Pathotype Diversity of *Phytophthora sojae* in Eleven States in the United States

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Abstract

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Pathotype diversity of *Phytophthora sojae* was assessed in 11 states in the United States during 2012 and 2013. Isolates of *P. sojae* were recovered from 202 fields, either from soil samples using a soybean seedling bioassay or by isolation from symptomatic plants. Each isolate was inoculated directly onto 12 soybean differentials; no *Rps* gene or *Rps* 1a, 1b, 1c, 1k, 3a, 3b, 3c, 4, 6, 7, or 8. There were 213 unique virulence pathotypes identified among the 873 isolates collected. None of the *Rps* genes were effective against all the isolates collected but *Rps*6 and *Rps*8 were effective against the majority of isolates collected in

the northern regions of the sampled area. Virulence toward *Rps*1a, 1b, 1c, and 1k ranged from 36 to 100% of isolates collected in each state, while virulence to *Rps*6 and *Rps*8 was less than 36 and 10%, respectively. Depending on the state, the effectiveness of *Rps*3a ranged from totally effective to susceptible to more than 40% of the isolates. Pathotype complexity has increased in populations of *P. sojae* in the United States, emphasizing the increasing importance of stacked *Rps* genes in combination with high partial resistance as a means of limiting losses to *P. sojae*.

Monitoring changes and shifts in a pathogen population's adaptation to resistance (*R*) genes is essential for long-term management of numerous plant diseases where host resistance is the primary means of managing a disease. Surveillance of pathogen population composition has helped scientists respond to the appearance of new pathogen strains where changes in virulence were observed and to more clearly understand the epidemiology of the pathogen. For example, the annual monitoring of *Puccinia triticina*, causal agent of leaf rust on wheat in the United States, that has documented shifts in pathogen virulence both within and among regions has enabled breeders to prepare for the movement of races to new regions that could otherwise threaten wheat production (Kolmer and Hughes 2013, 2014; Kolmer et al. 2003, 2004, 2005, 2006, 2008, 2009, 2013; Long et al. 2000, 2002). New individual virulences and combinations of virulences toward leaf rust *R* genes within the *P. triticina* population were detected in both the United States and Mexico (Huerta-Espino et al. 2008; Kolmer and Hughes 2014; Kolmer et al. 2008, 2009). Monitoring of *P. triticina* also demonstrated that indigenous overwintering populations of the pathogen can be the primary source of inoculum for some regions based on the unique virulence composition of populations observed within a region (Long et al. 2000, 2002). Most recently, the effectiveness of surveillance and screening of pathogen populations on differential lines was dramatically demonstrated with the discovery of a new race of *P. graminis* f. sp. *tritici*, Ug99, in Uganda that was virulent on almost all stem rust *R* genes (Singh et al. 2008, 2011).

Monitoring for changes in oomycete pathogen populations has detected not only shifts in virulence but also changes in sensitivity to fungicides and alterations in host range. For example, in the United States, shifts in sensitivity to the fungicide metalaxyl, pathogenicity

on black nightshade (*Solanum nigrum*), and the presence of sexual populations were detected in *Phytophthora infestans* (Daines et al. 2014; Fry et al. 1993; Seidl Johnson and Gevens 2014). Outbreaks of downy mildew on lima bean and spinach were attributed to new races of *Phytophthora phaseoli* (Davidson et al. 2008) and *Peronospora farinose* f. sp. *spinaciae* (Irish et al. 2007), respectively. Analysis of *Pseudoperonospora cubensis* populations associated with recent outbreaks of cucurbit downy mildew identified both fungicide insensitivity and new virulence pathotypes (Holmes et al. 2015).

A similar pattern of selection resulting in altered pathogen virulence has been observed in a soilborne pathogen, the soybean cyst nematode (SCN; *Heterodera glycines*), where repeated planting of soybean cultivars with only resistance derived from PI 88788 has resulted in selection of SCN populations that can reproduce on cultivars derived from this resistance source (Niblack 2005). This adaptation is circumvented by planting soybean cultivars derived from other resistant sources such as PI 548402 (Peking) or PI 437654 (Hershman et al. 2008; Niblack 2005; Niblack et al. 2008). *Phytophthora sojae*, the causal agent of root and stem rot of soybean, is no exception to this pattern of changing virulence. This soilborne pathogen is managed predominately by planting cultivars possessing resistance to *P. sojae* (*Rps*) genes and partial resistance (Dorrance et al. 2004; Schmitthenner 1985). Twenty-one *Rps* genes have been reported in the literature to date (Dorrance et al. 2004; Grau et al. 2004; Lin et al. 2013; Sugimoto et al. 2011; Sun et al. 2014; Zhang et al. 2013a,b), of which *Rps*1a, *Rps*1b, *Rps*1c, *Rps*1k, *Rps*3a, *Rps*6, and *Rps*8 have been deployed in soybean cultivars in the United States (Beuerlein et al. 2000; Slaminko et al. 2010) (A. E. Dorrance, unpublished data). Surveys of pathotypes (virulence diversity) within *P. sojae* populations in the United States have been conducted periodically on a state-by-state basis since the pathogen was first reported in the 1960s.

