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Phenotypic Expression of Resistance to *Phytophthora infestans* in Processing Potatoes in Argentina

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Abstract Ten potato cultivars were evaluated in Argentina for foliage and tuber susceptibility to *Phytophthora infestans*, the oomycete pathogen causing late blight. Pathogen isolates collected from two major potato production regions in the country were polymorphic for specific virulence and isozyme banding patterns, although diversity was found among and not within specific locations. Four of the cultivars were found to be highly resistant in foliage, having little disease in the field and greenhouse and race-specific resistance is suspected. One of these is Russet Burbank, which is highly susceptible in the United States. Cultivar Shepody was the most susceptible and was assigned a value of 8, which was used together with area under disease progress curve values to calculate scale values of the other cultivars on a 1 to 9 susceptibility scale (where 9 is highly susceptible). Cultivar Ranger Russet had values of about 2.5 and resistance could be race-non-specific. The other cultivars for which race incompatibility did not appear to be a problem had susceptibility values between 4 and 8. Foliage susceptibility values for greenhouse and field were correlated. Foliage and tuber susceptibility were also correlated, although with exceptions and only one cultivar, Innovator, was highly resistant in tubers. The two isolates chosen for assessment of foliage susceptibility in the greenhouse differed for aggressiveness in separate tests where components were measured; however, no cultivar-by-isolate interaction was measured. Cultivars with high levels of putative race-specific foliage resistance should be used with constant monitoring in Argentina.

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Resumen Se evaluaron diez cultivares de papa en Argentina para susceptibilidad de follaje y tubérculo a *Phytophthora infestans*, el oomiceto patógeno causante del tizón tardío. Aislamientos del patógeno colectados de dos regiones importantes productoras de papa en el país fueron polimórficos para virulencia específica y para patrones de bandas de isoenzimas, aunque la diversidad que se encontró fue entre y no dentro de localidades específicas. Se encontró que cuatro de los cultivares fueron altamente resistentes en follaje, con poca enfermedad en el campo e invernadero, sospechándose de resistencia específica a razas. Una de ellas es Russet Burbank, que es altamente susceptible en los Estados Unidos. El cultivar Shepody fue el mas susceptible, con un valor de 8, que se usó junto con los valores del área bajo la curva de progreso de la enfermedad para calcular valores de escala de los otros cultivares en una escala de susceptibilidad de 1 a 9 (donde 9 es altamente susceptible). El cultivar Ranger Russet tuvo valores cercanos a 2.5 y la resistencia pudo ser no específica para razas. Los otros cultivares, para los cuales la incompatibilidad de las razas no parecía ser problema, tuvieron valores de susceptibilidad entre 4 y 8. Se correlacionaron los valores de susceptibilidad del follaje para el invernadero y el campo. Así también se correlacionó la susceptibilidad de follaje y tubérculo, aunque con excepciones y solo un cultivar, Innovator, fue altamente resistente

en tubérculos. Los dos aislamientos escogidos para análisis de susceptibilidad del follaje en el invernadero difirieron en agresividad en pruebas separadas en las que se midieron los componentes; no obstante, no se midió la interacción cultivar-aislamiento. Los cultivares con altos niveles de supuesta resistencia del follaje específica a razas deberían de usarse con monitoreo constante en Argentina.

Keywords Aggressiveness · Host resistance · Late blight · Potato · *Solanum tuberosum* · *Phytophthora infestans*

Introduction

Potato (*Solanum tuberosum* L.) late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is a major constraint worldwide for the production of high quality seed, ware and processing potatoes. Once initiated, the disease can affect leaves, stems and tubers. Under conditions highly favourable for the pathogen, this disease can rapidly defoliate the crop and can also affect potato tubers when spores are washed into the soil by rainfall or irrigation. Both foliar and tuber infections can be controlled by applications of contact and systemic fungicides (Fry 1975; Kapsa 2002; Mantecon 1998) and/or the use of resistant cultivars (Huarte 2002; Micheletto et al. 1999; Micheletto et al. 2000).

In general, and particularly in years with heavy rainfall, potato crops in the two most important potato production areas of Argentina, the province of Cordoba and the south eastern part of the province of Buenos Aires (Caldiz 2006), suffer significant losses due to late blight (Carmona et al. 2003). Moreover, in Argentina more than 80% of the production area is represented by the varieties Spunta and Kennebec (Mantecón 1998), both of which are susceptible to late blight. This means that most potato agroecosystems in Argentina are dominated by large areas of genetically homogeneous, susceptible crop (Caldiz 2006). Losses occur due to both foliage and tuber blight (Mantecon 1998).

Unlike many other parts of South America (Fry et al. 2009), potato late blight in Argentina is associated with diversity in the pathogen population. Both A1 and A2 mating types have been recorded but one study found that 89% of the population was A2 (Van Damme and Ridao 1994). Greater aggressiveness and increased resistance to metalaxyl have been associated with the A2 mating type in Argentina (Van Damme et al. 1998). The association of only one mating type with these phenotypic characteristics would appear to indicate that there is little sexual recombination occurring. Otherwise, one would expect to find segregation for combinations of these characteristics and the two mating types among isolates. Of 48 isolates collected in the late 1990s, 85 % were virulent on 3 or 4 specific virulence differential plants (Van Damme et al. 1998)

Division Agronomia McCain and the Universidad Nacional de Mar del Plata (UNMdP) have developed late blight management strategies for Argentina based on cultural controls and timely application of available fungicides (Andreu and Caldiz 2006). These strategies could be further refined to include host resistance, but levels and stability of resistance must be well documented for strategies to be developed for candidate cultivars. Knowledge of resistance to both tuber and foliage blight is needed as these traits are not necessarily correlated (Platt and Tai 1998).

