Characterization of Components of Partial Resistance, *Rps2*, and Root Resistance to *Phytophthora sojae* in Soybean

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Accepted for publication 22 November 2006.

ABSTRACT

Mideros, S., Nita, M., and Dorrance, A. E. 2007. Characterization of components of partial resistance, *Rps2*, and root resistance to *Phytophthora sojae* in soybean. *Phytopathology* 97:655-662.

Phytophthora root and stem rot of soybeans caused by *Phytophthora sojae* is a serious limitation to soybean production in the United States. Partial resistance to *P. sojae* in soybeans is effective against all the races of the pathogen and is a form of incomplete resistance in which the level of colonization of the root is reduced following inoculation. Other forms of incomplete resistance include the single dominant gene *Rps2* and Ripley's root resistance, which are both race-specific. To differentiate partial resistance from the other types of incomplete resistance, the components lesion length, numbers of oospores, and infection frequency were measured in eight soybean genotypes inoculated with two *P. sojae* isolates. The *Rps2* and root-resistant genotypes had significantly lower oospore production and infection frequency compared with the partially

resistant genotype Conrad, while the root-resistant genotype also had significantly smaller lesion lengths. However, the high levels of partial resistance in Jack were indistinguishable from *Rps2* in L76-1988, based on the evaluation of these components. Root resistance in Ripley and *Rps2* in L76-1988 had similar responses for all components measured in this study. Partial resistance expressed in Conrad, Williams, Jack, and General was comprised of various components that interact for defense against *P. sojae* in the roots, and different levels of each component were found in each of the genotypes. However, forms of incomplete resistance expressed via single genes in Ripley and *Rps2* in L76-1988, could not be distinguished from high levels of partial resistance based on lesion length, oospore production, and infection frequency.

Additional keywords: *Glycine max*, host resistance, incomplete resistance, partial resistance.

The oomycete *Phytophthora sojae* Kaufmann & Gerdemann (*Phytophthora megasperma* Drechs. f. sp. *glycinea* Kuan & Erwin) causes *Phytophthora* root and stem rot of soybean (*Glycine max* L. Merr.) and is a limitation for soybean production around the world (56-58). Early in the season, this pathogen also causes pre- and post-emergence damping-off (18), and with heavy rains in midseason, susceptible cultivars yellow and wilt as the lateral roots are colonized. Plants which develop these stem rot symptoms usually wilt and die (16,34).

Phytophthora root and stem rot is predominantly managed through genetic host resistance via deployment of single resistance genes (*Rps*), but partial resistance to *P. sojae* also exists (34). Currently, 14 *Rps* genes have been reported at 8 loci, with a series of alleles at two of the loci (6,17,34). This management strategy has favored shifts in the pathogen population toward individuals adapted to specific *Rps* genes, thus increasing the number of races and the virulence composition among isolates (18,34). Currently, the combination of single resistance genes and partial resistance is recommended in order to avoid the boom and bust cycle that results from deployment of single resistance genes, but also to minimize yield losses when adaptation to *Rps* genes does occur (1,18).

There are different criteria used to classify plant resistance to pathogens (20). This study addresses phenotypic aspects of soybean resistance to *P. sojae*. Therefore, the reactions produced by *Rps* genes (except *Rps2*) will be considered as complete resistance because they do not allow pathogen development beyond

early stages of infection. Heitefuss (20) defined complete resistance as the absence of "development of the pathogen beyond early stages of infection that totally prevents its propagation." Conversely, Heitefuss (20) defined incomplete resistance as "limited development of the pathogen including some propagation and that may reduce spread of a disease in a host population." These definitions of complete and incomplete resistance were based on the terminology of Parlevliet (30). For this study, the effect of the soybean *Rps2* gene, "root resistance" and partial resistance, where limited colonization of the host occurs along with limited propagation of *P. sojae*, will be termed incomplete resistance (20,30). All of the reported *Rps* genes except *Rps2* completely arrest the growth of *P. sojae* through the hypersensitive response (HR) in the hypocotyl (18). The mechanisms of resistance associated with *Rps1a* and *Rps1b* genes include rapid cell death with necrosis beginning 4 hours after inoculation (hai), and cell wall appositions that encase the haustoria as soon as 4 hai (15). *Rps* gene resistance is characterized by a more rapid necrosis affecting fewer cells when compared with susceptible interactions (15,40,41,54). The presence of *Rps* genes in soybeans is usually determined using the hypocotyl inoculation technique. Plants are rated as resistant or susceptible depending on whether or not a lesion develops following inoculation of 7-day-old soybean seedlings with *P. sojae* (11).

Partial resistance is a form of incomplete resistance in the *P. sojae*-soybean system that results in reduced colonization by the pathogen. Partial resistance can be divided into specific components that limit the infection process (30,47). Several components of partial resistance such as infection frequency, latent period, spore production, incubation period, and others have been identified in other host-pathogen systems (10,12,19,30,32,52,59). Partial resistance to *P. sojae* in soybean is expressed and inherited

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doi:10.1094/PHYTO-97-5-0655

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quantitatively, effective against all the races of the pathogen, and has been shown to be polygenic (18,34,53). The exact number of genes involved in partial resistance is still unknown, although two quantitative trait loci (QTL) have been identified in 'Conrad' (7), and 4 QTL in the line V71-370 (36). In the field, genotypes with high levels of partial resistance have fewer plants that develop stem rot and have more consistent yields in the presence of the pathogen than those that do not have partial resistance (45,46). Partial resistance to *P. sojae* has also been referred to as rate-reducing resistance, field resistance, and tolerance in the soybean literature (18).

