Phytophthora sojae Races in Ohio Over a 10-Year Interval

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ABSTRACT

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During 1978 and 1980, 199 isolates of *Phytophthora sojae* were obtained from soil samples from 16 fields in northwest Ohio using a soybean leaf disk bait procedure, and 53 isolates were obtained from diseased soybean plants. During 1990 and 1991, 282 isolates of *P. sojae* were obtained from diseased soybean cv. Sloan or Amcor 89 seedlings planted in soil from 88 fields in northwest Ohio, and 27 isolates were obtained from field-grown plants with the *Rps*1-k resistance gene. All isolates were identified as to their race phenotypes by inoculation of differential soybean cultivars having *Rps*1-a, *Rps*1-b, *Rps*1-c, *Rps*1-d, *Rps*1-k, *Rps*3-a, or *Rps*6 resistance genes. *Rps*7 also was used for the 1990–1991 isolates. Race 7 was the most prevalent in 1978–1980, followed by races 9 and 3. In 1990, race 3 was most prevalent, followed by race 7, race 4, and race 1. In 1991, race 3 was most prevalent, followed by race 7, race 9, and new races with varied phenotypes that could defeat *Rps*1-k. In 1978–1980, 1990, and 1991, 7.5, 11.7, and 18.2%, respectively, of the *P. sojae* isolated were new races capable of defeating *Rps*1-k, the major gene used for control of Phytophthora rot of soybean in the northern midwest. Race numbers for five of these new races are proposed. It is concluded that race phenotypes that can defeat all existing *Rps* genes or gene combinations now available for control of Phytophthora rot are already present in soil. Time course for increase of these new races and alternative sources of resistance are discussed.

Phytophthora sojae M.J. Kaufmann & J.W. Gerdemann (Syn. *P. megasperma* Drechs. f. sp. *glycinea* T. Kuan & D.C. Erwin [9]), the causal agent of Phytophthora rot of soybean (*Glycine max* (L.) Merr.), is a highly variable pathogen. By 1992, 26 physiologic races had been described by their differential virulence on eight soybean genotypes (16,26,28). Description of one additional race, race 26, is in press (B. L. Keeling, *unpublished*). Additional virulence patterns have been found but not given race numbers (10,23,24). There are 13 *Rps* genes at seven loci that condition differential resistance to these races (19,28). None of the genes conditions resistance to all races, although one, *Rps*1-k, is effective against 21 races and has been widely deployed to control Phytophthora rot (19). In 1978–1980, Hobe (10) isolated *Phytophthora* nonselectively from 16 soils in Ohio using a leaf disk baiting procedure (4). Some isolates were found with cultivar reaction patterns different from described races, including several that were virulent on Kingwa (*Rps*1-k). In 1990, Schmitthenner (22) found root rot in fields of cultivars having the *Rps*1-k gene and isolated *P. sojae* cultures that were virulent on *Rps*1-k. In this paper, races found in Ohio soils in 1978–1980 are compared with those found in 1990–1991, and new *P. sojae* virulence phenotypes are described. Several new *P. sojae* races are proposed.

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MATERIALS AND METHODS

Media. A basal medium, VA, used for isolation, storage, and inoculum production, consisted of 40 ml of V8 juice, 1 g of sucrose, 0.2 g of yeast extract, and 0.01 g of cholesterol solidified with 20 g of agar per liter. The V8 juice was autoclaved with 0.6 g of $CaCl_2$ and filtered through diatomaceous earth prior to incorporation into the medium. Selective inhibitors added to VA for the PBNC medium for isolation from plants were 0.02 g of PCNB (Terrachlor 75 WP), 0.005 g of benomyl (Benlate 50W), 0.1 g of neomycin sulfate, and 0.01 g of chloroamphenicol. For isolation from bioassay seedlings, 0.02 g of iprodione (Rovral 50%) was added for the medium PBNIC. For isolation from bioassay leaf disks, 0.02 g of hymexazol (OAC-2582, 70% WP) was added to PBNC, and 0.009 g of rifampicin was substituted for chloroamphenicol in the PBNRH medium. Antibiotics were obtained from Sigma Chemical Corp., St. Louis, Missouri. All ingredients were added before autoclaving except rifampicin and iprodione, which were added after autoclaving in 5 ml of ethanol per liter of medium.

A second basal medium, lima bean broth (LBB), was used for zoospore and oospore production. The medium contained the extract of 50 g/L of frozen lima beans, prepared by autoclaving lima beans with water for 15 min at 121 C, macerating the lima beans, and filtering the extract and pulp through diatomaceous earth. Lima bean agar (LBA) was prepared by adding 20 g/L of agar to the LBB.

Isolation from diseased plants. Plants with well-defined stem lesions were collected from the field and stored at 3 C overnight. Small sections were taken from the edge of the stem lesions and placed on PBNC medium in 1978–1980 and PBNIC medium in 1990. Also, plants were collected with severe flood damage symptoms (wilting and dead root cortical tissues 10 days after heavy rains). Stem and roots were surface disinfested in 1% NaOCl and split, and necrotic tissue was removed from the inside of the tap root and placed on PBNIC. With all isolations, the agar medium was inverted over the diseased plant tissue pieces in the petri plate to reduce bacterial contamination.

