

Genotype, Race, Temperature, and Cultivar Effects on Reaction Type of Unwounded Soybean Hypocotyls Inoculated With Zoospores of *Phytophthora megasperma* f. sp. *glycinea*

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ABSTRACT

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Near-isogenic lines of soybean (*Glycine max*) were inoculated with race 1 or 2 of *Phytophthora megasperma* f. sp. *glycinea* (*Pmg*) by placing a droplet of zoospore suspension on hypocotyls of etiolated seedlings. At 48 hr after inoculation, the *Rps*₁ *Rps*₁, *Rps*₂ *Rps*₂, and *Rps*₅ *Rps*₅ genotypes differed in lesion length, necrosis rating, total glyceollin production, and glyceollin concentration in dissected tissue. Race 2 caused more necrosis, more total glyceollin, and greater localized glyceollin accumulation than did race 1, indicating that it is less compatible than race 1 in the reactions studied and

therefore less aggressive. Incubation at 25 C resulted in longer lesions than at 20 C in compatible reactions, but temperature did not significantly affect incompatible reactions. Genetic background had significant effects on necrosis rating and total glyceollin production in compatible reactions and on necrosis rating in incompatible reactions. Thus, the *Pmg*-soybean interaction is considerably more complex than previous studies have indicated.

Additional key words: resistance, susceptibility.

A method for inoculating unwounded, etiolated soybean hypocotyls (*Glycine max* (L.) Merr.) with zoospores of *Phytophthora megasperma* Drechs. f. sp. *glycinea* (Hildeb.) Kuan & Erwin (*Pmg*) was described previously (19). This method was used to test four races of *Pmg* for virulence simultaneously at adjacent sites on a single hypocotyl of soybean cultivar Altona. Since the responses were typical and not influenced by reactions at neighboring sites, it appeared that the technique should be useful in genetic studies of *Rps/rps* genes. However, in a preliminary evaluation of segregating material, we found that individual responses were not always clearly defined; there was some interference from reactions at adjacent sites, and consistent evaluations were not always achieved. This raised the possibility that there may be variations in reaction type, or that resistance is not uniformly expressed under all conditions.

The studies reported in this paper were designed to measure the influence of genotype, race, temperature, and genetic background on reaction type as indicated by lesion size, necrosis, and glyceollin accumulation.

MATERIALS AND METHODS

Three isolines of cultivar Harosoy, each carrying a different nonallelic *Rps* gene for resistance to *Pmg*, were used in one experiment. These isolines were developed through backcrossing by R. L. Bernard, Urbana, Illinois, as Harosoy 63 (Harosoy⁸ × Blackhawk), L70-6494 (Harosoy⁵ × D54-2437), and L62-904 (Harosoy⁶ × T240). Harosoy 63 is *Rps*₁ *Rps*₁ (2), L70-6494 is *Rps*₂ *Rps*₂ (4), and L62-904 is *Rps*₅ *Rps*₅ (6). A single replicate of the four isolines inoculated with race 1 or 2 of *Pmg* and incubated at 20 or 25 C was run on each of three dates.

*Rps*₁ isolines of cultivars Harosoy, Hawkeye, Clark, and Wayne were used in another experiment; these were Harosoy 63; Hawkeye 63, Clark 63, and L15, all developed through backcrossing by R. L.

Bernard (2,3,21). The four pairs of isolines were run as a four-replicate test on one date, inoculated with *Pmg* race 1, and incubated at 25 C.

Races 1 and 2 were used because these races give incompatible reactions with the *Rps* isolines being studied. An Ontario isolate of race 1 and a Mississippi isolate of race 2 routinely were grown on V-8 juice agar at 25 C in the dark. Procedures for zoospore production were as described previously (19). Numbers of zoospores in suspensions used for inoculum were adjusted to approximately 1×10^5 per milliliter by dilution with sterile distilled water.

Seedlings were grown and placed horizontally in glass trays for inoculation and incubation as described previously (19). Each genotype sample consisted of 10 seedlings, which were inoculated by placing one drop (approximately 10 μ l) of zoospore suspension on the upper part of the hypocotyl (about 2 cm below the cotyledons) by using an automatic microliter syringe. After incubation in the dark for 48 hr, lesions were measured, rated for necrosis, and excised for determination of glyceollin.

Lesions were measured along the length of the hypocotyl above and below the inoculation site with dividers and a millimeter ruler. In incompatible interactions, measurements were made to the end of the most extensive brown necrotic streaks where necrosis was not uniform over the full breadth of the hypocotyl. In compatible interactions, the limit of the water-soaking was assumed to be the boundary of the lesion; although not strongly marked, it was distinguishable as a transition zone. Necrosis was scored only on the area of the lesion that had received the initial inoculum droplet. An arbitrary scale of 1 (no visible browning) to 10 (intense browning of tissue) was used.