One potential concern associated with the evaluation of susceptibility in potato to *P. infestans* is the potential for a host-by-location interaction that may be due to several factors, including poor host adaptation in some locations (Forbes et al. 2005) and race-specific interactions (Andrivon et al. 2006). Race-specific interactions have also been detected for putative quantitatively inherited resistance (Flier et al. 2003). This has consequences for evaluating resistance, particularly in the laboratory or greenhouse, and the use of more than one pathogen genotype has been recommended (Flier et al. 2003).

In the past decade, the potato industry in Argentina has invested heavily in the development of existing and introduction of new cultivars that are suitable for processing purposes (Caldiz 2006). However, little is known of the expression of resistance to *P. infestans* of these materials in different locations or against different subpopulations of the pathogen that may exist in Argentina. Hence, the objective of this study was to quantify resistance to both foliage blight and tuber blight in new processing cultivars and test for potential host by pathogen interactions in the field and greenhouse. Field data were converted to scale values with simple scaling of the area under the disease progress curve (AUDPC) following the general approach of Hansen et al. (2005) with modifications proposed by Yuen and Forbes (2009).

Materials and Methods

Cultivars

Most potato cultivars evaluated in this study were developed or are currently used for processing, including established cultivars (Kennebec, Innovator, Santana, Shepody, Russet Burbank and Ranger Russet) and some recently introduced by the Argentinean processing industry (Bannock Russet, Gem Russet and Umatilla Russet). The cultivar Spunta, which is widely grown in Argentina for fresh consumption, was also included.

Isolates of *Phytophthora infestans*

Isolates of *P. infestans* were collected between 2000 and 2003 by field workers of McCain Argentina Division Agronomy from naturally infected potato plants in the two primary production areas; one area is located in the province of Buenos Aires (south-eastern part) and the second in the province of Cordoba. Twenty isolates were collected in the province of Buenos Aries (Table 1) from Bahia Blanca (n=2), Balcarce (n=9), Miramar (n=2), Tandil (n=2), Tres Arroyos (n=2) and Otamendi (n=3). Ten isolates were collected in the province of Córdoba (Table 1) from Córdoba (n=2), Villa Maria (n=2) and Villa Dolores (n=6).

Leaflets with single lesions were collected and brought directly to the laboratory for isolation. Pieces of infected tissue surrounding the lesion were placed on tuber slices of cv. Bintje and incubated in closed plastic boxes in darkness at 18ºC and 90 % relative humidity (RH) for 5 days or until new sporulation appeared. Small tufts of mycelium containing sporangia were transferred to fresh tuber slices of cv. Bintje on a regular bases (approximately weekly) to maintain a source of inoculum for foliage and tuber resistance trials in the greenhouse. To produce inoculum, sporangia were harvested in sterile water from the mycelium growing on the tuber slices. Zoospore release was stimulated by exposing the sporangial suspension to 4ºC for 6 h. After filtration through a 15 µ nylon filter cloth, suspensions of zoospores were quantified with a haemocytometer and adjusted via dilution.

Small tufts of mycelium were also transferred to Rye B agar (Caten and Jinks 1968) to create axenic cultures of each isolate. These were used for the characterization of isolates described below (isozymes, MT and specific virulence). Isolates were maintained at ambient temperature (20±1) on this medium and transferred monthly. Inoculum for the aggressiveness tests was also taken from Rye B agar. Inoculum preparation from Rye B agar was similar to that for tuber slices described above.

Characterization of Isolates

All isolates were characterized phenotypically for mating type, specific virulence on R gene differential plants and banding patterns of the isoenzymes glucose-phosphate-isomerase (Gpi) and peptidase (Pep). Mating types of the isolates were determined by pairing with A1 and A2 tester strains 2015 and 3016 (Van Damme and Ridao 1994) on 14 mm diameter leaf disks of cultivar Bintje, placed in 9 cm Petri dishes on 2% water agar. The tester strains were provided by the Instituto Nacional de Tecnología Agropecuaria (INTA, Balcarce, Argentina). Leaf disks were inoculated with the isolate to be tested and one of the tester strains by placing a small mycelial plug of each isolate 7 mm apart on the leaf disk in 10-µl droplet of tap water. Leaf disks were incubated at 15°C with 16 h of daylight for two weeks. After this period, leaf tissue was cleared in 70% ethanol at 50°C and microscopically examined for the presence of oospores.

The specific virulence pattern of each isolate was determined on a set of R-gene differential plants provided

Table 1 Location, marker information and frequency of isolates collected in the provinces of Cordoba and Buenos Aries in Argentina between 2000 and 2003

Province	Location[a]	Pathotype[b]	*Gpi*[c]	*Pep*[d]	Frequency	Phenotype ID
Buenos Aires	Bahía Blanca	2,3,4,6	86/100	100/100	2	B80
Buenos Aires	Balcarce	2,5,6,8,9	90/100	100/100	9	B26
Buenos Aires	Miramar	7,8,9	86/122	96/100	2	B76
Buenos Aires	Tandil (2) Tres Arroyos (2)	5,8,9	83/100	100/100	4	B70
Buenos Aires	Otamendi	2,5,6,8	86/86	100/100	3	B28
Córdoba	Córdoba (2) Villa Maria (2)	2,3,6,7,9	86/100	92/100	4	C50
Córdoba	Villa Dolores	2,3,6,7,8,9	100/100	100/100	6	C35
Total					30	

