Techniques

A Growth Chamber Test For Measuring Phytophthora Root Rot Tolerance in Soybean Seedlings

A. F. Olah and A. F. Schmitthenner

Research associate and professor, respectively, Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691.
Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. This work was also supported by USDA Special Soybean Grant 59-2392-0-2-024-0.
Journal Article 105-84.
Accepted for publication 6 December 1984 (submitted for electronic processing).

ABSTRACT

Olah, A. F., and Schmitthenner, A. F. 1985. A growth chamber test for measuring Phytophthora root rot tolerance in soybean seedlings. Phytopathology 75:546-548.

Soybean cultivars with high, moderate, and low tolerance to root rot caused by *Phytophthora megasperma* f. sp. *glycinea*, were grown in a growth chamber for 7 days, and then their taproots were inoculated with mycelial suspension. Six days later, elongation of the first internode and extent of tissue colonization in the hypocotyl were measured. The first internodes of the moderate- and low-tolerant lines were significantly shorter than those of uninoculated seedlings, and there were significant differences in hypocotyl tissue colonization among the three cultivars. Shortening of the first internode was not a reliable predictor of hypocotyl tissue colonization. Nonuniformity of root rot indicated that heterogeneity for tolerance may exist within cultivars, and high- or low-tolerant components can be selected for further evaluation after this nonlethal tolerance test.

Additional key words: *Glycine max*, resistance.

Tolerance of soybean (*Glycine max* [L.] Merr.) to root rot, incited by *Phytophthora megasperma* f. sp. *glycinea* Kuan and Erwin, (hereafter designated *P. megasperma*), is now well documented from studies in the field (1,9,11), greenhouse (11), and laboratory (2,9). In general, soybean cultivars can be classified along a continuum from high to low tolerance to *P. megasperma*. High-tolerant lines show little stunting and/or yield loss when roots are infected, whereas low-tolerant lines have severe plant or yield loss. Incorporation of race-specific resistance into susceptible cultivars has only a minor influence on the level of tolerance (12). The potential for increasing tolerance in breeding lines has recently been investigated, and the genetics of this tolerance mechanism appears to be quantitative (10). Further, tolerance to *P. megasperma* is race nonspecific, and production of glyceollin or other fungitoxic compounds in infected roots does not account for it (5,6).

Selecting for tolerance to *P. megasperma* in the field requires large amounts of space and time and the availability of a field heavily infested with races virulent to all soybean lines being tested. Additional irrigation is often necessary to ensure an adequate amount of water at critical infection periods. A growth chamber test with zoospores and continually flooded soil conditions has been demonstrated (2). However, inoculum density must be controlled to accurately select tolerance levels. A laboratory procedure (9), based on cotyledon inoculation, requires less time and space, but expertise is necessary to produce zoospores and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

inoculate the cotyledons of the plants. There is a need for a tolerance test analogous to the standard hypocotyl resistance test.

The purpose of this work was to devise a nonlethal growth chamber test that would allow for routine screening for root tolerance to *P. megasperma* and retention and cloning of plants for further study.

MATERIALS AND METHODS

Stock cultures of race 4 of *P. megasperma* were maintained at 10 C on V-8 juice agar. To preserve isolate aggressiveness, cultures were periodically inoculated into susceptible, low-tolerant soybeans (7) and reisolated from rotted hypocotyls. As described previously (6), ten-day-old cultures of *P. megasperma* on V-8 juice agar were used for inoculum. Seeds of the cultivars Voris 295, Sloan, and OX 20-8 (high-, moderate-, and low-tolerance, respectively) were germinated in damp vermiculite in plastic pots with bottom drainage in a growth chamber with 14-hr daylength (21 klux) at 25 C. Tolerance levels of these cultivars were determined previously by using standard field and greenhouse methods (11). Three days after planting, the pots were watered to excess with 25% Hoagland's nutrient solution. On day 6, the plants had upright cotyledons, but their primary leaves were not yet expanding. Lateral roots had developed along the taproot from the soil line downward to about 6 cm. Six-day-old plants were gently lifted from the vermiculite and the roots were rinsed with tepid water. Seedlings were arranged on slant boards (3,4,8), modified by the use of polyester greenhouse wicking material as absorbent backing, rather than perlite-filled bags. Boards with plants, were irrigated with 50 ml of nutrient and maintained standing in 25% nutrient solution to a depth of 10 cm and returned to the same growth chamber for inoculation 24 hr later. Nutrient solution was replaced every 2 days.

