Phenotypic Variation Within a Clonal Lineage of *Phytophthora infestans* Infecting both Tomato and Potato in Nicaragua

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ABSTRACT

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Late blight caused by *Phytophthora infestans* (Mont.) de Bary is a constraint to both potato and tomato crops in Nicaragua. The hypothesis that the Nicaraguan population of *P. infestans* is genotypically and phenotypically diverse and potentially subdivided based on host association was tested. A collection of isolates was analyzed using genotypic markers (microsatellites and mitochondrial DNA haplotype) and phenotypic markers (mating type, virulence, and fungicide sensitivity). The genotypic analysis revealed no polymorphism in 121 of 132 isolates of *P. infestans* tested. Only the Ia haplotype and the A2 mating type were detected. Most of the tested isolates were resistant to metalaxyl. The virulence testing showed variation among isolates of *P. infestans*. No evidence was found of population differentiation among potato and tomato isolates of *P. infestans* based on the genotypic and phenotypic analysis. We conclude that the Nicaraguan population of *P. infestans* consists of a single clonal lineage (NI-1) which belongs to the A2 mating type and the Ia mitochondrial DNA haplotype. Moreover, based on the markers used, this population of *P. infestans* does not resemble the population in countries from which potato seed is imported to Nicaragua or the population in neighboring countries. The data presented here indicate that the NI-1 clonal lineage is the primary pathogen on both potato and tomato, and its success on both host species is unique in a South American context.

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the main constraints to both potato and tomato crops in the northern highlands of Nicaragua, where weather conditions are conducive for the disease development from May to January. Despite this, no information has been available about the population biology and the underlying epidemiological implications of possible genotypic and phenotypic diversity of the pathogen in Nicaragua.

P. infestans is a diploid, hemibiotrophic, and heterothallic organism with two mating types designated A1 and A2. One hypothesis is that it originated and evolved in the central highlands of Mexico (26,41). However, recent studies have suggested a South American origin of *P. infestans* (28). Prior to the 1980s, worldwide populations of *P. infestans* were dominated by a single clonal lineage known as the US-1 "old" genotype, having the A1 mating type (20,25). In contrast, in the Toluca Valley in central Mexico, the A1 and A2 mating types were present in approximately equal frequencies and the populations of *P. infestans* were entirely different from populations in other locations (18,26). Since the mid-1980s, changes in the population structure of *P. infestans* outside Mexico have been reported (19). These changes brought about the displacement of the old genotype by a "new" genotype, which is characterized by both mating types and increased fitness and aggressiveness, as well as metalaxyl resistance (11). Moreover, the pathogen has been found to produce oospores under field conditions, which seem to be acting as initial inoculum in Northern Europe (2,3,37).

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Populations of *P. infestans* have been characterized using a series of genotypic and phenotypic markers. Phenotypically, populations of *P. infestans* have been distinguished through determination of the mating type, virulence spectrum, and metalaxyl resistance (21). The genotypic characterization of *P. infestans* has included the use of allozyme patterns, mitochondrial DNA (mtDNA) haplotype determination, random amplified polymorphic DNA, amplified fragment length polymorphism, and random fragment length polymorphism fingerprints with the probe RG57, and results from these studies can reveal high population diversity (17). In comparison with the previously mentioned markers, simple-sequence repeat (SSR) markers (also referred to as microsatellites) now seem to offer the greatest potential across a wide range of applications (9). Over the past 10 years, SSR markers have been developed for the study of *P. infestans* (34–36). Some advantages of SSR markers are their repeatability and that genotyping can be conducted directly from infected tissue without the need for pathogen isolation and culture. In addition, these are codominant markers, which provide better resolution when used with diploid organisms such as *P. infestans*.

In Latin America, *P. infestans* populations have been extensively studied. In the Toluca Valley in central Mexico, *P. infestans* reproduces sexually and the two mating types (A1 and A2) are found in approximately equal frequencies. In other countries of the subcontinent, *P. infestans* appears to reproduce primarily asexually, although both mating types have been found in the same host species. However, some of these data must be interpreted carefully due to revisions in the taxonomy of *P. infestans* and closely related species (42). Initial studies in Ecuador reported the presence of two clonal lineages (EC-1 and US-1) of the A1 mating type (16). However, further studies revealed the occurrence of the two mating types (A1 and A2) of *P. infestans* sensu

lato in *Solanum muricatum* (1) but the A2 isolate in that study was probably *P. andina* (42); in Peru, an A1 clonal population has been reported (44); in Brazil, the A1 and A2 mating types have been found in tomato and potato, respectively, but they have not been detected in the same field (46). In Uruguay, only the A2 mating type has been found (12); in Colombia, the A1 and A2 mating types have been found in the same host, *Physalis peruviana* (cape gooseberry), although no evidence of sexual recombination has been reported thus far (49); in Costa Rica, a clonal lineage of A1 mating type has been reported attacking potato; however, isolates of the A2 mating type have been also found in wild *Solanum* spp. (27). In Argentina, *Phytophthora infestans* populations seem to be more diverse when compared with other Latin American countries because both mating types have been found. However, Argentinean isolates of the A2 mating type have been found in a higher frequency and they showed greater aggressiveness and an increased resistance to metalaxyl compared with the A1 isolates (4). Clonality of *P. infestans* populations has been reported from Venezuela, where only the A1 mating type has been found (6).

