Development of Pure Lines of *Phytophthora sojae* Races

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ABSTRACT

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Single-oospore isolates were obtained from field isolates of *Phytophthora sojae* and from successive selfed generations and were evaluated using a hypocotyl-inoculation method for race phenotype on soybean (*Glycine max*) cultivars having *Rps*, *Rps*1, *Rps*1-c, *Rps*1-k, or *Rps*6 *Phytophthora*-resistance genes. Several races were found among S_1 (first generation) progeny of race 1 and 4; race 3 bred true. Single-oospore isolates unable to kill soybean seedlings also were found and were designated universally avirulent. Among S_1 progeny of race 1, the ratio of race 1 to avirulent was 3:1. There were no avirulent isolates among the progeny of race 3 and 4. Single-oospore lines with desired phenotypes were selected for further selfing from among the progeny having the least segregation. One race 1 line bred true for race 1 phenotype in the second generation. A second race 1 line segregated for race 1 and 4 and avirulent in early generations but bred true for the race 1 phenotype by the seventh generation. Avirulent isolates, which did not cause disease on the universally susceptible cultivar Williams, bred true by the third selfed generation. True-breeding race 4 was obtained after the third generation of selfing. This study demonstrated that some field isolates of *P. sojae* are heterokaryotic and/or heterozygous for virulence genotypes, and pure-breeding races could be developed by repeated selfing of oospore progeny.

The diploid, homothallic fungus *Phytophthora sojae* M.J. Kaufmann & J.W. Gerdemann (synonym *P. megasperma* Drechs. f. sp. *glycinea* T. Kuan & D.C. Erwin [11]) is the causal agent of root and stem rot of soybean (*Glycine max* (L.) Merr.). Several good reviews of Phytophthora root and stem rot are available (26,32).

Variation in pathogenicity and virulence of *P. sojae* has been studied widely (2,12,13). Hildebrand (12) reported that *Phytophthora* isolates from Ohio and Illinois were less virulent on soybeans than were isolates from Ontario, establishing the existence of strains in *P. sojae*. Hilty and Schmitthenner (13) noted variation in virulence and cultural characteristics among single-zoospore isolates. In their study, single-zoospore isolates of field isolates from different regions and within regions varied from nonpathogenic on all soybean cultivars tested to highly virulent on the susceptible cultivars, with no relationship between pathogenicity and cultural characteristics. Averre and Athow (2) reported that virulence was not associated with growth rates, oospore formation, or morphological characteristics in culture. They reported that highly virulent isolates killed susceptible cultivars without killing any seedlings of resistant cultivars. However, weakly virulent isolates produced either many lesions without killing the seedlings or no infection on susceptible cultivars. Morgan and Hartwig (23) first reported physiologic specialization in *P. sojae* and described races 1 and 2. Since this study, a total of 27 *P. sojae* races have been reported (16,31) based on differential virulence on 12 identified, race-specific resistance genes (1,24,32).

Hobe (14) reported that many single-zoospore isolates varied in virulence phenotypes from the original mass isolates, and some were entirely new races from the original. Tooley et al (30) reported the presence of diverse physiologic races of *P. sojae* in soybean-growing regions of Wisconsin in 1978 and 1980, in addition to a shift in frequency of virulent isolates between the two growing seasons. Thus, the high degree of potential pathogenic variability within natural populations of *P. sojae* casts doubt on the long-term effectiveness of *Rps* genes in soybean.

In 1985, Rutherford et al (25) found variation in virulence after successive single-zoospore propagations of *P. sojae* race 3 and 6. In addition, some isolates of race 1 and 3 lacked virulence on any soybean cultivar tested, yet these avirulent isolates retained their ability to infect soybean and elicit glyceollin production. Given the high level of variation among asexual isolates of *P. sojae* and other *Phytophthora* species (9), it is apparent that genetic purity of isolates is a concept, not a reality. Therefore, isolates obtained directly from soil or diseased samples, even by single-zoospore descent, may not be suitable for studying the genetics and molecular biology of *P. sojae*-soybean interactions.

Because *P. sojae* is vegetatively diploid (21), selfed progeny would show a Mendelian monohybrid segregation ratio of 3:1 (or 1:2:1) for dominant characteristics if the selfed isolate is heterozygous at a single locus. Selfing an isolate for seven generations with selection in each generation for particular traits would lead to a pure-breeding line homozygous at almost all loci (99%). On the other hand, if the characteristic is either dominant or recessive homozygous at a single locus, pure lines could be verified by two selfed generations. Establishing a collection of single-oospore isolates of different generations is laborious and time-consuming but necessary for genetic analysis.