Procedures for glyceollin determination were as described previously (20) except that the brown-necrotic tissue was excised from incompatible interactions and extracted separately from the remainder of the hypocotyl sections (40–50 mm long). A comparable amount of tissue was excised from compatible interactions. After extraction, the tissues were dried and weighed; glyceollin concentrations are expressed as micrograms per milligram of dried dissected tissue. Total glyceollin was obtained as the sum of the glyceollin from the dissected and remaining tissues.

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RESULTS

There were significant ($P=0.05$) effects of genotype and race on lesion length, necrosis rating, total glyceollin per inoculated hypocotyl, and glyceollin concentration in dissected diseased tissue (Table 1). The glyceollin concentration in all incompatible interactions was higher than in the compatible interactions. Lesions were significantly longer at 25 C, and at this temperature there was a trend towards a higher necrosis rating and higher glyceollin concentration in dissected diseased tissue than at 20 C.

The genotype \times race and genotype \times temperature mean squares for lesion length were the only significant interactions ($P=0.05$). With the *Rps*₁, *Rps*₂, and *Rps*₅ genotypes there were no significant temperature effects on lesion length, but with the *rps* genotype, lesion length was greater at 25 C than at 20 C (Table 2). Thus, temperature affected the length of water-soaked lesions in the compatible interaction, but it did not affect lesion length in incompatible interactions. Also, there were no significant race effects on lesion length with the *Rps*₁ and *Rps*₂ genotypes, but race 2 caused significantly shorter lesions than race 1 with the genotypes *Rps*₅ and *rps*. Necrosis scores showed no significant interactions of genotype \times race or genotype \times temperature. There was a trend toward a genotype \times temperature interaction in that the *Rps* genotypes scored 0.4 lower at 20 C than at 25 C and the *rps* genotype scored 0.6 higher at 20 C than at 25 C. Likewise, glyceollin concentration in dissected diseased tissue showed a trend toward a genotype \times temperature interaction caused by the differential

response of the *rps* genotype at 25 C (Table 2), whereas each of the three incompatible genotypes tended to have a higher concentration at 25 C than at 20 C.

*Rps*₁ and *rps*₁ genotype effects in plants with Harosoy, Hawkeye, Clark, and Wayne backgrounds are given in Table 3. Genotypes were significantly different ($P=0.05$) within each pair for each variable. There were no significant background effects on lesion length and glyceollin concentration in dissected diseased tissue for either of the two genotypes. In plants with the *rps*₁ genotype, cultivars Clark and Wayne had significantly lower necrosis ratings and less total glyceollin per inoculated hypocotyl than those of Harosoy and Hawkeye. In plants with the *Rps*₁ genotype, Clark and Wayne had significantly higher necrosis ratings than those of Harosoy and Hawkeye, but there were no differences in total glyceollin.

DISCUSSION

The present study indicates that the usefulness of our technique for testing the response of single F₂ seedlings to inoculation with several individual races as previously suggested (19) will depend on the choice of *Rps* gene, the genetic background, and the race of *Pmg* used. Combinations in which the expression of individual race-genotype interactions would be influenced by interactions at neighboring sites, due to the development of spreading lesions, cannot be effectively tested. Since the lower two-thirds of the hypocotyl is resistant to compatible as well as incompatible races

TABLE 1. Genotype, race, and temperature effects on reaction type of soybeans inoculated with zoospores of *Pmg* (one drop per hypocotyl); results are on a hypocotyl basis^a

	Lesion length (mm)	Necrosis rating ^b	Total glyceollin/inoculated hypocotyl (μ g)	Glyceollin (μ g/mg dry wt of dissected tissue)
Genotypes ^c				
Compatible				
<i>rps</i> ₁ <i>rps</i> ₁ <i>rps</i> ₂ <i>rps</i> ₂ <i>rps</i> ₅ <i>rps</i> ₅	26 D	4.0 B	0.03 B	23 A
Incompatible				
<i>Rps</i> ₁ <i>Rps</i> ₁ <i>rps</i> ₂ <i>rps</i> ₂ <i>rps</i> ₅ <i>rps</i> ₅	6 A	3.4 A	0.01 A	42 B
<i>rps</i> ₁ <i>rps</i> ₁ <i>Rps</i> ₂ <i>Rps</i> ₂ <i>rps</i> ₅ <i>rps</i> ₅	9 B	4.2 B	0.02 B	51 BC
<i>rps</i> ₁ <i>rps</i> ₁ <i>rps</i> ₂ <i>rps</i> ₂ <i>Rps</i> ₅ <i>Rps</i> ₅	13 C	6.2 C	0.04 C	60 C
Races ^d				
Race 1	16 N	3.9 M	0.02 M	38 M
Race 2	10 M	5.0 N	0.03 N	50 N
Temperatures ^e				
25 C	15 Y	4.5 Y	0.02 Y	46 Y
20 C	12 X	4.3 Y	0.02 Y	42 Y

^a Means in columns within genotypes, races, and temperatures not followed by the same letter are significantly different ($P=0.05$) by Duncan's multiple range test.

