Laboratory Method for Assessing Field Tolerance of Soybean Seedlings to *Phytophthora megasperma* var. *sojae*

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ABSTRACT

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Two- to four-day-old seedlings of soybean cultivars with known levels of field tolerance to Phytophthora root rot were planted in slit Styrofoam cups filled with vermiculite. The cups were placed in plastic trays, 17 × 25 × 6 cm, containing 100 g of steamed greenhouse potting mix and 200–250 ml water. Inoculum usually consisted of 4,000 zoospores obtained by flooding lima bean agar cultures of *Phytophthora megasperma* var. *sojae*. Plants were grown 15–20 days in a growth chamber at 28 C and a 12-hr photoperiod (17,000 lux). Disease was assessed by measuring dry weights and overall lengths of individual plants. With race 3, lengths of the cultivars Hark, Williams, and Agripro 26 were 36%, 43%, and 55%, respectively, of the uninoculated control plants. Dry weight measurements gave similar results. Results were comparable with race 7 and with a natural soil infested with race 4. The results agree with relative field tolerances reported previously. The response of the field tolerant cultivar Agripro 26 was maintained against eight races of the pathogen. The cultivars Woodworth, Agripro 25, SRF 307 P, and Wayne, also known to have field tolerance, showed less reduction in growth than did varieties without field tolerance.

Phytophthora root and stem rot incited by *Phytophthora megasperma* Drechs. var. *sojae* Hildeb. is one of the most important diseases of soybeans (*Glycine max* (L.) Merr.) in Michigan as well as other soybean-growing areas of the United States and Canada (6). The fungus attacks plants in all stages of growth, causing pre- and postemergent damping-off of seedlings and a root and stem rot that results in wilting and death of plants from the early stages of growth to maturity (6,8,19).

Resistance to the disease was found in 1957 (1) and was determined to be inherited through a single dominant gene, designated *Rps* (5). Resistant isolines were released to growers shortly after 1963. This was followed by the development of new physiological races of the pathogen (4,11,12,16,18), which complicates attempts to control the disease by means of race-specific resistance.

Inoculation methods have been developed to test for pathogenicity and to identify the reaction of different soybean cultivars to *P. megasperma* var. *sojae* (8,9); of these, the insertion of a small piece of mycelium into a longitudinal incision made in the hypocotyl of young plants (8) is most widely used. The hypocotyl inoculation method is reliable for identifying resistance conditioned by the *Rps* gene (9,21), but it does not detect intermediate reactions or types of resistance designated as field tolerance (21).

Field tolerance to Phytophthora root rot of soybean (13–15,21) differs from the race-specific, monogenic resistance commonly used in breeding resistant cultivars. Whereas field tolerance confers no resistance when plants are inoculated

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via hypocotyl wounds, they manifest less severe disease and yield better than fully susceptible cultivars in the field. Field tolerance also is considered to be expressed nonspecifically with respect to race of the pathogen (14,21). Whether field-tolerant soybean cultivars are tolerant in the sense of sustaining smaller losses of yield than other cultivars with equally severe infection is doubtful. Since actual resistance appears to be expressed (13–15), perhaps "field resistance" would be a less ambiguous term.

Apparently, field tolerance has so far been assessed only in field conditions. Efficiency in the identification and evaluation of field tolerance would be enhanced if laboratory or greenhouse techniques using young plants were available. Recently, some factors affecting the expression of field tolerance to *P. megasperma* var. *sojae* were studied (3).

The present investigation was undertaken to develop a laboratory method for assessing field tolerance in soybeans to *P. megasperma* var. *sojae*. A preliminary report has been published (7).

MATERIALS AND METHODS

Soybean varieties. The soybean cultivars used routinely were Hark, Williams, and Agripro 26, which have known levels of field tolerance to races 1, 3, and 7 of *P. megasperma* var. *sojae* (13–15). Hark has little field tolerance, Williams moderate field tolerance, and Agripro 26 greater field tolerance. Agripro 26 also contains the *Rps* gene for resistance to race 1. Other cultivars tested were Wayne, Harosoy 63, Agripro 25, Woodworth, SRF 307 P, Hodgson, OX-20-8, and Corsoy. Tracy, which is resistant to races 1 through 9, was used in some experiments.

