New Races 5 and 6 of Phytophthora megasperma var. sojae and Differential Reactions of Soybean Cultivars for Races 1 to 6

Jerry H. Haas and R. I. Buzzell

Plant Pathologist and Soybean Breeder, respectively, Agriculture Canada, Research Station, Harrow, Ontario, N0R 1G0, Canada. Present address of J. H. H.: Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

The technical assistance of L. G. Crawford, R. D. Walker and M. R. McLean is acknowledged. We are grateful to the Ontario Soya-Bean Growers' Marketing Board, Chatham, Ontario for financial support.

Accepted for publication 17 May 1976.

ABSTRACT

HAAS, J. H., and R. I. BUZZELL. 1976. New races 5 and 6 of Phytophthora megasperma var. sojae and differential reactions of soybean cultivars for races 1 to 6. Phytopathology 66:1361-1362.

Soybean (*Glycine max*) hypocotyl-inoculation tests for vertical resistance were used to differentiate two new pathotypes of *Phytophthora megasperma* var. *sojae* that were different from races 1 to 4 and are proposed as races 5 and 6. Eight different soybean resistance-susceptibility combinations were identified from differential cultivar responses to the six races.

Phytophthora root rot of soybeans [*Glycine max* (L.) Merr.] was first reported in the 1950's from several parts of the USA and Canada (2, 3, 4, 8). The isolates of *Phytophthora megasperma* Drechs. var. *sojae* Hildeb. (*Pms*) were considered to belong to a single pathotype (subsequently called race 1) virulent on soybean cultivars with the rps_1 gene and avirulent on those with Rps_1 (2).

Since 1963, race-1-resistant soybean cultivars have been grown widely, but new pathotypes have now appeared that are virulent on cultivars with the Rps_1 gene as well as those with rps_1 (6, 7). This paper reports the occurrence of two new pathotypes isolated from race-1-resistant plants growing in Ontario.

MATERIALS AND METHODS

Soil was obtained from two fields near Harrow during the summer of 1973. In one field (V), root rot had been observed in the race-1-resistant Beeson during 1971. In the other field which was at the Woodslee Soil Substation (W), root rot was present in 0X20-8, a race-1-resistant strain, during 1970, 1971, 1972, and 1973. Plantings of 0X20-8 were made in these soils in the greenhouse and one isolate morphologically similar to *Pms* (10) was obtained from affected plants in each of the soils. These isolates were designated "V" and "W". Subsequently, three additional *Pms* isolates were obtained from diseased 0X20-8 or Harosoy seedlings that had been growing in each of the V and W soils.

Soybean stem pieces from the margins of *Pms* lesions were surface sterilized in 0.6% sodium hypochlorite for 2-4 minutes and plated on Difco corn meal agar (CMA) with 100 μg/g pimaricin (9). Colonies resembling *Pms* were transferred to unamended CMA.

The hypocotyl-puncture method for testing virulence of *Pms* (4) was modified slightly to speed the inoculation procedure. Cultures (7 to 14 days old) on CMA were comminuted and loaded in a hypodermic syringe. A small quantity of the inoculum was extruded into a vertical cut in each hypocotyl of at least five seedlings per cultivar 7 to 8 days after planting. Each pot of inoculated plants was covered with a polyethylene bag and incubated in the greenhouse (minimum 18 C); shade was provided with brown paper or black polyethylene. After 4 to 5 days, plants were rated either resistant if they remained upright or susceptible if the hypocotyls were rotted and collapsed. Soybean cultivars exhibiting a variable reaction (some plants resistant and some susceptible) were retested several times.

RESULTS

Isolates V and W were virulent in cultivar Altona which is resistant to races 1 to 4 (1) indicating at least one new pathotype. In addition, isolate V was virulent in Arksoy but W was not. The two new pathotypes were provisionally named races 5 and 6, respectively, in accordance with the presently-used system for numbering races (3, 5, 6, 7). Cultures of these races have been forwarded to the American Type Culture Collection, Rockville, Maryland, and to the National Culture Collection, Agriculture Canada, Ottawa, Ontario.

Isolates of races 1 to 6, plus three additional V isolates and three additional W isolates, were inoculated into hypocotyls of eight soybean differentials (Table 1). All of the V isolates behaved as race 5 and all of the W isolates as race 6. The six races produced eight distinguishable reactions on the soybean differentials (Table 1). The reactions of other cultivars tested with the six races were as follows: Amsoy and Corosy were similar to Harosoy; Blackhawk and Mukden were similar to Harosoy 63; Arksoy, Higan, and Pickett 71 were similar to Mack; Toku was similar to Altona; Shinanomejiro was similar to P.I. 86.050; P.I. 86.465-1 and P.I. 103.091 were similar to P.I. 171.442; D60-9647, Harrel, and Nansemond were

TABLE 1. Differential responses of some soybean cultivars to hypocotyl inoculation with four previously described (races 1-4) and two new (races 5 and 6) pathotypes of *Phytophthora megasperma* var. *sojae*

Strain	Reaction[a]					
	Race 1[b]	Race 2[c]	Race 3[d]	Race 4[e]	Race 5[f]	Race 6[g]
Harosoy	S	S	S	S	S	S
Harosoy 63	R	R	S	S	S	S
Mack	R	R	R	S	S	R
Altona	R	R	R	R	S	S
P. I. 86.050	R	R	R	R	S	R
P. I. 171.442	R	R	R	R	R	S
Sanga	R	S	R	R	R	R
Tracy	R	R	R	R	R	R

[a]Reaction: S = plants killed, R = plants not killed.

