Traits of Soybean-Infecting *Phytophthora* Populations from Illinois Agricultural Fields

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ABSTRACT

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Phytophthora rot caused by *Phytophthora sojae* is a common and significant disease of soybean (*Glycine max*) in Illinois and throughout the Midwestern United States. The pathogenic characteristics of *P. sojae* populations in several Midwestern states have been reported recently, but pathogenicity and fungicide sensitivity traits of populations in Illinois were poorly understood. Isolates ($n = 121$) of soybean-infecting *Phytophthora* spp. were baited using susceptible cv. Sloan seedlings from soybean field soils with a history of seedling diseases in 24 counties across Illinois. The pathotype and race of isolates of *P. sojae* were characterized using 11 differential soybean cultivars in greenhouse tests using a hypocotyl inoculation method. Sensitivity to the fungicidal compounds metalaxyl and mefenoxam was tested with 63 isolates in vitro. Most (96%) of the *Phytophthora* isolates sampled from Illinois soybean fields were *P. sojae*, but 4% were an unidentified *Phytophthora* sp. as determined by phenotypic and genotypic traits. We present a preliminary description of another *Phytophthora* sp. from soybean fields in a restricted region of Illinois that is pathogenic and capable of killing soybean. Based on eight Rps gene differentials (Rps1a, 1b, 1c, 1d, 1k, 3a, 6, and 7), 22 virulence pathotypes of *P. sojae* were identified and 88% of all isolates were characterized to a defined race. The four most common races, which were 58% of all isolates, were races 1 (21%), 4 (15%), 33 (12%), and 28 (10%). Based on 11 differentials, (those noted above and Rps 2, 4, and 5), 31 virulence pathotypes were identified. The mean virulence complexities, which are the number of susceptible interactions on the sets of 8 and 11 Rps gene differentials, were 3.3 and 3.7, respectively. All isolates tested were sensitive to Apron XL, Allegiance, technical grade mefenoxam, and technical grade metalaxyl at 1.0 μg a.i./ml. The population of *P. sojae* is diverse and composed of multiple pathotypes and races in Illinois, and the results suggest that pathogen virulence partially explains poor performance of *Phytophthora*-resistant cultivars in many Illinois soybean fields.

Soybean (*Glycine max* (L.) Merr.) is cultivated on 10 to 11 million acres in Illinois. A common and prevalent disease that adversely affects soybean production in Illinois and throughout the Midwestern United States is Phytophthora rot, caused by *Phytophthora sojae* M. J. Kaufmann & J. W. Gerdemann (= *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin). In one survey, *P. sojae* was reported in 41% of soybean fields sampled in Illinois (31). Phytophthora rot kills soybean at all growth stages, from planting to maturity, and is among the most damaging soybean dis-

eases across the north-central region of the United States (5,25,26,33). The effect of this disease varies from year to year and among locations depending, in part, upon soil drainage and the occurrence of wet soil conditions early in the growing season.

Phytophthora rot of soybean is primarily managed with host resistance, soil drainage control, and fungicidal seed treatments such as metalaxyl (Allegiance, Gustafson LLC, Plano, TX) and mefenoxam (Apron XL; Syngenta Crop Protection, Inc., Greensboro, NC) (26). Resistant cultivars have been the cornerstone for management of Phytophthora rot of soybean. This disease, however, remains significant, in part because host resistance is not effective in many areas. Failures of resistance in soybean cultivars with the major Rps genes available commercially (Rps1a, 1c, and 1k) have been reported frequently by producers and crop advisors in Illinois.

P. sojae is a diverse pathogen with regard to its pathogenicity to soybean containing different resistance (Rps) genes (3,7,26). Races and virulence pathotypes within this species are described based on the reaction of sets of eight or more soybean differentials after inoculation using

standardized methods (3,7,26,27). The differential cultivars each contain a different major Rps resistance gene or allele. Approximately 55 different races have been defined based on eight differentials (10), although many additional pathotypes have been reported (3,16,18).

