

# Responses of Differential Soybean Cultivars to Hypocotyl Inoculation with *Phytophthora megasperma* f. sp. *glycinea* at Different Temperatures

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## ABSTRACT

Keeling, B. L. 1985. Responses of differential soybean cultivars to hypocotyl inoculation with *Phytophthora megasperma* f. sp. *glycinea* at different temperatures. *Plant Disease* 69:524-525.

The responses of differential soybean (*Glycine max*) cultivars (Harosoy, Sanga, Harosoy-63, Mack, Altona, PI 103091, PI 171442, and Tracy seedlings) were determined when hypocotyl-inoculated with mycelium of races 1, 2, 7, or 10 of *Phytophthora megasperma* f. sp. *glycinea* and maintained at 21, 24, 27, 32, or 38 C. Temperature affected host response in most cultivar-race combinations. The magnitude of the temperature effect varied with the cultivar-race combinations. Disease development was usually suppressed in cultivars susceptible to the pathogen at 21 C, and a significant number of plants of some cultivars resistant to the pathogen were susceptible at 27 or 32 C. Disease symptoms in inoculated seedlings did not develop at 38 C. The optimum temperature for classifying races of *P. megasperma* f. sp. *glycinea* on these differential cultivars was 24 C.

Additional key words: *Phytophthora megasperma* var. *sojae*, Phytophthora rot, race identification

Twenty-four physiologic races of *Phytophthora megasperma* Drechs. f. sp. *glycinea* Kuan & Erwin (Pmg)(5) (syn. *P. megasperma* Drechs. var. *sojae* Hildeb.) that cause Phytophthora rot (12) of soybeans (*Glycine max* (L.) Merr.) have been reported (3,6-11,14). A race of the pathogen is determined on the basis of reactions (resistant or susceptible) of differential soybean cultivars when wounded hypocotyls of seedlings are inoculated using a technique described by Kaufmann and Gerdemann (4).

In previous tests to classify isolates of Pmg to race, the reactions of the differential cultivars have been inconsistent in different tests using the same isolate and seed lot of the cultivar. These tests were done in a greenhouse with temperatures that varied from 20 to 24 C at night and as high as 38 C on cloudless days. A temperature effect was believed to be the reason for the erratic responses. Chamberlain and Gerdemann (2) found that hypocotyl-inoculated Harosoy-63 (resistant) seedlings were susceptible to Pmg when the hypocotyl and roots were

heated in a water bath at 44 C for 1 hr immediately before inoculation. Chamberlain (1) later reported that heat-treated (44 C) plants recovered resistance to Pmg within 24 hr at 28 C. Wyllie and Taylor (15) obtained 95% survival when the susceptible cultivar Harosoy was planted in infested soil maintained at 15-20 C and only 40% survival at 30-35 C after 6 wk. Ward and Lazarovits (13), using zoospores to inoculate etiolated soybean seedlings, reported increased susceptibility of the cultivar Altona to Pmg race 4 at 27.5 and 32.5 C.

The objective of this study was to determine the effects of different temperatures on the reactions of all differential soybean cultivars used to classify races of Pmg using the standard hypocotyl puncture inoculation technique and to determine the optimum temperature for race identification.

## MATERIALS AND METHODS

**Cultivars.** The differential soybean cultivars (Harosoy, Sanga, Harosoy-63, Mack, Altona, PI 103091, PI 171442, and Tracy), which are used to classify physiologic races of Pmg, were used in this study. The expected reactions of these cultivars to Pmg races 1, 2, 7, and 10 (3,6,8) are listed in Table 1. Seed of Harosoy, Harosoy-63, and Altona was obtained from R. I. Buzzell, Research Station, Harrow, Ontario, Canada. Seed of Sanga was obtained from A. F. Schmitthenner, Ohio Agricultural Research and Development Center, Wooster. Seed of Mack, PI 103091, PI 171442, and Tracy was produced at Stoneville, MS.

