Heat-Induced Susceptibility to Nonpathogens and Cross-Protection Against Phytophthora megasperma var. sojae in Soybean

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ABSTRACT

Of 17 species of fungi, all nonpathogens of soybean, four (*Ceratocystis fimbriata, Fusarium moniliforme, Gibberella zeae,* and *Helminthosporium turcicum*) killed heat-treated Harosoy soybean plants. These four also cross-protected Harosoy plants against *Phytophthora megasperma* var. *sojae*. Ten of the nonpathogens caused no infection on heat-treated plants; six of the 10 gave good cross-protection against *P. megasperma* var. *sojae*. Apparently, resistance to nonpathogens in soybeans is not governed by a single mechanism.

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Additional key words: phytoalexin, disease resistance.

Heat-induced susceptibility to nonpathogens of soybean has been demonstrated in soybean plants. Chamberlain & Gerdemann (1) showed that *Helminthosporium Sorokinianum* Sacc. ap. Sorok. (*H. sativum* Pam., King & Bakke) and *Phytophthora cactorum* (Leb. & Cohn) Schroet. killed heat-treated soybean plants, whereas three saprophytic fungi *Trichoderma viride* [Pers.] S. F. Gray, *Aspergillus niger* v. Tiegh., and *Chaetomium globosum* Kze. ex Fr. did not. These results led to speculation in regard to the susceptibility of heat-treated soybean plants to pathogens of other plant species and possible fundamental similarities between inter- and intraspecific resistance to pathogens.

Paxton & Chamberlain (5) have shown that *Phytophthora cactorum*, normally nonpathogenic on soybean, can protect the soybean plant against *P. megasperma* Drechs. var. *sojae* A. A. Hildeb. Since cross-protection involves the capacity to stimulate phytoalexin production, it was of interest to determine whether this capability is of general occurrence among pathogens acting upon a nonsusceptible host. The research described in this paper was twofold: (i) to further explore the heat-induced susceptibility of soybean to pathogens of other species; and (ii) to determine the ability of these fungi to protect the soybean plant against subsequent infection by *P. megasperma* var. *sojae*.

MATERIALS AND METHODS.–Soybean plants (*Glycine max* [L.] Merr.) 'Harosoy' were started in flats of builders' sand and transferred to beakers of water. They were treated and inoculated when they were 6 to 9 days old. Cultures of the various fungi were maintained on potato-dextrose agar or in a liquid medium made by autoclaving 5-10 soybean seeds in 100 ml distilled water for 30 min. Heat treatment consisted of immersing root and hypocotyl up to the cotyledonary node in a water bath at 50 C for 1 min or at 44 C for 60 min. Plants were inoculated by inserting bits of mycelium in a wound made in the hypocotyl with a half-spear needle. The wounds were covered with petrolatum to prevent drying. Each fungus was tested on untreated soybean plants to determine that it was not a natural pathogen on soybean. Plants were judged to be infected when browning spread a minimum of 1 cm beyond the inoculation wound. In the cross-protection experiments, Harosoy plants were inoculated with the nonpathogen and inoculated 2 to 3 days later in the same wound with *Phytophthora megasperma* var. *sojae*. Control plants were wounded with a sterile needle and inoculated in the same wound 2 to 3 days later with *P. megasperma* var. *sojae*.