^b Ratings: 1 = none, 10 = intense.

^c Average over two races and two temperatures.

^d Averaged over four genotypes and two temperatures.

^e Averaged over four genotypes and two races.

TABLE 2. Interaction effects of genotype, race and temperature on reaction type^a

Genotype	Race	Lesion length (mm) ^b			Glyceollin (μ g/mg dry wt of dissected tissue) ^c		
		25 C	20 C	\bar{x}	25 C	20 C	\bar{x}
Compatible							
<i>rps</i> ₁ <i>rps</i> ₁ <i>rps</i> ₂ <i>rps</i> ₂ <i>rps</i> ₅ <i>rps</i> ₅	R1	38	25	31 A	7	25	16 H
	R2	26	14	20 B	26	34	30 H
	\bar{x}	32 C	19 D		17 I	29 I	
Incompatible							
<i>rps</i> ₁ <i>rps</i> ₁ <i>rps</i> ₂ <i>rps</i> ₂ <i>Rps</i> ₅ <i>Rps</i> ₅	R1	16	17	16 E	62	46	54 J
	R2	10	9	9 F	76	56	66 J
	\bar{x}	12 G	13 G		69 K	51 K	

^a Within each pair of overall means those not followed by the same letter are significantly different ($P=0.05$).

^b Incompatible reactions had similar lesion length at both temperatures, whereas temperature had an effect on lesion length in the compatible reaction; races did not cause different lesion lengths with the *Rps*₁ and *Rps*₂ genotypes but did have an effect in the compatible and *Rps*₅ combination.

^c Each of the three incompatible genotypes tended to have a higher concentration of glyceollin at 25 C than at 20 C, but the compatible combination did not.

(13,14,16,20), only the uppermost 30–50 mm of the hypocotyl (depending on elongation) can be used to differentiate races. Thus, there is a limit to the distance by which inoculum droplets of individual races can be separated. However, when fairly discrete lesions are produced, as with Altona (19) and *Rps*₁ and *Rps*₂, it should be possible to test two-gene segregations by using two races.

Inoculation of intact hypocotyls with zoospores permits measurement of a range of responses within the categories of resistance and susceptibility that cannot be quantified as easily with other procedures. In most studies of the interaction between soybeans and races of *Pmg*, only two reaction types, resistant and susceptible, have been distinguished (eg, 5,12). In fact, there has been a preference for inoculation procedures involving hypocotyl wounding, in which susceptible plants are killed, and separation of the two categories is relatively straightforward (8). Variation in the expression of susceptibility has been noted in many studies beginning with those of Bernard et al (5), who observed that surviving infected plants of susceptible cultivars developed some necrosis. Variation in the expression of resistance has been observed rarely, except with the *Rps*₂ gene for which Kilen et al (12), who used mycelial inoculum and wounded hypocotyls, reported a range of symptoms. Some plants appeared completely healthy, some developed lesions at the inoculation site, and others were killed.

Our results indicate that there are differences in degree of expression of resistance as measured by lesion length and that the expression is influenced by the host genotype; ie, by race-specific genes for resistance, much more than by genetic background. The spread of the pathogen in the host, as indicated by lesion size, differed significantly between each of the incompatible genotypes. These observations have some similarities to those of Stuckey and Ellingboe (17), who found that elongation of secondary hyphae of *Erysiphe graminis* f. sp. *tritici* in wheat was influenced quantitatively by different host genes for incompatibility in a near-isogenic background. In terms of preventing the spread of race 1 or 2 of *Pmg*, and hence of ultimate damage to the soybean, *Rps*₅ appears to be a much less effective gene than *Rps*₁ or *Rps*₂. With *Rps*₁ there was little or no spread of the fungus beyond the

inoculum droplet.

