Soybean Phytoalexin, Hydroxyphaseollin, Induced by Ultraviolet Irradiation

M. A. Bridge and W. L. Klarman

Graduate Assistant and Associate Professor, respectively, Department of Botany, University of Maryland, College Park 20742.

Supported in part by USDA Grant 016-15-20. Scientific Publication No. A1805, Contribution No. 4600, Maryland Agricultural Experiment Station.

Accepted for publication 20 November 1972.

ABSTRACT

The phytoalexin, hydroxyphaseollin (HP), was detected in soybean hypocotyls 12 hr after ultraviolet irradiation (λ_{max} = 253 nm) when plants were maintained in darkness. Maximal concentrations of HP occurred 96 hr after irradiation and relatively high levels were still present after 216 hr. Hypocotyls from irradiated plants maintained 12 hr in darkness and subsequently in light contained approximately one-half as much HP as hypocotyls from plants kept in constant darkness following irradiation. Hypocotyls from plants placed in light immediately after irradiation contained almost no HP. The concentration of HP remained high in irradiated plants placed in darkness for 48 hr and subsequently placed in light for 48 hr. When genetically susceptible plants were irradiated, they became less susceptible to the soybean pathogen, *Phytophthora megasperma* var. *sojae*.

Phytopathology 63:606-609

Additional key words: Glycine max, resistance, photoreversibility.

Klarman & Sanford (7) isolated the phytoalexin produced by soybeans, and Sims et al. (8) identified it as 6α-hydroxyphaseollin (HP). Bridge & Klarman (1) reported that irradiation of soybean cotyledons with shortwave ultraviolet induced large quantities of phytoalexin which tended to be associated with a deep bronzing of irradiated surfaces. When cotyledons were placed in light immediately after irradiation, however, production of phytoalexin was much reduced. Hadwiger & Schwochau (3) found that relatively short exposures of pea pods to shortwave ultraviolet triggered the production of the phytoalexin, pisatin, in quantities detectable within 6 to 8 hr after irradiation.

The objectives of the present investigation were to determine: (i) quantities of HP induced in soybeans by ultraviolet irradiation, (ii) effects of visible light following ultraviolet irradiation, and (iii) if ultraviolet irradiation could increase disease resistance of soybean plants.

MATERIALS AND METHODS.–Soybeans, *Glycine max* (L.) Merr. 'Harosoy 63' (H63) and 'Harosoy' (H), were grown in plastic flats containing white quartz sand in a growth room maintained at ca. 25 C. Gro-Lux lamps located 70 cm above the flats supplied 3,230 lux of continuous light. Approximately 1.5% of radiant energy was in wavelengths less than 380 nm.

Ultraviolet irradiation was supplied by 46-cm General Electric germicidal lamps, No. G25T8 (λ_{max} = 253 nm). Otherwise, the experiment was conducted in total darkness. Two ultraviolet lamps were placed on either side of a rocking platform, and a flat containing 20 to 100, 6- to 7-day-old intact plants was placed on the platform which slowly tilted back and forth to insure uniform irradiation. Lamps were placed at the edges of the flat and were aimed directly at the hypocotyls. After irradiation, all plants either remained in darkness or were returned to the lighted growth room.

Hypocotyls were freeze-dried, ground in a Wiley Mill, and 0.2-g portions were blended in 100 ml of boiling distilled water. Homogenates were filtered through cheesecloth and centrifuged at 28,700 *g* for 1 hr. The supernatant was partitioned with an equal volume of freshly distilled diethyl ether in three aliquots, and pooled ether extracts were evaporated to dryness under vacuum. Quantities of HP were analyzed as the trimethylsilyl derivative by a gas-liquid chromatography (GLC) method of Keen et al. (5).

Hypocotyls of 8- to 9-day-old plants were inoculated with mycelium from 7- to 9-day-old cultures of *Phytophthora megasperma* Dresch. var. *sojae* A. A. Hildb. grown in clarified V-8 juice medium (2). A pad of several layers of cheesecloth was stuck to the center of a piece of plastic tape approximately 5-cm square, and enough mycelium was deposited on the center of the pad to cover ca. 4 cm^2. The tape containing the pad with mycelium was wrapped around the hypocotyl so that mycelium was in contact with the plant tissue. The cheesecloth pad was saturated with V-8 medium in which the fungus had been growing. All tapes were sealed as tightly as possible, and petroleum jelly was placed around the edges to prevent loss of moisture.