Some degree of pathogenic specialization of *P. infestans* to potato or tomato has been reported. In a study carried out in Ecuador, it was found that there were two different clonal lineages of *P. infestans* separated by their host adaptation. The EC-1 clonal lineage was found on potato, while US-1 was found mainly on tomato (43). Recently, studies in Brazil demonstrated that isolates of *P. infestans* belonging to the US-1 clonal lineage were adapted to tomato, while the isolates of the BR-1 clonal lineage were adapted to potato (46,48). In the United States, a study showed that potato and tomato populations of *P. infestans* had different genetic diversity and structure. Moreover, it was found that the tomato populations of *P. infestans* turned out to be more genetically diverse over time than potato populations (50). In contrast to this, a recent study carried out in Taiwan showed no host specificity on potato or tomato among *P. infestans* isolates from tomato (7). Thus, it is clear that there is no a general consensus about host specificity of *P. infestans* among scientific community.

Fig. 1. Map of Nicaragua showing the three northern departments where isolates of *Phytophthora infestans* were collected from 2007 to 2010. The number of isolates collected in each department is indicated on the map.

It is believed that cultivated potato (*S. tuberosum)* was introduced to Nicaragua in the early 1900s and, with it, the late blight pathogen. Troops from United States supposedly brought potato tubers for consumption, some of which fell into the hands of local people who began growing potato crops. Therefore it could be hypothesized that the first populations of *P. infestans* present in Nicaragua belonged to the old US-1 genotype of the A1 mating type and the Ib mtDNA haplotype (19). Moreover, this introduction would have occurred before the second worldwide migration of *P. infestans* that was thought to have taken place in the 1980s (24).

Unlike other countries, Nicaragua does not have a well-established program for potato seed production; therefore, the country depends on the import of potato seed to meet the grower's demand. The potato seed is frequently imported from European countries such as The Netherlands, North America (United States and Canada), and Guatemala. If *P. infestans* is carried on the seed potato, one might expect variation in the pathogen population. In Nicaragua, potato production is concentrated in three northern departments (the Nicaraguan equivalent of a province): Estelí, Jinotega, and Matagalpa, where climatic conditions are optimal for potato growth but also for late blight development. The disease also affects tomato in this region but the two crops are rarely grown in the same field. In this study, the hypothesis that the Nicaraguan population of *P. infestans* is genotypically and phenotypically diverse and potentially subdivided based on host association was tested. Thus, the strategy was to sample *P. infestans* in those regions of northern Nicaragua where both potato and tomato cultivation occurs along with the late blight pathogen. Isolates collected from both tomato and potato were analyzed using genotypic markers (SSR and mtDNA haplotyping) and phenotypic markers (mating type, virulence, and fungicide sensitivity).

MATERIALS AND METHODS

Sampling and isolation of *P. infestans*. Leaflets of potato and tomato with a single late blight lesion were collected from commercial production and experimental fields in northern Nicaragua from July 2007 to January 2010. In each department, five to seven locations were sampled, taking a different number of samples from each location (Fig. 1). In all, 13 tomato and 43 potato fields (56 fields in total) located in 18 sites in three northern departments of Nicaragua were sampled (Table 1). Leaflets with single lesions were washed with distilled water and dried with filter paper. Thereafter, they were individually placed abaxial side up in a sealed petri dish containing a layer of 1.5% water agar and incubated at 18°C to promote sporulation. When sporulation was observed, the mycelia with sporangia was transferred to a pea agar medium (13) amended with antibiotics (ampicillin at 0.2 g liter^{-1} and pimaricin at 10 mg liter^{-1}) and incubated at 18°C in darkness for a week. Plugs of agar with growing hyphal tips were cut from the colony margins and transferred to petri dishes with pea agar medium without antibiotics and incubated at 18°C for growth and sporulation. Axenic isolates were maintained on pea agar medium without antibiotics and transferred monthly to fresh medium. Additionally, 54 single lesion-blighted potato and tomato leaflets were preserved as dried material for DNA extraction.

