Virulence Composition of *Phytophthora sojae* in Michigan

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ABSTRACT

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Knowing the virulence composition of *Phytophthora sojae* is important for the management of Phytophthora root and stem rot of soybean. Plant samples were collected in Michigan from diseased plants in soybean fields with Phytophthora root and stem rot symptoms. Eighty-seven isolates of *P. sojae* were evaluated for virulence using 13 differential soybean cultivars. *Rps 3b*, *3a*, *1b*, *1k*, and *6* were resistant to 81, 77, 74, 69, and 66% of the isolates, respectively, while *Rps 1a* and *7* were resistant to only 12 to 13% of the isolates. Races 2, 25, 41, and 44 of *P. sojae* were identified among the isolates and reported for the first time in Michigan. Virulence formulae of 69 isolates did not match those of currently known races of *P. sojae*. Nine isolates were considered nonpathogenic. Incorporating *Rps* genes *1b*, *1k*, *3a*, *3b*, and *6* in soybean cultivars with good field tolerance, in conjunction with other control measures, should offer improved protection of soybeans from Phytophthora root and stem rot in Michigan.

Monitoring the race or virulence structure of a pathogen population is a key component in any management program using specific resistance (complete suppression of growth of a given pathogen by a cultivar). Race specific resistance has been a major element in the control of Phytophthora root rot (23). However, the appearance of new virulent races in response to continued use of resistant cultivars has led to documented failures of race specific resistance (25). *Phytophthora sojae* isolates are characterized to race using the hypocotyl inoculation method and differential reactions of soybean lines with 13 resistance genes at 7 loci (28). Breeders try to incorporate resistance to the most important races in their areas, if that knowledge and resistant germ plasm are available.

Other tactics used to control Phytophthora root rot have included tolerance (field resistance), fungicides (primarily metalaxyl), and cultural practices such as moldboard plowing and tile drainage. None of these have proved to be entirely effective by themselves (25). Based on this observation, Schmitthenner and Van Doren (25) advocated an integrated pest management (IPM) approach to control Phytophthora root rot. Availability of resistant cultivars and knowledge of the virulence structure of the pathogen in the target area

is vital if specific resistance is going to be an effective component of an IPM program.

Although soybeans grown in Michigan have been relatively free from Phytophthora root rot, the potential to affect yield exists. Lockwood and Chen (15) isolated races 1, 3, 4, and 6 of *P. sojae* and demonstrated the involvement of *P. sojae* in the root rot complex of soybeans in Michigan. Presently, information on the virulence structure of *P. sojae* and the occurrence and potential impact of Phytophthora root rot in Michigan soybean fields is not sufficient for farmers to make informed decisions when selecting cultivars. The objective of this project was to determine the virulence characteristics of *P. sojae* in Michigan.

MATERIALS AND METHODS

Collection of samples. Plant samples were collected from 60 soybean fields in 10 Michigan counties from 1993 to 1997. Soybean fields were walked in a zigzag pattern, and one to five samples per field were collected when symptomatic plants were found. Five to 10 plants collected at any one point in a single field constituted a sample. Plants were placed in plastic bags and stored for a maximum of 3 months at 5°C to maintain pathogen viability as samples were being processed. *P. sojae* races 1, 3, 4, 7, and 25, and differential soybean cultivars (Harlon *Rps1-a*, Harosoy 13xx *Rps1-b*, Williams 79 *Rps1-c*, PI 103 *Rps1-d*, Williams 82 *Rps1-k*, L76-1988 *Rps2*, L83-570 *Rps3-a*, PRX-146-36 *Rps3-b*, PRX145-48 *Rps3-c*, L85-2352 *Rps4*, L85-3059 *Rps5*, Harosoy 62xx *Rps6*, Harosoy *Rps7*, and Williams *rps*) representing different *Rps* genes and alleles were provided

by A. F. Schmitthenner of Ohio State University.