Another type of incomplete resistance in soybean is conferred by *Rps2*. This dominant single resistance gene was first reported more than 30 years ago in the soybean line D54-2437, derived from the genotype CNS (23). Interestingly, the genotype CNS and some of its derived lines have complete resistance in the field. A unique feature of the *Rps2* gene is that the resistance is expressed completely only in the roots and has an intermediate reaction in the hypocotyls, whereas other soybean R genes are all expressed in the hypocotyl (23). An intermediate reaction is one in which only 50% of hypocotyls will develop lesions following inoculation. The reaction of the *Rps2* has been previously described as a type of root resistance (34).

Root resistance has also been reported in soybeans that do not have *Rps2*. Soybean genotypes Ripley, York, and Ware have a susceptible reaction to hypocotyl inoculation and a resistant response when inoculated on the roots, but with different race specificity than *Rps2* (42,43). McBlain et al. (28) studied the inheritance of resistance to *P. sojae* in Ripley in a cross with the susceptible genotype Harper. In the population, resistance was inherited quantitatively with a heritability value of 0.54. In addition, they concluded that the genotype Ripley also has a race-specific root resistance similar to the resistance conferred by the *Rps2*, but with different race specificity (28).

The previously described studies in soybeans have highlighted the difficulty of distinguishing between high levels of partial resistance (non-race-specific) and incomplete forms of resistance due to a single gene such as *Rps2* (28,42). Incomplete resistance conferred by single genes with different race specificities has also been reported in other host pathogen systems. Incomplete R genes (*R2*, *R4*, *R10*, and *R11*) of potato against *P. infestans* can be mistaken for partial resistance and can only be differentiated using the appropriate isolates of the pathogen (9). In barley it has been shown that different genes for resistance to *Blumeria graminis* f. sp. *hedei* PM-R genes control different infection phenotypes called fast, intermediate, and slow-acting genes (24,25). Fast-acting genes do not allow fungal penetration through the process of epidermal cell death or other mechanisms. Intermediate acting genes limit hyphal development, and cell death was delayed until 24 to 72 hai. Finally, slow-acting PM-R genes allow robust hyphal growth for 72 hai, followed by cell death (24,25).

The use of parental lines with known specific components has been proposed as a way to improve the efforts of breeding for potato partial resistance to *P. infestans* (10,12). Unfortunately, there are limited studies of components of partial resistance in the soybean-*P. sojae* system and for the expression of resistance in roots in general. Lesion length was shown to be effective to model disease development, and to differentiate between genotypes with various levels of partial resistance (51), but it is not known if other components can be identified in soybean roots and what the contribution of each component is to the overall response to infection.

The hypothesis is that partial resistance, root resistance, and *Rps2*-mediated incomplete resistance, as they are expressed in soybeans, are all different mechanisms of incomplete resistance that can be differentiated by examining several components of partial resistance. Therefore the objectives of this study were to: (i) identify and determine the relationship of components of soy-

bean partial resistance to *P. sojae*; (ii) determine if lesion length, infection frequency, and oospore production are different among the incomplete resistance types (*Rps2*, root resistance, and partial resistance); and (iii) determine which component(s) most accurately differentiate(s) among these different types of incomplete resistance. For this study, eight soybean genotypes with various levels of partial resistance and different types of incomplete resistance were inoculated with a complex *P. sojae* pathotype (soybean genotypes with *Rps* genes are susceptible) to examine partial resistance (non-race-specific) and with a simple *P. sojae* pathotype (soybeans with *Rps7* are susceptible) to differentiate among types of incomplete resistance.

MATERIALS AND METHODS

***P. sojae* isolates and inoculum.** Two isolates of *P. sojae* from the collection of the soybean pathology laboratory at The Ohio State University were used: a simple pathotype OHR1 (vir. 7) and a complex pathotype 1.S.1.1 (vir. 1a, 1b, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8). To characterize the pathotypes of the isolates, the following differentials were used in this study: 'Williams' (universal susceptible); 'Harlon' (*Rps1a*), 'Harosoy 13XX' (*Rps1b*), 'Williams 79' (*Rps1c*); PI103091 (*Rps1d*); 'Williams 82' (*Rps1k*); L76-1988 (*Rps2*); L83-570 (*Rps3*); PRX 146-36 (*Rps3b*); PRX 145-48 (*Rps3c*); L85-2352 (*Rps4*); L85-3059 (*Rps5*); 'Harosoy 62XX' (*Rps6*) and 'Harosoy' (*Rps7*) (11). The pathogenicity of the isolates was maintained via inoculation and re-isolation from 'Sloan' using standard methodologies (33). Long-term storage of isolates was in 10% sterile glycerol in cryovials in liquid nitrogen (44). Oospores from approximately a 3- to 4-week-old culture grown on V8-juice agar were first transferred to cryovials and placed overnight at -80°C prior to liquid nitrogen as described by Tooley (44). For recovery, cryovials were submerged in a water bath at 25°C for 10 min and the entire contents were poured onto a plate of V8-juice agar or lima-bean agar.

