Tropical Plant Pathology, vol. 36, 6, 367-373 (2011)

www.sbfito.com.br

RESEARCH ARTICLE / *ARTIGO*

Foliar virulence of isolates of *Phytophthora infestans* sensu lato on detached leaves of two *Solanum betaceum* cultivars

Eliana Revelo, Gabriela Dorado, Luz Estela Lagos & Oscar Burbano-Figueroa

Universidad de Nariño, Ciudad Universitaria Torobajo, Pasto, Nariño, Colombia

Author for correspondence: Oscar Burbano-Figueroa, e-mail: burbano-figueroa.1@osu.edu

ABSTRACT

Foliar virulence of *Phytophthora infestans* isolates to two *Solanum betaceum* cultivars, Red and Yellow, was characterized using a detached leaf assay. Six *P. infestans* sensu lato isolates from *S. betaceum* belonging to at least two different populations were included (representing the EC-3 clonal lineage and a population that was not previously reported). Three isolates from potato (representing the EC-1 clonal lineage) were included in order to determine their ability to attack *S. betaceum* cultivars and whether there was difference in virulence between these and isolates from *S. betaceum*. Significant variation for virulence parameters was found between isolates, isolate-origins, and cultivars. Infection frequency, sporulation intensity, and incubation period allowed differentiation of EC-1 isolates from *S. betaceum* isolates. Isolates infecting both cultivars exhibited significantly higher values of infection frequency and sporulation intensity and lower latent period values. Cv. Red showed significantly higher values for area under the lesion expansion curve (AULEC), and final lesion size (FLS) and lower values for incubation period (IP) in comparison with cv. Yellow. These results demonstrated that there are two separate populations of *P. infestans* attacking *S. betaceum* each with specific virulence towards two different cultivars of *Solanum betaceum*.
Key words: detached leaves assay, EC-1 clonal lineage, EC-3 clonal lineage, late blight, tree tomato.

RESUMO

Virulência foliar de isolados de *Phytophthora infestans* sensu lato em folhas destacadas de duas cultivares de *Solanum betaceum*

A agressividade dos isolados de *Phytophthora infestans* sensu lato obtidos de duas cultivares de *Solanum betaceum*, Vermelha e Amarela, foi caracterizada em bioensio com folhas destacadas. Seis isolados de *S. betaceum* pertecendo a pelo menos duas populações diferentes foram incluídos (representando a linhagem clone EC-3 e uma população ainda não reportada). Três isolados obtidos de batata também foram incluídos para determinar se estes poderiam atacar cultivares de *S. betaceum* e se haveria diferença na agressividade entre isolados, origem dos isolados e cultivares. Freqüência de infecção, densidade populacional e o período de incubação permitiram a diferenciação do isolado EC-1 a partir dos isolados de *S. betaceum*. Isolados capazes de infectar os dois cultivares exibiram maior frequencia de infecção e densidade populacional e o menor período de latência. Um dos três isolados da população EC-1 não infectou folhas de *S. betaceum* cv. Vermelha, apresentando o maior valor de freqüência de infecção, maior área abaixo da curva de progresso da expansão de lesão (AACPEL) e tamanho final da lesão e o menor período de incubação. Estes resultados demonstraram a existência de duas populações distintas de *P. infestans* atacando *S. betaceum* com agressividades especificas para cada cultivar de *Solanum betaceum.*
Palavras-chave: agressividade foliar, bioensaio com folhas destacadas, linhagem clone EC-1, linhagem clone EC-3, requeima, tamarillo.

INTRODUCTION

Solanum betaceum, or tree tomato, is a fast growing tree originated in South America. Wild populations are known to occur in the Andean mountains of southern Bolivia and northeastern Argentina, the region regarded to be its center of origin (Bohs, 1991). Domestic populations have been introduced in many tropical and subtropical areas around the world. Two centuries ago *S. betaceum* was already grown as a crop in South Africa, India, Hong Kong, China, United States, Australia and New Zealand (Bohs, 1989). It is presently of particular relevance as a crop in the highlands of Southern and Central America and New Zealand. In Colombia, it is mainly cultivated in the Northwest and Southwest (particularly in the Nariño and Putumayo departments) and it became a vital element for the local economy, particularly for small farmers and is one of the most promising products under the classification of non-traditional export products (Duque & Morales 2005; MAG 2005a; MAG 2005b; MAG 2005c).