Surveys of the race and pathotype composition in populations of *P. sojae* in several U.S. states have been conducted in the past 10 to 20 years (1,3,11,16,18,22,28,34). The surveys have revealed different magnitudes of pathogenic diversity, depending on the state and year when they were conducted. In states where multiple surveys have been conducted during this time period, race diversity appears to be increasing over time. For example, in Minnesota, 18 races were identified in 2001 versus 5 races in 1986, and the virulence pathotype of 41% of the isolates did not match previously defined races (18). In Michigan, the virulence pathotype of 79% of isolates reported in 2001 did not match those of previously described isolates (16). In 2002, 12 races were reported from Iowa compared with only 1 race in 1966; furthermore, the percentage of isolates with virulence to Rps1k increased from 5% in 1994 to 49% in 2002 (22,34). Only race 1 was reported in Indiana before 1975, whereas eight races were reported in 1979 and 24 races in 1997 (1). In Ohio, where the most intensive surveys of *P. sojae* populations have been done over the past 20 years, 48 pathotypes were detected in 1990–91 compared with 55 in 1997–98. The number of fields in northwestern Ohio with populations virulent to Rps1k increased from 8.7 to 78.9% during the same time period (3,28). The reason for the increased pathogenic diversity in populations of *P. sojae* in these and other areas is unknown, but may have occurred in response to outcrossing and clonal evolution and concomitant selection pressure from deployment of different resistance genes (3,6,8,28).

Little was known or reported about the pathogenic diversity of *P. sojae* populations in Illinois. Since 1958, when Phytophthora rot was first described in Illinois (17), one survey of *P. sojae* has been reported (20). In that study, 35 isolates were characterized from 18 counties in Illinois, and seven pathotypes (races) were identified. Most (46%) of the isolates, as determined on the Williams set of differentials, were race 54 (virulent to Rps 1d and 7),

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and other races were 1, 4, 41, 43, 44, and 55. Only one isolate (3%) was virulent to Rps1k, and six isolates (17%) were virulent to Rps1c (19,20).

Continuing crop damage from *Phytophthora* rot of soybean in Illinois suggested that extensive assessment of virulence pathotypes and other phenotypic traits in the pathogen population was warranted. Previous reports from Illinois (19,20) suggested that populations of *P. sojae* that are virulent on the major Rps genes available in commercial cultivars, including Rps 1k and 1c, were uncommon. However, anecdotal reports from producers and crop consultants suggested that these major Rps genes were failing in farm fields much more frequently than the reported information on races and virulence pathotypes in Illinois indicated.

This project focused on characterizing populations of *P. sojae* from Illinois with the ultimate goal of increasing our knowledge of the population biology of this pathogen and improving management of *Phytophthora* rot of soybean. The objectives of this study were to (i) determine the virulence pathotypes of isolates of *P. sojae* from Illinois using sets of 8 and 11 Rps soybean differentials, (ii) determine sensitivity of the Illinois populations of *P. sojae* to the fungicides mefenoxam and metalaxyl, and (iii) determine the presence, characteristics, and distribution of other soybean-infecting *Phytophthora* spp. in Illinois. Numerous virulence pathotypes of *P. sojae* were identified in Illinois, many of which are virulent to major Rps resistance genes available in commercial cultivars. In addition, an unidentified soybean-infecting *Phytophthora* sp. was isolated and characterized from two Illinois counties. This information helps to explain the recurring problems with *Phytophthora* rot in Illinois and provides information of value to breeders and producers for development and selection of cultivars with resistance to this disease.

MATERIALS AND METHODS

Soil sampling and baiting assays. Soil samples ($n = 287$) were collected from 80 soybean fields with a history of *Phytophthora* rot or seedling disease problems in 32 Illinois counties. From one to eight soil samples per field were collected to a depth of 13 cm in 2001 and 2002. Soil samples were dried at 23°C for 3 to 5 days and stored at 6°C. To prepare the samples for baiting, subsamples of each soil sample were distributed into three paper cups, flooded for 1 h, the excess water was removed, and they were maintained in darkness in a saturated condition at 22°C for 10 days. Isolates of *Phytophthora* were then baited from the soil samples using a seedling bioassay with susceptible soybean cv. Sloan. Seedlings ($n = 6$) germinated in darkness for 3 days were pressed onto the surface of each saturated soil subsample,

maintained in darkness at 23°C, and then evaluated 5 to 10 days later for characteristic soft rot and brown lesions on the hypocotyls. All isolates were recovered from soil samples using the same method to avoid potential selection bias that could be imposed by isolating from arbitrarily collected, infected, field-grown cultivars. The baiting process was repeated once for soil samples that did not yield *P. sojae* in the initial baiting attempt.

