Journal of
Phytopathology

ORIGINAL ARTICLE

Virulence of *Phytophthora sojae* in the Pampeana Subregion of Argentina from 1998 to 2004

Pablo E. Grijalba and Marcela E. Gally

Agronomy Faculty, Phytopathology Department, University of Buenos Aires, Av. San Martín 4453, Buenos Aires, 1417, Argentina

Keywords
Phytophthora sojae, races, variability

Correspondence
P. E. Grijalba, University of Buenos Aires,
Buenos Aires, Argentina.
E-mail: grijalba@agro.uba.ar

Received: August 7, 2014; accepted:
December 15, 2014.

doi: 10.1111/jph.12369

Abstract

Phytophthora root rot is one of the most serious diseases of soybeans in Argentina. Surveys of commercial fields and trial plots of soybean were conducted throughout the northern Pampeana subregion (Argentina) between 1998 and 2004. A total of 193 isolates of *Phytophthora sojae* were collected and classified into races or virulence formulae. Among the 173 isolates tested on 8 differentials, 42 different pathotypes were detected, including 18 described races. Races 1, 4, 5, 7, 9, 13, 23 and 24 were found in both plants and soils, whereas races 2, 3, 6, 8, 11, 14, 15, 17, 43 and 44 were only isolated from plants. An additional 19 pathotypes were described from 20 isolates tested in Canada on the expanded set of 14 differential cultivars. Currently, all *Rps* genes/alleles associated with resistance have been defeated, indicating an increased complexity of virulence within the *P. sojae* populations in the region. The great increase in virulence complexity found in this study is most likely a result of a long period of continuous production of soybean cultivars with *Rps* genes and the extensive adoption of the no-tillage system.

Introduction

In Argentina, over 82% of soybeans (*Glycine max* (L.) Merr.) are produced in the northern Pampeana subregion (including the provinces of Córdoba (Cba), Entre Ríos (ER), central and southern Santa Fe (SFe), northern Buenos Aires (BA) and north-eastern La Pampa (Ploper 2004). *Phytophthora sojae* (Kaufmann & Gerdemann) causes seed rot, pre- and postemergence damping-off, and root and stem rot (Schmitthenner 1985). Phytophthora root rot (PRR) is regarded as one of the most serious diseases of soybean in Argentina. Reduction in soybean yield as a result of PRR was estimated at 92 000 metric tons in 1998 (Wrather et al. 2001). Since 1959, differences in virulence among isolates of *P. sojae* have been reported (Hildebrand 1959). With time, it has become more apparent that the natural populations of this pathogen are very complex and are composed of many described races and other additional virulence phenotypes, some of which can defeat all of the known *Rps* resistance genes. Currently, more than 55 races have been reported (Leitz et al. 2000; Anderson and Tenuta 2003) based on their reaction to 8–13 dominant resistance genes (Dorrance et al. 2003; Jackson et al. 2004; Nelson et al. 2008). Fourteen *Rps* genes have been mapped at eight loci in soybean (Burnham et al. 2003; Gordon et al. 2006). Two more genes have been described recently, *Rps*Yu25 (Sun et al. 2011) and another that is allelic to *Rps*1 or at a tightly linked locus in a gene cluster (Sugimoto et al. 2011).

Phytophthora root rot was first detected in Argentina in the 1970’s (A. F. Schmitthenner, *personal communication*). In 1978–1979, it was found in 15 localities in southern SFe and northern BA (Martinez and Ivancovich 1979). From 1989 to 1992, annual surveys of soybean fields carried out in the northeast of BA indicated that only race 1 was present in the area surveyed (Barreto et al. 1991, 1995). In the last 15 years, there has been a dramatic shift from conventional tillage to conservation tillage in Argentina. At present, more than 85% of the planted area is under minimum and no-tillage systems, and 50% is under soybean monoculture (Rossi 2004). The use of conservation tillage has been reported to increase damages caused by *Phytophthora sojae* in soybean

(Schmitthenner 1985; Workneh et al. 1999). In the 1994–1996 growing seasons, the disease showed a steady increase in prevalence, incidence and changes in pathogen virulence. Sixty-five per cent of 112 isolates obtained and tested during this period belonged to race 1, and 39% were avirulent on *Rps* 1-d and *Rps* 1-k (Barreto et al. 1998b). Race 4 was detected in 1996 in a soil sample from Los Molinos (SFe) (Barreto et al. 1998a). These results showed that some isolates possessed more than one virulence gene, which had not been reported previously, and changes in virulence variability of *P. sojae* were confirmed in the northern Pampeana subregion. The same year, virulence on *Rps* 1-c and *Rps* 2 was detected in ER (Vicentini 1996). In the 1997–1998 season, 53% of 71 isolates of *P. sojae*, obtained from different localities in northern BA, southern SFe and south-western Cba, belonged to race 1 (Gally et al. 1999). The evidence of increasing virulence of the pathogen in the area has led to integral studies of population virulence to provide information for breeding programs. The objective of this study was to characterize the virulence of populations of *P. sojae* in the Pampeana subregion of Argentina during the period from 1998 to 2004.