Isolation from diseased soybean plants. The Canaday-Schmitthenner medium (5) without hymexizol (which has the potential to inhibit sensitive strains of *Phytophthora*) was used to isolate *Phytophthora* from plant samples. Stems and roots of plants with well-defined symptoms were disinfected with 10% bleach for 10 min and thoroughly rinsed three to four times with sterile distilled water. Small sections of tissue were taken from the edges of advancing lesions and placed on the isolation medium in 100 × 15 mm petri plates. Necrotic tissue also was removed from plants with severe flood damage (following heavy rainfall), surface sterilized, rinsed, and placed on the medium. In all cases, the plant tissues were placed under the medium in order to limit oxygen availability and minimize bacterial contamination. As soon as hyphae of *Phytophthora* appeared in the medium, tips of the hyphae were excised and transferred to new plates to avoid contamination.

Production of single zoospore cultures. Single zoospore cultures were obtained following the method of Canaday and Schmitthenner (5). Fifteen 6-mm-diameter pieces from the edges of *P. sojae* colonies growing actively on dilute V8 juice agar were placed in 25 ml of quarter-strength V8 liquid medium (50 ml of V8 per liter of water) in petri plates. After 48 h, the culture medium was poured off and replaced with 25 ml of Chen and Zentmyer's salt solution (6) at pH 7.0. The solution was changed four times at 5-min intervals. The final salt solution was replaced with 20 ml of sterile deionized distilled water, and cultures were incubated under cool-white 40 W lights at room temperature. Sporangia formed 12 h after cultures were flooded. Cultures were placed in the refrigerator (4°C) for 4 h and then incubated at room temperature. Zoospores appeared within 1 h.

Zoospore concentration was estimated by placing 50 µl of each zoospore suspension in microprep preparation wells and staining with 25 µl of a lacto-phenol Trypan blue solution (27). The number of zoospores in each microprep preparation well was counted under a light microscope at ×250. Through serial dilution, the zoospore concentration was adjusted to 1 × 10³ per ml and plated onto quarter-strength V8 agar medium. Selected colonies of germinating zoospores were transferred to new plates to generate

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single-spore cultures. Cultures were stored up to 4 months in slants of dilute V8 agar medium (40 ml of V8 juice per liter of water) at 5°C until virulence and race tests were performed.

Identification. Two keys, one that groups species of *Phytophthora* by sporangial characteristics (29) and one that uses other characteristics (20) and other sources (17,22), were used in the identification of *Phytophthora* isolates. Specific parameters such as host range, growth rate, and oogonal size were used to delineate *P. sojae* from *P. megasperma* (10). An isolate (1819B) of *P. megasperma* from D. H. Scott of Purdue University was used for comparison.

Test for virulence and race determination. Eighty-seven isolates of *P. sojae* were tested for virulence and race determination. The hypocotyl injection method (9) was used to inoculate seedlings of the differential set of soybean cultivars described previously. Sets of 12 seeds of each of the differential cultivars were planted in vermiculite in 27 × 42 cm plastic trays in the greenhouse. Inoculum was prepared by growing cultures of the isolates and of previously known races of *P. sojae* on soft dilute V8 agar (9). Six-day-old seedlings were inoculated. After inoculation, plants were covered with clear plastic bags for 12 h to prevent the agar slurry and the wound from drying. Plants were grown in the greenhouse at 25°C in 14 h of light. Tests were done with three successive sets of 30 isolates each and the known races of *P. sojae*. Each test had five replications, arranged in a completely randomized manner, and was repeated three times. Reactions of the differential cultivars were evaluated 5 days after inoculation. Soybean seedlings with race-specific resistance remained healthy, with either restricted or no lesions. Susceptible seedlings developed large lesions and most had to be discarded. Based on the reactions of soybean differentials to known races of *P. sojae*, a differential was considered susceptible if 60% or more of the seedlings were killed, and resistant if 40% or less of the seedlings were killed. Intermediate reactions (40 to 60%) were not observed.

RESULTS AND DISCUSSION

Race identification of *P. sojae*. Eighty-seven isolates identified as *P. sojae* were characterized for virulence on 13 soybean

differentials. Nine isolates were identified as known races 2 (1b,7), 3 (1a,7), 4 (1a,1c,7), 25 (1a,1b,1c,1k,7), 41 (1a,1b,1d,1k,7), and 44 (1a,1d,7) (Table 1). Races

Table 2. Isolates of *Phytophthora sojae* with unique virulence formulae, from diseased soybean plants with *Phytophthora* root and stem rot symptoms

