Comparison of *Phytophthora sojae* populations in Iowa and Nebraska to identify effective *Rps* genes for Phytophthora stem and root rot management

Rashelle L. Matthiesen[1], Clarice Schmidt[1], Vinicius C. Garnica[2], Loren J. Giesler[3], and Alison E. Robertson.[1]

[1]Dept. Plant Pathology and Microbiology, Iowa State University, Ames IA 50011

[2]Dept. of Entomology and Plant Pathology, North Carolina State University, Raleigh NC 27695

[3]Dept. of Plant Pathology, University of Nebraska-Lincoln, Lincoln NE 68583

Abstract

Phytophthora stem and root rot (PSRR) of soybean, caused by the oomycete *Phytophthora sojae*, is prevalent in Iowa and Nebraska. Reducing losses to PSRR primarily relies on growing cultivars with specific resistance (*Rps)* genes. Predominant genes used in commercial soybean cultivars include *Rps* 1a, *Rps* 1c, *Rps* 1k, and *Rps* 3a. Knowing which *Rps* gene to deploy depends on knowledge of which genes are effective against the pathogen. From 2016 to 2018, 326 isolates of *P. sojae* from were recovered from fields in Iowa and Nebraska and classified into pathotypes based on their virulence on 15 soybean genotypes. A total of 15 and 10 pathotypes were identified in Iowa and Nebraska, respectively. Almost all isolates were virulent on *Rps* 1a, while over 70% of isolates were virulent on *Rps* 1c and *Rps* 1k. Only 2.3% of isolates from Iowa were virulent on *Rps* 3a. Among commercial soybean cultivars tested in the Illinois Soybean Variety trials from 2010 to 2020, *Rps* 1c was always the most frequently reported gene

followed by *Rps* 1k. In contrast, *Rps* 1a and *Rps* 3a were present in less than 10% and less than 5 % of the cultivars tested, respectively. Since many of the *P. sojae* isolates in our study were virulent on *Rps* 1a, *Rps* 1c, and *Rps* 1k, soybean cultivars with these genes are unlikely to provide protection against PSRR unless they have a high level of partial resistance.

Understanding pathogen diversity is important knowledge for developing disease management tools. One of the most effective tools for managing plant pathogens is host resistance, which. is governed by one or many genes. In some crop pathosystems, such as Phytophthora stem and root rot of soybean, leaf rust of wheat, and northern corn leaf blight of corn, specific genes in the pathogen interact with a corresponding gene in the host (Gevers 1975; Kolmer 1996; Ward 1990). This is known as the gene-for-gene interaction and was proposed by Flor (1955). Simply put, when a pathogen infects a host plant, the pathogen secretes an effector protein, encoded by an avirulence gene. If the effector is recognized by a protein encoded by a specific resistance gene in the host plant, the defense system of the host plant is triggered, and no disease develops. If, however, the resistance protein is not produced in the host plant or the pathogen does not secrete the effector, there is no recognition that infection is occurring and disease will develop. This gene-for-gene relationship has been exploited to characterize the diversity of pathogen populations both spatially (among fields and states) and temporally (across years) (Costamilan et al. 2013; Dorrance et al. 2003 2016; Grijalba et al. 2020; Kolmer 2019; Robertson et al. 2009; Ryley et al. 1998; Stewart et al. 2016; Weems and Bradley 2018; Xue et al. 2015). It is important to monitor changes in pathogen virulence (ability of the pathogen to cause disease on a specific resistance gene) to commercially deployed host resistance genes. Knowledge of pathogen

47 virulence provides information on the stability of the resistance genes in a region. This
48 information ensures effective resistance genes are utilized and thereby enables successful disease
49 management.
50
51 Phytophthora stem and root rot (PSRR), caused by *Phytophthora sojae*, is a soilborne pathogen
52 that ranks among the top five pathogens causing economic losses of soybean annually in the
53 United States (Allen et al. 2017). In Iowa and Nebraska, PSRR is widespread and tends to be
54 more of a problem in poorly drained soils and after periods of heavy precipitation (Dorrance et
55 al. 2009; Garnica and Giesler, 2020; Robertson et al. 2009). Losses vary widely from year to
56 year. Soybean pathologists in the two states estimate 7.1 million and 5.6 million bushels of
57 soybean were lost to PSRR in Iowa and Nebraska, respectively, from 2010 to 2019 (Crop
58 Production Network, 2020). Symptoms of the disease include damping off of seedlings, root and
59 basal stem rot, and blight of plants at any growth stage (Dorrance et al. 2008). This disease is
60 primarily managed with soybean cultivars that either contain: specific gene(s) that govern
61 resistance to *P. sojae* (*Rps* genes), multiple genes that provide incomplete resistance to the
62 pathogen (partially resistant), or both forms of resistance (Dorrance et al. 2004). More than 30
63 *Rps* genes have been identified (Van et al. 2020). In the United States, *Rps* 1a, *Rps* 1c, *Rps* 1k,
64 and *Rps* 3a have been deployed in many commercial soybean cultivars, while cultivars with *Rps*
65 1b, *Rps* 6, or *Rps* 8 are occasionally available (Slaminko et al. 2010; Dorrance et al. 2016). The
66 durability of the effectiveness of *Rps* genes, however, is estimated to be 8 to 20 years due to
67 changes in the virulence of *P. sojae* populations (Dorrance et al. 2003; Grau et al. 2004). Yan
68 and Nelson (2019) reported adaptation of *P. sojae* populations in North Dakota to *Rps* 1c and
69 *Rps* 1k from 1994 through 2015. Similarly, adaptations of *P. sojae* populations to *Rps* 1c, *Rps*

1k, and also *Rps* 1a have been detected in Iowa, Indiana, Michigan, Minnesota, and Ontario, Canada (Dorrance et al. 2016).

Phytophthora sojae is characterized into pathotypes (originally called races) based upon its virulence on a standard differential set of 8 to 14 soybean genotypes that each contain a different *Rps* gene, and a universal susceptible (Dorrance et al. 2004, 2008). Isolates of the pathogen are inoculated separately on each genotype, and the absence or presence of the disease is assessed approximately seven days after inoculation (Dorrance et al. 2008). Prior to 2000, a set of eight genotypes was used to officially classify *P. sojae* into 55 races (Grau et al. 2004). The identification of new *Rps* genes (Van et al. 2020) lead to the addition of several soybean genotypes to the standard differential set of eight genotypes originally used to characterize *P. sojae* isolates (Dorrance et al. 2008). Consequently, it became very difficult to assign race numbers to *P. sojae* isolates, since the number of potential races is 2^N, where N = the total number of *Rps* genes (Stewart et al. 2014). Therefore, an isolate of *P. sojae* is now classified as a pathotype (i.e., a virulence formula listing the *Rps* genes on which an isolate is able to cause disease) (Dorrance et al. 2008; Stewart et al. 2014).