The objective of this study was to obtain pure lines of *P. sojae* race 1, 3, and 4 through repeated selfing using a new simple method for single-oospore colony isolation. Virulence of progeny was tested at each generation. Brief reports of some of the results of these experiments have been published (5,6).

MATERIALS AND METHODS

Oospore production, harvesting, and germination. *P. sojae* race 1, 3, and 4 were isolated from diseased soybean plants in Ohio and were used for oospore production. A procedure was developed to produce and harvest contaminant-free, single oospores (3). Isolates were grown on lima bean agar (LBA) (extract of frozen lima beans, 50 g/L, filtered through diatomaceous earth and

solidified with 1.5% Bacto-agar). Twenty mycelial discs (4 mm in diameter) of each of the three races were cut from the edges of actively growing cultures and were placed in a 200-ml Erlenmeyer flask containing 20 ml of lima bean (LB) broth (extract of frozen lima beans, 50 g/L, filtered through diatomaceous earth). Cultures were incubated under cool-white fluorescent light (10 μmol cm^{-2} s^{-1}) 9 h per day at 24 C for a minimum of 30 days to produce mature oospores (15).

A portion of an isolate (15%) was transferred to 4 ml of sterile distilled water in a 5-ml vial and was mechanically sheared, aseptically, at 1,000 rpm for 60 s by a Tissue-Tearor (Biospec Products, Bartlesville, OK). The homogenized mixture of mycelium and oospores was incubated in 2,000 units/ml of β-glucuronidase (type H-1; Sigma Chemical Co., St. Louis, MO) at 37 C for 14 h in a sterile polyethylene, 15-ml centrifuge tube. The mixture was centrifuged at 1,900 *g* for 5 min and was washed five times with distilled water. After freezing at −20 C overnight, contents in the tube were thawed by immersion in water at 45 C. Immediately after thawing, the floating mycelial fragments were removed aseptically with a Pasteur pipette, and the oospores were pelleted by centrifugation.

Approximately 200 oospores were spread uniformly in a petri plate on 1.5% water agar containing 10 μg each of cholesterol and rifampicin per milliliter (Sigma). Cholesterol was added as a dimethyl sulfoxide solution prior to autoclaving, and rifampicin was added as a methanol-water solution (1:100 v/v) after autoclaving. Residual oospores were stored at −20 C for further use. Oospores began germinating 4 days after plating and formed individual colonies at 7 days. Single-oospore colonies were picked up from isolation plates at intervals of 2–3 days for 1 mo and were transferred to LBA. Those oospore colonies contaminated with bacteria or other fungi were purified using PBINC media, a dilute V8-juice agar (40 ml of extract autoclaved with 0.6 g of $CaCl_2$ filtered through diatomaceous earth and solidified with 15 g of Bacto-agar) supplemented with 0.04 g of pentachloronitrobenzene (0.054 g of Terrachlor; Uniroyal Chemical Company Inc., Middlebury, CT), 0.01 g of benomyl (0.02 g of Benlate, E. I. Du Pont de Nemours & Co., Inc., Wilmington, DE), 0.02 g of iprodione (0.04 g of Rovral, Rhone-Poulenc Ag Company, Research Triangle Park, NC), 0.1 g of neomycin sulfate, and 0.01 g of chloroamphenicol (Sigma) per liter of medium. All single-oospore isolates were stored on LBA plates at 8 C after 10-days growth at 24 C.

Numbering of single-oospore isolates. *P. sojae* field isolates of race 1, 3, and 4 were designated as R1, R3, and R4, respectively. Single-oospore isolates derived from these races were given consecutive numbers indicating the number of selfed generations. Each decimal point in the isolate designation represents one selfed generation, and each number to the right of the decimal point indicates the specific isolate. For example, R4.3.46.62 is the 62nd third generation (S_3), single-oospore isolate derived from the 46th, second generation (S_2), oospore isolate derived from the third oospore isolate of the first generation (S_1) of field isolate R4 (S_0).

