Evaluation of Major Ancestors of North American Soybean Cultivars for Resistance to Three *Pythium* Species that Cause Seedling Blight

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Abstract

Pythium seedling blight, which can be caused by a number of *Pythium* spp., is a disease that affects soybean (*Glycine max*) in the United States and Canada. *Pythium ultimum* var. *ultimum*, one of the most common pathogenic species, is favored by cool, wet conditions in early spring and causes seed decay, root rot, and seedling damping-off. In all, 102 major ancestors of modern North American cultivars and “first progeny” cultivars developed directly from ancestral lines were evaluated for resistance to *P. ultimum* var. *ultimum* and two other species of *Pythium* in greenhouse assays. Several ancestors and first progeny cultivars, as well as the resistant check Archer, had varying levels of partial resistance to an Illinois isolate of *P. ultimum* var. *ultimum*. In a subsequent experiment,

four of the most resistant lines (PI 84637, Maple Isle, Fiskeby III, and Fiskeby 840-7-3) and the susceptible cultivar Kanro were screened for resistance against isolates of *P. irregulare* and *P. sylvaticum*, and resistance to *P. ultimum* var. *ultimum* was confirmed. The lines that were partially resistant to *P. ultimum* var. *ultimum* in the first experiment were also partially resistant to *P. irregulare* and *P. sylvaticum*. The *P. ultimum* var. *ultimum* isolate was the most aggressive of the three isolates, followed by the *P. irregulare* and *P. sylvaticum* isolates. Modern cultivars descended from the soybean lines with partial resistance to these pathogens could be useful sources of resistance to *Pythium* seedling blight if they are found to have similar levels of resistance.

Pythium seedling blight of soybean (*Glycine max* (L.) Merr.) can be caused by several different species of *Pythium* (Broders et al. 2007, 2009; Dorrance et al. 2004; Jiang et al. 2012; Rojas et al. 2017a). *Pythium* spp. are soilborne oomycetes that generally are most damaging when cool temperatures and rainfall follow planting (Broders et al. 2007; Dorrance et al. 2004). Symptoms caused by *Pythium* spp. that infect soybean include seed rot, preemergence seedling rot, root rot, and damping-off, which can result in poor emergence and sparse plant stands (Rothrock et al. 2015; Schlub and Lockwood 1981).

Pythium ultimum var. *ultimum*, *P. irregulare*, and *P. sylvaticum* are pathogenic on soybean and other field crops across U.S. soybean production regions (Broders et al. 2007, 2009; Brown and Kennedy 1965; Dorrance et al. 2004; Grau et al. 2004; Griffin 1990; Jiang et al.

2012; Rojas et al. 2017a; Rupe et al. 2011; Zhang and Yang 2000). *P. ultimum* var. *ultimum* grows best in temperatures of 5 to 35°C, with temperatures below 23°C most favorable for infection. *P. irregulare* grows in temperatures between 1 and 35°C, with 30°C being the optimal temperature, whereas *P. sylvaticum* grows optimally at 25°C but can grow at a minimum of 5°C to a maximum of 35 to 40°C (van der Plaats-Niterink 1981). *P. ultimum* was the most abundant of 29 *Pythium* spp. isolated from infected seedlings collected from 125 fields in North Dakota during 2011 and 2012, representing 21% of 2,675 isolates (Zitnick-Anderson and Nelson 2015). *P. ultimum* var. *ultimum* was also the most abundant species isolated from soybean fields in Ontario, Canada, in those same years, representing 56.7% of the isolates, followed by *P. sylvaticum* (29.8%), in a survey conducted by Marchand et al. (2014). Of the two varieties of *P. ultimum* that are pathogenic on soybean in the United States, *P. ultimum* var. *sporangiiferum* is rarer than var. *ultimum* (Lévesque and De Cock 2004), though Jiang et al. (2012) recovered the same number of isolates of both in a survey conducted in Illinois. Lévesque and De Cock (2004) also found that based on internal transcribed spacer sequences, the two varieties of *P. ultimum* were in the I clade, whereas *P. irregulare* and *P. sylvaticum* were classified in the F clade of the genus *Pythium*. *P. ultimum* was also the third most abundant *Pythium* spp. that Kirkpatrick et al. (2006b) recovered from soybean plants from flooded fields in eastern Arkansas. Variation in the virulence and aggressiveness of *P. ultimum* isolates and populations has not been thoroughly investigated but available data indicate that variation exists and affects the expression of host resistance (Francis and St. Clair 1997).

The optimal planting date for maximizing soybean yield potential is 1 May in the U.S. Midwest (Robinson et al. 2009), and between mid-May and late May in Canada. Therefore, the trend of planting earlier in the growing season in northern production regions to maximize soybean yields (Hu and Wiatrak 2012) is likely to increase *Pythium*-caused disease due to the higher likelihood of cool temperatures and abundant precipitation occurring (Broders et al. 2007; Rojas et al. 2017b). Management of diseases caused by *Pythium* spp. and other soilborne oomycetes requires a combination of improved soil drainage, tillage, crop rotation, resistant host plants, and fungicide seed treatments (Grau et al. 2004); however, the ability of *P. ultimum* var. *ultimum* to infect many of the field crops grown in the

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north-central United States and Canada reduces the efficacy of crop rotation.

Soybean resistance to *Pythium* spp. is uncommon but resistance to *P. ultimum* has been reported for the cultivars Archer, Dare, and Semmes and in the breeding line V81-141 (Bates et al. 2008; Griffin 1990; Keeling 1974; Kirkpatrick et al. 2006a). Archer was subsequently found to also be resistant to *P. aphanidermatum*, *P. irregulare*, and *P. vexans* (Bates et al. 2008; Rosso et al. 2008), and Semmes also has resistance to *P. debaryanum* (Keeling 1974). In a screening of 1,289 maturity group (MG) 000 to IV accessions from the United States Department of Agriculture (USDA) Soybean Germplasm Collection for resistance to *P. ultimum* in the field, Bernard et al. (1998) identified 60 accessions with resistance and another 52 that had heterogeneous reactions. Resistance of Plant Introduction (PI) 424354 to *P. irregulare* was discovered by Ellis et al. (2013). Although high levels of resistance to *Pythium* spp. have not been reported, it is possible that some modern North American cultivars inherited a moderate level of resistance from one or more of their ancestors; however, resistance to *P. ultimum* var. *ultimum* has not been thoroughly investigated.

On the basis of the pedigrees of 258 public soybean cultivars released in North America between 1947 and 1988, Gizlice et al. (1994) identified PI ancestors and “first progeny” from the USDA Soybean Germplasm Collection that constituted the genetic base of the North American public cultivars. The term “ancestor” referred to progenitors that had not been developed using manual hybridization, whereas the first progeny were lines and cultivars developed through manual hybridization, and for which no intermediates between them and at least one ancestor currently exist in the USDA Soybean Germplasm Collection. Gizlice et al. (1994) estimated that 91 first progeny constituted more than 99% of the North American soybean genetic base. These consisted of 5 breeding lines, 8 older cultivars, and 78 modern cultivars. The 91 first progeny lines have been evaluated for resistance to several soybean pathogens, including *Macrophomina phaseolina* (Pawlowski et al. 2015), *Fusarium virguliforme* (Mueller et al. 2003), *Rhizoctonia solani* (Bradley et al. 2001), and multiple soybean viruses (Wang et al. 2005).

The objectives of this study were to test soybean germplasm accessions that Gizlice et al. (1994) identified as the genetic base of North American soybean cultivars for resistance to *P. ultimum* var. *ultimum*, and to determine whether any lines with resistance to *P. ultimum* var. *ultimum* are also resistant to other pathogenic species of *Pythium*.