DNA extraction. Two approaches were used to extract DNA for mitochondrial haplotyping and microsatellite analysis, depending on whether the sample was stored as lyophilized mycelium or as dried leaflets. Individual pieces of lyophilized mycelium were placed in a 2-ml polypropylene vial containing six glass beads and homogenized in a FastPrep preparation shaker (Precellys 24; Bertin Technologies). DNA was extracted following the protocol provided with the Wizard Genomic DNA purification kit: protocol for plant tissue (Promega Corp.) for isolating genomic DNA from plant tissue. Dried leaflets of potato and tomato infected with *P. infestans* were homogenized for DNA extraction as described for

lyophilized mycelium. DNA from dried leaflets was extracted using a cetyltrimethylammonium bromide (CTAB) procedure (22), with the exception that 3% CTAB was used.

Genotypic analysis. *mtDNA haplotyping.* mtDNA haplotyping was carried out using a method described earlier (29), with slight modifications. The annealing temperature was increased to 63°C and the primer pairs P2 and P4 were used at a concentration of 0.4 µM.

Microsatellite analysis. Seven SSR primers were used for the microsatellite analysis of 132 isolates of *P. infestans*: Pi4B, PiG11 (35), Pi16, Pi70, PiD13, Pi63, and Pi04 (36). Forward primers 4B, Pi16, D13, and Pi04 were labeled with 6-FAM (TAG Copenhagen), whereas G11, Pi70, and Pi63 were labeled with NED (Applied Biosystems). Polymerase chain reaction (PCR) amplifications were performed in 15 µl containing approximately 10 ng of genomic DNA, 0.2 mM dNTPs, 0.4 µM each forward and reverse primers, ThermoRed DNA polymerase (Saveen & Werner AB) at 0.04 U μl^{-1}, and 1× reaction buffer Y (containing 2 mM $MgCl_2$) supplied by the manufacturer. For the primers for locus 4B and Pi70, 4 mM $MgCl_2$ was used. The PCR conditions were as follows: an initial denaturation at 94°C for 3 min; followed by 30 or 33 cycles (dependent on the primers) at 94°C for 30 s, 30 s of annealing temperature of 50 to 62°C dependent on the primers, and elongation at 72°C for 1 min; and final extension at 72°C for 25 min (GeneAmp PCR System 2700; Applied Biosystems). The annealing temperature and the number of cycles for each primer were as follows: 50°C and 33 cycles for primer D13, 58°C and 33 cycles for primers Pi4B and Pi70, 60°C and 30 cycles for Pi63, 60°C and 33 cycles for Pi16, and 62°C and 30 cycles for primers PiG11 and Pi04. For dried leaflet samples, the annealing temperature and the number of cycles for primer Pi63 were 58°C and 33 cycles, respectively. Separation of the amplified fragments was done using an ABI 3730*xl* DNA analyzer at Uppsala Genome Center, Rudbeck Laboratory, Uppsala University in Sweden. The fragment length of the fluorescently labeled fragments was visualized and scored using the software GeneMarker version 1.6 (Softgenetics). The allele sizes were adjusted to the sizes obtained at SCRI (36) by comparison with reference isolates, kindly supplied by Drs. Lee and Cooke, SCRI.

Phenotypic analysis. *Mating type determination.* Mating type determination was made using tester isolates of known mating type (A1 or A2) and the unknown Nicaraguan isolates. Mycelial plugs (0.5 cm in diameter) of each were placed in petri dishes containing rye pea agar (38) and incubated at 20°C in the dark. Cultures were examined for oospore formation in the zone of interaction. After 2 weeks, the mating type of the unknown isolate was recorded as the opposite of the tester isolate with which it formed oospores. In the mating type assays, 248 Nicaraguan isolates of *P. infestans* were tested.

Fungicide sensitivity, and virulence tests. The fungicide sensitivity of the isolates to metalaxyl-M and propamocarb hydrochloride (propamocarb-HCl) was determined using the floating leaf disc method (38,47). For virulence testing, a procedure described earlier (38) was followed. Only potato plants with resistance genes were used for virulence tests, because no tomato differentials were available. Mean number of virulence factors per isolate (*Ci*) and race (*Cp*) was calculated (5). The *Ci* and *Cp* were separately calculated for the potato and tomato isolates. Moreover, to detect differences among potato and tomato isolates, a *t* test procedure with the *Ci* and *Cp* values was performed.