The diversity of *P. sojae* in Midwestern states of the U.S. has been characterized several times since the pathogen was first reported in Indiana in 1957 (Bernard et al. 1957; Dorrance et al. 2003, 2016; Niu 2004; Stewart et al. 2016; Yang et al. 1996). Surveys have shown the pathogen continues to diversify, and new pathotypes are reported with each survey. In Iowa, 17 pathotypes were identified from 99 fields sampled in 2001 to 2003 using eight genotypes (Niu 2004), compared to 37 pathotypes recovered from 35 fields that were sampled in 2012 to 2013, using 10

genotypes (Dorrance et al. 2016). In Nebraska, three comprehensive studies documenting the virulence diversity of *P. sojae* have been conducted since early 1980s. From 1980 to 1981, seven pathotypes were identified in six eastern counties (White et al. 1983). The number of genotypes used in this study to pathotype the isolates was not specified. Schimelfenig et al. (2005) identified 11 pathotypes on eight genotypes in soil and plant samples recovered from 52 fields with a history of seedling disease from 2000 to 2004, while 10 pathotypes were recovered from six fields using 10 genotypes in 2012 to 2013 (Dorrance et al. 2016). Moreover, the complexity, defined as the number of *Rps* genes on which an isolate is pathogenic, continues to increase in the Midwest (Dorrance et al. 2016).

From 2016 to 2018, a survey of soybean fields in Iowa and Nebraska was conducted as part of a larger study examining *P. sojae* populations within the north central region of the United States. Since Iowa and the primary soybean region of Nebraska have similar production zones, soil, precipitation, maturity group use, and even grower's seed brand preferences, data regarding *P. sojae* populations could be useful to breeding companies to provide effective resistance genes. Therefore, this study was conducted to (i) characterize the pathotype diversity in each state and (ii) compare diversity and complexity of pathotypes between states. In addition, changes in *Rps* gene deployment in commercial soybean cultivars from 2010 to 2018 was reported to elucidate any relationship between *Rps* gene deployment and virulence diversity. Data from this study could identify which *Rps* genes would be more effective for PSRR management in each state.

Methods

Soil sampling

In Iowa, 26 soybean fields in 21 counties were arbitrarily selected and sampled for *P. sojae* (Fig. 1A). Ten fields were sampled in 2016 and 16 fields in 2017 during the growing seasons. In a low-lying area of each field, soil samples were collected from 10 soil sampling sites, 20 to 25 m apart from each other (approximately 1000 m^2). At each site, approximately 1 L of the top 20 cm of soil was dug with a shovel and placed in a plastic bag. Soil samples were transported to Iowa State University and stored at 4ºC until they were processed for baiting of *P. sojae*. In Nebraska, soil samples were collected from soybean fields during the 2017 and 2018 growing seasons. In total, 39 fields in 23 counties were sampled to represent *P. sojae* populations endemic to the soybean-producing areas of the state (Fig. 1B). Soil cores were collected across various points in a field using a sampling probe at 15 to 20 cm deep to form a composite soil sample of approximately 300 cm^3.

Plant sampling

Phytophthora sojae was also recovered from symptomatic plants submitted to the Plant and Pest Diagnostic Clinic at the University of Nebraska-Lincoln from 2016 through 2018. Fresh stem sections with distinctive dark-brown lesions were surface sterilized with 0.5% sodium hypochlorite solution for 30 s, washed under tap water, and triple rinsed in distilled water before placed in laminar flow hood to dry. Stem sections were then split in half and cut into 1-cm-longpieces, which were placed onto PBNIC selective media (Dorrance et al. 2008). A single colony of *P. sojae* was then transferred to V8 juice medium and stored until pathotype characterization.

Baiting and isolation

Standard methods for baiting and isolation of *P. sojae* similar to that described in Dorrance et al. (2008) were used. Briefly, for soil samples collected in Iowa, each soil sample was manually passed through a sieve and then air-dried on a bench in the laboratory. For soil samples from Nebraska, each sample was air dried in the greenhouse and ground into fine soil particles using a soil grinder Romer Series II Mill (Romer Labs, Inc., Newark, DE, USA). Soil samples from each field were thoroughly mixed and combined, and then placed into ten polystyrene squat cups (355 ml) or plastic pots (200 ml) with four 3-mm holes on the bottom of each cup for drainage. The cups were flooded with deionized water for 24 h, and then drained for approximately 24 to 48 h. After draining, cups were placed in plastic bags and incubated at 25°C in the dark for 14 days. At this time, the plastic bags were removed and each pot was planted with 5 seeds of the *P. sojae*-susceptible cultivar Sloan and the seeds were covered with coarse vermiculite. Three days after planting, cups were flooded again with deionized water for 24 h and then removed from trays and placed in a growth chamber at 25°C with a 16 h photoperiod. Cups were watered with deionized water daily. Seedlings with PSRR symptoms (brown to tan lesions on the hypocotyl, brown root lesions, and damping-off) were observed 7 to 10 days after the second flooding and removed for isolation of *P. sojae*. If no isolates were recovered in the first baiting, all remaining ungerminated seeds and asymptomatic seedlings were removed from the cups. One or two subsequent baitings were performed using the same soil sample by replanting the cups with five soybean seeds (cultivar Sloan), and processed as described above.

Symptomatic seedlings were washed with sterilized distilled water. Tissue, containing the leading edge of a lesion, was excised and transferred to *Phytophthora*-selective media (PBNIC) (Dorrance et al. 2008) and incubated at 22°C in the dark. Characteristic mycelia of *P. sojae* that

grew from tissue samples were excised and transferred to media containing PBNIC or DV8++ [DV8 media containing neomycin sulfate (0.10 g L^{-1}) and chloramphenicol (0.01 g L^{-1}] (Dorrance et al. 2008; Matthiesen et al. 2016). After 2 to 7 days at 22°C in the dark, cultures were examined under the microscope and those that appeared to be *P. sojae* were transferred to a new 100 mm x 15 mm Petri dish containing 20 mL of DV8++. All isolates were identified as *P. sojae* morphologically (Waterhouse 1963) and those collected in Nebraska were placed on PDA to evaluate mycelial growth (Dorrance et al. 2008). Further identification was conducted by sequencing the ITS region (Cooke et al. 2000) of four isolates that had slow growth on 20% clarified V8 agar.