No. of <i>Rps</i> genes on which an isolate was virulent	No. of isolates	Virulence formula ^a
3	1	1a,1b,1k
3	4	1a,3c,7
3	1	1a,4,7
3	1	1a,5,7
4	1	1a,ab,3c,7
4	4	1a,1c,3c,7
4	1	1a,2,3c,7
5	1	1a,1c,1d,3c,7
5	1	1a,2,3c,4,7
5	1	1a,2,3c,5,7
5	2	1a,3c,5,6,7
6	1	1a,1b,1c,3b,6,7
6	1	1a,1c,1d,3a,3c,7
6	1	1a,1c,1k,3c,6,7
7	1	1a,1b,1c,1d,1k,5,7
7	1	1a,1b,1c,2,3b,5,7
7	2	1a,1c,1d,2,3c,4,7
7	1	1a,1c,1d,3c,4,5,7
7	1	1a,1b,2,3a,5,6,7
7	1	1a,1c,2,3a,3b,5,7
7	1	1a,1d,1k,3c,4,5,6
7	1	2,3a,3c,4,5,6,7
8	1	1a,1b,1d,1k,3a,3b,3c,7
8	1	1a,1c,1d,1k,3c,5,6,7
8	1	1a,1d,1k,2,3c,4,5,7
8	1	1a,1c,1k,2,3a,4,5,7
8	3	1a,1c,1d,2,3c,4,5,7
8	1	1a,1c,1d,2,3c,5,6,7
8	1	1a,1c,2,3c,4,5,6,7
8	1	1a,2,3a,3c,4,5,6,7
8	1	1a,1c,1d,3c,4,5,6,7
9	1	1a,1b,1d,1k,3a,3c,5,6,7
9	1	1a,1b,1c,1d,1k,2,3a,3b,7
9	2	1a,1b,1d,2,3a,3c,5,6,7
9	1	1a,1c,1d,1k,3c,4,5,6,7
9	2	1a,1c,1d,1k,2,3c,4,5,7
9	2	1a,1c,1d,2,3c,4,5,6,7
9	1	1a,2,3a,3b,3c,4,5,6,7
9	1	1a,1d,2,3a,3c,4,5,6,7
10	1	1a,1b,1c,1d,1k,2,3a,3c,6,7
10	1	1a,1b,1c,1d,1k,3c,4,5,6,7
10	1	1a,1b,1d,1k,2,3c,4,5,6,7
10	1	1a,1c,1d,1k,2,3c,4,5,6,7
11	1	1a,1b,1d,1k,2,3b,3c,4,5,6,7
11	1	1a,1b,1c,1d,1k,3b,3c,4,5,6,7
11	1	1a,1b,1c,1d,2,3a,3b,4,5,6,7
11	1	1a,1c,1d,1k,2,3a,3b,3c,4,5,7
11	1	1a,1c,1d,1k,2,3a,3c,4,5,6,7
12	1	1a,1b,1c,1d,1k,2,3a,3c,4,5,6,7
12	5	1a,1b,1d,1k,2,3a,3b,3c,4,5,6,7
13	2	1a,1b,1c,1d,1k,2,3a,3b,3c,4,5,6,7

^a List of ineffective *Rps* genes and alleles.

Table 1. Races of *Phytophthora sojae* identified among isolates obtained from diseased soybean plants in Michigan soybean fields over the 1993 to 1997 growing seasons

Race	Virulence formula ^a	<i>Rps</i> genes and alleles						No. identified
		1a	1b	1c	1d	1k	7	
2	1b,7		S				S	1
3	1a,7	S ^b					S	2
4	1a,1c,7	S		S			S	2
25	1a,1b,1c,1k,7	S	S	S		S	S	2
41	1a,1b,1d,1k,7	S	S		S	S	S	1
44	1a,1d,7	S			S		S	1

^a Ineffective *Rps* genes and alleles (1a, 1b, 1c, 1d, 1k, 7).

^b 60% or more of hypocotyl inoculated seedlings were killed.

3 and 4 were previously identified in Michigan (15), and races 2, 25, 41, and 44 are reported for the first time in Michigan. Sixty-nine isolates had virulence reactions that did not match those of currently known races of *P. sojae* (1, 2, 4, 13, 24) (Table 2). Two isolates were pathogenic on all of the soybean differentials. Nine isolates incited disease in 40% or less of the seedlings of each soybean differential, including cv. Williams (*rps*), and were classified nonpathogenic.