Two different *Phytophthora* species, *andina/ infestans*, have been reported as coexisting on this host in the *Solanum betaceum* growing area, however the taxonomic status of *P. andina* remains uncertain (Cardenas et al., 2011). The name *Phytophthora. infestans* will be used for the isolates/populations discussed in the present publication in this document in its sensu lato (s.l.) definition. EC-3 populations of *P. infestans* s.l. were proposed as a new candidate specie, *P. andina* (Oliva et al., 2010) and are involved in disease on *Solanum betaceum* crops in Ecuador

(Adler et al., 2004). Members of this clonal lineage belong to the A1 mating type and are characterized by Gpi (86/100) and Pep (76/100) allozyme genotypes, and the Ia mtDNA haplotype. EC-3 populations are closely related with the *Anarrhichomenum* complex and genetically are distinct from *P. infestans* populations from potato and tomato. These populations are regarded as possibly having a palaeoendemic origin and their presence/arrjval in South America is still puzzling (Adler et al., 2004). Alleles and mitochondrial haplotypes of this population had not been previously reported for other worldwide populations, which supports this explanation. EC-1 clonal lineage coexists with EC-3 in the same region and are the dominating populations on crops of *Solanum tuberosum* and *Solanum lycopersicum* (Gomez-Alpizar et al., 2008, Oliva et al., 2010; Cardenas et al., 2011).

Regardless of economic importance, there are no commercially available cultivars of *S. betaceum* with well-recognized pathogen resistance. In the Southwest region of Colombia, two semi-domesticated cultivars or landraces are widely grown. They are easily distinguishable by fruit color and are known as cv. Yellow and cv. Red. Additionally, they can be differentiated based on texture, leaf-shape and organoleptic characteristics of the fruit. Local farmers in the Colombian Southwest attribute different levels of late blight resistance to the Yellow and Red cultivars. However, resistance of these cultivars against *P. infestans* populations has not been measured.

The objective of this study was to test the hypothesis that isolates of *P. infestans s.l.* associated with *S. betaceum* (EC-3, Sb-isolates) differs in terms of virulence from those associated with *S. tuberosum* (EC-1, St-isolates). For this purpose, five-virulence parameters were measured (infection frequency, sporulation, disease intensity, area under lesion expansion curve, final lesion size and incubation period) in a detached leaf assay using two cultivars/landraces of *S. betaceum*. Additionally, putative differential resistance of these cultivars was compared using the virulence parameters calculated during the detached leaf assay.

MATERIALS AND METHODS

Isolates of *P. infestans*

Three EC-1 isolates and five EC-3 from *S. betaceum* were selected from the collection of the Genetica de Patosistemas Group (GENPAT) at the Universidad de Nariño (Table 1). These isolates were collected from potato and tree tomato growing regions in the departments of Nariño and Putumayo during 2007. They were isolated from typical sporulating lesions (Figure 1) using Sb-agar, a medium consisting of: 100 mL/L of tree tomato juice (425 g of fruit to 500 mL), 100 mL/L of pea broth (225 g of boiled pea during 30 min, filtered and dissolved up to 500 mL), agar (18 g/L) supplemented with rifampicin (0.02 g/L), mycostatin (0.05 g/L), ampicillin (0.10 g/L) and benomyl (Benlate 50%) (0.1 g/L). Colonies were incubated at room temperature (approximately 15°C during two weeks) and after initial growth mycelium from colonies was transferred to plates of Sb-agar. Sporangia suspension (10^5 sporangia/mL) was obtained by flooding 2-week-old cultures on Sb-agar plates with 1.5 mL sterile water. Zoospores were released by chilling the sporangia suspension at 4°C for an hour and used for inoculation.