Root rot of soybean caused by *P. sojae* was first observed in Indiana in 1948 and Ohio in 1951 (Bernard et al. 1957; Schmitthenner 1985) but the causal agent was not described until 1958 (Kaufmann and Gerdeman 1958). This soilborne pathogen was detected in more than 50% of the soil samples collected in Ohio, Minnesota, and Missouri in survey findings from 1995 and 1996 (Workneh et al. 1999). The first races of *P. sojae* were identified by Morgan and Hartwig (1965). Subsequent state-based surveys reported continued shifts in

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pathogen virulence as well as increases in complexity. In Indiana, virulence to *Rps1a* was prevalent during a survey conducted from 1973 to 1979 (Laviolette and Athow 1981), while virulence to *Rps1k* was found shortly after its deployment in 1984 (Abney et al. 1997; Schmitthenner et al. 1994). *P. sojae* isolates collected from fields in Michigan during 1993 to 1997 and tested on 13 differential soybean cultivars had a great range of pathotype diversity, with 73 different pathotypes identified among 87 isolates (Kaitany et al. 2001). Despite this diversity, *Rps* genes *1b*, *1k*, *3a*, and *6* conferred resistance to more than 65% of these isolates and, thus, were recommended for use in limiting losses to Phytophthora root and stem rot (Kaitany et al. 2001). In Ohio, on the same 13 differential cultivars, 202 pathotypes were identified among 429 isolates collected from 82 fields during the period for 1997 to 1999. *R* genes *1c*, *3a*, and *6* conferred resistance to more than 50% of the isolates collected in the study (Dorrance et al. 2003a). In Illinois, virulence to *Rps1a* was prevalent in the *P. sojae* population in surveys completed during 1997 and 2001 to 2002 (Leitz et al. 2000; Malvick and Grunden 2004). Interestingly, all of the *Rps* genes, except *Rps7*, conferred resistance to only 21% of the 121 *P. sojae* isolates collected during 2001 to 2002 in Illinois. Surveys from Iowa reported a greater number of pathotypes of *P. sojae* in 1994 compared with 1966, when virulence to *Rps7* was first discovered, although virulence to *Rps1a* was still the most common (Yang et al. 1996). In North Dakota, where soybean acreage has expanded rapidly into areas not previously planted to soybean, *P. sojae* has limited distribution, and the total number of pathotypes and complexity are limited. The most recent survey recovered isolates from only 5 of 20 counties that were sampled, and virulence to *Rps1a* was the most common virulence detected among 157 isolates (Nelson et al. 2008).

Procedures for determining *P. sojae* pathotype diversity based on a standardized set of *Rps* gene differentials for assessing isolate pathogenicity have been widely published (Dorrance et al. 2004, 2008; Pazdernik et al. 1997; Schmitthenner and Bhat 1994; Stewart and Robertson 2012). Interestingly, even though *P. sojae* is a root pathogen, the presence or absence of an *Rps* gene can readily be assessed through inoculations of the soybean hypocotyl with either mycelia or zoospores (Dorrance et al. 2004; Gijzen et al. 1996). Both etiolated and nonetiolated seedlings have been used and respond similarly. A differential series originally developed during the 1970s (Bernard et al. 1991) remains in use today with only minor modification. When differentials putatively representing the same *Rps* gene were inoculated with a standard set of isolates, several of the cultivars responded differently. This was not unexpected because some of these differentials were derived from different sources of resistance. Based on these results, a subset of differentials from multiple maturity groups

was recommended to provide for uniform results across studies (Dorrance et al. 2004).

The importance of surveys for assessing pathotype diversity among *P. sojae* populations cannot be overemphasized. Early surveys in the United States demonstrated that virulence to multiple *R* genes was already common in some regions, prior to the deployment of specific genes in resistant cultivars (Schmitthenner et al. 1994; Tooley et al. 1982; Ward 1990; Xue et al. 2015), while the results of more recent surveys suggest that *P. sojae* pathotype populations are adapting to deployment of *Rps R* genes (Abney et al. 1997; Anderson et al. 2012; Dorrance et al. 2003a; Kaitany et al. 2001; Nelson et al. 2008). Findings similar to those in the United States have been reported from other soybean regions affected by *P. sojae* (Barreto et al. 1995; Costamilan et al. 2013; Cui et al. 2010; Ryley et al. 1998; Sugimoto et al. 2006). Because deployment of *Rps R* genes remains the most effective and economical means of managing *P. sojae*, our objectives were to assess current pathotype prevalence and diversity among fields in soybean-growing states in the North Central Region to determine: (i) if the most commonly deployed *Rps* genes (*Rps1c*, *Rps1k*, *Rps3a*) were still effective; (ii) if “newer” (*Rps8*) or less commonly deployed *Rps* genes (*Rps6*) have potential for management of *P. sojae*; (iii) if “old” *Rps* genes, those previously deployed during the 1960s and ‘70s (*Rps1a*, *Rps1c*), could potentially be “recycled”; and (iv) if the complexity of the population continues to increase throughout the region.

Materials and Methods

Sample collection and baiting procedures: In fall 2012 and 2013, soil samples were collected in soybean fields in 11 northern soybean-producing states: Iowa, Indiana, Illinois, Kansas, Michigan, Minnesota, Missouri, Nebraska, New York, Ohio, and South Dakota. The number of fields sampled varied among states (Table 1) because the soil samples were collected from fields with a history of Phytophthora root and stem rot or with a history of stand establishment problems. At each field, 10 separate soil samples, approximately 1 liter in total volume, were collected from an area of 7.62 by 53.4 m. Soil baiting for *P. sojae* was conducted in the greenhouse following the procedure of Dorrance et al. (2008). After collection, the soil samples were dried separately; then, each sample was finely ground using a soil grinder. After grinding, each sample was dispensed into a separate plastic pot (15 cm in diameter). The pots were placed in deionized, nonchlorinated water in water baths in the greenhouse overnight at approximately 24 to 27°C, then removed and placed on the greenhouse bench to allow excess water to drain for approximately 24 to 48 h. After draining, the pots were placed in plastic bags and incubated at room

Table 1. Number of fields sampled, number of fields where *Phytophthora sojae* was recovered, number of isolates of *P. sojae* that were collected, and pathotypes that were identified in each state along with indices of diversity

