Suppression of Rishitin and Phytuberin Accumulation and Hypersensitive Response in Potato by Compatible Races of Phytophthora Infestans

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ABSTRACT

A compatible or susceptible interaction is characterized by the suppression of both necrosis and accumulation of rishitin and phytuberin in the potato tuber. An alteration of cellular response in the host during the compatible interaction suppressed the ability of the host to respond normally to a subsequent infection by an incompatible race. Conversely, once the host was inoculated with an incompatible race, suppression from a subsequent infection with a compatible race did not occur. An incubation period of 12 hr is sufficient to either establish suppression or elicit the hypersensitive host response. A compatible interaction also suppressed the hypersensitive host response to sonicated homogenates of the fungus. Phytopathology 61:178-181.

Additional key words: thin-layer chromatography, gas-liquid chromatography, mechanism for susceptibility.

Earlier studies established that the hypersensitive response of potato tubers to inoculation with incompatible races of *Phytophthora infestans* (Mont.) d By. included rapid cell necrosis and the accumulation of two terpenoids, rishitin and phytuberin (2, 3, 4, 5, 6). Sonicated homogenates of the fungus elicited the hypersensitive response in all cultivars (2, 5). This paper supports the hypothesis that susceptibility is the result of a suppression of the resistance response.

MATERIALS AND METHODS.—*Inoculation, incubation, and extraction of potato slices.*—The cultivars Kennebec (R_1) and WV-13-8 (R_1R_4) were inoculated with a suspension of zoospores, approx 40,000-50,000/ml, of race 4 (resistant interaction) and race 1.2.4 (susceptible interaction).

A sonicated suspension of 10 g fungus/100 ml water was dispersed over the tuber slices. The treatments received by slices are listed in Table 1. In all cases, the combined 1st and 2nd mm of tuber surface were harvested. Techniques for the growth of the fungus, preparation of inoculum, inoculation, incubation, and extraction were identical to those previously described (5, 6). Extracts of tuber tissue were adjusted to a concn of 5 g dry wt/ml.

Estimation of rishitin and phytuberin by thin-layer chromatography.—Extracts were applied to channels on Silica Gel-G plates (gel 250 μ thick) and developed in cyclohexane:ethyl acetate (1:1, v/v) for estimation of rishitin, and in hexane:acetone (95:5, v/v) followed by cyclohexane:ethyl acetate (4:1, v/v) for estimation of phytuberin. Plates for treatments of individual cultivars were sprayed at the same time with vanillin-sulfuric acid reagent (5) to estimate rishitin and phytuberin.

Quantitative analyses of rishitin and phytuberin.—Extracts (100-200 μliters) were applied to Silica Gel-G plates and developed in cyclohexane:ethyl acetate (1:1, v/v). Gel in bands extending 1 cm on either side of the rishitin (R_F 0.31) and phytuberin (R_F 0.64) was removed and extracted (5).

An aerograph Hy-Fi Model 600 gas chromatograph, equipped with glass column containing diethylene glycol-adipate packing and flame ionization detector was utilized for all gas-liquid chromatography (GLC) analyses. Phytuberin was eluted in 7.6 min, but rishitin required 28.3 min; therefore, blockage of the hydroxyl groups of rishitin was necessary. The bis trifluoroacetyl rishitin derivative was prepared by adding 100 μliters of trifluoroacetic anhydride to the dried sample and allowing the mixture to stand in a water bath at 35 C for 15 min. After evaporation of excess anhydride, the sample was dissolved in cyclohexane and injected into the chromatograph. The rishitin derivative had a retention time of 1.6 min.

Methyl stearate was selected as an internal standard because of its retention time of 5.4 min. One to 6 μliters of a cyclohexane solution containing 2.0 μg methyl stearate/μliter solvent were injected into the instrument. Recovery from the total thin-layer chromatography (TLC) GLC process for rishitin and phytuberin was 95 ± 5% and 85 ± 3%, respectively. The following GLC materials and conditions were used: Carrier gas (N_2), H_2, and O_2—50, 23, and 240 cc/min, respectively; Injection port temp, 250 C; Detector, 190 C; Oven temp—190 C isothermal; Attenuation setting —max sensitivity; Glass column—6.0 ft × 2 mm ID; Packing—15% Hi-EFF-2AD on Chromosorb Q (60-80 mesh) (Applied Science Labs, Inc.).