Isolation from soil with the leaf disk method. In 1978, 30 soil samples were collected from each of nine soybean fields; and in 1980, five soil samples were collected from each of seven soils in northwest Ohio. Soil was sieved through a 456-μm screen, and two 5-g subsamples were removed from each soil and stored in plastic bags at 5 C. One subsample from each soil was used to determine the percentage of soil moisture. The other subsamples were combined and placed in a pressure membrane apparatus to determine the soil moisture for a water potential of -15 kPa at a bulk density of 1.25. Subsamples were taken from each soil (equivalent to 126.6 g dry weight) and adjusted to a water potential of -15 kPa by mixing with ice. The amount of ice added depended on the soil moisture percentage at the time of collection. All subsamples from one field were adjusted the same way. The moisture-adjusted soil samples were stored in double plastic bags for 3 mo at 25 C in a windowless room. The soil samples then were packed in polyvinylchloride (PVC) tubing. One end of the tubing was covered with nylon cloth, 0.1- to 0.15-mm-mesh, and black plastic. Soil, 101 g, was poured into the cylinder, which was 5 cm diameter and 5 cm deep, and tapped with a metal plunger until the soil was level with the top of the cylinder, thus achieving the 1.25 bulk density. The packed cylinders were placed in a double plastic bag, frozen for 1 wk at -5 C, thawed for 1 wk at

5 C, and incubated for 1 wk at 25 C in a windowless room.

Phytophthora was isolated from soil with the leaf disk technique described by Canaday and Schmitthenner (4). Nine grams of soil were removed from the PVC tubing, placed in a 50-ml beaker and flooded with 25 ml of nonsterile distilled water. Scum that formed on the water surface was removed using paper tissue. Twenty leaf disks, 7 mm diameter, freshly cut with a paper punch from the unifoliolates of 7-day-old Amsoy soybean seedlings, were floated immediately on the surface of the water. After 90 min, the floating leaf disks were removed, blotted, and placed on PBNRH medium. The plates were examined after 1 wk. *Phytophthora* colonies growing from the edge of the leaf disk, identified by their characteristic mycelial growth, were transferred to PBNC media, which was inverted over the transfer to form a bubble. Bacteria-free cultures were isolated from the surface of the culture at the edge of the colony after 1 wk. Cultures were stored on VA medium at 11 C until virulence on soybean was evaluated.

Isolation from soil with the soybean seedling bioassay method. Six soil samples were collected from each of 67 fields in northwest Ohio in 1990 and 23 fields in 1991. Each sample consisted of two adjacent cylinders, 10.5 cm diameter and 15 cm long, removed with a cup cutter. Soil was air-dried and passed through a burr mill with burrs spaced 2 mm apart. Soil was stored dry at room temperature until assays were made. Approximately 1,200 g of air-dried soil was placed in a 15-cm-diameter plastic pot. Pots were flooded until the soil was saturated, then drained and allowed to dry until cracks appeared. Pots were then sealed in plastic bags and incubated at room temperature, ca 25 C, in a windowless room. Total time from flooding at room temperature was 2 wk. The surface 1 cm of soil from each pot was tilled. Fifteen seeds of the soybean cultivar Sloan, susceptible to all races of *P. sojae,* or Amcor 89 with the *Rps*1-k resistance gene, were placed in the surface 1 cm of soil. The soil surface was covered with 250 ml of coarse vermiculite saturated with water, and the pots were sealed in plastic bags. After 3 days, when the seeds had germinated and the roots were approximately 5 cm long, the soil was flooded for 24 hr, then drained and incubated at 25 C in the greenhouse during winter or at ambient temperature outside during summer on the north side of a building. Two weeks after planting, *Phytophthora* was isolated on PBNIC medium from damped-off seedlings and stored as described above.

Isolation of single-zoospore cultures. Single-zoospore isolates were obtained from some of the cultures isolated in 1978–1980, and from the new virulence phenotypes found in 1990 and 1991. Ten disks, 2 mm diameter, from the edge of VA cultures were placed in 25 ml of LBB in a 125-ml flask. Cultures were incubated for 48 hr at 25 C. The liquid culture was replaced by 25 ml of Chen-Zentmyer salt solution (5) four times at 15 min intervals, then with 25 ml of sterile distilled water. Zoospores were formed after 5 hr at 25 C. Zoospores were diluted 10-, 100-, and 1,000-fold with distilled water, and 0.2 ml from each dilution was spread on the surface of PBNC medium. The medium was inverted in the plate. Single-zoospore colonies were selected after 3 days at 25 C and stored on VA at 11 C until evaluated for virulence.

Isolation of single-oospore cultures. Single-oospore isolates were obtained from the new virulence phenotypes found in 1990 and 1991 using a method modified from Bhat et al (2). Four agar culture plugs, 0.2 cm^2, were placed in 5 ml of LBB in a 25-ml screw-capped, autoclavable scintillation vial. Cultures were incubated 30 days on the laboratory bench at room temperature, ca 24 C, without light control. The culture was then transferred to a 5-ml screw-capped bottle, and 2,000 units of β-Glucuronidase (Type H-1, Sigma) per milliliter of distilled water were added to bring the volume to 4 ml. The culture was macerated for 1 min at 1,000 rpm using a Tissue-Tearor (Biospec Products, Bartlesville, OK). The macerated culture was incubated for 12 hr at 37 C, then transferred to a 15-ml centrifuge tube and centrifuged 10 min at 3,000 rpm in a bench-top centrifuge. The oospore and mycelium pellet was washed five times with 13 ml of distilled water and centrifuged at 2,000 rpm for 5 min between washes. The oospore and mycelium pellet was resuspended in 10 ml of distilled water and frozen at −20 C for 24 hr, then thawed at 45 C. Mycelial debris on the surface of the suspension was removed with a pasteur pipette, and the oospores were centrifuged to concentrate. Approximately 200 oospores were spread on the surface of 1.5% water agar containing 10 μg/ml of cholesterol and 10 μg/ml of rifampicin. Plates of oospores were incubated on the laboratory bench, ca 24 C. Single-oospore colonies were selected after 1 wk and stored on LBA.