Soybean seedlings were inoculated with either zoospores or a mycelial slurry of *P. sojae*. Zoospores were produced by transferring five plugs (6 mm) onto non-clarified V8-juice agar plates. Four days later plates were flooded with 15 ml of sterile deionized water (pH 6 to 7) for approximately 14 to 18 hours. Level of pH in water has been shown to be critical; at pH levels lower than 6.0, zoospores will burst (A. Dorrance et al., unpublished data). Water in plates was then removed and fresh water added every 30 min for a total of 7 times, with a final incubation for 3 h at 26°C (31). Zoospores were collected and counted with a hemacytometer. The concentration of zoospores was adjusted to 1×10^4 zoospores per ml of water.

The mycelial slurry was prepared from 7-day-old cultures of *P. sojae* growing on dilute lima-bean agar (15 g agar/liter) at 26°C (33). The colonized agar was macerated through a 60-ml syringe without a needle. This mycelial slurry was then transferred to a 10-ml syringe with a needle (18-gauge $\times$ 2.54 cm).

Soybean genotypes. A collection of genotypes consisting of cultivars, breeding lines, and differentials was chosen to represent the different types of resistant reactions: *Rps* genes, partial resistance, *Rps2*, and root resistance. The genotypes, pedigrees, and resistance to *P. sojae* are listed in Table 1.

Lesion length. The slant board assay was used to assess lesion length (7,29). Briefly, seeds of the eight genotypes were planted in 946 cm³ polystyrene containers (Dart 32TJ32, Mason, MI) with the following layers: (i) an ~2-cm layer of coarse vermiculite (Therm O-Rock #1A, New Eagle, PA) at the bottom of the containers; (ii) 15 cm of fine vermiculite (Therm O-Rock #4, New Eagle, PA); and (iii) 25 seeds, with another ~2 cm layer of coarse vermiculite. The containers were placed in a growth chamber with temperatures between 22 and 26°C, and a 14-h photoperiod using both fluorescent and incandescent light bulbs. Seven-day-old seedlings were removed from the containers and the vermiculite

washed from the roots in running tap water. Eight to ten plants were aligned on a polyester cloth that was placed on top of a thick, 0.5-mm absorbent cotton wicking pad on a plastic tray (27 × 35 cm) that had the raised side (2 cm) of one end removed. With a scalpel, a wound of 5 mm was scraped on the tap root 2 cm below the stem/root interface. A 1-cm³ of the mycelial slurry of *P. sojae* was then applied over the wound with a syringe. Trays were stacked and bound together with a large rubber band, and placed in 25-liter plastic containers with 2 liters of deionized water in the bottom. Roots were between the polyester cloth and were not in direct contact with water. The plastic containers were maintained for 7 days in a growth chamber with temperatures between 22 and 26°C, and a 14-h photoperiod using both fluorescent and incandescent light bulbs.

Lesion lengths were measured from the site of inoculation toward the cotyledon on each seedling. Previous reports have shown no significant differences in lesion length for measurements below the inoculation site (49). To clearly assess the presence of the pathogen, roots and hypocotyls were sliced open to expose the root stele. For each genotype, ten plants within a tray were the experimental unit. There were three replicates in each experiment and the experiment was repeated three times. The experimental design was a split plot design where isolate type was the main factor (25-liter plastic container) and genotype was the subfactor.

Number of oospores. Resistance to reproduction of *P. sojae* was measured by counting the number of oospores that formed in a 1-cm section of the root immediately below the inoculation site. These root pieces were collected from the same seedlings assayed for lesion length and were placed in 80% ethanol (14). The root tissue was fixed by heating in a microwave for 5 min on the high setting (~100°C) and then placed on the lab bench at approximately 25°C for one hour. Ethanol was poured off and the root sections were rinsed once in deionized water and then placed in commercial bleach (5.25% NaClO). Vials containing the root samples were placed in a drying oven for 5 min at 60°C. Root samples were then kept at 4°C until oospores were counted.

The root pieces of each genotype were placed on a glass slide with water and a cover slip. The mount was pressed to an approximately one- to two-cell depth to ensure that all the oospores could be observed under a compound microscope. Oospores were counted in 1-cm root pieces from four or six plants from each tray. The average for each tray was used for the statistical analysis. There were three replicates within each experiment and the whole experiment was repeated three times. The experimental design was a split plot design where isolate type was the main factor (25-liter plastic container) and genotype was the subfactor.