Each experiment was conducted at least two, and usually three or more times.

RESULTS.–*Ultraviolet-induction of HP.*–H63 plants were irradiated for 30 min and placed in darkness. After 48 hr, hypocotyl extracts were prepared and examined by GLC. The chromatogram contained a series of peaks which were similar to those reported by Keen et al. (5) for HP obtained from fungal-induced soybeans.

Effects of duration of ultraviolet on HP concentration.–H63 plants irradiated for period of 1,

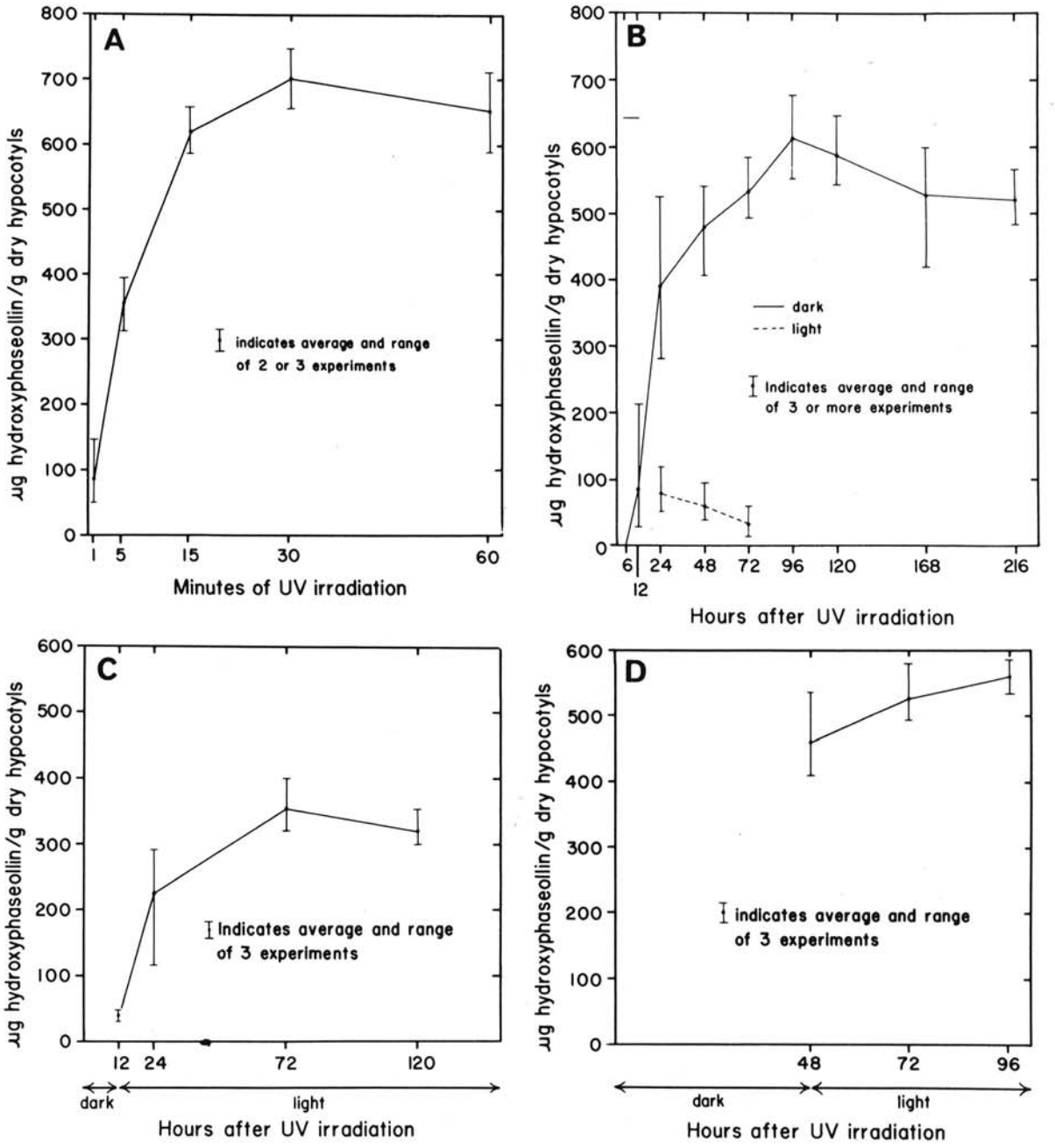

Fig. 1 (A-D). Quantities of hydroxyphaseollin from H63 soybean hypocotyls irradiated with ultraviolet (UV) for **A)** 1, 15, 30, and 60 min and maintained in darkness for 2 days; **B)** 30 min and continuously maintained in either darkness or light; **C)** 30 min and maintained in darkness for 12 hr and subsequently in light; and **D)** 30 min and maintained in darkness for 48 hr and subsequently in light.

5, 15, 30, and 60 min were placed in darkness. After 48 hr, extracts were prepared from each group of hypocotyls and concentrations of HP were determined by GLC. Concentration of HP increased with duration of irradiation up to 30 min (Fig. 1-A).

Rate of production and breakdown of HP in darkness.—H63 plants were irradiated for 30 min and placed in darkness. Hypocotyls were harvested at 6, 12, 24, 48, 72, 96, 120, 168, and 216 hr after irradiation, and extracts were prepared. No HP was detected in extracts from hypocotyls harvested 6 hr after irradiation, but hypocotyls harvested after 12 hr contained 84 μg HP/g dry tissue (Fig. 1-B). Concentrations of HP increased rapidly between 12