Isolation, culturing, and characterization of *Phytophthora* isolates. Sections of symptomatic seedlings from the baiting assays were disinfested with sodium hypochlorite solution and plated on semi-selective Canaday-Schmitthenner medium (CSM) containing hymexazol at 50 µg/ml a.i. (Tachigaren, 70% WP; Gustafson LLC; 13,21,27). After 3 to 6 days of growth, hyphal-tip isolations were taken from emerging *Phytophthora* growth. The following features were used to confirm the identity of 8-day-old cultures of *P. sojae*: isolation from a soybean host; homothallic production of oospores; robust mycelial growth on CSM; microscopic measurements of oogonium diameter and pleroticity (15 oogonia measured per isolate); fertilization of oogonia by a single paragonous antheridium; ellipsoid, indeterminate, nonpapillate sporangia; and differentiation of zoospores within the sporangium (7,26).

Five isolates of an unknown *Phytophthora* sp. from two Illinois counties were distinct from *P. sojae* based on the features described above and also were characterized with additional tests. Growth rates were compared with *P. sojae* on V8 juice agar (V8A) and Difco potato dextrose agar (PDA) at 25°C in darkness (26). Isolates of the unknown and *P. sojae* (one isolate each) were tested with the Alert *Phytophthora* enzyme-linked immunosorbent assay (ELISA) test kit (Neogen Corp., Lansing, MI). DNA was extracted from 3-day-old mycelium of three isolates of the unknown and two isolates of *P. sojae* using a FastDNA kit (Qbiogene/Bio101, Carlsbad, CA). The internal transcribed spacer (ITS)1, 5.8s gene, and ITS2 region of nuclear ribosomal DNA was amplified by polymerase chain reaction (PCR) using primers ITS1 and ITS4 (30). Both strands of PCR products (approximately 800 bp in length) were sequenced by the University of Illinois Biotechnology Center. BLAST searches for similar sequences were done on the GenBank database.

All isolates were transferred to V8A and maintained at 5°C until use in pathotype tests. In most cases, one isolate was tested per field, with the exception of 10 fields where we tested four to six isolates per field. Each isolate used for pathogenicity testing originated from a distinct, single soil sample. To determine if single zoospore isolates had the same virulence pathotype as parental isolates obtained directly from plants, 11 single zoospore

progeny subcultures were prepared from eight isolates and the pathotype was tested on eight soybean differentials as described below.

Pathogenicity testing. Pathogenicity tests were conducted with 121 isolates of *P. sojae*. The tests included 11 differential soybean cultivars with different Rps genes, one susceptible control cultivar, and one cultivar with a combination of Rps 1a and 7: (i) Union (Rps1a), (ii) Harosoy13 (Rps1b), (iii) L75-3735 (Rps1c), (iv) Harosoy16 (Rps1d), (v) Williams82 (Rps1k), (vi) L76-1988 (Rps2), (vii) L83-570 (Rps3a), (viii) L85-2352 (Rps4), (ix) L85-3059 (Rps5), (x) L89-1581 (Rps6), (xi) L93-3258 (Rps7), (xii) Sloan (susceptible), and (xiii) Harosoy 63 (Rps1a & 7). The seed of the differentials was increased in field plots at Urbana, IL in 2002 from a small quantity of seed recommended and provided by T. S. Abney (United States Department of Agriculture-Agricultural Research Service [USDA-ARS], West Lafayette, IN) and R. Nelson (USDA-ARS, Urbana, IL). Five 7-day-old seedlings of each differential cultivar in pots containing vermiculite were inoculated with each isolate using a hypocotyl slit inoculation method (27). This experiment was set up as a randomized block design and was replicated two additional times. Immediately after inoculation, inoculated plants were maintained in high humidity and darkness at 24°C for 14 to 16 h. Thereafter, inoculated plants were kept in a greenhouse at 24°C with a 14-h photoperiod and scored 7 days post inoculation as susceptible (dead) or resistant (healthy). Races 1, 4, 7, and 28 (from A. Dorrance, Ohio State University) and an agar control were used as standard checks with all inoculation trials. Differential cultivars were scored susceptible if at least 60% of the plants in the three replicate pots were killed after inoculation. In most cases, over 80% of the inoculated plants were killed. The races of isolates were characterized by comparison with previously defined virulence patterns on eight differentials (10).