Materials and Methods

Inoculum production. Isolate 12Py391 of *P. ultimum* var. *ultimum*, originally collected in 2012 from a soybean field in Fayette County, IL, was used in the assays. In a preliminary experiment conducted to compare the aggressiveness of six different *P. ultimum* var. *ultimum* isolates on soybean seedlings, 12Py391 was determined to be the most aggressive (data not shown). To prepare inoculum, millet (*Panicum miliaceum*) seed were soaked for 12 h in tap water and drained. The millet seed were then autoclaved at 121°C for 60 min in an autoclavable bag (89 by 63.5 cm), allowed to cool to room temperature, then autoclaved a second time for 60 min. Once cooled to room temperature, two 5-day-old cultures of *P. ultimum* var. *ultimum* isolate 12Py391 grown on potato dextrose agar in 89-mm-diameter Petri dishes were added to each bag of autoclaved millet seed. The inoculated millet seed were incubated for 1 week at room temperature (21 to 25°C) to allow the pathogen to colonize the seed, and the bags were gently massaged at day 4 to evenly distribute the colonized seed in the bag. The inoculum was then dried with forced air at 25°C for 2 days and ground using a Model 60GM Grinding Mills grinder (The C.S. Bell Co., Tiffin, OH) to ensure that the inoculum was a uniform size (1 to 2 mm in diameter).

Evaluation of North American ancestral soybean lines for resistance to *P. ultimum* var. *ultimum*. In all, 102 germplasm accessions from the USDA Soybean Germplasm Collection were screened for resistance to the 12Py391 isolate in the first experiment (Table 1).

These included 90 of the North American ancestral lines identified by Gizlice et al. (1994), with 58 ancestors and 32 first progeny. These will be referred to collectively as “ancestral lines”. Archer and 10 of its progenitors were also tested, as well as two cultivars known to be susceptible to *Pythium* spp., Jack and Sloan (Dorrance et al. 2004; Ellis et al. 2013). The cultivar Sioux was not included in the screening because seed were not available (McLachlan 2016). Seed were obtained from the USDA Soybean Germplasm Collection in Urbana, IL. Archer, an MG I cultivar, was included because of its resistance to Arkansas isolates of several *Pythium* spp., including *P. ultimum*, *P. aphanidermatum*, and *P. irregulare* (Bates et al. 2008; Rosso et al. 2008; Urrea et al. 2017). The 10 ancestors of Archer were chosen on the basis of pedigree information available at the SoyBase (<https://soybase.org/uniformtrial>) and Germplasm Resources Information Network (<https://www.ars-grin.gov/>) websites.

Ten seeds of the same soybean genotype were planted in plastic pots (approximately 15 cm in diameter) that contained 1.6 liters of steam-pasteurized soil of a sandy loam mix (two parts torpedo sand and one part soil). Prior to planting, 5 g of *P. ultimum* var. *ultimum* inoculum (described above) was uniformly spread in a layer 5 cm below the soil surface and 2.5 cm below the seed. Each pot served as an experimental unit, with three replications of each soybean line tested. A noninoculated control pot also was included to confirm the seed viability for each soybean line but was not replicated. Pots were arranged in a completely randomized design, and the experiment was repeated once. Greenhouse temperatures ranged from 21°C in the daytime to 16°C at night. No artificial lighting was used, and the natural photoperiod during the experiment was approximately 14 h. Plants were watered until the soil was moist once each day. The layer-inoculation technique used and the method used to prepare the inoculum were modifications of methods described by Kirkpatrick et al. (2006a) and Mueller et al. (2003), and were described in greater detail by McLachlan (2016).

Two weeks after planting, data were collected for plant stand per pot, shoot dry weight per plant, and root dry weight per plant. Plant stand was measured as the number of plants per pot that emerged from the soil. Soil was washed away from the roots of the plants, and the stems were cut with scissors at the soil line. Shoots and roots were dried with forced air at 25°C for 3 days before being weighed. Total weights of the roots and shoots from each pot were divided by the plant stand number to calculate the mean root or shoot dry weight per plant.

Evaluation of selected soybean lines for reactions to two other *Pythium* spp. Four soybean lines that showed varying levels of partial resistance to *P. ultimum* var. *ultimum* in the ancestral genotype screen were subsequently screened with isolates of *P. irregulare* and *P. sylvaticum*, in addition to the *P. ultimum* var. *ultimum* 12Py391 isolate that was used in the ancestral line screen. Kanro, a line that was susceptible to the 12Py391 isolate in the previous experiment, was also included in the assay as a susceptible check. The *P. irregulare* isolate, 12Py354, was collected from a soybean field in Gallatin County, IL in 2012, and *P. sylvaticum* isolate 12Py353 was collected from a soybean field in Fayette County, IL in 2012. All three *Pythium* spp. have been reported to be pathogenic on soybean (Broders et al. 2007, 2009; Jiang et al. 2012; Rojas et al. 2017a).

PI 84637, Maple Isle, and Fiskeby III were included because they had some of the highest relative plant stands and relative shoot and root dry weights compared with their noninoculated controls after being challenged with *P. ultimum* var. *ultimum* in the previous ancestral soybean screening experiment. Fiskeby 840-7-3, also known as Holmberg 840-7-3, was chosen to represent a moderately resistant line because it was close to the median of the cultivars that were significantly different from the ratings of the most susceptible lines. All four of the resistant lines were in MG II or an earlier MG. Kanro (PI 548356) was used as the susceptible check because it was highly susceptible in the previous experiment and because it is also in MG II.

This factorial experiment was conducted as a completely randomized design, with three *Pythium* spp. and five soybean lines. Each

species–soybean line combination had six inoculated replications and one noninoculated control. The experiment was repeated once, and the experimental conditions and methods were the same as described previously. After 2 weeks of growth, data for plant stand per pot, shoot dry weight per pot, and root dry weight per pot were collected. Shoots and roots of inoculated replications and the noninoculated controls were dried on a whole-pot basis. The weight of tissue per pot was divided by the number of plants to calculate mean weight per plant (McLachlan 2016).

Statistical analyses. Data were analyzed using SAS (version 9.4; SAS Institute Inc., Cary, NC). Plant stands from inoculated pots were compared with their noninoculated controls and expressed as a percentage relative to those. The average weight per plant of the shoot or root was then compared with the average weight per plant part of the noninoculated control, and was expressed as a percentage relative to the corresponding control. In all replicated experiments, analysis of variance was conducted using the mixed-model procedure (PROC MIXED). For each repeated experiment, the data from each experiment were pooled and analyzed together because there was no significant ($P \leq 0.05$) soybean line–experiment interaction. Cultivar (i.e., genotype) was considered a fixed variable and experiment was considered random. Relative means from the lines in the ancestral screen were tested for significant differences using Fisher's protected least significant difference (LSD) test at $\alpha = 0.05$ with the PDMIX800 macro (Saxton 1998). Correlations between plant stand, root weight, and shoot weight were tested with the Spearman's Rank Correlation (PROC CORR). In the soybean line–*Pythium* spp. experiment, the pathogen–soybean line interaction was significant ($P \leq 0.05$); therefore, relative means from the species and soybean lines were compared using Fisher's protected LSD with the PDMIX800 macro. Correlations between plant stand, root weight, and shoot weight were tested using the Spearman's Rank Correlation as well.

Results

Evaluation of ancestral soybean lines for resistance to *P. ultimum* var. *ultimum*. Significant differences among the soybean lines were found for plant stand ($P < 0.0001$), root dry weight ($P < 0.0001$), and shoot dry weight ($P < 0.0001$) ratios for inoculated plants relative to those of noninoculated plants. The 12Py391 isolate had a detrimental effect on seedling emergence of all of the lines, including the partially resistant check Archer (Fig. 1). Plant stands ranged from 0 to 58% of the noninoculated controls, with an LSD of 0.18 (Table 2). Forty-five lines had plant stands that were less than 10% of their noninoculated controls, illustrating the aggressiveness of the isolate used. Root dry weights ranged from 0 to 93% of noninoculated controls, and shoot dry weights ranged from 0 to 94% (Table 2). Considerable variation among plants within inoculated treatments resulted in LSDs of 0.43 and 0.44 for root and shoot dry weights, respectively; therefore, those data were considered to be less informative measurements of resistance and susceptibility than plant stand ratios.

Out of the 102 accessions evaluated in the ancestral lines experiment, only 4 lines (Harosoy, Maple Isle, Delmar, and PI 84637) had significantly higher plant stands than both Jack and Savoy, the susceptible controls (Table 2; Fig. 1). PI 84637 also had the highest root dry weight compared with its noninoculated control (0.93), and Harosoy was the only line among the other three lines that had a root dry weight significantly lower than that of PI 84637. The shoot dry weight ratios of all four lines were also not significantly lower than those of Fiskeby III (0.94) or Jogun (0.94), the lines with the highest weights relative to their noninoculated controls. PI 84637 was the only one of the four lines that is not a cultivar developed in North America, and Delmar was the only one from an MG higher than II (Table 1).