Virulence evaluation of cultures. Isolates were classified into races by their differential virulence on a known set of cultivars containing different *Rps* genes. In 1978–1980, soybean cultivars used for race determination were Amsoy 71 (*Rps*1-a + *Rps*7), D60-9647 (*Rps*1-b), Mack (*Rps*1-c), PI 103091 (*Rps*1-d), Kingwa (*Rps*1-k), PI 171442 (*Rps*3-a), and Altona (*Rps*6). Amsoy was used as a universal suscept. In some tests, Sanga, Vickery, or Rockford were substituted for D60-9647, Mack, or Altona, respectively, because of a seed shortage. In 1990 and 1991, cultivars used were Sloan (*rps*), Harlon (*Rps*1-a), Harosoy 13XX (*Rps*1-b), Williams 79 (*Rps*1-c), PI 103091 (*Rps*1-d), Williams 82 (*Rps*1-k), PI 171442 (*Rps*3-a), Altona (*Rps*6), and Harosoy (*Rps*7). In some tests, Chapman was substituted for PI 171442.

In 1978–1980, virulence was evaluated using the hypocotyl inoculation method (11) modified by covering the wound with vaseline instead of placing the plants in a moist chamber for 24 hr after inoculation. In 1990, the hypocotyl injection method (8) used was modified by covering inoculated plants overnight with plastic to maintain high humidity after inoculation rather than covering wounds with vaseline. VA cultures, 7- to 14-days-old, of all isolates were used for inoculation of 10, 7- to 10-day-old plants for each interaction. Inoculated plants were incubated 1 wk in the greenhouse at 25 C under fluorescent light (210 $\mu E \cdot cm^{-2} \cdot s^{-1}$) or in ambient temperature outside during the summer on the north side of a building under indirect sun light (300 $\mu E \cdot cm^{-2} \cdot s^{-1}$). Generally, seedlings were either 100% healthy or 100% dead. Occasionally, only partial kill occurred with some interactions. These intermediate interactions were repeated three times. If intermediate reactions always occurred, the isolates were considered unclassifiable and listed as variable in the data. New race phenotypes obtained during 1990–1991 were evaluated four times as mass cultures. Also, multiple single zoospore and single oospore cultures were obtained from one selected culture of each of these new race phenotypes and evaluated for virulence. Known isolates of race 1 and race 4 were evaluated on Sloan, Harlon, Williams 79, and Williams 82 periodically to verify that the procedures and environment were suitable to detect cultivar and race differences.

RESULTS

Identity and prevalence of races of *P. sojae*. The differential virulence of published *P. sojae* races on cultivars with eight different *Rps* genes is listed in Table 1 by both race number and virulence formula. The virulence formulas differ from those proposed by Tooley et al (24) by listing only the ineffective genes. The cultivar Williams 82, with the *Rps*1-k gene, was added to the traditional differentials. The original descriptions of races 12, 14, 17, and 19 are listed, as modified by additional information provided later for *Rps*1-d or *Rps*1-k (14,28). All other interactions are as listed by Ward (28), Wagner and Wilkinson (26), or B. L. Keeling (*unpublished*).

In 1978, 175 isolates were obtained from the soil of nine fields. In 1980, 24 and 54 isolates were obtained from soil samples and diseased plants, respectively, from seven fields. Virulence

phenotypes of isolates from soil and plants in 1978–1980 are summarized in Table 2. Among the 1978 soil isolates, race 7 was the most prevalent, consisting of 52.3% of the cultures. Races 3 and 9 constituted 9.6 and 18.1%, respectively. Undescribed virulence phenotypes constituted 5.0% of the cultures. These are listed sequentially in Table 3 with the prefix 'H', starting with the number after the last numbered race at the time the research was completed. Hobe's isolates were not tested on Harosoy, so their reaction on *Rps*7 is unknown. A number of these new races were virulent on Kingwa, the source of *Rps*1-k, the predominant resistance gene in modern cultivars.

Table 1. Virulence formulas and reaction of differential soybean cultivars to described races of *Phytophthora sojae*

Race[w]	Virulence formula[x]	Susceptibility of *Rps* genes[y]							
		1-a	1-b	1-c	1-d	1-k	3-a	6	7
1	7								S[z]
2	1b,7		S						S
3	1a,7	S							S
4	1a,1c,7	S		S					S
5	1a,1c,6,7	S		S				S	S
6	1a,1d,3a,6,7	S			S		S	S	S
7	1a,3a,6,7	S					S	S	S
8	1a,1d,6,7	S			S			S	S
9	1a,6,7	S						S	S
10	1b,3a,7		S				S		S
11	1b,6,7		S					S	S
12	1a,1b,1c,1k,3a	S	S	S		S	S		
13	6,7							S	S
14	1c,7		S						S
15	3a,7						S		S
16	1b,1c,1k		S	S		S			
17	1b,1d,3a,6,7		S		S		S	S	S
18	1c			S					
19	1a,1b,1c,1d,1k,3a		S	S	S	S	S		
20	1a,1b,1c,1k,3a,7		S	S	S		S		S
21	1a,3a,7	S					S		S
22	1a,1c,3a,6,7	S		S			S	S	S
23	1a,1b,6,7	S	S					S	S
24	1b,3a,6,7		S				S	S	S
25	1a,1b,1c,1k,7	S	S	S		S			S
26	1b,1d,3a,6,7		S		S		S	S	S
27	1b,1c,1k,3a,6,7		S	S		S	S	S	S

[w] Numerical races according to Ward (28) and Wagner and Wilkinson (26), except for races 6 (8) and 14 (11), where the original descriptions were used as modified by addition of *Rps*1-d interaction (15), and race 26, which was provided by R. L. Keeling (*unpublished*). All races are virulent on universal suscepts such as Williams or Sloan (*rps*).

