Races of *Phytophthora megasperma* f. sp. *glycinea* in Wisconsin

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ABSTRACT

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Studies were undertaken to determine the range of physiologic races of *Phytophthora megasperma* f. sp. *glycinea* in Wisconsin in 1978 and 1980 and to investigate the relationship between race frequency and cultivar selection. In 1978, race 3 was isolated at the highest frequency, whereas races 1, 4, 7, 8, and 9 were isolated less frequently. The use of virulence formulae (effective/ineffective host genes) was necessary in 1980 to describe the virulence of the isolates because the variation encountered could not be described under the current numeric classification scheme. Previously undescribed races were found more frequently than were races 3, 9, 1, 5, and 8 and were detected in decreasing frequency on cultivars containing race-specific resistance conferred by the Rps_1^c allele, the Rps_1 allele, and those with no race-specific resistance, respectively. The results suggest that selection pressure exerted by cultivars containing race-specific resistance has resulted in increased virulence in the pathogen population and cast doubt on the long-term effectiveness of race-specific resistance.

Phytophthora root and stem rot of soybean (*Glycine max* (L.) Merrill) caused by *Phytophthora megasperma* f. sp. *glycinea* (Pmg) is important throughout the soybean growing regions of the United States and Canada. It may reduce yields by more than 50% by killing plants or reducing root and shoot growth of plants that are not killed (17).

Hildebrand (9) in his early description of the disease found isolates from Ontario that were more virulent than those from Ohio and Illinois; he also found that isolates differed in growth characteristics at temperature ranges of 25–32.5 C. Hilty and Schmitthenner (10) found differences in virulence among isolates from different sources as well as among those from the same origin. Averre and Athow (3) also found differences in virulence among 31 isolates from the United States and Canada.

These reports provided evidence that an array of genetic variation existed within Pmg, but no distinct physiologic races (pathotypes) were evident. It has become evident that natural populations of Pmg are made up of many physiologic races (8,13,14,18,20,22). Control of Phytophthora root and stem rot in Wisconsin has been achieved by planting soybean cultivars with the Mukden-type resistance to races 1 and 2 (allele Rps_1) (4).

In 1974, however, Pmg was isolated from the resistant cultivar Amsoy 71 from a field in southeastern Wisconsin (E. W. Hanson, *personal communication*). New races other than the original race 1 were identified in Wisconsin in 1977 from a field in which cultivars containing the Mukden resistance had recently failed (6). The prevalence and severity of Phytophthora root rot in Wisconsin increased progressively since 1974, developing into a major epiphytotic in southeastern Wisconsin in 1978. The detection of new races in scattered geographic areas indicates a natural selection for biotypes that exist in local populations rather than the dissemination of Pmg between regions. Because of different cultural practices, climatic conditions, and soybean cultivars grown, the race complex of different geographic regions may differ and needs to be documented in each specific region. The intent of this study was to determine the range of physiologic races of Pmg that are present in Wisconsin and whether race frequencies are correlated with specific soybean cultivar selection. This knowledge is important in advising soybean growers on cultivar selection and in advising plant breeders, soybean seed producers, and agronomists on the current status of Phytophthora root and stem rot in Wisconsin.

MATERIALS AND METHODS

Isolation. Plants exhibiting symptoms of Phytophthora root rot were obtained by field surveys, from cultivar evaluation plots, and from diseased soybean plants submitted to the Department of Plant Pathology, University of Wisconsin, for diagnosis. Soybean stem pieces (3–5 mm) from the margins of lesions were surface disinfected in 1% sodium hypochlorite for 1–2 min, blotted on paper towels, and plated on a modified V-8 juice agar medium (21). Colonies that were morphologically similar to Pmg were transferred to cornmeal agar for further use.

Pathogenicity tests and race determination. Isolates were grown on unamended V-8 juice agar for 7 days.

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Ten-day-old seedlings were inoculated using a variation of the technique described by Kaufmann and Gerdemann (12). A small mycelium-agar plug was cut from the margin of a colony of Pmg and inserted into a 3-mm longitudinal slit on the hypocotyl 1 cm below the cotyledons. The wound was covered with petroleum jelly to prevent desiccation. The inoculated seedlings were rated as living or dead after 5 days of incubation.