Seeds were surface-sterilized (5% sodium hypochlorite for 2 min), thoroughly rinsed with distilled water, and germinated for 2 to 4 days at 28 C, in plastic trays (17 × 25 × 6 cm) containing 3:2 vermiculite and water (v/v). The seeds were first soaked for 2 hr in water through which air was bubbled to promote uniform germination. Seedlings with hypocotyls 5–7 cm long were selected for use.

Pathogen isolates. *P. megasperma* var. *sojae* races 1, 2, 3, 4, 5, 7, 8, and 9 were used. Races 1 through 5 were obtained from A. F. Schmitthenner of Ohio State University, and races 7, 8, and 9 were obtained from F. A. Laviolette of Purdue University. Each isolate was maintained on lima bean agar (Difco Laboratories, Detroit, MI) or on Schmitthenner's selective medium (17) at 24 ± 1 C and was subcultured in lima bean agar bimonthly.

Production of inoculum. Lima bean agar cultures were flooded several times with distilled water to induce production of zoospores (2). Zoospore concentration was determined with a hemacytometer or by the microsyringe method (10). Desired inoculum densities were made by serial dilutions of zoospore suspensions of known concentration.

Detection of field tolerance. Five to 10 soybean seedlings 2–4 days old were transplanted in slit 200-ml Styrofoam cups filled with premoistened coarse vermiculite. Vertical slits about 50 × 2 mm were made in the bottom half of the cups by using a hot needle. The cups were placed in plastic trays, usually 17 × 25 × 6 cm, containing 100 g of steamed greenhouse mix and 200–250 ml of water. Unless stated otherwise, inoculum consisted of 4,000 zoospores per tray (16–20/ml of water). Soil was infested 2–3 days after direct seeding or at transplanting. The plants were grown 15–20 days in a growth chamber at 28 C and with a 12-hr photoperiod. The light source consisted of cool-white fluorescent lamps and incandescent light bulbs (17,000 lux). During the first 3 days the volume of water in each tray was kept constant. From then until completion of the experiment, enough water was added daily to maintain flooded conditions.

The criteria for assessing reaction of soybean seedlings to *P. megasperma* var. *sojae* were overall plant length and dry weight or, in the case of high zoospore concentrations, incidence of killed plants. The lengths of individual plants were measured from the apical meristem to the tip of the main root. Plants from each cup were oven-dried 12 hr at 80 C and their weights determined. Although growth of both roots and shoots was reduced by the pathogen, combined values were less variable than shoot or root lengths alone. The appearance of the roots was not used to assess disease, because visual differences among some of the varieties were not clear-cut.

Statistical analysis. Values are means of two or three replications, each of which is the average length or dry weight of five or 10 plants. Significant differences among treatments were identified using Tukey's *w* procedure or the Student-Newman-Keul's test (20). Experiments were repeated at least once to verify the results.

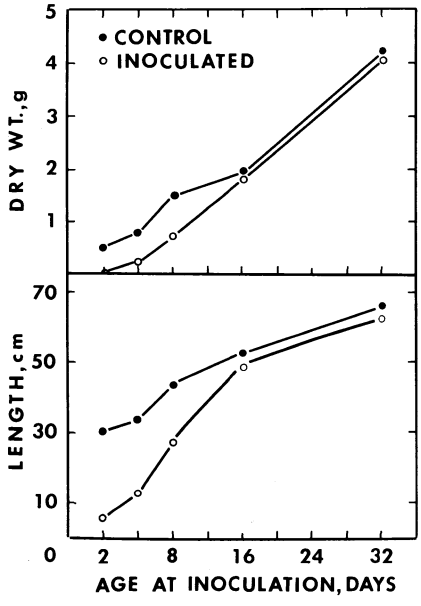

Fig. 1. Effect of age of seedlings at time of inoculation on disease reaction of Hark soybeans to race 3 of *Phytophthora megasperma* var. *sojae*. Least significant ranges ($P = 0.05$) by Tukey's *w* procedure were 0.28 g for dry weights and 4.5 cm for lengths.