[x] Listing of genes defeated by a race.

[y] 1-a In cv. Harlon, 1-b in Harosoy isoline 13XX, 1-c in cv. Williams 79, 1-d in PI 103091, 1-k in cv. Williams 82, 3-a in PI 171442, 6 in cv. Altona, and 7 in cv. Harosoy.

[z] Susceptible or compatible interaction.

Table 2. Percentage of race phenotypes identified in 1978–1980, 1990, and 1991 in Ohio

Race[r]	Virulence formula	Races isolated (%)					
		1978–1980		1990			1991
				Seedling bioassay		Field	Seedling
		Soil[s]	Field plants[t]	Sloan[u]	Amcor 89[v]	plants[w]	bioassay[x]
1	7	3.0	20.8	14.7	5.0	0	3.0
3	1a,7	9.6	28.3	28.2	27.5	9.3	24.2
4	1a,1c,7	3.5	1.9	8.8	5.0	14.0	4.5
5	1a,1c,6,7	1.0	7.5	4.1	0	0	0
7	1a,3a,6,7	55.8	15.1	22.4	7.5	14.0	18.2
8	1a,1d,6,7	2.5	0	4.7	2.5	2.3	1.5
9	1a,6,7	18.1	9.4	2.9	2.5	0	18.2
25	1a,1b,1c,1k,7	0	0	0	2.5	9.3	0
Rare	Variable	1.4	3.8	4.1	0	0	4.5
New	Variable	5.0	13.2	4.7	22.5	28.0	18.2
Avir[y]	00	0	0	3.5	20.0	14.0	3.0
Ambiguous[z]		0	0	2.4	5.0	9.3	3.0

[r] Based on differential reactions listed in Table 1. Rare races were 2, 13, 14, 15, 21, or 22. New races are other phenotypes with virulence patterns different from races 1–27, many of which defeated *Rps*1-k.

[s] Based on 199 isolates obtained using the leaf bait method (10) from 103 soil samples from six fields.

[t] Based on 54 isolates from 54 diseased plants from seven fields.

[u] Based on 167 isolates from damped-off Sloan seedlings growing in six soil samples from each of 65 fields.

[v] Based on 40 isolates from damped-off Amcor 89 (*Rps*1-k resistance gene) seedlings growing in six soil samples from each of 65 fields.

[w] Based on 41 isolates obtained from flood-damaged plants, of which 27 were Amcor 89, Pella 86, Resnik, or Williams 82 with the *Rps*1-k resistance gene.

[x] Based on 75 isolates from damped-off Sloan or Amcor 89 seedlings growing in six samples from each of 23 soils.

[y] Does not cause disease on cv. Amsoy or cv. Sloan.

[z] Isolates nonclassifiable for virulence since they varied each of three times tested.

Table 3. Virulence formulas and reaction of differential soybean cultivars to new races of *Phytophthora sojae* found in Ohio

Race[u]	Virulence formula[v]	Susceptibility of cultivars with different *Rps* genes[w]							
		1-a	1-b	1-c	1-d	1-k	3-a	6	7
S28	1a,1b,1k,7	S[x]	S			S			S
S29	1a,1b,1k,6,7	S	S			S		S	S
S30	1a,1b,1k,3a,6,7	S	S			S	S	S	S
S31	1b,1c,1d,1k,6,7		S	S	S	S		S	S
S32	1b,1k,6,7		S			S		S	S
H17	1a,1c,1d	S		S	S				NT[y]
H18	1a,1b,3a,6	S	S				S	S	NT
H19	1a,1d	S			S				NT
H21	1a,1b,1d,6	S	S		S			S	NT
H22	1a,1c,1d,3a,6	S		S	S		S	S	NT
H23	1a,1b,1d,3a,6	S	S		S		S	S	NT
H25	1b,1d		S		S				NT
H26	1a,1c,1d,6	S		S	S			S	NT
H28[z]	1a,1b,1d,3a,6	S	S		S		S	S	NT
H29	1a,1k	S				S			NT
H30	1a,1c,1k	S		S		S			NT
H31	1a,1c,1k,6	S		S	S	S		S	NT
H32	1a,1k,6	S				S		S	NT

[u] S series from A. F. Schmitthenner (*unpublished*), sequence starts with the last published race (26); H series from Hobe (10). H20, 24, 27, and 33 were not included because they were identical to race 23, 6, H19, or H23, respectively. H34–38 were not included because they were separated from races 1, 4, 5, 8, or 9 by reaction on PI 68708, which is not an accepted differential.
[v] Listing of genes compatible to race.
[w] 1-a In cv. Harlon, 1-b in Harosoy isoline 13XX, 1-c in cv. Williams 79, 1-d in PI 103091, 1-k in cv. Williams 82, 3-a in PI 171442, 6 in cv. Altona, and 7 in cv. Harosoy.
[x] Susceptible or compatible interaction.
[y] Not tested on cv. Harosoy.
[z] Differs from H23 by incompatible reaction on cv. Tracy.