Infection frequency. A modified slant board assay was used to measure infection frequency. Seeds of the eight soybean genotypes were sown in polystyrene containers with vermiculite as described above. Seven-day-old seedlings were rinsed in running tap water and ten seedlings were aligned on top of a 10 × 5-cm

strip of Parafilm that was placed on the polyester cloth which was on top of a wicking pad. The roots were inoculated by placing a 10-μl drop of water containing 1×10⁴ zoospores per ml at five positions on the root. The first drop was placed on the interface between the stem and the root, and four more inoculation positions were 2 cm below each other. Following inoculation the plants were incubated for 20 min at approximately 25°C to favor zoospore encystment and penetration. Plants were covered with the polyester cloth and stacked as in the regular slant board assay in 25-liter plastic containers as described above.

Data for infection frequency was collected 3 days after inoculation by slicing the root and counting the number of water-soaked lesions at each of the five inoculation positions. The number of lesions that developed out of the 50 total inoculation sites was collected for the ten plants on each tray. The infection frequency among the inoculation positions was also determined as the proportion for each position within each tray. Each experiment was in a split-split plot design where isolate was considered as the main factor, genotype as the subfactor, and position of inoculation as the sub-subfactor. There were three replicates in each experiment and the whole experiment was repeated three times.

Data analysis. Homogeneity of variances was tested using Levene's test in Minitab release 14.2 (Minitab Inc., State College, PA). Variance infection frequency data was homogenized using an arcsine square root transformation, and oospore counts and lesion lengths were log₁₀-transformed. Each component of resistance was analyzed with Proc MIXED of SAS V.9.1 (SAS Institute Inc., Cary, NC). Replicates within experiment and repetition of the experiments were considered random factors, while isolates, genotype, and their interactions were considered fixed factors. For factors with significant differences ($P = 0.05$), Tukey-Kramer multiple range test was used for comparisons of least square means (27). Tukey-Kramer multiple range test was chosen for this analysis due to the large number of mean comparisons as well as some missing values (27,37).

The rank of the genotypes for each component of resistance and independent isolate was obtained using Proc RANK in SAS. Spearman correlations were done using Proc CORR in SAS to determine if there were relationships among the components of partial resistance for the eight genotypes inoculated only with the complex pathotype of *P. sojae* (1.S.1.1), because of the non-race-specific interaction.

RESULTS

When all eight genotypes and *P. sojae* isolates were compared, they were significantly different ($P < 0.0001$) from each other for all the components of resistance measured in this study (Table 2). The interaction of isolate by host genotype was also significant ($P < 0.0001$) for all the components. This significant interaction was expected due to isolate-specific resistance genes to *P. sojae*

TABLE 1. Type of incomplete and *Rps* gene resistance expressed to simple and complex pathotypes of *Phytophthora sojae* in soybean genotypes used in this study

Genotype	<i>Rps</i> gene	Incomplete resistance	<i>Rps</i> gene response to <i>P. sojae</i> ^z		Pedigree; (Literature cited)
			Simple (OHR1)	Complex (1.S.1.1)	
General	<i>Rps1k</i>	High partial resistance	R	S	Voris311 × Resnik; (48)
Jack	<i>rps</i>	High partial resistance	I	I	Fayette × Hardin; (26,48)
Williams	<i>rps</i>	Medium partial resistance	S	S	Wayne × L57-0034 (Clark × Adams); (48,49)
Conrad	<i>rps</i>	Medium partial resistance	S	S	A3127 × Tri-Valley Charger (Williams in background); (48,49)
Ox20-8	<i>Rps1a</i>	Low partial resistance	R	S	M405 × Harosoy 63; (8,49)
Sloan	<i>rps</i>	Low partial resistance	S	S	M59-120 × IVRE × 4731; (7,48)
L76-1988	<i>Rps2</i>	(Williams isolate with <i>Rps2</i>)	I	S	Williams (6) × (Harosoy (5) × D54-2437 [CNS background]); (3)
Ripley	<i>rps</i>	Root resistance	I	S	Hodgson × V68-1034 (CNS in background); (28,42)

^z *Rps* gene, race-specific resistance response of seedlings after inoculation with *P. sojae* using a modified hypocotyl inoculation technique. R (resistant) = zero to two seedlings developed lesions; I (intermediate) = three to six seedlings developed lesions; and S (susceptible) = seven to eight seedlings developed lesions. The pathotypes of the isolates were OHR1 (vir. 7) and 1.S.1.1 (vir. 1a, 1b, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8).

isolate OHR1 in genotypes with *Rps1a*, *Rps1k*, *Rps2*, and root resistance, but these same genotypes are susceptible to the complex isolate 1.S.1.1.

Lesion length. For the R gene response to the simple pathotype (OHR1), estimated mean lesion sizes were 1.0, 1.6, and 1.6 mm for General (*Rps1k*), Ox20-8 (*Rps1a*), and Ripley (root resistance), respectively (Table 3). Lesion size of *Rps2* expressed in L76-1988 (3.1 mm) was significantly larger than General (*Rps1k*), but not larger than Ox20-8 (*Rps1a*). The lesion lengths of Jack were not significantly different from the L76-1988 (*Rps2*), from Ox20-8 (*Rps1a*), or from Ripley (root resistance) genotypes, but Jack had significantly larger lesions than did General (*Rps1k*). Sloan was highly susceptible to both the simple and complex *P. sojae* pathotypes (23.0 and 29.3 mm, respectively).