Resistant genotypes also differed in intensity of necrosis, total glyceollin, and (for two of them) localized accumulation of glyceollin. Intensity of necrosis and glyceollin production were greatest with *Rps*₅, which also resulted in the greatest lesion length. Hence, it appears in this instance that necrosis and glyceollin production, which are commonly used indicators of incompatibility, do not correlate closely with resistance to spread of the pathogen. Previous work also indicates that glyceollin production and necrosis may be correlated more closely with one another than with resistance (13,20). In view of these results, assumptions that fungal cell-wall preparations that stimulate glyceollin production are necessarily mediators of resistance may not be justified (10,11).

Our results indicate that, based on lesion length, race 2 differs from race 1 both in the compatible combination and in the incompatible combination with *Rps*₅. Differences between the effects of *Rps*₁ and *Rps*₂ were not expressed (or measurable); lesions were restricted and similar in size. The differences observed between *Pmg* races 1 and 2 are not related to specificity, but can be explained in terms of aggressiveness as discussed by Vanderplank (18). Similar nonspecific differences have been reported previously for the *Pmg*-soybean interaction. Averre and Athow (1) observed that some isolates produced nonlethal lesions on susceptible cultivars compared with isolates that killed seedlings. Hilty and Schmitthenner (7) observed differences in disease severity in roots inoculated with zoospores of different isolates of *P. megasperma* var. *sojae*. Keen (9) reported more hydroxyphaseollin production stimulated by race 2 than by race 1 in incompatible reactions with *Rps*₁. Also, races differed in ability to penetrate hypocotyl epidermal walls and (similarly) these differences were not related to specificity (15).

In this study, race 2 is less compatible than race 1 on the basis of necrosis and glyceollin production and it is less aggressive than race 1, as indicated by lesion length. This is especially interesting, because the tendency of being less aggressive is expressed in the compatible combination in the absence of a specific gene for resistance to races 1 and 2. It suggests that a sufficient decrease in aggressiveness of the pathogen could result in incompatibility. Averre and Athow (1) and Hilty and Schmitthenner (7) reported that some *Pmg* isolates failed to infect Harosoy. Microscopy (14,16) has revealed that even in compatible-type lesions many of the individual interactions between host cells and hyphae are incompatible. It can be visualized that, with decrease in pathogen aggressiveness, numbers of such incompatible interactions could be increased until the lesion became effectively incompatible.

On the basis of the material studied, the phenotypic expression of resistance/susceptibility interaction is influenced by the *Rps* genes, the aggressiveness of the *Pmg* race, and to a lesser degree by genetic background and incubation temperature. Previous work has shown that small changes in tissue maturity also alter the expression of the reaction (13,20). These findings, together with the observation that even susceptible lesions contain a high proportion of incompatible cell-hyphal interactions (14,16), indicate that each observed reaction type is the result of a complex set of interactions between host and pathogen.

TABLE 3. Interactive effects of genotype and selected cultivars on reaction type 48 hr after inoculation of unwounded soybean hypocotyls with zoospores^a of *Pmg* race 1

Parameters and genotypes	Reaction ^b	Cultivar ^c			
		Harosoy	Hawkeye	Clark	Wayne
Lesion length^d					
<i>rps</i> ₁ <i>rps</i> ₁	C	38 A	39 A	39 A	39 A
<i>Rps</i> ₁ <i>Rps</i> ₁	I	7 A	9 A	8 A	8 A
Genotype L.S.D. (<i>P</i> = 0.05) = 3.2					
Necrosis rating^e					
<i>rps</i> ₁ <i>rps</i> ₁	C	2.8 B	3.2 B	2.2 A	2.2 A
<i>Rps</i> ₁ <i>Rps</i> ₁	I	4.0 A	4.9 B	5.8 C	5.7 C
Genotype L.S.D. (<i>P</i> = 0.05) = 0.7					
Total glyceollin^f					
<i>rps</i> ₁ <i>rps</i> ₁	C	0.03 B	0.03 B	0.02 A	0.02 A
<i>Rps</i> ₁ <i>Rps</i> ₁	I	0.02 A	0.02 A	0.02 A	0.02 A
Genotype L.S.D. (<i>P</i> = 0.05) = 0.007					
Glyceollin^g					
<i>rps</i> ₁ <i>rps</i> ₁	C	9 A	10 A	8 A	8 A
<i>Rps</i> ₁ <i>Rps</i> ₁	I	40 A	31 A	39 A	43 A
Genotype L.S.D. (<i>P</i> = 0.05) = 12.4					

^a One drop of inoculum per hypocotyl.

^b C = compatible; I = incompatible.

^c Within rows, means followed by same letter are not significantly different (*P* = 0.05) according to Duncan's multiple range test.

^d Millimeters.

^e Ratings: 1 = none, 10 = intense.

^f Micrograms per hypocotyl.

^g Micrograms per milligram (dry weight) of dissected tissue.

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