Root Reduction and Stem Lesion Development on Soybeans by Phytophthora megasperma var. sojae

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ABSTRACT

Phytophthora megasperma var. *sojae (Pms)* reduced the root system of soybean *(Glycine max)* in the presence and absence of stem lesions. *Pms*-susceptible cultivars grown in *Pms*-infested soil in the greenhouse and field had no stem lesions, but plant heights and yields were significantly less than those of similarly treated *Pms*-resistant cultivars. In the greenhouse, *Pms* reduced dry weights of roots but not plant heights of 10-day-old resistant plants. Lesions developed on stems of *Pms*-susceptible plants in the greenhouse when inoculum was placed adjacent to plants at 1 or 4 cm below the soil line, but not at 9 cm. Root and shoot dry weights increased with depth of inoculum placement.

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Phytophthora megasperma Drechs. var. *sojae* A. A. Hildeb. *(Pms)* causes stem lesions and eventual death of susceptible soybean [*Glycine max* (L.) Merr.]. Susceptible cultivars can become infected at any stage of growth (4, 10, 11, 12). Stunting or general unthriftiness of soybeans in the field has been

observed (2, 3, 4, 6, 8, 9) but not definitely attributed to *Pms* because of the lack of stem lesions. The cause of this stunting and the effect of inoculum placement on the development of stem lesions have not been examined. This paper describes the effect of *Pms* on root systems of resistant and susceptible soybean plants in the greenhouse and field, and the effect of inoculum placement on root reduction and stem lesion development on susceptible cultivars in the greenhouse.

MATERIALS AND METHODS.–Race 1 of *P. megasperma* var. *sojae (Pms)* was used throughout this study (7). Inoculum discs of *Pms* were prepared by soaking 12-mm sterilized cellulose discs in sterile soybean-hypocotyl extract (35 g hypocotyl tissue/liter water), then placing them on the surface of V-8 juice (Campbell)/$CaCO_3$ (5) agar plates inoculated with *Pms*. The soybean cultivars used were: *Pms*-susceptible Amsoy (AS) and Harosoy (HS); field-tolerant Wayne (WT); and *Pms*-resistant (to race 1) Amsoy-71 (AR), Harosoy-63 (HR), and Wayne (WR) (1). Resistant and susceptible cultivars are isogenic cultivars differing by one gene for resistance to *Pms* (1).

We established plots in a field shown to be infested with *Pms* by isolating the fungus from roots of susceptible soybeans chosen at random from border rows throughout the growing season. Seed of the six cultivars were planted in a randomized, complete-block design with each block 6.1 m long. A seedling rate of 7 seed/0.31 m and spacing of 91-cm centers was used. Approximately 18 weeks after planting, height and yield were measured using 10 plants of each cultivar chosen at random from each of four blocks. No stem lesions were observed.

The two greenhouse experiments were conducted using a randomized, complete-block design in a chamber maintained at 23 to 27 C with a 14-hr day maintained with 600 ft-c of artificial illumination (fluorescent tubes). There were two trials of each experiment. Two-day-old seedlings were transplanted from moist vermiculite (Terralite brand) into 15-cm clay pots filled with an autoclaved (3 hr, 15 psi and 120 C) 1:1 mixture of sandy loam soil and silica sand.

For the first experiment, 15 AS and 15 AR seedlings were planted with one seedling/pot. Three 15 X 150 mm glass test tubes were placed in the soil to depth of 10 cm and 2 cm from each seedling. Test tubes were removed from six pots of each cultivar at 8 or 18 days. Either two or four inoculum discs were placed in each of the three holes which were then filled with soil. Pots containing discs without inoculum served as controls. Clay saucers under each pot were filled twice daily in addition to a surface watering to maintain a high soil moisture. Height measurements were taken after 6 weeks, the plants removed, and the roots washed under tap water. A 1-cm segment of hypocotyl and four 1-cm segments of root tissue from each plant were surface-sterilized in a 0.2% sodium hypochlorite solution for 5 min and assayed for *Pms* by plating on modified cornmeal agar (1 liter of prepared Difco cornmeal agar containing 10 mg pimaricin, 20 mg streptomycin, and 100 mg penicillin). The remainder of the roots and aboveground portions were dried for 4 days at 70 C, and dry weights recorded.

The second experiment was similar to the first, except that 15 pots were used to transplant two 2-day-old seedlings of AS, and test tubes were placed at depths of either 1, 4, or 9 cm adjacent to the seedlings. The test tubes were removed after 8 days, and single discs with or without (control) inoculum were placed in the bottom of each hole, which was then filled with soil. One set of pots had inoculum discs placed at all three depths. There were three pots for each treatment.