RESULTS.—*Symptoms.*—A system for the designation of treatments is given in Table 1. For example, R *12 hr* S indicates inoculation with race 4 (incompatible) followed in 12 hr by inoculation with race 1.2.4 (compatible). Symptoms were recorded and the tissue harvested 72 hr after a single treatment or 72 hr after a second treatment for slices receiving two treatments.

The 36- and 72-hr aged controls had normal periderm formation and no visible contamination. The 72-hr susceptible control was as the aged controls, except for flecking on the top mm. A strong hypersensitive re-

TABLE 1. Treatments received by potato slices inoculated with *Phytophthora infestans*

Abbreviation	Treatment
R, S	Inoculated with an incompatible race (race 4) or compatible race (1.2.4) and incubated 72 hr
R *12 hr* S, S *12* hr R	Inoculated, incubated 12 hr, reinoculated and incubated 72 hr
Aged *12 hr* R, Aged *12 hr* S	Aged 12 hr, inoculated and incubated 72 hr
R *24 hr* S, S *24 hr* R	Inoculated, incubated 24 hr, reinoculated, and incubated 72 hr
Aged *24 hr* R, Aged *24 hr* S	Aged 24 hr, inoculated and incubated 72 hr
R *24 hr* Homog, S *24 hr* Homog	Inoculated, incubated 24 hr, treated with sonicated homogenate of fungus, and incubated 72 hr
Aged *24 hr* Homog	Aged 24 hr, treated with sonicated homogenate of fungus, and incubated 72 hr
S *36 hr* R	Inoculated, incubated 36 hr, reinoculated, and incubated 72 hr
Aged *36 hr* R	Aged 36 hr, inoculated, and incubated 72 hr

sponse with heavy flecking and necrosis into the third mm occurred in the 72-hr resistant control.

R *12 hr* S had more necrotic browning in the first and second mm than Aged *12 hr* R and S *12 hr* R and the treatments were rated R *12 hr* S >> Aged *12 hr* R > S *12 hr* R in order of decreasing necrosis. The surface of the third mm of R *12 hr* S had sparse necrosis. The treatment R *24 hr* S had greater necrotic browning in the first and second mm than Aged *24 hr* R or S *24 hr* R, and the treatments were rated R *24 hr* S > Aged *24 hr* R >> S *24 hr* R in order of decreasing necrosis. Treatment R *24 hr* S had heavy flecking on the surface of the third mm, whereas S *24 hr* R had only sparse flecking. S *24 hr* R tissue was softer than that of the other treatments. Aged *24 hr* Homog had more necrotic browning and was firmer than the S *24 hr* Homog. The compatible race not only suppressed host response to an incompatible race, but also suppressed a sudden response to an inducer present in the sonicated homogenate. When races 4 and 1.2.4 were grown side by side on lima bean agar there was no indication of antagonism. This, plus the suppression of response to sonicated homogenates, minimize the possibility that suppression is due to an antagonism between the races.

Estimation of rishitin, phytuberin, and unknown.— Comparison of rishitin and phytuberin accumulation is given for all treatments with Kennebec (Table 2) and WV-13-8 (Table 3). The susceptible controls accumulated little or no rishitin or phytuberin. A trace of rishitin was found in the WV-13-8 72-hr aged control and in the WV-13-8 susceptible control.

With 12 and 24 hr between aging and inoculation or consecutive inoculations, rishitin accumulated in the order R ——— S > Aged ——— R > S ——— R. Phytuberin accumulated as above with Kennebec, but there was little or no difference between Aged *12 hr* R and R *12 hr* S or Aged *24 hr* R and R *24 hr* S of WV-13-8. Inoculation with a compatible race before treatment

TABLE 2. Estimation of rishitin, phytuberin, and unknown in Kennebec potato slices after aging, treatment with fungal homogenate, inoculation or consecutive inoculations with *Phytophthora infestans*[a]