All isolates were A2 mating type, which was assessed after pairing of the isolates with tester strains (A_1) and (A_2) on leaf disks of cultivar Bintje

[a] Location within the province where the isolates were collected. *Numbers in parentheses* represent the frequency when a phenotype was found in more than one location

[b] Pathotypes determined with R-gene differential plants containing genes R_1 to R_{11}

[c] *Gpi*=Glucose-6-phosphate isomerase

[d] *Pep*=Peptidase

by the International Potato Center (Lima, Peru). The set contains 11 identified R genes from *Solanum demissum* either individually or in different combinations (Black et al. 1953; Toxopeus 1961). After multiplication and cultivation for 25 days in vitro, plantlets of the differentials were transplanted in 1 L plastic pots containing substrate composed of vermiculite (5% by volume), worm humus (20%), peat (40%) and organic matter (35%). The plants were grown in a growth chamber at 25ºC with a photoperiod of 16 hours produced by Philips Fluorescent TL70 and Philips Tungsten 60 W bulbs, at a radiation level of 60 Wm^{-2}. For race determination of isolates, detached leaflets of each of the differential plants were placed abaxial side up on 2% water agar in 20 cm diameter plastic Petri dishes. The leaflets were inoculated with a *P. infestans* sporangial suspension (7.0×10^3 sporangia mL^{-1}) with an atomizer. Immediately after inoculation, the leaflets were incubated in a growth chamber at 15ºC in darkness for 24 hours. After this period, incubation continued in a growth chamber at 15ºC and 16 hours light period by fluorescent tubes (type 33, Philips) at a radiation level of 12 Wm^{-2}. Lesions were assessed at 4, 5 and 6 days after inoculation. The disease reaction was considered compatible when sporulation was clearly visible on an R-gene differential leaflet.

Isoenzyme banding patterns for Gpi and Pep were determined for all isolates as described by Goodwin et al. (1995a) and Daayf and Platt (2000). Following separation of the sample by electrophoresis and activity staining, allozyme alleles were designated by the numbers representing their percent mobility relative to previously established standards (Tooley et al. 1985). When the banding pattern was atypical (difference in bands intensity or number), the assay was repeated at least once.

Based on frequency of phenotypes (Table 1), one isolate from each location was chosen: one B26 phenotype from Buenos Aires and one C35 phenotype from Cordoba. These two phenotypes were the most frequently encountered in their respective areas. Prior to use in the foliage and tuber resistance assays, the two isolates were compared for aggressiveness parameters on tubers and foliage of cultivar Bintje.

Aggressiveness of Isolates on Tubers and Foliage

Tubers of plants grown in the greenhouse as previously described were used for this assay. The assay was done in three replicates; each replicate (e.g., one experimental unit) consisted of 80 undamaged tubers. Tubers were placed in plastic crates and inoculated with a *P. infestans* sporangial suspension (2.0×10^4 sporangia ml) with an atomizer until runoff. Inoculated tubers were kept moist by enclosing the crates in plastic bags, which were then maintained at 15°C and 80% relative humidity. After 14 days, infection frequency (IF) was measured as the percentage of tubers per crate with symptoms of blight. In addition, 10 randomly selected diseased tubers per crate were cut longitudinally and disease development was scored according to a 1 to 5 disease severity scale, from which a severity index (SI) was calculated (Flier et al. 2001). IF data were transformed using Gregory's multiple infection transformation (Gregory 1948). The transformed data can be interpreted as a relative measurement of infection efficiency (IE). Finally, a tuber blight index (TBI) was calculated as $TBI=-Log_e$ (IE x SI)

Leaf tissues of greenhouse grown plants (grown as described above) were used for this assay. The assay was done in three replicates; each replicate (experimental unit) consisted of 50 14-mm leaf disks. Thus, a total of 150 leaf disks per isolate were inoculated. A single leaf disk was placed abaxial side up in a flat-bottom well of a 24-well tissue culture plate with cover (Linko cat. 76-033-05, Flow laboratories, USA). Prior to adding the disks, one ml of 2 % water agar was added to each well such that each disk was lying on agar. Inoculation was done by placing a 10-µl droplet of sporangial suspension (2×10^3 sporangia ml^{-1}) in the centre of each disk. Plates with the inoculated leaf disks were placed in plastic trays, which were then enclosed in a transparent plastic (polythene) bag to avoid desiccation. Trays were placed in the dark in a growth chamber at 15°C. After 24 h, droplets were removed with the help of filter paper and incubation was continued at 18°C and 16 h photoperiod for 8 days. Readings started 72 h after inoculation and were repeated at intervals of approximately 12 h.

Latent period (LP10) was calculated as the period in hours after inoculation until 10% of the disks had sporulation. The cumulative percentage of infected disks was plotted on time. The maximal curve growth rate (MGR) was calculated as the first derivative of the fitted curve at the point of inflexion. This parameter indicates the time needed to reach maximum percent infection. Foliar infection efficiency (FIE) was based on the percentage of infected leaf disks after 8 days. Sporulation intensity (SPOR) was calculated as the natural logarithm of the average number of sporangia per cm^2 infected leaf tissue, based on 30 affected leaf disks per isolate. Sporulation for each replicate was measured in three sub samples of 0.5 ml using a Coulter counter. A composite aggressiveness index (AI) was calculated for each isolate using the formula $AI=1/LP10\times MGR\times FIE\times SPOR\times10^4$ (Flier et al. 2001).