RESULTS

Sampling and isolation of *P. infestans*. Of the sampled isolates, 84% (209 isolates) were isolated from blighted potato leaflets and 16% (39 isolates) were isolated from blighted tomato leaflets and fruits. All of the 248 collected isolates were tested for mating type, and a subset of 132 isolates was used for micro-

TABLE 1. Origin, mating type, mitochondrial DNA haplotype, and simple-sequence repeat (SSR) fingerprinting pattern of *Phytophthora infestans* isolates collected from 2007 to 2010 in northern Nicaragua[a]

Department	Location[b]	Crop	N-of-I[c]	Mating type	Haplotype[d]	SSR[e]
	El Jobo (P)	Potato	10	A2	nd	nd
	La Laguna (G,P)	Potato	21	A2	Ia (13)	M (13)
Estelí	La Tejera (G,P)	Potato	9	A2	Ia (3)	M (3)
	Miraflor (G,P)	Potato	39	A2	Ia (37)	M (34), V (3)
	Sesteo (P)	Potato	23	A2	nd	nd
	Tisey (G,P)	Potato	34	A2	Ia (22)	M (20), V (2)
Subtotal	6		136	136	75	75
	Chagüite Grande	Tomato	12	A2	nd	nd
	El Canal (G)	Tomato	7	A2	Ia (7)	M (7)
	El Mojón (P)	Potato	3	A2	nd	nd
Jinotega	El Mojón (G)	Tomato	1	A2	Ia (1)	M (1)
	Las Colinas (P)	Tomato	4	A2	nd	nd
	La Galia (P)	Potato	10	A2	nd	nd
	La Parranda (G)	Potato	5	A2	Ia (5)	M (5)
	Tomatoya (P)	Tomato	5	A2	nd	nd
Subtotal	7		47	47	13	13
	Aranjuez (G)	Potato	1	A2	Ia (1)	M (1)
	El Arenal (G)	Potato	17	A2	Ia (17)	M (11), V (6)
Matagalpa	La Fundadora (G)	Potato	29	A2	Ia (18)	M (18)
	La Fundadora (G,P)	Tomato	10	A2	Ia (3)	M (3)
	Sitio Viejo (G)	Potato	5	A2	Ia (5)	M (5)
	Yucul (P)	Potato	3	A2	nd	nd
Subtotal	5	…	65	65	44	44
Total	18	…	248	248	132	132

[a] Abbreviation: nd = not determined or not included in the analysis.

[b] Isolates collected from locations marked with the letters G and P were used for genotypic (G) and phenotypic (P) analyses. In some cases, the isolates were collected from the same location for both analyses (G,P).

[c] Number of isolates collected from 2007 to 2010 in the main potato growing areas of northern Nicaragua.

[d] Mitochondrial DNA haplotype. Numbers in parenthesis indicate the number of isolates that were tested.

[e] SSR = simple sequence repeats (also known as microsatellites). M = monomorphic for SSR markers. Numbers in parenthesis indicate the number of isolates that were included in the analysis. V = variants, those isolates that showed a one-step difference at loci G11 and Pi16. For instance, in location Miraflor, two isolates had a one-step difference at locus G11 and one isolate showed a one-step difference at locus Pi16. On the other hand, in other locations, such as Tisey and El Arenal, variation was observed only at locus G11.

satellite analysis and mtDNA haplotyping. In all, 98 isolates (82 from potato and 16 from tomato) were tested for fungicide sensitivity and used in virulence tests. Isolates for genotypic and phenotypic analyses were collected from 11 and 12 locations, respectively (Table 1).

Genotypic analysis. Overall, SSR genotyping using the set of seven primers (4B, G11, Pi16, Pi70, D13, Pi63, and Pi04) revealed no polymorphism in 121 of 132 isolates of *P. infestans* from Nicaragua. The only exception to this was two rare genotypes that showed one-step difference at the loci Pi16 and G11 when compared with the commonly found genotype. Differences at the loci Pi16 and G11 were found in 1 and 10 potato isolates, respectively, representing 0.7% for the locus Pi16 and 7.6% for the locus G11 of the total isolates tested. These 11 potato isolates were collected from three different locations (El Arenal, Miraflor, and Tisey). Otherwise, all the other isolates from potato and tomato belonged to a single multilocus genotype. This dominant genotype was heterozygous for almost all the analyzed loci (4B, 205/213; G11, 132/156; D13, 98/108; Pi63, 148/157; and Pi04, 166/170), except for loci Pi16 (176/176) and Pi70 (192/192). Minor variants of this genotype were found, with 176/174 at Pi16 (1 isolate) and 132/154 at G11 (10 isolates). mtDNA haplotyping revealed that all 132 isolates tested had the Ia haplotype (Table 1). No evidence was found of population differentiation among potato and tomato isolates of *P. infestans* based on the SSR fingerprinting patterns and mtDNA haplotyping.