Plant stand ratios in the inoculated pots of Habaro (0.41), Chico (0.40), Hodgson (0.38), PI 54615-1 (0.37), Fiskeby III (0.37), and Richland (0.37) were significantly higher than that of Jack (0.19) but not Sloan (Table 2). The root and shoot dry weight ratios for all of these were also relatively high; thus, it is possible that they have some resistance to the isolate used. All five of these accessions are in MG II or a lower MG. Chico and Hodgson are cultivars from

Minnesota; Habaro, PI 54615-1, and Richland are germplasm accessions from East Asia; and Fiskeby III is a cultivar that was developed in Sweden.

The Spearman rank correlation showed that plant stand was positively correlated with both root dry weight ($P < 0.0001$) and shoot dry weight ($P < 0.0001$), and that root dry weight was positively correlated with shoot dry weight ($P < 0.0001$) (Table 3). Despite this overall trend, several lines with some of the highest root and shoot dry weights had comparatively poor seedling emergence. For example, Jogun (PI 548352) had the highest relative shoot dry weight ratio (0.95) and a relatively high root weight ratio (0.71) but inoculated pots had a low plant stand relative to noninoculated pots (0.23). A similar discrepancy between poor plant stands and relatively high dry weight ratios was also observed for Mejiro (PI 80837), Novosad-ska Bela (PI 248404), Fiskeby 840-7-3 (PI 438477), Aoda (PI 548301), and T204 (PI 548195), as well as for Archer, the check with putative partial resistance (Table 2). PI 54615-1 is in MG III and Aoda is in MG IV but the other lines are from earlier MG.

Relative to its progenitors, inoculated Archer plants had numerically higher root and shoot weights than Adams (PI 548502), and a numerically higher plant stand than Adams, Amsoy (PI 548506), BSR-101 (PI 548519), and Clark (PI 548533) (Table 2). Harosoy had a higher relative plant stand than Archer and similar root and shoot dry weights; thus, it would be a good candidate for the source of Archer's resistance to *Pythium* spp. Blackhawk was not among the lines with the most resistance to the *P. ultimum* var. *ultimum* isolate used in this study, even though it is derived from Richland (Cober and Voldeng 2012) and is in the pedigree of Archer.

Reactions of four partially resistant ancestral lines to three *Pythium* spp. Interactions between the five selected soybean lines and the three *Pythium* spp. were significant for plant stands ($P < 0.0001$), root dry weights ($P < 0.0001$), and shoot dry weights ($P < 0.0001$) of inoculated treatments relative to noninoculated treatments. PI 84637, Maple Isle, Fiskeby III, and Fiskeby 840-7-3 were all partially resistant to the isolates of *P. ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum* used, thus confirming that these four lines are partially resistant to the 12Py391 isolate. Plant stand was again positively correlated with shoot and root dry weights (Table 4), which were also positively correlated with one another.

The isolates of the three *Pythium* spp. used in the screen varied in aggressiveness toward the four partially resistant soybean lines challenged in this experiment. The *P. ultimum* var. *ultimum* 12Py391 isolate was the most aggressive, causing, on average, a 33% reduction in plant stand, a 46% reduction in mean root weight, and a 24% reduction in shoot weight (Table 5). The *P. irregulare* isolate was the second most aggressive isolate, causing, on average, a 22% reduction in relative mean plant stand, a 33% reduction in root weights, and a 26% reduction in shoot weights (Table 5). The *P. sylvaticum* isolate was the least aggressive of the three isolates, failing to reduce any of the measured disease reaction parameters by more than 7% (Table 5). The relative plant stand and shoot weight means for the susceptible soybean line Kanro were significantly lower than those of the partially resistant lines inoculated with the *P. ultimum* var. *ultimum* isolate. The relative mean plant stand of Kanro was also significantly lower than the stands of the partially resistant lines inoculated with the *P. irregulare* isolate. In contrast, when Kanro plants were challenged with the *P. sylvaticum* isolate, the mean stand counts and the root and shoot dry weights were above 0.90 relative to the noninoculated control.

Discussion

Despite the abundance of *Pythium* pathogens of soybean, resistance to *Pythium* spp. has received much less attention than *Phytophthora sojae* resistance in both research efforts and soybean breeding programs (Rojas et al. 2017b). To our knowledge, this is the first study to specifically evaluate the major ancestors of North American soybean cultivars for resistance to *Pythium ultimum* var. *ultimum* and other *Pythium* spp. The foreign germplasm accessions and first progeny cultivars that Gizlice et al. (1994) identified as the genetic foundation of North American soybean breeding prior to 1989 served

as a logical starting point for investigating sources of *Pythium* resistance. However, a few points about the Gizlice et al. (1994) study should be borne in mind. One is that it was conducted more than two decades before the present study, and at a time when cultivars from private companies were just beginning to dominate soybean seed sales in the United States. Because the pedigrees of proprietary cultivars are seldom divulged, it is not possible to estimate the extent to which the findings of Gizlice et al. (1994) apply to currently grown cultivars. Another point to keep in mind is that some of the cultivars in the Gizlice et al. (1994) pedigree analyses were relatively recent releases at the time that study was conducted; thus, their genetic contribution to cultivars released after 1988 may have been substantially greater than it had been to pre-1989 cultivars. This is particularly true

for cultivars bred for Canada, where soybean production underwent very rapid expansion between 1971 and 2000 (Cober and Voldeng 2012).

Bernard et al. (1998) had previously screened 656 germplasm accessions from MG 000 to IV for resistance to *P. ultimum* in a 1964 field test, and another 633 accessions were screened in 1965 and 1966. Those assays were limited to accessions with PI numbers of 266807 or lower that had been acquired by the USDA Soybean Germplasm Collection before 1961. Fewer than 5% of the 1,289 accessions screened were rated as resistant to *P. ultimum*, and the reactions of another 4% were heterogeneous (<https://www.ars-grin.gov/>). A large percentage of the resistant accessions originated from either northeast China, the Korean peninsula, or northern Japan.

Table 1. Ancestral and first progeny lines, the cultivar Archer, and ancestors of Archer assayed for their reactions to an Illinois isolate of *Pythium ultimum* var. *ultimum*