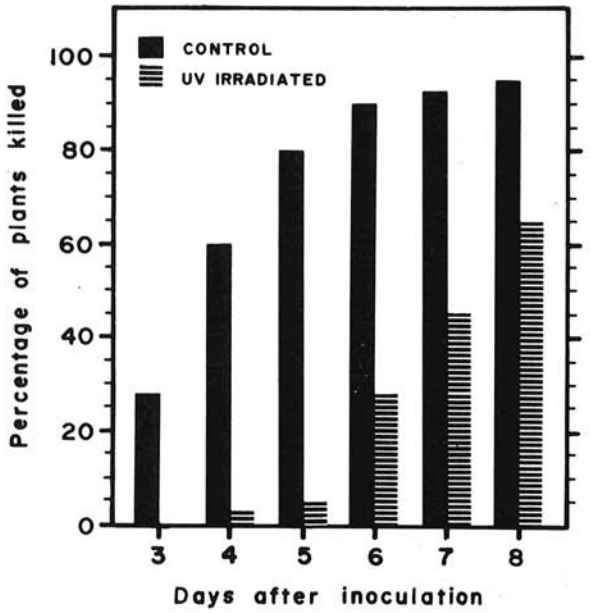

Fig. 2. Percentage of ultraviolet (UV) irradiated and nonirridiated soybean plants killed by *Phytophthora megasperma* var. *sojae*. All plants were placed in darkness immediately following treatment and maintained in darkness for 2 days before and 8 days following inoculation.

and 24 hr and more slowly from 24 to 96 hr. The concentration of HP decreased slowly after 96 hr, but 216 hr after irradiation, hypocotyls still contained ca. 500 μg HP/g dry tissue.

Photoreversibility of ultraviolet-induction of HP.–H63 plants were irradiated for 30 min and placed immediately in the lighted growth room. Extracts prepared from hypocotyls harvested 24, 48, and 72 hr after irradiation all contained very low concentrations of HP (Fig. 1-B). A second group of H63 plants were irradiated for 30 min, maintained in darkness for 12 hr, and removed to the lighted growth room. Extracts prepared from hypocotyl samples collected 12, 24, 72, and 120 hr after irradiation contained 37, 216, 342, and 309 μg HP/g dry tissue, respectively (Fig. 1-C). A third group of H63 plants was irradiated for 30 min, placed in darkness for 48 hr and subsequently placed in the lighted growth room. Extracts prepared from hypocotyls collected 48, 72, and 96 hr after irradiation contained 443, 513, and 543 μg HP/g dry tissue, respectively (Fig. 1-D).

