Races of *Phytophthora sojae* in Iowa Soybean Fields

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ABSTRACT

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During 1991 to 1994, *Phytophthora sojae* was isolated from plants or soils in 50 Iowa counties. Eighty-two isolates from soil and 124 isolates from diseased soybean plants were collected and tested. Of the 206 isolates tested, 178 were classified into races; the other 28 isolates could not be classified due to intermediate reactions. Isolates represented races 1, 2, 3, 4, 8, 13, 15, and 25. Race 3 was the dominant race from samples of diseased plants, consisting of 30 and 50% of isolates in 1991 and 1994, respectively. Race 1 was the dominant race from soil samples, ranging from 34 to 45% over the years. One new race (virulence formula = 1a,1c,1d,1k,7) was identified. Our results suggest that *P. sojae* has greater diversity in virulence than in 1966 when race 1 was first found in Iowa.

Phytophthora root rot (PRR) of soybean caused by *Phytophthora sojae* M. J. Kaufmann & J. W. Gerdemann (syn. *P. megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin) was first noted in Indiana in 1948 and subsequently was found in Ohio in 1951 (15). Since then, the disease has been identified in all soybean-growing regions of the United States and Canada. With the release of PRR-resistant soybean cultivars, physiological races of *P. sojae* were identified (6,8,9,16). Recently, races 38 and 39 have been identified in Arkansas (5). Surveys for *P. sojae* races have been conducted in several soybean-producing states in the North Central region of the United States (8,9,16,19). Studies have shown that *P. sojae* races differ geographically. For example, races in Indiana or Ohio (9,16) were more diverse than those in Nebraska (19).

In Iowa, Tachibana et al. (17) reported that race 1 was first found in 1966 in two counties, and results of their survey from 1966 to 1973 indicated race 1 was the only race present. Since then, PRR has frequently been observed in cultivars with race-specific resistance, suggesting the occurrence of races other than race 1 in Iowa. However, no studies of races have been done since the report of race 1 in 1966, in spite of the fact that approximately 25% of early season damping-off in Iowa is caused by *P. sojae* (12) and Iowa is the second-largest soybean-producing state in the United States. This paper reports the presence and distribution of races of *P. sojae* isolated from soil or diseased soybean plants from 1991 to 1994 in Iowa.

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MATERIALS AND METHODS

Isolation from plants. In the spring and early summer of 1991 and 1994, soybean plants with PRR symptoms were collected from soybean fields in 57 Iowa counties. The number of fields per county varied from one to four. Stem pieces were cut from the borders of stem lesions and washed with tap water for 1 h. The pieces were then surface disinfected in 0.05% NaOCl for 1 min before being plated on the selective media, PsSM (15) or V8-PARP (1 liter V8 basal medium with the addition of 10 ppm pimaricin, 250 ppm ampicillin, 10 ppm rifampicin, and 100 ppm PCNB [pentachloronitrobenzene]). The plates were incubated in the dark at room temperature. Hyphal tips from colonies with growth characteristics similar to those of *P. sojae* were transferred to the selective medium for further purification and subsequent identification.

Isolation from soil. In 1992 and 1993, soil samples were randomly collected from soybean fields in 23 Iowa counties. Twenty- to 30-g subsamples were placed in petri dishes and flooded with distilled water. Germinated soybean seeds (cvs. Williams or Sloan) were placed on the surface of the flooded soils and incubated in the dark at room temperature for 3 to 7 days. Pieces of the soybean radicle were then removed, surface sterilized, blotted dry, and plated on PsSM.

In 1994, soil samples were randomly collected from soybean fields in 22 Iowa counties in cooperation with the Iowa Department of Agricultural Statistics. *P. sojae* was isolated from the soil samples by a modified leaf-disk-bioassay method (3, 16). Soil (60 cm^3) was spread over an 8-cm column of sterile soil in 500-ml perforated cups. The samples were wetted to saturation and drained for 4 days on greenhouse benches. The samples were flooded, and 10 6-mm-diameter leaf disks (susceptible cv. Sloan) were floated on the surface. After 24 h, the leaf disks were surface sterilized with 0.05% NaOCl for 30 s and plated onto a selective medium containing 40 mg of hymexazol per liter (11). Plates were incubated in the dark for 4 days before examination. Hyphal tips from colonies with growth characteristics similar to those of *P. sojae* were transferred to selective medium for further purification.

