# Detection of Phytoalexin in Soybean Roots

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ABSTRACT

Phytoalexin ($PA_k$) production was demonstrated in fibrous roots of soybean (*Glycine max*). Amsoy, Harosoy, and Harosoy-63 seedlings were transplanted at 2, 6, and 12 days following germination into sterile, distilled water without or with zoospores of *Phytophthora megasperma* var. *sojae*. Analyses of water suspensions at 3 and 7 days following inoculation confirmed the presence of $PA_k$ only in suspensions containing zoospores. Phytopathology 61: 584-585.

*Additional key words: Phytophthora* root rot, soybean resistance.

Phytoalexin ($PA_k$) production by resistant soybean (*Glycine max* L.) cultivars provides a defense mechanism against infection by *Phytophthora megasperma* Drechs. var. *sojae*, A. A. Hildeb. (1, 2). $PA_k$ is a yellow-green, fluorescing material with an $R_F$ of 0.56 in the organic phase of a butanol:acetic acid:water solvent (5). It has an ultraviolet absorption maximum at 489 nm (2). Its production and reactions were studied only in soybean hypocotyls (2, 5).

We developed two systems for studying host-parasite relations in soybean roots, and determined the capability of roots to produce $PA_k$. A portion of this work was published as an abstract (6).

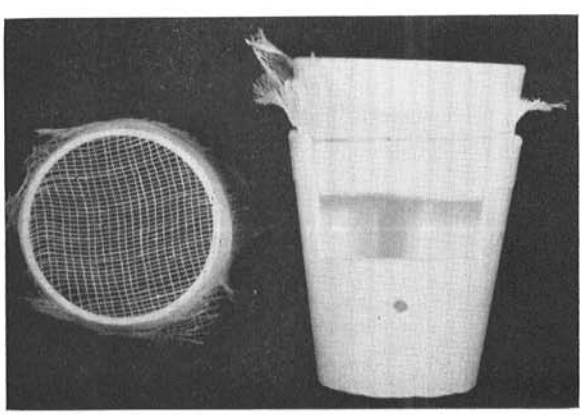

**Fig. 1.** Technique for collecting phytoalexin from roots of soybean seedlings inoculated with *Phytophthora megasperma* var. *sojae*, using two styrofoam cups; (Right) cutaway view; (Left) end view of top section with cheesecloth covering over cut end.

Race 1 of *P. megasperma* var. *sojae* (*Pms*), the susceptible cultivars, Harosoy (HS) and Amsoy (AS), and the resistant cultivar, Harosoy-63 (HR) were used in this study. Inoculum was prepared for root inoculations using the technique of Ho & Hickman (3) for zoospore production. Roots of AS, HS, and HR seedlings were inoculated with zoospore suspensions by either of two systems: (i) zoospores were contained in a central petri dish (90 nm) while 2-day-old seedlings were laid in a larger (140 nm) surrounding dish, allowing only the roots to extend into the zoospore suspension; and (ii) a "double-cup" system (6) (Fig. 1), whereby the bottom of one cup (9-oz styrofoam) was removed and the cut surface then covered with a layer of cheesecloth. Seeds were then placed on the cloth, covered with moistened vermiculite, and allowed to germinate by setting this container in a whole cup containing water. When seedlings were 6 and 12 days old, the water in the outside cup was replaced by zoospore suspensions in contact with the roots protruding through the cheesecloth. Seedling roots were allowed to grow for 10 days in sterile, distilled water without or with a zoospore suspension. Effects of the fungus on fibrous roots could conveniently be observed at will by either system. The double-cup system also was used to determine if phytoalexin was produced by roots treated with sodium hypochlorite. HS and HR seedlings, 6 days old, were treated with 0.525% sodium hypochlorite solution for 3 min by a distilled-water rinse. The roots above the water level were examined after 4 days for the presence of $PA_k$.

We noted that when AS and HS seedlings were transplanted into either system, infection of all seedlings occurred within 8-10 days. HR, treated similarly, remained noninfected up to 10 days. Cheesecloth wicks placed through a 5-mm hole in the side of each cup with one end in the water suspension, showed a reddish-brown coloration at 3 days and a dark-brown color at 6-7 days at the opposite end of the wicks for the three cultivars. No color was noted on wicks in noninoculated controls. Chromatographic and spectrophotometric analyses of the wicks, and water suspensions from inoculated roots of the three cultivars, confirmed the presence of $PA_k$ (2). No $PA_k$ was found in the water from controls. *Pms* was isolated (6) only from root segments of the AS and HS seedlings, and never from HR seedlings or control seedlings.

$PA_k$ also was found in the water in which seedlings treated with sodium hypochlorite were grown. The $PA_k$ collected at the wick tips was similar to that recovered from hypocotyls (5). All previous studies report production of $PA_k$ in hypocotyl tissue (1, 2, 5). Our studies show that $PA_k$ also is produced in fibrous roots of soybean seedling cultivars either resistant or susceptible to *Pms*. The difference between resistant and susceptible soybeans, in their reaction to infection by *Pms* in the hypocotyl, is the quantitative production of $PA_k$, with more $PA_k$ produced by the resistant plant (2). Our findings agree with this report. We isolated the fungus only from roots of inoculated, sus-

ceptible plants. It appears that $PA_k$ is as important in the roots of resistant plants as in the hypocotyls. Fibrous roots, as well as stems, produce $PA_k$, and thus may play a significant role in the root-rot phase of the disease as compared to the lower stem blight phase (4). Since $PA_k$ is water-soluble, the diluting effect of water could have prevented the recognition of the characteristic reddish-brown color of $PA_k$ in the roots earlier.

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