Pathotyping

Isolates from both Iowa and Nebraska were pathotyped at Iowa State University on a set of 14 genotypes and one *P. sojae*-susceptible cultivar (Table 1). Soybean seeds were placed on germination paper (#38 cut to 30cm x 60cm, Anchor Paper, St. Paul MN), the germination paper was rolled, and then placed in a plastic box (12.7cm H x 35cm L x 21cm W) containing 2 L of deionized water. The boxes of germination rolls with seeds were placed in a controlled environmental room at 25ºC with 16h photoperiod (Nebraska isolates) or in the dark at room temperature (22ºC) (Iowa isolates). After 7 days, the germination paper was unrolled to expose the hypocotyls of the seedlings for inoculation. For each soybean genotype, 10 1-week-old seedlings were inoculated 1 cm below the cotyledons by injecting approximately 200-400 µl of mycelial slurry of an isolate into the hypocotyl using an 18-guage needle (Dorrance et al. 2008). The germination paper with the inoculated seedlings was then re-rolled and placed back into the plastic box containing water and returned to the controlled environmental room at 25ºC with 16h

photoperiod (Nebraska isolates) or grown in the dark at room temperature (22ºC) (Iowa isolates). For both procedures, the pathogenicity of each isolate was evaluated 7 days after inoculation. Reactions were scored as resistant or susceptible based on the presence of lesions on the hypocotyl of less than 20% or more than 70% of the seedlings, respectively. Pathotype tests were repeated once.

Survey of Rps *genes present in commercial soybean cultivars*

The frequency of various *Rps* genes present in commercial soybean cultivars was determined using data from the Illinois Soybean Variety trials conducted from 2010 through 2020 (Esgar et al. 2010, 2011, 2012; Joos et al. 2013, 2014; Ames et al. 2015, 2016; Joos et al. 2017, 2018, 2019, 2020). These trials are conducted annually at 13 sites in Illinois and compare the performance (yield, lodging, maturity, height, and shattering) of private and public cultivars from seed companies in Illinois and the surrounding states. Although similar trials were done in Iowa and Nebraska, information regarding *Rps* genes present in the cultivars tested was not included. Since similar soybean maturity groups, and consequently similar cultivars, are grown in Iowa, Illinois, and Nebraska, the Illinois Soybean Variety trial data was reasoned to be a useful resource for determining frequency of various *Rps* genes present in commercial soybean cultivars. In each year, the number of cultivars that were reported to contain *Rps* 1a, *Rps* 1c, *Rps* 1k, or *Rps* 3a was calculated as a percent of the total number of cultivars grown in the trials for that year. Only the presence of these *Rps* genes was reported, and not all cultivars tested in the trials reported the presence of a *Rps* gene.

Data analysis

Only those isolates that had consistent pathotypes in both pathotype test experimental runs were included in the analysis. Isolate virulence data were analyzed for each state individually using the "HaGis" R package (McCoy et al. 2019). Pathotype virulence data was compared between states, frequency distributions of the virulence of Iowa and Nebraska isolates to specific *Rps* genes were produced, and the complexity of the pathotype of isolates from both states were described. A paired-sample *t* test was done to compare the mean complexity between states. Differences were considered statistically significant at $P<0.05$. Furthermore, the following diversity indices were calculated using the "HaGis" R package (McCoy et al. 2019) in R 3.2.2 (R core team 2019): Simple diversity index (proportion of distinct pathotypes relative to the number of isolates collected) where 0 = no diversity and 1 = infinite diversity; Gleason's index which also indicates pathotype richness within a population, but is less sensitive to sample size than the Simple Index. Dorrance et al. (2016) reported Gleason index's ranging from 0.4 to 22.94. Shannon's index, which is an indication of the evenness of distribution of pathotypes within a sample and usually ranges from 1.5 (less diversity) to 3.5 (more diversity); Simpson index (proportion of pathotypes relative to the total number of pathotypes) where 0 = no diversity and 1 = infinite diversity; and Evenness index (relative abundance of the different pathotypes composing the sample) which ranges from 0 to 1, where 1 represents an even distribution of the frequency of each pathotype within the population.

Results

Altogether, 65 fields from 45 counties in Iowa and Nebraska (Table 2, Fig. 1) representing approximately 30 percent of soybean acreage in the two states

(https://www.nass.usda.gov/Statistics_by_State/index.php) were sampled. A total of 326 isolates of *P. sojae* were recovered (Table 2). The number of isolates recovered from each field in Iowa varied from five to 17 and those in Nebraska varied from one to seven. In addition, there were nine isolates of *P. sojae* recovered from mature PSRR symptomatic plants in Nebraska.

Pathotype diversity and complexity.

Consistent pathotypes were recorded for 258 Iowa and 68 Nebraska isolates (Table 2). Of the isolates from Iowa, 100% were virulent on *Rps* 1a and *Rps* 7, while 95.6% and 97.1% of the Nebraska isolates were virulent on these two *Rps* genes (Fig. 2). Over 70% of isolates from both states were virulent on *Rps* 1b, *Rps* 1c, *Rps* 1k, and *Rps* 2, while 35.1% and 17.6% of the isolates from Iowa and Nebraska, respectively, were virulent on *Rps* 3b (Fig. 2). Very few isolates from Iowa (2.3%) and none from Nebraska were virulent on *Rps* 3a (Fig. 2). No Iowa isolates were virulent on *Rps* 3c, *Rps* 4, *Rps* 6, and *Rps* 8; however, a few (less than 2.0%) of the Nebraska isolates were virulent on at least one of these genes (Fig. 2). A total of 15 and 10 pathotypes were identified in Iowa and Nebraska, respectively (Table 1 and Supplementary Table 1). Six of the pathotypes identified were alike across states, while nine pathotypes were unique to Iowa and four unique to Nebraska (Supplementary Table 1).

The complexity of the pathotypes recovered in each state differed slightly in this study with mean complexity estimated at 5.5 and 6.5 in Iowa and Nebraska, respectively ($P<0.001$; Fig. 3). Of the Iowa isolates, 41.3% of the isolates were virulent on six *Rps* genes, followed by 26.7% of the isolates on three *Rps* genes (Fig. 3). In Nebraska, 58.8% of isolates were virulent on seven *Rps* genes, followed by 14.7% of isolates on six *Rps* genes (Fig. 3). Six isolates that were