Lesion size for Jack was significantly smaller (4.9 mm) for the complex pathotype (1.S.1.1), compared with all but one of the other genotypes, but similar in size (3.7 mm) to the simple pathotype (OHR1) (Table 3). For the complex pathotype (1.S.1.1) no race-specific responses were measured among any genotypes. Ox20-8 was the most susceptible to the complex pathotype (1.S.1.1), with an average lesion length of 40.7 mm, which was significantly dif-

ferent from Conrad, Jack, General, L76-1988 (*Rps2*), and Ripley. No significant differences in lesion size were found among the responses of Williams, Conrad, General, L76-1988 (*Rps2*), and Ripley to the complex *P. sojae* pathotype.

When genotypes with non-race-specific response to both the simple and complex pathotypes were compared, the results were mixed (Table 3). Williams and Conrad inoculated with the complex pathotype resulted in significantly larger lesion length ($P \leq 0.05$), while Sloan and Jack were not significantly different.

Number of oospores. An average of less than one oospore per 1-cm root section developed in genotypes with *Rps1a* (Ox20-8) and *Rps1k* (General) following inoculation with the simple *P. sojae* pathotype (Table 3). Few oospores (<5) were found in the genotypes with incomplete race-specific resistance *Rps2* (L76-1988) and root resistance (Ripley). The root-resistant genotype (Ripley) and Jack did not differ from the *Rps2* genotype (L76-1988). The remaining three genotypes with *rps* genes (Sloan, Williams, and Conrad), had significantly more oospores than did genotypes with *Rps* genes (General, Ox20-8, and L76-1988), as did the root-resistant genotype, Ripley.

TABLE 2. Analysis of variance (ANOVA)^w for three components of resistance to two *Phytophthora sojae* isolates among eight soybean genotypes, including race-specific and non-race-specific interactions

Effect	Lesion length ^x		Oospores/cm ^y		Infection frequency ^z	
	F	P > F	F	P > F	F	P > F
Isolate	439.72	<0.0001	395.61	<0.0001	107.02	<0.0001
Genotype	12.96	<0.0001	9.70	0.0002	23.94	<0.0001
Isolate*genotype	38.63	<0.0001	25.37	<0.0001	26.00	<0.0001
Position	--	--	--	--	19.95	<0.0001
Isolate*position	--	--	--	--	1.82	0.1236
Genotype*position	--	--	--	--	1.99	0.0020
Isolate*genotype*position	--	--	--	--	0.86	0.6688

^w Proc MIXED of SAS (ver. 9.1, SAS Institute, Cary, NC) was used for ANOVA.

^x Lesion length was measured (millimeters) from the top of the inoculation site to the edge of the symptomatic tissue 7 days after inoculation.

^y Mean number of oospores from four to six 1-cm root pieces collected immediately below the inoculation site 7 days after inoculation.

^z Infection frequency was measured as the proportion of successful infections from five inoculation points on the roots of 10 seedlings.

TABLE 3. Estimated means for each component of resistance of eight soybean genotypes inoculated with simple and complex pathotypes of *Phytophthora sojae*

Incomplete resistance <i>Rps</i> gene, genotype	Infection frequency ^u					Lesion length ^v					Oospores/cm ^w				
	Simple pathotype ^x		Complex pathotype			Simple pathotype		Complex pathotype			Simple pathotype		Complex pathotype		
	R ^y	p	R	p	Diff ^z	R	mm	R	mm	Diff	R	#	R	#	Diff
Low partial resistance															
<i>Rps</i> 1a, Ox20-8	8	0.06 c	1	0.81 a	**	6	1.6 cd	1	40.7 a	**	7	0.1 c	2	181.9 ab	**
<i>rps</i> , Sloan	1	0.75 a	2	0.68 ab	ns	1	23.0 a	2	29.3 a	ns	1	99.2 a	1	293.3 a	**
Medium partial resistance															
<i>rps</i> , Williams	2	0.63 a	3	0.64 ab	ns	2	9.8 b	3	15.6 ab	**	2	25.2 ab	3	97.8 b	**
<i>rps</i> , Conrad	3	0.60 a	4	0.60 ab	ns	3	6.3 b	4	12.0 b	**	3	16.0 ab	4	53.6 ab	**
High partial resistance															
<i>rps</i> , Jack	4	0.39 b	7	0.38 c	ns	4	3.7 bc	8	4.9 c	ns	4	1.7 bc	8	3.5 c	ns
<i>Rps</i> 1k, General	7	0.08 c	5	0.56 bc	**	7	1.0 d	7	10.5 bc	**	8	0.0 c	6	48.8 ab	**
<i>Rps</i> 2															
<i>Rps</i> 2, L76-1988	5	0.33 b	5	0.56 bc	**	5	3.1 bc	6	11.5 b	**	5	0.8 c	4	70.2 ab	**
Root resistance															
<i>rps</i> , Ripley	6	0.26 b	6	0.51 bc	**	6	1.6 cd	5	11.8 b	**	6	0.3 c	7	24.0 bc	**

^u Infection frequency was measured as the proportion of successful infections of five inoculation points on the roots of 10 seedlings. Ten plants were in trays in a slant board assay. There were three repetitions (trays), and the whole experiment was repeated three times. Proportions of each tray were arcsine square root-transformed prior to analysis. Different letters in the same column indicate a significant difference based on Tukey-Kramer multiple range test.