Accession	Line name	Maturity group	Province of origin	Country of origin	Description
FC 31745	...	VI	Unknown	Unknown	Ancestor
FC 33243	Anderson	IV	Unknown	Unknown	Ancestor
PI 54615-1	No. 55	III	Jilin	China	Ancestor
PI 65338	Botanical Garden No. 4	II	Heilongjiang	China	Ancestor
PI 71506	No. 94	IV	Jiangsu	China	Ancestor
PI 80837	Mejiro	IV	Unknown	Japan	Ancestor
PI 84631	S-56	III	Kyonggi	South Korea	Ancestor
PI 84637	S-62	II	Kyonggi	South Korea	Ancestor
PI 84946-2	(Kandokon)	IV	Pusan	South Korea	Ancestor
PI 86972-1	Pakute	II	Cholla Puk	South Korea	Ancestor
PI 88788	...	III	Liaoning	China	Ancestor
PI 88811	Pakute	IV	Pyongan Puk	North Korea	Ancestor
PI 91110-1	...	I	Heilongjiang	China	Ancestor
PI 96983	...	V	Hwanghae Puk	North Korea	Ancestor
PI 159925	Glycine H	VIII	Lima	Peru	Ancestor
PI 171450	Kisaya	III	Kagoshima	Japan	Ancestor
PI 171451	Kosamame	VII	Kanagawa	Japan	Ancestor
PI 180501	Strain No. 18	0	Unknown	Germany	Ancestor
PI 200492	Komata	VII	Shikoku	Japan	Ancestor
PI 240664	Bilomi #3	X	Unknown	Philippines	Ancestor
PI 248404	Novosadska Bela	0	Serbia	Yugoslavia	Ancestor
PI 317335	Koganejiro	I	Hokkaido	Japan	Ancestor
PI 360955A	Fiskeby V	0	Ostergotland	Sweden	First progeny
PI 360955B	(Fiskeby V)	0	Ostergotland	Sweden	First progeny
PI 438471	Fiskeby III	0	Ostergotland	Sweden	First progeny
PI 438477	Fiskeby 840-7-3	0	Ostergotland	Sweden	First progeny
PI 508269	Stafford	IV	Virginia	United States	First progeny
PI 513382	Glenwood	0	Minnesota	United States	First progeny
PI 535807	Crockett	VIII	Texas	United States	First progeny
PI 542402	Chico	0	Minnesota	United States	First progeny
PI 548169	T117	IV	Illinois	United States	Ancestor
PI 548178	T145	III	Illinois	United States	Ancestor
PI 548193	T201	IV	Iowa	United States	Ancestor
PI 548195	T204	IV	Illinois	United States	Ancestor
PI 548237	T260H	VII	North Carolina	United States	Ancestor
PI 548298	A.K. (Harrow)	III	Northeast China	China	Ancestor
PI 548301	Aoda	IV	Hokkaido	Japan	Ancestor
PI 548302	Bansei	II	Hokkaido	Japan	Ancestor
PI 548307	Blackeye	0	Heilongjiang	China	First progeny
PI 548311	Capital	0	Northeast China	China	First progeny
PI 548318	Dunfield	III	Jilin	China	Ancestor
PI 548325	Flambeau	0	Unknown	Russia	Ancestor
PI 548336	Habaro	I	Khabarovsk	Russia	Ancestor
PI 548342	Higan	IV	Tokyo	Japan	Ancestor
PI 548348	Illini	IV	Heilongjiang	China	Ancestor
PI 548352	Jogun	III	Hamgyong Puk	North Korea	Ancestor
PI 548356	Kanro	II	Pyongyang	North Korea	Ancestor
PI 548359	Kingwa	IV	Beijing	China	Ancestor
PI 548360	Korean	II	Unknown	North Korea	Ancestor
PI 548362	Lincoln	III	Unknown	China	First progeny
PI 548379	Mandarin (Ottawa)	0	Heilongjiang	China	Ancestor

(Continued on next page)

Surprisingly, PI 86437 (Line S-62), which had among the highest levels of resistance to the *P. ultimum* var. *ultimum* isolate used in our study, had been rated as susceptible to *P. ultimum* in the 1964 field evaluations. Bernard et al. (1998) did not provide many details about the methods used to determine and rate reactions to *P. ultimum* in their field tests but it seems unlikely that *P. ultimum* would have been the only soybean pathogen in the soil, and the presence of other pathogenic soil organisms could have affected apparent *P. ultimum* resistance. Although it is also possible that the *P. ultimum* pathotypes in the field where the Bernard et al. (1998) screening was conducted were different from the 12Py391 isolate used in our assays, the 12Py391 isolate was chosen because it was more aggressive than other *P. ultimum* var. *ultimum* isolates that we tested. The lack of any seedling emergence from 16 lines in the inoculated treatment demonstrated the aggressiveness of this Illinois isolate.

The ancestral lines and first progeny that appeared to have resistance to the 12Py391 isolate can be classified into three groups. Lines in the first group had plant stand ratios significantly higher than those of both Sloan and Jack (susceptible controls), whereas the ratios of the lines in the second group were only significantly higher than that of Jack. A third group consisted of lines with relatively low plant stand ratios but whose surviving plants had high root and shoot dry weights compared with noninoculated plants. Further investigations are needed to determine whether the third group has a different mode of resistance that is more effective postemergence than pre-emergence. We are relatively confident in concluding that the lines in the first group are truly resistant but believe that resistance of lines in the second and third groups needs to be confirmed.

Of the four lines with plant stand ratios significantly higher than those of both Sloan and Jack, Harosoy and Maple Isle had the highest

Table 1. (Continued from previous page)

Accession	Line name	Maturity group	Province of origin	Country of origin	Description
PI 548382	Manitoba Brown	0	Unknown	Unknown	Ancestor
PI 548383	Mansoy	III	Heilongjiang	China	Ancestor
PI 548391	Mukden	IV	Liaoning	China	Ancestor
PI 548402	Peking	III	Beijing	China	Ancestor
PI 548406	Richland	II	Jilin	China	Ancestor
PI 548409	Sato	IV	Hokkaido	Japan	Ancestor
PI 548411	Seneca	II	Northeast China	China	Ancestor
PI 548438	Arksoy	VI	Pyongyang	North Korea	Ancestor
PI 548444	Biloxi	VIII	Zhejiang	China	Ancestor
PI 548445	CNS	IX	Jiangsu	China	Ancestor
PI 548456	Haberlandt	V	Pyongyang	North Korea	Ancestor
PI 548457	Hahto	VI	Fukushima	Japan	Ancestor
PI 548461	Improved Pelican	VIII	Unknown	China	First progeny
PI 548463	Laredo	IV	Shaanxi	China	Ancestor
PI 548469	Mammoth Yellow	VII	Unknown	Japan	Ancestor
PI 548477	Ogden	VI	Unknown	Unknown	First progeny
PI 548484	Ralsoy	VI	Pyongyang	North Korea	Ancestor
PI 548485	Roanoke	VII	Jiangsu	China	Ancestor
PI 548488	S-100	V	Heilongjiang	China	Ancestor
PI 548493	Tokyo	III	Kanagawa	Japan	Ancestor
PI 548494	Volstate	VII	Unknown	Unknown	First progeny
PI 548528	Protana	II	Indiana	United States	First progeny
PI 548548	Delmar	IV	Delaware	United States	First progeny
PI 548559	Emerald	IV	Delaware	United States	First progeny
PI 548561	Hodgson	I	Minnesota	United States	First progeny
PI 548587	Kim	III	Iowa	United States	First progeny
PI 548595	Maple Isle	0	Ontario	Canada	First progeny
PI 548599	Monroe	I	Ohio	United States	First progeny
PI 548603	Perry	IV	Indiana	United States	First progeny
PI 548604	Pershing	IV	Missouri	United States	First progeny
PI 548623	Vansoy	0	Ontario	Canada	First progeny
PI 548626	Wabash	IV	Indiana	United States	First progeny
PI 548633	Wye	IV	Maryland	United States	First progeny
PI 548657	Jackson	VII	North Carolina	United States	First progeny
PI 548663	Dowling	VIII	Texas	United States	First progeny
PI 548697	Majos	VIII	South Carolina	United States	First progeny
PI 548983	Tracy	VI	Mississippi	United States	First progeny
PI 553048	Vance	V	Virginia	United States	First progeny
PI 567790	Curtis	VI	Louisiana	United States	First progeny
PI 546487	Archer	I	Iowa	United States	Resistant check
PI 548519	BSR-101	I	Iowa	United States	Archer pedigree
PI 548506	Amsoy	II	Iowa	United States	Archer pedigree
PI 548527	Calland	III	Indiana	United States	Archer pedigree
PI 548502	Adams	III	Iowa	United States	Archer pedigree
PI 548573	Harosoy	II	Ontario	Canada	Archer pedigree
PI 548504	Altona	0	Manitoba	Canada	Archer pedigree
PI 548516	Blackhawk	I	Iowa	United States	Archer pedigree
PI 548533	Clark	IV	Illinois	United States	Archer pedigree
PI 518671	Williams 82	III	Illinois	United States	Archer pedigree
PI 540556	Jack	II	Illinois	United States	Susceptible check
PI 548616	Sloan	II	Iowa	United States	Susceptible check

survival rates in inoculated pots. This is interesting because Harosoy (MG II) is an ancestor of Maple Isle (MG 0) (Cober and Voldeng 2012; Voldeng et al. 1985). Maple Isle originated as a selection from a single backcross of Harosoy *e3* to PI 194641. Because Harosoy *e3* is an early-maturing isolate of Harosoy (Bernard et al. 1988; Voldeng et al. 1985), it is conceivable that Maple Isle could have inherited resistance to *P. ultimum* var. *ultimum* from Harosoy, which we tested in our study because it is also an ancestor of Archer (Cianzio et al. 1991). Although Archer was not among the most resistant lines to the 12Py391 isolate, it is partially resistant to other pathogenic species and isolates of *Pythium* (Bates et al. 2008). Harosoy was selected from a backcross between Mandarin (Ottawa) and A.K. (Harrow). Surprisingly, neither of these ancestral lines exhibited any resistance to the 12Py391 isolate in this study; therefore, the source of *Pythium* resistance in Harosoy is a mystery unless some combination of minor resistance genes inherited from both of its parents was effective when combined in the genetic background of Harosoy. Chico, an MG 00 cultivar from Minnesota that appeared to have some resistance in our assays, also has Harosoy in its pedigree, by way of both Evans and Corsoy (Orf et al. 1985). Evans was also a parent of the first progeny cultivar Glenwood, which was not resistant to the 12Py391 isolate (Table 2).