Resistance induced by ultraviolet.–Twenty, 6- to 7-day-old H soybean plants, susceptible to *P. megasperma* var. *sojae*, were irradiated for 30 min and placed in darkness. Similar nonirradiated plants were placed in darkness as controls. After 2 days, hypocotyls of both groups were inoculated with mycelium of *P. megasperma* var. *sojae*. All plants remained in darkness after inoculation and were examined at daily intervals. The experiment was performed twice and results were averaged. Four days after inoculation, 60% of the nonirradiated control plants were dead, but only 2.5% of irradiated plants had been killed. Sixty-five percent of the irradiated and 95% of the nonirradiated plants were dead 8 days after inoculation (Fig. 2). Death of nonirradiated control plants was characterized by a soft rot of the hypocotyl causing it to collapse. Symptoms of infected irradiated plants more closely resembled those of vascular wilt than of stem rot; cotyledons and epicotyls wilted, but hypocotyls did not rot or collapse.

DISCUSSION.–Shortwave ultraviolet irradiation is an excellent inducer of HP. Unlike biological and chemical stimuli, it can be defined in terms of quality, quantity, and duration making it an especially appropriate tool for time-studies of production of phytoalexin and its breakdown in living plants.

The concentration of HP in irradiated soybean tissue was dependent on the length of time plants remained in darkness following ultraviolet irradiation. No HP could be detected in hypocotyls until 12 hr after the irradiation; an interval similar to that encountered with other induction systems (4, 6). Maximal concentrations of HP occurred 96 hr after irradiation and thereafter decreased slightly. This apparent decrease in HP concentration after 96 hr was probably due to the continued increase in diameter of hypocotyls following irradiation, as irradiated hypocotyls ceased to elongate but continued to increase in diameter. The quantity of tissue per hypocotyl thus continually increased after irradiation, but the number of cells originally exposed to irradiation did not.

Irradiation of susceptible plants caused them to become more resistant to attack by *P. megasperma* var. *sojae*. Fungal invasion of the cortex did not occur as rapidly in irradiated as in nonirradiated plants. Irradiation probably induced the production of HP in the epidermis and cortex, and thereby prevented or delayed penetration and infection. Irradiated plants which did become infected were wilted – indicating colonization of the vascular tissue. Penetration might have occurred in an area of incomplete ultraviolet coverage or an area where injury occurred during the inoculation process.

LITERATURE CITED

1. BRIDGE, M., & W. L. KLARMAN. 1970. Ultraviolet induction of an antifungal chemical in soybeans. Phytopathology 60:1013.
2. CASTRO, F. JESUS, G. A. ZENTMYER, & W. L. BELSER, JR. 1971. Induction of auxotrophic mutants in Phytophthora by ultraviolet light. Phytopathology 61:283-289.
3. HADWIGER, L. A., & M. E. SCHWOCHAU. 1971. Ultraviolet light-induced formation of pisatin and phenylalanine ammonia lyase. Plant Physiol. 47:588-590.
4. KEEN, N. T. 1971. Hydroxyphaseollin production by soybeans resistant and susceptible to Phytophthora megasperma var. sojae. Physiol. Plant Pathol. 1:265-275.

5. KEEN, N. T., J. J. SIMS, D. C. ERWIN, E. RICE, & J. E. PARTRIDGE. 1971. 6α-hydroxyphaseollin: an antifungal chemical induced in soybean hypocotyls by Phytophthora megasperma var. sojae. Phytopathology 61:1084-1089.
6. KLARMAN, W. L., & F. HAMMERSCHLAG. 1972. Production of the phytoalexin, hydroxyphaseollin, in soybean leaves inoculated with tobacco necrosis virus. Phytopathology 62:719-721.
7. KLARMAN, W. L., & J. B. SANFORD. 1968. Isolation and purification of an antifungal principle from infected soybeans. Life Sci. 7:1095-1103.
8. SIMS, J. J., N. T. KEEN, & V. K. HONWAD. 1971. The structure of hydroxyphaseollin, an induced antifungal compound from soybeans. Phytochemistry 11:827-828.