Materials and Methods

Region surveyed

Between 1998 and 2004, soybean production areas were surveyed in the northern Pampeana subregion (32°29′S to 34°32′S, Fig. 1). These areas comprised 20 localities within BA, Cba, ER and SFe provinces. Samples were obtained mainly from commercial soybean fields and some trial plots. All fields were planted with soybean and were at R1-R5 growth stages (Fehr and Caviness 1977) at sampling in January, February and early March. In 2000, the survey was conducted at the V1–V2 growing stages. Areas sampled were intentionally selected based on reports of the presence of wilting and dead plants in cooperation with breeders, extension personnel and individual farmers. Soybean plants, with typical wilting and dark brown stem lesions, and soil from around the roots of plants with root rot were collected. After removing whole symptomatic plants (10–15 plants from each lot) including the root system, stem sections (20–40 cm long within the upper margin of the lesion) were cut to remove leaves and upper branches. Sections and roots were stored in plastic containers with ice at approximately 10°C until processed (within 48 h). At least 15 individual soil samples collected in each lot were transported to the laboratory in closed plastic containers with ice. The samples were stored for no more than 5 months at 25°C prior to use.

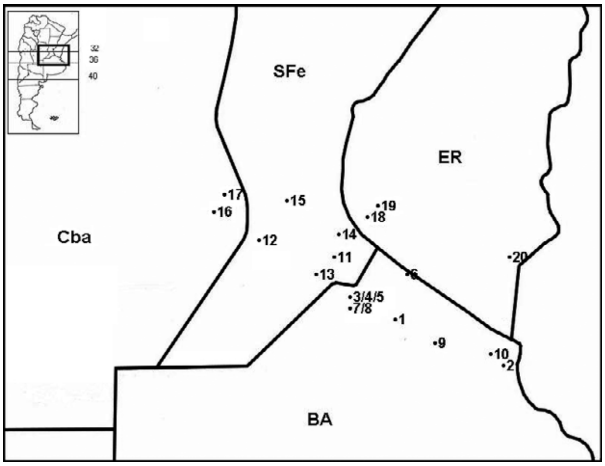

Fig. 1 Distribution of *Phytophthora sojae* in Argentina. Numbers represent the 20 localities where isolates were collected. Letters represent Argentine provinces: BA (BUENOS AIRES) 1 Arrecifes; 2 Field plot; 3 Pergamino; 4 El Socorro; 5 M. Ocampo; 6 Ramallo; 7 Rojas; 8 R. Obligado; 9 San Antonio de Areco; 10 Pilar. SFe (SANTA FE) 11 Bigand; 12 Godeken; 13 Hughes; 14 Muñoz; 15 Las Rosas. Cba (CORDOBA) 16 Marcos Juarez; 17 Saira. ER (ENTRE RIOS) 18 Victoria; 19 Villa. Teresita; and 20 Gualeguaychu.

Isolation from diseased plants

Diseased stems were thoroughly washed in distilled water to remove soil particles, soaked in 0.5% sodium hypochlorite solution for 30 seconds, rinsed in distilled water and blotted-dried with paper towels. In 2001, stem isolations were made after washing with distilled water, without using the sodium hypochlorite solution. Isolations were made from the advancing margin of stem and branch lesions, after the epidermis was removed. Five to seven sections of vascular tissue (0.3–0.5 cm) were cut out and transferred to Petri plates on diluted V-8 juice medium (VA) (Schmitthenner et al. 1994). VA consists of 40 ml of V8 juice in water (first autoclaved with 0.6 g of CO_3 Ca and filtered through diatomaceous earth), 1.0 g of sucrose, 0.2 g of Difco yeast extract, 0.01 g of cholesterol and 20 g of Difco Bacto agar/litre. To suppress fungi and bacterial growth, a solution of PCNB (0.02 g), benomyl (0.005 g), neomycin sulphate (0.1 g) and chloramphenicol (0.01 g) was added after the VA medium was autoclaved and cooled to 60°C. In 2001, isolations were made on diluted lima bean agar (LBA, extract from 25 g/l of frozen lima beans) plus the same fungicides and antibiotics as used for the VA medium. Stem sections were placed under the medium in the Petri plate to seal the tissue in a bubble for additional bacterial control. The isolations were held in an incubator at 23–25°C in darkness for 7–10 days. Hyphal tips reaching the surface of the medium were transferred to fresh VA or LBA media without inhibitors for further purification and identification, and maintained on VA slants.