^v Lesion length was measured (millimeters) from the top of the inoculation site to the edge of the symptomatic tissue 7 days after inoculation. Ten plants were in trays in a slant board assay. There were three repetitions (trays), and the whole experiment was repeated three times. Data were transformed using log10 value prior to analysis (PROC MIXED of SAS), and data presented in the table are back-transformed values of expected lesion length based on the least square mean. Different letters in the same column indicate a significant difference based on Tukey-Kramer multiple range test.

^w Oospore numbers were determined by counting the total oospores in a 1-cm root piece below the inoculation point on four to six plants 7 days after inoculation. Ten plants were on each tray in a slant board assay. There were three repetitions (trays) and the whole experiment was repeated three times. Data were transformed using log10 value prior to the analysis (PROC MIXED of SAS), and data presented in the table are back-transformed values of the expected number of oospores based on the least square mean. Different letters in the same column indicate a significant difference based on Tukey-Kramer multiple range test.

^x The simple pathotype was OHR1 (vir. 7); and the complex pathotype was 1.S.1.1 (vir. 1a, 1b, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8).

^y R = rank in the column.

^z Diff = difference between isolates, based on Tukey-Kramer multiple range test. ** = significant difference, and ns = not significant.

In the susceptible interaction with the complex *P. sojae* pathotype (1.S.1.1) various groups of genotypes could be distinguished based on the number of oospores (Table 3). The first group consisted of Sloan and Ox20-8, in which the highest numbers of oospores developed. In the second group, large numbers of oospores developed in root sections of Conrad, Williams, General, and L76-1988, although not significantly different from Sloan or from Ripley. Fewer oospores developed in root sections of Ripley, which was significantly different from Sloan. The fewest number of oospores developed in Jack, which was significantly different from all other genotypes except Ripley (root resistance), with an estimated mean of only 3.5 oospores per 1-cm root section.

In a comparison of the genotypes without race-specific resistance, there were significantly more oospores formed in roots inoculated with the isolate with the complex pathotype compared with the simple pathotype, with one exception (Table 3). For the genotype Jack, very few oospores formed.

Infection frequency. In the resistant response to the simple *P. sojae* pathotype (OHR1), the *Rps1a* (Ox20-8) and the *Rps1k* genotypes (General) had the lowest infection frequencies, with 0.06 and 0.08, respectively (Table 3). These values were significantly lower than all the other genotypes in this study. The *Rps2* genotype (L76-1988) and the root-resistant genotype (Ripley) had infection frequencies of 0.33 and 0.26, respectively, which was significantly greater than the *Rps1a* and *Rps1k* genotypes. The *Rps2* genotype (L76-1988) infection frequency was not significantly different from that of either the root-resistant genotype (Ripley) or Jack. Sloan had the highest infection frequency (0.75), which was not significantly different from that of Conrad or Williams, which had infection frequencies of 0.60 and 0.63, respectively.

Ox20-8 had the highest infection frequency when inoculated with the complex pathotype (1.S.1.1) (Table 3). Sloan, Williams, Conrad, General, Ripley, and L76-1988 did not show significantly different infection frequencies. Jack had the lowest level of infection frequency (0.38) against the complex pathotype of *P. sojae*, which was significantly different from Williams and Conrad, which showed medium levels of partial resistance.

Inoculation position on the root affects infection frequency. Significant differences ($P < 0.0001$) in infection frequencies were found among soybean genotypes and inoculation positions for both *P. sojae* isolates (Tables 2 and 4). The interaction of genotype by position for both isolates was significant ($P = 0.002$). There were significantly fewer infections for Jack at the stem/root zone compared to positions below this site. Although it was not statistically significant, for all of the genotypes, the most successful infections occurred at positions four and five (Table 4).

Correlation of the components of partial resistance. The correlation of components for partial resistance was evaluated with only the complex *P. sojae* pathotype (1.S.1.1) for the non-race-specific response (Table 5). Lesion length was significantly correlated with infection frequency, with a coefficient of correlation of 0.61 (Table 5). Lesion length and the number of oospores were not significantly correlated, nor was infection frequency significantly correlated with the number of oospores.

Rank changes for the components of partial resistance. Based on the smallest lesion size, fewest oospores, and lowest infection frequency, Jack had the highest levels of partial resistance expressed in this study (Table 3). The most susceptible genotype, Ox20-8, ranked first for both infection frequency and lesion length with the complex pathotype. Sloan (with low levels of partial resistance) did not differ from Williams and Conrad for infection frequency but Conrad was significantly more resistant than Sloan for lesion length. Ripley was the second most resistant genotype for infection frequency (sixth) and oospore counts (seventh), but ranked fifth for lesion length (Table 3).