Virulence evaluation. Differential soybean cultivars, Altona (*Rps*6), Amsoy 71 (*Rps*1), Sloan (*rps*), Vickery (*Rps*1-c), Williams (*rps*), and Williams 82 (*Rps*1-k), were used to evaluate the virulence of the S_1 of *P. sojae* race 1 and 4 field isolates. Virulence of S_1 single-oospore isolates from race 3 was evaluated only on cultivars Amsoy 71, Williams, Williams 79 (*Rps*1-c), and Williams 82. Cultivars containing *Rps*1, *Rps*1-c, *Rps*1-k, or *Rps*6 were resistant to race 1; cultivars containing *Rps*1-c, *Rps*1-k, or *Rps*6 were resistant to race 3; and cultivars containing *Rps*1-k or *Rps*6 were resistant to race 4. Sloan and Williams were susceptible to all these races. Race differentiation of the second and succeeding generations was determined by differential reaction on Williams, Williams 79 and 82, and Amsoy 71.

Virulence of isolates was evaluated with a modified hypocotyl inoculation method (10,32). A test unit consisted of 10–12 plants of a differential in one 10-cm-diameter pot containing coarse vermiculite. Each differential was planted in a separate pot. All differentials used in a test were inoculated by an isolate at the same time. *P. sojae* isolates were grown on LBA for 7 days at 24 C, were macerated by passage through an 18-gauge needle at the end of a 10-ml syringe. The upper portions of hypocotyls of 7-day-old plants were split longitudinally with the syringe tip for about 1 cm and approximately 200 μl of inoculum (375 cfu/ml) was deposited in and around the slit. Inoculated plants were covered with plastic sheets for 12 h to maintain high humidity and afterward were incubated at an ambient temperature of 25 C under light (Metalarc R, Sylvania, Manchester, NH; 90 μmol cm^{-2} s^{-1}) for 14 h per day. After a week, dead plants were counted. Isolates were designated as virulent on a particular soybean cultivar if more than 50% of the seedlings were killed in 7 days (18). Otherwise, the isolate was considered an avirulent race on that cultivar.

An attempt was made to evaluate a minimum of 30 progeny in the second and subsequent generations because this number would be sufficient to detect a recessive phenotype for single-locus segregation. However, not all isolates produced the same number of germinating oospores, and 30 progeny were not always available for evaluation. Therefore, only isolates exhibiting growth similar to field isolates with prolific oospore production, limited segregation for undesired phenotypes, and a clear avirulence (no plants killed) or virulence (all plants killed) were selected for further selfing. The selfing was continued until an isolate showed no further variation for its race phenotype. The minimum time difference between two successive selfed generations was 60 days.

RESULTS

Virulence of S_1 single-oospore isolates. A total of 110 S_1 single-oospore isolates derived from a *P. sojae* race 1 field isolate were evaluated for virulence on six soybean cultivars (Table 1). The reaction of 73 of the isolates was similar to race 1. Of the remaining 37 S_1 isolates, 25 were unable to kill any of the cultivars, including universally susceptible Sloan and Williams, and were designated universally avirulent (4). A hypersensitive response was observed on hypocotyls inoculated with avirulent isolates; the resulting necrotic symptoms were indistinguishable from the typical incompatible interaction of field races on resistant cultivars. The ratio of a race 1 phenotype to avirulent was 73:25, which did not differ significantly from 3:1 ($P = 0.95 < \chi^2 = 0.014 < 0.90$). The virulence of three isolates was similar to a race 4 phenotype. Altona, Sloan, and Williams were susceptible to eight isolates that were indistinguishable from race 13, 17, or 24 based on the differentials tested. One isolate had an undescribed virulence as it attacked a cultivar with *Rps*1-k but not those with *Rps*1, *Rps*1-c, or *Rps*6. The ratio of virulent to avirulent on universally susceptible cultivars was 85:25, which did not differ significantly from 3:1 ($P = 0.70 < \chi^2 = 0.303 < 0.50$).

All 39 S_1 single-oospore isolates from the race 3 field isolate were race 3. Of the 109 S_1 single-oospore isolates from the race 4 field isolate, 89 were of the parental virulent phenotype (Table 1). Four isolates had a race 1 phenotype. Ten isolates were virulent on all cultivars evaluated, except Altona, which placed them either in race 12, 19, 20, or 25. Altona, Amsoy 71, and Vickery, but not Williams 82, were susceptible to five isolates, placing the five isolates in either race 5 or 22. One isolate was virulent on all six cultivars and therefore, was an undescribed race. No avirulent single-oospore isolates were obtained from the race 4 field isolate.