State	Number of ^a				Indices of diversity ^b			
	Samp	Recov	Iso	Path ^c	Simple	Simpson	Gleason	Shannon
Iowa	36	35	130	37	0.28	0.91	7.40	2.87
Indiana	26	13	38	27	0.71	0.96	7.15	3.08
Illinois	76	45	67	48	0.72	0.99	11.18	3.75
Kansas	2	2	12	2	0.17	0.55	0.40	0.69
Michigan	3	3	7	3	0.43	0.67	1.03	0.96
Minnesota ^d	31	14	57	49	0.86	0.99	11.87	3.83
Missouri	1	1	3	2	...	...	...	...
Nebraska	7	6	14	10	0.71	0.89	3.41	2.06
New York	1	1	6	5	0.83	0.93	2.23	1.56
Ohio	92	77	510	144	0.28	0.96	22.94	4.02
South Dakota	5	5	29	18	0.62	0.96	5.05	2.74
Totals	249	202	873	345	...	...	...	...

^a Number of fields sampled (Samp), fields from which *P. sojae* was recovered (Recov), Isolates (Iso), and pathotypes (Path).

^b Diversity indices were calculated using formula presented in Groth and Roelfs (1987) using the spreadsheet program HaGiS (Hermann et al. 1999).

^c Pathotypes were identified using the hypocotyl inoculation technique on a soybean differential series consisting of ‘Williams’ (universal susceptible), ‘Harlon’ (*Rps1a*), ‘Harosoy 13XX’ (*Rps1b*), ‘Williams79’ (*Rps1c*), PI103091 (*Rps1d*), ‘Williams82’ (*Rps1k*), L76-1988 (*Rps2*), L83-570 (*Rps3a*), PRX146-36 (*Rps3b*), PRX 145-48 (*Rps3c*), L85-2352 (*Rps4*), Harosoy 62XX (*Rps6*), ‘Harosoy’ (*Rps7*), and PI 399073 (*Rps8*).

^d Two fields from North Dakota in the Red River Valley were included in the overall Minnesota totals.

temperature in the dark for 2 weeks. After 2 weeks, each pot was planted with 15 to 20 seeds of 'Sloan' soybean (*rps* and low partial resistance) and covered with coarse vermiculite. The seed had been disinfested by immersion in sodium hypochlorite (0.05%) for 30 s, then rinsed in sterile, distilled water prior to planting. Three days after planting, when the seed had germinated, the pots were flooded a second time and placed in water baths for 24 h, then removed and placed on a greenhouse bench. Deionized water was added to the pots twice daily to allow for continued seedling development. Seedlings with characteristic symptoms of root lesions and damping-off were observed most often within 1 to 2 weeks after the second flooding. In some cases, where no seedlings developed symptoms, a second baiting step was initiated by removing the plants from the first baiting, replanting with new seed, and flooding 3 days after planting.

Isolation of *P. sojae*. All seedlings with expanding brown to tan lesions on the hypocotyl or roots were collected and washed with sterilized, distilled water. The lesions were then excised and plated on a Phytophthora selective media (PBNIC) containing Benlate (50% benomyl; 0.01 g liter⁻¹), pentachloronitrobenzene (0.054 g liter⁻¹), neomycin sulfate (0.10 g liter⁻¹), chloramphenicol (0.01 g liter⁻¹), Rovral (50% iprodione; 0.04 g liter⁻¹) and hymexazole (20 mg liter⁻¹ added after autoclaving) in V8 agar (Dorrance et al. 2008). Characteristic mycelia of *P. sojae* were excised and transferred onto fresh petri dishes containing PBNIC agar media. The cultures were examined under the microscope and mycelia that appeared to be *P. sojae* were transferred to vials of lima bean agar or V8 juice agar and stored at 15°C until evaluated.

When diseased soybean plants were collected in the sampled fields, isolates of *P. sojae* were obtained by culturing directly from the plants. Plants with symptomatic chocolate-brown lesions were washed in running tap water to remove soil and debris. Pieces of stem tissue were then cut from the lesion margin into 0.6-cm lengths and surface disinfested for approximately 30 s in flowing, chlorinated tap water. The stem pieces were placed on PBNIC selective media and isolations were made as described previously.

To verify that the isolates recovered were *P. sojae*, all isolates, both those obtained from stem tissue and those isolated through soil baiting, were examined for morphology and size of oospores, absence of sporangia in lima bean agar, and absence of growth on full-strength potato dextrose agar. Genomic DNA was extracted from mycelia grown on dilute V8-juice broth with a Qiagen plant mini kit (Qiagen, Inc.), and the internal transcribed spacer (ITS) region was amplified and sequenced using ITS1/ITS4 primers (White et al. 1990). Amplicons were sequenced at the Molecular and Cellular Imaging Center at the Ohio Agricultural Research and Development Center in Wooster. Sequence data were compared with known sequences deposited in the National Center for Biotechnology Information.

Inoculum preparation. Isolates identified as *P. sojae* were grown on dilute lima bean agar (agar at 12 g liter⁻¹) (Dorrance et al. 2008) for 7 days. A mycelial slurry of *P. sojae* was prepared for each isolate by placing colonized agar into a 12-cm³ syringe, which was then passed through to a second 12-cm³ syringe immediately prior to inoculation of the soybean differentials.