Host Resistance in Foliage—Field Assays

Two experiments were conducted near Balcarce, the area from which phenotype B26 was collected (Table 1), during the summer growing seasons (mid-October to late February) of

2004/05 and 2005/06. The trials were done at the McCain Argentina Experimental Field and irrigated with a forward advance irrigation system. The plots consisted of 4 rows (0.85 m between rows), each 6 m long (20 m^2), and there were 3 replications in a randomized block design. In both seasons plant density was 5.8 plants m^2, similar to commercial crops grown in the area. Disease occurred from natural infection and there was a favourable climate for late blight. Disease severity, estimated visually as percent foliage affected, was evaluated every 4 days for approximately 40 days after appearance of first symptoms.

Susceptibility to *P. infestans* in foliage was quantified by first calculating the AUDPC using the midpoint method (Campbell and Madden 1990) and converting this to the RAUDPC (Fry, 1978). Susceptibility scale values were calculated from RAUDPC values as described by Yuen and Forbes (2009). To calculate scale values cultivar Shepody (susceptible), was assigned a value of 8 based on previous knowledge of its susceptibility level. Scale values were estimated using the one-standard model in which no susceptibility (or complete resistance) is represented by absence of disease symptoms (Yuen and Forbes, 2009).

Host Resistance in Foliage—Greenhouse Assays

Seed tubers of the candidate cultivars were planted at 10 cm depth in 7 L plastic pots containing substrate (described above). During plant growth, the temperature ranged between 20–24ºC and 500 W by high-pressure sodium lamps were used to provide a 14–10 hday-night cycle. Three experiments were done during 2003 and 2004, with each experiment consisting of three repetitions of 50 plants each (5 plants per cultivar). Plants at 7–11 weeks after planting, near the time of flowering, were inoculated until run-off with a *P. infestans* sporangial suspension (2.0×10^3 sporangia ml) with an atomizer. Afterwards, each inoculated plant was transferred for 24 h to a dark chamber at 16ºC and 90 % relative humidity. The plants were then placed in a greenhouse at 18–20ºC and 80% relative humidity for the duration of the experiment. Disease severity was estimated visually as the percentage foliage showing symptoms. Disease severity was recorded daily from 4 to 11 days after inoculation and the daily severity readings were integrated into the AUDPC. This was then converted to the RAUDPC (Fry 1978) and subsequently to scale values based on an arbitrary value of 8 for Shepody as described above.

Host Resistance in Tubers—Whole Tuber Assay

In preparation for this assay, field- harvested tubers were stored at 10ºC and 90% relative humidity for three months. At the time of evaluation, they were washed in distilled water and disinfested by immersion in 5% sodium hypochlorite for 5 min, then dried. For the resistance assessment, 30 undamaged tubers from each cultivar were inoculated by spraying them with 50 ml of a sporangial suspension (2.0×10^3 sporangia ml^{-1}). Inoculated tubers were kept moist by enclosing the crates in plastic bags, which were then maintained at 15°C and 80% relative humidity. After 20 days of incubation, the percentage of infected tubers was recorded, based on visual examination of symptoms. The cortical resistance of tubers was evaluated using an invasive ability index (IAI) as described Flier et al. (2001). Ten randomly selected diseased tubers per cultivar were cut longitudinally and the IAI was assessed for each individual tuber using the following scale: 0=no symptoms, 1=<2,5% of cut area with symptoms, 2=2,5–10% cut area with symptoms, 3=10–25% cut area with symptoms, 4=25–50% cut area with symptoms and 5=>50% cut area with symptoms. All experiments were replicated three times and results represent the average of the three experiments.

Host Resistance in Tubers—Tuber Slice Assay

A tuber slice assay adopted from Bathia and Young (1985) and Dorrance and Inglis (1998) was used. Undamaged tubers of similar size were taken from 10ºC storage eight weeks after harvest, surface disinfested for 5 min as described above, rinsed in tap water and wiped dry with tissue paper. Two, one-cm thick slices were cut from the central part of a single tuber and placed in a Petri dish, ten slices per cultivar. A single 10-µl droplet of inoculum was applied in the middle on the upper side of each individual slice. The inoculated tuber slices were incubated for 8 days in closed Petri dishes containing humid filter paper, which were maintained in dark growth chamber at 15ºC. The percentage of necrotic tissue on the tuber slice surface was evaluated as an indicator for the area of invaded tissue. In addition, the mycelia coverage index (MCI) was estimated using a linear scale of 0=no mycelium to 9=completely covered with mycelium. Treatments were replicated 3 times and results represent the average of the three experiments.

Data Analysis

To evaluate the possible interaction of host phenotype with either field environment (between the two trials) or host phenotype (in the greenhouse trials), analysis of variance (AOV) was done in which an interaction term was tested. The data for the greenhouse assessment of susceptibility were analyzed in an AOV for which the following linear model was fit to the data: $x_{ij} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \chi_k + \varepsilon_{ij}$, where x_{ij} is the RAUDPC for the ijth cultivar, τ_i is the effect of the ith experiment (i=1, 2) β_j is the effect of the jth pathogen isolate (j=1, 2), $\tau\beta_{ij}$ is the interaction of

Table 2 Aggressiveness on potato tubers and on potato foliage of two isolates of *P. infestans* used to evaluate susceptibility in potato cultivars in Argentina