The virulence data were analyzed using the HaGiS spreadsheet for automatic Hagood-Gilmour calculation (12). The spreadsheet calculated octal codes for each virulence pathotype, virulence complexity, and the Shannon diversity index. Octal codes, using a reversed octal system, were established based on virulence (1) or avirulence (0) in the following triplets of Rps genes: 1a, 1b, 1c; 1d, 1k, 2; 3a, 4, 5; and 6, 7 (12). The virulence pattern within each triplet was given a single numerical octal code based on the pattern of virulence as follows: 000 (= 0), 100 (= 1), 010 (= 2), 110 (= 3), 001 (= 4), 101 (= 5), 011 (= 6), and 111 (= 7). For example, an isolate with the virulence pathotype 1a, 1c, 1k, and 7 was described by the octal code 5202.

Fungicide sensitivity testing. Sixty-three isolates of *P. sojae* selected arbitrar-

ily from 21 counties in Illinois were tested for sensitivity to technical and commercial formulations of mefenoxam (Apron XL LS; Syngenta Crop Protection, Inc.), and technical and commercial formulations of metalaxyl (Allegiance FL; Gustafson LLC). The technical grade compounds were dissolved in dimethyl sulfoxide and the commercial products were dissolved in sterile, deionized H₂O prior to amending dilute lima bean agar (LBA; 26). Plugs of mycelium (4 mm in diameter) were removed from the margins of 7-day-old colonies grown on V8A and transferred to amended LBA. Each isolate was placed on three replicate plates of amended LBA. Initial trials included each compound at 0, 1.0, and 5.0 µg a.i./ml, and thereafter the tests were conducted with 0, 0.1, 0.5, and 1.0 µg/ml. The diameter of each colony of *P. sojae* on the surface of the amended LBA was measured in two directions separated by 90° on each plate after 8 days of growth at 24°C in a 10-h photoperiod. Mean colony diameter of each isolate grown on LBA amended with each concentration was compared with mean diameter of the same isolate grown concurrently on the nonamended medium (2,23). Percent growth for each isolate on the amended LBA relative to the controls was calculated and plotted as frequency distributions for each fungicide concentration.

RESULTS

Isolation and characterization of *Phytophthora* isolates from soil. *P. sojae* ($n = 121$ isolates) was isolated from 24 of 32 Illinois counties sampled (Fig. 1). All 121 isolates matched the features of *P. sojae*. Soybean production in the 24 counties represents 30% of the total soybean acreage in Illinois in 2002, and includes the eight (of nine total) Illinois crop-reporting districts with the largest acreage of soybean. This pathogen was isolated from 42% of all soil samples tested. Two replicate baiting assays were necessary to isolate *P. sojae* from 20% of the soil samples. Hymexazol was required in the medium to separate *Phytophthora* spp from *Pythium* spp., which often infected seedlings in the baiting assays.

Isolates of an unknown *Phytophthora* sp. that are pathogenic to soybean and distinct from *P. sojae* were isolated in the baiting assays at a frequency of 70% from soybean fields in two east-central Illinois counties with a history of soybean seedling disease. Seven unknown *Phytophthora* sp. isolates that all appear to be the same species and three confirmed *P. sojae* isolates were obtained from Champaign and Douglas counties. The unknown isolates have coenocytic hyphae, oospores, and a sporangial shape characteristic of *Phytophthora* spp. The unidentified species tested positive as *Phytophthora* in the Alert ELISA test and is homothallic, monoclinal, and fertilized in an amphigynous

manner by antheridia enveloping a short pedicel. The unknown isolates produced larger oogonia (average 48.6 µm) than *P. sojae* (average 36.2 µm) and grew five times faster on PDA and 1.5 times faster on V8A than *P. sojae* (3,17). DNA sequences of the ITS region of the unknown isolates most closely matched *P. medicaginis* and *P. cryptogea* based on significant sequence alignments; and the other species in the top 25 matches were *P. drechsleri* and *P. erythrosetica*. The ITS sequence of the *P. sojae* matched *P. sojae*

in GenBank. The unknown isolates killed from 0 to 60% of plants of the 11 differential cultivars used for pathotype testing; however, the pathogenicity characteristics were inconsistent and did not match any established races of *P. sojae* (10).

