Effect of the R-3 Gene in Resistance of the Wauseon Potato Tuber to Phytophthora infestans

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ABSTRACT

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Tuber tissue of potato cultivar Wauseon, which has R_1R_3 resistance genes, was compatible with *Phytophthora infestans* race 1, even though incompatibility was predicted from its foliar reaction to that pathogen. When challenged by *P. infestans* race 4, Wauseon tuber slices developed a penetrating necrosis and showed a high accumulation of the terpenoids, phytuberin, and katahdinone. The susceptibility of Wauseon tubers to race 1 exemplify van der Plank's contention that the tubers of R_3 potato cultivars may not be as resistant as is its foliage to this race. In the compatible interaction of Wauseon with race 1, production of rishitin is almost equal to that produced during the hypersensitive response of Kennebec (R_1) to race 4. This suggests that rishitin is not the primary factor involved in the resistance of potato tubers to races of *P. infestans*.

Sato et al. (4) demonstrated that the terpenoid, rishitin, accumulates in incompatible interactions of potato tubers having resistance genes R_1, R_2, R_3, and R_4 with race 0 of *Phytophthora infestans*. Rishitin and phytuberin (7) and lubimin (3) also have been found to accumulate in incompatible host-pathogen interactions, whereas little or none of these compounds accumulates during compatible interactions. More recently, spiroveta-1(10),11-diene-2-one (given the trivial name katahdinone by us and referred to as solavetivone by the British workers) has been found in potato tubers stressed with *P. infestans* (8) or *Erwinia carotovora* (2).

The international system for designating the relation of genes and races of *P. infestans* (1) predicts that the potato cultivar Wauseon, an R_1R_3 type, would differ from Kennebec, an R_1 cultivar, in its reaction to race 1 of the fungus. The genetic constitution of Wauseon should provide an incompatible interaction with race 1, and that of Kennebec would be expected to be compatible. Nevertheless, that system is based entirely on foliage responses and van der Plank (5) has indicated that the tubers of the R_3 type may be susceptible to races that do not attack the foliage.

In this study, we have examined and compared the foliar and tuber tissue responses of potato cultivars Wauseon and Kennebec toward races 1 and 4 of *P. infestans*.

MATERIALS AND METHODS

Preparation, inoculation, incubation, and extraction of potato slices.—Tubers of Kennebec (R_1) and Wauseon (R_1R_3) were surface-sterilized in 1% NaClO solution on a reciprocating shaker for 15 minutes, lightly flushed with distilled H_2O and cut into 4-mm-thick slices that averaged 60 mm in diameter with a food slicer that had been flushed with 70% ethanol. Each slice was flushed with 20-30 ml of sterile distilled H_2O, placed in a sterile petri dish, and inoculated, by pipetting 1.0-1.3 ml of a suspension of sporangia (~15,000 sporangia/ml) of *P. infestans* R-1 (compatible foliar host response) or R-4 (incompatible foliar host response). Race 1 was obtained from K. Deahl, ARS, Beltsville, Md., and race 4 was provided by H. Van Etten of Cornell University. Control slices of both cultivars received 1 ml of distilled H_2O. The slices were incubated at 20 C. At intervals during 6 days of exposure, six slices were removed from each cultivar-race interaction, weighed, freeze-dried, reweighed for dry weight, and extracted.

The dry tissue was ground with 125 ml of methylene chloride (nanograde quality), filtered through a medium-porosity sintered glass Büchner funnel, and the residue twice more extracted by stirring each time with 100 ml of the solvent. The combined filtrates were taken to dryness on a rotary evaporator at reduced pressure. The residue was taken up in approximately 10 ml of methanol (reagent grade) which was reduced to less than 1 ml with a stream of N_2 and then centrifuged. The clear supernatant was carefully removed and measured with a Hamilton syringe.