Race testing. Cultivars and lines used for race tests during this study are given in Table 1. From 1991 to 1993, soybean seeds of the differential cultivars were maintained at Iowa State University. Seeds for the 1994 test were obtained from A. F. Schmitthenner at Ohio State University. Eleven seeds of each differential cultivar were planted in 10-cm-diameter pots and placed in a greenhouse at 20 to 27°C. Mycelium from 7- to 10-day-old cultures grown on oatmeal agar was inserted into hypocotyls of 10 8- to 10-day-old seedlings. The seedlings were then placed in a mist chamber for 24 to 48 h at 24°C. Reactions of the differentials were evaluated 4 to 5 days after inoculation.

A differential was classed as susceptible if >70% of the seedlings were killed and resistant if <30% of the seedlings were killed. Seedling mortality ranging from 30 to 70% was considered an intermediate reaction. The tests were repeated for all isolates that gave intermediate reactions and, when the intermediate reaction was consistent, isolates were considered not classifiable. If reaction types indicated a

Table 1. Differential soybean cultivars and lines used for *Phytophthora sojae* race tests

Gene[a]	Cultivars/lines	
	1991 to 1993	1994
rps	Williams	Williams
*Rps*1-a	BSR 101	L75-61
*Rps*1-b	Sanga	L77-1863
*Rps*1-c	Mack	Williams 79
*Rps*1-d	PI 103.091	PI 103.091
*Rps*1-k	Kingwa/ Williams 82	Williams 82
*Rps*3	PI 171.442	L83-570
*Rps*6	Altona	Altona
*Rps*7	Harosoy	Harosoy
*Rps*1-a and *Rps*7	Harosoy 63	Harosoy 63

[a] *rps* is susceptible; others indicate resistance genes.

new race, the differential test was repeated three additional times to confirm the reaction.

RESULTS AND DISCUSSION

Soil or plant samples were collected from 57 Iowa counties, with samples from 50 counties in the major soybean-production areas of the state yielding isolates of *P. sojae* (Fig. 1; Table 2). Eighty-two isolates from soil and 124 isolates from diseased soybean plants were tested. Of the 206 isolates tested, 178 were identified to race (Table 2).

Race test for isolates from plants. In 1991, 22 *P. sojae* isolates collected from 13 soybean fields in eight counties were classified to race. Thirty-two percent of the isolates were classified as race 3, while 27% of the isolates had intermediate reactions that were unidentifiable to race. One isolate was classified as race 2, the only time during the study that this race was found.

In 1994, 102 isolates were collected from soybean plants in 20 fields in 18 counties. Eighty-six of these isolates were identified to race, with 50% classified as race 3 (Table 2). Races 8, 13, and 15 were identified for the first time in Iowa. About 16% of isolates had intermediate reactions and some of these isolates gave intermediate reactions on all eight resistant alleles used in the race differentiation tests. Intermediate reactions in the race differentiation tests may be due to variation in the environment, heterokaryosis, or heterogeneity of the fungus (2,14).

Race test for isolates from soil. In 1992 and 1993, all 42 isolates collected from 32 fields in 23 counties were classified. Races 1, 3, 4, and 25 were found, with frequencies of 34.4, 29.3, 31.4, and 5%, respectively (Table 2). Race 25, first reported in Indiana in 1986 (10), was found for the first time in Iowa. In 1994, 40 isolates were collected from soils in 22 counties, and race 1 was identified as the dominant race. Race 3, which accounted for 50% of the isolates from plant samples, represented 20% of isolates from soil samples. Races 8 and 25 were also detected from soil samples in 1994.

The frequency of occurrence of races of *P. sojae* in soil samples differed from that in plant samples. Race 3 was recovered most frequently from plant samples, while race 1 was isolated at the highest frequency from soil samples. Schmitthenner et al. (16) reported that race 7 was dominant (55%) in soil samples in Ohio while races 1 and 3 (20.8 and 28.3%, respectively) were the dominant races recovered from plants. A new race was identified in our tests. The new *P. sojae* race was isolated from plant samples in 1991 and had a virulence formula of 1a,1c,1d,1k,7.

The dominant races found in Iowa are similar to those found in neighboring states. In 1980, when PRR was considered a new disease in eastern Nebraska, a survey by White et al. (19) found that race 1 was the dominant race in that region; Tachibana et al. (17) reported similar results in 1975 in Iowa. A 1987 report in South Dakota (4) and a 1984 report in Minnesota (7), as well as our survey, showed that race 3 was the dominant race for isolates from diseased plants. Tooley et al. (18) in 1982 also reported that race 3 was the dominant race, with races 7, 8, and 9 isolated less frequently, in Wisconsin.