[b]Culture 77—single zoospore isolate from Harosoy grown in soil collected in 1962 and repeatedly planted with Harosoy.

[c]Culture 281—from K. L. Athow, Purdue Univ., W. Lafayette, Indiana, who obtained it from B. L. Keeling, Stoneville, Mississippi.

[d]Culture 191—from A. F. Schmitthenner as his isolate 595, previously numbered 573, and used in describing race 3 (6).

[e]Culture 319—from F. W. Schwenk as the type-culture of race 4 (7).

[f]Culture 81—isolate "V" plus three other isolates from field V (see text).

[g]Culture 113—isolate "W" plus three other isolates from field W (see text).

similar to Sanga; and Kingwa and Toyosuzu were similar to Tracy. With the exception of P.I. 86.050, the cultivars listed in Table 1 were accepted as differentials for identifying races of *Pms* by soybean workers from Arkansas, Illinois, Indiana, Kansas, Michigan, Mississippi, Missouri, Ohio, and Ontario, meeting at Harrow on 2 July 1975.

DISCUSSION

Resistance or susceptibility of soybean cultivars to Phytophthora rot usually is tested by a hypocotyl-puncture technique (4). Races 2-4 were defined on the basis of stem necrosis on a set of differential cultivars inoculated by insertion of mycelium from agar cultures into seedling hypocotyls (5, 6, 7). This technique bypasses the normal site of pathogen ingress, the roots, and tests the ability of the fungus to infect the stem. This method has been compared with inoculation by soil infestation (6), and the two methods gave similar results; the soil infestation method requires more time and space, and disease escapes are more frequent. In our hypocotyl inoculation tests, disease escapes were infrequent; however, some cultivars exhibited a variable reaction. These lines either were from apparently mixed seed lots or the susceptible reaction resulted from bacteria-incited rotting of the hypocotyl. The latter rotting occurred only occasionally; thus subsequent inoculations with the same cultivars generally gave unambiguous results. To obtain unambiguous results with certain cultivars, it was necessary to select a specific seed lot.

The occurrence of several different races of *Pms* which attack race-1-resistant cultivars complicates the attempts to control Phytophthora rot by means of race-specific resistance. Considerable genetic variability for virulence must be present in the fungus: we speculate, on the basis of the limited information presently available, that there are at least five virulence genes involved in these six races. In order to develop a rational approach for control of this disease, further study of the soybean-pathogen system is needed.

LITERATURE CITED

1. ATHOW, K. L., F. A. LAVIOLETTE, and T. S. ABNEY. 1974. Reaction of soybean germplasm to four physiologic races of Phytophthora megasperma var. sojae. Plant Dis. Rep. 58:789-792.
2. BERNARD, R. L., P. E. SMITH, M. J. KAUFMANN, and A. F. SCHMITTHENNER. 1957. Inheritance of resistance to phytophthora root and stem rot in the soybean. Agron. J. 49:391.
3. HILDEBRAND, A. A. 1959. A root and stalk rot of soybeans caused by Phytophthora megasperma Drechsler var. sojae var. nov. Can. J. Bot. 37:927-957.
4. KAUFMANN, M. J., and J. W. GERDEMANN. 1958. Root and stem rot of soybean caused by Phytophthora sojae n. sp. Phytopathology 48:201-208.
5. MORGAN, F. L., and E. E. HARTWIG. 1965. Physiologic specialization in Phytophthora megasperma var. sojae. Phytopathology 55:1277-1279.
6. SCHMITTHENNER, A. F. 1972. Evidence for a new race of Phytophthora megasperma var. sojae pathogenic to soybean. Plant Dis. Rep. 56:536-539.
7. SCHWENK, F. W., and T. SIM. 1974. Race 4 of Phytophthora megasperma var. sojae from soybeans proposed. Plant Dis. Rep. 58:352-354.
8. SUHOVECKY, A. J. 1955. A Phytophthora root rot of soybeans. Ph.D. Diss., Ohio State Univ., Columbus. (Doctoral Diss. Ser. Public. No. 14,500. Univ. Microfilms, Ann Arbor, Michigan). 83 p.
9. TSAO, P. H. 1970. Selective media for isolation of pathogenic fungi. Annu. Rev. Phytopathol. 8:157-186.
10. WATERHOUSE, G. M. 1970. The genus Phytophthora de Bary. Mycol. Pap. No. 122. Commonw. Mycol. Inst., Kew, Surrey, England. 59 p.