Pathogenic characteristics of *P. sojae* isolates. Twenty-two virulence pathotypes were identified based on a set of eight differential cultivars with common Rps genes (1a, 1b, 1c, 1d, 1k, 3a, 6, and 7) that are used to define races (1,3,10,26,34; Tables 1 and 2). Of these, 12 pathotypes

Fig. 1. Illinois counties (shaded) where isolates of *Phytophthora sojae* were obtained from soil samples.

matched previously defined races and 10 pathotypes (15 isolates) were not characterized to any race based on published race definitions (10). The four most common races identified, which composed 58% of all isolates studied, were race 1 (pathotype Rps7; 21% of isolates), 4 (pathotype 1a, 1c, and 7; 15% of isolates), 33 (pathotype Rps 1a, 1c, 1d, 1k, and 7; 12% of isolates), and 28 (pathotype 1a 1b, 1k, and 7; 10% of

isolates). None of the other pathotypes composed more than 5% of the total collection of isolates tested. Relatively few (16%) of the isolates were virulent to Harosoy 63 with a combination of Rps 1a and 7, whereas 58 and 90% of the isolates were virulent to cultivars with single Rps genes 1a and 7, respectively.

Additional differentials and other tests revealed different types of pathogenic di-

versity. Based on the 11 differential lines used, 31 different virulence pathotypes were identified among the 121 isolates (Table 1). The octal code representation for the 31 virulence phenotypes also is shown in Table 1. The number of virulence phenotypes ranged from 3 to 11 and the Shannon diversity index ranged from 1.0 to 2.08 for isolates within each of the eight cropping districts sampled (Table 2). Virulence complexities up to 8 were found among the 121 isolates, with the most frequent complexities being 2, 4, 5, and 7 (Fig. 2).

Single zoospore subcultures were prepared from eight isolates of *P. sojae* to compare virulence pathotype of parental mass isolates and progeny single zoospore isolates. The pathotype reaction on the set of eight differentials was the same as the parental mass isolate for four of the single zoospore progeny isolates, three of the single zoospore isolates were virulent on one or two additional Rps genes compared with the parental isolate, and one single zoospore isolate was virulent to one fewer Rps gene than the parental isolate (*data not shown*). Pathogenic diversity of multiple isolates collected from separate soil samples within 10 individual fields ranged from one to three pathotypes per field as determined with 8 differentials and two to five pathotypes per field base on 11 differentials (Table 3).

Fungicide sensitivity testing. All isolates of *P. sojae* tested ($n = 63$) were sensitive to Apron XL, Allegiance, technical grade mefenoxam, and technical grade metalaxyl at 1.0 µg/ml a.i. (Fig. 3). Sensitivity among the isolates was similar to all four fungicidal compounds, although there were minor differences in sensitivity noted (Fig. 3). Although all isolates were similar in sensitivity at 1.0 µg/ml and above (for >1.0 µg/ml, *data not shown*), the isolates differed with an increased range of sensitivity to all compounds at 0.5 and 0.1 µg/ml. Isolates would have been considered resistant if growth on amended media was >40% of growth on unamended media (23).

DISCUSSION

This is the most comprehensive study reported on the traits of populations of soybean-infecting *Phytophthora* spp. in Illinois soybean fields. It provides a glimpse of the characteristics of soybean-infecting *Phytophthora* populations across the state of Illinois, and reports three key general results. First, greater pathogenic diversity in *P. sojae* populations was found across Illinois and in individual fields than was known previously (19,20). These new results on distribution and occurrence of pathotypes and races may explain many of the failures of common Rps genes that have been reported in Illinois soybean fields. Second, we detected no evidence of low sensitivity to metalaxyl or mefenoxam

Table 1. Virulence pathotypes, races, and octal codes identified among 121 isolates of *Phytophthora sojae* from Illinois soybean fields