254 virulent on 8 *Rps* genes were recovered in Nebraska, while four and three isolates from Iowa
255 were virulent on nine and 10 *Rps* genes, respectively (Fig. 3).
256
257 The percent of isolates that were virulent on *Rps* 1a, *Rps* 1b, *Rps* 1c, *Rps* 1k, and *Rps* 3a in Iowa
258 increased in this survey compared with the surveys done from 1991 to 1994 and 2012 to 2013
259 (Supplementary Fig. 1). In this survey however, no isolates with virulence to *Rps* 6 or *Rps* 8 were
260 detected in Iowa, while they had been detected in the earlier surveys. Similarly, in Nebraska, the
261 prevalence of isolates that were virulent on *Rps* 1a, *Rps* 1b, and *Rps* 1k increased and those that
262 were virulent for *Rps* 1c, *Rps* 3a, *Rps* 6, and *Rps* 8 decreased compared to the 2000 to 2004 and
263 2012 to 2013 surveys (Supplementary Fig. 2).
264
265 Comparison of the calculated diversity indexes between the two states should be done with
266 caution, since sample sizes (the number of isolates evaluated) varied greatly (5.5-fold more
267 isolates from Iowa versus Nebraska). Even so, indices were similar between states (Table 2).
268 Simple diversity was low for both states indicating the proportion of distinct pathotypes relative
269 to the total number of isolates collected was low, with the value for Iowa less than half of that for
270 Nebraska (Table 2). Low values for both states were also calculated for the Gleason diversity
271 index, which indicated the richness of pathotypes among the isolates (Table 2). The Shannon
272 Diversity index, which measures relative differences in pathotypes among the isolates was
273 greater for Iowa compared to Nebraska (2.04 versus 1.64) (Table 2). The evenness scores for
274 Iowa and Nebraska isolates were similar (0.75 and 0.71, respectively) and indicated the number
275 of pathotypes of isolates within each community was not similar (Table 2). Simpson's index,

which gives a measure of pathotype richness and evenness, was close to 1 for both states (Table 2). The closer the value to 1, the more diverse the population.

Survey of Rps *genes present in commercial soybean cultivars*

The number of cultivars tested in the Illinois Soybean Variety trials varied from 236 cultivars in 2019 to 588 cultivars in 2010 (Fig. 4). The percentage of cultivars reported to have a *Rps* gene also varied from year to year (Fig. 4). Across all years however, *Rps* 1c was always the most frequently reported gene followed by *Rps* 1k (Fig. 4). The *Rps* 1c gene was present in approximately 28.0% of cultivars tested in 2014 and 2020, and more than 50% of cultivars in 2011, 2012, 2013, 2017, and 2018 (Fig. 4). In general, the frequency of the *Rps* 1k gene in cultivars tested decreased from 2010 (24.3%) to 2018 (7.2%); however, in 2019 and 2020, 11.0% and 14.8%, respectively, of cultivars were reported to have the gene (Fig. 4). The *Rps* 1a gene was present in less than 10% of cultivars tested from 2010 through 2020, while *Rps* 3a was present in less than 5 % of the cultivars tested, and was not reported in any cultivars in 2018 to 2020 (Fig. 4).

Discussion

This study compared the pathotype diversity of *P. sojae* from Iowa and Nebraska to other similar studies in the region (Dorrance et al. 2016; Schimelfenig et al. 2005; Stewart et al. 2016; White et al. 1983; Yang et al. 1996). Similar to previous studies, the pathogen was recovered from all fields sampled in Iowa and Nebraska, suggesting it is endemic in commercial soybean fields throughout both states. The Iowa isolates were categorized into 15 pathotypes, which is half of the number of pathotypes that were reported for Iowa in the Dorrance et al. (2016) study,

299 which was conducted three to four years previously, while the Nebraska isolates were
300 categorized into 10 pathotypes, which is similar to the Dorrance et al. (2016) study. Although the
301 number of pathotypes in populations of the pathogen in Iowa and Nebraska have not increased
302 since 2013, the complexity of the pathotypes has increased. Pathotype complexity of Iowa
303 isolates increased (5.5 versus 3.6) compared to that reported in Dorrance et al. (2016).
304
305 We tested virulence of Iowa and Nebraska isolates on 15 soybean genotypes, which is more than
306 the eight to 10 genotypes used in previous studies (Dorrance et al. 2016; Yang et al. 1996), but
307 similar to another study done in Iowa where 14 genotypes were used (Robertson et al. 2009). In
308 that study, 23 pathotypes were present in two fields that were intensively sampled, whereas the
309 current study sampled 26 fields. Surprisingly, we detected fewer pathotypes among the Iowa
310 isolates in this study compared to these previous studies, despite using more genotypes in the
311 differential set. Nevertheless, in both states the frequency of isolates that were virulent on *Rps*
312 1a, *Rps* 1c, and *Rps* 1k, remained high while the frequency of isolates virulent on all other genes
313 evaluated remained comparatively low, as has been reported in previous studies (Dorrance et al.
314 2016; Robertson et al. 2009; Schimelfenig et al. 2005; White et al. 1985; Yang et al. 1996). One
315 explanation for the reduced number of pathotypes detected in Iowa in this survey compared to
316 other surveys could be due to where the soil was collected for baiting. In this survey, soil was
317 collected from fields arbitrarily selected across Iowa; whereas, in Dorrance et al. (2016), soil
318 samples were collected from fields with a history of PSRR or stand establishment issues.
319 Another explanation may be a function of the fitness of the pathotype. Plates were checked daily
320 for signs of mycelium growing from symptomatic tissue. Mycelium that is first noticed growing
321 out from symptomatic tissue is typically the first and only mycelium to be isolated. Therefore,

322 pathotypes that are faster growing may have been recovered more frequently than slower
323 growing pathotypes. Niu (2004) reported differences in mycelial growth rate, zoospore
324 production, and infective ability of 16 isolates representing five pathotypes (races) of *P. sojae.*
325
326 Diversity indices allow differences between populations to be compared (McCoy et al. 2019). In
327 this study, all of the diversity indices, except the Simple Index, were slightly higher for Iowa
328 than Nebraska, indicating more pathotype diversity in Iowa. Since considerably more isolates
329 were collected in Iowa than Nebraska (258 isolates vs 68 isolates, respectively), there was a
330 greater chance of detecting unique pathotypes (greater richness) and thus affecting a diversity
331 index. In Iowa, 15 pathotypes were detected compared to 10 pathotypes in Nebraska. That
332 Nebraska had a greater Simple index than that of Iowa is because a comparatively higher number
333 of pathotypes were detected among the lower number of isolates collected from Nebraska. Thus,
334 the Simple Index indicated greater diversity in Nebraska, although since the values for both
335 states were both close to zero, diversity was still relatively low. The Gleason, Shannon, and
336 Simpson indices of diversity for isolates of *P. sojae* recovered from Iowa were similar to those
337 reported in other studies (Dorrance et al. 2016; Stewart et al. 2016); however, the Simple index
338 was also lower in this study compared to the Dorrance et al. (2016) study. Similarly, we
339 evaluated considerably more isolates in this study (258 isolates) compared with the Dorrance et
340 al. (2016) and Stewart et al. (2016) studies (130 and 47 isolates, respectively), but detected
341 proportionally fewer pathotypes (15 vs 37 and 10, respectively). Even so, comparison of the
342 calculated diversity indices among studies should be done with caution, since diversity indices
343 are dependent on sample size, evenness and richness, and, furthermore, there are subtle
344 differences in the characteristics of diversity they report (Daly et al. 2018).