Table 4. Location of races of *Phytophthora sojae* isolated in northwest Ohio in 1978–1980

County	Field no.	Isolates (no.)	Races identified[x]
1978 Soil isolates[y]			
Fulton	1	79	2, 3, 4, 6, 7, 9, 23, and 5 new
Defiance	2	34	6, 7, and 2 new
Wood	3	30	1, 3, 4, 5, 6, 7, 9, and 1 new
Wood	8	8	1, 3, 8, 9
Ottawa	4	13	3, 7, 9, and 1 new
Erie	5	4	7, 13
Williams	6	2	4
Huron	7	5	7, 9, and 1 new
1980 Soil isolates[y]			
Wood	3	4	8, 9
Erie	5	1	7
Putnam	9	5	1, 5, 6, 9
Putnam	10	1	1
Paulding	11	5	7
Williams	12	4	7, 9
Henry	13	4	4, 7, 8
1980 Plant isolates[z]			
Wood	3	5	3, 6, 7, 9, and 1 new
Erie	5	7	1, 2
Putnam	9	10	1, 3, 6, 14, and 1 new
Putnam	10	10	3, 5, and 2 new
Paulding	11	7	1, 3, 7, and 1 new
Williams	12	10	3, 7, 9, and 1 new
Henry	13	4	4, 5

[x] Based on differential virulence to *Rps* genes 1-a, 1-b, 1-c, 1-d, 1-k, 3-a, and 6 as summarized by Ward (28). Isolates designated new have a different virulence pattern from races 1–27.
[y] Isolated from soil using the leaf disk method (4).
[z] Isolated from diseased soybean plants using a selective medium.

Races obtained from individual fields in 1978–1980 are summarized in Table 4. The larger the number of isolates tested from a field, the larger the number of different races identified. The race that predominated in one field did not necessarily predominate in others (*data not shown*). For example, over all soil and plant isolations, race 7 was most prevalent in fields 1 and 2, and race 3 in fields 3 and 10. Races 7 and 9 also were prevalent in field 3, and race 7 predominated in field 4. Races 1 and 3 were most prevalent in field 9, and races 3 and 9 in field 12.

In 1990, three sets of *P. sojae* were evaluated. Forty-one isolates were obtained from flood-damaged soybeans, 167 from Sloan seedlings planted in soil samples collected from 65 fields of northwest Ohio, and 40 from Amcor 89 seedlings planted in the above soils. The data are summarized in Table 2. The most prevalent virulent phenotype was race 3, followed by race 7, race 4, and race 1. Races 5, 13, 14, 15, and 22 were isolated infrequently from Sloan. Race 25, not previously reported from Ohio, and five new phenotypes virulent to *Rps*1-k also were found. The differential reactions of these new races are listed in Table 3 with the prefix 'S'. Most common races were isolated from all three sources, but the incidence of races virulent on *Rps*1-k was higher from the flooded field plants and the Amcor 89 seedlings.

The distribution of races by county in 1990 is summarized in Table 5. As with the 1978 collections (Table 4), the greatest numbers of races were obtained from the counties from which the greatest numbers of isolates were obtained. No one race predominated over all counties. Race 3 was most prevalent in seven counties, race 7 in four counties, race 1 in three counties, and race 9 in one county. Races 3 and 7 were both present in nine counties. Ten new race phenotypes were found in four counties.

In 1991, 75 isolates were obtained by bioassay of 23 soil samples with either cv. Sloan or Amcor 89. The results are summarized in Table 2. All commonly isolated races were found except race 5. Race 3, race 7, race 9, and new races that were virulent on *Rps*1-k were most prevalent. The new race phenotypes found were the same as encountered in 1990.

Prevalence of new race phenotypes of *P. sojae*. Five isolates of race 25, new

to Ohio, were obtained from Delaware, Erie, Ottawa, and Putnam counties in 1990. Of the new race phenotypes to which *Rps*1-k was ineffective (Table 2) in 1990, two isolates were obtained from a field of Pella 86 and one from Williams 82 in Delaware county, five isolates from a field of Amcor 89 in Erie county, one isolate from a field of Pella 86 in Putnam county, and one isolate from Resnik in Wyandot county. Six isolates were recovered from damped-off cv. Sloan bioassay seedlings from one soil sample from Crawford County, one isolate from Defiance County, three isolates from Huron County, five isolates from two soil samples in Ottawa County, and five isolates from one soil sample in Seneca County. Four isolates were obtained from damped-off bioassay Amcor 89 seedlings from one soil sample from Huron County, five isolates from three soil samples from Ottawa County, and two isolates from one soil sample in Paulding County.

In 1991, three isolates of new race phenotypes were obtained from damped-off cv. Sloan using the seedling bioassay from Ottawa County, three isolates from Hancock County, two isolates from Paulding County, and one isolate from Defiance County. Three isolates were obtained from damped-off bioassay cv. Amcor 89 from a soil sample from Ottawa county, six isolates from two soil samples from Hancock county, and four isolates from Defiance county. Over the 2-yr period, a total of 70 isolates to which *Rps*1-k was ineffective were found from 14 fields in 12 counties and represented six different virulence phenotypes.

Verification of new race phenotypes of *P. sojae*. Mass cultures of putative new races were evaluated four times for virulence. One typical isolate of each new phenotype was selected and assigned a race number. The predominant new race phenotype, S28 in Table 3, with the virulence formula 1a,1b,1k,7, was designated race 28. Other unique isolates S29 (1a,1b,1k,6,7), S30 (1a,1b,1k,3a,6,7), and S31 (1b,1c,1d,1k,6,7) were designated race 29, race 30, and race 31, respectively.

Virulence of both single-zoospore and single-oospore isolates of each new race was evaluated. For race 28, 15 out of 19 single-zoospore and 29 out of 30 single-oospore cultures had the same reaction as did the parent culture. For race 29, all 29 single-zoospore and 29 out of 30 single-oospore cultures had the same reaction as the parent culture. For race 30, 59 out of 60 single-zoospore and all 30 single oospore cultures had the same reaction as the parent culture. Race 31 was more variable. Among 32 single-zoospore isolates tested, 10 had the same reaction as the parent culture, but 22 were avirulent on *Rps*1-c and *Rps*1-d. Isolates with this new virulence formula of 1b,1k,6,7 were designated race 32. Among 20 single-oospore cultures tested, 16 had the reaction of race 31 and four had the reaction of race 32. New race phenotypes reported by Hobe (10) were neither evaluated on Harosoy nor saved in culture and therefore could not be verified and named as new races.

