# Coordinate Production of Hydroxyphaseollin and the Yellow-Fluorescent Compound $PA_k$ in Soybeans Resistant to Phytophthora megasperma var. sojae

N. T. Keen and J. D. Paxton

Associate Professors, Departments of Plant Pathology, University of California, Riverside 92502; and University of Illinois, Urbana 61801, respectively. We thank A. F. Schmitthenner and F. W. Schwenk for fungus isolates, and E. E. Hartwig for soybean seed.

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ABSTRACT

Eight incompatible soybean-*Phytophthora megasperma* var. *sojae* combinations resulted in higher rates of production of $PA_k$ and hydroxyphaseollin (HP) than did eight compatible combinations. The two compounds were produced at rates similar to each other in all combinations, which indicated that they may be under coordinate metabolic control in the inoculated soybean plants.

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*Additional key words*: phytoalexins, monogenic resistance, antifungal compounds.

Monogenic resistance in soybean [*Glycine max* (L.) Merr.] hypocotyls to *Phytophthora megasperma* Drechs. var. *sojae* A. A. Hildb. results in restriction and then death of the invading fungus (1, 3, 9, 11, 14). Concomitantly, resistant-reacting plants undergo activated biosynthesis of several secondary metabolites, among which are the antifungal pterocarpan, hydroxyphaseollin (HP) (4, 6, 8, 9), and a yellow-fluorescent compound, called $PA_k$ (2, 3, 8, 10, 11, 14). Although these chemicals have been reported to be produced more rapidly in resistant than in susceptible soybean plants, no conjunctive quantitative analyses for both compounds have been performed. We therefore tested the hypothesis that hydroxyphaseollin and $PA_k$ are produced under coordinate control in soybean hypocotyls inoculated with *P. megasperma* var. *sojae*.

Soybean plants were grown as previously described (6) and inoculated when 5 to 7 days old with *P. megasperma* var. *sojae* mycelium grown for 2 to 5 days on pea broth medium (6). Race 1 (P900) and race 3 (P892) isolates were kindly supplied by A. F. Schmitthenner, Ohio Center for Agricultural and Research Development, Wooster (12). The race 4 isolate (P972) was obtained from F. W. Schwenk, Kansas State University, Manhattan (13) and the race 2 isolate (P406) was available from previous work (4). Reactions on the differential soybean varieties (Table 1) confirmed those in the published reports (4, 12, 13). Plants were inoculated in the hypocotyls by the previously described wounding method (4), and harvested at various intervals after inoculation for extraction of chemicals.

Hydroxyphaseollin levels in the hypocotyls were determined by slight modification of the TLC/UV method (5). After extraction of hypocotyls with ethanol,

TABLE 1. Concentrations of hydroxyphaseollin (HP) and the yellow-fluorescent compound $PA_k$ in resistant or susceptible soybean hypocotyls inoculated with various races of *Phytophthora megasperma* var. *sojae*[a]

| Cultivar | Fungus race | Visible plant response[b] | $PA_k$[c] (μg/g) | HP[c] (μg/g) |
|---|---|---|---|---|
| Harosoy | 1 | S | 180 | 190 |
| | 2 | S | 200 | 360 |
| | 3 | S | 200 | 300 |
| | 4[d] | S | 170 | 190 |
| Harosoy 63 | 1 | R | 1100 | 540 |
| | 2 | R | 1000 | 730 |
| | 3 | S | 250 | 280 |
| | 4[d] | S | 160 | 140 |
| D60-9647 | 1 | R | 1100 | 610 |
| | 2 | S | 160 | 140 |
| | 3 | R | 680 | 440 |
| | 4[d] | R | 370 | 480 |
| Semmes | 1 | R | 790 | 590 |
| | 2 | R | 770 | 680 |
| | 3 | R | 900 | 500 |
| | 4[d] | S | 110 | 130 |

[a]Six-day-old plants were inoculated and harvested for analyses after 50 hours.

[b]Susceptible (S) = extensive rot and water soaking; resistant (R) = little or no rot with brown-red pigment at inoculation point.

[c]Wounded but noninoculated plants contained less than 40 μg/g $PA_k$ and less than 30 μg/g HP.

[d]Data were taken from separate experiments.