Isolation from soil

Soil subsamples were bulked, sieved through a 0.5-cm (mesh-size) screen and mixed to obtain a single composite sample of approximately 2–3 kg. Each composite sample was stored in a sealed polyethylene bag for no more than 6 months at laboratory temperature. Each soil was dispensed into a shallow tray with bottom drainage, flooded for 1 h and then drained. Soils were incubated at 25°C for 10 days, approximately when the soil moisture reached field capacity, and, following incubation, the sample was placed into a plastic bag to maintain moisture. The soybean seedling baiting technique was used to isolate *P. sojae* from the soil (Schmitthenner et al. 1994). Eight to ten 2-day-old pregerminated soybean seeds of Williams or Sloan genotypes were transplanted into 10-cm-diameter plastic pots filled with the infested soil, and covered with a wet, coarse mix vermiculite and perlite (1:1 v/v). When the cotyledons reached the surface, generally 48 h after transplanting, the soil was flooded for 24 h and drained and pots were incubated in a cool room at 20–25°C. Isolation of *Phytophthora* was made from the hypocotyls of damped-off seedlings. Before isolation from the hypocotyls, root tissues of damped-off seedlings were stained with lacto-fuchsin or lactophenol-trypan blue to verify the presence of oospores under a compound microscope.

Culture identification and storage

Cultures were characterized by colourless, coenocytic mycelium with irregular hyphal swellings and identified by the abundant production of homothallic smooth-wall spherical oospores. Pure cultures were transferred to slants of VA for storage and LBA for maintenance at room temperature. In both cases, cultures were kept in an unlighted incubator. To maintain aggressiveness, the isolates were inoculated into susceptible lines (cv. Sloan or Williams) using the inoculum layer technique at 4-month intervals (Ohal et al. 1985; Schmitthenner et al. 1994).

Virulence evaluation of cultures

Pathotypes or virulence formulas were used to describe virulence patterns based on reactions to a known set of soybean genotypes containing eight different *Rps* genes (Schmitthenner et al. 1994; Dorrance et al. 2004). The different genes included HARO 12 (*Rps* 1-a), HARO 13 (*Rps* 1-b), Corsoy 79 (*Rps* 1-c), HARO 16 (*Rps* 1-d), HARO 15 (*Rps* 1-k), HARO 3272 (*Rps* 3-a + *Rps* 7), HARO 6272 (*Rps* 6+*Rps* 7) and Harosoy (*Rps* 7). The susceptible controls were Haro (1-7) 1, Williams and Sloan. Some isolates obtained from 2002 to 2004 were evaluated on an expanded set of 14 differential cultivars, in addition to the aforementioned differentials, which included L82-1449 (*Rps* 2-7), HARO 33 (*Rps* 3b), HARO 34xx (*Rps* 3c), HARO 4272 (*Rps* 4), HARO 5272 (*Rps* 5), the *Rps* 8 gene (PI 399073) and 3 experimental lines, Haro Nez (*Rps* 744), Ox 939 (*Rps* Ox939) and X570-20-2-1 (*Rps* Ox940), with 3 putative genes/alleles at Harrow (Canada) (Table 1). The soybean cultivars (differentials) were obtained from A.F. Schmitthenner at the Ohio Agricultural Research and Development Center, and T. Anderson at Agriculture Canada, Research Station, Harrow, Ontario. Some of these differentials might not be recommended due to inconsistencies in the results (Dorrance et al. 2004). *Phytophthora* virulence was evaluated using the hypocotyl inoculation technique (Schmitthenner et al. 1994). Inoculum was produced on VA or LBA media (1.2% agar) in Petri plates in a cabinet without light at 23–25°C. A 7- to 12-day-old colony of each isolate was macerated by passage through a syringe and was then reloaded into the syringe. Longitudinal slits (of approximately 1 cm long) were made with a 16-gauge needle on hypocotyls of 10 7-day-old plants of each differential growing in coarse vermiculite and perlite (1:0.5, v/v), and approximately 300 μl of macerated culture was placed on each slit. The plants were incubated in new plastic containers with lids to maintain high humidity (98% RH) during the initial incubation period. After 20–24 h, plants were removed and incubated in a cool room chamber at 20–25°C for 6 days. Plants were watered daily, and care was taken to keep the soil moist. Symptom development was rated 3 days after inoculation in susceptible differentials. The final assessment was completed 5 days after inoculation. The virulence pattern was identified on the basis of a susceptible (70% or more seedlings killed) or a resistant (30% or less seedlings killed) reaction. Seedling mortality from 31 to 69% was considered an intermediate reaction (Anderson and Buzzell 1992; Ryley et al. 1998). Inoculation tests were conducted twice.