Number	Virulence pathotype (11 genes) ^a	Race (8 genes) ^b	Octal code (11 genes) ^c	Number of isolates
1	7	1	0002	19
2	5,7	1	0042	6
3	1a,7	3	1002	4
4	1a,1d,7	44	1102	4
5	1a,1d,5,7	44	1142	2
6	1a,2,7	3	1402	2
7	1b,1d,1k,5,6,7	UD	2343	1
8	1b,1d,2,3a,4,5,6	UD	2571	2
9	1b,1d,2,3a,4,5,6,7	17	2573	5
10	1b,1d,1k,2,7	UD	2702	1
11	1b,1d,1k,2,6,7	UD	2703	1
12	1b,1d,1k,2,5,7	UD	2742	4
13	1a,1b,1k,7	28	3202	11
14	1a,1b,1k,5,7	28	3242	1
15	1a,1b,1d,1k,7	41	3302	2
16	1a,1c,7	4	5002	13
17	1a,1c,1d,7	43	5102	5
18	1a,1c,1d,6,7	UD	5103	1
19	1a,1c,1d,5,7	43	5142	2
20	1a,1c,1k,7	UD	5202	1
21	1a,1c,1d,1k,7	40	5302	1
22	1a,1c,2,7	4	5402	4
23	1a,1c,2,4,7	4	5422	1
24	1a,1c,1d,2,5,7	43	5542	1
25	1b,1c,1k,7	UD	6202	2
26	1a,1b,1c,7	47	7002	1
27	1a,1b,1c,1k	UD	7200	1
28	1a,1b,1c,1k,7	25	7202	2
29	1a,1b,1c,1d,1k,7	33	7302	15
30	1a,1b,1c,1d,1k,2,6,7	UD	7703	1
31	Avirulent	0	0000	5

^a Virulence pathotype, which is a list of ineffective Rps genes among 11 soybean Rps gene differentials (1a, 1b, 1c, 1d, 1k, 2, 3a, 4, 5, 6, and 7).

^b Race based on interaction with eight soybean Rps gene differentials (1a, 1b, 1c, 1c, 1k, 3a, 6, and 7). UD = undefined races of *P. sojae*.

^c Octal codes were established based on virulence or avirulence in the following triplets of Rps genes: 1a, 1b, 1c; 1d, 1k, 2; 3a, 4, 5; and 6, 7.

Table 2. Number of isolates of *Phytophthora sojae*, number of virulence pathotypes, and Shannon diversity index for isolates from eight different cropping districts in Illinois

Cropping district	No. of isolates tested	No. of virulence pathotypes		Shannon diversity index	
		8 genes ^a	11 genes ^b	8 genes ^a	11 genes ^b
Northwest	7	4	5	1.35	1.55
Northeast	18	10	10	2.00	2.00
West	27	9	11	1.84	2.08
Central	5	5	5	1.61	1.61
East	30	8	10	1.68	1.87
West-southwest	7	3	4	1.00	1.28
East-southeast	12	5	5	1.47	1.67
Southwest	15	5	6	1.40	1.67
Totals	121	22	31	2.70	3.08

^a Number of virulence pathotypes and Shannon diversity index based on interactions with eight soybean Rps gene differentials (1a, 1b, 1c, 1d, 1k, 3a, 6, and 7).

^b Number of virulence pathotypes and Shannon diversity index based on interactions with 11 soybean Rps gene differentials (1a, 1b, 1c, 1d, 1k, 2, 3a, 4, 5, 6, and 7).

in Illinois, although these compounds have been used for a number of years as seed treatments to manage *Phytophthora* and *Pythium* seed and seedling diseases. We also report for the first time that *Phytophthora sojae* is not the only *Phytophthora* sp. that infects and kills soybean plants in Illinois. Another as yet unknown *Phytophthora* sp. was isolated from infected soybeans that can kill seedlings.

This study was focused on determination of pathogenic diversity in populations of *P. sojae* in Illinois soybean fields. Numerous producers and crop consultants in Illinois had reported failure of resistance in commercial cultivars with Rps1c and 1k resistance genes. Prior to this study, however, only one isolate with virulence to Rps1k from one county, and five isolates with virulence to Rps1c from four counties, had been reported in Illinois (19,20). Results from this study reveal that many more counties in Illinois have populations of *P. sojae* that are virulent to Rps 1k and 1c. Isolates that are virulent to Rps 1c and 1k were isolated from 8 and 10 counties, respectively. Twelve races were identified based on eight differentials (1a, 1b, 1c, 1d, 1k, 3a, 6, and 7), which is a common set of differentials used to define races of *P. sojae* (10). However, another 15 of the isolates were not characterized into any race based on published race definitions (10). The use of 11 differentials revealed additional pathogenic diversity (i.e., 31 total different virulence pathotypes) that was not detected with the 8 differentials. The pathotype diversity varied among cropping districts in Illinois. These results suggest that the *P. sojae* population in Illinois is more pathogenically diverse than was previously known, and it will be a challenge to maintain effective major-gene resistance to *P. sojae*.