Virulence assay

Clonal plants from Red and Yellow cvs. were used for the detached leaf assay. These plants were grown under field conditions in the Sibundoy Valley (Vereda El Cascajo), department of Putumayo. Plants without visible symptoms of any disease were selected for this assay. Three fully expanded healthy leaves were assigned randomly to each isolate-cultivar combination. Each leaf was washed in tap water, towel-dried and placed with their abaxial side upwards on lids of Petri dishes (20 cm diam) floating inside transparent plastic boxes containing a layer of water internally. These boxes were used as humidity chambers for inoculation and incubation. Each leaf was inoculated with four 25 µL droplets of the previously described sporangia suspension. Droplets were placed at each side of the main vein between the second and third veins and the penultimate and antepenultimate vein. They were maintained at 4°C for

TABLE 1 - Isolates of *Phytophthora infestans* from Southwest Colombia included in aggressiveness evaluations on detached *Solanum betaceum* leaves

Isolate	Haplotype	Possible clonal lineage	Host	Place of origin
St128	Iia	EC-1	*S. tuberosum*	Cabrera, NAR
St151	IIa	EC-1	*S. tuberosum*	Catambuco, NAR
St152	IIa	EC-1	*S. tuberosum*	Catambuco, NAR
Sb120	Ia	EC-3	*S. betaceum*	Nariño, NAR
Sb121	Ia	EC-3	*S. betaceum*	Genoy, NAR
Sb127	Hsb-I	-	*S. betaceum*	Genoy, NAR
Sb154	Hsb-I	-	*S. betaceum*	Genoy, NAR
Sb701	Ia	EC-3	*S. betaceum*	Santiago, PUT
Sb704	ND	ND	*S. betaceum*	Santiago, PUT

FIGURE 1 - Intense growth and sporulation of *Phytophthora infestans* on successive rings over a leaf of *Solanum betaceum* cv. Yellow - sample collected in municipality of Santiago, Putumayo.

12 h. After inoculation, leaves were kept in the humidity chambers for seven days at room temperature. The experiment was repeated twice.

Measurement of virulence-related variables

Leaves were analyzed every 24 h for a week. Parameters measured were infection frequency (IF), sporulation intensity (SI), area under lesion expansion curve (AULEC), final lesion size (FLS) and incubation period (IP). IF was estimated as the proportion of inoculated sites that developed sporulating lesions after seven days. Lesion size was recorded daily using a digital photographic camera and measured using the software Tpsdig version 1.37. (F. James, Rohlf, Ecology & Evolution, SUNY, Stony Brook, NY), which was also used for estimation of AULEC and FLS. Each leaf and each lesion in a digital picture were outlined and saved as a JPG file and analyzed by Tpsdig software. Resulting pixels were converted to measurement units (cm) using a scale pattern inside of each picture. AULEC was computed in an MS Excel file from daily measurement of lesion size during seven days. IP was calculated as the number of days between inoculation and the lesion development. SI was estimated as the number of sporangia produced per cm^2 of lesion area 7 days after inoculation. To perform this measurement, sporangia were washed from lesions with 0.5 ml of demineralized sterilized water and counted under a microscope on 3 aliquots of the resulting suspensions using a haemocytometer.

SI, AULEC and FLS were not normally distributed; log10 transformations resulted in sufficient normality of the data. These are presented as mean log10. The relationship between components was estimated through Pearson's correlations coefficient. Data was subjected to analysis of variance using the general linear models of Statgraphics Software 5.1 (Statistical Graphics. Corp. 1994-2000). Data was pooled only when the hypothesis of equal variances was not rejected. The null hypothesis of equal variances was tested at the 95% confidence level using the Levene's test.

RESULTS

Wide spectra of virulence were exhibited between isolates ranging from incompatibility reactions to quantitative differences in infection (Table 2). A significant effect of cultivar, isolate and isolate-origin was generally detected for virulence parameters ($P<0.05$), but interaction effects were not significant (Table 3 and 4). Statistical differences between the virulence parameters of isolates from *S. betaceum* and *S. tuberosum* on *S. betaceum* indicates specific virulence of Sb-isolates based on a high IF and SI, and a low IP on both cultivars ($P<0.05$) (Table 4). However, when individual isolates were analyzed, the weakest pathogenic Sb isolates could not be distinguished from potato isolates. This effect was clear for cv. Yellow, the relatively resistant cultivar, where only isolate 704 could be distinguished from potato isolates (Table 2). Highly significant ($P < 0.001$) correlations were found between IF and LP, SI and AULEC, SI and FLS; and AULEC and FLS (Table 5). Isolates from *S. betaceum,* as a group, were more aggressive than those from *S. tuberosum* on the two tested cultivars considering IF, SI and IP parameters without influence of each cultivar (Cultivar × Isolate origin > 0.05) (Table 4). However, some *S. betaceum* isolates had indistinguishable virulence as compared to potato isolates. No isolate exhibited specific virulence on any of the cultivars (Table 4). Only one strain among the three obtained from *S. tuberosum* was not infective (non-compatibility reaction) in either of the two cultivars of *S. betaceum* (Table 2).