The diversity of *P. sojae* races in Iowa soybean fields does not appear to be as great as in the eastern United States (8,9, 16) or in southeastern Canada (1). Before 1975, only race 1 had been reported in Iowa (17). However, from 1973 to 1979, races 1, 3, 4, 5, 7, 8, and 9 were found in Indiana (8), and in 1978 to 1980, these races were also reported in Ohio. Many new races were originally reported in Indiana (7,8,9) and Ohio (16). These regions seem to have more *P. sojae* races than are found in Iowa, based on these reports (1,10,16). Race 7, which was reported to constitute 18.1% of the classified isolates in Indiana as early as 1981 (9), was not found in Iowa. A later study in Ohio showed that race 7 constituted 14 to 18% of isolates from plants and 55.8% of isolates from soils (16).

By 1975, PRR had been found in 49 counties in Iowa (17), but only race 1 of *P. sojae* was identified. Soybean cultivars resistant to *P. sojae* race 1 were available for planting in Iowa as early as 1973 (17). PRR has been known to occur on Iowa soybean cultivars with the *Rps*1 and the *Rps*1-c resistant alleles in the last few years. Results presented here suggest that the diversity of *P. sojae* isolates found in Iowa today is greater than in 1966. The isolates found in our study can attack several commonly used PRR-resistant alleles. The increase in diversity of races may be due to selection pressure from the use of race-specific resistant cultivars. The occurrence of race 25, which was isolated from four counties, may be the result of increased use of the *Rps*1-k gene. In 1994, we obtained isolates of race 25 from seedlings in a 12-ha field in Cass County. This field had been farmed without tillage for 8 years and planted with Williams 82, a cultivar with the

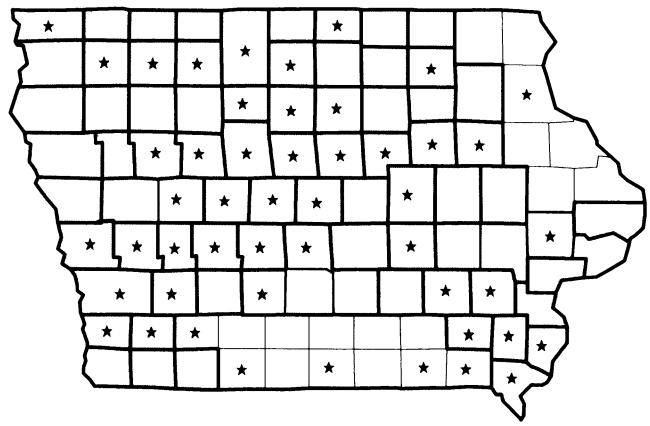

Fig. 1. Iowa counties where *Phytophthora sojae* was isolated in soybean fields from 1991 to 1994. Shaded counties are those having greater than 50,000 acres of soybean.

Table 2. Percentage of *Phytophthora sojae* races isolated from diseased plants or soil from Iowa soybean fields during 1991 to 1994

		Total isolates (%)			
		1991	1992 and 1993	1994	
Race	Virulence formula[a]	Plant[b]	Soil[c]	Plant[b]	Soil[c]
1	7	13.8	34.4	16.7	45
2	1b,7	4.6	0	0	0
3	1a,7	32	29.3	50	20
4	1a,1c,7	18	31.4	9.8	20
8	1a,1d,6,7	0	0	0.98	2.5
13	6,7	0	0	2	0
15	3a,7	0	0	1	0
25	1a,1b,1c,1k,7	0	5	1	2.5
New	1a,1c,1d,1k,7	4.6	0	0	0
Intermediate reaction	27	0	16	10	
Isolates tested (no.)		22	42	102	40
Counties sampled (no.)		8	23	18	22

[a] See Schmitthenner (15) for details.
[b] Isolates obtained from diseased plants.
[c] Isolates obtained from soils of soybean fields.

*Rps*1-k gene. About 80% stand reductions occurred in this field.

In recent years, no severe PRR outbreaks have been observed in Iowa, perhaps because of the widespread use of PRR-resistant cultivars. In a 1986 survey, 21 of the 62 most commonly grown cultivars/brands in Iowa were resistant to *P. sojae* race 1 (13). Of these 21 cultivars/brands, 43% were resistant to race 3 and 19% were resistant to race 4. In a similar survey from 1992, 26 of 62 commonly grown soybean cultivars/brands were resistant to *P. sojae* race 1; of these 26 cultivars/brands, 18 (69%) were also resistant to *P. sojae* race 3, and 10 (38%) were also resistant to *P. sojae* race 4. A 1995 survey (X. B. Yang, *unpublished*) showed that 82 of 168 PRR-resistant cultivars/brands used in Iowa had the *Rps*1-k gene. Changes in diversity of PRR races in Iowa are expected as more race-specific resistant alleles are deployed.

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