DISCUSSION

In this study, partial resistance was characterized using a complex *P. sojae* isolate that produced a susceptible interaction on all soybean genotypes (18), thus eliminating the race-specific response of *Rps1a*, *Rps1k*, *Rps2*, and Ripley's root resistance. Significant differences among the soybean genotypes were found for the three components of soybean partial resistance to *P. sojae* which were evaluated. The susceptible soybean genotype Sloan was similar to genotype Conrad in response to the isolate with the

TABLE 5. Spearman correlation coefficients for lesion length^w, oospores/cm^x, and infection frequency^y in eight soybean genotypes inoculated with a complex isolate of *Phytophthora sojae* (1.S.1.1)

Component	Lesion length	Oospores/cm
Infection frequency	0.61 ^z	0.14
Lesion length	1	0.30

^w Infection frequency was measured as the proportion of successful infection of five inoculation points on the roots of 10 plants 3 days after inoculation.

^x Lesion length was measured (millimeters) from the top of the inoculation site to the edge of the symptomatic tissue 7 days after inoculation.

^y Oospore numbers were determined by counting the total oospores in 1-cm root pieces below the inoculation point on four to six plants 7 days after inoculation.

^z $P \leq 0.01$.

TABLE 4. Effect of inoculation position on infection frequency proportion^y on the roots of eight soybean genotypes inoculated with two *Phytophthora sojae* isolates

Position	Genotype ^z							
	Conrad	General	Jack	L76-1988	Ox20-8	Ripley	Sloan	Williams
one	0.46 a	0.21 a	0.15 a	0.37 a	0.34 a	0.25 a	0.60 a	0.50 a
	A	B	B	AB	AB	B	A	A
two	0.62 a	0.23 a	0.24 ab	0.49 a	0.42 a	0.29 a	0.80 a	0.63 a
	A	B	B	AB	AB	B	A	A
three	0.65 a	0.33 a	0.49 bc	0.49 a	0.39 a	0.44 a	0.80 a	0.69 a
	BC	D	BCD	BCD	CD	BCD	A	AB
four	0.63 a	0.36 a	0.59 c	0.42 a	0.42 a	0.49 a	0.70 a	0.69 a
	AB	B	AB	B	B	AB	A	A
five	0.65 a	0.39 a	0.50 c	0.45 a	0.42 a	0.45 a	0.67 a	0.70 a
	A	A	A	A	A	A	A	A

^y Soybean roots were inoculated by placing a 10 µl drop of water containing 1×10^4 zoospores/ml at five positions on the root. Position one was at the interface between the stem and the root, and four more positions were 2 cm below each other. Infection frequency proportions are the means of three experiments with three replicates of 10 plants in each experiment. Data were arcsine($\sqrt{x}$) transformed prior to analysis; presented data are back-transformed (expected infection efficiency based on the least square means).

^z Different lowercase letters in the same column indicate significant difference ($P \leq 0.05$) in infection frequency within the same genotype. Different upper case letters below the number in the same row indicate significant difference in infection frequency within the same inoculation position according to Tukey-Kramer multiple range test.

complex pathotype for infection frequency and for the number of oospores; but it had significantly larger root lesions. Sloan was also similar to the genotype Williams for infection frequency and lesion length but had significantly higher number of oospores. These results suggest that partial resistance expressed in the genotypes Williams and Conrad is composed of more than one factor which affects lesion length and oospore production. These results also suggest that high levels of soybean partial resistance to *P. sojae* can be the result of combinations of various mechanisms of resistance where the different components influence the total level of partial resistance. For example, Jack, which is considered to be a high partial resistance genotype, had the lowest infection frequency, fewer number of oospores, and smallest lesion size.

In addition, low coefficients of correlation were found among the components of partial resistance, but lesion length was significantly correlated with infection frequency. This correlation suggests that studies of partial resistance in the soybean-*P. sojae* system that assay lesion length as a tool to identify partial resistance, will also capture some aspects of infection frequency, but may not capture some aspects of oospore production. Low coefficients of correlation for components of partial resistance and changes in the ranking of genotypes have been reported in many foliar-host pathogen interactions, such as wheat infected with *Septoria nodorum* (19) or with *Microdochium nivale* (5), and *Solanum* species infected with *P. infestans* (10).

An additional finding of this study is that L76-1988, an isolate of the genotype Williams with the *Rps2* gene, was not significantly different from Williams for any of the components of resistance studied with the complex pathotype. This confirms earlier reports that no residual effects were found in the presence of defeated *Rps* genes (60). These results provide additional evidence that partial resistance in soybeans is not the result of residual effects of *Rps* genes, whether they confer complete or incomplete resistance, and suggests that partial resistance appears to be a separate mechanism of resistance from the known *Rps* genes.