345 Several studies have suggested widespread deployment of *Rps* genes in commercial soybean
346 cultivars have contributed to the high frequencies of isolates with virulence to the genes
347 deployed (Dorrance et al. 2016; Jackson et al. 2004; Schmitthenner et al. 1994; Yan and Nelson
348 2019; Yang et al. 1996). Certainly, data from the Illinois Soybean Variety trials support this
349 suggestion since *Rps* 1c and *Rps* 1k have been extensively incorporated into commercial soybean
350 cultivars over the past decade; 42.8% and 17.2% of all cultivars tested, respectively. Thus,
351 detection of higher frequencies of isolates virulent on these genes could be a function of these
352 genes being available in many conventional soybean cultivars. Regarding *Rps* 3a, approximately
353 2% of isolates tested in this study were virulent on this gene, which is less than that reported by
354 Dorrance et al. (2016) in their study. Only 1.4% of cultivars evaluated in the Illinois Soybean
355 Variety trials were reported to have *Rps* 3a. Consequently, the low frequency of *P. sojae* isolates
356 with virulence to *Rps* 3a may be a function of low selection pressure. In contrast, *Rps* 1a has
357 been deployed in few (5%) cultivars over the same time period and yet almost all of the isolates
358 tested in this study (323 of 324 isolates) were virulent on *Rps* 1a. Other studies have also
359 reported high frequencies of isolates that are virulent on *Rps* 1a (Dorrance et al. 2016, Robertson
360 et al. 2009; Yang et al. 1996). *Rps* 1a was the first resistance gene against *P. sojae* deployed in
361 commercial soybean cultivars in the north central U.S. (Grau et al. 2004). Since almost all the
362 isolates were virulent on *Rps* 1a, this implies that there are few, if any, fitness costs associated
363 with loss of the avirulence gene *Avr* 1a in *P. sojae.*
364
365 Our data suggest populations of *P. sojae* in Iowa and Nebraska have adapted to *Rps* 1a, *Rps* 1c,
366 or *Rps* 1k since nearly 70% to 100% of isolates recovered from fields in Iowa and Nebraska were
367 virulent on these three genes. Indeed, the frequency of isolates from Iowa that are virulent on *Rps*

1c and *Rps* 1k has increased since the last survey in 2012 to 2013 (Dorrance et al. 2016). Although the frequency of virulence on *Rps* 1k in Nebraska has increased and the frequency of virulence on *Rps* 1c was slightly lower, this may be because fewer isolates (14 isolates) were collected in the 2012 to 2013 survey (Dorrance et al. 2016). Despite the high frequency of *P. sojae* isolates that are virulent on *Rps* genes commonly used in commercial soybean cultivars (*Rps* 1c and *Rps* 1k), in the past five years, widespread outbreaks of PSRR have not been reported in either Iowa or Nebraska. Reasons for this include unfavorable conditions for infection and disease development (Robertson et al. 2009; Rojas et al. 2017), widespread use of seed treatments that reduce damping off (Dorrance et al. 2009; Garnica and Giesler 2019) and therefore loss of crop stand, or high partial resistance in cultivars being planted.

Data from our study suggests incorporating *Rps* 3a into commercial cultivars could be very useful for reducing losses to PSRR in Iowa and Nebraska, since so few isolates were virulent on the *Rps* 3a differential line (PI 171442) used. Dorrance et al. (2016) also suggested that *Rps* 3a would be a good candidate for incorporation into commercial cultivars, since less than 15% of the isolates in that study were virulent on the gene. Interestingly, no cultivars with *Rps* 3a were tested in the Illinois Soybean Variety trials in 2018, 2019, and 2020. Since the durability of a *Rps* gene is estimated to be 8 to 20 years (Dorrance et al. 2003; Grau et al. 2004), stacking *Rps* genes has also been suggested to prolong the life of a *Rps* gene (Dorrance et al. 2016; Yan and Nelson 2019). Between 2010 and 2020, of the more than 4000 cultivars entered into the Illinois Soybean Variety trials, only 13 cultivars were reported to have stacked *Rps* genes; either *Rps* 1a and *Rps*1c (2 cultivars), *Rps* 1c and *Rps*1k (3 cultivars), *Rps* 1c and *Rps* 3a (6 cultivars), and *Rps* 1k and *Rps*3a (2 cultivars).

Data from this study corroborates previous work reporting that the diversity and complexity of *P. sojae* in the north central U.S. continues to increase. Effective management of PSRR in Iowa and Nebraska will rely on strategic deployment of *P. sojae*-resistant cultivars. Since many of the *P. sojae* isolates in our study were virulent on *Rps* 1a, *Rps* 1c, and *Rps* 1k, cultivars with these genes may not provide adequate protection against PSRR unless the cultivars also have a high level of partial resistance. Multigenic partial resistance is effective against all pathotypes of the pathogen (Schmitthenner 1985). Although partial resistance is only expressed after the first true leaves have emerged (Dorrance et al. 2007), cultivars with partial resistance can still be infected by the pathogen. However, the level of root colonization is reduced and consequently disease development is slower and a soybean plant is still productive, albeit yields may be reduced. Commercial soybean cultivars with partial resistance are available and this information, along with *Rps* gene information, is usually presented in seed company catalogs. Cultivars with *Rps* 3a should reduce PSRR; however, to maintain durability of this gene, stacking *Rps* 3a with another *Rps* gene and/or partial resistance is recommended. The Illinois Soybean Variety trials data listed varieties with two *Rps* genes; however, zero to less than one percent of the varieties tested in any one year from 2010 to 2018 contained stacked genes. Therefore, these data suggest varieties with stacked genes are not easily available. No data for partial resistance was available in the Illinois Soybean Variety trials data, so it was difficult for us to determine the percent of varieties that had a *Rps* gene stacked with partial resistance. Alternatively, rotating *Rps* genes with each other or partial resistance may also help with *Rps* durability. In the *P. nicotianae*-tobacco pathosystem, rotating single-gene resistance and cultivars with high levels of partial resistance mitigated race shifts of the pathogen (Sullivan et al. 2010). However, rotating *Rps* genes in the *P. sojae*-soybean

pathosystem needs to be explored further since Stewart et al. (2014) reported that changes in pathotype of *P. sojae* were not a function of the type of resistance deployed. Another option to maintain durability of *Rps* genes could be seed treatments to protect against damping off caused by *P. sojae* (Dorrance et al. 2009; Garnica and Giesler 2019).

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Table 1. Soybean differential set used to determine the pathotypes of isolates of *Phytophthora sojae* recovered from Iowa and Nebraska from 2016 to 2018.