PI 84637 was obtained by the U.S. National Plant Germplasm System in 1929 from the Suigen Experiment Station in Gyeonggi-do (Kyonggi) province, which surrounds Seoul in the northwestern

corner of what is now the Republic of Korea (<https://www.ars-grin.gov/>). It is not clear, however, whether this MG II accession is originally from that region, because W. J. Morse, one of the two collectors from the USDA Bureau of Plant Industry, wrote in 1930 that Korean, Japanese, and Manchurian varieties were being evaluated at the Suigen station (Shurtleff and Aoyagi 2017). Morse also reported that hybridization, as well as selection to purify lines, was being used in Suigen at that time. The assignment of the Suigen AES number S-62 to PI 84637 suggests that, whatever the origin of this line, it had been selected for its performance in that region of the Korean peninsula. According to information at the Germplasm Resource Information Network website (<https://www.ars-grin.gov/>), PI 84637 was rated as susceptible to *P. ultimum* in 1964 field tests conducted by Bernard et al. (1998). It is interesting to note, however, that at least 13 other soybean accessions that had been collected in Gyeonggi or Seoul at the same time as PI 84637 had either resistant or heterogeneous reactions to *P. ultimum* in field assays that Bernard et al. (1998) conducted in 1965 and 1966. PI 424354, a line that Ellis et al. (2013) reported to have resistance to a *P. irregulare* isolate, originates from Chungcheongnam province, which lies immediately to the south of Gyeonggi province. Because PI 424354 was not considered by Gizlice et al. (1994) to be one of the North American ancestral lines, it was not evaluated in the present study.

The last of the four lines with the highest plant stand ratios was the first progeny Delmar, an MG IV cultivar released by the University of Delaware in 1967. This line differed from the other three in being

Fig. 1. Symptoms of *Pythium* seedling blight on **A**, the North American soybean ancestral line PI 84637; **B**, the partially resistant check Archer; **C**, the susceptible check Jack; and **D**, Harosoy. All genotypes were inoculated with the 12Py391 isolate of *Pythium ultimum* var. *ultimum* from Illinois.

from a later MG and in being adapted to a lower latitude. It was derived from the cross C 799 × F. C. 33243 (Crittenden and Cole 1967). The pedigrees of the parents were not described in the release but the maternal parent was presumably a breeding line, and the paternal parent was apparently an off-type MG IV plant selected from a field of Lincoln in 1954 (<https://www.ars-grin.gov/>). Lincoln was developed in Illinois from a cross between Mandarin and Manchu, two accessions from Heilongjiang Province in northeastern China, where several other ancestral lines tested in this study had also been collected. Neither Mandarin nor Manchu were in this study but Mandarin (Ottawa), an earlier-maturing selection from Mandarin, was not resistant to the 12Py391 isolate. As mentioned earlier, Mandarin (Ottawa) is an ancestor of Harosoy and, therefore, a progenitor of many Canadian cultivars (Cober and Voldeng 2012). Considering that all of the most resistant ancestors and first progeny except Delmar are in lower MG, it is possible that the resistance genes in Delmar were inherited from one or more ancestors from northern China.

Of the lines that had plant stand ratios significantly greater than Jack but not greater than Sloan, Chico (late MG 00) was previously mentioned for having two Harosoy-derived lines in its pedigree (Orf et al. 1985). Richland, another ancestral line with putative resistance to the 12Py391 isolate, was the variety name given to an MG II line descended from a 1927 selection from PI 70502 (<https://www.ars-grin.gov/>). PI 70502 was collected in China's Jilin province, which borders present-day North Korea. Like Chico, the MG I cultivar Hodgson was developed and selected in Minnesota, and also has the Harosoy-derived cultivar Corsoy in its pedigree (Lambert and Kennedy 1975). Hodgson also has Lincoln and Richland in its pedigree, as well as PI 180501, an accession originally from Manchuria that had been selected in Germany.

Ancestral lines that had high root and shoot dry weight ratios but relatively low plant stand ratios included Mejiro (PI 80837), Fiskeby III, Fiskeby 840-7-3, T204 (PI 548195), Aoda (PI 548301), Jogun (PI 548352), and Richland. The putative resistance of these lines needs to be confirmed. Fiskeby 840-7-3, Fiskeby III, Mejiro, and Jogun each contributed less than 1% to the total genes in pre-1989 cultivars but Richland had contributed 8.2% of the genes to public cultivars overall and 11.3% to the northern gene pool (Gizlice et al. 1994). With the major expansion of soybean production in Ontario and in the north-central states of the United States since the 1980s, however, it is likely that the relative contributions of early-maturing lines such as Fiskeby 840-7-3 and Fiskeby III to modern cultivars with earlier maturities is higher. Mejiro, Aoda, and T204 are in MG IV, while the other lines were in earlier MG.

The results of the experiment in which four resistant lines and one susceptible line were inoculated with isolates of *P. ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum* confirmed that PI 84637, Maple Isle, Fiskeby III, and Fiskeby 840-7-3 are resistant to the 12Py391 isolate of *P. ultimum* var. *ultimum* (Table 5), and showed that these four lines were also more resistant than Kanro to an Illinois isolate of *P. irregulare*. The comparatively low aggressiveness of the *P. sylvaticum* isolate in the present study resulted in similar levels of disease on the susceptible check; thus, it is not possible to assess whether the resistance of the other four lines is also effective against *P. sylvaticum*, which Rojas et al. (2017a) found to be the most abundant *Pythium* spp. in soybean fields across Ontario, Canada, and 11 major soybean-producing U.S. states. The apparent weak aggressiveness of the *P. sylvaticum* isolate in the present study was surprising, considering that *P. sylvaticum* isolates caused considerable disease in assays conducted by Rojas et al. (2017a); however, data from their study also indicated that *P. sylvaticum* isolates caused more disease at 13 than at 20°C. Because the inoculated millet seed in this experiment were incubated at 21 to 25°C and the greenhouse assay temperatures ranged from 16 to 21°C, it is possible that the apparent aggressiveness of the isolate would have been higher at lower temperatures. In any case, the data from this experiment demonstrated that at least some of the accessions with resistance to the 12Py391 isolate of *P. ultimum* var. *ultimum* also have resistance to other pathogenic species of *Pythium*. Further assays are needed to determine the extent and level of resistance to different species and isolates.

The pedigrees of many cultivars developed by breeding programs in Canada and the north-central United States include one or more lines selected by Sven Holmberg of Algot Holmberg & Söner AB in the town of Fiskeby, in Ostergötland, Sweden (Elovson 1997; Holmberg 1973). A diagram in Cober and Voldeng (2012) shows the presence of Fiskeby 840-7-3 (PI 438477) in the ancestry of numerous public cultivars released in Ontario between the mid-1970s and 2000. These cultivars include Maple Arrow (released in 1976), which was grown commercially for at least 13 years, and which is itself in the pedigrees of many other more recent cultivars. Fiskeby 840-7-3 was also an indirect ancestor of Maple Glen, which was released in 1988 and was grown commercially for at least 15 years, and of Maple Isle, which was partially resistant to *P. ultimum* var. *ultimum* in the present study. Maple Isle is a selection from a cross between PI 194641, another accession developed in Sweden, and Harosoy (Voldeng et al. 1985); thus, *Pythium* resistance could have been inherited from more than one of its progenitors. In addition to being an ancestor of a number of Canadian cultivars, Harosoy is in the pedigree of Archer (Cianzio et al. 1991; Cober and Voldeng 2012). Fiskeby III was one of the parents of Maple Ridge, a cultivar subsequently used in breeding programs in Minnesota and North Dakota (<https://soybase.org/>). The results of this study suggest the possibility that some high-yielding contemporary cultivars and breeding lines descended from the cold-tolerant Fiskeby lines identified through our assays could potentially be useful sources of resistance to *Pythium* root rot and damping-off.

