Diversity and Frequency of Races of *Phytophthora megasperma* f. sp. *glycinea* in Soybean Fields in Essex County, Ontario, 1980–1989

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ABSTRACT

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A survey of races of *Phytophthora megasperma* f. sp. *glycinea* was conducted in Essex County, Ontario, between 1980 and 1989. Races 1, 3, 4, 5, 6, 7, 8, 9, 13, and 21 comprised 2.7, 24.2, 13.7, 3.5, 0.2, 32.5, 0.2, 16.5, 1.3, and 0.2%, respectively, of 705 isolates. Genes for resistance to root rot in soybean (*Glycine max*) cultivars recommended in the area between 1976 and 1989 were identified. The diversity of races of *P. m. glycinea* could be explained on the basis of the genotypes for resistance recommended in the survey area. The frequency of occurrence of races supports the theories of directed and stabilizing selection of plant pathogens based on putative genotypes of *P. m. glycinea* races in the area.

Root rot of soybean (*Glycine max* (L.) Merr.) caused by *Phytophthora megasperma* f. sp. *glycinea* T. Kuan & D. C. Erwin was first observed in Essex County, Ontario, on the cultivar Harosoy in 1954 (7). The disease was controlled by the release of Harosoy 63 and other cultivars with the *Rps1-a* gene for resistance in the 1960s. In 1973, *P. m. glycinea* was isolated from *Rps1-a* cultivars, and races 3, 4, 5, and 6 were identified in Essex County in surveys in 1973–1976 (5). Races 3, 4, 5, 7, and 9 were identified in the county in 1979 with a full set of differential cultivars (1). A number of new races compatible with the *Rps1-a* genotype were identified during the same period in the north central soybean-producing areas of the United States (17). Introduction of soybean cultivars with multiple-race resistance to prevalent races in the United States in the late 1970s resulted in additional race changes. A slight increase in the prevalence of *P. m. glycinea* race 4 was noted in the last two years of an extensive survey in Indiana following the release of cultivars with multiple-race (*Rps1-c*) resistance (10). A change in races was detected in Minnesota after the introduction of resistant cultivars (9), and in South Dakota a shift in prevalence from race 3 to race 4 was noted when cultivars resistant to race 3 were grown (6). Race 4 increased in prevalence in Ohio following the introduction of cultivars with the *Rps1-c* gene (13). Such race changes are not unexpected. The diversity of *P. m. glycinea* isolates from soil was demonstrated by Hobe (8), who identified a number of new races from soil by a leaf-baiting technique. In addition, widely virulent isolates that were different from reported races were detected in surveys in Wisconsin (15) and in New York (14) following the introduction of cultivars with the *Rps1-c* gene for resistance.

The objectives of this study were to determine the incidence of races of *P. m. glycinea* in Essex County over a long term and to relate the observed frequencies of races to the *Rps* genes in soybean cultivars recommended for the area and to putative genes for virulence in the pathogen.

MATERIALS AND METHODS

Field survey. Soybean fields of loam or clay-loam in Essex County were sampled each survey year in late May and early June. Fields were selected on the basis of grower cooperation and to obtain a broad distribution within the county. Individual fields were not selected for sampling on a repeated basis. Cropping history was not considered in field selection; however, all fields were planted with soybeans at the time of sampling. Soil was collected with a trowel adjacent to soybean rows in a 50- × 100-m area in each field. Soil was sampled adjacent to diseased plants, if they were present. A bulk sample of about 2 kg of soil collected at each location was stored at 3 C prior to processing. Soil was mixed and placed in fiber pots 10 cm in diameter. Pots were watered daily beginning 3 days before planting. Seeds of the soybean cultivars Kentland, Jewel, or Williams, susceptible to all known races of *P. m. glycinea*, were pushed gently into the soil surface and covered with 1 cm of steamed sand. After planting, pots were kept in a growth room at 24–26 C with a 14-hr day length and a light intensity of 112 $\mu E \cdot m^{-2} \cdot s^{-1}$. Sections of hypocotyls and cotyledons of plants with symptoms of root rot caused by *P. m. glycinea* were surface-disinfested for 2–3 min in a 1.25% NaOCl solution and plated on Difco lima bean agar (LBA) amended with 50 μg/ml of streptomycin sulfate. One to five isolates of *P. m. glycinea* per field were transferred to LBA slant tubes and stored at 15 C. Pots were replanted at 3-wk intervals until sufficient isolates were obtained. Races were initially identified with the standard set of soybean differentials, which includes Harosoy, Sanga, Harosoy 63, Mack, Altona, PI 171.442, PI 103.091, and Tracy (10); Harosoy isolines (4) were used as they became available. For example, in 1989, differentials and major genes for resistance included Harosoy (*Rps7*) (Anderson and Buzzell, *unpublished*), Harosoy 63 (*Rps1+Rps7*), HARO 13 (*Rps1-b*), OX682 (*Rps1-c+Rps7*), HARO 15XX (*Rps1-k+Rps7*), OX642 (*Rps1-d*), HARO 32XX (*Rps3-a+Rps7*), and HARO 62XX (*Rps6+Rps7*), and Kentland or Williams as susceptible controls. Differentials were grown in a 50:50 (v/v) mix of steamed sand-peat moss for 10 days in a growth room as described previously. Isolates of *P. m. glycinea* were cultured for 10–14 days at 25 C on 0.7% LBA (11 g in 1,000 ml of H_2O) in darkness. Inoculum was mixed with a spatula in the culture plate, transferred to a 5- or 10-ml syringe, and applied to wounded hypocotyls (1). Inoculated plants were covered with a plastic bag for 24 hr under the normal day-night cycle. Plants were rated as resistant (<30% plants killed) or susceptible (>70% of plants killed) 5 days after inoculation.