Virulence of second and subsequent selfed single-oospore isolates. Three S_1 isolates of race 1 were selfed. Progeny from R1.47 segregated for race 1 and 4 in the S_2 (Table 2). A race 1 selection from this line, R1.47.48, had progeny that were all race 1 in the S_3 and was considered a pure line. Race 1 isolates R4.1 and R4.3, originally obtained from a field isolate of race 4 (Table 1), had progeny that segregated for race 1 and 4 and avirulent in the S_2. Two isolates from the R4.1 and 12 isolates from the R4.3 lines were advanced to the S_3, and their progeny all segregated for race 1 and 4 or avirulent. The race 1 isolate from R4.3, selected for selfing, segregated for virulent to avirulent at 74:29 in the S_2 ($P = 0.50 < \chi^2 = 0.547 < 0.30$) and at 70:32 in the S_3 ($P = 0.20 < \chi^2 = 2.209 < 0.10$) neither of which differed

TABLE 1. Virulence of single-oospore isolates obtained in a first selfed generation (S_1) of *Phytophthora sojae* race 1 and 4 field isolates

No. of S_1 isolates	Race	Reaction on soybean cultivar: Sloan (*rps*)	Williams (*rps*)	Amsoy 71 (*Rps*1)	Vickery (*Rps*1-c)	Williams 82 (*Rps*1-k)	Altona (*Rps*6)
Race 1							
...	R1 parent	V[a]	V	A	A	A	A
73	R1	V	V	A	A	A	A
25	Avirulent	A	A	A	A	A	A
8	R13, 17, or 24[b]	V	V	A	A	A	V
3	R4	V	V	V	V	A	A
1	R?[c]	V	V	A	A	V	A
Race 4							
...	R4 parent	V	V	V	V	A	A
89	R4	V	V	V	V	A	A
10	R12, 19, 20, or 25[b]	V	V	V	V	V	A
5	R5 or 22[b]	V	V	V	V	A	V
4	R1	V	V	A	A	A	A
1	R?[c]	V	V	V	V	V	V

[a] V = Virulent (compatible interaction), > 50% of the plants killed; A = avirulent (incompatible interaction), > 50% of the plants killed.
[b] Race 5, 12, 13, 17, 19, 20, 22, 24, and 25 were not distinguishable based on the partial set of differentials used.
[c] Previously undescribed race.

TABLE 2. Virulence of single-oospore isolates of *Phytophthora sojae* race 1 over seven selfed generations, S_1–S_7

Generation	Parent	No. of progeny isolates with specific-race phenotypes[a]: Race 1	Race 4	Avirulent
S_1	R1.47[b]	34	19	0
	R4.1	35	7	15
	R4.3	74	0	29
S_2	R1.47.48	54	0	0
	R4.1.1	19	3	10
	R4.1.6	83	0	25
	R4.3.7	18	3	12
	R4.3.10	17	0	11
	R4.3.16[c]	14	1	5
	R4.3.17	5	0	1
	R4.3.20	11	0	7
	R4.3.30[c]	22	0	9
	R4.3.38	21	0	13
	R4.3.46[c]	70	0	32
	R4.3.122	24	0	11
	R4.3.154	11	13	12
	R4.3.182	17	5	15
	R4.3.193	17	2	15
S_3	R4.3.16.26	29	0	26
	R4.3.30.14[c]	21	0	9
	R4.3.30.98[c]	21	0	9
	R4.3.46.62[c]	28	1	1
S_4	R4.3.30.14.8	27	6	19
	R4.3.30.14.20	2	1	1
	R4.3.30.14.24	28	3	8
	R4.3.30.98.1	14	1	7
	R4.3.30.98.6	14	5	12
	R4.3.30.98.9	3	6	8
	R4.3.30.98.11	27	8	14
	R4.3.30.98.14	10	6	9
	R4.3.30.98.27	12	10	9
	R4.3.46.62.15	13	23	9
	R4.3.46.62.24[c]	39	6	1
	R4.3.46.62.27	19	2	2
	R4.3.46.62.30	19	7	2
S_5	R4.3.46.62.24.10	44	0	0
	R4.3.46.62.24.19	44	0	4
	R4.3.46.62.24.21[c]	41	0	0
S_6	R4.3.46.62.24.21.9	32	0	0