On day 7 the polyester cloth covering the roots was pulled back and a 1-cm scrape-wound was made into the stele with a single-edge razor blade 2-cm below the hypocotyl-root junction in the area of developing lateral roots. Two drops of inoculum from a syringe were placed directly on the wound. Control plants received only a scrape-wound and V-8 agar suspension. Roots were again covered with polyester cloth and the slant-boards, maintained standing in 10-cm of nutrient solution, were returned to the growth chamber. At inoculation, the root tips had not yet reached the level of the nutrient solution.

Tolerance evaluation was made 6 days after inoculation. At that time, length of the first internode was measured. Preliminary experiments indicated that control-wounded and control-unwounded plants of the three cultivars did not significantly differ in first-internode length. Thus, the use of control-unwounded plants was discontinued. Taproots and hypocotyls were split to expose rotted interior tissues and the extent of tissue colonization upward through the taproot and into the hypocotyl was measured.

Immediately after colonization measurements, plants were retained for propagation in the following way. The hypocotyl and roots were severed just below the cotyledonary node. A second cut was made 1-cm above the cotyledons and a third longitudinal to the axis between the cotyledons. This provided a top node with leaves and two separated cotyledons with a small piece of nodal tissue. The top was placed in a test tube with 1% nutrient solution, and cotyledons were arranged in petri dishes on filter paper with enough 1% nutrient to keep the paper damp. After 2 wk in dim light, the plants were transferred to a soil mixture in a lighted mist chamber for 5 days and then to a growth chamber or greenhouse. A soil drench containing 1 mg of metalaxyl (*N*-[2,6-dimethylphenyl]-*N*-[methoxyacetyl]-alanine methyl ester) per milliliter was used to treat plants that had developed disease symptoms.

TABLE 1. Comparison of first-internode length and amount of upward tissue colonization in soybean cultivars wound-inoculated with *Phytophthora megasperma* f. sp. *glycinea* (*Pmg*) on day 7 and measured on day 13[a]

Cultivar	Disease tolerance	Tissue colonization (mm)	Length of first internode (mm)	
			Pmg	Control
OX 20-8	low	30[b]	44	75
Sloan	moderate	21	52	75
Voris 295	high	7	75	75
LSD at $P = 0.05$		2.01	5.51	
LSD at $P = 0.01$		3.68	7.84	

[a]Tap roots were inoculated with a suspension of virulent race 4 of *Pmg* in the area of developing lateral roots approximately 20 mm below the hypocotyl-root junction.

[b]Values shown are the mean of three experiments × two replications for a total of 400 plants per cultivar tested.

RESULTS AND DISCUSSION

Three cultivars of soybean, susceptible to race 4 of *P. megasperma*, were taproot inoculated at 7 days and incubated for 6 days on slant boards in a growth chamber. First-internode length and extent of tissue colonization in the taproot were measured (Table 1). The first internodes of all three cultivars had essentially the same length as the first internode in the control plants. Internode length of the inoculated, high-tolerant cultivar (Voris 295) did not differ significantly from the control, whereas the moderate-and low-tolerant cultivars (Sloan and OX 20-8) had significantly shorter mean internode lengths ($P = 0.01$). Tissue colonization was significantly different ($P = 0.01$) in the three cultivars.

Linear regression analysis was performed by using the first-internode length as a predictor of tissue colonization. The relationship was significant only for low-tolerant OX 20-8. The r^2 values were low, (0.0–7.3%, adjusted for degrees of freedom) indicating that measurement of the first internode in inoculated plants would not be a useful predictor of tolerance level.

Disease progress was not uniform within cultivars. A large percentage of plants of the high-tolerant line had no, or only slight, rotting the hypocotyl-root junction. The low-tolerant cultivar had a high percentage of plants that had colonization well into the hypocotyl and occasionally into the cotyledons. The nonuniformity of colonization for each line is depicted in the histograms of Fig 1. We have seen this same general pattern of