Pathotype evaluation of *P. sojae*. The pathotype of each isolate was determined using the hypocotyl inoculation technique (Dorrance et al. 2008) at the University of Illinois, Iowa State University, The Ohio State University, and the University of Minnesota on the following differentials: 'Williams' (universal susceptible, *rps*), 'Harlon' (*Rps1a*), 'Harosoy 13XX' (*Rps1b*), 'Williams79' (*Rps1c*), PI103091 (*Rps1d*), 'Williams82' (*Rps1k*), L76-1988 (*Rps2*), L83-570 (*Rps3a*), PRX146-36 (*Rps3b*), PRX 145-48 (*Rps3c*), L85-2352 (*Rps4*), Harosoy 62XX (*Rps6*), 'Harosoy' (*Rps7*), and PI 399073 (*Rps8*). In Minnesota the differential set was modified and 'McCall' was used as the universal susceptible. The seed of all of these differentials (except McCall) was grown in Ohio and maintained at The Ohio State University. Ten 1-week-old seedlings of each soybean differential cultivar were inoculated in the hypocotyl by injecting approximately 100 µl of mycelial slurry of an isolate into the stem using an 18-gauge needle. In Ohio, inoculated seedlings were covered with plastic overnight to maintain high humidity, after which they were

uncovered and maintained in the greenhouse at temperatures of 23 to 28°C. In Minnesota, inoculated seedlings were initially maintained for 24 h in a growth chamber in darkness at 25°C, with mist applied for 60 s every 30 min. This was followed by an 8-day period with a day-night cycle of 12 h of light and 12 h of darkness at 25 and 23°C, respectively. Following inoculation, the plants were watered daily. The pathogenicity of each isolate was evaluated after 7 (Ohio) to 8 (Minnesota) days. Reactions were scored as resistant, intermediate, or susceptible with <20, 21 to 70, or >80% of the seedlings with lesions, respectively. A susceptible interaction was designated for *Rps2* only when all seedlings were killed to eliminate possible false positives that can occur with the intermediate resistance reaction of this differential (Kilen et al. 1974; Lohnes et al. 1996).

Pathotype virulence, description, and analysis. Isolate virulence data were summarized for each state individually and for all states combined using the Habgood-Gilmour Spreadsheet (HaGiS) program (Herrmann et al. 1999). This program was also used to analyze pathotype virulence data within and among states, produce frequency distributions of isolate virulence to specific *Rps* genes, describe the complexity of the pathotype virulence (the number of *Rps* genes with which an isolate has a susceptible interaction), and calculate a number of frequently used diversity indices. Because of the large number of pathotypes isolated and identified in the survey, an octal nomenclature was utilized as an efficient, convenient method to summarize this data (Dorrance et al. 2003a; Gilmour 1973; Goodwin et al. 1990; Habgood 1970) rather than the lengthy, complex pathotype descriptions (Gilmour 1973). This format also facilitates use of the HaGiS program (Herrmann et al. 1999) to analyze the data for frequency distributions of virulence (the number of differentials with which an isolate has a susceptible interaction), determine isolate complexities, and calculate diversity indices.

The reverse octal format previously described for *P. sojae* (Dorrance et al. 2003a) *Rhynchosporium secalis* (Goodwin et al. 1990), and *P. infestans* (Goodwin et al. 1995) was used to identify the isolate pathotypes. In this format, the differentials are arranged in groups of three, and each group of three differentials is coded as one octal digit. Octal numbers were assigned based on the susceptible or resistant responses of each differential within the set, with 0 indicating a resistant response following inoculation and 1 indicating a susceptible response. The intermediate ratings were not used. The soybean *P. sojae* differentials were grouped into octal digits as follows: the first octal digit contained *Rps1a*, *Rps1b*, and *Rps1c*; the second octal digit contained *Rps1k*, *Rps2*, and *Rps3a*; the third octal digit contained *Rps3b*, *Rps3c*, and *Rps4*; and the fourth octal digit contained *Rps6*, *Rps7*, and *Rps8*. Octal digits were assigned as follows: 000 = 0, 100 = 1, 010 = 2, 110 = 3, 001 = 4, 101 = 5, 011 = 6, and 111 = 7. Simple diversity and Shannon, Gleason, and Simpson diversity indices were calculated for both the complete collection of isolates and for diversity within each state using the HaGiS program (Herrmann et al. 1999).

Results

In all, 1 to 31 fields were sampled for *P. sojae* in Indiana, Kansas, Michigan, Minnesota, Nebraska, New York, and South Dakota. Sampling in Iowa, Illinois, and Ohio was more intensive, with 36, 76, and 83 fields sampled, respectively (Table 1). From a total of 202 fields across all states, 870 isolates of *P. sojae* were recovered and evaluated for pathotype on 11 differentials (Table 1). More than 50% of the isolates collected were virulent on *Rps1k* or *Rps7*, while more than 40% of the isolates were virulent on *Rps1a*, *Rps1b*, or *Rps1c* (Fig. 1). Of the isolates collected, 15% were virulent on *Rps3a*, *Rps3b*, and *Rps3c*. Less than 12% of the isolates were virulent on *Rps4* or *Rps6* and less than 4% were virulent on *Rps8*. Approximately 85% of the pathotypes identified in the samples were isolated two or more times. Twelve pathotypes made up 50% of the isolates recovered in the region (Table 2). However, prevalence was not related to pathotype complexity. The 12 most common pathotypes ranged in complexity from those virulent on a single *Rps* gene to pathotypes virulent on seven *Rps* genes. Of these 12 pathotypes, 8 were virulent on two or more *Rps* genes and 5 were virulent on three or more *Rps* genes.

The pathotypes collected in the survey ranged widely in complexity (Fig. 2) and were frequently virulent on more than one *Rps* gene. On average, the isolates collected were virulent on at least three *Rps* genes. However, 61% of the isolates were virulent on two to five *Rps* genes, while 15% of the isolates were virulent on at least six *Rps* genes and more than 7% were virulent on seven or more *Rps* genes. Two isolates were virulent on 10 *Rps* genes and a single isolate was virulent on all genes in the differential set.