Phenotypic analysis. All of the isolates collected from different locations in northern Nicaragua were characterized as the A2 mating type (Table 1). In total, 96 isolates (including both potato and tomato isolates) were resistant to metalaxyl (98%), 1 showed an intermediate reaction (1%), and 1 was sensitive to metalaxyl (1%) (Table 2; Fig. 2). The latter two were potato isolates. In all, 53 (54%) were able to sporulate in propamocarb-HCl at 10 mg/liter, 27 isolates (28%) sporulated in propamocarb-HCl at 100 mg/liter, 18 isolates (18%) grew only in the control, and no isolate was able to sporulate at 1,000 mg/liter (Fig. 2).

The results from the virulence testing showed variation among isolates of *P. infestans* from Nicaragua. Among the 82 potato isolates, 31 races were found. The most frequent race in the potato isolates was R1.2.3.4.5.6.7.10.11 (14 isolates), followed by R1.2.3.4.6.7.10.11 (9 isolates), R1.3.4.5.7.10.11 (7 isolates), R1.3.4.7.11 (6 isolates), and R1.3.4.7 (5 isolates). Fifteen races were represented by a unique isolate, whereas the remaining races (11) were represented by two or four isolates (Table 2). Among the 16 tomato isolates, 11 races were identified; that is, almost 1 race per isolate tested. The most frequent races found in tomato

TABLE 2. Race structure and response to metalaxyl-M of *Phytophthora infestans* isolates collected from potato and tomato plants during 2008 and 2009 in the main potato- and tomato-growing areas of Nicaragua

Locations[a]	Races[b]	RM[c]	N-of-I[d]
LC	R2.3 (T)	R (1)	1
LG	R2.11 (P)	R (1)	1
LL	R3.7 (P)	R (1)	1
MF	R3.11 (P)	R (1)	1
LF, TM	R1.3.4 (T)	R (2)	2
EJ, LL	R1.3.7 (P)	R (2)	2
EJ	R3.4.7 (P)	R (1)	1
LG, MF	R3.4.11 (P)	R (2)	2
ST	R3.7.11 (P)	R (1)	1
LF, LL, ST, TM	R1.3.4.7 (P [5]), (T [3])	R (8)	8
LF, ST, YC	R1.3.4.11 (P [2]), (T [1])	R (3)	3
TM	R2.3.7.11 (T)	R (1)	1
ST	R3.4.7.11 (P)	I (1)	1
LL	R1.2.3.4.7 (P)	R (1)	1
LT	R1.3.4.5.11 (P)	R (1)	1
LL	R1.3.4.5.7 (P)	R (1)	1
EJ, LC, LF, LL, LT, ST, TM	R1.3.4.7.11 (P [6]), (T [3])	R (9)	9
ST	R2.3.4.6.11 (P)	R (1)	1
LG	R3.4.7.10.11 (P)	R (1)	1
LF	R1.2.3.4.6.7 (T)	R (1)	1
LT, ST	R1.2.3.4.7.11 (P)	R (2)	2
TM	R1.3.4.5.7.11 (T)	R (1)	1
TY	R1.3.4.5.10.11 (P)	R (2)	2
ST	R1.3.4.6.7.11 (P)	R (1)	1
MF, TY	R1.3.4.7.10.11 (P)	R (4)	4
ST	R1.2.3.4.5.6.11 (P)	R (1)	1
LG, LT	R1.2.3.4.6.7.11 (P)	R (2)	2
LG, TY	R1.2.3.4.7.10.11 (P)	R (2)	2
EJ, ST, TY	R1.3.4.5.7.10.11 (P)	R (6) S (1)	7
TY	R1.3.4.6.7.10.11 (P)	R (1)	1
ST, TY	R1.2.3.4.5.6.7.11 (P)	R (4)	4
LT, ST	R1.2.3.4.5.6.10.11 (P)	R (2)	2
LF	R1.2.3.4.5.7.10.11 (T)	R (1)	1
EJ, LF LG, LT, MF, ST, TY	R1.2.3.4.6.7.10.11 (P [9]), (T [1])	R (10)	10
LT, ST	R1.3.4.5.6.7.10.11 (P)	R (2)	2
EM	R1.2.3.4.5.6.7.9.11 (P)	R (1)	1
EJ, LF, LT, MF, ST, TY	R1.2.3.4.5.6.7.10.11 (P [14]), (T [1])	R (15)	15
Total	…	98	98

[a] EJ = El Jobo, EM = El Mojón, LC = Las Colinas, LF = La Fundadora, LG = La Galia, LL = La Laguna, LT = La Tejera, MF = Miraflor, ST = Sesteo, TM = Tomatoya, TY = Tisey, and YC = Yucul.

[b] The host origin of the isolates belonging to each race is indicated by the letter P (potato plants) and T (tomato plants); numbers in brackets indicate the number of isolates sampled from each host plant and used for virulence testing.

[c] RM = response to metalaxyl-M; R = resistant, I = intermediate resistant, and S = susceptible. Numbers in parenthesis indicate the number of isolates in each category. The susceptible isolate was collected in location Tisey.