Table 1 Distribution of virulence of *Phytophthora sojae* isolated in the northern Pampeana subregion (Argentina) from 1998 to 2004, tested on 8 *Rps* genes or 14 *Rps* genes, and three experimental lines (* Tested in Harrow – Canada)

Virulence formulae[a]	Race	No of isolares[b]	Area
7	1	41pl-16 s	MO-R-A-SA-LR-Go-H-Mu-S-MJ-FA-Gu-Vi
6,7	13	8pl-2s	MO-Pi-Go-ES-MJ
3a,7	15	2pl	MO-Go
3a,6,7		6pl	MO-Bi-Go-ES
1a,7	3	2pl	Pe-ES
1a,6,7	9	13pl-7s	ES-Go-RO
1a,1c,7	4	15pl-3s	MO-R-ES-Ra-Pi-RO-VT-Vi
1a,3a,6,7	7	3pl	MO-Go
1a,1c,6,7	5	1pl-1s	S-Vi
1a,1d,7	44	1pl	MO
1a,1d,6,7	8	1pl	RO
1a,1d,3a,6,7	6	1pl	Mu
1a,1b,6,7	23	3pl-3s	ES-Bi-Go-RO
1a,1b,1c,6,7		1pl	ES
1a,1b,1d,6,7		2pl	RO
1a,1b,1d,1k,6,7		1pl-2s	ES-Go-RO
1a,1c,1d,7	43	3pl	MO-Pe
1a,1c,1d,3a,7		1pl	MO
1a,1c,1k,6,7		2pl	B
1a,1c,1d,1k,6,7		1pl	Pe
1a,1d,1k,7		3pl	MO
1b,7	2	9pl	MO-R-A-SA-LR-B
1b,6		1s	ES
1b,6,7	11	1pl-1s	Pi-MJ
1b,1c,1d,6,7		1pl	ES
1b,1d,6,7		1pl-2s	Go-RO
1b,1d,3a,6,7	17	3pl	Mu-Gu
1b,3a,6,7	24	4pl	MO-Pi-LR
1c,7	14	2pl-2s	MO-Pe
1c,6,7		1s	Pe
1c,1d,7		1s	Pe
1c,1d,6,7		1s	Pe
1c,1d,1k,6,7		1pl	Pe
1c,3a,7		1pl	ES
1c,3a,6,7		1pl	ES
1d,3a,7		1pl	MO
1k,7		1s	Vi
Tested in Canada			
1a,1c,1d,7	43	1pl	Ra
1a,1c,1d,3b,7		1pl	Pi
1a,1b,1c,1d,1k,2,3b,3c,4,5,6,7		1pl	VT
1a,1b,1c,1d,1k,3b,4,5,6,7,940		1pl	Vi
1b,1d,2,3b,3c,4,5,6,7		1pl	Mu
1b,1d,1k,3b,4,7,940		1pl	Gu
1b,1d,1k,2,3a,3b,3c,4,5,6,7,8,944,939,940		1pl	Mu
1b,1d,2,3a,3b,3c,4,5,6,7,8,944,939,940		1pl	SA
1b,1k,2,3b,3c,5,6,7,940		1pl	H
1b,1k,2,3a,3b,3c,4,5,7		1pl	SA
1b,3a,3b,5,7,8,940		1pl	Vi
1b,3b,5,7		1pl	A
1k,3b,4,5,7		1pl	Vi
1k,2,3b,5,6,7		1pl	Vi
1k,3b,7,940		1pl	FA

(continued)

Table 1 (continued)

Virulence formulae[a]	Race	No of isolares[b]	Area
2,3b,4,5,7		1pl	Vi
3b,7		1pl	Vi
3b,4,5,6,7		1pl	H
3b,5,6,7		1pl	A

[a] Listing of defeated *Rps* alleles (1a, 1b, 1c, 1d, 1k, 3a, 6, 7) and 3b,3c,4,5,8,939, 940, 944.

[b] pl, Plant; s, Soil; MO, Manuel Ocampo; R, Rojas; A, Arrecifes; Pe, Pergamino; ES, El Socorro; Ra, Ramallo; SA, San Antonio de Areco; Pi, Pilar; LR, Las Rosas; B, Bigand; S, Saira; Go, Godeken; H, Hughes; Mu, Muños; RO, Rafael Obligado; MJ, Marcos Juarez; FA, FAUBA; Gu, Gualeguaychu; VT, Villa Teresita; Vi, Victoria.

If intermediate reactions occurred both times, the isolates were considered race unclassifiable (Schmitthenner et al. 1994).

Results

During the 6 years of the study, a total of 50 soybean fields were selected and sampled for Phytophthora root rot in the northern Pampeana subregion. Seventy-eight of the surveyed fields were commercial soybean fields with a long history of soybean production, and the remaining fields were soybean trial plots. *P. sojae* was isolated in 96% of the suspected infected fields. A total of 193 isolates of *P. sojae* were recovered from 1998 to 2004 in the surveyed region, 75% from diseased plants and 25% from soils. All the isolates proved to be pathogenic and were tested in the susceptible differentials Haro (1–7)1, Williams or Sloan cultivars.