Fiskeby III and Fiskeby 840-7-3 are closely related to each other. Fiskeby 840-7-3 is from a cross of 201-14-20 × 680+993+994 (Müncheberg), and 201-14-20 is a sib of Fiskeby III (Bernard et al. 1988; Gizlice et al. 1994). Both were developed by Sven Holmberg in Sweden (Shurtleff and Aoyagi 2015). Although we do not know whether Maple Isle is directly related to Fiskeby 840-7-3 and Fiskeby III, it was derived from a cross to Holmberg 744-2, another line from the same breeding program at Fiskeby. Because Fiskeby III and Fiskeby 840-7-3 were not significantly different from each other in any of the pathogen screens, it is possible that they carry similar resistance genes. Although we do not know the origin of the *Pythium*-resistant ancestors of these lines, Holmberg collected seed of cold-tolerant landraces from Hokkaido and adjacent islands of the Okhotsk Sea, in northern Japan, in 1939 to 1940, and from the northern Amurskaja region of eastern Siberia (Holmberg 1973; Shurtleff and Aoyagi 2015). The 680+993+994 (Müncheberg) parent of Fiskeby 840-7-3 was developed at the Kaiser-Wilhelm-Institut für Züchtungsforschung in northeastern Germany, and might have also contributed useful resistance genes. Fiskeby III is also noteworthy for its tolerance to a number of abiotic stress conditions (Do et al. 2018).

Incorporation of early maturity and cold tolerance traits from the Swedish lines into the existing Canadian germplasm led to the development of cultivars that enabled the geographic expansion of soybean production northward and eastward in Ontario, and into Quebec and the Maritime provinces (Shurtleff and Aoyagi 2010; Voldeng et al. 1997). The abundance of *P. ultimum* found in the recent survey by Marchand et al. (2014) of soybean fields in Ontario suggests the possibility that resistance to *P. ultimum* var. *ultimum* could have been an important component of the “cold tolerance” that was transferred into at least some cultivars in the historically important Maple series of Canadian soybean cultivars from several Swedish lines developed by the soybean breeding program at Fiskeby. Because of the critical importance of cold tolerance and adaptation to latitudes with a short growing season, it seems likely that many modern cultivars adapted to Canada also have Swedish germplasm in their pedigrees. It is interesting, however, that both accessions of Fiskeby V (PI 360955A and PI 360955B) were part of the 16 lines with no seedling emergence when challenged with the 12Py391 isolate of *P. ultimum* var. *ultimum*, even though the noninoculated pots from these lines had greater than 90% germination.

Because Maple Isle was the only cultivar from the Maple series that was tested for resistance in our assays, it would be interesting to investigate whether other cultivars in the Maple series

(i.e., Maple Arrow, Maple Amber, Maple Donovan, Maple Presto, and Maple Ridge) have similar levels of resistance to *Pythium* pathogens. All of these cultivars have the line Fiskeby 840-7-3 as a parent, except for Maple Isle (with Holmberg 744-2) and Maple Ridge, which had Fiskeby III as its maternal parent. The degree of genetic relationship between Holmberg 744-2 and Fiskeby 840-7-3 is not known to us. Most of the cultivars in the Maple series were developed from crosses to Harosoy, Harosoy 63, or Amsoy (Bernard et al.

1988); thus, they share progenitors with Archer. Harosoy and Amsoy were included in the first experiment, in which they had above-average dry root and shoot weights, though Harosoy had a significantly greater plant stand than Amsoy (Table 2).

It was not surprising that none of the ancestral lines tested had high levels of resistance to the aggressive *P. ultimum* var. *ultimum* isolate used in this study, because high levels of resistance to *Pythium* pathogens appear to be uncommon in soybean (Rupe et al. 2011). This

Table 2. Relative mean plant stands and root and shoot dry weights of the 102 soybean lines used in the ancestral experiment that were screened for resistance against *Pythium ultimum* var. *ultimum*^v

Accession	Cultivar name	Plant stand	Root dry weight ^w	Shoot dry weight ^w
FC 31745	...	0.06 P-U	0.12 Q-V	0.22 R-BB
FC 33243	Anderson	0.09 N-U	0.19 J-V	0.25 Q-BB
PI 54615-1	No. 55	0.37 B-G	0.53 B-L	0.63 A-Q
PI 65338	Botanical Garden No. 4	0.08 N-U	0.56 A-I	0.54 C-T
PI 71506	No. 94	0.17 I-T	0.42 B-S	0.62 A-R
PI 80837	Mejiro	0.25 D-M	0.69 A-C	0.86 A-D
PI 84631	S-56	0.02 S-U	0.03 UV	0.08 X-BB
PI 84637	S-62	0.47 AB	0.93 A	0.87 A-C
PI 84946-2	(Kandokon)	0.13 J-U	0.54 B-K	0.53 C-U
PI 86972-1	Pakute	0.06 Q-U	0.33 C-V	0.10 W-BB
PI 88788	...	0.02 S-U	0.17 K-V	0.03 AABBB
PI 88811	Pakute	0.04 R-U	0.27 E-V	0.31 N-BB
PI 91110-1	...	0.35 B-H	0.60 A-F	0.71 A-L
PI 96983	...	0.02 S-U	0.17 K-V	0.16 S-BB
PI 159925	Glycine H	0 U	0 V	0 BB
PI 171450	Kisaya	0.29 C-J	0.46 B-Q	0.43 G-Z
PI 171451	Kosamame	0.15 I-U	0.47 B-Q	0.50 C-V
PI 180501	Strain No. 18	0.18 I-S	0.56 A-H	0.55 B-T
PI 200492	Komata	0.06 P-U	0.42 B-S	0.33 J-BB
PI 240664	Bilomi #3	0.10 M-U	0.19 I-V	0.38 G-BB
PI 248404	Novosadska Bela	0.35 B-G	0.72 AB	0.71 A-M
PI 317335	Koganejiri	0.22 E-O	0.14 O-V	0.34 J-BB
PI 360955A	Fiskeby V	0 U	0 V	0 BB
PI 360955B	(Fiskeby V)	0 U	0 V	0 BB
PI 438471	Fiskeby III	0.37 B-F	0.67 A-D	0.94 AB
PI 438477	Fiskeby 840-7-3	0.28 C-K	0.58 A-F	0.76 A-G
PI 508269	Stafford	0.17 I-T	0.57 A-G	0.53 C-U
PI 513382	Glenwood	0.12 K-U	0.47 B-Q	0.69 A-N
PI 535807	Crockett	0.02 TU	0.17 K-V	0.17 S-BB
PI 542402	Chico	0.40 BCD	0.52 B-L	0.69 A-N
PI 548169	T117	0.12 M-U	0.41 B-S	0.73 A-J
PI 548178	T145	0.17 I-T	0.63 A-E	0.47D-Y
PI 548193	T201	0.15 I-U	0.31 D-V	0.54 C-T
PI 548195	T204	0.30 C-I	0.65 A-D	0.84 A-F
PI 548237	T260H	0.05 R-U	0.20 G-V	0.30 N-BB
PI 548298	A.K. (Harrow)	0.08 N-U	0.21 G-V	0.31 N-BB
PI 548301	Aoda	0.30 C-I	0.64 A-D	0.72 A-K
PI 548302	Bansei	0.15 I-U	0.40 B-U	0.62 A-Q
PI 548307	Blackeye	0 U	0 V	0 BB
PI 548311	Capital	0.10 M-U	0.16 L-V	0.29 O-BB
PI 548318	Dunfield	0.02 TU	0.07 R-V	0.14 U-BB
PI 548325	Flambeau	0.13 K-U	0.31 D-V	0.47 D-Y
PI 548336	Habaro	0.41 BC	0.40 B-T	0.56 A-S
PI 548342	Higan	0 U	0 V	0 BB
PI 548348	Illini	0.07 O-U	0.27 E-V	0.51 C-V
PI 548352	Jogun	0.23 E-N	0.71 AB	0.95 A
PI 548356	Kanro	0 U	0 V	0 BB
PI 548359	Kingwa	0 U	0 V	0 BB
PI 548360	Korean	0 U	0 V	0 BB
PI 548362	Lincoln	0.04 R-U	0.24 F-V	0.38 G-BB

(Continued on next page)

^v Soybean lines include 90 ancestral and first progeny lines, the resistant check Archer, 10 lines in Archer's pedigree, and 2 susceptible checks of Jack and Sloan. Each of the inoculated replications was compared with its noninoculated control. Fisher's protected least significant difference (LSD) was used for LS means separation.