Soybean Line	*Rps* gene
Harlon	1a
Harosoy 13XX	1b
Williams 79	1c
PI 103091	1d
Williams 82	1k
L76-1988	2
L83-570	3a
PRX 146–36	3b
PRX 145–48	3c
L85-2352	4
L85-3059	5
Harosoy 62XX	6
Harosoy	7
PI-399073	8
Williams	Sus.

Table 2. Number of counties and fields sampled in Iowa and Nebraska (2016 to 2018), number of fields where *Phytophthora sojae* was recovered, number of isolates, and number of pathotypes in each state, and indices of diversity.

Number of[y]	Iowa	Nebraska
Counties	21	22
Fields	26	39
Isolates pathotyped	258	68
Pathotypes	15	10
Indices of diversity[z]		
Simple	0.06	0.15
Gleason	2.52	2.13
Shannon	2.04	1.64
Simpson	0.83	0.71
Evenness	0.75	0.71

[y] Number of counties and fields sampled, fields from which *P. sojae* was recovered, isolates of *P. sojae* recovered, isolates with consistent pathotype across 2 runs, and pathotypes.

[z] Indices of diversity: Simple diversity index (proportion of distinct pathotypes relative to the number of isolates collected); Gleason's index (indication of pathotype richness); Shannon's index (indication of the evenness of distribution of pathotypes within a sample); Simpson index (proportion of pathotypes relative to the total number of pathotypes); and Evenness index (relative abundance of the different pathotypes composing the sample).

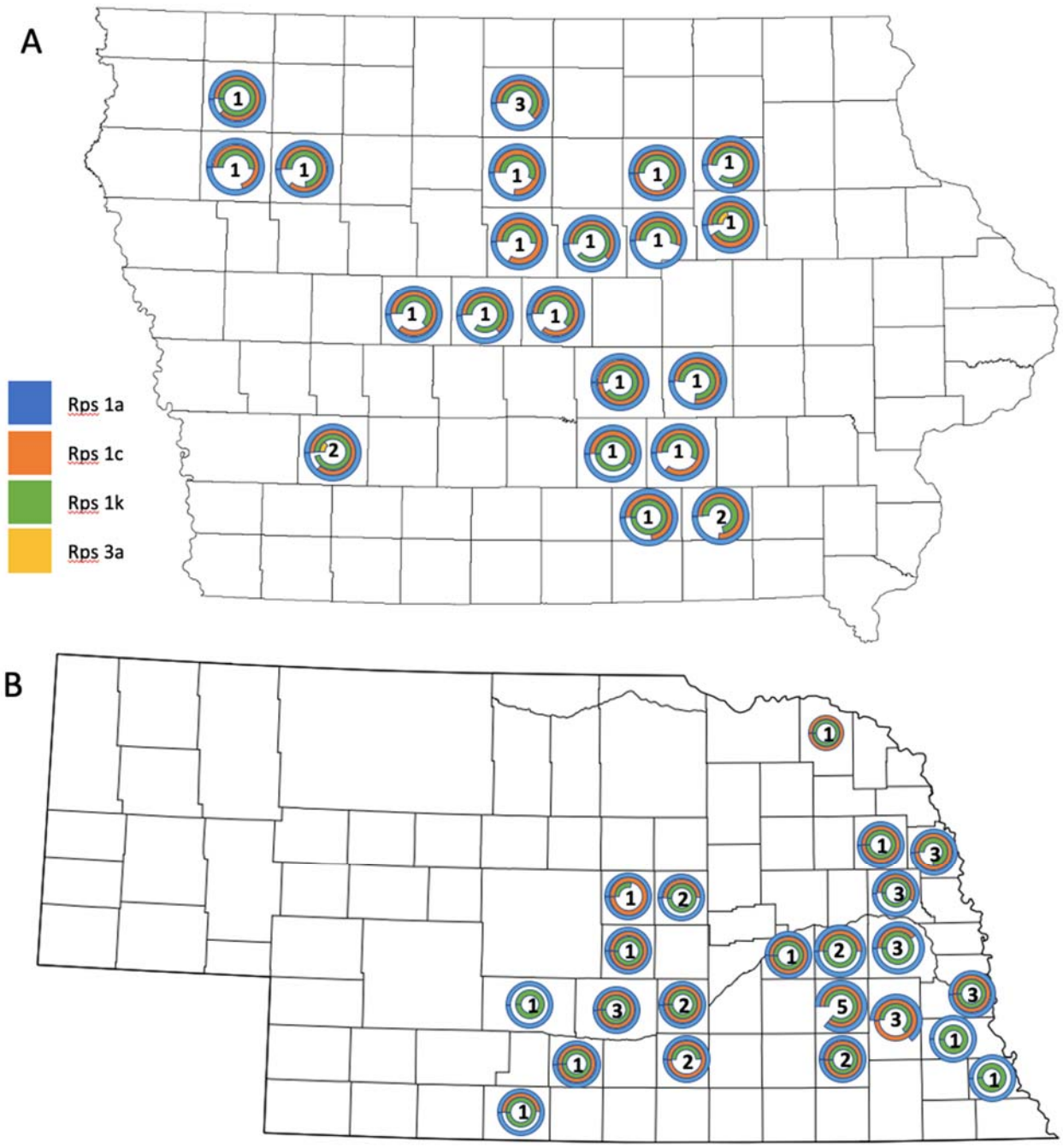


Figure 1. Maps of Iowa (A) and Nebraska (B) indicating counties from which soil samples were collected from commercial soybean fields to isolate *Phytophthora sojae* from 2017 to 2018. Donut charts represent proportion of isolates recovered from the soil samples that were virulent on *Rps* 1a, *Rps* 1c, *Rps* 1k and *Rps* 3a. Number in the center of the chart represents the number of fields sampled in that county.