**Races.** Test plants were inoculated with Pmg races 1, 2, 7, or 10. Races 1 (isolate 74-1), 2 (isolate 65-C), and 10 (isolate 78-7) were isolated from diseased

soybean plants growing in experimental plots at Stoneville. Race 7 (isolate SS-80-10-10) was supplied by A. F. Schmitthenner.

## Inoculum and method of inoculation.

Inoculum was produced by growing each isolate in 100 ml of a semisolid cornmeal medium (2.5 g of Difco cornmeal agar in 1 l of water) for 10 days at 24 C. The test seedlings were inoculated using a modification of the wound-inoculation technique described by Kaufmann and Gerdemann (4). A spear-shaped needle was dipped through a 10-day-old culture of the fungus in semisolid medium to pick up strands of hyphae. The needle was then inserted through the hypocotyl 1 cm below the cotyledons.

**Test procedure.** Plants (10-12 per pot) were grown in sand in 8.5-cm-diameter plastic pots in a greenhouse at 22-28 C. Nine days after seeding, the plants were placed in a controlled-environment chamber maintained at temperatures of 21, 24, 27, 32, or 38 C ( $\pm 1$ ) with 12 hr of alternating dark and light ( $225 \mu\text{E m}^{-2} \text{s}^{-1}$ ). A combination of fluorescent and incandescent light was used. Relative humidity was maintained at  $50 \pm 5\%$ . After 24 hr (10 days after seeding), the plants were removed from the chamber and inoculated. Pots containing the inoculated plants were placed in a metal container  $56 \times 38 \times 13$  cm deep and water was added to a depth of 1 cm. The container was closed to form a moist chamber, using a plastic sheet secured with a large elastic band. The container was then placed in a controlled-environment chamber at the desired temperature for 16-17 hr without light. The temperature within the box did not vary from the temperature in the controlled-environment chamber. After the moist-chamber treatment, the plants were removed from the metal container and returned to the controlled-environment chamber. Disease reactions of the plants were recorded 5 days after inoculation. Ten to 12 plants of each cultivar were inoculated in each test. Unless stated otherwise, the treatments were replicated eight times and the data were analyzed for variance.

## RESULTS AND DISCUSSION

Temperature affected the responses of some of the differential cultivars after wound inoculation of the hypocotyl with certain races of Pmg (Table 1). A susceptible response is expected when

Contribution in cooperation with Mississippi Agricultural and Forestry Experiment Station, Stoneville.

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Accepted for publication 14 December 1984.

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Harosoy, Sanga, PI 171442, and Tracy are inoculated with race 10 of the pathogen. However, fewer than 50% of the plants of these cultivars responded with a Pmg-susceptible reaction at 21 C. Races 1, 2, and 7 were accurately classified at 21 C.

The eight differential cultivars inoculated with the four Pmg races responded as expected at 24 C with the exception of the cultivar Tracy. Tracy responded to race 10 with 33–89% susceptible in different replications. These and other unpublished results suggest that Tracy may be composed of genotypes that react differently to Pmg race 10.

Most of the cultivars with an expected resistant response to Pmg were less resistant at 27 and 32 C than at other temperatures. Cultivar-race combinations most affected by higher temperatures were Sanga, Harosoy-63, Altona, and PI 103091 inoculated with race 1, Harosoy-63 inoculated with race 2, PI 103091 and Tracy inoculated with race 7, and Altona inoculated with race 10. The effect of higher temperatures (27 and 32 C) was not consistent for all cultivar-race combinations. For example, the expected response of Harosoy-63 inoculated with race 1 or 10 is resistant; however, at 32 C, 90% were susceptible when inoculated with race 1 and only 22% were susceptible when inoculated with race 10. The response of Mack inoculated with either of the four races was not appreciably affected by different temperatures.

For some cultivar-race combinations, the percentage of susceptible plants was reduced at 32 C compared with those at 27 C, i.e., Harosoy, Sanga, Altona, and PI 103091 inoculated with race 2.