In 1998, 60 isolates were obtained from diseased plants and three from soil in five cities. In 1999, 35 and 30 isolates were obtained from plants and soil, respectively, from five cities. In 2000, only 4 and 7 isolates were obtained from plants and soil, respectively, from two cities. In 2001, no isolates were obtained; however, in 2002 and 2003, 8 isolates were obtained from plants in each year, from two and one locality, respectively. In 2004, 27 and 11 isolates were obtained from plants and soil, respectively, from four localities. Virulence phenotypes of isolates from soil and plants and their distribution in 1998–2004 are summarized in Table 1.

In recent years, the number of identified pathotypes has increased dramatically, which has made the number system of classification into races cumbersome (Dorrance et al. 2004), but both virulence formulas and race number have been included because Argentine breeders and farmers are accustomed to the number system. Among the 173 isolates tested on the eight differentials, 42 different pathotypes were detected, including 18 described races. Undescribed virulence phenotypes constituted 24% of the strains. Sixty-one pathotypes were identified, 18 of which fit previously described races; race 1 was prevalent, comprising 25% of the isolates and was present in 12 of the 20 cities surveyed, followed by race 13 which made up 5% of the isolates and was present in only four localities. A higher virulence variability was found in BA and SFe where most of isolates were obtained. The second most prevalent races were race 4 in BA and race 2 in SFe. Race 9 was the third most prevalent in both provinces (Fig. 1). In BA, races 1, 2, 3, 4, 7, 8, 9, 11, 13, 14, 15, 23, 24, 43 and 44 were identified; in SFe, races 1, 2, 5, 6, 7, 9, 13, 15, 17, 23 and 24 were identified; in ER, races 1, 4, 5 and 17 were identified; and in Cba, races 1, 5, 11 and 13 were identified. Races 1, 4, 5, 7, 9, 13, 23 and 24 were found both in plants and soils, whereas races 2, 3, 6, 8, 11, 14, 15, 17, 43 and 44 were only isolated from plants. An additional 19 pathotypes were described from 20 isolates tested in Canada on the expanded set of differentials (Table 1). The frequency of isolates compatible on *Rps* genes in soybean is illustrated in Fig. 2. All of the eight differentials were defeated, showing that *Rps* 7 was most frequently susceptible. The majority of the isolates tested in Argentina were virulent on *Rps* 1a (41.5%) or *Rps* 6 (46.1%), and 21.5% of the isolates also showed virulence on *Rps* 1b. Only 6% of the isolates exhibited virulence to the resistance gene *Rps*1k. Within the expanded set, the isolates tested in Canada were virulent on *Rps* 3b (91.3%) or *Rps* 5 (78.3%), and 56.5% of the isolates also showed virulence on *Rps* 4. Fewer than 18% of the isolates exhibited virulence to the resistance gene *Rps* 8 and the putative genes/alleles of the Ox744 and Ox939 lines.

Discussion

The results of this survey support the hypothesis that in Argentina, like in other soybean regions, *P. sojae* is a highly variable pathogen (Schmitthenner et al.

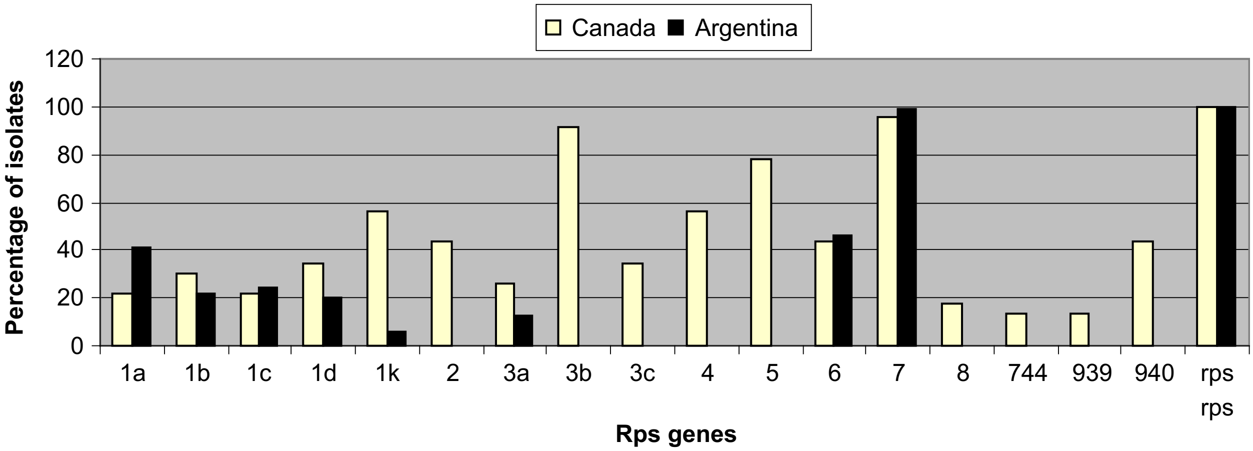

Fig. 2 Percentage of the *Phytophthora sojae* isolates collected in Argentina with a compatible interaction with *Rps* genes. White: tested in Harrow (Canada) on 15 differential cultivars, and three experimental lines. Black: tested in Argentina on eight different *Rps* genes.