IF and IP were strongly inversely correlated, however SI was not correlated with IF or IP (Table 5). In the case of incubation period, basically the most virulent isolates (the highest values for these three parameters) differed significantly from the other isolates, regardless of whether they were Sb or St-isolates. Some weakly aggressive isolates from *S. betaceum* were grouped together with *S. tuberosum* isolates. Besides, each cultivar of *S. betaceum* was severely infected by different isolates in concordance with these three parameters. On cv. Yellow isolate 704 exhibited the most significant values for all these variables, and on cv. Red isolates 154 and 120 exhibited the most significant values. The cultivars had a significant effect on several disease parameters, but this is not regarded as enough to differentiate highly aggressive isolates. Isolates virulence differed on each cultivar. This was particularly clear for *S. betaceum* isolate Sb121 which was able to attack cv. Yellow but had an incompatible reaction with cv. Red.

Quantitative resistance differences were observed between cultivars. Cv. Red exhibited significantly higher values for AULEC and FLS and lower values for IP as compared with cv. Yellow (Table 6). Based on the results obtained for these parameters, cv. Red can be considered more susceptible to the pathogen. Differences in resistance

TABLE 2 - Aggressiveness components of *Phytophthora infestans* isolates inoculated on *Solanum betaceum* leaves

	Mean infection frequency (Ratio)		Mean log10 ((sporangia per cm² lesion) + 1)		Mean log10 area under lesion expansion curve (AULEC) (cm²)		Mean log10 final lesion size (ratio)		Mean incubation period (days)	
Isolate	**Yellow**	**Red**	**Yellow**	**Red**	**Yellow**	**Red**	**Yellow**	**Red**	**Yellow**	**Red**
128*	0.00 a	0.00 a	0.00 a	0.00 a					8.00 b	8.00 c
151*	0.11 a	0.33 ab	3.09 a	5.93 bc	-1.29 bc	-1.34 a	-0.84 abc	-1.70 a	7.56 b	6.56 bc
152*	0.33 ab	0.33 ab	2.78 a	2.78 ab	0.21 c	-0.31 ab	-0.79 abc	0.07 ab	6.33 b	6.56 bc
120	0.22 a	1.00 c	3.92 ab	9.84 c	-3.29 a	-1.50 a	-3.21 A	-1.59 a	6.89 b	3.50 a
121	0.08 a	0.00 a	2.63 a	0.00 a	-2.97 ab	-	-2.85 ab	-	7.75 b	8.00 c
127	0.17 a	0.75 bc	2.29 a	7.62 bc	0.06 c	0.46 ab	0.75 C	0.40 ab	7.50 b	5.00 ab
154	0.17 a	1.00 c	4.54 ab	10.22 c	-0.46 c	-0.56 a	-1.46 abc	-0.54 a	7.17 b	3.00 a
701	0.42 ab	0.67 bc	6.18 ab	6.97 bc	-1.01 c	1.53 b	-0.94 bc	1.72 b	6.75 b	6.08 bc
704	0.75 b	0.67 bc	10.51 b	6.60 bc	-0.64 c	0.18 ab	-0.93 bc	0.21 ab	4.00 a	4.42 ab

Within cultivars, values followed by the same letter do not differ significantly (LSD P<0.05). *Isolates from *Solanum tuberosum*

TABLE 3 - ANOVA for aggressiveness parameters caused by isolates of *Phytophthora infestans* on leaves of two *Solanum betaceum* cultivars

	Infection frequency				Log10 (Sporulation intensity + 1)				Log10 Area under lesion expansion curve				Log Final lesion size				Incubation period			
	DF	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**
Cultivar	1	1.0135	10.76	0.0022	1	31.8901	2.06	0.1589	1	7.2963	9.15	0.0064	1	9.6102	8.91	0.0071	1	19.0316	9.93	0.0032
Isolate	8	0.4170	4.43	0.0007	8	53.9932	3.50	0.0041	7	3.3963	4.26	0.0045	7	3.8229	3.54	0.0114	8	10.6013	5.53	0.0001
Cultivar X Isolate	8	0.1768	1.88	0.0927	8	19.3603	1.25	0.2962									8	3.8083	1.99	0.0747