[d] N-of-I = number of potato and tomato isolates used in fungicide sensitivity tests and in virulence testing using the potato differential set of resistance (*R*) genes (*R1* to *R11*).

isolates were R1.3.4.7. (three isolates) and R1.3.4.7.11 (three isolates) followed by R1.3.4 (two isolates). The remaining tomato races were each represented by a unique isolate (Table 2). Both potato and tomato isolates overcame resistance gene R1 at the same proportion (88%). The remaining resistance genes were overcome at different proportions depending on the source (tomato or potato) of the isolate tested. Only one potato isolate collected during 2008 was able to overcome resistance gene R9 and no isolate examined was able to overcome the resistance gene R8 (Fig. 3). The number of virulence factors in each isolate was 2 to 9 in both potato or tomato isolates. Among the potato isolates, 17 (grouped in four races) were found to have eight virulence factors. The *Ci* and *Cp* were 6.4 and 5.5, respectively, for potato isolates whereas, for tomato isolates, *Ci* and *Cp* were 5.0 and 5.4, respectively. The *Ci* was higher than *Cp* in potato isolates, indicating that complex races predominate within potato populations of *P. infestans* from Nicaragua. The number of tomato isolates was low compared with the number of potato isolates but there was a tendency of a higher number of simpler races in the tomato isolates. Nonetheless, the *t* test procedure showed no significant differences between potato and tomato isolates for the *Ci* and *Cp* values (data not shown).

DISCUSSION

Genotypic diversity within populations of *P. infestans* from Nicaragua was expected due to the fact that potato seed is imported from the Netherlands, Canada, the United States, and Guatemala. Contrary to this initial hypothesis, the *P. infestans* population from Nicaragua seems to belong to a single clonal lineage having the A2 mating type and the Ia mtDNA haplotype. Our results indicate that the Nicaraguan clonal lineage of *P. infestans* does not originate from seed imported from The Netherlands or other European sources, because the allele with the size 132 bp found at the locus G11 has not been recorded in European populations (D. Cooke, *personal communication*). This clonal lineage is not US-8, either, because that clonal lineage has a different genotype based on these microsatellite loci (D. Cooke, *personal communication*). The allele size 132 bp at G11 has been found in a *P. infestans* strain from Mexico (www.euroblight.net) and has been recorded from A1 tomato isolates from the United States such as US-11 and US-12 (D. Cooke, *personal communication*), suggesting a New World origin of the Nicaraguan population. In studies carried out in Venezuela using 4B and G11 SSR markers (6) and Colombia using 4B and D13 markers (49), a similar low genotypic diversity was found among the tested *P. infestans* isolates. In Central America, the Nicaraguan population of *P. infestans* is of different mating type than the *P. infestans* populations in the neighboring countries. Transfer of agricultural products occurs over the borders of Nicaragua, Costa Rica, and Honduras and one might expect that isolates of *P. infestans* population from these countries might have entered Nicaragua or vice versa. Nonetheless, there is no indication from the data presented here that such a transfer has taken place. The Costa Rican population of *P. infestans* belongs to two clonal lineages with the A1 mating type in potato and the A2 mating type in wild *Solanum* spp. (27), while the *P. infestans* population from Honduras belongs to a clonal lineage having the A1 mating type (15). We are not aware of any publications about the population genetic structure of *P. infestans* from Guatemala and El Salvador.

These data suggest that *P. infestans* populations have experienced a major shift since their first appearance in Nicaraguan potato fields. The Ia and IIb mtDNA haplotypes have been found in herbarium specimens from Nicaragua dating from 1954 and 1956, respectively (39). In the present study, however, the IIb mtDNA haplotype was not found, indicating that it was completely replaced by the Ia mtDNA haplotype.

Despite low genotypic variability, the Nicaraguan population of *P. infestans* was found to be variable with regard to virulence spectra and fungicide resistance. This is in agreement with the results from a similar study conducted in Northern China, in which low genotypic diversity was observed, while the virulence spectra turned out to be highly variable. Moreover, in that study, it was