Among the isolates collected, the greatest number of pathotypes ($n = 144$) was identified in the samples from Ohio, which also yielded the greatest number of isolates ($n = 510$). The next largest group of isolates ($n = 130$), collected in Iowa, yielded 37 pathotypes. Fewer isolates were obtained in both Illinois and Minnesota; however a larger proportion of the isolates from these two states were discrete pathotypes (Table 1).

In this survey, virulence to *Rps1a*, *1b*, *1c*, and *1k* was present in 40% of the isolates collected in all of the states surveyed, with the exception of Kansas (Fig. 3), where these genes would be effective in the two fields that were sampled, and Michigan, where only 30% of the samples were virulent to *Rps1c*. In contrast, virulence to *Rps3a* was less than 15%, except for Illinois and Kansas. A similar pattern for reduced virulence was also less than 15% for *Rps6*, with the exception of Illinois, Minnesota, Nebraska, and New York. In Illinois, 40 and 25% of isolates were virulent on *Rps3a* and 6, respectively. Virulence toward *Rps3a* was present in 50% of the six isolates recovered in Kansas. In Minnesota and Nebraska, virulence on *Rps6* was present in 45 and 30% of isolates, respectively. Interestingly, more than 40% of the 57 *P. sojae* isolates collected in Minnesota had virulence to *Rps6* while less than 2% of the isolates were virulent to *Rps3a*. Virulence to *Rps8* was present in less than 10% of isolates from all states.

Three indices of diversity were calculated for the data (Table 1). Depending on the index under consideration, diversity varied widely among the states. Although each index was informative of a particular aspect of the number, distribution, virulence, and complexity of the pathotypes recovered in the sampling process, the value calculated should be considered with caution (Grünwald et al. 2003). Sample sizes (both the number of fields sampled and the number of isolates collected) varied widely among states (Table 1), which directly influences the number of possible pathotypes. Simple diversity (the proportion of distinct pathotypes relative to the number of isolates collected) was greatest in Minnesota and New York, followed by Indiana and Illinois. Gleason's index (an indication of phenotypic richness) was greatest for Illinois, Minnesota, and Ohio, with the value for Ohio roughly double that of collections of *P. sojae* from other states. Intermediate values (5.05 to 7.40) were calculated for Iowa, Indiana, and South Dakota. Shannon's index (an indication the evenness of distribution of virulence

phenotypes within a sample) was greatest for Ohio, followed by Minnesota, Illinois, and Indiana.

Discussion

This is the first regional-scale analysis of the *P. sojae* population in the United States since races of *P. sojae* were first identified in the United States in 1965. Previously, surveys had been conducted on a state-by-state basis. Uniform simultaneous sampling provides a comprehensive overview of pathotype prevalence and offers insight into the possible efficacy of cultivar resistance as a strategy for managing *Phytophthora* seed, seedling, root, and stem rot. The prevalence of virulence to *Rps1a*, *Rps1c*, and *Rps1k* has continued to increase in the states from which soil samples were collected. Virulence toward these *Rps* genes was reported shortly after their deployment and has since been identified frequently in state-by-state surveys. The increasing incidence of virulence to *Rps1c* and *Rps1k* has been attributed to selection for virulence in response to widespread deployment of these *Rps* genes (Jackson et al. 2004; Ryley et al. 1998; Schmitthenner et al. 1994; Yang et al. 1996). Virulence to these genes was present in earlier surveys but not to the degree that it was observed in this current survey.

The pathotypes that were reported from the United States and Ontario, Canada during 1960 to 1980 possessed virulences to relatively few *Rps* genes and, thus, were of low complexity (Kennedy 1984; Tooley et al. 1982). However, reports during the 1990s and 2000s indicated that *P. sojae* pathotype composition was increasing in complexity (Anderson and Buzzell 1992; Dorrance et al. 2003a; Leitz et al. 2000; Nelson et al. 2008; Ryley et al. 1998; Schmitthenner et al. 1994; Wagner and Wilkinson 1992; Xue et al. 2015; Yang et al. 1996; Zhang et al. 2010). In Iowa, Ohio, Minnesota, North Dakota, and Ontario, where this has been documented, complexity in *P. sojae* populations has continued to increase (Anderson et al. 2012; Dorrance et al. 2003a; Nelson et al. 2008; Xue et al. 2015; Yang et al. 1996). This would also indicate that increasing virulence was not resulting in decreased pathogen fitness and was not limiting pathogen growth and survival. In spite of this, many of the pathotypes differed by only one or two susceptible reactions on the differentials. Many of the simplest pathotypes remain common despite deployment of increasing numbers of *Rps* genes. For instance, although *Rps* genes have been deployed widely throughout the surveyed states for over 50 years, race 1, octal code 0001 (pathogenic on *Rps7*) was still common among the isolates from Iowa, Ohio, Kansas, and South Dakota. In addition, what would have been classified as race 0, octal code 0000 isolates that were avirulent to all of the differentials except the universal susceptible, Williams, were identified in Illinois, Indiana, Ohio, Minnesota, and South Dakota. Both morphological and molecular techniques confirmed that these isolates were, in fact, *P. sojae*.

The one exception was the isolates recovered from Ohio soils, where the complexity has declined from 4.06 to 2.71 since the last survey. This is most likely due to the large number of isolates ($n = 80$) recovered from 34 different locations, which was 15.7% of the isolates from Ohio that were only pathogenic on the universal susceptible. Most of these isolates were baited from soils during 2012, following a drought. Most of these soils sample were baited twice by replanting and flooding to recover *P. sojae*. There is the possibility that these isolates represent "older" pathotypes from oospores formed possibly in previous years or with the long-term drought, if there were epigenetic changes that occurred that could influence the virulence pathotype pattern. Schmitthenner (1985) referred to the longevity of this pathogen in soil and the need to understand how this can affect epidemics. Clearly, this is still an area that requires more research. It should be noted that, at each of the locations where isolates were recovered with this very simple pathotype (0000), there were other isolates with much greater complexity.