Identification of cultivar genotypes. Cultivars in maturity groups I, II, and III recommended for Essex County were tested to determine the presence of genes for resistance to *P. m. glycinea*. In Canada, soybean cultivars must be registered for sale of seed by cultivar name. Subsequently, cultivars are recommended for production in certain areas. Thus, in Essex County, recommended cultivars represent the majority of cultivars being grown. Cultivars grown between 1976 and 1983 were tested for resistance to *P. m. glycinea* races 1, 3, 4, 7, and 9. Those cultivars grown between 1983 and 1989 were tested with races 5, 8, 12, 13, 17, and 21 to identify single dominant genes (3). Cultivars were inoculated with isolates of additional races if the pattern of response did not fit the expected pattern for single-gene

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resistance. Cultivars were grown, inoculated, and rated as described for the *P. m. glycinea* race differentials.

Avirulence-virulence genes. The inheritance of *P. m. glycinea* cultivar-specific virulence is unknown, but Layton and Kuhn (11) indicate that avirulence is dominant to virulence, and that a gene-for-gene system should occur. In order to study cultivar (*Rps*) effects on races in the survey area, we determined the presence of putative virulence and avirulence genes that would match 15 of the known *Rps* genes. Of these, *Rps1-a, Rps1-b, Rps1-c, Rps1-d, Rps1-k, Rps3-a, Rps3-b, Rps3-c, Rps4, Rps5,* and *Rps6* are listed in Buzzell and Anderson (2); *Rps?* (Harosoy) listed in Ward (17) is now designated *Rps7*; and *Rps?* (Nezumisaya), *Rps?* (OX939), and *Rps?* (OX940) are reported in Rennie et al (12).

RESULTS

During the 10-yr period of 1980–1989, 10 races of *Pmg* were identified from a total of 705 isolates (Table 1). These were races 1, 3, 4, 5, 6, 7, 8, 9, 13, and 21. A relatively small number of isolates could not be characterized, because of inconsistent reactions, even with repeating the inoculation of the differential soybean lines. The incidence of race 1 varied between 0 and 7% during the survey, and race 3 increased from 12% in 1980 to 28% in 1989. Races 4 and 5 increased in frequency, beginning in 1983. Two isolates were identified as race 6 in 1989. In the initial screening, these isolates were virulent on OX642 (*Rps1-d*) compared to isolates of race 7 that were avirulent on OX642; however, after storage for 6 mo on LBA, race 6 isolates became avirulent on OX642 and indistinguishable from race 7. Races 7 and 9 that were prevalent in 1980 decreased in frequency by 1989. In 1989, races 3 and 4 accounted for 56% of isolates of *P. m. glycinea*.

The results of this survey combined with previous surveys indicated that over the 17-yr period from 1973 to 1989, races 1, 3, 4, and 5 increased in frequency, and races 6–9 decreased (Table 2).