Treatment	Rishitin	Phytuberin	Unknown[c]
Fresh tissue	—[b]	—	—
36-hr aged control	—	—	—
72-hr aged control	—	—	+—
R control	+++	++	+—
S control	—	—	+++
R *12 hr* S	+++	+++	+—
S *12 hr* R	+	—	+++
Aged *12 hr* R	++	++	+—
Aged *12 hr* S	—	—	++
R *24 hr* S	++++	+++	+—
S *24 hr* R	+—	—	+++
Aged *24 hr* R	+++	+	+—
Aged *24 hr* S	—	—	++
S *24 hr* Homog	++	+	+++
R *24 hr* Homog	++++	+++	+—
Aged *24 hr* Homog	++++	+++	+—
S *36 hr* R	+—	—	+++
Aged *36 hr* R	++	++	+—

[a] Relative estimations valid only for a single compound. Estimations based on color reaction after spraying with vanillin-sulfuric acid reagent.
[b] Compound not detected.
[c] Unknown component has R_F 0.71 when separated on Silica Gel-G plates with cyclohexane:ethyl acetate (1:1, v/v), and appears dark blue after spraying with vanillin-sulfuric acid reagent.

with the sonicated fungus suppressed the accumulation of rishitin and phytuberin and necrosis. The discoloration associated with the hypersensitive response was directly related to the accumulation of rishitin and phytuberin.

The third column in Tables 2 and 3 indicates the

TABLE 3. Estimation of rishitin, phytuberin, and unknown in WV-13-8 potato slices after aging, treatment with fungal homogenate, inoculation or consecutive inoculations with *Phytophthora infestans*

Treatment	Rishitin	Phytuberin	Unknown[c]
Fresh tissue	—[b]	—	—
36-hr control	—	—	+—
72-hr control	+	—	+
R control	++++	++	+—
S control	+—	—	+++
R *12 hr* S	++++	++	+
S *12 hr* R	—	—	+
Aged *12 hr* R	+++	++	+
Aged *12 hr* S	—	—	++
R *24 hr* S	++	+—	++
S *24 hr* R	—	—	+++
Aged *24 hr* R	+	+—	+
Aged *24 hr* S	—	—	++
S *24 hr* Homog	+	—	+++
R *24 hr* Homog	+++	++	+—
Aged *24 hr* Homog	+++	+—	—
S *36 hr* R	—	—	++++
Aged *36 hr* R	++	—	+—

[a] Relative estimations valid only for a single compound. Estimations based on color reaction after spraying with vanillin-sulfuric acid reagent.
[b] Compound not detected.
[c] Unknown component has R_F 0.71 when separated on Silica Gel-G plates with cyclohexane:ethyl acetate (1:1, v/v), and appears dark blue after spraying with vanillin-sulfuric acid reagent.

TABLE 4. Suppression of rishitin and phytuberin accumulation in Kennebec potato slices by a compatible race of *Phytophthora infestans*[a]

Treatment[b]	µg/g dry wt	
	Rishitin	Phytuberin
Fresh tissue	—[b]	—[b]
Aged 36 hr	—	—
Aged 72 hr	—	—
R	118	5
S	—	—
R *12 hr* S	217	20
S *12 hr* R	42	3
Aged *12 hr R*	130	6
Aged *12 hr S*	—	—
R *24 hr* S	334	23
S *24 hr* R	56	2
Aged *24 hr* R	238	4
Aged *24 hr* S	—	—
S *24 hr* Homog	52	2
Aged *24 hr* Homog	415	13
S *36 hr* R	9	2
Aged *36 hr* R	76	5

[a] Analyses by thin-layer and gas-liquid chromatography of combined first and second mm of slices.
[b] Compound not detected.

relative accumulation of an unknown component at R_F 0.71 in cyclohexane:ethyl acetate (1:1, v/v) and R_F 0.34 in hexane:acetone (95:5, v/v) followed by cyclohexane:ethyl acetate (4:1, v/v). The unknown was dark blue on silica gel after spraying with vanillin-sulfuric acid reagent and heating at 120 C for 3 min. It was in low quantities in aged controls and incompatible interactions and in high quantities in compatible interactions and interactions initially inoculated with the compatible race.