Isolate[Γ]	Tuber aggressiveness[Δ]				Foliage aggressiveness [E]				
	IF	SI	IE	TBI	LP10	MGR	FIE	SPOR	AI
C35	90[a]	4.0[a]	1.5[a]	1.8[a]	80[a]	31.4[a]	0.99[a]	5.38[a]	21[a]
B26	40[b]	1.25[b]	0.5[b]	0.5[b]	120[b]	45.5[b]	9.74[b]	4.89[a]	14[b]

Columns with the same letter are not statistically different at 5% according to Tukey's test

[Γ] Isolates originated from two potato growing regions in Argentina. See Table 1 for more information on isolates

[Δ] Tuber aggressiveness components: IF=infection frequency; SI=severity index; IE=infection efficiency; and TBI=tuber blight index. See Materials and Methods for more information

[E] Foliage aggressiveness components LP10=latent period, MGR=maximal growth rate, FIE=foliar infection efficiency; SPOR=sporulation intensity and AI=aggressiveness index. See Materials and Methods for more information

cultivar and experiment, χ is the effect of the kth block and ε_{ij} is the residual error. For our analysis, the three samples within each experiment were considered sub-samples and analysis of variance was run on the means of the three samples, with the blocks being represented by experiments.

A similar model was used for the field data except that here the error variance was estimated with the replications in the field design and there was no term for isolate. The primary objective of the field data AOV was to test the experiment-by-cultivar interaction. To evaluate the potential for race-specific resistance based on phenotypic data, we used the method of Andrivon et al. (2006). The apparent infection rate (r) was calculated using a logit transformation of percent infection. This was then used to calculate the parameters Δa, and Δt, as follows: $\Delta a = a_i - a_s$, where a_1 and a_s are the slopes of the transformed disease progress curves for the genotype of interest and the standard susceptible genotype (Shepody), respectively; and $\Delta t = t_{0i} - t_{0s}$, where t_{0i} and t_{0s} are the dates of appearance of the first visible symptoms on the genotype of interest and the standard susceptible genotype (Shepody), respectively.

Race specific resistance is then suspected when both Δa, and Δt are greater than 0, i.e., when disease initiates in the genotype of interest after it does in the susceptible control, but the disease progress rate is higher in the former, once disease does start.

Table 3 Foliage susceptibility to *Phytophthora infestans* and disease progress parameters in 10 Argentinean potato cultivars measured in two greenhouse trials and two field trials

Cultivars	Greenhouse Scale[a]		Field Scale[b]		Disease initiation Δa[c]		Disease rate Δt[d]		R Type[e]
	B26	C35	A	B	A	B	A	B	C
Shepody	8.00	8.00	8.03	7.97	0	0	0	0	H
Kennebec	4.55	4.99	6.14	6.08	8	8	−.53	−.14	H
Gem Russet	3.87	4.04	6.24	6.69	0	0	−.42	−.12	H
Spunta	6.24	7.12	5.28	6.07	8	8	−.48	−.11	H
Bannock Russet	3.49	4.38	4.66	5.51	8	8	−.48	−.12	H
Ranger Russet	1.12	1.19	2.54	2.47	12	12	−.56	−.19	V?
Russet Burbank	2.23	3.19	0.46	0.59	16	16	−.65	−.3	V
Santana	1.11	1.53	0.36	0.34	12	12	−.67	−.29	V
Umatilla Russet	0.59	0.80	0.14	0.07	20	32	−.69	−.34	V
Innovator	0.01	0.00	0.00	0.00	40	40	−.69	−.34	V
LSD[h]	1.80	0.46	0.40	0.38					

[a] Foliage susceptibility in two greenhouse trials with artificial inoculation using two isolates, B26 and C35 (see Table 1). See Materials and Methods for more information on calculation of scale values

[b] Susceptibility in two field trials done in Balcarce Argentina in 2004/05 (A) and 2005/06 (B) with natural infection

[c] Delta *a* represents the difference in days between disease initiation in that cultivar and disease initiation in the susceptible control, Shepody

[d] Delta *t* represents the difference between the disease rate of the cultivar being evaluated and the rate of the susceptible cultivar Shepody

[e] The resistance type was estimated based on the ensemble of disease progress measures, where H=horizontal, V=vertical, ?=unknown and V?=suspected vertical (Andrivon et al. 2006); C refers to data from both field trials, 2004/05 and 2005/06

Results

Characteristics of Isolates

Seven phenotypes were identified among the thirty isolates evaluated (Table 1). There was very strong evidence of geographic substructuring as most localities had their own unique phenotype; only in two cases was the same phenotype found in two localities. All isolates were A2 mating type.

The two isolates used to evaluate susceptibility in potato cultivars differed in their patterns of specific virulence. Isolate C35 (race 2,3,6,7,8,9) was virulent on differentials carrying six R genes, while isolate B26 (race 2,5,6,8,9) was virulent on differentials carrying five. Isolate C35 was more aggressive than B26 for all parameters measured both on tubers and foliage (Table 2) and in some cases differences were extreme. For example, infection efficiency (IF) on tubers was 90% with C35 but only 40% with B26. Similarly, LP10, a measure of latent period on foliage, was only 80 h for C35 but 120 h for B26. The aggressiveness index of C35 was 21, while it was only 14 for B26.

Foliage Resistance of Cultivars Measured in Greenhouse and Field

There were clear differences in the levels of susceptibility of the ten cultivars both in the greenhouse and field (Table 3). In both types of assay, Shepody was the most susceptible cultivar. Based on the field scale values, it was fairly easy to separate cultivars into groups. Shepody, given the arbitrary value of 8, was followed by four cultivars with scale values between 5 and 6: Kennebec, Gem Russet, Umatilla Russet and Bannock Russet. Ranger Russet had a value of about 2.5 and as such was relatively less susceptible than the others and could possibly have race-non-specific resistance. All the other cultivars had values below 1 and possibly were incompatible with the pathogen population. This hypotheses is also supported by the disease initiation and disease rate parameters (Table 3).