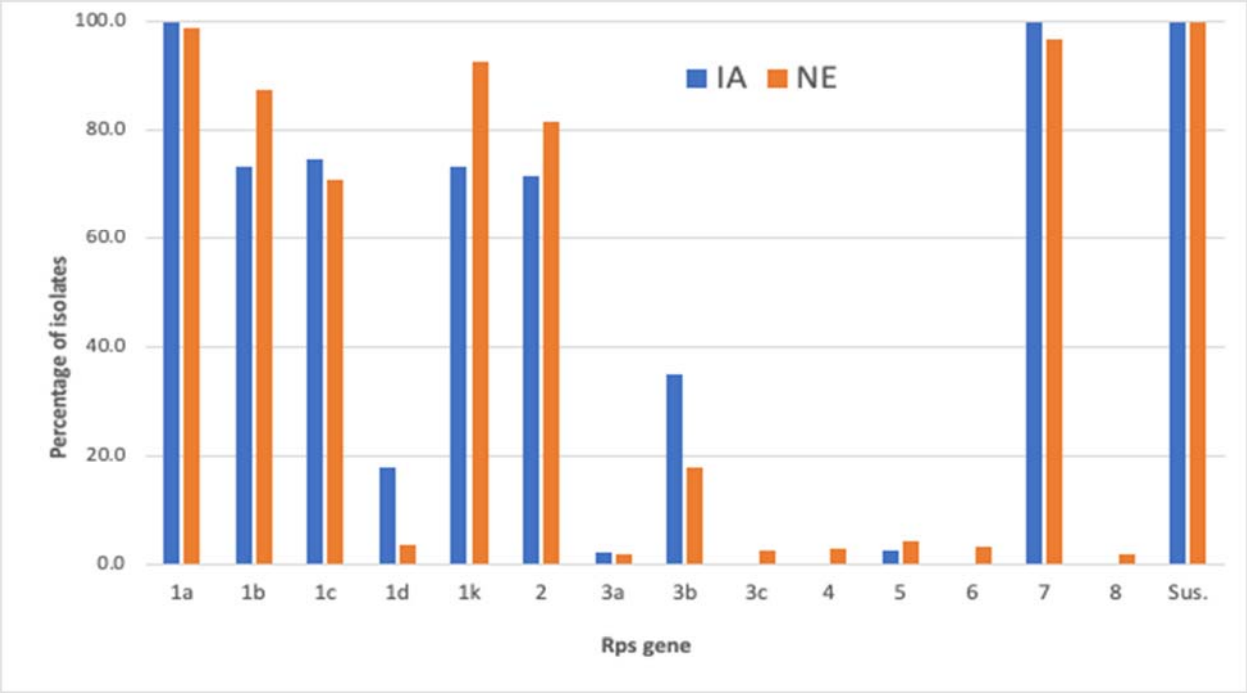


Figure 2. Percentage of *Phytophthora sojae* isolates recovered in a survey of soybean fields in Iowa and Nebraska in 2016 to 2018 that were virulent on a specific *Rps* gene.

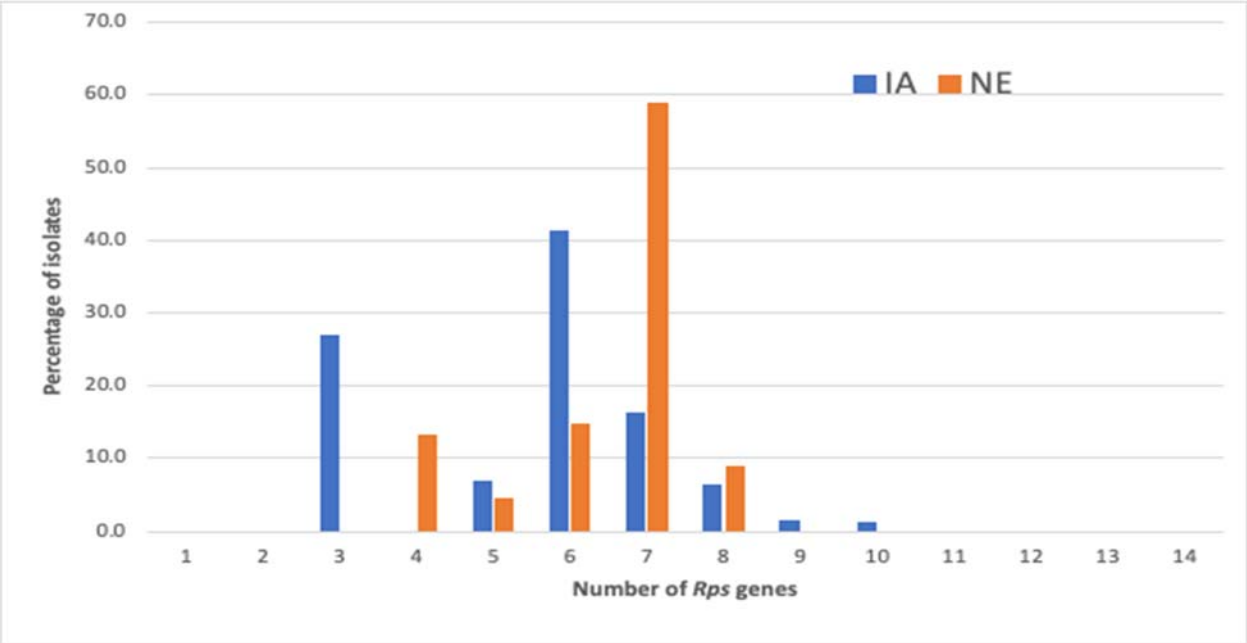


Figure 3. Complexity (the number of *Rps* genes on which an isolate is pathogenic) of *Phytophthora sojae* isolates recovered in a survey of soybean fields in Iowa and Nebraska in 2016 to 2018. Figure shows the percentage of isolates that were pathogenic on 1 to 14 *Rps* genes tested.