In Minnesota and South Dakota, deployment of *Rps3a* would be effective because virulence to this gene was observed at very low incidence in the survey, whereas virulence to *Rps6* occurred only infrequently in South Dakota. Surprisingly, greater than 40% of isolates recovered in Minnesota were virulent on *Rps6*, although this

Fig. 1. Proportion of *Phytophthora sojae* isolates collected in an 11-state survey between 2012 and 2013 that are virulent on a particular soybean *Rps* gene.

Rps gene is present in only a very limited number of soybean cultivars adapted in the state. These two *P. sojae* *R* genes, *Rps3a* and *Rps6*, may be candidates for rotation back into breeding programs in Iowa, Indiana, Michigan, New York, and Ohio. In these states, virulence to both *Rps* genes was present in only 10% or fewer of the isolates collected. Previous surveys in Indiana (Abney et al. 1997; Laviolette and Athow 1981) reported virulence toward *Rps6* at 32 and 16% of the population whereas, in this survey, it was lower. Having the ability to rotate genes would be a great benefit to breeders and the seed industry.

Several novel *Rps* genes have been identified since 2010 (Lin et al. 2013; Sugimoto et al. 2011; Sun et al. 2014; Zhang et al. 2013a,b)

and numerous sources of resistance have been identified (Dorrance and Schmitthenner 2000; Kyle et al. 1998) (A. E. Robertson, unpublished data). These novel genes from plant introductions greatly increase the number of potential sources of resistance that are available for management of *P. sojae*. However, most of these *Rps* genes are located in very complex regions of the soybean genome and can be associated with deleterious traits. Introgression of these *Rps* genes from land races and plant introductions into elite germplasm in soybean breeding programs will be very beneficial but will take time. Beginning a coordinated approach to rotate novel *Rps* genes along with previously utilized *Rps* genes (*Rps1c*, *1k*, *3a*, and *6*) may enhance and extend the usefulness of *R*-gene-mediated resistance for *P. sojae*.

Table 2. Prevalence and ranking of predominant pathotypes of *Phytophthora sojae* isolated from soybean seedlings in a soil bioassay or directly from plants collected from fields in a 11-state survey conducted during 2012 to 2013^a

Pathotype ^b	Virulence formulae ^c	Number of isolates	Isolates (%)
0	0000	88	10.1
1a,1b,1c,1k,3a,7	7101	79	9.1
7	0001	56	6.4
1k	0100	36	4.1
1b,1k,7	2101	36	4.1
1a,1b,1k,7	3101	24	2.8
1a,1b,1c,1k	7100	23	2.6
1c,1k	4100	20	2.3
1b,1k	2100	16	1.8
1a	1000	15	1.7
1a,1c,1k	5100	15	1.7
1c	4000	12	1.4
1a,1b,1c,1k,3b,7	7501	12	1.4
1k,7	0101	11	1.3
3a,7	0201	11	1.3
1a,7	1001	11	1.3
1a,1c,7	5001	11	1.3
1a,1c,1k,7	5101	11	1.3
3b	0400	9	1
1a,1b,7	3001	9	1
1c,7	4001	9	1
1a,1c	5000	9	1
1b,1c,1k,7	6101	9	1
1a,1b,1c,1k,3a,7	7301	9	1
3c	0010	8	0.9
1a,1k,7	1101	8	0.9
1a,1b,1c,7	7001	8	0.9
1a,1k	1100	7	0.8
1b,1k	3100	7	0.8
1b,1c,1k	6100	7	0.8
1a,1b,1k,3b,7	3501	6	0.7
1c,1k,7	4101	6	0.7

(continued in next column)

^a Pathotypes are those identified two or more times in the survey. Isolates were baited with susceptible Sloan from soil collected in soybean-growing areas of Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, Nebraska, New York, Ohio, and South Dakota.

^b Pathotypes were identified using the hypocotyl inoculation technique on soybean *P. sojae* differentials 'Williams' (universal susceptible), 'Harlon' (*Rps1a*), 'Harosoy 13XX' (*Rps1b*), 'Williams79' (*Rps1c*), PI103091 (*Rps1d*), 'Williams82' (*Rps1k*), L76-1988 (*Rps2*), L83-570 (*Rps3*), PRX146-36 (*Rps3b*), PRX 145-48 (*Rps3c*), L85-2352 (*Rps4*), Harosoy 62XX (*Rps6*), 'Harosoy' (*Rps7*), and PI 399073 (*Rps8*).

^c Virulence formulae are presented as octal four-digit codes, where the differentials were grouped as follows: *Rps1a*, *Rps1b*, and *Rps1c* - the first octal digit; *Rps1k*, *Rps2*, and *Rps3a* = the second octal digit; *Rps3b*, *Rps3c*, and *Rps4* = the third octal digit; and *Rps6*, *Rps7*, and *Rps8* = the fourth octal digit. Octal numbers were assigned based on the susceptible or resistant responses of each differential within the set, with 0 indicating a resistant response following inoculation and 1 indicating a susceptible response. The intermediate ratings were not used. Octal digits were assigned as follows: 000 = 0, 100 = 1, 010 = 2, 110 = 3, 001 = 4, 101 = 5, 011 = 6, and 111 = 7.