1994; Drenth et al. 1996; Ryley et al. 1998; Dorrance et al. 2003; Jackson et al. 2004). Currently, all *Rps* genes/alleles known for resistance are susceptible, indicating an increased complexity of virulence within the *P. sojae* populations in the region. No isolate was pathogenic on all the soybean differentials, unlike results obtained in Michigan (Kaitany et al. 2001) and Arkansas (Henry and Kirkpatrick 1995) in the USA. Barreto et al. (1998b) reported an increase in virulence from 1992 to 1997; however, prior to this study, the most common pathotype reported in the Pampeana region of Argentina was virulent only on *Rps*7 (race 1). Although race 1 is still prevalent (25% of the isolates tested belonged to this race, present in 12 localities surveyed), the level of complexity has dramatically increased. Race 13, never before detected, was the second most prevalent race in 1999, indicating that *Rps* 6 is one of the most frequently deployed genes. The *Rps* 1k gene, which has been universally used in breeding programs due to its effectiveness against 21 physiologic races (McBlain and Schmitthenner 1991) and is still highly effective in Brazil (Costamilan et al. 2013), was defeated by 11 isolates from different localities. This result indicates that Argentinean and Brazilian *P. sojae* populations most likely do not share the same origin. In contrast, although there is no strong evidence, Argentinean populations seem to have a common origin with the US population, because the development of the disease in both countries has been similar. The most commonly deployed resistance genes in the US soybean cultivars were *Rps*1a, *Rps*1c or *Rps*1k genes (Slaminko et al. 2010). In Argentina, resistance to *P. sojae* began to be included in breeding programs in the 1980's; genes *Rps*1a and *Rps*1c were incorporated in 1984 and *Rps*1k in 1991 (Rossi and Nari 1995). However, it is not still possible to establish a direct relationship between the genes released and the distribution of their effectiveness as it occurred in the USA. Recently, Stewart et al. (2014) reported that changes in pathotype structure were not necessarily a function of the type of resistance being deployed. *P. sojae* has the potential to shift pathotypes or to evolve new genotypes very quickly; however, it has not been possible to demonstrate that the shift is a function of the *Rps* gene used. Therefore, the overuse of an *Rps* gene does not necessarily appear to be the primary reason for changes in pathotype structure of *P. sojae*.

Genetic intraspecific variability among *P. sojae* isolates from the same geographic origin was also observed in Argentina (Gally et al. 2007) and in the USA (by Dorrance et al. (2003) in Ohio, by Jackson et al. (2004) in Arkansas, by Malvick and Grunden (2004) in Illinois and by Nelson et al. (2008) in North Dakota). As reported in Brazil by Costamilan et al. (2013), the great increase in virulence complexity found in this study is most likely a result of a long period of continuous production of soybean cultivars with *Rps* genes and the extensive adoption of the conservation soil tillage system in both countries. Selection pressure has most likely been induced by cultivars with increasing virulence to a greater number of *Rps* genes, possibly enhanced by a local indigenous wild-type population (Drenth et al. 1996). This conclusion is supported by the fact that the pathogen was found in undisturbed soils, which were never previously cropped (Pilar locality, BA province).

The possibility of stacking resistance genes *Rps*1k with *Rps*3a or *Rps*8 (neither of these two have been used to control this disease in Argentina) would most likely be highly effective as suggested by Kaitany et al. (2001) and Cochran and Abney (1999). Figure 2 may indicate other gene combinations that would be effective against most races. However, in this process, care should be taken to combine these *Rps* genes in cultivars with high levels of partial or non-specific-race resistance to avoid selection pressure that could lead to a change in the virulence composition of the pathogen (Dorrance and St. Martin 2000; Grijalba and Barreto 2002). In recent years, a number of plant pathologists and breeders have expressed preference for the use of partial resistance, described as the ability of plants to survive pathogen infection without displaying severe disease symptoms or yield loss (Tooley and Grau 1984; Burnham et al. 2003; Dorrance et al. 2003; Zhang et al. 2010). The data resulting from this research are essential for breeding programs, leading to a selection of effective *Rps* gene programs for cultivar development, which, combined with high levels of partial resistance in conjunction with other control measures, should provide improved protection from PRR.

Acknowledgements

The authors would like to thank Dr. Terry Anderson (former researcher Harrow, Ontario- Canada) and Ing. Agr. Dora Barreto (former researcher IMYZA-INTA-Argentina). Both of them are retired at present, but this research would have been impossible without their great knowledge and hard work during the six years in which the research was carried out. We also appreciate the technical assistance of Juan Carlos Torres (IMYZAINTA) and María Flor Feuermann.

