulation, was previously reviewed (Hartman et al., 2005). Reduced sporulation also has been used to classify partial resistance to rust in flax (Rashid and Bernier, 1991). Other quantitative traits like latent period and uredinial size have been used to classify wheat lines for partial resistance to leaf rust (Rime et al., 2005). For faba bean (*Vicia faba* L.) and soybean rust, uredinial numbers and not size were proposed to be better attributes for evaluating partial resistance (Bonde et al., 2006; Herath et al., 2001).

When comparing uredinial counts in live and fixed tissue, counts in fixed tissue generally were greater, indicating that uredinia were not fully developed and may have sporulated in time or they were suppressed and were not going to sporulate. Even though the correlation between the two variables was significant, the results between the two variables did not always show the same differences within a genotype. For example, among the genotypes with red-

brown lesions, PI 561377 had greater fixed uredinial counts than zero with all of the isolates but only had greater than zero with two isolates when live uredinia were counted. In addition, among the genotypes with tan lesions, Dwight and MT-1 were not different from Williams 82 on the basis of live uredinial counts but had fewer fixed uredinial counts than Williams 82. On the other hand, PI 459025B produced more live uredinial counts than Williams 82 but had less fixed uredinial counts than Williams 82. Since the two variables, live versus fixed uredinial counts, were not the same magnitude difference within each genotype, additional research is needed to evaluate how these variables interact over time. This is especially important when considering or defining attributes of partial resistance.

In conclusion, a differential response was not observed in our test that challenged 28 soybean genotypes with six U.S.-collected isolates of *P. pachyrhizi*. Many of the claimed rust resistant sources that we tested were not resistant in our test, indicating that these isolates contained virulence genes that were not observed when these genotypes were first reported to be resistant to *P. pachyrhizi.* Additional screening of isolates over time and locations is needed to better understand the spectrum of virulence and diversity of the U.S.-collected isolates so that resistance screening and breeding programs may be more effective in developing lines with broad-based resistance.

Acknowledgments

We thank the United Soybean Board and the Illinois Soybean Association for partial support for this research, and Lawrence Boyce who assisted in counting the uredinia in fixed tissue. We would also like to thank Drs. Isakeit, Marois, Moore, and Mueller for sending infected samples; Drs. Bandyopadhyay, Nelson, and Wang for providing seed; and Theresa Herman and Curt Hill for their suggestions.

References

Bonde, M.R., S.E. Nester, C.N. Austin, C.L. Stone, R.D. Frederick, G.L. Hartman, and M.R. Miles. 2006. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomiae* isolates. Plant Dis. 90:708–716.

Bromfield, K.R. 1984. Soybean rust. Monogr. 11. Am. Phytopathological Soc., St. Paul, MN.

Bromfield, K.R., and E.E. Hartwig. 1980. Resistance to soybean rust and mode of inheritance. Crop Sci. 20:254–255.

Calvo, E.S., R.A.S. Kiihl, A. Garcia, A. Harada, and D.M. Hiromoto. 2008. Two major recessive soybean genes conferring soybean rust resistance. Crop Sci. 48:1350–1354.

Chakraborty, N., J. Curley, R.D. Frederick, D.L. Hyten, R.L. Nelson, G.L. Hartman, and B.W. Diers. 2009. Mapping and confirmation of a new allele at *Rpp1* from soybean PI 594538A conferring RB lesion–type resistance to soybean rust. Crop Sci. 49:783–790.

Hartman, G.L. 1995. Highlights of soybean rust research at the Asian Vegetable Research and Development Center, p. 19–28, *In* J. B. Sinclair and G. L. Hartman (ed.) Proc. of the Soybean Rust Workshop, Urbana, IL. 9–11 Aug. 1995. College of Agricultural, Consumer, and Environmental Sciences, National Soybean Research Lab., Urbana, IL.

Hartman, G.L., M.R. Miles, and R.D. Frederick. 2005. Breeding for resistance to soybean rust. Plant Dis. 89:664–666.