Qualitative thin-layer chromatographic analysis for terpenoids.—Aliquots (8-15 μliters) of the extracts were applied to Silica Gel-G plates (250 μm thick) which were developed with cyclohexane:ethyl acetate (1:1, v/v) (7). The dried plates were sprayed with $CHCl_3$ saturated with $SbCl_3$, and examined cold and after heating at 110 C for 5 minutes. Standards of rishitin, phytuberin, katahdinone, and lubimin were isolated from infected tubers and slices and identified by mass spectroscopy and nuclear magnetic resonance. Purity was determined by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

Quantitative analysis for rishitin, phytuberin,

katahdinone, and lubimin.—A Varian Aerograph Model 1520 gas chromatograph, equipped with two glass columns, each 184 cm × 3.2 mm, packed with 3% OV-1 and 3% EGSP-Z on Gas Chrom Q, respectively (all obtained from Applied Science Laboratories, State College, Pa.), and a flame ionization detector, was employed in the gas-liquid chromatographic analysis of the extracts. Peak integration was carried out on the eluate of the OV-1 column. The above EGSP-Z column and 3% QF-1 on Variport 30 packed in a 184 cm × 3.2 mm glass column were used to confirm the identification of the peaks. Co-chromatography with standards established the identity of the peaks. The columns were programmed at 4 degrees per minute from 115-225 C. Injection port temperature 180 C; detection temperature 230 C; carrier gas (He) 20 ml/minute (OV-1); 40 ml/minute (EGSP-Z); and 30 ml/minute (QF-1). With the apparatus and procedures described the four terpenoids were quantitated with a reasonable degree of accuracy in amounts as low as 0.05 μg.

TABLE 1. Amounts of certain terpenoids produced after inoculating Kennebec tuber slices with two different races of *Phytophthora infestans*

P. infestans race and hours of exposure	Terpenoids detected (μg/g dry weight of tissue)			
	Rishitin	Phytuberin	Katahdinone	Lubimin
Race 4				
24	3.8	trace	trace	n.d.[b]
46	5.55	trace	trace	3.7
68	19.6	trace	2.4	7.1
96	26.5	1.3	9.5	20.9
140[a]	26.8	3.7	15.8	12.9
Race 1				
24	trace	n.d.	trace	n.d.
48	0.44	n.d.	trace	trace
68	1.12	trace	trace	0.85
91	2.07	trace	trace	0.83
140	1.88	trace	trace	0.50

[a]None of the four terpenoids was detected in the H_2O control slices after 140 hours.
[b]Abbreviation n.d. = none detected.

TABLE 2. Amounts of certain terpenoids produced after inoculating Wauseon tuber slices with *Phytophthora infestans*

P. infestans race and house of exposure	Terpenoids detected (μg/g dry weight of tissue)			
	Rishitin	Phytuberin	Katahdinone	Lubimin
Race 4				
23	7.04	0.87	trace	trace
47	20.2	3.48	0.70	3.13
72	33.8	46.5	12.7	7.8
96	44.4	146	66.4	17.4
119	24.2	42.8	78.5	6.9
Race 1				
47	5.1	trace	1.9	trace
72	12.5	1.85	3.28	2.67
96	20	2.0	4.58	1.90
132[a]	18.1	trace	7.56	trace

[a]After 132 hours 3.53 μg of rishitin per gram dry weight of tissue was found in the H_2O control slices. None of the other terpenoids was detected.

RESULTS

When inoculated with R-4, the Kennebec slices exhibit the typical necrotic flecking on the uppermost millimeter, whereas the Wauseon slices developed deep necrotic lesions that penetrated to one-half the depth of the slice in the same time period. However, the latter had maintained slice integrity at the termination of the experiment (6 days).

Inoculation of Kennebec slices with R-1, however, produced a deep browning of the slice, softening, and finally tissue liquefaction. Race R-1 of *P. infestans* inoculated on Wauseon slices also caused browning and loss of tissue integrity, but to a lesser degree than on Kennebec.

Analyses by TLC revealed rishitin in all of the *P. infestans*-challenged slices of the time study except for Kennebec inoculated with R-1. Although Wauseon slices exposed to R-1 showed the outward symptoms of a susceptible host response, clear evidence was observed for the presence of rishitin at 48, 72, 96, and 132 hours after inoculation. Results of quantitative GLC analysis of the time-accumulation study are given in Tables 1 and 2. Wauseon slices after 4 days had accumulated twice as much rishitin when inoculated with R-4 (44 μg/g dry weight) as with R-1 (20 μg/g dry weight). The Kennebec slices produced small amounts of rishitin during the susceptible response with R-1. For the resistant response of Kennebec with R-4, the rishitin found was somewhat less (27 μg/g dry weight) than that observed in the hypersensitive response of Wauseon with this race.