Pathogenicity traits are important for describing *P. sojae* populations for two key reasons. First, pathogenicity traits are variable and provide numerous data points for measuring diversity. Second, they are critical for disease management because race-specific resistance has been fundamental for management of *Phytophthora* rot. The value of pathogenicity trait information increases if it is studied over time to determine when and where populations are changing. Compared with the research on the populations of *P. sojae* done in several Midwestern states, minimal work has been done in Illinois and no record of changes in race or pathotype structure over time exist. Indiana, Iowa, Michigan, Minnesota, and Ohio have conducted multiple surveys of *P. sojae* over the last 10 to 20 years (1,3,16,18,22,31). These studies suggest that virulence diversity is increasing over time. The level of diversity found in Illinois suggests that the same trend may be occurring in Illinois. In spite of these descriptive studies, the mechanisms driving the changes in pathogenic traits in popula-

tions of *P. sojae* remain largely unknown. We know that *P. sojae* is a highly variable organism. A common hypothesis suggests that increasing pathogenic diversity results from selection pressure by deployment of different resistance genes, perhaps in combination with changes in tillage (3,8,15,28,32). In addition, our results showing variation for virulence among parental strains and single zoospore progeny agree with Rutherford et al. (24), who suggested that variation for virulence may occur asexually. Understanding of the factors that generate and propagate pathogen virulence may improve management of *Phytophthora* rot and our understanding of the population biology of *P. sojae*.

The race system has long been used to define different virulence phenotypes of *P. sojae* (26). However, the utility of the race system is decreasing to the point where it often cannot be used. The number of defined races is large, creating challenges for research, communication, and especially

for management recommendations. At least 55 races have been defined based on eight differential lines (10). Moreover, many virulence phenotypes of *P. sojae* that have been reported from multiple studies, as well as the study reported here, cannot be defined based on published race definitions (3,16,18). Another related system for representation of virulence data for plant pathogens has been developed using an octal system based on incompatible or compatible interactions for each differential within sets of three differentials (3,9,12,14). The resulting octal codes for each virulence phenotype provide a relatively simple method to concisely define the pathotype, while at the same time providing information on interactions with specific genes and indicating relationships among pathotypes (e.g., an octal code of 5302 is more closely related to 5422 than 1002). This system was used to describe pathogenic variation among isolates of *P. sojae* from Ohio (3). Although races and

Fig. 2. Frequency distribution of virulence complexities, which is the number of ineffective Rps genes for individual isolates among 121 isolates of *Phytophthora sojae* from Illinois based on 11 differential genes.

Table 3. Number of virulence pathotypes of *Phytophthora sojae* in 10 individual Illinois soybean fields as determined by pathogenicity tests with sets of 8 and 11 soybean Rps gene differentials

Field code	County	No. of isolates	No. of pathotypes	
			8 gene differentials ^a	11 gene differentials ^b
G2	Crawford	4	2	3
F6	Ford	4	1	2
DR1	Jackson	4	1	2
A4	Lawrence	4	1	2
O1	Ogle	5	3	5
D5	Perry	5	1	2
BCS	St. Clair	6	2	2
K8	Vermillion	4	2	4
H3	Will	5	3	3
L1	Will	4	3	4

^a Number of virulence pathotypes based on interactions with eight soybean Rps gene differentials (1a, 1b, 1c, 1d, 1k, 3a, 6, and 7).

^b Number of virulence pathotypes based on interactions with 11 soybean Rps differentials (1a, 1b, 1c, 1d, 1k, 2, 3a, 4, 5, 6, and 7).

Fig. 3. Sensitivity of 63 isolates of *Phytophthora sojae* from Illinois to Apron XL, technical grade mefenoxam (Mef.), Allegiance FL, and technical grade metalaxyl (Met.) based on growth during exposure to these compounds at **A**, 1.0, **B**, 0.5, and **C**, 0.1 µg a.i./ml relative to growth in the absence of these compounds in dilute lima bean agar. Data for Apron XL, Tech. Mef., Allegiance, and Tech. Met. appear in order from left to right in the sets of four bars.

pathotypes can be compared among studies from different locations, octal codes reported for pathotypes from different areas (e.g., Ohio and Illinois) cannot be compared unless the codes are based on the same distinct sets of differentials in each study. A solution for these problems is to abandon the race system and define pathogenic traits for isolates based on the virulence pathotype, which is essentially a list of susceptible Rps genes. Using pathotype in place of race has been discussed previously (3,29). The pathotype system not only circumvents the frustration for researchers of trying to characterize every isolate to race, which often cannot be done, but also provides a system that allows for widespread, uniform application and interpretation that is much easier to use by scientists and by producers for selection of cultivars with appropriate resistance genes.