In 1978, the soybean cultivars Corsoy (rps_1) and Steele (Rps_1) were selected for pathogenicity tests and preliminary race determination. Seedlings were grown in a vermiculite planting medium in 10-cm-diameter plastic pots in a growth chamber maintained at 28/23 C (day/night) with a 16-hr photoperiod (2,000 fc).

Isolates were determined to be pathogenic if inoculated seedlings were killed. Isolates that killed only Corsoy seedlings were determined to be race 1 and were held in storage for future needs. Isolates that killed both Corsoy and Steele were determined to be a physiologic race other than race 1 and were advanced for further evaluation. The uniform set of seven differential soybean cultivars as described by Laviolette and Athow (14) (except that PI 86972-1 was substituted for PI 171.442) was used to make race determinations of the isolate collection. Growth of seedlings, inoculation, and incubation conditions were the same as those described above. In 1980, all isolates were tested directly on the seven differential cultivars as well as on the cultivar Tracy (Rps_1^b Rps_3; resistant to races 1–9) without a preliminary evaluation on Corsoy and Steele.

Location and number of Pmg isolates tested. In 1978, 92 isolates were obtained from diseased plants from 24 locations in south central and southeastern Wisconsin. Cultivar evaluation plots at two additional locations (designated A and B) yielded another 117 Pmg isolates. Thus, 209 isolates from 26 Wisconsin locations were characterized as to race in 1978. In 1980, 48 isolates were obtained from 18 locations in south central and southeastern Wisconsin.

RESULTS

In 1978, Pmg races 1, 3, 4, 7, 8, and 9 were detected from 26 locations in Wisconsin at a frequency of 13, 65, 7, 5, <1, and 10% of the 209 isolates evaluated, respectively (Tables 1 and 2). To our knowledge, this is the first report of races 7 through 9 other than in Indiana (14).

Races 1, 3, 4, 7, 8, and 9 were detected in 35, 73, 31, 15, 4, and 19% of the 26 locations sampled in 1978, respectively. Race 1 was always detected on cultivars susceptible to race 1 at each location except for location A (Table 2), which had a history of being planted to cultivars containing the Mukden resistance (Rps_1) to races 1 and 2.

Table 1. Frequency of physiologic races of *Phytophthora megasperma* f. sp. *glycinea* isolated from soybeans grown at 24 Wisconsin locations in 1978

Host genotype	Locations (no.)	Isolates (no.)					
		Race 1	Race 3	Race 4	Race 7	Race 9	Total
rps_1	5	16	4	2	0	0	22
Rps_1	9	0	23	7	1	1	32
Unknown	10	7	19	6	3	3	38
Total	24	23	46	15	4	4	92

Table 2. Frequency of physiologic races of *Phytophthora megasperma* f. sp. *glycinea* recovered from soybean cultivars at locations A and B in 1978

Host genotype	Cultivars (no.)	Isolates (no.)						
		Race 1	Race 3	Race 4	Race 7	Race 8	Race 9	Total
				Location A				
rps_1	8	0	31	0	2	0	7	40
Rps_1	13	0	50	0	2	1	9	62
				Location B				
rps_1	3	5	1	0	0	0	0	6
Rps_1	3	0	7	0	2	0	0	9
Total	27	5	89	0	6	1	16	117

At location A, almost identical frequencies of the four races isolated were obtained from soybean cultivars with or without the Mukden resistance (Rps_1). Races 3, 7, 8, and 9 comprised 78, 5, 0, and 18% of the 40 isolates from eight cultivars containing no race-specific resistance (rps_1). The same races respectively comprised 81, 3, 2, and 15% of the 62 isolates from 13 cultivars containing the Mukden resistance (Rps_1).

At location B and at the 14 additional locations from which isolations were made from cultivars with and without the Mukden resistance, a different trend in race frequency was apparent. Although race 3 predominated on resistant cultivars (Rps_1), it was race 1 that predominated on the cultivars susceptible to race 1 (rps_1). On resistant cultivars, races 1, 3, and 7 comprised 0, 78, and 22% of the nine isolates tested (Table 2). A similar trend was apparent at the 14 additional locations in which cultivars with or without the Mukden resistance were grown (Table 1). Races 1, 3, 4, 7, and 9 comprised 0, 72, 22, 3, and 3%, respectively, of the 32 isolates from nine locations in which resistant cultivars were grown. The same races, respectively, comprised 73, 18, 9, 0, and 0% of the 22 isolates from four locations in which susceptible cultivars were grown.

