

# Four New Physiologic Races of *Phytophthora megasperma* f. sp. *glycinea*

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## ABSTRACT

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Hypocotyls of differential soybean (*Glycine max*) cultivars were inoculated to identify four new physiologic races of *Phytophthora megasperma* f. sp. *glycinea* (syn. *P. megasperma* var. *sojae*) that differ from races 1-16. These new physiologic races are proposed as races 17-20.

Phytophthora rot of soybean (*Glycine max* (L.) Merr.), caused by *Phytophthora megasperma* Drechs. f. sp. *glycinea* Kuan and Erwin (6) (syn. *P. megasperma* Drechs. var. *sojae* Hildeb.), was first reported in Ohio in 1955 (13) and subsequently reported in other areas of the United States and Canada (1,3,4). Physiologic specialization in the pathogen was discovered in 1965, when Morgan and Hartwig (8) identified race 2 in Mississippi. Race 3 was reported in 1972 (9), race 4 in 1974 (11), races 5 and 6 in

1976 (2), races 7-9 in 1977 (7), and races 10-16 in 1979 (5).

In this report, four new physiologic races recovered from diseased soybean plants growing in the Mississippi Delta of Mississippi and Arkansas are proposed as races 17-20. The reactions of eight differential cultivars to races 1-20 of the pathogen are listed in Table 1.

## MATERIALS AND METHODS

Producer fields of soybeans in the alluvial plain of the Mississippi River between the Mississippi-Tennessee border and Vicksburg, MS, and experimental soybean nurseries in Stoneville, MS, have been surveyed annually (1967-1981) for Phytophthora rot. An estimated 2.4 million ha of soybeans are grown in this area. Plants with symptoms of Phytophthora rot (12) were collected, and the pathogen was isolated. Isolates of the new races of *P. megasperma* f. sp. *glycinea* described in this report were cultured from diseased soybean plants collected in 1976, 1977, 1978, and 1980.

Pieces of stem or root tissue taken from the margin of diseased areas on plants were surface-disinfected in 0.5% sodium hypochlorite and 10% ethyl alcohol for 1

min, rinsed in sterile, distilled water, and plated beneath a layer of selective culture medium. I prepared the selective medium by mixing together and autoclaving for 20 min 40 ml of V-8 juice, 0.6 g of calcium carbonate, 0.2 g of yeast extract, 1.0 g of sucrose, 10 mg of cholesterol, 20 mg of 50% benomyl, 27 mg of 75% pentachloronitrobenzene, 100 mg of neomycin sulfate, 30 mg of chloramphenicol, and 20 g of agar made up to 1 L with distilled water (10).

Fungal colonies growing through the selective medium were transferred to slants of Difco cornmeal agar (CMA) for maintenance. I tentatively identified isolates by comparing colony characteristics on the CMA medium to known isolates of *P. megasperma* f. sp. *glycinea*. Morphologically, the isolates were indistinguishable from *P. megasperma* f. sp. *glycinea* as described by Hildebrand (3).

The races were identified on the basis of a resistant (no effect) or a susceptible (plants killed) reaction of seven differential host cultivars—Harosoy, Sanga, Harosoy 63, Mack, Altona, PI103091, and PI171442—to inoculation. The reactions of cultivars Tracy and Kingwa and of breeding line L77-2015 (Clark<sup>6</sup> × Kingwa) were also determined. About 10 plants of each cultivar were inoculated with each isolate per test, and tests were repeated four times.

Plants used in these tests were grown in sand in 8.5-cm plastic pots in a greenhouse at 22-28 C. Eight to 10 days after seeding, the seedlings were inoculated with 10-day-old cultures of the pathogen grown in semisolid CMA (2.5 g

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**Table 1.** Responses of differential soybean cultivars to physiologic races 1–20 of *Phytophthora megasperma* f. sp. *glycinea*<sup>a</sup>

Differential cultivar	Physiologic race																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17 <sup>b</sup>	18 <sup>c</sup>	19 <sup>d</sup>	20 <sup>e</sup>
Harosoy	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	R	R	S
Sanga	R	S	R	R	R	R	R	R	R	R	S	S	R	R	R	S	R	R	S	S
Harosoy 63	R	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	S
Mack	R	R	R	S	S	R	R	R	R	R	R	S	R	S	R	S	R	S	S	S
Altona	R	R	R	R	S	S	S	S	S	R	S	R	S	R	R	R	S	R	R	R
PI103091	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R	R	S	R	S	R
PI171442	R	R	R	R	R	S	S	R	R	S	R	S	R	R	S	R	S	R	S	S
Tracy	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	S	R	S	S

<sup>a</sup> Races 1–16 have been previously reported. S = susceptible; R = resistant.

<sup>b</sup> Culture 76-46, isolated from an unknown soybean cultivar in Chico County, AR.

<sup>c</sup> Culture 78-15, isolated from a breeding line (Lee 68 × Mack) in Washington County, MS.

<sup>d</sup> Culture 80-1, isolated from Tracy; culture 77-4, isolated from D77-1492; and culture 77-56, isolated from D60-12058, in Washington County, MS.

<sup>e</sup> Culture 80-3, isolated from D55-1492 in Washington County, MS.

of CMA in 1 L of water) at 21–24 C. A modification of the hypocotyl inoculation technique of Kaufmann and Gerdemann (4) was used to inoculate the plants. A spear-shaped needle was dipped through a culture of the fungus in the CMA semisolid medium to pick up strands of mycelium. The needle was then inserted through the hypocotyl of a plant about 1 cm below the cotyledons. The mycelium was deposited on and in the wound when the needle was withdrawn.

Pots of inoculated plants were placed in a metal container 56 cm long by 38 cm wide by 13 cm deep, and water was added to a depth of 1 cm. A plastic sheet was secured around the container with a large elastic band to form a moist chamber. The container was then placed in a controlled-environment chamber at 24±1 C for 16–17 hr without light. The plants were then removed from the container and returned to a controlled-environment chamber at 24±1 C and exposed to 12-hr periods of alternating darkness and light (225 μE/m<sup>2</sup>/sec). The disease reactions of the cultivars were recorded 5 days after inoculation.

## RESULTS AND DISCUSSION

The differential responses to the four new physiologic races (17–20) are listed in Table 1. The results of inoculation tests were generally uniform. Occasional off-type reactions could be attributed to a seed mixture or escape. The only ambiguous reaction encountered was with the differential cultivar Sanga inoculated with race 17 (culture 76-46).

Of 36 Sanga plants inoculated with race 17, 23 were resistant, four were intermediate in resistance and developed a large lesion, and nine were susceptible. Sanga was judged to be resistant on the basis of this ratio (3:1).

The soybean cultivar Kingwa and the breeding line L77-2015 (Clark<sup>6</sup> × Kingwa) were resistant to race 17 (culture 76-46) and to race 18 (culture 78-15). Both were susceptible to race 19 (cultures 80-1, 77-56, and 77-4) and race 20 (culture 80-3). The cultivar Tracy was resistant to race 18 but susceptible to races 17, 19, and 20.

This and previous research (5,8) demonstrate the presence of 13 physiologic races of *P. megasperma* f. sp. *glycinea* that attack soybeans in the Mississippi Delta of Arkansas and Mississippi. Race 4 has also been isolated from diseased soybeans in this area (*unpublished*). Knowledge of the presence of these races and the identification of parental lines resistant to these races will be used in a breeding program designed to minimize the danger of these races to soybean production.

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