Cultivar scale values were similar for the two field trials (Fig. 1a) but, nonetheless, the experiment-by-cultivar interaction was significant in the AOV ($P<0.0015$). This interaction represented minor deviations from a linear correlation between the two field trials (e.g., see cultivars Kennebec and Spunta, Fig. 1a) and did not result in a change of rankings between the two years. Susceptibility was also consistently measured in the two greenhouse trials, each done with a different isolate. The pathogen-by-cultivar interaction was not significant in the analysis of variance for the greenhouse. Overall, the greenhouse trials gave results similar to those in the field in that the scale value for most cultivars was the same ($r=0.99$); however, there were

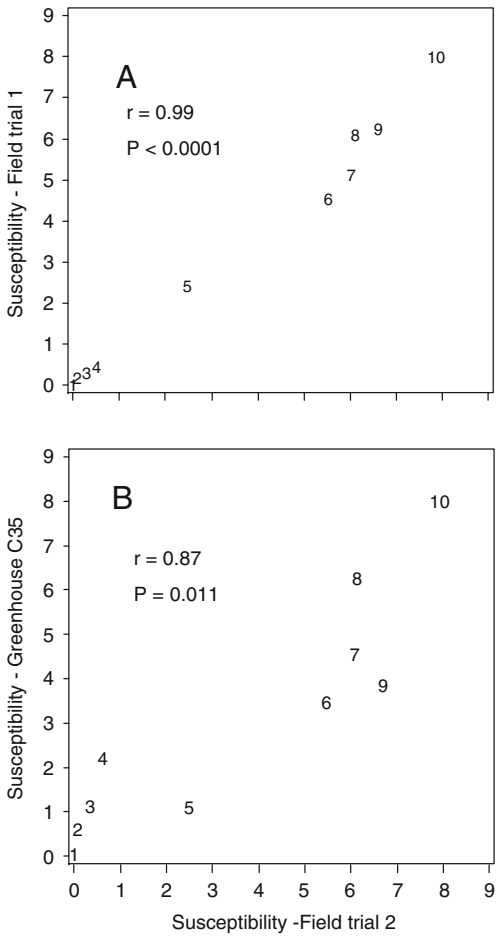

Fig. 1 Scatter plot of levels of susceptibility of ten potato cultivars to *Phytophthora infestans,* evaluated in Argentina in the field and in the greenhouse. Cultivars are compared for two field trials conducted in separate years (**A**) and for one greenhouse trial and one field trial (**B**). Cultivar code: 1=Innovator; 2=Umatilla Russet; 3=Santana; 4 Russet Burbank; 5=Ranger Russet; 6=Bannock Russet; 7=Spunta; 8=Kennebec; 9=Gem Russet; and 10=Shepody

exceptions (Fig. 1b). For example, Russet Burbank was found to have little disease in both field trials, having a susceptibility scale value below 1 in both experiments, but had scale values between 2 and 3 in the greenhouse experiments with two isolates (Table 3 and Fig. 1b). In contrast, Ranger Russet had scores of about 2.5 in the field and only slightly over 1 in the greenhouse. Kennebec was also more susceptible in the field than in the greenhouse (Table 3). Overall, susceptibility values were higher for isolate C35 than for B26, which was consistent with results from the aggressiveness tests.

Tuber Resistance of Cultivars Measured with Whole Tubers and Tuber Slices

Shepody was also the most susceptible cultivar for tuber blight for all indicators, particularly for percent of tubers

showing symptoms: 99.8 and 85.3 with isolates C35 and B26, respectively (Table 4). The ranking of cultivars was similar with both isolates, although severity was consistently higher with C35, which had been found to be more aggressive in assays (Table 2). No symptoms or signs were detected with either isolate on cultivar Innovator. Results were similar using the tuber slice assay, with the possible exception of cultivar Ranger Russet, which was more susceptible than in the whole tuber assay.

Correlations among variables used to measure susceptibility to tuber infection and symptom expression were generally high and all were significant at $P=0.05$ except one (Table 5). With isolate C35, the invasive ability index (IAI) was more closely correlated with tuber slice variables than was the percentage of tubers infected. With isolate B26 this was not the case as both whole-tuber variables were equally correlated with measurements made on tuber slices.

Tuber blight resistance and foliage resistance were significantly correlated in our study. A correlation ($r=0.61$, Spearman rank correlation) was measured when susceptibility values of the first year trial and colonization with mycelium on tuber slices with isolate B26 were compared, that was significant at $P=0.059$ (Fig. 2). However, as exceptions, the three cultivars, Umatilla Russet, Russet Burbank and Santana, had moderate levels of tuber blight but almost no foliage blight.

Discussion

In this study, we found large differences in the level of susceptibility among the ten cultivars evaluated. The RAUDPC of Shepody was 0.82 in one field study (data not shown), indicating that it reached 100% infection relatively early in the evaluation process. On the other end of the susceptibility spectrum, the cultivar Innovator was completely resistant in all trials.

This study suffers from the same limitations that affect late blight foliage resistance evaluations done anywhere except Europe; there is no widely used system for comparing results from one location to another. We attempt to begin rectifying that problem by employing a method proposed by Hansen et al. (2005) and modified by Yuen and Forbes (2009). In our application of the method, the cultivar (Shepody) was used to anchor the scale to the RAUDPC, by giving Shepody a value of 8.