TABLE 4 - ANOVA for aggressiveness parameters testing the effect of host-isolates of *Phytophthora infestans* on leaves of two *Solanum betaceum* cultivars

	Infection frequency				Log10 (Sporulation intensity + 1)				Log10 Area under lesion expansion curve				Log Final lesion size				Incubation period			
	DF	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**	**DF**	**MS**	**F**	**P>F**
Cultivar	1	0.4720	3.55	0.0653	1	15.9465	0.81	0.3714	1	8.4841	5.81	0.0230	1	10.8828	6.19	0.0193	1	14.4439	4.60	0.0366
Host-isolate	1	1.0389	7.81	0.0073	1	136.3060	6.95	0.0110	1	1.0964	0.75	0.3580	1	1.9206	1.09	0.3053	1	18.1453	5.78	0.0198
Cultivar X Host-isolate	1	0.1837	1.38	0.2454	1	0.4666	0.02	0.8780												

TABLE 5 - Pearson's correlation coefficients for the relationships between infection frequency, sporulation intensity, area under the lesion expansion curve (AULEC) and final lesion size of *Phytophthora infestans* isolates on detached leaves of *Solanum betaceum*

	Infection frequency		Log (Sporulation intensity + 1)		Log AULEC		Log Final lesion size	
Infection frequency	1							
Log (Sporulation intensity + 1)	0.074	0.697[a]	1					
Log AULEC	0.287	0.124	-0.673	0.000	1			
Log Final lesion size	0.183	0.333	-0.659	0.000	0.941	0.000	1	
Incubation period	-0.865	0.000	-0.140	0.462	-0.095	0.617	0.055	0.771

[a]P-value.

TABLE 6 - Resistance of *S. betaceum* cultivars against *P. infestans* measured as virulence components during a detached leaf assay

Cultivars	AULEC			Log Final Lesion Size			Incubation period		
	Mean	Limits		Mean	Limits		Mean	Limits	
Yellow	0.0417	0.0148	0.0686	0.2019	-0.4348	0.8385	6.8827	6.3587	7.4068
Red	0.1114	0.0823	0.1406	1.3743	0.6838	2.0649	5.6790	5.1106	6.2474

between cultivars were totally associated to parameters related with lesion expansion (AULEC, FLS and IP). These parameters were previously reported as responsible for host-differentiation in field assays (Carlisle et al., 2002). However with exception of IP, these parameters were not associated to host-differentiation of *S. betaceum* isolates and EC-1 individuals. IF and SI were the critical parameters determining host-specificity.

DISCUSSION

This study proposed to test the hypothesis that *P. infestans* s.l. individuals isolated from *S. betaceum* and *S. tuberosum* have a range of different levels of virulence on two *S. betaceum* cultivars. We confirmed this hypothesis and found a range of levels of virulence among such isolates (Table 2 and 3) as has been found for studies on other *P. infestans* populations. Also differential virulence on *S. betaceum* of isolates of *P. infestans* from *S. betaceum* and *S. tuberosum* (EC-1 clonal lineage) was found. This quantitative host-specificity can be considered an additional example of differential virulence previously reported in *P. infestans* s.l., in which host-isolates are best adapted to their original host (Garry et al., 2005; Legard et al., 1995; Oyarzun et al., 1998; Suassuna et al., 2004; Vega-Sanchez et al., 2000). These quantitative differences were represented on IF, SI and IP. Previous reports from Uganda and Kenya (Vega-Sanchez et al., 2000) did not show any significant difference in IF between host-isolates and their hosts (*S. lycopersicum* and *S. tuberosum*). However, low values of IP and high values of IF can explain pathogen fitness on host (Lebreton et al., 1999).

Pathogen sporulation is an important component of pathogen aggresiveness. If an individual in a population sporulates more abundantly, it obtains more opportunities for establishing its genes in the genetic pool of the population (Lebreton et al., 1999; Suassuna et al., 2004). High sporulation levels are typically found for pathogenic fungi inoculated on their original host and it is a characteristic of its field fitness and capacity for dissemination through the host population. Field studies of *P. infestans – S. tuberosum* relationships have shown that late blight epidemics are linked to high sporulation capacity of *P. infestans*, a feature typical of a polycyclic disease (Lebreton et al., 1999; Spielman et al., 1992). Sb-isolates showed a highly significant SI compared with St-isolates. Regardless of isolate or isolate-origin, a significant effect of cultivar on SI was detected, but different isolates had the highest values on each cultivar. SI did not show any correlation with other virulence parameters and can be considered an independent feature able to determine host species-specificity.