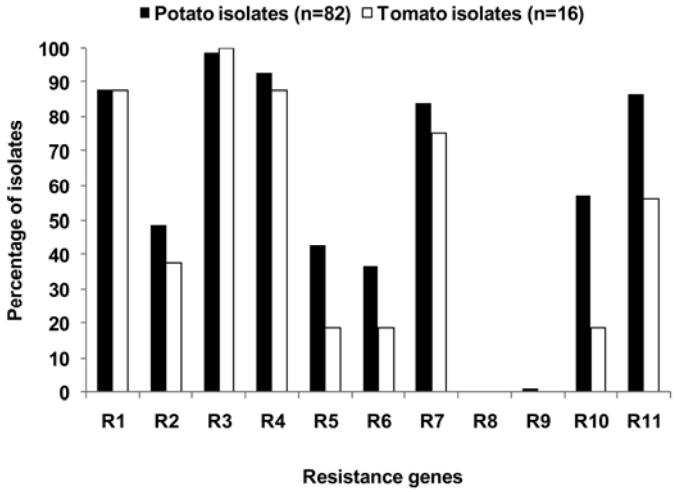

Fig. 3. Percentages of *Phytophthora infestans* isolates from Nicaragua overcoming resistance (*R*) genes (*R1* to *R11*) from samples taken during 2008 and 2009 in potato (black bars) and tomato (white bars) fields.

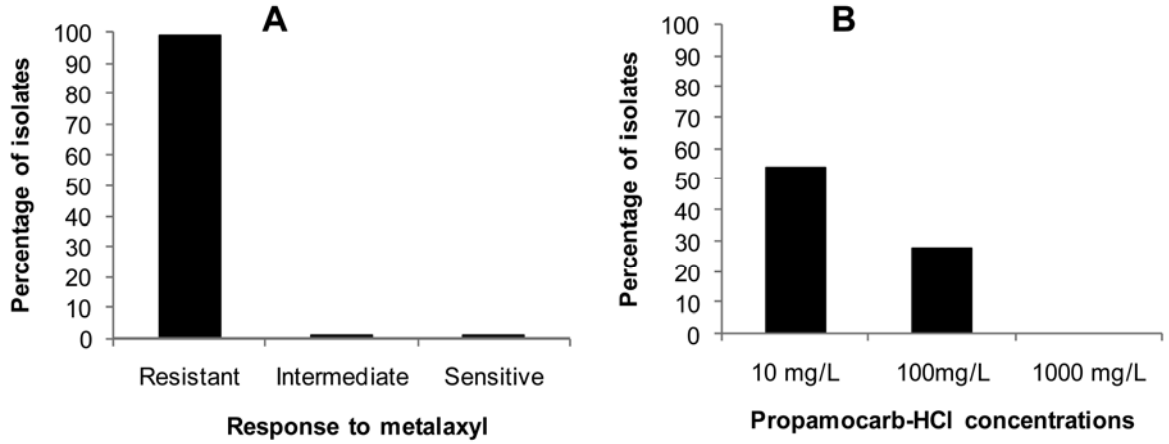

Fig. 2. Response of *Phytophthora infestans* isolates from Nicaragua (sampled in 2008–09) to **A,** phenylamide fungicide metalaxyl and **B,** propamocarb-HCl. Samples were taken from tomato and potato fields in different locations in northern Nicaragua.

also found that some of the tested isolates were virulent to all resistance (*R*) genes (32). However, unlike the Chinese population of *P. infestans*, the Nicaraguan one could not overcome all of the *R* genes present in the traditional differential set of potato clones, and the most complex and most common races of *P. infestans* population overcame eight and nine resistance genes, respectively. A complex race structure and high virulence diversity have been detected in other parts of the world (12,38,44). The resistance genes *R1, R3, R4*, and *R7* are the ones most frequently overcome by the Nicaraguan potato isolates. The same trend was observed among tomato isolates. This is in agreement with the Euroblight data base of 4,349 isolates, where virulence frequencies of >80% are reported for genes *R1*, *R3*, *R4*, *R7*, and *R11* (www.euroblight.net). Race complexity observed in *P. infestans* isolates from Nicaragua can have arisen as a result of the selection pressure imposed by potato cultivars carrying different *R* genes. For example, 'Santé' potato, which is the preferred cultivar among Nicaraguan potato growers, is known to carry the resistance genes *R1* and *R10* (14), and this may be responsible, in part, for the high frequency of virulence to these resistance genes. The appearance of new races is related to mutations in avirulence (*Avr*) genes encoding effector proteins in such a way that the effectors are not able to be recognized by the R protein (32). Because the *Avr* genes are localized in a hypervariable region of the genome (33), this could explain the high virulence diversity in isolates with the same SSR multilocus genotype.