Assuming a gene-for-gene system of pathogen recognition exists for *P. m. glycinea* and soybean, putative genes for avirulence-virulence that would match the known *Rps* genes were tabulated for races of *P. m. glycinea* that occur in Ontario (Table 3). In this set, race 1 has one virulence gene; races 3 and 13 have two; race 4 has three; and races 5, 6, 7, 8, 9, and 21 have four or more.

The presence of all races of *P. m. glycinea* identified in this and previous surveys can be explained on the basis of compatible reactions with one or more of the soybean cultivars available for Essex County.

Table 1. Races of *Phytophthora megasperma* f. sp. *glycinea* in Essex County, Ontario, detected in surveys of 1980–1989

Year	Number of fields	Number of isolates	Race (% of total isolates)										
			1	3	4	5	6	7	8	9	13	21	?[a]
1980	20	100	2	12	0	0	0	27	0	57	2	0	0
1981	16	84	1	23	0	0	0	49	0	20	6	0	1
1983	20	88	7	18	10	3	0	40	0	12	0	0	7
1985	49	198	1	30	9	2	0	52	0	5	0	1	3
1987	26	85	5	34	35	5	0	14	0	0	0	0	7
1989	30	150	0	28	28	11	1	13	1	5	0	0	13
$\bar{x}$			2.7	24.2	13.7	3.5	0.2	32.5	0.2	16.5	1.3	0.2	5.2

[a] Isolates of questionable identity.

Table 2. Prevalent races of *Phytophthora megasperma* f. sp. *glycinea* in Essex County, Ontario, in 3- or 4-yr periods, 1973–1989

Survey years	Number of isolates	Race (% of total isolates)				
		1	3	4	5	6–9
1973–1976[a]	146	1.0	13.5	11.9	1.7	72.9
1979,[b] 1980–1981	236	1.0	19.3	0.7	1.3	74.7
1983–1985	286	4.0	24.0	9.5	2.5	54.5
1987–1989	235	2.5	31.0	31.5	8.0	17.0

[a] Buzzell et al (5).
[b] Anderson (1).

Table 3. Putative genes for avirulence and virulence in *Phytophthora megasperma* f. sp. *glycinea* for races collected from soils in Essex County, Ontario, 1973–1989

Genes for resistance in soybean	Races of *P. m. glycinea*[a]									
	1	3	4	5	6	7	8	9	13	21
Rps1-a	−	+	+	+	+	+	+	+	−	+
Rps1-b	−	−	−	−	−	−	−	−	−	−
Rps1-c	−	−	+	+	−	−	−	−	−	−
Rps1-k	−	−	−	−	−	−	−	−	−	−
Rps1-d	−	−	−	−	+	−	+	−	−	−
Rps3-a	−	−	−	−	+	+	−	−	−	+
Rps3-b	−	−	−	−	−	−	−	−	−	−
Rps3-c	−	−	−	+	+	+	+	+	−	−
Rps4	−	−	−	+	+	+	+	+	−	−
Rps5	−	−	−	−	+	+	−	−	−	+
Rps6	−	−	−	+	+	+	+	+	+	−
Rps7	+	+	+	+	+	+	+	+	+	+
Rps? (Nezumisaya)	−	−	−	−	−	−	−	−	−	−
Rps? (OX939)	−	−	−	−	−	−	−	−	−	−
Rps? (OX940)	−	−	−	+	−	−	−	+	−	−
Avirulence-virulence gene ratio	14:1	13:2	12:3	8:7	7:8	8:7	10:5	9:6	12:2	10:4

[a] − = Avirulence gene in pathogen, which results in an incompatible reaction with the *Rps* allele. + = Virulence gene in pathogen, which results in a compatible reaction with the *Rps* allele.

DISCUSSION

From 1967 to 1976, only cultivars with *Rps1-a* resistance were recommended for cultivation in Essex County. Beginning with 1977, cultivars were recommended according to ratings of field tolerance to Phytophthora rot. From 1977 to 1981, all soybean cultivars recommended in the survey area (Table 4) were compatible with all races, except for those cultivars with *Rps1-a* that were incompatible with race 1 found in the 1979 race survey (1). Evidently races 1 and 13 isolated in 1980 and 1981 were maintained on those cultivars that were susceptible to all races of *P. m. glycinea*. Corsoy 79 with *Rps1-c* was released in 1982, and additional cultivars with *Rps1-c* became available in subsequent years. Cultivars with *Rps1-k* and *Rps6* were not planted extensively during the survey and were not considered to have influenced the frequency of races.

