A New Physiologic Race of *Phytophthora megasperma* f. sp. *glycinea*

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ABSTRACT

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A new physiologic race of *Phytophthora megasperma* f. sp. *glycinea* was recovered from diseased soybeans (*Glycine max*) in the Delta of Mississippi in 1982. Isolates of the new race were recovered from soybeans grown at two locations (Stoneville and near Shelby, MS). The differential soybean cultivars Harosoy 63, Mack, and PI 103091 were resistant. Harosoy, Sanga, Altona, PI 171442, and Tracy were susceptible. This new race is proposed as race 24.

Phytophthora rot of soybeans (*Glycine max* (L.) Merr.) caused by *Phytophthora megasperma* Drechs. f. sp. *glycinea* Kuan & Erwin (5) (*Pmg*) (syn. *P. megasperma* Drechs. var. *sojae* Hildeb.) has been recognized since 1955 (10). Physiological specialization in the pathogen was reported in 1965 after race 2 was discovered (7). Twenty-one additional races of *Pmg* have since been characterized (4,6,11).

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In 1982, twenty-three isolates of *Pmg* were recovered from diseased soybean plants collected from production fields and experimental plots in the Delta of Mississippi. Twenty of these were identified as known races of *Pmg*; however, three isolates, designated 82-9, 82-28, and 82-30, differed pathologically from previously described races of the pathogen. Isolate 82-9 was obtained from plants of the breeding line D55-1492 growing in Sharkey clay in Washington County near Stoneville, MS, and isolates 82-28 and 82-30 were obtained from Tracy-M plants also growing in Sharkey clay in a production field near the north end of Sunflower County, east of Shelby, MS. The reactions of isolates 82-9, 82-28, and 82-30 on the host differential cultivars were evaluated to determine if they constitute a new physiologic race.

MATERIALS AND METHODS

Diseased soybean plants symptomatic of Phytophthora rot (9) caused by *Pmg* were collected from production fields and experimental plots, placed in plastic bags, and kept on ice until sectioned. Sections of stem tissue taken from the transition zone between diseased and healthy tissue were surface-disinfected in an aqueous solution containing 0.5% sodium hypochlorite and 10% ethyl alcohol for 1 min, rinsed in sterile water, and placed beneath a layer of selective culture medium in petri plates (15 × 60 mm). The plates were incubated at 21 C until the fungus grew through the selective medium (3–4 days).

The selective medium consisted of 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 (8). The medium was sterilized by autoclaving for 20 min at 16 psi before pouring into petri plates.

Fungal colonies growing through the selective medium were transferred to slants of V-8 juice agar for maintenance. Cultures were stored at 15 C in an unlighted incubator. Isolates were tentatively identified by comparing colony characteristics with known isolates of *Pmg*. Morphologically, the isolates were indistinguishable from *Pmg* as described by Hildebrand (2).

Test 1 (hypocotyl inoculation). Pathogenicity of isolates was characterized on the basis of a resistant (no effect) or a susceptible (plant killed) reaction of the

differential soybean cultivars Harosoy, Sanga, Harosoy 63, Mack, Altona, PI 103091, PI 171442, and Tracy. Hypocotyls of 10 plants of each cultivar were inoculated with each isolate per test. Tests were repeated five times on different dates.

Plants were grown in sand in 8.5-cm plastic pots in a greenhouse at 22–28 C. Ten days after planting the seed, seedlings were inoculated with 10-day-old cultures of the pathogen grown in semisolid cornmeal agar (CMA) (2.5 g of CMA in 1 L of water) at 21 C. Seedlings were inoculated using a modification of a technique described by Kaufmann and Gerdemann (3). 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 1 cm below the cotyledons. The mycelium was deposited on and within the wound when the needle was withdrawn.

After inoculation, the plants were placed in metal containers 56 × 38 × 13 cm deep and water was added to a depth of 1 cm. A plastic sheet was secured over the top of 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 hr without light. The plants were then removed from the container and placed on a bench in the greenhouse. The disease reaction was recorded 6 days after inoculation.

Test 2 (hydroponic culture). The reaction of the differential cultivars to these isolates was also determined in hydroponic culture using zoospores as inoculum. Seed of the differential cultivars were germinated in vermiculite at 20 ± 1 C with 12 hr of alternating light (about 10,000 ft-c) and dark. After 5 days, the seedlings were transferred to holes 5 mm in diameter punched 50 mm apart in sheets of green Styrofoam 25 mm thick. The Styrofoam sheets were floated on a 25% Hoagland's nutrient solution, about 15 cm deep. Twenty-four hours after the seedlings were placed in the nutrient solution, they were inoculated with *Pmg* zoospores of the desired isolate at a rate of 25 zoospores per seedling. The zoospores of *Pmg* were produced using a modification of the technique described by Eye et al (1). Mycelial disks (3 mm in diameter) cut from 5-day-old culture on lima bean agar (LBA) were transferred to new plates of LBA (10 ml per 90-mm plate). Four disks were arranged symmetrically on each plate. After an incubation period of 5 days at 21 C without light, the cultures were flooded four times at 30-min intervals with 10 ml of sterile distilled water at 21 C. The water remained on the cultures for the 30-min intervals. The plates were flooded a fifth time after an additional 30 min with 10 ml of sterile distilled water at 5 C. The cultures were then incubated for about 16 hr at 21 C without light. Water covering the fungus colonies was then collected, and zoospores were counted with the aid of a hemacytometer. After the proper dilutions of the spores were made, they were mixed thoroughly into the nutrient solution containing the test plants. Ten plants of each differential cultivar were inoculated.

RESULTS AND DISCUSSION

Climatic conditions during 1982 were generally unfavorable for the development of Phytophthora rot of soybeans in the Delta of Mississippi. Consequently, despite repeated searches, only 23 isolates of *Pmg* were obtained. All of these were isolated from soybean plants growing in irrigated fields. Twenty of the isolates were previously described races. Of these, 10 were race 1, six were race 19, two were race 2, and one each was of races 10 and 13.

The reaction of the differential soybean host cultivars, when inoculated with isolates 82-9, 82-28, and 82-30 obtained in 1982, differed from their reaction to the 23 previously described *Pmg* races. The cultivars Harosoy 63, Mack, and PI 103091 were resistant. Harosoy, Sanga, Altona, PI 171442, and Tracy were susceptible. The reaction of these isolates on the differential cultivars differs from that of race 10 only on Altona. Altona is susceptible to these isolates and resistant to race 10. On the basis of the reaction of the differential host cultivars to these isolates, they are proposed as race 24. Identical results were obtained when the differential host cultivars were inoculated using either the hypocotyl or hydroponic inoculation technique.

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