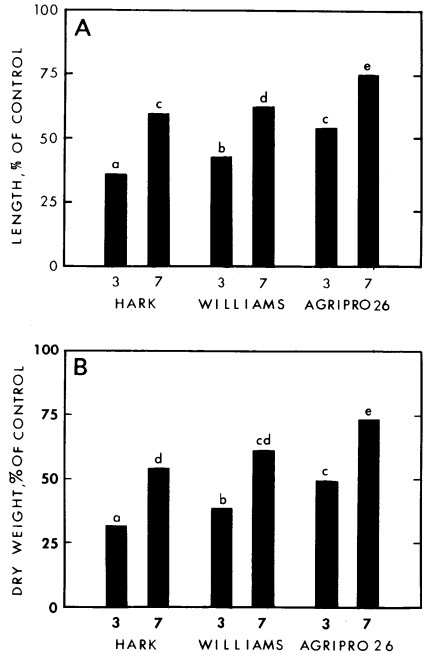

Fig. 2. Effect of races 3 and 7 of *Phytophthora megasperma* var. *sojae* on growth of the soybean cultivars Hark, Williams, and Agripro 26: **(A)** total plant length, **(B)** dry weight. Bars topped by the same letters do not differ significantly ($P = 0.05$) by the Student-Newman-Keul's test.

RESULTS

Effect of seedling age. To determine the effect of the soybean plant's age on infection by *P. megasperma* var. *sojae*, seedlings of the cultivar Hark at ages 2–32 days were inoculated with 30,000 zoospores of race 3 per 39 × 34 × 10 cm tray (40–50 zoospores/ml) in the greenhouse. Dry weights and lengths of plants were measured 8 days after inoculation.

Mean lengths and weights of inoculated plants were significantly less ($P = 0.05$) than those of controls when inoculated at ages up to 8 days (Fig. 1). Thereafter, differences narrowed and were not significant, even though root infection of inoculated seedlings was still evident. The results indicate that plants inoculated at 16 days of age or older show increased resistance and that maximum effects of disease are expressed when younger plants are inoculated.

Effect of zoospore inoculation of seedlings. The effect of races 3 and 7 of *P. megasperma* var. *sojae* on the growth of

Hark, Williams, and Agripro 26 soybeans was tested. Agripro 26 was less affected than the other two cultivars by both races of the pathogen (Fig. 2). Williams was intermediate in response and Hark was most strongly affected. With race 3, total plant lengths of Agripro 26, Williams, and Hark were 55%, 43%, and 36%, respectively, of the lengths of uninoculated control plants. With race 7, corresponding lengths were 74%, 66%, and 59%. A similar trend was observed when dry weights were measured. These results indicate that the field tolerant cultivar Agripro 26 has greater ability to withstand the infection caused by two races of this pathogen than the more susceptible cultivar Hark. Williams, which also has demonstrated field tolerance in the field, though less than that of Agripro 26, showed an intermediate response.

The cultivars Agripro 25, Woodworth, Wayne, SRF 307 P, and Harosoy 63 also were tested with zoospores of races 3 and 7; all have shown some degree of field tolerance (13–15). The cultivars Hodgson, Hark, and OX-20-8, which are fully susceptible, were included for comparison. Inoculum consisted of 10,000 zoospores of either race 3 or 7 per tray (40–50 zoospores/ml water).

The lengths of the eight cultivars represent a gradient in response to infection by *P. megasperma* var. *sojae* (Table 1). Growth of Woodworth, SRF 307 P, and Agripro 25 was least affected (67–73% of control); growth of Wayne and Harosoy 63 was somewhat more affected (58–62%). Growth of Hark and Hodgson was reduced nearly by half (51–54%) and that of OX-20-8, a known highly susceptible variety, was reduced even more (40–46%). There were no obvious differences between the effects of the two races of the pathogen.

An experiment was done to determine the response of the cultivars Hark, Williams, Agripro 26, and Tracy to races 1–5 and 7–9 of the pathogen at a higher concentration of inoculum (20,000 zoospores per tray; 80–100/ml of water).

Table 1. Effect of zoospores of races 3 and 7 of *Phytophthora megasperma* var. *sojae* on overall lengths of eight soybean cultivars susceptible to the pathogen by hypocotyl inoculation

Cultivar	Length, % of control[z]	
	Race 3	Race 7
Woodworth	73.4 a	67.0 a
SRF 307 P	72.7 a	65.3 ab
Agripro 25	67.2 ab	66.8 abc
Wayne	61.9 bc	58.8 bc
Harosoy 63	61.3 bcd	58.3 bc
Hark	53.7 cd	52.1 bcd
Hodgson	51.2 d	52.2 cd
OX-20-8	40.2 e	45.6 d

[z] Means in the same column followed by the same letter are not significantly different ($P = 0.05$) by Tukey's w procedure.