Hartman, G.L., J.B. Sinclair, and J.C. Rupe. 1999. Compendium of soybean diseases. APS Press, St. Paul, MN.

Herath, I.H.M.H.B., F.L. Stoddard, and D.R. Marshall. 2001. Evaluating faba beans for rust resistance using detached leaves. Euphytica 117:47–57.

Killgore, E., and R. Heu. 1994. First report of soybean rust in Hawaii. Plant Dis. 78:1216.

Levy, C. 2005. Epidemiology and chemical control of soybean rust in southern Africa. Plant Dis. 89:669–674.

McLean, R.J. 1979. Histological studies of resistance to soybean rust, *Phakopsora pachyrhizi* Syd. Aust. J. Agric. Res. 30:77–84.

Miles, M.R., R.D. Frederick, and G.L. Hartman. 2006. Evaluation of soybean germplasm for resistance to *Phakopsora pachyrhizi*. Plant Health Progr. doi 10.1094/PHP-2006-0104-01-RS.

Miles, M.R., W. Morel, J.D. Ray, J.R. Smith, R.D. Frederick, and G.L. Hartman. 2008. Adult plant evaluation of soybean accessions for resistance to *Phakopsora pachyrhizi* in the field and greenhouse in Paraguay. Plant Dis. 92:96–105.

Parlevliet, J.E. 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17:203–222.

Patzoldt, M.E., R.K. Tyagi, T. Hymowitz, M.R. Miles, G.L. Hartman, and R.D. Frederick. 2007. Soybean rust resistance derived from *Glycine tomentella* in amphiploid hybrid lines. Crop Sci. 47:158–161.

Paul, C., and G.L. Hartman. 2008. Evaluation of sources of soybean rust resistance using detached leaves. Phytopathology 98:S123.

Pham, T.A., M.R. Miles, R.D. Frederick, C.B. Hill, and G.L. Hartman. 2009. Differential responses of resistant soybean genotypes to ten isolates of *Phakopsora pachyrhizi*. Plant Dis. 93:224–228.

Rashid, K.Y., and C.C. Bernier. 1991. The effect of rust on yield of faba bean cultivars and slow rusting populations. Can. J. Plant Sci. 71:967–972.

Rime, D., C. Robert, H. Goyeau, and C. Lannou. 2005. Effect of host genotype on leaf rust (*Puccinia triticina*) lesion development and urediniaspore production in wheat seedlings. Plant Pathol. 54:287–298.

Schneider, R.W., C.A. Hollier, W. H.K., M.E. Palm, J.M. McKemy, J.R. Hernandez, L. Levy, and R. DeVries-Paterson. 2005. First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. Plant Dis. 89:774.

Smith, D., C. Paul, T.A. Steinlage, M.R. Miles, and G.L. Hartman. 2007. Isolation, purification, and characterization of *Phakopsora pachyrhizi* isolates, *In* Proc. of the 2007 National Soybean Rust Symp., Louisville, KY. 12–14 Dec. 2007. Am. Phytopathological Soc., St. Paul, MN.

Twizeyimana, M., P.S. Ojiambo, T. Ikotun, C. Paul, G.L. Hartman, and R. Bandyopadhyay. 2007. Comparison of field, greenhouse, and detached-leaf evaluations of soybean germplasm for resistance to *Phakopsora pachyrhizi*. Plant Dis. 91:1161–1169.

Twizeyimana, M., P.S. Ojiambo, T. Ikotun, J.L. Ladipo, G.L. Hartman, and R. Bandyopadhyay. 2008. Evaluation of soybean germplasm for resistance to soybean rust (*Phakopsora pachyrhizi*) in Nigeria. Plant Dis. 92:947–952.

Yorinori, J.T., W.M. Paiva, R.D. Frederick, L.M. Costamilan, P.F. Bertagnolli, G.L. Hartman, C.V. Godoy, and J. Nunes, Jr. 2005. Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001–2003. Plant Dis. 89:675–677.