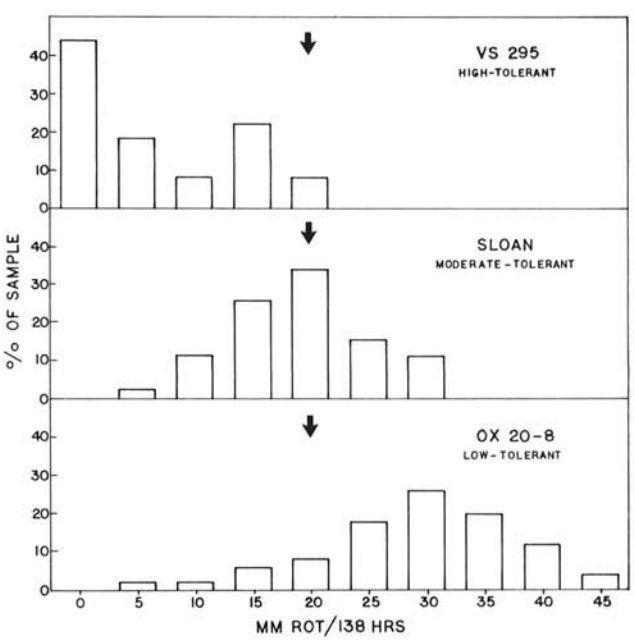

Fig. 1 Percentage distribution of length of tissue colonization caused by *Phytophthora megasperma* f. sp. *glycinea* on 13-day-old soybeans. The uppermost portion of the inoculation zone is at 0. The approximate location of the hypocotyl-root junction is shown by the arrow. Each histogram taken from three experiments times two replicatons for a total of 400 plants.

nonuniform rotting in other soybean cultivars that we have investigated (6). Because the root was scrape-wounded before inoculation, we do not believe that plants without tissue colonization were escapes due to unsuccessful infection. Additionally, plants that had colonization only to the hypocotyl-root junction appeared to contain a mechanism that retarded the advance of the fungus, perhaps similar to that described by Tooley and Grau (9).

More than 90% of the severed tops and 80% of the severed cotyledons formed new roots or shoots, respectively, and these were grown to maturity. Less than 5% of the developing plants had disease symptoms and these were successfully treated with metalaxyl. This procedure establishes single-plant lines that can be used for further genetic and disease evaluation. We are now testing these lines to determine whether we have been able to select for high- and low-tolerant components within lines.

LITERATURE CITED

1. Buzzell, R. I., and Anderson, T. R. 1982. Plant loss response of soybean cultivars to *Phytophthora megasperma* f. sp. *glycinea* under field conditions. Plant Dis. 66:1146-1148.
2. Jimenez, B., and Lockwood, J. L. 1980. Laboratory method for assessing field tolerance of soybean seedlings to *Phytophthora megasperma* var. *sojae*. Plant Dis. 64:775-778.
3. Kendall, W. A., and Leath, K. T. 1974. Slant-board culture methods for root observations of red clover. Crop Sci. 14:317-320.
4. Leath, K. T. and Kendall, W. A. 1978. Fusarium root rot of forage species: Pathogenicity and host range. Phytopathology 68:826-831.
5. Olah, A. F., Schmitthenner, A. F., and Walker, A. K. 1982. The role of glyceollin in soybean root tolerance to Phytophthora root rot. (Abstr.) Phytopathology 72:967.
6. Olah, A. F., Schmitthenner, A. F., and Walker, A. K. 1985. Glyceollin accumulation in soybean lines tolerant to *Phytophthora megasperma* f. sp. *glycinia*. Phytopathology 75:542-546.
7. Schmitthenner, A. F., and Hilty, J. W. 1962. A method for studying postemergence seedling root rot. Phytopathology 51:177-179.
8. Schmitthenner, A. F., and Walker, A. K. 1979. Tolerance versus resistance for control of Phytophthora root rot of soybeans. Pages 35-44 in: Proc. Ninth Soybean Seed Research Conference. H. D. Loden and D. Wilkenson, eds. Am. Seed Trade Assoc., Washington, DC. 91 pp.
9. Tooley, P. W., and Grau, C. R. 1982. Identification and quantitative characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean seedlings. Phytopathology 72:727-733.
10. Walker, A. K., and Schmitthenner, A. F. 1982. Developing soybean varieties tolerant to Phytophthora rot. Pages 67-78 in: Proc. Twelfth Soybean Seed Research Conference. H. D. Loden and D. Wilkenson, eds. Am. Seed Trade Assoc., Washington, DC. 124 pp.
11. Walker, A. K., and Schmitthenner, A. F. 1984. Comparison of field and greenhouse evaluations for tolerance to Phytophthora rot in soybean. Crop Sci. 24:487-489.
12. Walker, A. K., and Schmitthenner, A. F. 1984. Heritability of tolerance to Phytophthora rot in soybean. Crop Sci. 24:490-491.