References

Anderson TR, Buzzell RI. (1992) Inheritance and linkage of the *Rps*7 gene for resistance to *Phytophthora* rot of soybean. Plant Dis 76:958–959.

Anderson TR, Tenuta A. (2003) Phytophthora rot. In: Bailey KL, Gossen BD, Gugel RK, Morrall RAA (eds) Diseases of Field Crops in Canada. Saskatchewan, Saskatoon, Canada, CPS Press.

Barreto D, Stegman B, Fortugno C. (1991) *Phytophthora megasperma* f. sp. *glycinea*: detección y virulencia del patógeno. Actas Primera Reunión Nacional de Oleaginosas, 10-11 Octubre 1991. Rosario, Argentina, pp 304–311.

Barreto D, Stegman B, Fortugno C. (1995) Races of *Phytophthora sojae* in Argentina and reaction of soybean cultivars. Plant Dis 79:599–600.

Barreto D, Anderson T, Gally M, Grijalba P. (1998a) Evaluation of *Phytophthora sojae* from Argentine soybean fields (Abstr.). In: BSPP. (ed) Proc. 7th International Congress of Plant Pathology, 9-16 Agosto 1998. Edimburgo, Escocia 1: 6.72.

Barreto D, Grijalba P, Gally M, Vallone S, Ploper D. (1998b) Prevalencia de *Phytophthora sojae* en la Región Pampeana Norte de Argentina, caracterización de razas y reacción de cultivares. Fitopatol Bras 23:54–57.

Burnham KD, Dorrance AE, Francis DM, Fioritto RJ, St. Martin SK. (2003) *Rps* 8, a new locus in soybean for resistance to *Phytophthora sojae*. Crop Sci 43:101–105.

Cochran AJ, Abney TS. (1999) *Rps* gene combinations needed to control diverse pathotypes of *Phytophthora sojae*. Phytopathology 89:S104.

Costamilan LM, Clebsch CC, Soares RM, Seixas CDS, Godoy CV, Dorrance AE. (2013) Diversity of *Phytophthora sojae* pathotypes from Brazil. Eur J Plant Pathol 135:845–853.

Dorrance AE, St. Martin S. (2000) *Phytophthora sojae*: Is it time for a new approach? APSnet Features. Internet Resource: http://www.apsnet.org/publications/ apsnetfeatures/Pages/PhytophthoraSojae.aspx (verified Jul 17, 2012).

Dorrance AE, McClure SA, de Silva A. (2003) Pathogenic diversity of *Phytophthora sojae* in Ohio soybean fields. Plant Dis 87:139–146.

Dorrance AE, Jia H, Abney TS. (2004) Evaluation of soybean differentials for their interaction with *Phytophthora sojae*. Internet Resource: http://www.plantmanagementnetwork.org/pub/php/research/2004/psojae/ (verified Oct 20, 2014).

Drenth A, Whisson SC, Maclean DJ, Irwin JAG, Obst NR, Ryley MJ. (1996) The evolution of races of *Phytophthora sojae* in Australia. Phytopathology 86:163–169.

Fehr WR, Caviness CE. (1977) Stages of soybean development. Ames, IA, USA, Spec. Rep.80. Iowa Agric. Home Econ. Exp. Stn. Iowa State Univ.

Gally M, Grijalba P, Barreto D. (1999) Investigación sobre *Phytophthora sojae* (campaña 1997/98): Relevamiento, aislamiento y prueba de material genético. Actas X Jornadas Fitosanitarias Argentinas, 1-9 abril de 1999. San Salvador de Jujuy, Argentina, pg 48.

Gally M, Ramos AM, Dokmetzian D, Lopez SE. (2007) Genetic variability of *Phytophthora sojae* isolates from Argentina. Mycologia 99:813–819.

Gordon SG, St. Martin SK, Dorrance AE. (2006) *Rps*8 maps to a resistance gene rich region on soybean molecular linkage group F. Crop Sci 46:168–173.

Grijalba P, Barreto D. (2002) Comparison Between Methods to Evaluate Reaction of Soybean Cultivars To Phytophthora Root Rot. Actas II Congreso Brasilero de soja-

Mercosoja, 03-06 junio 2002. Foz do Iguazu, Brasil, p. 79.

Henry RN, Kirkpatrick TL. (1995) Two new races of *Phytophthora sojae*, causal agent of Phytophthora root and stem rot of soybean, identified from Arkansas soybean fields. Plant Dis 79:1074.

Hildebrand AA. (1959) A root and stalk rot of soybean caused by *Phytophthora megasperma* Drechsler var. *sojae* var. nov. Can J Bot 37:927–957.

Jackson TA, Kirkpatrick TL, Rupe JC. (2004) Races of *Phytophthora sojae* in Arkansas soybean fields and their effects on commonly grown soybean cultivars. Plant Dis 88:345–351.

Kaitany RC, Hart LP, Safir GR. (2001) Virulence composition of *Phytophthora sojae* in Michigan. Plant Dis 85:1103–1106.