RESULTS AND DISCUSSION.–Even in the absence of stem browning and lesion development, *Pms* can have an adverse effect on susceptible soybeans. *Pms*-resistant plants grown in a field plot shown to be infested with race 1 of *Pms* were significantly taller than *Pms*-susceptible plants (Table 1). This stunting confirms previous observations (2, 3, 4, 6, 8). In addition, seed yields of AS and HS were significantly reduced below those of AR and HR cultivars. The reduction of plant height and yield in

TABLE 1. The effect of *Phytophthora megasperma* var. *sojae (Pms)* race 1 on mean[a] plant height and yield of six soybean cultivars grown in field plots

Cultivars	Reaction to Race 1 of *Pms*	Plant height[b] (cm)	Plant yield[b] (g)
Amsoy	Susceptible	76 d	99 b
Amsoy-71	Resistant	94 b	138 a
Harosoy	Susceptible	75 d	87 c
Harosoy-63	Resistant	93 b	153 a
Wayne-T	Field tolerant	84 c	132 a
Wayne-R	Resistant	102 a	129 ab

[a] Mean of four replications of 10 plants/treatment.
[b] Cultivar means with the same letter are not significantly different at the 5% level according to the Duncan's multiple range test.

TABLE 2. Mean[a] plant height and dry weight of roots and shoots of soybean seedlings either susceptible or resistant to race 1 of *Phytophthora megasperma* var. *sojae (Pms)* 6 weeks after inoculation. Two ages of plants and two inoculum levels were tested

Cultivar	Plant age (days)	Inoculum discs/hole	Plant height (cm)	Dry weights (g)	
				Roots	Shoots
Amsoy		0	46 ab	2.0 a	2.9 ab
Amsoy	10	2	25 d	0.3 c	0.9 d
Amsoy	10	4	27 d	0.4 c	1.2 cd
Amsoy	20	2	43 bc	0.8 bc	2.8 ab
Amsoy	20	4	39 c	0.8 bc	2.2 bc
Amsoy-71		0	48 ab	2.5 a	3.2 a
Amsoy-71	10	2	49 a	1.6 ab	2.8 ab
Amsoy-71	10	4	44 abc	1.7 ab	2.4 ab
Amsoy-71	20	2	48 ab	1.8 ab	2.9 ab
Amsoy-71	20	4	45 abc	2.0 a	3.3 a

[a] Mean of six replications.
[b] Means with the same letters are not significantly different at the 5% level according to Duncan's multiple range test.

TABLE 3. Mean[a] plant height and dry weight of roots and shoots of Amsoy[b] soybean seedlings 6 weeks after placing inoculum of race 1 *Phytophthora megasperma* var. *sojae* in the soil at three depths

Inoculum depth (cm)	Plant height (cm)[c]	Dry weights (g)[c]	
		Roots	Shoots
Control	42 a	2.5 a	3.4 a
1	25 b	0.3 b	1.3 c
4	31 b	0.5 b	1.9 bc
9	32 b	0.8 b	2.2 b
1+4+9	23 c	0.3 b	1.4 c

[a] Mean of six replications for plant height and dry weight measurements.

[b] Amsoy susceptible to race 1 *Pms*.

[c] Means with the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

the absence of obvious stem lesions suggested that *Pms* significantly affected the root systems.

In the greenhouse, the mean plant height and the dry weights of roots and shoots of 10-day-old seedlings were significantly lower than those of the AS controls (Table 2). Infection of soybean seedlings less than 10 days old in the field has been reported (4, 10, 11, 12). Amsoy plants inoculated 20 days after planting were affected less than 10-day plants, but *Pms* did cause a significant reduction in height at higher inoculum level and in dry root weights for both inoculum levels. The lack of a significant difference in height at the lower inoculum level may be due to the shorter exposure time to the inoculum. There were no significant differences in plant height or dry weights between inoculated and noninoculated AR seedlings. In both trials, only a single plant developed a stem lesion. *Pms* was isolated from this hypoctyl lesion and from roots of all AS seedlings growing in infested soil, but not from AS controls. *Pms* was not isolated from hypocotyl tissues of any other seedlings, nor from any tissues of the AR seedlings.

In the second greenhouse experiment, when inoculum was placed at 1 cm or in combination of 1, 4, and 9 cm, stem lesions developed on 11 of 24 plants. At 4 cm, 2 of 12 plants developed lesions. No stem lesions developed on seedlings with inoculum placement at 9 cm. The mean plant height and dry weights of roots and shoots of all plants growing in infested soil were significantly lower than the control regardless of inoculum placement (Table 3). Plants grown in soil infested with a combination of the three placement depths were significantly shorter than those grown in soil with a single inoculum depth. Root and shoot dry weight tended to increase with increase in depth of inoculum placement. *Pms* was isolated from all stem lesions and root pieces of seedlings grown in infested soil, but not from control plants or stem tissues without lesions. Significant height reductions of plants growing in soil infested at the 9-cm depth were evidently due to root reduction.

Pms can reduce the root system and dry weight of aboveground parts of soybean seedlings with or without the production of a stem lesion. Stem lesions are more apt to occur when inoculum is located close to the soil surface.

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