The temperatures used in these experiments were lower than the 44 C Chamberlain and Gerdemann (2) found to inhibit phytoalexin production in inoculated resistant Harosoy-63 plants. Thus, the inhibition of phytoalexin production is not believed to be the reason for increased susceptibility of resistant cultivars at 27 and 32 C. The optimum temperature for growth of Pmg is 28 C, and little difference in growth of the fungus occurs from 25 to 30 C (9). The apparent diminished resistance of cultivars in this study may be caused by maintaining the inoculated plants at a temperature at or near that optimum for fungal growth. No plants were susceptible at 38 C when inoculated with race 1, 2, 7, or 10. No growth of Pmg occurred at 35 C (9), thus disease development in inoculated susceptible cultivars was not expected at 38 C.

It is concluded that the response of hypocotyl-wound-inoculated plants is influenced by the temperature at which they are maintained, even within limits previously believed acceptable. The magnitude of the temperature effect is not the same for all cultivar-race combinations, and 24 C is the optimum temperature for classifying isolates of Pmg to race.

**Table 1.** Responses of differential soybean cultivars hypocotyl-inoculated with four races of *Phytophthora megasperma* f. sp. *glycinea* and maintained at different temperatures

	Differential cultivars*							
	Harosoy	Sanga	Harosoy-63	Mack	Altona	PI 103091	PI 171442	Tracy
<b>Percentage of plants susceptible to race 1</b>								
Temp. (C)								
21	70 a <sup>x</sup>	0 a	0 a	0 a	0 a	0 a	0 a	0 a
24	100 b	0 a	0 a	0 a	0 a	0 a	0 a	0 a
27	94 b	27 b	7 a	1 a	14 b	9 a	0 a	1 a
32	95 b	91 c	90 b	4 a	62 c	42 b	5 a	4 a
38	0	0	0	0	0	0	0	0
Expected response <sup>y</sup>	S <sup>z</sup>	R	R	R	R	R	R	R
<b>Percentage of plants susceptible to race 2</b>								
Temp. (C)								
21	72 a	91 a	0 a	0 a	0 a	0 a	0 a	0 a
24	91 b	96 a	1 a	0 a	0 a	0 a	0 a	0 a
27	95 b	100 a	29 b	18 b	33 b	27 b	6 a	11 b
32	62 b	89 a	72 c	0 a	16 b	18 b	0 a	0 a
38	0	0	0	0	0	0	0	0
Expected response	S	S	R	R	R	R	R	R
<b>Percentage of plants susceptible to race 7</b>								
Temp. (C)								
21	97 a	0 a	99 a	4 a	95 a	3 a	97 a	0 a
24	100 a	0 a	100 a	0 a	100 a	0 a	100 a	0 a
27	100 a	26 b	97 a	16 a	100 a	48 b	100 a	31 b
32	91 a	67 c	95 a	0 a	99 a	78 c	100 a	48 c
38	0	0	0	0	0	0	0	0
Expected response	S	R	S	R	S	R	S	R
<b>Percentage of plants susceptible to race 10</b>								
Temp. (C)								
21	38 a	40 a	0 a	0 a	0 a	0 a	49 a	12 a
24	81 b	81 b	0 a	0 a	0 a	0 a	73 b	57 b
27	89 b	85 b	4 a	6 a	8 a	10 a	94 c	92 c
32	82 b	91 b	22 b	10 a	46 b	26 b	98 c	98 c
38	0	0	0	0	0	0	0	0
Expected response	S	S	R	R	R	R	S	S

\*Ten to 12 plants per treatment, eight replicates (only four replicates at 38 C, data not included in the statistical analysis).

<sup>x</sup>Means in the same column for individual races followed by the same letter are not statistically different ( $P=0.05$ ) according to Duncan's multiple range test (STE = 3.9).

<sup>y</sup>The expected response is the reaction of cultivars to a race according to published descriptions.

<sup>z</sup>S = susceptible and R = resistant to *P. megasperma* f. sp. *glycinea*.

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