Leitz RA, Hartman GL, Pederson WL, Nickell CD. (2000) Races of *Phytophthora sojae* in Illinois. Plant Dis 84:487.

Malvick DK, Grunden E. (2004) Traits of soybean-infecting *Phytophthora* populations from Illinois agricultural fields. Plant Dis 88:1139–1145.

Martinez C, Ivancovich A. (1979) Presencia de *Phytophthora megasperma* var. *sojae* en la pampa húmeda. En: Actas VII Reunión Técnica Nacional de Soja, 03-06 junio 1979. Santa Fe, Argentina, p. 3.

McBlain BA, Schmitthenner AF. (1991) Evaluations of recurrent selections for *Phytophthora* tolerance. Ohio State University, Ohio Agric. Res. Dev Ctr. Research Bulletin 1187.

Nelson BD, Mallik I, McEwen D, Christianson T. (2008) Pathotypes, distribution, and metalaxyl sensitivity of *Phytophthora sojae* from North Dakota. Plant Dis 92:1062–1066.

Ohal AF, Schmitthenner AF, Walker AK. (1985) Glyceollin accumulation in soybean lines tolerant to *Phytophthora megasperma* f. sp. *glycinea*. Phytopathology 75:542–546.

Ploper LD. (2004) Economic importance of and control strategies for the major soybean diseases in Argentina. In: Moscardi F. (ed) Proc 7th World Soybean Research Conference, 4th International Soybean Processing and Utilization Conference, III Congresso Mundial de Soja, 29 February–5 March 2004, Foz de Iguazu, Brasil, pp 155–156.

Rossi RL. (2004) Current status of the soybean production and utilization in Argentina. In: Moscardi F. (ed) Proc 7th World Soybean Research Conference IV International Soybean Processing and Utilization Conference. III Congreso Brasileiro de Soja. February 29 to March 5, 2004. Foz Do Iguazu, Brazil, pp 38–49.

Rossi RL, Nari C. (1995) Genes de resistencia a *Phytophthora sojae* en líneas avanzadas y cultivares de soja (glycine max) en la Argentina. Actas Primer Congreso Nacional de Soja-Segunda Reunión Nacional de Oleaginosos. 24–27 de Octubre de 1995. Pegamino, Argentina, pp 65–71.

Ryley MJ, Obst NR, Irwin JAG, Drenth A. (1998) Changes in the racial composition of *Phytophthora sojae* in Australia between 1979 and 1996. Plant Dis 82():1048–1054.

Schmitthenner AF. (1985) Problems and progress in control of Phytophthora root rot of soybean. Plant Dis 69:362–368.

Schmitthenner AF, Hobe M, Bhat RG. (1994) *Phytophthora sojae* races in Ohio over a 10-year interval. Plant Dis 78:269–276.

Slaminko TL, Bowen CR, Hartman GL. (2010) Multi-year evaluation of commercial soybean cultivars for resistance to *Phytophthora sojae*. Plant Dis 94:368–371.

Stewart S, Abeysekara N, Robertson AE. (2014) Pathotype and genetic shifts in a population of *Phytophthora sojae* under soybean cultivar rotation. Plant Dis 98:614–624.

Sugimoto T, Yoshida S, Kaga A et al. (2011) Genetic analysis and identification of DNA markers linked to a novel *Phytophthora sojae* resistance gene in the Japanese cultivar Waseshiroge. Euphytica 182:133–145.

Sun S, Wu XL, Zhao JM, Wang YC, Tang QH, Yu DY, Gai JY, Xing H. (2011) Characterization and mapping of *Rps*Yu25, a novel resistance gene to *Phytophthora sojae*. Plant Breeding 130:139–143.

Tooley PW, Grau CR. (1984) Field characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology 74:1201–1208.

Vicentini R. (1996) La Fitóstora y el cancro del tallo: dos enfermedades destructivas de la soja de aparición reciente en Entre Ríos. Serie Extensión No 12. Estación Experimental Paraná: 41-48.

Workneh F, Tilka GL, Yang XB, Faghihi J, Ferris JM. (1999) Regional assessment of soybean brown stem rot, *Phytophthora sojae*, and *Heterodera glycines* using area-frame sampling: prevalence and effects of tillage. Phytoptathology 89:204–211.

Wrather JA, Anderson TR, Arsyad DM, Tan Y, Ploper LD, Porta-Puiglia A, Ram HH, Yorinori JT. (2001) Soybean disease loss estimates for the top ten soybean-producing countries in 1998. Can J Plant Pathol 23:115–121.

Zhang SZ, Xu PF, Wu JJ, Xue AG, Zhang JX, Li WB, Chen C, Chen WY, Lv HY. (2010) Races of *Phytophthora sojae* and their virulences on soybean cultivars in Heilongjiang, China. Plant Dis 94:87–91.