Although the Wauseon/R-1 interaction yielded as much rishitin as that of Kennebec/R-4, the former produced only a small amount of phytuberin, katahdinone, and lubimin. The hypersensitive response

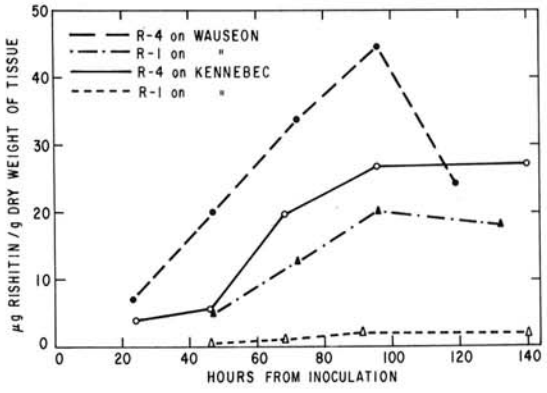

Fig. 1. Rate of accumulation of rishitin in Wauseon and Kennebec tuber slices inoculated with *Phytophthora infestans* races 1 and 4.

of Kennebec to R-4 failed to produce levels of phytuberin and katahdinone that were commensurate with that of the Wauseon/R-4 interaction. During the first 96 hours, phytuberin was the most abundant terpenoid (of the four determined) in the Wauseon slices (146 μg/g dry weight). At 120 hours, phytuberin had diminished sharply (42.8 μg/g dry weight), and katahdinone had increased to become the most abundant terpenoid (78.5 μg/g dry weight).

DISCUSSION

Although the foliage of Wauseon, an R_1R_3 cultivar, is resistant to both *P. infestans* R-1 and R-4, its tubers appear to be susceptible to R-1. The tissue, however, is more slowly disrupted than that of Kennebec by this race. The level of rishitin found in the disrupted Wauseon tissue was well above that normally observed in a susceptible interaction although it was only about half of that present in slices inoculated with R-4. The tuber tissue inoculated with R-1 also failed to accumulate significant quantities of phytuberin, katahdinone, or lubimin. The production of rishitin has been regarded as a biochemical response accompanying rapid cell death in a hypersensitive reaction. The extent of rishitin and phytuberin accumulation has been described as a parameter by which a resistant- and susceptible interaction can be differentiated (7). In this context, Wauseon tubers are to be regarded susceptible to R-1. Even the "resistant" response to R-4 is suspect. Although the interaction produces large amounts of terpenoids, including rishitin, the penetrating necrosis suggests visible manifestations of susceptibility. This is reminiscent of Varns' et al. (6) observation that the sprouts of cultivars inoculated with compatible races formed deep necrotic lesions accompanied by notable accumulations of rishitin and phytuberin. This finding supports van der Plank's contention regarding foliage versus tuber behavior of the R_3 genotype to challenge by races of *P. infestans* (5). Since leaves and stems contain identical genetic information as tubers, other factors related to the latter must have had an overriding influence that prevented the tissues from expressing effective resistance to the parasite.

It should be noted that the accumulation of rishitin which occurred in the hypersensitive response of Kennebec tuber slices inoculated with R-4 was approximately the level found in the disrupted tissue of the Wauseon/R-1 interaction (Tables 1 and 2). Moreover, the rate of accumulation of this compound was quite similar in both cases (Fig. 1). However, since the whole tissue slice was analyzed in compatible and incompatible interactions, the actual concentration of rishitin at the site of expressed resistance might be substantially higher in incompatible interactions. A possible additive or synergistic role for an appreciable level of lubimin in the Kennebec slices after the first three days cannot be clearly ascertained. However, its delayed accumulation would not suggest that it played an effectual role in the resistance mechanism.

From this study and previous observations (8), it appears that the accumulation of katahdinone is related to the extent of necrosis resulting from infection and is largely associated with the necrotic tissue. This would be commensurate with finding it increasing with progressing interaction time although the other stress metabolites which were measured either have ceased to accumulate or have diminished.

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