Adler et al. (2004) reported that *in-leaf* test, petota isolates were unable to infect *S. betaceum,* an example of host-incompatibility. However, in this research, individuals of the clonal lineage EC-1 (belonging to the petota section) were able to infect and sporulate at low levels on *Solanum betaceum* leaves, in a clear example of host-specificity. Nevertheless, a simultaneous-field research using PCR detection of *P. infestans* never detected EC-1 clonal lineage on *S. betaceum* (Mideros-Bastidas MF, Burbano-Figueroa O, Lagos-Mora LE, 2009 - unpublished data). Host-incompatibility was observed in isolate cases for isolates obtained from *S. tuberosum* and *S. betaceum*. Quantitative host-specificity and incompatibility reactions observed for isolates obtained from *Solanum betaceum* shows how diverse pathogen virulence and host-response mechanisms of *Solanum betaceum – P. infestans* s.l. interaction can be. Isolates virulence differed on each cultivar, a condition that was evident for *S. betaceum* isolate Sb121 which was able to attack the cv. Yellow but showed an incompatible

reaction on cv. Red. This is in general the typical behavior of a population of *P. infestans* facing different cultivars of potato with race non-specific resistance (Day & Shattock 1997).

Phytophtora infestans s.l. populations have clonal structure in South America, but differences in virulence between isolates within populations that infect *S. tuberosum* and *S. lycopersicum* were previously reported. In this study, isolates within the clonal lineage EC-1 (potato-specific) show variability, but the variability in virulence between isolates from this clonal lineage and *S. betaceum* isolates is larger. The highly aggressive Sb-isolates included isolates with an unknown mitochondrial haplotype (Hsb-I). Isolates from *S. quitoense* in Nariño have a similar pattern (Mideros-Bastidas MF, Burbano-Figueroa O, Lagos-Mora LE, 2009 - unpublished data). There is clear evidence that the populations of *P. infestans* s.l. on *S. betaceum* are not restricted to the clonal lineage EC-3, but could include new clonal lineages with new mitochondrial haplotypes and differential virulence levels. This study and a simultaneous field study (Mideros-Bastidas MF, Burbano-Figueroa O, Lagos-Mora LE, 2009 - unpublished data) demonstrated that occurrence of this new strain is not a casual event. It is a common event, which implies the presence of two aggressive clonal lineages associated with an epidemic event. Isolates inside these clonal lineages show variability but not significant differences between them (data not shown) for all components measured. This new haplotype is widely distributed and have virulence at a level similar to the previously reported EC-3. This is the first report of two specific aggressive populations of *P. infestans* s.l. attacking *S. betaceum* simultaneously (*P. andina* populations). Exceptions to the one-host/one-pathogen hypothesis were previously reported. In Ecuador pear melon (*Solanum muricatum*) is generally attacked by the clonal lineage US-1, however on some locations is attacked by EC-2. Both populations showed high levels of virulence and correspond to two different genetic populations with different mating types and consequently there is a high risk of sexual recombination (Ordoñez et al., 2000). The mating type of the new haplotype is unknown, but if this is the case, we are facing a similar scenario involving sexual populations of the pathogen.

Quantitative differences in virulence related to host-specificity can be explained by reproductive isolation associated with different environments where these crops are grown and the pathogen survives. *Solanum betaceum* is grown mainly in midland areas (between 1500 – 2000 m.s.l.) while *S. tuberosum* is preferably grown at higher altitudes (>2500 m.s.l.). Additionally, presence of inoculum at high altitudes on *S. betaceum* is low and not related to epidemic events. Possibly, Sb-populations are intolerant to high altitudes. In the interandean valleys the context is different, areas of these two crops overlap and different populations of the pathogen coexist. However, an EC-1 isolate has never been isolated from *S. betaceum*. Other studies have shown that in the absence of inoculum, an epidemic can be initiated by a non-specific pathogen. However, in the case of *S. betaceum* certain conditions like its perennial nature and the high relative humidity of growing areas allow maintenance of high and constant inoculum levels where there are small probabilities for infection by a non-specific population.