^w Relative weights used were on a per-plant basis of the inoculated replications to their noninoculated control.

^x Resistant check.

^y Susceptible check.

^z Overall mean of inoculated replications for plant stand, dry root weight, or dry shoot weight.

might be due to the biology and genome of *Pythium* spp., which differ from *Phytophthora* pathogens in being adapted to a necrotrophic lifestyle and in lacking RxLR effectors (Adhikari et al. 2013; Glazebrook 2005; Lévesque et al. 2010; van West et al. 2003). In contrast, pathogenic *Phytophthora* spp. carry genes for hundreds of RxLR effectors that are thought to be associated with entry into host cells and suppression of host defense responses. Recognition of some RxLR effectors by plant immune receptors results in programmed

cell death and resistance to incompatible races of *Phytophthora* spp. (Adhikari et al. 2013; Laviolette et al. 1979). It is possible that the absence of this class of effectors in *Pythium* pathogens allows them to evade the type of gene-for-gene interactions that characterize relationships between *Phytophthora sojae* races and soybean plants carrying major *Rps* resistance genes, thus avoiding a strong host defensive reaction. Although Rosso et al. (2008) found that a single major resistance gene on soybean chromosome 13 (linkage group F)

Table 2. (Continued from previous page)

Accession	Cultivar name	Plant stand	Root dry weight ^w	Shoot dry weight ^w
PI 548379	Mandarin (Ottawa)	0.08 N-U	0.24 F-V	0.45 F-Y
PI 548382	Manitoba Brown	0.08 N-U	0.51 B-O	0.61 A-R
PI 548383	Mansoy	0.03 S-U	0.06 R-V	0.33 K-BB
PI 548391	Mukden	0.10 M-U	0.42 B-S	0.52 C-V
PI 548402	Peking	0.02 TU	0.17 K-V	0.17 S-BB
PI 548406	Richland	0.37 B-G	0.61 A-E	0.70 A-N
PI 548409	Sato	0.28 C-L	0.55 B-J	0.65 A-P
PI 548411	Seneca	0.07 O-U	0.49 B-P	0.35 J-BB
PI 548438	Arksoy	0 U	0 V	0 BB
PI 548444	Biloxi	0.08 N-U	0.17 K-V	0.36 H-BB
PI 548445	CNS	0.22 F-P	0.55 B-J	0.76 A-H
PI 548456	Haberlandt	0 U	0 V	0 BB
PI 548457	Hahto	0.02 S-U	0.05 S-V	0.12 V-BB
PI 548461	Improved Pelican	0.10 M-U	0.46 B-Q	0.40 G-AA
PI 548463	Laredo	0.09 N-U	0.40 B-U	0.66 A-P
PI 548469	Mammoth Yellow	0 U	0 V	0 BB
PI 548477	Ogden	0.23 E-N	0.40 B-U	0.69 A-N
PI 548484	Ralson	0 U	0 V	0 BB
PI 548485	Roanoke	0.02 TU	0.15 M-V	0.10 W-BB
PI 548488	S-100	0.03 S-U	0.04 T-V	0.09 X-BB
PI 548493	Tokyo	0.02 TU	0.14 N-V	0.04 Z-BB
PI 548494	Volstate	0 U	0 V	0 BB
PI 548528	Protana	0.04 R-U	0.27 E-V	0.26 R-BB
PI 548548	Delmar	0.49 AB	0.57 A-G	0.69 A-N
PI 548559	Emerald	0.11 M-U	0.51 B-M	0.66 A-P
PI 548561	Hodgson	0.38 B-E	0.46 B-Q	0.50 C-X
PI 548587	Kim	0 U	0 V	0 BB
PI 548595	Maple Isle	0.57 A	0.61 A-E	0.68 A-O
PI 548599	Monroe	0.06 Q-U	0.04 T-V	0.08 Y-BB
PI 548603	Perry	0.11 M-U	0.35 B-V	0.56 A-S
PI 548604	Pershing	0.16 I-T	0.55 B-J	0.59 A-R
PI 548623	Vansoy	0.12 M-U	0.49 B-P	0.43 G-AA
PI 548626	Wabash	0.03 S-U	0.20 H-V	0.32 M-BB
PI 548633	Wye	0.22 F-P	0.53 B-K	0.45 F-Y
PI 548657	Jackson	0.12 L-U	0.33 C-V	0.32 L-BB
PI 548663	Dowling	0.05 R-U	0.47 B-Q	0.33 K-BB
PI 548697	Majos	0 U	0 V	0 BB
PI 548983	Tracy	0.04 R-U	0.08 R-V	0.22 R-BB
PI 553048	Vance	0.04 R-U	0.17 K-V	0.16 T-BB
PI 567790	Curtis	0.05 R-U	0.27 E-V	0.37 G-BB
PI 546487	Archer ^x	0.35 B-G	0.59 A-F	0.75 A-I
PI 548519	BSR-101	0.09 M-U	0.32 D-V	0.63 A-Q
PI 548506	Amsoy	0.09 M-U	0.43 B-R	0.46 E-Y
PI 548527	Calland	0.29 C-I	0.48 B-Q	0.56 A-T
PI 548502	Adams	0 U	0 V	0 BB
PI 548573	Harosoy	0.58 A	0.55 B-J	0.85 A-E
PI 548504	Altona	0.21 G-Q	0.55 B-J	0.48 C-X
PI 548516	Blackhawk	0.29 C-J	0.37 B-U	0.64 A-Q
PI 518671	Williams 82	0.28 C-L	0.51 B-N	0.75 A-H
PI 548533	Clark	0.12 M-U	0.27 E-V	0.36 I-BB
PI 540556	Jack ^y	0.19 H-R	0.39 B-U	0.43 G-Z
PI 548616	Sloan ^y	0.25 D-M	0.12 R-V	0.42 G-AA
LSD ($P \leq 0.05$)	...	0.18	0.43	0.44
Standard error	...	0.058	0.148	0.145
Overall mean ^z	...	0.140	0.323	0.399

from Archer named *Rpa1* conferred a high level of resistance to *Pythium aphanidermatum* following hypocotyl inoculations, Urrea et al. (2017) reported that two independent quantitative trait loci (QTL) were associated with Archer's resistance to seed and root rot caused by *P. aphanidermatum*. Mahuku et al. (2005) found that resistance to *P. ultimum* var. *ultimum* in common bean (*Phaseolus vulgaris*) was also conditioned by a single dominant gene.

It was also not surprising that the lines with partial resistance were in MG 00 to IV, and had originated from cool, temperate regions of several different countries (<https://www.ars-grin.gov/>), because *P. ultimum* var. *ultimum* is adapted to cool conditions and can be found around the world (van der Plaats-Niterink 1981). Recent surveys have confirmed that *Pythium* pathogens, particularly *P. ultimum* var. *ultimum* and *P. sylvaticum*, are abundant in the rhizosphere of soybean fields in Ontario and the upper Midwest and north-central United States (Marchand et al. 2014; Rojas et al. 2017a,b; Zitnick-Anderson and Nelson 2015).