Quantitation of rishitin and phytuberin.—Samples from treatments of Kennebec were analyzed, and the results are shown in Table 4.

Prior inoculation with the compatible race always decreased the ability of the host to elicit a hypersensitive response or accumulate rishitin and phytuberin after subsequent inoculation with an incompatible race. The small differences in rishitin and phytuberin levels between S *12 hr* R and S *24 hr* R suggest that the compatible race requires only 12 hr of incubation to markedly suppress the host response.

DISCUSSION.—A suppression of the hypersensitive response and rishitin and phytuberin accumulation occurred in compatible interactions and in compatible interactions subsequently inoculated with an incompatible race. Conversely, once the host had been inoculated with an incompatible race, suppression from a subsequent infection with a compatible race did not occur. If reversibility of the initial response was rapid, the marked differences in symptoms and sesquiterpenoid accumulation between R ——— S and S ——— R would not have been seen. A 12-hr incubation period can either establish the suppression or elicit the host response. Due to the small number of cells penetrated by the fungus relative to the total number of host cells on the slice surface and the limited growth of the organism during a 12-hr period, rapid conveyance of information is required for the conditioning of host cells. If the information directly from the fungus or indirectly from infected cells is chemical, it must have sufficient mobility to influence quickly a large number of neighboring uninfected cells. The suppression of response to sonicated homogenates by a compatible race is further evidence that the fungus influences cells neighboring to those it penetrated.

Slices receiving treatment S *36 hr* R were spongy, with large areas of aerial mycelia. Aged *36 hr* R tissue remained firm but less necrotic than the Aged *12* or *24* hr R. The decrease in sensitivity with increased aging prior to inoculation may be due to a physical barrier to penetration caused by suberization. Allen & Kuć (1) demonstrated the accumulation of the steroid glycoalkaloids α-solanine and α-chaconine in potato slices after aging. The lack of α-solanine and α-chaconine accumulation during rishitin synthesis in incompatible interactions (3) suggests a common use of the acetate-mevalonate pathway. If rishitin is assumed to be partly the cause or result of the hypersensitive response, then 24-36 hr of aging would orient biosynthesis in favor of the steroid glycoalkaloids. Since the production of the steroid glycoalkaloids and rishitin appears to be competitive, it could take another 30 to 40 hr to reverse the system so that the aged tissue could synthesize rishitin in quantities comparable to unaged tissue. Should the above considerations be valid, only the latter portion of the 72-hr incubation period with aged tissue would be effective for a hypersensitive response and rishitin and phytuberin accumulation.

The similar levels of phytuberin found in R *12 hr* S and R *24 hr* S indicate that the additional 12 hr of incubation did not increase phytuberin accumulation. Interference by the compatible race should decrease the accumulation of terpenoids, especially in the R *12 hr* S treatment. Actually, an increase in phytuberin and rishitin accumulation over the Aged *12 hr* R occurred. A compatible interaction when preceded by an incompatible interaction did not suppress the hypersensitive response, and the terpenoids accumulated. This inability to suppress host response may be caused by several factors. A prior incompatible interaction may cause the accumulation of a toxic factor in the slice which kills or contains the compatible fungus. The toxic factors may include rishitin and phytuberin, since their accumulation is detected in the top mm of slices 30-40 hr after inoculation and they reach fungitoxic levels within 48-72 hr (5, 6). On the other hand, the high level of rishitin and phytuberin in R *12 hr* S may be due to the inability of the compatible race to interfere with the host response due to prior host recognition of the incompatible race. The fungus cannot alter this recognition, and the host now recognizes the compatible race as incompatible. The number of recognizable spores has therefore doubled in the R ——— S as compared to the Aged ——— R, and an increase in penetrated cells has occurred.

Tubers reacted hypersensitively to a heat-stable factor from sonicated homogenates of both races. This suggests that the compatible interaction depends upon

the ability of the fungus to interfere with host response to this factor. We propose that a blocking action or interference is achieved by a race only after adaptation to a specific host tissue. An unadapted race (incompatible) would lack information for interference. The host could therefore respond to the fungal trigger secreted during cellular penetration and/or hyphal growth with an incompatible or resistant interaction.

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