Table 2. (continued from preceding column)

Pathotype ^b	Virulence formulae ^c	Number of isolates	Isolates (%)
1a, 1b, 1c,1k,6,7	7141	6	0.7
1b	2000	5	0.6
1b,1c,1k,3b,7	6501	5	0.6
1a,1b,1c,1k,3a,7,8	7303	5	0.6
1a,1b,1c,1k,3a,3b,3c,7	7711	5	0.6
3c,7	11	4	0.5
4	20	4	0.5
1k,4,6	160	4	0.5
1a,1b,3b,7	3401	4	0.5
1a,1b,1c	7000	4	0.5
1a,1b,1c,1k,3b	7500	4	0.5
1a,1b,1c,1k,3a,3b,7,8	7703	4	0.5
4,6,7	0061	3	0.3
1b,7	2001	3	0.3
1a,1b	3000	3	0.3
1a,1b,1k,3c,7	3111	3	0.3
1c,3b	4400	3	0.3
1a,1b,1c,1k,3c,7	7111	3	0.3
1a,1b,1c,1k,3c,4,6,7	7171	3	0.3
1a,1b,1c,1k,3a,3c,4,6,7	7311	3	0.3
1a,1b,1c,1k,3a,3c,6,7	7351	3	0.3
1a,1b,1c,1k,3a,3b,7	7701	3	0.3
3c,4,6,7	0071	2	0.2
3a	0200	2	0.2
3a,3c,4,6,7	0271	2	0.2
3b	0401	2	0.2
1k,3b	0501	2	0.2
1a,3c	1010	2	0.2
1b,1k,4	2120	2	0.2
1b,1k,3a,3c,6	2141	2	0.2
1b,1k,3a,3c,6	2371	2	0.2
1b,1k,3b	2501	2	0.2
1a,1b,3c,6	3151	2	0.2
1a,1b,1k,3c,4,6	3171	2	0.2
1a,1b,3a	3200	2	0.2
1a,1b,6	3240	2	0.2
1a,1b,1k,3a	3300	2	0.2
1a,1b,1k,3a,7	3301	2	0.2
1a,1b,1k,3a,3b,7,8	3703	2	0.2
1a,1b,1k,3a,3b,3c	3711	2	0.2
1c,4	4020	2	0.2
1c	4041	2	0.2
1c,3c,4,6	4071	2	0.2
1c,1k,3b	4501	2	0.2
1c,1k,3a,3b	4701	2	0.2
1a,1c,1k,6	5141	2	0.2
1a,1c,1k,3b	5501	2	0.2
1b,1c,1k,3c	6111	2	0.2
1a,1b,1c,1k,3c,6,8	7353	2	0.2
1a,1b,1c,1k,3b,3c	7511	2	0.2
1a,1b,1c,1k,3b,6	7541	2	0.2

It was surprising to see the presence of virulence toward *Rps8* in almost all of the states where fields were sampled. Cultivars with this gene are still in development or have been planted on a very limited area. It will be critical for cultivars with *Rps8* to also have high levels

Fig. 2. Complexity of pathotypes of *Phytophthora sojae* (the number of differentials with which a specific isolate has a susceptible response) that were collected in an 11-state survey between 2012 and 2013.

of partial resistance to avoid the “vertifolia effect” (Van der Plank 1963) from occurring. Susceptibility to *P. sojae* can be reduced or limited when virulence to an *Rps* gene does develop, if cultivars have high partial resistance (Dorrance et al. 2003b; Schmitthenner 1985; Tooley and Grau 1984).

There have been other reports of virulence in *P. sojae* populations in the United States prior to any *Rps* gene deployment (Tooley et al. 1982). Large percentages of the *P. sojae* populations from Indiana, Michigan, and Ohio had virulence for *Rps1d*, *Rps3b*, *Rps3c*, *Rps4*, and *Rps5* without any exposure to these *Rps* genes (Abney et al. 1997; Dorrance et al. 2003a; Kaitany et al. 2001; Schmitthenner et al. 1994). As early as 2000 in Minnesota, Kurlle and El-Araby (2001) reported finding isolates of *P. sojae* that had virulence to all of the known *Rps* genes that were in cultivars at the time, and isolates with these virulences can be found in most locations if sampling was intensive.

When the complexity of pathotypes observed in this survey is compared with results from earlier surveys, the number virulent on multiple genes continues to increase. This is apparent when the results of surveys conducted in Iowa, Indiana, Michigan, Minnesota, New York, Ohio, and Ontario at intervals of 10 to 20 years are compared for either pathotype complexity or prevalence of individual pathotypes (Table 3). Increasing pathotype or race complexity has also been documented in other pathogen populations such as *R. secalis* and *Blumeria graminis* collected from barley (Zhan et al. 2012), *P. infestans* from potato (Montarry et al. 2010), and *Mycosphaerella graminicola* (Yang et al. 2013). A more troubling finding is the

Fig. 3. Proportion of isolates of *Phytophthora sojae* collected in each state that are virulent on soybean *Rps* genes 1a, 1b, 1c, 1k, 3a, 6, and 8. Abbreviations: IA = Iowa, IN = Indiana, IL = Illinois, KS = Kansas, MI = Michigan, MN = Minnesota, NE = Nebraska, NY = New York, OH = Ohio, and SD = South Dakota.