With only one reference cultivar as we have done here, and by forcing the intercept through zero, the process becomes a simple scaling exercise as the slope parameter becomes the assigned scale value (8 in this case) divided by the measurement value (the RAUDPC of Shepody in each experiment). However, this is not the case if the intercept is not forced through zero, and/or if more than one reference cultivar is used; then a regression line must be estimated (Yuen and Forbes, 2009).

Table 4 Susceptibility of ten Argentinean potato cultivars to tuber infection by two isolates of *Phytophthora infestans* measured in whole tubers and tuber slices

Cultivars	Whole tuber				Tuber slices			
	Isolate C35[h]		Isolate B26		Isolate C35		Isolate B26	
	TB[i]	IAI[k]	TB[i]	IAI[k]	Nec.[m]	CM[p]	Nec. [i]	CM[p]
Shepody	99.80[a]	5.00[a]	85.30[a]	3.80[a]	85.40[a]	9.30[a]	80.50[a]	7.20[a]
Spunta	85.20[ab]	4.00[b]	70.20[b]	2.70[b]	75.80[b]	8.50[a]	50.20[c]	5.30[b]
Bannock Russet	83.30[ab]	4.00[b]	65.30[bc]	2.00[c]	65.80[bc]	8.50[a]	50.50[c]	4.40[c]
Kennebec	80.40[b]	2.00[d]	60.20[c]	1.70[c]	60.30[c]	4.10[d]	4.20[f]	2.50[e]
Gem Russet	70.60[c]	4.20[b]	62.30[c]	3.00[b]	75.40[b]	8.30[a]	60.30[b]	4.20[c]
Santana	70.50[c]	3.20[c]	60.40c	3.00[b]	60.20[c]	7.20[b]	50.20[c]	5.30[b]
Russet Burbank	60.60[d]	3.20[c]	45.20[d]	2.20[c]	68.50[bc]	7.40[b]	30.20[d]	3.40[d]
Umatilla Russet	37.20[e]	1.10[e]	20.10[e]	0.50[d]	5.80[e]	2.20[e]	0.80[f]	1.10[f]
Ranger Russet	35.80[e]	1.20[e]	25.60[e]	0.70[d]	40.20[d]	6.20[c]	20.20[e]	2.20[e]
Innovator	0.00[f]	0.00[f]	0.00[f]	0.00[e]	0.00[f]	0.00[f]	0.00[g]	0.00[g]

All experiments were replicated three times and results represent the average of the three experiments

Columns with the same letter are not statistically different at 5% according to Tukey's test

[h] For information on isolates see Table 1

[i] TB=percentage of tubers showing symptoms of blight

[k] IAI=invasive ability index and is calculated based on a 0–5 scale where (0=no symptoms and 5≥50% cut area with symptoms

[m] Percentage necrotic tissue on the tuber slice surface

[p] Cm=colonization with mycelium and is based on a 0 to 9 scale where 0=no mycelium and 9=completely covered with mycelium

Table 5 Spearman's rank correlation of measure of tuber susceptibility to *Phytophthora infestans* in ten Argentinean potato cultivars measured in two trials, each with a different isolate of the pathogen

	Whole tuber				Tuber slices			
	TB[a] -C35[b]	IAI[c]-C35	TB-B26	IAI-B26	Nec[d]-C35	CM[e]-C35	Nec-B26	CM-B26
TB-C35	1.00							
IAI-C35	0.79	1.00						
TB-B26	0.93	0.91	1.00					
IAI-B26	0.61[f]	0.86	0.78	1.00				
Nec-C35	0.80	0.91	0.85	0.77	1.00			
CM-C35	0.78[f]	0.93	0.91	0.74	0.87	1.00		
Nec-B26	0.67	0.96	0.86	0.86	0.78	0.90	1.00	
CM-B26	0.79	0.84	0.89	0.89	0.74	0.84	0.83	1.00

[a] *TB* Percentage of tubers showing symptoms of blight

[b] Number after dash represents isolate used in trial; for information on isolates see Table 1

[c] *IAI*=invasive ability index and is calculated based on a 0–5 scale where (0=no symptoms and 5≥50% cut area with symptoms

[d] Percent necrotic tissue on the tuber slice surface

[e] Cm=colonization with mycelium and is based on a 0 to 9 scale where 0=no mycelium and 9=complete colonization

[f] This is the only correlation not significant at $P=0.05$

Overall, the method would appear to be highly applicable to the Argentina situation and Shepody, a well known cultivar in the southern cone, is a likely candidate for a susceptible control. Yuen and Forbes (2009) give reasons for selecting a scale in which susceptibility increases with severity, which is the approach also adopted here. Those authors used Bintje as a scale anchor, giving it a value of 8. We have been unable to find a study in which both cultivars Shepody and Bintje were compared in the field for late blight resistance, which would allow us to confirm or potentially adjust the value of 8 given to Shepody. Other studies demonstrate, however, that both Shepody (Jenkins and Jones 2003) and Bintje (Andrivon et al. 2006; Vleeshouwers et al. 2000) are highly susceptible cultivars. The assignation of 8 to each is recognition that they are very susceptible but that something more susceptible may be found. In the trial by Jenkins and Jones (2003) some cultivars had slightly higher AUDPC values than Shepody.