[a] Based on the reaction of single-oospore isolates on 10 plants each of four soybean cultivars with *Rps*1 (Amsoy 71), *Rps*1-c (Williams 79), *Rps*1-k (Williams 82), or *rps*1 (Williams) alleles. An isolate was considered virulent if more than 50% of the seedlings were killed.
[b] The first number indicates the reaction of the field isolate, and the following numbers indicate the single-oospore isolates selected in the S_1, S_2, S_3, S_4, S_5, and S_6, respectively.
[c] Isolates from which race 1 progeny were selected for additional selfing.

TABLE 3. Virulence of single-oospore isolates of *Phytophthora sojae* race 4 over four selfed generations, S_1–S_4

Generation	Parent[b]	No. of progeny isolates with specific-race phenotypes[a]: Race 1	Race 4	Race X	Avirulent
S_1	R1.1[c]	0	115	0	0
	R1.2	0	6	0	0
	R4.3[d]	74	0	0	29
	R4.36	0	64	0	0
	R4.63	0	63	0	0
S_2	R1.1.18	0	30	0	0
	R1.1.36	0	35	0	0
	R1.1.83	0	9	0	0
	R4.3.16[d]	14	1	0	5
	R4.63.17	0	25	16	0
	R4.63.33	0	33	1	3
	R4.63.58	0	44	4	0
S_3	R4.3.16.25[e]	3	15	0	14

[a] Based on the reaction of single-oospore isolates on 10 plants each of the soybean differential cultivars. Race 1 was virulent only on cv. Williams (*rps*); race 4 was virulent on Williams, Amsoy 71 (*Rps*1), and Williams 79 (*Rps*1-c); race X was virulent on Williams, Amsoy 71, Williams 79, and Williams 82 (*Rps*1-k); and avirulent was not virulent on any of the cultivars. An isolate was considered virulent if more than 50% of the seedlings were killed.
[b] Parents were all race 4 with the exception of one isolate indicated to be race 1.
[c] The first number indicates the reaction of the field isolate, and the following numbers indicate the single-oospore isolates selected in the S_1, S_2, and S_3, respectively.
[d] The race 1 isolate was included because it was the parent of race 4 isolate R4.3.16.25.
[e] Race 4 segregant from race 1 isolate R4.3.16.

significantly from a 3:1 ratio. S_4 progeny from R4.3.46.62, selected for further selfing, still segregated for race 1 or 4 or avirulent, but the ratio of virulent to avirulent, 29:1, differed significantly from 3:1 ($\chi^2 = 7.51$, $P < 0.01$). Progeny from S_4 isolate R4.3.46.62.24 also segregated, but the ratio of virulent to avirulent, 45:1, also differed significantly from a 3:1 ratio ($\chi^2 = 12.79$, $P < 0.01$). This isolate was selected for further selfing. S_5 isolate R4.3.46.62.24.21 produced progeny that were all race 1 oospores. One S_6 isolate from this line, R4.3.46.62.24.21.9, was selfed, and all progeny were race 1. It was concluded that the latter was homozygous for the race 1 virulence phenotype. Selfing of other isolates was abandoned at various generations beyond the S_3 because proportions of race 4 and avirulent progeny to race 1 progeny were higher than in the progeny of the R4.3.46.62 line selected (Table 2).

A homozygous race 4 phenotype was attained in two generations of selfing. Two race 4 S_1 isolates from a field isolate of race 1, R1.1 and R1.2, were selfed, and all progeny were race 4 (Table 3). Three S_2 isolates, R1.1.18, R1.1.36, and R1.1.83, when selfed had progeny that were all race 4. The R1.1 isolate was considered to be homozygous for the race 4 phenotype. Segregation of progeny of isolate R4.63 was more complicated. All S_2 progeny were race 4. Progeny of three S_2 isolates, R4.63.17, R4.63.33, and R4.63.58, segregated for race 4, an undetermined race virulent on *Rps*1-k, and avirulent. Selfing of this isolate line was not continued. Selfing of S_1 isolate R4.3 also produced variable results. Isolate R4.3 was race 1. One S_2 selection, R4.3.16, when selfed had one race 4 isolate, R4.3.16.25, that when selfed had race 1 and 4 and avirulent progeny. Selfing of this isolate line was discontinued.