Interestingly virulence of isolates of a new population associated to *S. betaceum* was similar to the established clonal lineage EC-3. This result supports the idea that EC-3 is not the dominant population on *S. betaceum*. An intensive characterization of the population of Sb-isolates can allow determination of how these pathogens coexist on the same host. Displacement of a clonal lineage of *P. infestans* by a new one is a well-documented process on potato and tomato. Both crops are seasonal and in a particular area potato or tomato can be rapidly rotated or replaced by another crop. When potato and tomato are rotated, a previously non-adapted pathogenic population can adapt to the new host and begin an epidemic event. But in the case of *S. betaceum,* where inoculum and host are constant during a long time, the emergence of a new population cannot be explained in that way. *Solanum betaceum* exhibits a high diversity with landraces or semi domestic cultivars adapted to specific regions. Probably this new pathogen population is specific for a cultivar or geographic area. Pathogen-populations adapted to specific cultivars and changes in the population caused by the incorporation of new cultivars had been analyzed for *P. infestans* attacking potato (Sacristan & Garcia Arenal 2008). A similar approach can be used for analyzing the pathogen populations attacking *S. betaceum* and develop a suitable strategy for future breeding program.

Our results showed statistically significant quantitative differences in virulence between St-isolates and Sb-isolates on two *Solanum betaceum* cultivars possibly resulting in host-specificity. Considering the overlapping area of these crops this host-specificity must be maintained by reproductive isolation. Occurrence of a novel haplotype from *S. betaceum* and its high virulence shows that clonal populations associated to each host are dynamic. Subsequent studies including more isolates and using intra-populational molecular markers are necessary, and will allow determination of possible relationships between pathogenicity and population structure.

This study only included two cultivars and a restricted number of isolates. Isolates from *S. betaceum* are difficult to recover and only a small proportion of them survived the isolation and purification steps. It is necessary to include a high number of isolates and a robust marker able to find intra-population variability inside clonal lineages EC-1, EC-3 and the new population Hsb-1. Association between virulence and genotype is not consistent between studies but is positive in some cases (Carlisle et al., 2002; Miller et al., 1998). Probably EC-3 and Hsb populations can exhibit different behavior and this is a unique opportunity to analyze two different clonal populations of *P. infestans* competing on a heterogeneous host. In this research we have used the most broadly and

commonly used cultivars, but it is possible to differentiate at least five cultivars and at least one wild-related species. Some of these cultivars are highly valued by the farmers but are highly susceptible to the disease. A future study using a larger number of isolates and cultivars would offer a broader and more clear perspective of genetic structure and virulence of *P. infestans* populations associated with *S. betaceum*. It could also allow for investigation of host-specificity between these cultivars and the determination of whether the prevalent virulence is quantitatively determined. This information could also be used to determine the resistance range of cultivars/landraces used by local farmers.

ACKNOWLEDGEMENTS

This work was supported financially by the ViceRectoria de Investigaciones, Postgrados y Relaciones Internacionales and Departmento de Biologia of Universidad de Nariño, Colombia.

REFERENCES

Adler NE, Erselius LJ, Chacón MG, Flier WG, Ordoñez ME, Kroon L, Forbes GA (2004) Genetic diversity of *Phytophthora infestans* sensu lato in Ecuador provides new insight into the origin of this important plant pathogen. Phytopathology 94:154-162.

Bohs L (1989) Ethnobotany of the genus *Cyphomandra* (Solanaceae). Economic Botany 43:143-163.

Bohs L (1991) Crossing studies in *Cyphomandra* (Solanaceae) and their systematic and evolutionary significance. American Journal of Botany 78:1683-1693.

Cárdenas M, Grajales A, Sierra R, Rojas A, González-Almario A, Vargas A, Marín M, Fermín G, Lagos L, Grunwald N (2011) Genetic diversity of *Phytophthora infestans* in the Northern-Andean region. BMC Genetics 12:1-13.

Carlisle DJ, Cooke LR, Watson S, Brown AE (2002) Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. Plant Pathology 51:413-423.

Day JP, Shattock RC (1997) Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. European Journal of Plant Pathology 103:379-391.