The results on pathotype diversity reported in this study differ from those reported previously from Illinois (19,20). *P. sojae* was isolated from 42% of all soil samples tested in this study; which compares with an isolation frequency of 12% reported previously from Illinois (19,20). Many virulence pathotypes were identified in this study that were not reported previously in Illinois. In this study, 121 isolates of *P. sojae* were obtained from 24 counties, and the most common races were race 1 (pathotype Rps7), 4 (pathotype 1a, 1c, and 7), and 33 (pathotype Rps 1a, 1b, 1c, 1k, and 7). Previously, 35 isolates were characterized from 18 counties (19,20), and the most common races were 54 (pathotype 1d and 7), 44 (pathotype 1a, 1d, and 7), and 43 (pathotype 1a, 1c, 1d, and 7). Different materials and methods used in the two studies may account for the differences in results. For example, different soil sample selection strategies, sampling locations, baiting methods, and differential cultivars were used to obtain and determine the race of isolates. More intensive sampling and a larger number of isolates (121 versus 35) also may have been significant. Schmitthenner et al. (28) reported that the number of races increased in Ohio as the number of isolates tested increased. We may have observed the same phenomenon in Illinois.

Although host resistance is the key strategy for managing *Phytophthora* rot, treatment of seed with fungicides also is important for disease management. Metalaxyl and mefenoxam in the form of Allegiance FL and Apron XL are widely used as seed treatments for control of *Phytophthora* seed and seedling infections. Metalaxyl has been used to manage *Phytophthora* rot in Illinois since the mid-1980s; and, although mefenoxam has been used for a shorter time period, it has the same mode of action. The population of *P. sojae* was sensitive to these compounds at the 1.0 µg/ml level; however, variation for sensitivity was noted at 0.5 and 0.1 µg/ml, sug-

gesting that variation in sensitivity at low concentrations occurs. These data provide information on the background level of sensitivity to metalaxyl and mefenoxam that will be useful for monitoring potential change in Illinois over time. There were no major differences for sensitivity to these four compounds, which is as expected due to their common modes of action. The results also corroborate reports from the field that suggest there is no practical level of resistance to these compounds. However, we must consider that this survey of isolates represents a small sample from a limited number of fields and we cannot discount the possibility that resistance may be developing in areas not sampled. Resistance in *P. sojae* probably has been slow to develop due to the minor selection pressure exerted by these compounds when they are used as seed treatments. The risk of resistance developing to metalaxyl and mefenoxam may be low based on the data and usage pattern in Illinois.

P. sojae has been considered to be the primary *Phytophthora* sp. causing root and stem rot of soybeans. We report and describe another *Phytophthora* sp. that infects and kills soybean plants in Illinois. This unknown *Phytophthora* sp. was isolated from infected soybean plants and can kill soybean seedlings in greenhouse conditions. This unidentified species was isolated from 2 of the 32 counties (Champaign and Douglas counties in east-central Illinois) that were included in this survey, but it was especially common in Douglas county (66% of total isolates). The unknowns were not *P. sojae*, based on phenotypic traits and ITS sequence analysis. We could not yet assign these unknown isolates to any described species of *Phytophthora*. This unknown species may be important to understand more completely if Rps genes are not consistently effective against this pathogen and it becomes more widespread. Isolates of unknown soybean-infecting *Phytophthora* spp. also have been reported from soybean fields in Indiana, Ohio, and Ontario (*personal communication*), and research is planned to compare and identify the unknowns from several states.

In summary, this study reveals critical information about characteristics of *Phytophthora* populations in Illinois soybean fields and provides data useful for further studies on population biology and disease management. *Phytophthora* rot is a significant, widespread, and recurring problem in the Midwestern United States. The population of *P. sojae* is diverse pathogenically in Illinois, and many more pathotypes and races exist than were known previously. This suggests that the populations have adapted, and may continue to adapt, to new resistance genes. Long-term management

of *Phytophthora* rot in Illinois and elsewhere will depend on appropriate development and deployment of cultivars with effective Rps resistance genes, partial resistance or tolerance, tillage, seed treatments, and effective soil drainage.

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