In our study, there was a significant correlation between greenhouse and field evaluations of foliage resistance ($r=87$, $P=0.0011$). The literature on correlation between field and other types of evaluation (whole plant, detached leaf, leaf disk) was reviewed by Vleeshouwers et al. (2000). They found that correlations are common but generally higher using whole plant assays (as we did) than detached leaf assays. They argued that the high humidity of containers generally used with detached leaves tends to reduce the resistance reaction. Using whole plants, Vleeshouwers et al. (2000) had correlation coefficients similar to ours, although they only used two cultivars.

In our correlation analysis of foliage susceptibility in the field with one measure of tuber susceptibility, the coefficient was 0.61 and the level of significance was $P=0.059$. Visual examination of the relationship, however, demonstrated that there was some level of agreement for cultivars with foliage susceptibility scale values between 2 and 8. Thus it would appear that if R genes were active for foliage infection (those with foliage susceptibility values below 1, Figs. 1 and 2), they were not active in tubers. There is little evidence in the literature for correlation between suscepti-

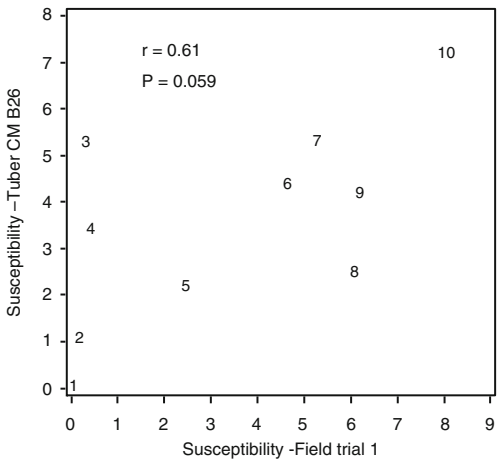

Fig. 2 Scatter plot of resistance scale readings from the field (2004/05, Table 2) and tuber blight incidence readings based on fungal colonization of tuber slices with isolate B26 (Table 4). A good correlation was evident for most cultivars; however three, Umatilla Russet (1), Russet Burbank (2) and Santana (3), had moderate levels of tuber blight but almost no foliage blight

bility in foliage and in tubers (Dorrance and Inglis 1998; Porter et al. 2004). Jenkins and Jones (2003) found significant but weak correlations between susceptibility in foliage and tubers, when both were measured in the field. R-square values (regression analysis) were 0.41 and 0.39 in two yearly trials, which correspond to a correlation coefficient similar to what we found (Fig. 2).

The role of host susceptibility in disease management may be most clearly envisioned in its potential relationship to fungicide dependency. It has been almost three decades since Fry (1978) demonstrated a quantitative relationship between susceptibility and the amount of fungicide needed. It is now fair to question the degree to which resistant cultivars have been used to reduce fungicide application in farmers' fields. Recent work in the US and Europe has continued to demonstrate the capacity for reduced fungicide application when less susceptible cultivars are used (Kirk et al. 2005; Naerstad et al. 2007), but there is little evidence of this occurring in farmers' fields. It would appear that the problem resides in the difficulty in diffusing resistant cultivars, as most production is still dominated by highly susceptible material.

A first step toward using resistant cultivars, however, is accurate characterization of the phenotypic expression and, when possible, genetic composition of resistance. We have provided a characterization of phenotypic expression of resistance in 10 cultivars that can be useful in the improvement of fungicide usage, once resistant cultivars are adopted. We also propose that our use of an interval scale will facilitate comparison among experiments. We did not study the genetic composition of the resistance, but some inferences can be made from the data. It would appear that field observations of foliage resistance were affected by major gene interactions, for which this pathosystem is notorious. Four of the cultivars we tested had RAUDPC values near 0 (not shown) and scale values below 1 (Table 3). One of these, Russet Burbank, is known as susceptible in the US and Europe. The other three, Innovator, Ranger Russet and Santana, had moderate levels of tuber blight. Major genes in foliage are not always expressed in tubers.

The method developed by Andrivon et al. (2006) was used to evaluate the disease progress data for the presence of a race-specific resistance (partial or complete incompatibility). We found the parameters Δa, and Δt to be very informative about the dynamics of disease development but could not strictly use the decision rules provided in the original paper. Those assume very high disease progress rates in cultivars with major genes once a compatible isolate infects. We did not find this as the four cultivars we suspect of having race-specific resistance never developed much disease. The little disease that was observed (except Innovator which remained completely resistant) could have resulted from partial resistance provided by the major gene, or development of disease in older, senescent leaves. Regardless, each parameter provides a new dimension to the phenotypic assessment of resistance, and assisted us in arriving at the general conclusion that the four cultivars mentioned have such extreme levels of resistance that a specific gene-for-gene (effector-R gene) interaction should be suspected. Resistance of this type has typically not been durable in this pathosystem and those cultivars could eventually have significantly higher levels of disease through selection of virulent pathogen genotypes from within the pathogen population. Further testing of these materials is warranted, as well as surveillance should they be adopted for commercial use.

We found differences in pathogen aggressiveness in the two isolates we examined, but there was little evidence of pathogen-by-cultivar interaction. This is in contrast to recent studies in Europe, where host by pathogen interactions were found for cultivars with putative race-non-specific resistance (Flier et al. 2003). This could reflect either the small sample size in our study (only two isolates were compared) or that the pathogen population is less diverse in Argentina than in Europe. Given the small number of markers we used this hypothesis is difficult to test, but the location-driven structure of the isolates we found, and the fact they are all A2 mating type, would seem to suggest that the population in Argentina is primarily clonal. High levels of diversity for specific virulence have been found within clonal populations (Goodwin et al. 1995b).

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