RESULTS.–The following nonpathogens of soybean were included in the heat-treatment tests: *Alternaria solani* (Ell. & G. Martin) Sor.; *Aspergillus niger* v. Tiegh.; *Botryosphaeria ribis* Gross. & Dug.; *Ceratocystis fagacearum* (Bretz) Hunt; *Ceratocystis fimbriata* Ell. & Halst.; *Ceratocystis ulmi* (Buisman) C. Moreau; *Cladosporium paeoniae* Pass.; *Diplodia zeae* (Schw.) Lev.; *Dothiorella ulmi* Verrall & May; *Endothia parasitica* (Murr.) P. J. & H. W. Anderson; *Fusarium moniliforme* Sheldon; *Gibberella zeae* (Schw.) Petch; *Gnomonia platani* Kleb.; *Gnomonia quercina* Kleb.; *Helminthosporium turcicum* Pass.; *Monilinia fructicola* (Wint.) Honey; and *Verticillium albo-atrum* Reinke & Berth. Of these 17 microorganisms, only four (*Ceratocystis fimbriata, Fusarium moniliforme, Gibberella zeae,* and *Helminthosporium turcicum*) showed appreciable pathogenicity on heat-treated plants (40-90% killed), and three showed slight pathogenicity (5-20% infected). None showed evidence of pathogenic activity on untreated plants.

In the cross-protection trials, observations were made 7 and 10 days after the challenge inoculation with *P. megasperma* var. *sojae*. Fourteen of the fungi gave a high degree of protection (50-100%) against killing by *P. megasperma* var. *sojae*, and 10 of these gave a similar degree of protection against infection by the challenge fungus. Table 1 shows the mean results of three tests for each fungus. The "percentage protected plants" was calculated by using the number of dead plants in the control instead of 100 to compensate for escapes from infection.

DISCUSSION.–Resistance to nonpathogens in

TABLE 1. The effect of prior inoculation of soybean plants with nonpathogens of soybean on subsequent susceptibility to *Phytophthora megasperma* var. *sojae*

Nonpathogen, 1st inoculation[a]	% of plants Killed	Infected	% Protected[c]
Alternaria solani	25	45	74
Control (wounded only)[b]	95	95	
Aspergillus niger	25	50	74
Control	95	95	
Botryosphaeria ribis	40	45	58
Control	95	100	
Ceratocystis fagacearum	30	50	57
Control	70	70	
Ceratocystis fimbriata	0	33	100
Control	73	87	
Ceratocystis ulmi	30	70	57
Control	70	70	
Cladosporium paeoniae	45	60	53
Control	95	100	
Diplodia zeae	40	55	43
Control	70	80	
Dothiorella ulmi	19	36	80
Control	93	97	
Endothia parasitica	15	20	83
Control	90	95	
Fusarium moniliforme	2	12	95
Control	55	88	
Gibberella zeae	10	17	87
Control	77	87	
Gnomonia platani	57	67	41
Control	97	97	
Gnomonia quercina	60	80	29
Control	85	95	
Helminthosporium sorokinianum	3	20	95
Control	67	77	
Helminthosporium turcicum	27	40	65
Control	77	87	
Monilinia fructicola	40	47	43
Control	70	80	
Verticillium albo-atrum	30	70	57
Control	70	70	

[a] All inoculations challenged 2-3 days later with *P. megasperma* var. *sojae.*

[b] All controls were wounded but not inoculated.

[c] % Protected = % killed in control − % killed in "protected" plants ÷ % killed in control.

soybean is not governed by a single mechanism. *Ceratocystis fimbriata, Fusarium moniliforme, Gibberella zeae,* and *Helminthosporium turcicum* apparently act essentially like *Phytophthora cactorum*; heat treatment induces susceptibility, and previous inoculation gives local protection against infection by *P. megasperma* var. *sojae.* With *P. cactorum,* heat-induced susceptibility is accomplished by suppressing or reducing phytoalexin production (1, 3), and resistance is induced by stimulating the production of phytoalexin (4, 5). Fungus-host interaction for the other organisms suggests some other mode of action, since 10 of them caused no infection on heat-treated plants, but 6 of the 10 gave good cross-protection against *P. megasperma* var. *sojae.* A partial suppression of phytoalexin production or some other factor may be the answer. The work of Keen (3) and of Chamberlain & Paxton (2) and Paxton & Chamberlain (6) suggests the possibility of two kinds of phytoalexin produced by the *Phytophthora*-soybean interaction. Whether this is true for the interaction between nonpathogens and soybean remains to be determined.

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