Variability within field isolates of *P. sojae*. Race reaction of selected mass cultures isolated in 1978–1980 and single-zoospore isolates derived from them are summarized in Table 6. Out of 52 single-zoospore cultures tested, 25 had a different virulence pattern than the mass culture from which they were obtained.

Race phenotype expressed by mixed cultures of *P. sojae*. The effect of a mixture of races was evaluated for virulence phenotype (Table 7). Race 7 was virulent on Amsoy 71 regardless of the proportion of avirulent race 1 mixed with it.

DISCUSSION

The incidence of different *P. sojae* races in Ohio (Table 2) was similar to that of Indiana (15,16) and Ontario (1). Race 1 is still a major component of the *P. sojae* population, except in Alabama (6) and New York (23). Race 1 appears to make up a larger proportion of *P. sojae* in the western part of the midwest United States, where Phytophthora rot damage is more recent. Race 3 is the most frequently reported race from diverse

Table 5. Distribution of races of *Phytophthora sojae* isolated from northwest Ohio in 1990–1991

County	Fields *P. sojae* (no.) Absent	Present	Isolates[y] (no.)	Races identified in order of prevalence[z]
1990 Isolates from cv. Sloan bioassay				
Allen	1	1	1	7
Crawford	1	7	26	3, 7, 5, 4, 8, 14, 22
Defiance	0	6	23	7, 3, 4, 1, 8, new, avir
Erie	0	1	4	1, 7
Hancock	1	3	8	3, 7, 1
Hardin	4	1	3	3, 7
Huron	1	1	7	3, 7, ambiguous
Marion	0	4	12	7, 1, 3, new, ambiguous
Ottawa	1	4	28	3, 1, new, 7, 9, 4, 13, 15
Paulding	2	2	9	3, 7, 8, 9, 1, 4
Seneca	0	6	33	1, 3, 4, 7, 13
Van Wert	3	3	3	3, 8
Williams	0	2	3	7, 5
Wood	3	2	3	9, 3
Wyandot	1	2	7	1, 4, 3
1990 Isolates from Amcor 89 bioassays				
Crawford	6	2	3	7, 3
Defiance	1	5	6	3, avir, 4, new
Erie	0	1	1	4
Hancock	3	1	2	avir
Hardin	3	2	5	avir, 3, new, ambiguous
Ottawa	2	3	12	new, 1, avir, 9, 25, ambiguous
Paulding	2	2	4	new, 3, 8
Van Wert	5	1	3	3
Wood	4	1	2	3
Wyandot	2	1	1	7
1990 Isolates from field isolations				
Delaware	0	3	4	new, 25, avir
Erie	0	1	8	new, 25
Henry	0	1	1	avir
Huron	0	1	3	3, new
Paulding	0	1	3	7, avir
Putnam	0	3	10	4, 7, avir, 25, new
Seneca	0	1	2	new
Wood	0	1	2	8, new
Wyandot	0	6	8	3, avir, 4, 7, 8, new
1991 Isolates from Sloan and Amcor 89 bioassays				
Allen	1	0		
Defiance	1	1	9	new, 8, avir
Hancock	2	2	15	new, 3, 7, 9
Hardin	2	1	1	4
Ottawa	0	3	29	new, 3, 7, 9, 1, avir, 4
Paulding	0	1	2	new
Putnam	1	1	9	3, 9, 7
Van Wert	1	2	6	7, 3, 9
Wood	2	2	4	9, 3, 7

[y] Isolates from damped-off soybean cv. Sloan seedlings planted in six subsamples of 1,200 g of soil from each field.

[z] Based on differential reactions listed in Table 1. New isolates are different from races 1–27. Avir isolates did not kill any of the soybean cultivars inoculated. Ambiguous isolates gave variable results each time tested.

geographic regions (1,7,13,15,16,24). Race 4, virulent on cultivars with *Rps*1-a and *Rps*1-c, is more prevalent in Minnesota, South Dakota, and Ontario (1,7,13). It is a minor component of the *P. sojae* population in Ohio (Table 3), Indiana, and Wisconsin (15,16,24), even though cultivars with *Rps*1-c have been widely grown in these states.

Race 7 appears to be almost as prevalent as race 3 in Ohio (Table 3), Indiana, and Ontario (1,15,16), but has not been reported from Minnesota, South Dakota, Nebraska, New York, or Alabama (1,6,7, 13,15,23,29), and was isolated less frequently in Wisconsin (24). In Ohio, both race 3 and race 7 are frequently encountered in the same soil (Tables 4 and 5). Race 8 or race 9 is prevalent in Ohio (Tables 2), Ontario, Indiana, and Wisconsin (1,13,15,24). Races 7, 8, and 9 are virulent on *Rps*1-a; and their incidence increased at about the same time as race 3, which is virulent only on *Rps*1-a. It is difficult to explain how races 7, 8, and 9, with additional virulence genes compared to Race 3, have become significant components of *P. sojae* populations in Ohio. Cultivars with *Rps*1-d, *Rps*3a, or *Rps*6, with differential reactions to races 7, 8, or 9, respectively, have never been widely grown in the areas where these races are prevalent. In contrast, races 4 and 5, with virulence genes for frequently planted *Rps*1-c cultivars, have not increased as expected in Ohio (Table 2), Indiana, and Wisconsin (15,16,24), but have become prevalent in Ontario, Minnesota, and South Dakota (1,7,13). Races 2 and 6 were not encountered in this investigation. One isolate each of races 13, 14, and 15 was found, but none of the other races first described in the southern United States (11,12). In general, races were isolated nonselectively from seedling baits in Ohio and Ontario, but from infested plants from other locations. The disparities in distribution of races might reflect the cultivar source of the isolates or the cropping history of the field from which isolates were obtained, but such information is not available.