Currently, metalaxyl-containing products are rarely applied in Nicaragua, if at all. Despite this, a high percentage of the tested isolates were resistant to metalaxyl. In other places around the world, a high percentage of *P. infestans* isolates resistant to metalaxyl has also been found (8,12,45). In contrast, in a study carried out recently in Scandinavian countries, a decrease in the proportion of metalaxyl-resistant isolates compared with the early 1990s was observed (38). This decline in the proportion of isolates resistant to metalaxyl was attributed to a limited use of this fungicide in these countries. Resistance to phenylamide fungicides, such as metalaxyl, can naturally occur as a result of random mutation (10,23). Nonetheless, preexisting resistant individuals increase in frequency as a result of the selection pressure imposed by fungicide application (23,31). In Nicaragua, the appearance and increase of metalaxyl-resistant isolates probably occurred in the 1980s and early 1990s, when the potato production areas increased in size and metalaxyl-based fungicides were used frequently and indiscriminately. Therefore, this could have led to a directional selection toward resistance which persists in the current clonal Nicaraguan population of *P. infestans*. This could also result in a reduction in genotypic diversity, as has been reported in other studies (31). In spite of the infrequent use of metalaxyl, the phenotypic trait of metalaxyl resistance remains at a high frequency in the Nicaraguan population of *P. infestans*. This may be explained by a clonal population, which will retain unnecessary traits longer than a sexually reproducing population.

In this study, the sensitivity of Nicaraguan isolates of *P. infestans* against propamocarb hydrochloride was also tested. This fungicide is used by Nicaraguan potato growers formulated alone or as a mixture with other fungicides with different modes of action. As was pointed out earlier, no evidence of resistance to propamocarb-HCl was found when these isolates were exposed to the highest concentration (1,000 mg/liter) of the fungicide. Potato growers in Nicaragua apply propamocarb-HCl at a dose of 1,083 mg/liter active ingredient (a.i.) when formulated alone and 564 mg/liter a.i. when formulated as a mixture with another fungicide. Although 28% of the tested isolates were able to sporulate in propamocarb-HCl at a concentration of 100 mg/liter a.i., the lower fungicide dose applied by potato growers in the field is five times greater than that in which sporulation was detected. There are some reports from other parts of the world of *P. infestans* isolates resistant to propamocarb-HCl (38,40).

In the present study, based on the SSR analysis and mtDNA haplotyping, no genotypic difference was found between potato and tomato isolates of *P. infestans*. In studies addressing host specificity of *P. infestans* using phenotypic and genotypic markers, it has been found that populations on tomato often are different from those on potato. Tomato isolates from South America are associated with the US-1 clonal lineage (43,48). On the other hand, one United States study reported that tomato isolates were more genetically diverse than those from potato (50). The data presented here indicate that the NI-1 clonal lineage has completely replaced the old genotypes and is the primary pathogen on both potato and tomato. For this reason, the success of NI-1 clonal lineage on both host species is unique in a South American context. No aggressiveness tests were carried out in this study and, therefore, it is not known whether there are differences between potato and tomato isolates with regard to aggressiveness on the two host plants. Inclusion of the differentials for determining tomato races of *P. infestans* may have provided information as to any specialization but these were not available at the time of this study.

Although migrations of both mating types of *P. infestans* have made sexual reproduction of the pathogen theoretically possible, clonal lineages seem to dominate in most Latin American countries. This may be due to the fact that, in some Latin American regions (except the Toluca valley in Mexico), the climate is mild, there is a continuous potato production throughout the year, and there are more host plants available for the pathogen (including native solanaceous plants). It is also possible that, in these regions dominated by clonal lineages of *P. infestans*, antagonistic microbial processes limit the survival of oospores. In contrast, in the Toluca valley in central Mexico, there is substantial variation due to sexual reproduction (30). The high variation resulting from sexual reproduction of the pathogen could be due to a number of factors, including more seasonal production of potato in the Toluca valley versus continuous production in other Latin American countries. Moreover, the unique weather conditions (cool and dry climate) prevailing in the Toluca valley seems to favor the survival of the sexual structures (oospores) of *P. infestans* and foster sexual reproduction of the pathogen in that part of the world (30,51).

In conclusion, the current Nicaraguan population of *P. infestans* consists of a single clonal lineage (NI-1), which belongs to the A2 mating type and the Ia mtDNA haplotype. Moreover, based on SSR markers, this population does not resemble the *P. infestans* population of those countries which provide the potato tuber seed planted in Nicaragua. With regard to mating type, the Nicaraguan population of *P. infestans* is different from that of neighboring countries such as Costa Rica and Honduras. However, the Nicaraguan population of *P. infestans* is highly variable regarding race composition and has a high frequency of individuals resistant to metalaxyl. Unlike other countries in Latin America, this clonal lineage appears to dominate in both potato and tomato crops. The tools used in this study were not able to distinguish between the potato and tomato isolates, and it is not known if there are differences in aggressiveness between these isolates, though they share a common ancestry. Moreover, important issues such as survival of *P. infestans* from season to season, sources of primary inoculum that initiate epidemics at the beginning of the growing season as well as their competition, and the role of the native solanaceous plants as a reservoir of inoculum remain unknown and could clarify the reasons behind the survival and persistence of this clonal lineage despite probable incursions by other genotypes.

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