In 1980, virulence formulae (effective/ineffective host genes) (5,7) were used to describe the virulence of groups of Pmg isolates on soybean cultivars carrying identified resistance genes (Table 3). The formulae were necessary because the variation encountered could not be described with the current numeric system of categorizing physiologic races. Alleles that confer resistance or susceptibility to races 1–16 have been determined for four of the seven differential cultivars plus the cultivar Tracy (2,14,15,19): Harosoy (unknown), Harosoy 63 (Rps_1), Sanga (Rps_1^b), Mack (Rps_1^c), Altona (unknown), PI 103.091 (unknown), PI 86972-1 (Rps_3), and Tracy (Rps_1^b Rps_3).

Table 3. Virulence formulae for previously described races of *Phytophthora megasperma* f. sp. *glycinea*

Race	Virulence formula[a]
1	1, 1b, 1c, 3, Alt, 103/Har
2	1, 1c, 3, Alt, 103/1b, Har
3	1b, 1c, 3, Alt, 103/1, Har
4	1b, 3, Alt, 103/1, 1c, Har
5	1b, 3, 103/1, 1c, Alt, Har
6	1b, 1c/1, 3, Alt, 103, Har
7	1b, 1c, 103/1, 3, Alt, Har
8	1b, 1c, 3/1, Alt, 103, Har
9	1b, 1c, 3, 103/1, Alt, Har
10	1, 1c, Alt, 103/1b, 3, Har
11	1, 1c, 3, 103/1b, Alt, Har
12	1, Alt, 103, Har/1b, 1c, 3
13	1, 1b, 1c, 3, 103/Alt, Har
14	1, 1b, 3, Alt, 103/1c, Har
15	1, 1b, 1c, Alt, 103/3, Har
16	1, 3, Alt, 103, Har/1b, 1c

[a] Alt indicates the reaction of the cultivar Altona, 103 indicates the reaction of PI 103.091, and Har indicates the reaction of the cultivar Harosoy.

Thus, virulence formulae could be used to characterize isolates of Pmg (Table 3) and would be especially useful for isolates that do not fit the virulence pattern of isolates that have been numerically designated. In these formulae, numbers and letters refer to the corresponding resistance genes identified in the host (ie, "1b" refers to Rps_1^b, "3" refers to Rps_3, etc.). Because identified genes have not yet been fully characterized in differential cultivars Harosoy, Altona, and PI 103.091, the names of the cultivars have been abbreviated for use in the virulence formulae (7).

By 1980, a different trend in race frequencies had become apparent (Table 4). Races 1, 3, 5, 8, 9, and previously undescribed races were detected from 18 Wisconsin locations at a frequency of 2, 19, 2, 2, 17, and 58% of the 48 total isolates evaluated, respectively (Table 4). Races 1, 3, 5, 8, 9, and previously undescribed races were detected in 1, 7, 1, 1, 8, and 10 fields, respectively from the 18 locations. Previously undescribed races were most frequently detected on cultivars containing the Arksoy resistance (Rps_1^c), followed by cultivars containing the Mukden resistance (Rps_1). One such isolate was also obtained from the cultivar Corsoy, which contains no identified race-specific resistance (rps_1).

DISCUSSION

The studies reported here show that diverse new physiologic races of *P. megasperma* f. sp. *glycinea* were present throughout the primary soybean-growing regions of Wisconsin. Data from 1978 indicated that, although the originally described race 1 was predominant in causing disease on cultivars containing no race-specific resistance, race 3 was predominant on cultivars containing the Mukden resistance to races 1 and 2 (gene Rps_1). Races 4, 7, 8, and 9 were isolated less frequently.

The large differences in race frequencies noted among cultivars with and without the Mukden resistance at all but location A in 1978 are attributed to selection pressure put on the pathogen population by the planting of these resistant cultivars. The results indicated that race 1 was predominant in fields with a history of being planted to cultivars susceptible to race 1. However, once resistant cultivars were planted, races emerged that were capable of overcoming the Mukden resistance. This was apparently the case at location A, from which race 1 was isolated at a low frequency in 1977 (6) but not at all in 1978.