We did expect Archer to have a higher level of resistance, because it had been reported by Bates et al. (2008) to have some resistance against *P. ultimum*, *P. aphanidermatum*, *P. irregular*, and *P. vexans*. Based on root and shoot dry weight ratios, Archer showed partial resistance to the 12Py391 isolate of *P. ultimum* var. *ultimum* (Table 2). The *P. ultimum* isolate that Bates et al. (2008) and Kirkpatrick et al. (2006a) used to challenge Archer was a 1999 isolate from Arkansas. We do not know how the pathotype of the 12Py391 isolate compares with that of the Arkansas isolate but, because *P. ultimum* var. *sporangiferum* is less common, it seems likely that the Arkansas isolate would have also been the variety *ultimum*. Different assay methods or reaction rating methods, or differences in the aggressiveness of the isolates, could have also contributed to the differences observed in the apparent resistance of Archer. Urrea et al. (2017) recently discovered two QTL associated with the resistance of Archer to *P. aphanidermatum*; therefore, it will be interesting to see whether those are also involved in resistance to other *Pythium* spp. The locations of these QTL (on chromosomes 4 and 7) differed from the chromosome 13 location of the dominant *Rpa1* gene identified by Rosso et al. (2008) using a hypocotyl inoculation assay. Urrea et al. (2017) hypothesized that the different results might have been due to their use of infested vermiculite and seed plating assays that measure seed and root rot rather than plant survival, which is used to assess resistance following hypocotyl inoculation. It would also be interesting to know whether the QTL for resistance to seed and root rot caused by *P. aphanidermatum* are associated with *P. ultimum* var. *ultimum* resistance in any of the lines that appeared to be resistant in the present study.

A single isolate was used to represent each of the three *Pythium* spp. in the second experiment. We do not know how well those

Table 3. Spearman rank correlations between relative plant stand, root dry weight, and shoot dry weight in the ancestral screen experiment^z

Variables	Plant stand	Root dry weight	Shoot dry weight
Plant stand	1	0.82638	0.8136
Root dry weight	...	1	0.8782
Shoot dry weight	...	...	1

^z Weights used were on a per-plant basis. All correlations are significant ($P \leq 0.0001$).

Table 4. Spearman rank correlations between pairs of resistance evaluation trait ratios in assays with isolates of *Pythium ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum*^z

Variables	Plant stand	Root dry weight	Shoot dry weight
Plant stand	1	0.59	0.34
Root dry weight	...	1	0.59
Shoot dry weight	...	...	1

^z Weights used were on a per-plant basis. All correlations are significant ($P \leq 0.0001$).

isolates represented the average pathogenicity of North American populations of those species but the 12Py391 isolate was more aggressive than some other *P. ultimum* var. *ultimum* isolates in preliminary assays. Our observation that *P. ultimum* var. *ultimum* was more aggressive than *P. irregulare*, which was more aggressive than *P. sylvaticum*, should therefore be confirmed using other isolates. The weak aggressiveness of the *P. sylvaticum* isolate used, even toward Kanro, makes it unclear whether all five lines are actually resistant or if soybean is simply not a good host for this species or isolate.

Soybean disease caused by *Pythium* pathogens can occur as a seed rot phase or a root rot phase, and some host resistance genes may not necessarily provide protection from both (Rupe et al. 2011). Different types of assays have been developed to assess resistance to disease of seed, seedlings, or both. The ability of a pathotype to colonize seed from different soybean genotypes can be measured using Petri plate assays (Dorrance et al. 2004). Some types of seedling resistance to *Pythium* spp. can be measured using a hypocotyl inoculation assay of the type that Rosso et al. (2008) used to assess *P. aphanidermatum* resistance inherited from Archer. This method, which was originally developed for *Phytophthora sojae* assays, was found to be effective and more consistent than a sand/cornmeal inoculum layer method that was also tested by Rosso et al. (2008). We were unable to obtain consistent differences in reactions, however, when we tested the hypocotyl inoculation method with the 12Py391 isolate and cultivars (data not shown).

The inoculum-layer assay used in the present study revealed variation among soybean lines for both seedling emergence and seedling growth and, thus, provided information about levels of seed rot and stunting of seedlings in pots with inoculum. In our assays, the seed were separated from the layer of inoculum by 2.5 cm of potting soil, preventing direct contact between the inoculum and the seed. In contrast, Bates et al. (2008) conducted inoculum layer emergence assays in which the seed were placed directly onto the inoculum, and an establishment assay in which a 1.8-mm layer of pasteurized soil separated the seed from the inoculum. This thin barrier likely exposed seed to the pathogen considerably earlier than the 2.5-cm layer used in our assays, which could influence observed levels of premergence damping-off. Because soil saturation and flooding often

Table 5. Relative means for resistance evaluation traits measured on plants of four soybean lines inoculated with *Pythium ultimum* var. *ultimum*, *P. irregulare*, and *P. sylvaticum* compared with noninoculated controls of the same line^z

<i>Pythium</i> spp. and lines screened	Plant stand	Root dry weight	Shoot dry weight
<i>P. ultimum</i> var. <i>ultimum</i>			
PI 84637	0.82 A	0.62 A	0.83 A
Maple Isle	0.61 B	0.58 AB	0.76 A
Fiskeby III	0.60 B	0.56 AB	0.76 A
Fiskeby 840-7-3	0.67 AB	0.42 BC	0.71 A
Kanro	0.13 C	0.27 C	0.41 B
Standard error	0.062	0.086	0.069
<i>P. irregulare</i>			
PI 84637	0.96 A	0.94 A	0.82 A
Maple Isle	0.73 B	0.64 B	0.66 A
Fiskeby III	0.75 B	0.59 B	0.80 A
Fiskeby 840-7-3	0.70 B	0.50 B	0.69 A
Kanro	0.33 C	0.55 B	0.74 A
Standard error	0.052	0.065	0.083
<i>P. sylvaticum</i>			
PI 84637	0.97 A	0.96 AB	0.94 AB
Maple Isle	0.94 A	0.99 A	0.91 B
Fiskeby III	0.94 A	0.98 AB	0.96 A
Fiskeby 840-7-3	0.84 B	0.94 AB	0.95 AB
Kanro	0.93 A	0.92 B	0.98 A
Standard error	0.024	0.020	0.028

^z Fisher's least significant difference was used to determine significant differences. Means followed by the same letter within a column are not significantly different ($P \leq 0.05$). Weights used were on a per-plant basis.

exacerbate soybean pre- and postemergence damping off caused by *Pythium* pathogens (Kirkpatrick et al. 2006a), the composition of the soil or soil mix used in inoculum-layer assays could also influence the severity of disease and rot observed in assays. As mentioned earlier, temperature differences in assays conducted by different research groups may have also contributed to differences in the perceived aggressiveness of different isolates and species of *Pythium*. Although each type of assay has its merits and disadvantages, the inclusion of noninoculated or mock-inoculated control treatments and known susceptible hosts in each assay provides a critical indication of the success of each experiment and assay method to distinguish between resistant and susceptible plants.

Statistical sensitivity in future assays could likely be improved to decrease the large standard errors that we had in the present study, particularly for root and shoot dry weight ratios, by increasing the number of inoculated replications per line or the number of plants per replication. In addition, experimental error resulting from random differences in the rate of water absorption, germination, and emergence of seed in the same pot should be reduced, if possible, because variation in emergence rates results in broader differences in root and shoot dry weights among noninoculated control plants. Aggressiveness levels among and within *Pythium* spp. vary (Broders et al. 2007; Radmer et al. 2017), which contributes to variation in seedling emergence and vigor.

From this study, we conclude that sources for resistance to *P. ultimum* var. *ultimum* exist in the genetic base of North American soybean cultivars released prior to 1989, and that some of those might also have resistance to other *Pythium* spp. These resistant accessions originated from several different countries but germplasm accessions originating from northeastern China, Korea, and northern Japan, or North American cultivars derived from them, might have a higher than average probability of being resistant, though some of the lines we screened from those regions in this study were susceptible. Recently developed, early-maturing cultivars descended from Fiskeby III or the Fiskeby 840-7-3 line might also prove to be good sources of *Pythium* resistance genes. We also showed that some of the same lines with partial resistance to *P. ultimum* var. *ultimum* were partially resistant to an isolate of *P. irregulare*, another common soil pathogen of soybean grown in North America. More research is needed to determine whether the same or different genes are responsible for partial resistance to different species of *Pythium*, and whether the resistant lines identified here are also resistant to other *Pythium* spp. and isolates. Although seed treatments with fungicides that protect soybean seed and seedlings from *Pythium* pathogens as well as fungi have become common for seed marketed in North America, resistance to *Pythium* pathogens could help to protect seedlings after their roots grow beyond the activity zone of the seed fungicides and when environmental conditions are particularly favorable for *Pythium* spp. pathogens.

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