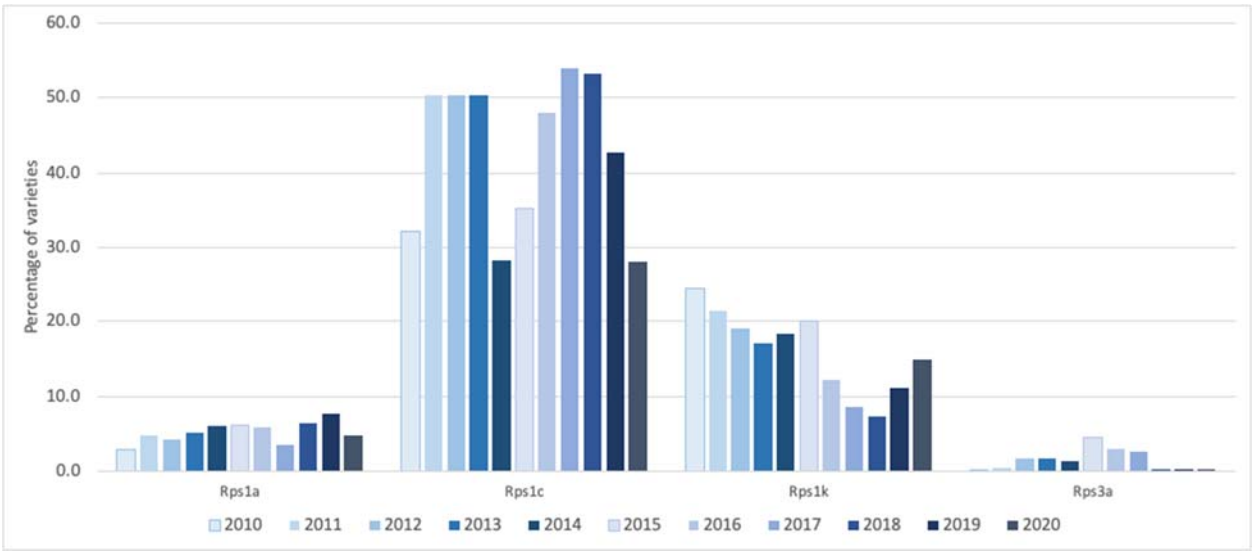
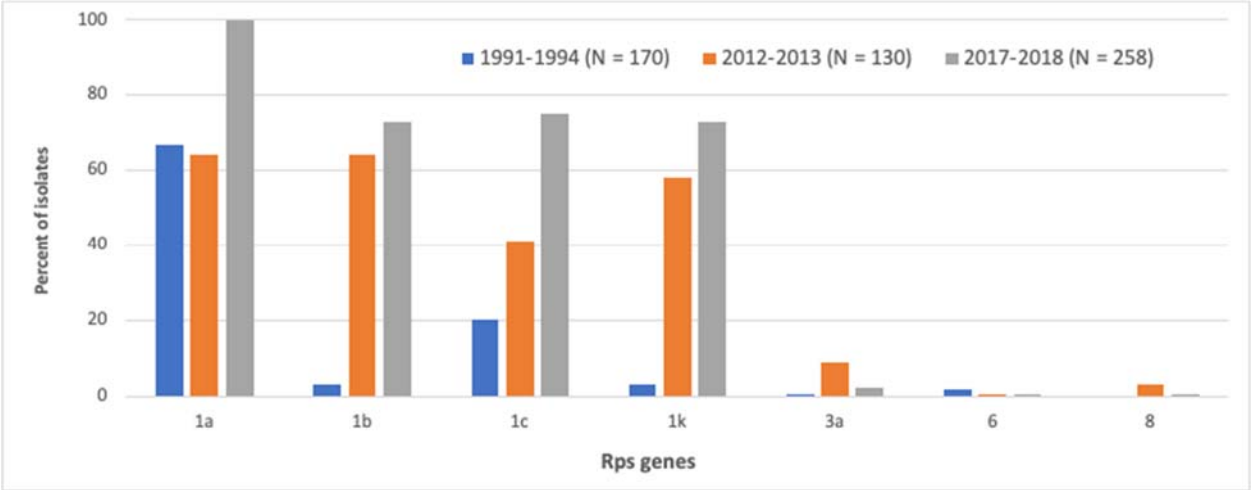


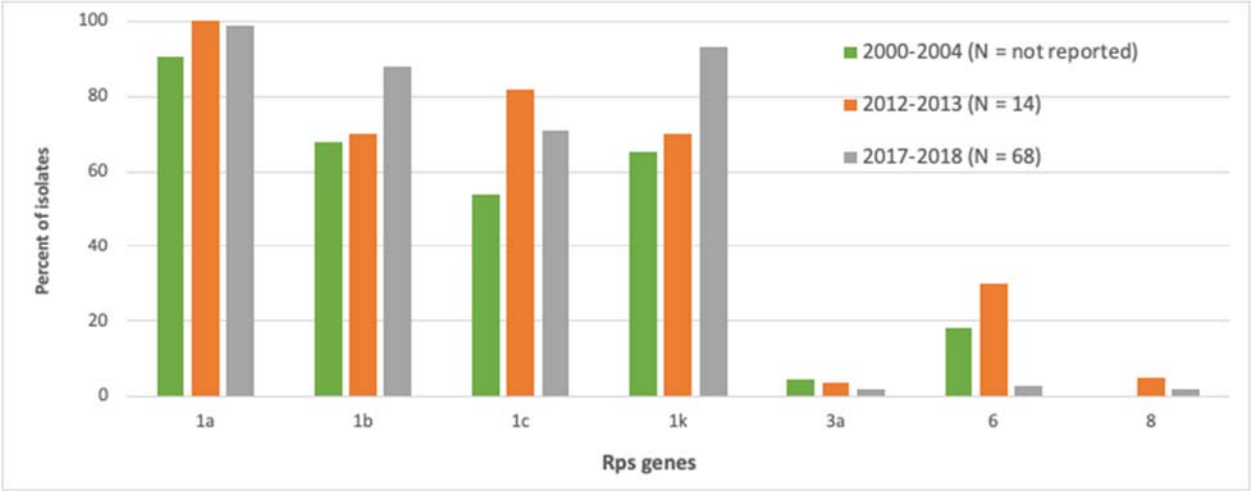
Figure 4. Percentage of soybean varieties with *Rps* 1a, *Rps* 1c, *Rps* 1k, and *Rps* 3a tested in the Illinois Variety Testing trials from 2010 to 2020. *Rps* genes were not reported for all varieties tested.

Supplementary Table 1. Pathotypes of *Phytophthora sojae* recovered in a survey of soybean fields in Iowa and Nebraska in 2016-2018 in order of frequency recovered. Pathotypes in bold were common across states.

Iowa	Nebraska
1a, 1c, 7	**1a, 1c, 7**
1a, 1b, 1k, 2, 7	**1a, 1b, 1k, 2, 7**
1a, 1b, 1c, 1k, 2, 7	**1a, 1b, 1c, 1k, 2, 7**
1a, 1b, 1k, 3b, 7	**1a, 1b, 1k, 3b, 7**
1a, 1b, 1k, 2, 3b, 7	**1a, 1b, 1k, 2, 3b, 7**
1a, 1b, 1c, 1k, 2, 3b, 7	**1a, 1b, 1c, 1k, 2, 3b, 7**
1a, 1b, 1c, 1k, 7	1a, 1b, 1c, 1k, 2, 5, 7
1a, 1b, 1c, 1d, 1k, 2, 3b, 7	1b, 1c, 1k, 2
1a, 1b, 1c, 1d, 1k, 2, 7	1b, 1k, 2, 4, 6, 7
1a, 1b, 1d, 1k, 2, 3b, 7	1a, 1b, 1k, 7
1a, 1b, 1d, 1k, 2, 7	
1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 5, 7	
1a, 1b, 1c, 1k, 2, 3a, 3b, 5, 7	
1a, 1b, 1c, 1d, 1k, 2, 3b, 5, 7	
1a, 1b, 1d, 1k, 2, 3a, 3b, 5, 7	



Supplementary Figure 1. Comparison of pathotype diversity based on the percentage of isolates that were pathogenic on a given *Rps* gene for isolates of *Phytophthora sojae* collected in this survey (2016-2018) to two earlier surveys, 1991-1994 (Yang et al. 1996) and 2012-2013 (Dorrance et al. 2016) in Iowa.



Supplementary Figure 2. Comparison of pathotype diversity based on the percentage of isolates that were pathogenic on a given *Rps* gene for isolates of *Phytophthora sojae* collected in this survey (2016-2018) to two earlier surveys, 2000-2004 (Schimelfenig et al. 2005) and 2012-2013 (Dorrance et al. 2016) in Nebraska.