A homozygous universally avirulent phenotype also was attained in two generations of selfing. Two S_1 segregants, R4.1 and R4.3, had both race 1 and avirulent progeny when selfed (Table 4). Three S_2 selections, R4.1.3, R4.3.34, and R4.3.42, when selfed had progeny that were all avirulent. Five R4.1.3 selections when selfed had progeny that were all avirulent. It was concluded that all the R4.1.3 selections were homozygous for the avirulent phenotype.

During the virulence evaluation of *P. sojae* selfed progeny, we noticed that some single-oospore isolates in each of the several generations took 4–5 days to kill susceptible soybean seedlings, instead of the usual 2–3 days; these were considered less aggressive *P. sojae* isolates. Mycelium of such isolates proliferated internally, causing brown discoloration and wilting of soybean seedlings without causing massive rot.

DISCUSSION

This is the first report of selfing *P. sojae* to obtain races that are true-breeding for virulence. In the present study, S_1 progenies from race 1 and 4 consisted of many races in addition to parental types; race 3 bred true. Siblings from race 1 and 4 isolates each contained an undescribed race. Variation in virulence among the S_1 isolates could be the result of a mixture of races, heterokaryosis, and/or the heterozygous nature of the field isolates. Variation in virulence among single-zoospore isolates has been reported (13,14,25). Therefore, variation in isolates can be generated both asexually and sexually. As a result, field isolates may not be suitable for studying the genetics and molecular biology of host-pathogen interactions.

Variation in virulence observed during successive single-oospore selfed generations of race 1 and 4 could have resulted from parasexuality. It has been postulated that heterokaryosis is one of the mechanisms by which variation in *P. infestans* (8) and *P. capsici* (29) is brought about. Heterokaryon formation has been forced in *P. sojae* (17) and reported in inoculated plants (19). However, the incidence of heterokaryon formation in *P. sojae* has been reported as low (20).

Rutherford et al (25) suggested that variation among five successive single-zoospore propagations was probably not the result of heterokaryon formation by means of somatic crossing over followed by mutation because of the high frequency of variants after random single-zoospore isolations. In the present study, the frequency of heterokaryon formation by random mutation in virulence genes during selfing would be very low, and the likelihood of mutated nuclei among hundreds of thousands of other nuclei in the thalli undergoing meiosis and taking part in oospore formation can be ruled out unless mutation occurs at the beginning of the initial few mitotic divisions after oospore germination. Even if some mutation takes place in genes for virulence, the probability of selecting the single-oospore isolates for progeny analysis from oospores carrying mutated virulence genes is very low. Thus, it is highly unlikely that variation among single-oospore isolates in the second and subsequent generations was the result of segregation of components of heterokaryons.

The data obtained for virulence segregation in the first, second, and subsequent generations could be explained readily by a recombination of genes resulting from repeated selfing. *Phytophthora* species, like those of other fungi belonging to the class Oomycetes, are diploid (27). The genetics of species in this genus might be similar to that of higher plants. One *P. sojae* race 1 line (field isolate; S_0) segregated for race 1 virulence and avirulence in a 3:1 Mendelian monohybrid ratio in the S_1, S_2, and S_3. Through selection it was possible to reduce this segregation in the S_4, S_5, and S_6 and to obtain isolates that bred true for race 1 virulence. We hypothesize that this race 1 isolate is heterozygous at a locus conditioning race 1 virulence, and that virulence is dominant over avirulence at this locus. A second race 1 line did not segregate and appeared to be homozygous in the S_2. The universally avirulent condition was homozygous recessive, and pure lines were readily selected. Isolates of race 4 selected for selfing appeared to be homozygous for the race 4 virulence genotype in the S_2 and S_3. No race 3 phenotypes were obtained from race 1 and 4. Only race 3 was obtained from field isolates of race 3, indicating that this field race was homozygous for race 3 virulence. We cannot determine from these data if virulence is dominant or recessive for race 3 and 4. The inheritance pattern of virulence in *P. sojae*, dominant for race 1 and probably recessive for race 3 and 4, may be similar to that of *P. infestans*, reported by Spielman et al (28), in which virulence was dominant for race 2 and 4 and was recessive for race 3. Thus, in nature, *P. sojae* could be heterokaryotic for different races and either heterozygous or homozygous (dominant or recessive) for virulence. Crosses between races have been made, and the results support these hypotheses (3).