A shift in pathogen virulence appears to have occurred between 1978 and 1980. Data obtained in 1980 indicated the emergence of previously undescribed races of more complex virulence, which were present in increasing frequencies on cultivars possessing the genes rps_1, Rps_1 (Mukden resistance), and Rps_1^c (Arksoy resistance), respectively. Cultivars containing multirace resistance derived from the cultivar Arksoy (Rps_1^c) were released in 1979, but it was known that race 4, capable of attacking these cultivars, was present in Wisconsin (Table 1). However, the planting of cultivars with the Rps_1^c allele did not result in increased frequencies of races 4 and 5; instead, previously undescribed physiologic races were frequently isolated.

In light of evidence from Hobe and Schmitthenner (11), who found many new races in soil but not isolated from diseased plants grown in that soil, it seems likely that many or all of the new virulence combinations we detected from diseased plants existed in the soil at low frequencies. Eight isolates were obtained that were not virulent on Tracy but were virulent on both Sanga and PI 86972-1. Because Tracy carries the two resistance alleles Rps_1^b and Rps_3 that are carried singly by Sanga (Rps_1^b) and PI 86972-1 (Rps_3) (2), Tracy may contain an additional resistance gene or genes not yet identified. Not surprisingly, an isolate obtained from the cultivar Steele grown in a field plot artificially infested with a combination of races 1 and 4 was virulent on Tracy (Tooley and Grau, *unpublished*), indicating that such potential virulence exists in the pathogen population.

In addition to further documenting the high degree of pathogenic variability within natural populations of Pmg, the above evidence casts doubt on the long-term effectiveness of race-specific resistance. If such measures are attempted, a new or expanded set of single-gene differential soybean cultivars is needed for further race categorization. Such a set should include the recently characterized Rps_4 gene (1).

With the number of races steadily increasing, it has become difficult to make race characterizations based on the presently accepted race identification scheme (13). In addition, race designations per se do not give direct genetic information about host resistance or pathogen virulence (5). Instead, the use of virulence formulae (7) has been employed as a superior alternative method of characterization that has been used for pathotypes in other host-pathogen systems (7,16,23) in which pathogen variability has been great. We think that the use of virulence formulae will prove valuable as a method for describing future virulence frequencies of Pmg.

Table 4. Virulence formulae and frequencies of 48 *Phytophthora megasperma* f. sp. *glycinea* isolates recovered from diseased soybean plants from 18 Wisconsin locations in 1980

Virulence formula[a]	Isolates (no.) on host genotype				
	rps_1	Rps_1	Rps_1^c	Unknown	Total
1, 1b, 1c, 3, Alt, 103, / Har (race 1)	0	0	0	1	1
1b, 1c, 3, Alt, 103/1, Har (race 3)	2	3	2	2	9
1c, 3, Alt, 103/1, 1b, Har	1	0	0	0	1
1b, 1c, 3, 103/1, Alt, Har (race 9)	1	6	0	1	8
1b, 1c, 3, Alt/1, 103, Har	0	1	0	0	1
1b, 3, 103/1, 1c, Alt, Har (race 5)	0	0	1	0	1
1b, 1c, Alt/1, 3, 103, Har	0	1	0	0	1
1b, Alt, 3/1, 1c, 103, Har	0	0	1	0	1
1c, 3, 103/1, 1b, Alt, Har	0	2	0	1	3
1b, 1c, 3/1, Alt, 103, Har (race 8)	0	1	0	0	1
1b, 1c/1, 3, Alt, 103, Har	0	1	0	0	1
1b, 103/1, 1c, 3, Alt, Har	0	1	0	0	1
1b, 3/1, 1c, Alt, 103, Har	0	0	2	0	2
1c, 3/1, 1b, Alt, 103, Har	0	2	0	0	2
3, 103/1, 1b, 1c, Alt, Har	0	0	3	0	3
1b/1, 1c, 3, Alt, 103, Har	0	0	2	0	2
1c/1, 1b, 3, Alt, 103, Har	0	4	0	0	4
103/1, 1b, 1c, 3, Alt, Har	0	0	1	0	1
3/1, 1b, 1c, Alt, 103, Har	0	0	2	0	2
/1, 1b, 1c, 3, Alt, 103, Har	0	0	3	0	3
Total	4	22	17	5	48

[a] Alt indicates the reaction of the cultivar Altona, 103 indicates the reaction of PI 103.091, and Har indicates the reaction of the cultivar Harosoy.

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