Although the hypocotyl injection test may bypass some natural defense mechanisms that have been described as field tolerance (19), all isolates tested on the differentials were isolated from soybean plants with symptoms of Phytophthora root and stem rot. This suggests a broad range of *P. sojae* virulence capable of causing disease on soybean in Michigan.

Reaction of soybean differentials to isolates of *P. sojae*. RPX-146-36 (*Rps* 3b), L83-570 (*Rps* 3a), Harosoy 13XX (*Rps* 1b), Williams 82 (*Rps* 1k), and Harosoy 62XX (*Rps* 6) were resistant to 81, 77, 74, 69, and 66% of the isolates, respectively (Table 3). According to the 2000 Michigan Soybean Performance Report (7), *Rps* genes 1a, 1c, 1k, 3, 6, and 7 are incorporated either singly or in combinations (1b+3, 1c+3, and 1k+6) in many cultivars currently planted in Michigan. Previously, these genes were reported to convey resistance against most of the currently known races of *P. sojae*. In this study, *Rps* 1a and 7 were resistant to only 12 and 13% of the isolates (Table 3). This may be the result of long-term use of these genes in the state. Incorporating *Rps* 3b, 3a, 1b, 1k, and 6 singly or in combinations in soybean cultivars should provide improved genetic protection against most of the races present in Michigan soybean fields and minimize the risk of yield loss. Due to race shifts, however, and the presence of rare but compatible races of the pathogen, virulence has

been known to increase and result in disease within 8 years of continued deployment of cultivars with a narrow line of genetic defense (18). Because of this risk, non-race-specific genetic protection in soybeans against *P. sojae* (19,21,26) has become more attractive, particularly when deployed as part of an IPM program. Using soybean lines with both field tolerance and genes conferring resistance to the majority of virulent races of *P. sojae* that are common in the state may be beneficial due to the difficulty of predicting weather conditions conducive to Phytophthora root rot and the broad range of virulence of *P. sojae* observed in this study. Highly variable and virulent pathotypes of *P. sojae* have been reported elsewhere. Henry and Kirkpatrick (11) reported an isolate of *P. sojae* that was pathogenic on all eight soybean differentials tested. Hobe and Schmitthenner (12) isolated previously uncharacterized pathotypes of *P. sojae* directly from soil, suggesting the existence of a large diversity of virulence in *P. sojae* populations. Since outcrossing (in addition to clonal evolution) appears to produce new races in *P. sojae* (3,8,14,16), Forster et al. (8) predicted that a very large number of *P. sojae* races eventually would be identified. The virulence diversity exhibited by the isolates in this study lends support to this prediction. It is possible, therefore, that a large number of *P. sojae* races or the appearance of novel pathotypes as observed in this work may in the future make ineffective the *Rps* genes currently used to protect soybeans from Phytophthora root and stem rot. Therefore, it is necessary to intensify the search for new genes for resistance to Phytophthora root rot, to increase use of other control tactics, and to understand the mechanisms involved in the soybean-Phytophthora pathosystem. Nevertheless, incorporating *Rps* 1b, 1k, 3a, 3b, and 6 in soybean cultivars with good field tolerance, and in conjunction with other control measures, should provide improved protection of soybeans from Phytophthora root and stem rot.

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Table 3. Ranked responses of *Rps* genes and alleles to virulent isolates of *Phytophthora sojae* from soybean plants with Phytophthora root and stem rot symptoms

Gene ^a	Cultivar	% isolates non-pathogenic on cv. ^b
<i>Rps</i> 3b	PRX 146-36	81
<i>RPS</i> 3a	L83-570	77
<i>Rps</i> 1b	Harosoy 13xx	74
<i>Rps</i> 1k	Williams 82	69
<i>Rps</i> 6	Harosoy 62xx	66
<i>Rps</i> 2	L76-1988	61
<i>Rps</i> 4	L85-2352	60
<i>Rps</i> 5	L85-3059	55
<i>Rps</i> 1c	Williams 79	54
<i>Rps</i> 1d	PI 103	54
<i>Rps</i> 3c	PRx145-48	45
<i>Rps</i> 1a	Harlon	22
<i>Rps</i> 7	Harosoy	21
<i>rps</i>	Williams	0

^a *rps* is susceptible; *Rps* indicates resistance.

^b % of virulent isolates.

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