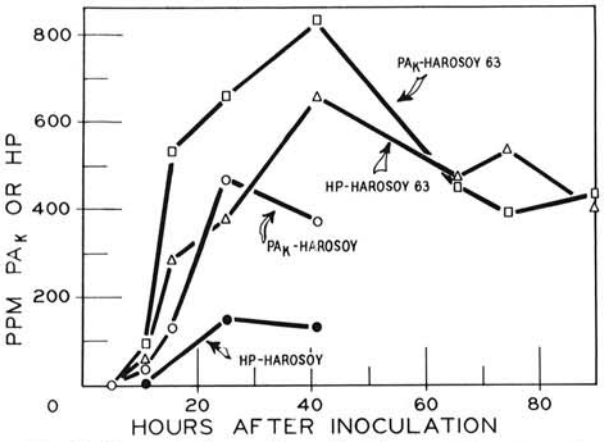


**Fig. 1.** Concentrations of a yellow-fluorescent compound called $PA_k$ and hydroxyphaseollin (HP) in Harosoy and Harosoy 63 soybean hypocotyls at various intervals after inoculation with race 1 of *Phytophthora megasperma* var. *sojae*. Plants were 5 days old when inoculated. Wounded, but non-inoculated, hypocotyls contained less than 22 $\mu$g/g $PA_k$ and less than 20 $\mu$g/g hydroxyphaseollin. Inoculated Harosoy hypocotyls were not harvested after 40 hours postinoculation because of extensive decomposition by the fungus.

followed by filtration and drying of the crude extracts, they were dissolved in 0.1 ml ethyl acetate/g fresh weight. Then 30 $\mu$liters were applied to silica gel $GF_{254}$ thin-layer chromatography plates, chromatographed, and the HP eluted prior to quantitation by absorbance at 285 nm (5).

$PA_k$ was quantitated by placing seven-to-ten hypocotyl sections in vials with 5 ml 0.05 M potassium phosphate (pH 7.5)/g fresh weight, and extracting in a boiling water bath for 30 minutes. Concentrations of $PA_k$ in the resulting extracts were directly estimated spectrophotometrically after Paxton and Chamberlain (11), but at 494 nm. To correct for interfering substances, the numerical mean of absorbances at 450 and 550 nm was subtracted from that at 494 nm to obtain the $PA_k$ concentration. All data for hydroxyphaseollin and $PA_k$ were expressed as $\mu$g/g on a fresh weight basis.

Hydroxyphaseollin and $PA_k$ were first detected in resistant, inoculated Harosoy 63 hypocotyls at 10-12 hours after inoculation (Fig. 1), and both chemicals were produced rapidly thereafter for approximately 40 hours, followed by a decline. The two chemicals were also produced in susceptible Harosoy hypocotyls, but at slower rates than in Harosoy 63, and levels did not increase after 24 hours when rotting of the tissues by the fungus became severe.

Eight incompatible soybean-*P. megasperma* var. *sojae* combinations resulted in greater accumulation of $PA_k$ and HP than did eight compatible host-pathogen combinations (Table 1). This confirms previous results with several of the combinations, and the data also show that the newly described races 3 and 4 lead to lower levels of HP and $PA_k$ in susceptible Harosoy 63 than the incompatible races 1 and 2 (Table 1). The data are therefore consistent with the conclusion that susceptibility to *P. megasperma* var. *sojae* in soybeans may be associated with repressed production of HP and $PA_k$ and resistance with derepressed production. Although the molecular mechanisms underlying these specific host-parasite interactions are not known, the present observations are consistent with the hypothesis that differential rates of production of $PA_k$ and HP are causally related to the expression of resistance and susceptibility.

Earlier data (3) disclosed the presence of $PA_k$ in susceptible, inoculated soybean hypocotyls at 4 hours after inoculation. However, in this work it was not detected until approximately 10 hours in either resistant or susceptible plants. The basis for this discrepancy is probably the lower detection limits in the earlier work, and the fact that different techniques were used to produce and detect $PA_k$.

The production of $PA_k$ and HP may be under coordinate metabolic control in the soybean hypocotyl, since the curves for accumulation of HP and $PA_k$ were similar (Fig. 1). In addition to these compounds, the isoflavonoids daidzein, coumestrol, and sojagol also appear to be produced at approximately the same time as HP and $PA_k$ in resistant-responding soybean hypocotyls (7). Although certain of these compounds do not appear to be involved with the restriction of fungus growth, it may be suggested that the resistant reaction involves a general and coordinate derepression of terminal isoflavonoid biosynthesis. Elucidation of the chemical structure of $PA_k$ will further test the validity of this hypothesis by disclosing whether its biosynthesis likely involves the isoflavonoid pathway.

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