Specific Elicitors of Plant Phytoalexin Production: Determinants of Race Specificity in Pathogens?

Abstract. *Race 1 cultures of the phytopathogenic fungus* Phytophthora megasperma *var.* sojae *produced a specific elicitor of the soybean phytoalexin hydroxyphaseollin that resulted in higher production of the phytoalexin on disease-resistant Harosoy 63 soybeans than in the near-isogenic susceptible cultivar Harosoy. Race 3 of the fungus, which gives susceptible reactions on both soybean cultivars, did not produce the race 1 specific elicitor.*

Derepressed production of the antifungal phytoalexin hydroxyphaseollin (HP) appears to be the basis for resistance of certain soybean [*Glycine max* (L.) Merr.] cultivars to incompatible (*1*) races of *Phytophthora megasperma* var. *sojae* (*2, 3*). In contrast, infection of plants by compatible (*1*) races results in repressed production of HP (2 to 5 percent of the rate of incompatible combinations). I have found that race 1 and race 2 cultures of the fungus produce metabolites which, in cell-free bioassays on soybean plants, elicit higher levels of HP in the monogenic disease-resistant cultivar Harosoy 63 than in the near-isogenic susceptible cultivar Harosoy. I have proposed (*4*) to call these substances specific elicitors (*5*) and hypothesize that they may determine the resistance or susceptibility of soybeans to various races of *P. megasperma* var. *sojae*.

Phytophthora megasperma var. *sojae* races 1, 2, and 3 were used, and their disease reactions on Harosoy (H) and Harosoy 63 (H63) soybeans are shown in (*2*). The fungus races were cultured on a sucrose-asparagine synthetic medium (*6*) in standing culture. Cell-free fluids from cultures on this medium could not be bioassayed directly for elicitor activity due to interference by the high nutrient content. The medium was therefore decanted from cultures in the logarithmic growth phase and replaced with pea broth medium (*3*), and the cultures were incubated for 12 to 72 additional hours. The cell-free replacement culture fluids were then directly bioassayed.

Elicitor bioassays were performed by applying drops of crude replacement culture fluids or partially purified elicitor preparations into hypocotyl wounds on H and H63 plants 8 to 12 days old and incubating the plants for 36 to 48 hours at about 100 percent relative humidity. The wounded areas of the hypocotyls were then harvested and the HP was extracted and quantitated (*7*).

Soybean broth gave little HP production in either H or H63 in the elicitor bioassay (Table 1), but replacement culture fluids from *P. megasperma* var. *sojae* elicited considerable HP. Fluids from races 1 and 2 produced more HP in H63 than in H, consistent with the response of these cultivars to the living fungi (*2*); race 3, which gives a compatible reaction on both H and H63 (*2*), yielded culture fluids that produced the same HP levels in both cultivars (Table 1). These data indicated that races 1 and 2 produced a specific hydroxyphaseollin elicitor (or elicitors) for H63 soybeans, while race 3 did not. The well-known nonspecific phytoalexin elicitor actinomycin D led to production of HP in the bioassay, but, as expected, did not show any cultivar specificity (Table 1).

The specific elicitor activity from race 1 appeared to pass through dialysis membranes, although considerable nonspecific elicitor activity did not dialyze. When Bio-Gel P-2 columns (2.5 by 115 cm) equilibrated with water were used to fractionate concentrated crude culture fluids from race 1 grown on synthetic medium, the bulk of the specific elicitor activity occurred at or shortly after the column void volume. However, when columns were equilibrated with 10 m*M* potassium phosphate, *p*H 7.5, race 1 specific elicitor activity eluted as a distinct ultraviolet light–absorbing peak in fractions 42 to 44, after fractions 29 to 37, which contained sugars and amino acids (Fig. 1, Table 1). Culture fluids from race 3 treated in the same way produced a much smaller

Table 1. Plant reactions to living fungus [C = compatible and I = incompatible (*1*)] and hydroxyphaseollin (HP) produced in near-isogenic H and H63 soybean hypocotyls treated with soybean broth, soybean broth replacement culture fluids from *Phytophthora megasperma* var. *sojae*, actinomycin D, or partially purified race 1 elicitor.