Complete and incomplete resistance due to *Rps* genes was evaluated on the interaction with the simple pathotype that produces a race-specific response by *Rps1a*, *Rps1k*, *Rps2*, and the root resistance genotype Ripley. Expression of *Rps* genes in soybean following inoculation with *P. sojae* has been reported to produce cell wall appositions and cell death in a hypersensitive response (15,40). In this study, the R gene response of *Rps1a* and *Rps1k* genes were expressed to the simple *P. sojae* pathotype and had significantly lower values than those genotypes with medium partial resistance (Conrad and Williams) for all the components of resistance measured. Low infection frequency, very small lesions, and few oospores per root section developed. The presence of oospores in resistant genotypes has been observed by other researchers, and it has been debated whether inoculum builds or is maintained even when resistant soybeans are planted (2,38). If these oospores are viable, this would provide evidence that even effective *Rps* genes do not completely arrest inoculum production (34), and thus provide *P. sojae* with a strong selection pressure that may, in part, explain the continuous appearance of more complex isolates (13,21,35).

Rps2 incomplete resistance to the simple *P. sojae* pathotype (OHR1) was expressed as intermediate values for all the components of resistance studied. These values were greater than those measured for the *Rps* gene response for infection efficiency (Ox20-8, *Rps1a*; General, *Rps1k*) and for lesion length (General, *Rps1k*). Previous reports have called the *Rps2* a type of root resistance because it confers complete levels of resistance only in the roots of the plant but not in the hypocotyls (18,34). This study indicated that levels of suppression of disease components measured with the *Rps2* genotype in the root were similar to that with other genotypes with high levels of partial resistance, such as Jack. This type of reaction at the phenotypic level is similar to that described for the slow-acting resistance genes of barley that

confer incomplete resistance to *Blumeria graminis* f. sp. *hordei*. In that situation the response of barley genes *Mlk* and *Mlp* allows the formation of small colonies with limited sporulation (25). There are also reports of race-specific incomplete resistance genes to oomycetes. For example the genes *R2*, *R4*, *R10*, and *R11* in potato confer an incomplete type of resistance to *P. infestans* (9). In addition, the *RB* gene from *Solanum bulbocastanum* provides an incomplete resistance phenotype in a transformed potato variety that is known to be susceptible (39). The Arabidopsis gene *RPPI-WsB* confers only incomplete resistance to *Pernospora parasitica* even though it is a member of a complex R gene locus and the other members of the *RPPI* family confer complete resistance (4). Timing for the onset of the hypersensitive reaction and cell death has been suggested as one of the mechanisms for these intermediate phenotypic responses (22,24,25,50).

The genotype Ripley has race-specific root-resistance to the simple *P. sojae* isolate and produced an intermediate response to all the components of resistance measured, similar to the *Rps2* response. These results are similar to those reported by McBlain et al. (28), in which Ripley developed small, 2-mm lesions. The difficulty encountered in this previous study to detect a major gene in the cross between Ripley and Harper was probably due to the assumption that soybeans with R genes will rarely produce lesions in these root inoculations (ie., the assumption that R genes produce an HR, not a susceptible lesion) (28).

Partial resistance expressed in the genotype Conrad to the simple *P. sojae* pathotype had higher infection frequency and more oospores than both the *Rps2* and the root-resistant genotype. However, Jack, which has high levels of partial resistance (26), did not differ from the *Rps2* genotype when inoculated with the simple isolate (OHR1). This emphasizes the difficulty in differentiating between high levels of partial resistance and single genes that confer incomplete resistance.

Differences among inoculation positions on soybean roots have been found previously. Stössel et al. (40) reported that the root/hypocotyl interface is more resistant than hypocotyls of etiolated plants. Their cytological evaluation demonstrated that *P. sojae* did not colonize the root/hypocotyl interface (40,41) and that these differences were attributed to age-related resistance because the root/hypocotyl interface cells were 2 to 3 days older (40,41,55). The results of our study of infection frequency also showed that with most of the genotypes tested, the root/stem interface is more resistant than the lower root region, albeit only the cultivar Jack had significant differences based on Tukey-Kramer multiple range test. In our study, there was no forced etiolation of any tissue, thus confirming that the root/stem interface is more resistant to infection than other root tissues. Age differences can be a possible source of variation among the results, but in order to prove this, inoculation of the same region of tissue in plants of different age would be required.

In summary, results from this study showed that various components could contribute to produce a partial resistance phenotype for defense against *P. sojae* in soybean roots. However, the high levels of partial resistance in Jack were indistinguishable from the *Rps2* and root resistance reaction for many of these same components. Both the *Rps2* and the root resistance phenotypes reacted similarly to the reaction of barley slow resistance genes to *Blumeria graminis* f. sp. *hordei* and to the reaction of potato partial resistance genes to *P. infestans*, in that all produce incomplete types of resistance.

QTL for partial resistance have been associated with lesion length (7,36) in two soybean genotypes. Future studies should evaluate if different QTL may be found that are associated with the remaining components of resistance. However, the methodology to measure infection frequency and the number of oospores at the moment restricts the number of lines that can be phenotyped in any one experiment. Molecular tools which quantify the number of oospores would greatly expedite studies which map QTL associated with resistance to reproduction.

ACKNOWLEDGMENTS

We thank J. L. Zambrano, T. Smith, and S. A. Berry for technical assistance; and C. H. Sneller for valuable discussions during the preparation of this study. Salaries and research support were provided by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University, Wooster. This work was supported in part by NSF Plant Genome grant DBI-0211863, and by Ohio's Soybean Producers' check-off dollars through the Ohio Soybean Council.

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