Table 3. Comparison of pathotype diversity which is based on percentage of virulence (susceptibility) to a given *Rps* gene and complexity of isolates of *Phytophthora sojae* collected in each state in 2012 to 2013 (this study) compared with results obtained in earlier surveys^a

State	Fld	Year	Iso	1a	1b	1c	1k	3a	6	8	CX	Cited
Iowa	71	1991–94	170	67	3	20	3	0.6	2	NT	2.67	Yang et al. 1996
	35	2012–13	130	64	64	41	58	9	0	3	3.58	This study
Illinois	80	2001–02	121	75	50	51	44	7	10	NT	NA ^b	Malvick and Grunden 2004
	76	2012–13	67	48	41	42	34	30	18	6	4.43	This study
Indiana	NA	1973–79	1,099	83	0	3	0	18	32	NT	1.14	Laviolette and Athow 1981
	NA	1993	300	67	15	12	17	8	16	NT	1.90	Abney et al. 1997
	13	2013	38	53	58	63	71	8	8	3.0	3.74	This study
Michigan	60	1993–97	87	88	26	44	31	23	34	NT	3.91	Kaitany et al. 2001
	31	2013	7	100	86	30	86	0	0	0	4.00	This study
Minnesota	90	2000	71	34	29	41	39	32	34	NT	NA ^b	Kurle and El-Araby 2001
	14	2012	57	65	61	60	68	18	47	5.3	4.40	This study
New York	1	1983	5	100	80	60	NT	80	1.2	NT	4.2	Tooley et al. 1982
	1	2012	6	67	67	67	67	0	17	0	3.87	This study
Ohio	57	1997–99	429	83	50	46	52	33	27	NT	4.06	Dorrance et al. 2003a
	77	2012–14	510	36	37	38	56	13	8	2.7	2.71	This study
Ontario	161	1980–1989	705	91.9	38.1	18.1	38.3	34.4	553.6	NT	NA	Anderson and Buzzell 1992
	205	2010–12	358	93.4	38.1	49.4	38.3	18.9	51.3	NT	NA	Xue et al. 2015

^a Fld = number of fields, Iso = number of isolates, CX = average complexity, NT = *Rps8* not included as a differential, and NA = not available in cited reference.

^b Complexity is the number of differentials with which a specific isolate has a susceptible interaction and was not calculated or calculated on different combination of *Rps* genes in the original article.

observation that new pathotypes developed in *M. graminicola* recovered from a wheat cultivar with high levels of quantitative resistance in the absence of specific *R* genes. In addition, isolates obtained in this situation were more aggressive than those recovered from a susceptible cultivar (Andrison et al. 2007; Yang et al. 2013). A similar phenomenon was observed when a soybean line considered to be partially resistant to *P. sojae* was planted repeatedly in a 4-year crop sequence. Pathotypes recovered following the partially resistant cultivar were among the most complex recovered from six rotation sequences (Stewart et al. 2014). This may have implications for long-term management of disease resistance sources, especially when the same crop is produced continuously without rotation, an increasingly common crop sequence in soybean production. Yang et al. (2013) proposed that turn-over or rotation of host resistance may be needed to prevent the emergence of pathogen populations with increased virulence.

The development of complex races or pathotypes is commonly attributed to selection of virulent pathotypes in response to deployment of single-gene resistance in commonly planted cultivars. However, other mechanisms may be involved in the increasing number of *P. sojae* pathotypes. Rutherford et al. (1985) observed the development of new pathotypes following single-spore transfers in vitro. Stewart et al. (2014) observed that the number, complexity, and virulence of pathotypes increased when cultivars with high levels of partial resistance were planted. In their study, the effect of continuous 4-year sequences of a partially resistant cultivar having no *rps* gene was compared with continuous planting for 4 years of a cultivar with the *Rps1k* gene. Eight pathotypes were recovered after 4 years of the partially resistant cultivar whereas two pathotypes, neither with virulence to *Rps1k*, were recovered in the continuous *Rps1k* sequence (Stewart et al. 2014). In 4-year sequences of a cultivar with no *Rps* gene or annual rotations of a partially resistant cultivar with either a cultivar having either no *Rps* gene or a cultivar with the *Rps1k* gene, six, six, and eight pathotypes, respectively, were recovered after the fourth year. None of the pathotypes recovered were virulent on *Rps1k*. However, virulence to *Rps1k* was recovered after alternate-year rotations of a cultivar with no resistance and a cultivar with the *Rps1k* *R* gene. Their conclusion was that presence of an *Rps* gene was not the reason for changes in pathotype structure in this field.

In this study, where the seedling hypocotyl inoculation assay was used to determine pathogenicity, aggressiveness was not evaluated and would be very difficult to measure. The likelihood of either increased virulence complexity or increased aggressiveness in populations of *P. sojae* will be dependent on the fitness costs associated with

these changes. At this time, there is no evidence to suggest that these isolates may or may not have changes in fitness. This is a question that deserves further research.

In the states that were surveyed, all of the *Rps* genes provided protection to some proportion of the *P. sojae* isolates that were evaluated. The distribution of virulences varied considerably and the proportion in neighboring states was dissimilar. There is a strong potential that newer (*Rps8*) or uncommon (*Rps6*) *Rps* genes can provide effective management of *P. sojae* but this should be deployed with partial resistance. It appears that virulence to *Rps1a* and *Rps1b* may be “fixed” and very common in the U.S. populations of *P. sojae*. However, based on comparisons with previous surveys in many of these states, *R* genes *Rps3a* and *Rps6* may be candidates for reintroduction into breeding programs. The effect on overall pathogen fitness that may accompany the gain of virulence to these genes should be a focus of future studies. Finally, where comparisons could be made, a gain of virulence or increasing complexity does appear to have occurred, especially for those genes in the *Rps1* locus (*Rps1a*, *1b*, *1c*, and *1k*).

Although the complexity and virulence of *P. sojae* populations continues to increase, occurrence of virulence on a specific *Rps* gene was present in only a proportion of fields sampled (Dorrance et al. 2003a; Robertson et al. 2009). Thus, cultivar selection is still essential for long-term management of *Phytophthora* seedling, root, and stem rot and to prevent losses to *P. sojae* when environmental conditions that are favorable for infection occur. Assessing pathotype prevalence in pathogen populations is a cumbersome but essential task that is necessary to ensure the immediate effectiveness of resistance breeding and to avoid boom and bust cycles in cultivar development in the long term.

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