Treatment	Harosoy (H)		Harosoy 63 (H63)	
	Reaction	HP (ppm)	Reaction	HP (ppm)
Soybean broth control		8 ± 6		4 ± 3
Race 1 fluids	C	16 ± 10	I	81 ± 27
Race 2 fluids	C	35 ± 18	I	75 ± 22
Race 3 fluids	C	50 ± 5	C	50 ± 14
Actinomycin D (1 μg/ml)		30 ± 8		27 ± 12
Race 1 P-2 column effluent*		22 ± 12		105 ± 24

* Fractions 42 to 44, diluted 1 : 20 with water.

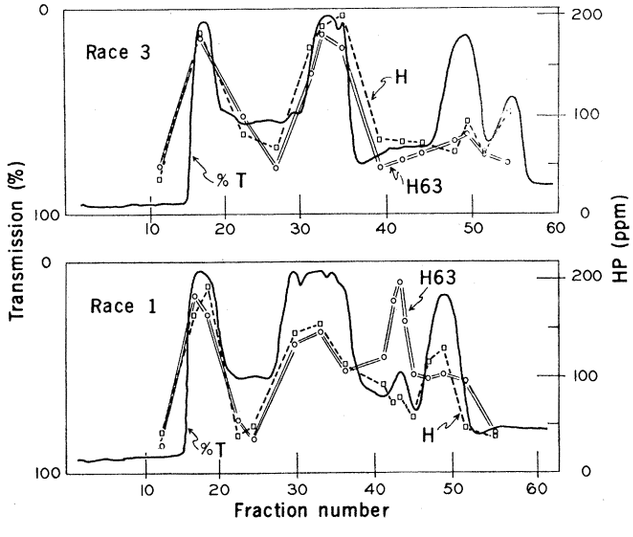

Fig. 1. Fractionation of concentrated crude culture fluids from races 1 and 3 of *Phytophthora megasperma* var. *sojae* on a column of Bio-Gel P-2 eluted with 0.01*M* potassium phosphate, *p*H 7.5. The column was continuously monitored for percentage transmission (%*T*) at 265 nm, and 12.5-ml fractions were collected, diluted 1:10 with water, and bioassayed for HP elicitor activity on H and H63 soybeans. From each race 500 ml of culture fluids was processed.

or negligible peak on the P-2 column at the position of the race 1 elicitor, and no specific elicitor activity was present in these fractions (Fig. 1). The column results disclosed the presence of several ultraviolet-absorbing peaks from races 1 and 3 that contained nonspecific elicitor activity, but only the one specific elicitor peak from race 1 was consistently observed with potassium phosphate elution.

Further attempts to purify the race 1 specific elicitor from P-2 column fractions have thus far been unsuccessful, in part due to a gradual loss of activity in the pooled fractions and to the apparent absence of charge for the specific elicitor. The peculiar behavior of the specific elicitor activity on the P-2 columns with or without salt suggests that the race 1 specific elicitor may be adsorbed on proteins and the polyacrylamide gel matrix.

A glucan elicitor of hydroxyphaseollin production in soybeans has been isolated from culture fluids of *P. megasperma* var. *sojae* by Ayers *et al.* (*8*). This metabolite is likely not the race 1 specific elicitor detected here since it has not been shown to have specific elicitor activity. Furthermore, I have not detected anthrone reactive material in the race 1 specific elicitor peak from Bio-Gel P-2 columns.

The detection of specific hydroxyphaseollin elicitors from race 1 but not race 3 cultures of *P. megasperma* var. *sojae* constitutes an additional independent proof that derepressed HP production is indeed the basis for resistance in soybeans to incompatible races of the fungus. Despite the failures thus far encountered in isolation and chemical characterization of the race 1 specific elicitor, the data presented here are consistent with the hypothesis that metabolites of pathogenic origin can indeed specifically predicate disease resistance or susceptibility through a differential effect on plant biosynthesis of phytoalexins such as HP. Since race 3 of *P. megasperma* var. *sojae* presumably evolved from race 1, and specific elicitor activity was not detected from race 3 crude culture fluids or P-2 column fractions, it is enticing to speculate that the genetic-chemical basis for race 3 evolution was loss of the ability to make the specific elicitor typical of race 1.