A large number of undescribed race phenotypes was obtained in this investigation by baiting soil with either leaf disks or soybean seedlings (Table 4). Tooley et al (23,24) also reported widely divergent virulence types in Wisconsin and New York. Apparently, *P. sojae* exists in soil in many virulence forms that are abundant enough to be isolated with baits or seedlings. They did not develop as a result of widespread production of cultivars with new resistance genes, and many appear to have unnecessary virulence genes. They constituted approximately 7.5% of the population sampled in 1978–1980, 11.7% in 1990, and 18.2% in 1991. Most of these new race phenotypes were virulent on the major resistance gene, *Rps*1-k, widely deployed for control of this disease.

It is concluded that *P. sojae* is a highly variable pathogen and exists in soil in a wide variety of virulence phenotypes to which most *Rps* genes are ineffective. The prospects for increase in these new race phenotypes sufficient to cause widespread damage on cultivars with *Rps*1-k are unknown. Race 1 was the only known race in Ohio from the time Phytophthora rot was first identified in 1954 until 1972. Resistance to race 1 was identified in 1957 in some cultivars that were produced on a limited scale, but cultivars with race 1 resistance were not widely grown in Ohio until the release of Harosoy 63, Lindarin 63, and Clark 63. Then the *P. sojae* race picture changed dramatically; races 3, 4, 5, 6, 7, 8, and 9 all were detected between 1972 and 1979 as the consequence of widespread production of cultivars with just the *Rps*1-a gene. The second resistance gene, *Rps*1-c, widely used to replace *Rps*1-a resistance, only provides some disease control in Minnesota (13) and Ohio because of the increase of races 4 and 5. The time cycle for loss of disease control with *Rps*1-a and *Rps*1-c was 8–10 yr for each gene from the time of introduction of resistant cultivars with these genes. The third cycle of resistance was initiated with Williams 82 (*Rps*1-k) to control races 1–9, but compatible race phenotypes had already been found in soil (10). If race increase patterns are to be repeated, we can expect widespread loss of control in cultivars with *Rps*1-k within a few years.

It is not known if these new races are capable of increasing in the field in competition with established races. These new races occasionally were obtained from damped-off seedlings of a universally susceptible cultivar, indicating that they are abundant enough to infect plants in competition with more common races. Virulent races of *P. sojae* apparently can infect susceptible plants even though they are a minor component of a race mixture. Race 7 (virulent) initiated infections in a cultivar with *Rps*1-a when mixed with race 1 (avirulent) (Table 7) in proportions as low as 10%. It was concluded that virulence will be expressed over avirulence in race mixtures. Ward (27) has confirmed this principle in more extensive research with different races and cultivars using mixtures containing 5% compatible zoospores. Apparently zoospores of a compatible race can readily infect soybeans in the presence of incompatible race zoospores.

Vanderplank (25) has conjectured that increase in virulence is generally accompanied by loss of aggressiveness or fitness. Pathogenic aggressiveness in the

Table 6. Virulence of single zoospores cultures obtained from field races of *Phytophthora sojae*

	Field cultures[w]		Single-zoospore cultures[y]	
Source[v]	Race[x]	Isolates (no.)	Different[z] (no.)	Races
Soil	3	1	1	9
	4	2	1	4,5
	5	1	1	3
	7	18	5	7,9,H23,H24,H28
	9	4	2	3,9,H21
	17	1	0	17
Plants	1	7	1	1,2
	3	8	6	3,7,9,H27,H29
	4	1	1	1
	5	1	1	5,9
	7	6	3	1,7,9
	9	4	2	3,9,H27
	23	1	1	9,H23

[v] Soil isolates were obtained using the leaf disk bait method (4) in 1978. Plant isolates were obtained from diseased plants in the field in 1980.
[w] Isolates were maintained as mass cultures.
[x] Based on virulence on cultivars with different *Rps* genes as listed in Table 1 for races 1–9. Prefix H designates new races found by Hobe (10).
[y] Single zoospores randomly selected from zoospores formed by a field culture.
[z] Number of single zoospore isolates having a different virulence pattern than the original field race.

Table 7. Reaction of selected soybean cultivars to mixtures of races 1 and 7 of *Phytophthora sojae*

Soybean cultivar	*Rps* gene	All race 1	All race 7	Reaction of ratio race 1:race 7				
				9:1	4:1	1:1	1:4	1:9
Amsoy	*rps*	S[z]	S	S	S	S	S	S
Amsoy 71	*Rps*1-a + *Rps*7	R	S	S	S	S	S	S
Vickery	*Rps*1-c	R	R	R	R	R	R	R
Kingwa	*Rps*1-k	R	R	R	R	R	R	R

[z] S = all plants killed; R = no plants killed.

P. sojae-soybean interaction can be measured as the rate of rot in wound-inoculated roots (20). Using this criterion, races that are more virulent (possess more virulence genes) than race 1 appear to be as aggressive as race 1 (A. F. Schmitthenner, *unpublished*). The most commonly isolated new race in Ohio, race 28, with the virulence formula of 1a,1b,1k,7, is virulent on only four resistance genes and is thus equally virulent to race 7, which is well-established in Ohio. Thus, lack of aggressiveness would not prevent an increase of these new races. However, considerable time could lapse before they become prevalent enough to cause serious damage.

It is concluded that resistance may not be as effective in controlling Phytophthora rot in the future as it is now unless new genes for resistance are found, or existing genes are pyramided to control more races. *Rps*1-d and *Rps*3-b are not being used at present. Combinations of *Rps*1-c and *Rps*3-a have been very effective in the southern United States but have not been widely used in the north. Examination of Table 1 may indicate other gene combinations that would be effective against the most races.