Disease in this experiment was more severe than at lower inoculum concentrations and was assessed by the percentage of plants killed after 15 days. The pooled mean percentages of plants killed by the eight races of the pathogen were 2.5% for Agripro 26, 48.7% for Williams, and 56.2% for Hark. The means for Hark and Williams did not differ significantly ($P = 0.05$), but that for Agripro 26 was significantly lower. These results suggest that at high inoculum concentrations, cultivars with intermediate field tolerance such as Williams may give a fully susceptible response. The number of plants (10 for each race and cultivar combination) was insufficient to distinguish differences in virulence or host-specificity among the races.

Effect of natural inoculum. To determine the effect of natural inoculum of *P. megasperma* var. *sojae* on growth of the field tolerant cultivars Agripro 26 and Williams, soil from a field naturally infested with race 4 was sieved and diluted with sterile sand. Each dilution was separately flooded with sterile water and incubated for 3 days at 24 C to promote oospore germination. Seedlings of the cultivars Hark, Williams, Agripro 26, and Tracy were transplanted into cups containing vermiculite and placed in the flooded soil dilutions. Control cups were placed in flooded sand. Lengths of individual plants were measured after 15 days in a growth chamber.

Agripro 26 and Williams were less affected than the more susceptible variety Hark (Fig. 3). Agripro 26 showed less reduction in length than Williams. Tracy, which is known to be resistant to race 4, showed the least reduction in length of all cultivars. The four cultivars differed significantly ($P = 0.05$) from each other at all inoculum densities. A concentration of 20 parts of infested soil to 80 parts of sand was enough to induce severe disease, especially on Hark soybeans, and allowed clear separation of the field tolerant cultivars Agripro 26 and Williams from the susceptible Hark.

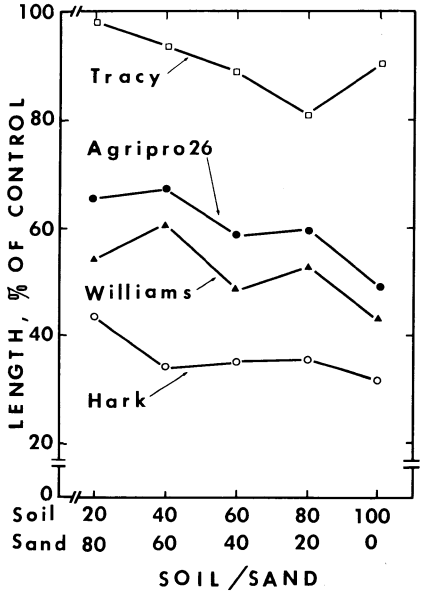

Fig. 3. Effect of *Phytophthora megasperma* var. *sojae* race 4 in a naturally infested soil on the total lengths of field tolerant soybean cultivars. Any two varietal means differ significantly ($P = 0.05$) at any given inoculum concentration.

DISCUSSION

Measurements of length and dry weight of soybeans inoculated via the root system with zoospores at controlled densities or with naturally infested soil produced results corresponding to reported field tolerance of the same cultivars in field performance tests (13–15, 21). Growth of Agripro 26 and Williams was less affected by races 3 and 7 than was growth of Hark. In addition, disease was less severe in Woodworth, Agripro 25, and SRF 307 P (all with demonstrated field tolerance) than in Hark and OX-20-8 (known to be susceptible). The resistant response of Agripro 26 was maintained against the eight races of *P. megasperma* var. *sojae* tested. Wayne showed a greater reduction in growth than its reported field tolerance would suggest. This may have been because it grew less vigorously than other cultivars under the test conditions.

Our results were obtained in a growth chamber under controlled light and temperature and with specified concentrations of zoospores as inoculum. However, close control of light and temperature may not be necessary since comparable results were obtained in the greenhouse following the same procedures (Jimenez and Lockwood, *unpublished data*). The concentration of inoculum must be controlled within reasonable limits since high levels overcame resistance to *P. megasperma* var. *sojae* in some soybean cultivars (2). When the concentration of zoospores was increased to 20,000 per tray (80–100/ml), the moderately field tolerant cultivar Williams showed a fully susceptible reaction.

We suggest that the method described might be useful as an initial screen for field tolerance in soybeans. If only high levels of field tolerance are sought, such as that demonstrated by Agripro 26, zoospore concentrations high enough to kill the more susceptible cultivars can be used and data can be taken on the basis of surviving plants, thus obviating the need to measure plant lengths.

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