Selfing of race 1 isolate-line R4.3.30 was discontinued in the S_5. Progeny from this line had a higher proportion of race 4 segregants than had the progeny from line R4.3.46. Segregation for separate loci for race 1 and 4 virulence probably was occurring in isolate-line R4.3.30. For the same reason, selfing of race 4 isolates R4.36 and R4.63 was discontinued. Progeny of these isolates had a high incidence of race X segregants; those of isolate-line R1.1 had none. Race X differed from race 4 in that it killed Williams 82, which contains the *Rps*1-k resistance gene. Loci for both race 4 and X were probably segregating in isolate-lines R4.36 and R4.63. More selfed generations would have been needed to develop pure lines of race 1 from R4.3.30 or of race 4 from R4.36 or R4.63.

It is evident from these data that considerable variation occurs

TABLE 4. Virulence of avirulent single-oospore isolates of *Phytophthora sojae* obtained from race 1 over four selfed generations, S_1–S_4

Generation	Parent[b]	No. of progeny isolates with specific-race phenotypes[a]		
		Race 1	Race 4	Avirulent
S_1	R4.1[cd]	35	7	15
	R4.3[d]	74	0	29
	R1.29	0	0	39
	R1.35	0	0	14
S_2	R4.1.3	0	0	113
	R4.3.34	0	0	31
	R4.3.42	0	0	41
S_3	R4.1.3.14	0	0	33
	R4.1.3.22	0	0	14
	R4.1.3.53	0	0	43
	R4.1.3.81	0	0	30
	R4.1.3.102	0	0	57
	R4.3.42.33	0	0	1

[a] Based on the reaction of single-oospore isolates on 10 plants each of the soybean differential cultivars. Race 1 was virulent only on cv. Williams (*rps*); race 4 was virulent on Williams, Amsoy 71 (*Rps*1), and Williams 79 (*Rps*1-c); and avirulent was not virulent on any of the cultivars. An isolate was considered virulent if more than 50% of the seedlings were dead 1 wk after hypocotyl inoculation.

[b] Parents all were avirulent except for the two race 1 isolates.

[c] The first number indicates the reaction of the field isolate, and the following numbers indicate the single-oospore isolates selected in the S_1, S_2, and S_3, respectively.

[d] Race 1 isolates were sources of the universally avirulent isolates.

among single-oospore isolates from field races. Some isolates are homozygous for virulence at all loci tested. Some may be homozygous at one virulence locus but heterozygous at others. If virulence loci act independently, then lack of homozygosity may not be important. However, if action of loci is dependent (i.e., some loci are epistatic to others), then homozygosity at all loci is important as a starting point for genetic evaluation of virulence. The pure lines of race 1 and 4 selected from R4.3.46 or R1.1, respectively, are presumed to be homozygous at all virulence loci. However, they were evaluated on only four differentials; it is possible that heterozygosity for another unevaluated race phenotype could exist in these isolates.

During the course of progeny analysis, some single-oospore isolates were recovered that were unable to kill universally susceptible cultivars that had no race-specific resistance genes. These *P. sojae* isolates were considered universally avirulent. Isolates with similar interactions also have been referred to as nonpathogenic or avirulent (12,13,22,25). The avirulent isolates produced in the present study were similar to those reported previously. They were morphologically similar to the virulent isolates from which they were derived in that they grew normally, produced abundant zoospores, produced restricted, brown, hypersensitive lesions, and accumulated glyceollin on inoculated plants (4). Rutherford et al (25) suggested the universally susceptible cultivar Wayne probably has an *Rps* gene that has yet to be identified. Absence of disease could be the result of a lack of aggressiveness (7,25), an acquisition of genes in culture that elicit host-defense reactions, a loss of genes that suppress resistance, or a loss of basic compatibility in the avirulent isolates (25). We suggest the avirulent isolates found in our study were genetically homozygous recessive for lack of virulence at the race 1 virulence locus. The pathogenic capabilities of these avirulent isolates have been published elsewhere (4).

Crosses between the races have been made using the pure lines developed in this study. Segregation data obtained from the F_2 from hybrids (F_1) of these crosses will be used to determine the relationships of virulence to avirulence among races of *P. sojae*. The pure lines obtained also will be useful for investigating the biochemical mechanisms of variation in virulence among races.

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