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References and Notes

1. Incompatible host-parasite combinations result in resistant plant reactions with no occurrence of disease; compatible combinations give susceptible plant reactions to the pathogen.
2. The responses of various resistance genotypes of soybeans to three known races of *P. megasperma* var. *sojae* are as follows (C = compatible response; I = incompatible):

Soybean cultivar	Race		
	1	2	3
Harsoy (H)	C	C	C
Harosoy 63 (H63)	I	I	C
D60-9647	I	C	I
Semmes	I	I	I

3. N. Keen, J. Sims, D. Erwin, E. Rice, J. Partridge, *Phytopathology* **61**, 1084 (1971); N. Keen, *Physiol. Plant Pathol.* **1**, 265 (1971); W. Klarman and J. Sanford, *Life Sci.* **7**, 1095 (1968); J. Sims, N. Keen, V. Honwad, *Phytochemistry* **11**, 827 (1972); N. Keen, A. Zaki, J. Sims, *ibid.*, p. 1031.
4. N. Keen, J. Partridge, A. Zaki, *Phytopathology* **62**, 768 (1972).
5. Known mechanisms for the hypersensitive resistant reaction in higher plants are the permeability decompartmentalization mechanism and the production of antibiotic phytoalexins. Little is known about elicitors of the former mechanism, but many substances are recognized that elicit phytoalexin production in plants. Of these, the great majority are defined as nonspecific elicitors since they exhibit no known differential effects on various cultivars of a plant species; specific elicitors are metabolites, presumably of pathogenic origin, that elicit differential phytoalexin production on various host cultivars similar to the fungus race that produces them.
6. The medium consisted of the following, in the amounts specified per liter: sucrose, 15 g; asparagine, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 1 mg; $CaCl_2 \cdot 2H_2O$, 10 mg; thiamin-HCl, 1 mg; K_2HPO_4, 1.04 g; KH_2PO_4, 1.90 g; β-sitosterol, 20 mg; $ZnSO_4 \cdot 7H_2O$, 1 mg; $CuSO_4 \cdot 5H_2O$, $NaMoO_4 \cdot 2H_2O$, and $MnCl_2 \cdot 2H_2O$, each 0.02 mg; and $CaCO_3$, 3 g.
7. N. Keen and B. Kennedy, *Physiol. Plant Pathol.* **4**, 173 (1974).
8. A. Ayers, J. Ebel, P. Albersheim, abstract of paper presented at the 66th annual meeting of the American Phytopathological Society, Vancouver, Canada, 1974.
9. Supported by NSF grant GB-35531. I thank A. F. Schmitthenner for fungus cultures and E. E. Hartwig for seed of certain of the soybean cultivars.

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Halogenated Hydrocarbons in New Orleans Drinking Water and Blood Plasma

Abstract. *Volatile organics from New Orleans drinking water and pooled plasma were collected on a solid phenyl ether polymer and analyzed by gas chromatographic and mass spectrometric techniques. Thirteen halogenated hydrocarbons were identified in the drinking water. Five halogenated compounds were found in the plasma. Tetrachloroethylene and carbon tetrachloride were found in both the plasma and the drinking water. Considerable variation in the relative concentrations of the halogenated hydrocarbons was noted from day to day in the drinking water.*

In recent years concern has been expressed about the quality of water available at the lower end of the Mississippi River. Several qualitative studies have identified halogenated hydrocarbons in local drinking water (*1*). Isolation procedures such as reverse osmosis, carbon adsorption, and solvent extraction have been commonly employed (*2*), in addition to total trapping techniques (*3*). Over 50 percent of the compounds isolated in the above studies were described as moderately toxic to very toxic, and two compounds as extremely toxic (*1*). Many studies have been conducted on the toxicity of halogenated hydrocarbons (*4*), and it is well known that such compounds as vinyl chloride (*5*) and chloromethyl ether (*6*) are carcinogenic whereas carbon tetrachloride and chloroform (*7*) are suspected carcinogens. Halogenated hydrocarbons will tend to accumulate in various tissues of animals and man (*8*). Instances of cancer, excluding skin cancer, in the New Orleans vicinity have been reported to be above the national average (*9*). More recent studies on cancer mortality have supported these earlier findings (*10*). It is our purpose here to report a new and more rapid procedure to identify the major halogenated hydrocarbons in New Orleans drinking water and further to correlate, by the use of the same method, such observations with low-molecular-weight halogenated hydrocarbons in blood plasma from local residents.

Volatile organics were eluted from water and blood plasma samples by heating to 95°C under a stream of ultrapure helium. The helium stream was passed through a series of glass condensers to eliminate the bulk of water vapor. The volatile organics were trapped on poly(*p*-2,6-diphenylphenylene)oxide adsorbant having a mesh size of 35/60 (Applied Science Laboratories, Inc., State College, Pennsylvania) attached to the end of a condenser train. At the end of a 1-hour trapping period, the 1 g of polymer containing the adsorbed organics was transferred from the collection reservoir