Duque C, Morales AL (2005) El aroma frutal de Colombia. Bogota. Universidad Nacional de Colombia.

Garry G, Forbes GA, Salas A, Cruz M, Perez WG, Nelson RJ (2005) Genetic diversity and host-differentiation among isolates of *Phytophthora infestans* from cultivated potato and wild solanaceous hosts in Peru. Plant Pathology 54:740-748

Gomez-Alpizar L, Hu CH, Oliva R, Forbes G, Ristaino JB (2008) Phylogenetic relationships of *Phytophthora andina*, a new species from the highlands of Ecuador that is closely related to the Irish potato famine pathogen *Phytophthora infestans*. Mycologia 100:590-602.

Lebreton L, Lucas JM, Andrivon D (1999) Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. Phytopathology 89:679-686.

Legard DE, Lee TY, Fry WE (1995) Pathogenic specialization in *Phytophthora infestans*: aggressiveness on tomato. Phytopathology 85:1356-1361.

MAG. Ministerio de Agricultura, Ganaderia, Acuacultura y Pesca. Ecuador. Estudio de Competitividad del tomate de arbol. Ministerio de Agricultura, Ganaderia, Acuacultura y Pesca. Ecuador. Estudio de Competitividad del tomate de arbol. 2005a. http://www.sica.gov.ec/agronegocios/productos%20para%20invertir/frutas/tomate%20arbol/Estudio%20de%20Competitividad%20Tomate%20de%20Arbol.pdf. (August 4, 2010)

MAG. Ministerio de Agricultura, Ganaderia, Acuacultura y Pesca. Ecuador. Estudio de Prefactibilidad del tomate de arbol. Ministerio de Agricultura, Ganaderia, Acuacultura y Pesca. Ecuador. Estudio de prefactibilidad del tomate de arbol. 2005b http://www.sica.gov.ec/agronegocios/productos%20para%20invertir/frutas/tomate%20arbol/epftomarbol.pdf. (August 4, 2010)

MAG. Ministerio de Agricultura, Ganaderia, Acuacultura y Pesca. Ecuador. Tomate de arbol / Tamarillo / Tree tomato / Sweer Tomato. Ministerio de Agricultura, Ganaderia, Acuacultura y Pesca. Ecuador. Tomate de arbol / Tamarillo / Tree tomato / Sweer Tomato. 2005c. http://www.sica.gov.ec/agronegocios/Biblioteca/Convenio%20MAG%20IICA/productos/tomate_arbol_mag.pdf. (August 4, 2010)

Miller JS, Johnson DA, Hamm PB (1998) Aggressiveness of isolates of *Phytophthora infestans* from the Columbia Basin of Washington and Oregon. Phytopathology 88:190-197.

Oliva, RF, Kroon L, Chacón G, Flier WG, Ristaino JB, Forbes GA. (2010) *Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands. Plant Pathology 59:613-625.

Ordoñez ME, Hohl HR, Velasco JA, Ramon MP, Oyarzun PJ, Smart CD, Fry WE, Forbes GA, Erselius LJ (2000) A novel population of *Phytophthora*, similar to *P. infestans*, attacks wild *Solanum* species in Ecuador. Phytopathology 90:197-202.

Oyarzun PJ, Pozo A, Ordoñez ME, Doucett M, Forbes GA (1998) Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. Phytopathology 88:265-271.

Sacristan S, Garcia Arenal F (2008) The evolution of virulence and pathogenicity in plant pathogen populations. Molecular Plant Pathology 9:369-384.

Spielman LJ, McMaster BJ, Fry WE (1992) Relationships among measurements of fitness and disease severity in *Phytophthora infestans*. Plant Pathology 41:317-324.

Suassuna ND, Maffia LA, Mizubuti ESG (2004) Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*. Plant Pathology 53:405-413.

Vega-Sanchez ME, Erselius LJ, Rodriguez AM, Bastidas O, Hohl HR, Ojiambo PS, Mukalazi J, Vermeulen T, Fry WE, Forbes GA (2000) Host adaptation to potato and tomato within the US-1 clonal lineage of *Phytophthora infestans* in Uganda and Kenya. Plant Pathology 49:531-539.

TPP 34 - Received 8 October 2009 - Accepted 28 December 2011
Section Editor: Eduardo S.G. Mizubuti