Descriptions of some *P. sojae* races have changed since they were first reported. For example, reaction of race 6 (8) was extended by addition of the differential line, PI 103091 (14). Reactions for races 12, 14, 17, and 19 given by Ward (28) differ from the original reaction type (11,12). Reactions of races 12, 16, 19, and 20 were extended to include *Rps*1-k. Race 14 is now considered virulent to *Rps*1-a and therefore identical to race 4, whereas Keeling (11) indicated avirulence to this genotype. An isolate with the original virulence phenotype for race 14 was found in Ohio as a part of this investigation. Some of the inconsistencies could have resulted from using Harosoy 63, which has both *Rps*1-a and *Rps*7 alleles, as a differential. Also, stability of race reaction in field isolates may be a problem. Some isolates always gave a consistent reaction, whereas reactions of other isolates have changed. In our race survey, we found that some cultures that initially were considered new race phenotypes had different virulence patterns after storage and retesting. Isolates of races 10, 12, 16, and 19 from two sources maintained in culture for long periods of time no longer give the expected race reaction (A. F. Schmitthenner, *unpublished*). Such changes reflect the instability of virulence in mass cultures of *P. sojae*.

Differential cultivars used for *P. sojae* race identification have changed with time. The original set of differentials consisted of Harosoy, Harosoy 63, Sanga, Mack, PI 103091, PI 171442, Altona, and Tracy. These cultivars were subsequently found to have *Rps*7, *Rps*7 + *Rps*1-a, *Rps*1-b, *Rps*1-c, *Rps*1-d, *Rps*3-a, *Rps*6, and *Rps*1-b + *Rps*3-a, respectively. Hobe (10) substituted Amsoy and Amsoy 71 for Harosoy and Harosoy 63, respectively, in the 1978–1980 tests because seeds of the latter were not generally available. Amsoy 71 is resistant to races 1 and 12 (*data not shown*) and was assumed to be an acceptable substitute for Harosoy 63. The reaction of Amsoy to race 12 is unknown, and it may or may not have *Rps*7 even though one of the parents was Harosoy. Therefore, the reaction of Hobe's isolates (10) against *Rps*7 is unknown. Unfortunately, authentic cultures of race 12 are not available (A. F. Schmitthenner, *unpublished*), and reaction of Amsoy to this virulence phenotype cannot be tested until a suitable substitute is obtained. Sanga, Vickery, and Rockford were substituted for D60-9647, Mack, and Altona, respectively, because they gave the same reaction to known races and were readily available. Kingwa, subsequently found to carry *Rps*1-k, was added to the differential set as another type of resistance.

For the 1990 and 1991 evaluations, all available cultivars with *Rps*1-a were tested with races 1 and 12, and only Harlon was susceptible to race 12 but resistant to race 1 (*data not shown*). Consequently, this cultivar was substituted for Harosoy 63 to eliminate a double gene combination from the differentials. Harosoy 13XX, Williams 79, and Williams 82 were substituted for Sanga, Mack, and Kingwa, respectively, because they were more readily available and known to have *Rps*1-b, *Rps*1-c, and *Rps*1-k, respectively. Sloan was added to detect nonpathogenic isolates. Sloan is susceptible to all *P. sojae* races (*data not shown*). For future race identifications, it should be possible to use the Williams isolines being developed by R. L. Bernard, University of Illinois (*personal communication*) or Harosoy isolines being developed by R. I. Buzzell at Harrow, Ontario (*personal communication*), but complete sets are not yet available.

Variable or ambiguous reactions were occasionally found with tests of a large number of field isolates of *P. sojae* repeated over time. Such variable isolates were designated as unclassifiable. The traditional method of handling unclassifiable isolates is to continue testing until a consistent reaction is obtained. A second option would be to select single-zoospore isolates from the cultures that give a consistent reaction. However, single-zoospore isolates may not have the same race phenotype as parent cultures (Table 6). Furthermore, selection of a single-zoospore isolate does not necessarily ensure race stability. Rutherford et al (21) reported race changes following several successive single-zoospore propagations. Race 27 was selected from the fifth consecutive single-zoospore propagation from race 16 (27). A third option would be to select single-oospore isolates. Bhat et al (2) have demonstrated heterogeneity within field isolates of *P. sojae*. They were able to select pure lines following successive single-oospore propagations. This inconsistent race reaction with time in storage seems to be more pronounced with the new race phenotypes.

The simplest explanation for variation in virulence with time is that mass cultures could be mixtures of races. Mixtures could result in several ways. Layton and Kuhn (17) demonstrated coinfection of soybeans with two compatible races and with one compatible and one incompatible race. Mixtures of races could be isolated from diseased tissues and persist in culture. Also, it is possible that coinfection could result in heterokaryon formation (17,18) or outcrossing (3). During normal culturing and culture storage procedures, vegetative segregants from heterokaryons or segregants from hybrid oospores could become large enough components of cultures to express a different virulence phenotype. Only 5–10% of a virulent component may be necessary in a mixture to induce a compatible interaction (Table 7; 27). However, both Layton and Kuhn (17) and Long and Keen (18) have reported heterokaryon formation to be a rare phenomenon.

It is concluded that simple race mixtures or mixtures resulting from segregation of heterokaryon or hybrid oospore progeny could account for changes in race reaction of field cultures over time. It is probable that heterogeneity was established prior to isolation and that selection of virulence variants occurred during subsequent culturing. Research now is in progress to eliminate this variation by selection of single-zoospore or single-oospore cultures that exhibit a stable race phenotype.

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