Shifts in frequency of races can be partially explained by shifts of compatible-incompatible genotypes recommended as cultivars. Because the *P. m. glycinea* isolates were obtained from random fields in each survey (1979–1989), and the history of soybean production and cultivar usage are not known, the effects of specific causes cannot be established. However, some general observations may be made. The predominant use of soybean cultivars with *Rps1-a* following the introduction of Harosoy 63 in 1963 must have resulted in a decrease in the prevalence of race 1 to undetectable levels and an increase

Table 4. Number of cultivars with genes for resistance to root rot caused by *Phytophthora megasperma* f. sp. *glycinea* in soybean cultivars recommended for cultivation in Essex County, Ontario, 1976–1989

Year	Number of cultivars	Number of cultivars with genes						
		Rps1-a	*Rps1-c*	*Rps1-k*	*Rps6*	*Rps7*[a]	*rps*[b]	Unknown[c]
1977	10	8	0	0	0	NT[d]	2	0
1978	11	8	0	0	0	NT	3	0
1979	13	6	0	0	0	NT	7	0
1980	17	5	0	0	0	NT	12	0
1981	18	6	0	0	0	NT	12	0
1982	19	5	1	0	0	NT	13	0
1983	24	6	1	0	0	10	15	2
1984	27	6	3	0	0	10	15	3
1985	32	6	4	0	0	11	21	1
1986	30	8	6	0	1	10	15	1
1987	36	10	6	0	1	12	17	3
1988	34	10	6	0	1	7	15	3
1989	43	15	8	2	1	11	13	3

[a] Some cultivars contain more than one *Rps* allele.
[b] *rps* at all known *Rps* loci.
[c] Genotype could not be determined because of intermediate responses to inoculation resulting in 31–69% plant death, or the responses did not correspond to known genotypes.
[d] NT = not tested.

in the prevalence of races 3, 4, 5, and 6–9 in 1973–1976 (Table 2). The recommendation, beginning in 1977, of field-tolerant cultivars, some of which were *rps*, could have resulted in the increase of race 1 to detectable levels in subsequent surveys (Table 2). The widespread use of Corsoy 79 as a major cultivar in Essex County after 1982 could have resulted in increases in races 4 and 5 (Table 2).

None of the *Rps* alleles given in Table 3 would result in directed selection favoring race 3 versus races 6–9 or race 4 versus race 5; thus the recommended cultivars should not have differentially affected race 3 compared to races 6–9 and race 4 compared to race 5. Therefore, the frequency of races can be partially explained by compatible genotypes, but additional factors are affecting the survival of individual races. In addition, the development of several races of *P. m. glycinea* that were compatible with the *Rps1-a* gene for resistance after it was released has never been adequately explained. Races 3, 7, and 9 were reported as prevalent races in several soybean production areas, including Indiana (10), Ontario (1), and Ohio (8). Given the theory that genes for virulence are deleterious (16), development of race 3 was expected because it has only two genes for virulence, based on currently known genes for resistance; but races with complex genotypes, such as 7 and 9 with seven and six virulence genes, respectively, are unexpected (Table 3). It is possible that races 7 and 9 arose from frequent mutations similar to those that are known to occur in other host-pathogen systems (16). The increase in race 3 and the decrease in races 6–9 (Table 2) may be the result of stabilizing selection. Once present in an area, a race would be subject to both directed selection by host genotype and stabilizing selection by pathogen genotype (16). Stabilizing selection might also explain why race 4, with three virulence genes, is isolated more frequently than race 5, with seven virulence genes, even though both races are compatible with *Rps1-c*. Laviolette and Athow (10) suggested that race 4 was more competitive than race 5 in Indiana. The low frequency of race 13, with only two virulence genes, may result from the large number of cultivars with the *Rps1-a* gene, which results in an incompatible reaction with race 13.

Widely virulent isolates of *P. m. glycinea* obtained after the release of cultivars with *Rps1-c* in Wisconsin (15) and New York (14) were not identified in this survey, but there was an increase in isolates that could not be characterized, because of inconsistent or intermediate responses on differential cultivars. It is possible that new races detected by leaf baiting in Ohio (8) were not identified in our study, because we obtained isolates from root infections.

In summary, the occurrence of races of *P. m. glycinea* identified in this survey could be explained on the basis of host genotype in the survey area. The frequency of races over a 17-yr period appears to be subject to directed and stabilizing selection sensu Vanderplank (16).

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