

Host Specificity of *Phytophthora infestans* on Tomato and Potato in Uganda and Kenya

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Introduction

Host specificity of *Phytophthora infestans*, the potato late blight (LB) pathogen, has obvious epidemiological consequences in areas where two or more potential hosts grow in close proximity. If inoculum can pass readily from one host to the other, or if more than one host is cultivated, then disease management activities must take both hosts into consideration. In addition to that practical consideration, specificity of *P. infestans* to potato (*Solanum tuberosum*) and tomato (*S. lycopersicon*) is an interesting phenomenon for study because it appears to be caused by quantitative differences in epidemic components, rather than an ability to cause disease (Oyarzun et al., 1998). Elucidation of the mechanisms that govern this type of host specificity could provide new insights into the nature of host-pathogen relations.

In spite of numerous studies on host specificity of *P. infestans* to potato and tomato, several aspects remain unclear. Many isolates attacking tomato and potato in some regions of the Netherlands (Fry et al., 1991) and in North America (Goodwin et al., 1995a; Legard et al., 1995) could not be distinguished by restriction fragment length polymorphism (RFLP) fingerprint, dilocus allozyme genotype, or mating type. That implies they might be of the same pathogen population. Furthermore, many isolates collected from tomato in North

America were highly aggressive on potato in a detached leaf assay (Legard et al., 1995), again indicating that one population attacks both hosts. In contrast, the same genetic markers have shown that distinct genotypes are associated with each host in Brazil (Brommonschenkel, 1988), northwest Mexico (Goodwin et al., 1992a), the Philippines (Koh et al., 1994), one region of the Netherlands (Fry et al., 1991), France (Lebreton and Andrivon, 1998), and Ecuador (Oyarzun et al., 1998).

Studying the population of *P. infestans* in North America, Legard et al. (1995) concluded that pathogenic aggressiveness on tomato evolved from within the potato-aggressive populations. All tomato-aggressive genotypes were still aggressive on potato, so aggressiveness on tomato was not associated with any measurable loss in aggressiveness on potato. However, this model of evolution toward aggressiveness on tomato is not universally accepted. Turkensteen (1973) argued that if a genotype of *P. infestans* were able to acquire equal aggressiveness on both hosts with no loss of fitness, it would rapidly replace other genotypes that are aggressive on only one host. The presence of host-specific populations in many parts of the world supports the hypothesis that dually aggressive genotypes do not readily occur, or if they occur they are less fit than those occurring on one host are. Geographical separation of hosts could theoretically lead to separate pathogen populations. In Ecuador, however, tomatoes and potatoes growing in close proximity to one another were found to have separate pathogen populations (Oyarzun et al., 1998).

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In cases where host specificity has been corroborated with molecular markers, the pathogen genotypes were from different clonal lineages. This adds an unquantified variable, because it is not known if the highly specialized forms of the pathogen evolved sympatrically, or were introduced from separate sources. An ideal system to study host specificity would involve two host-specific populations that evolved sympatrically. Potatoes and tomatoes are grown together or in close proximity to one another on small farms in Uganda and Kenya. Our preliminary investigation (unpublished data) indicated that isolates of *P. infestans* from that region may provide such a system. The objective of the research reported here was to test the hypothesis that potato and tomato populations from Kenya and Uganda differ but belong to the same clonal lineage. We also discuss evidence supporting sympatric evolution of host-specific populations in this region.

Materials and Methods

Collection of isolates

Data reported here come from two collection events. The first took place in March 1995 in Kenya and Uganda. A sample of 14 isolates from tomato and 10 from potato were collected in Kenya; 7 isolates from tomato and 8 from potato were collected in Uganda. The specific regions within countries were recorded (data available upon request). They are not reported here because we did not find variability associated with collection site. A second collection trip was made in Kenya and Uganda in late 1997 and early 1998. Fifty-eight isolates were collected on this trip, all from potato.

P. infestans was isolated from potato leaves by trapping the isolates in potato tuber slices (Forbes et al., 1997). *P. infestans* from tomato grew poorly on tuber slices and was, therefore, isolated by placing 1 cm² pieces of infected tissue on a selective medium (Oyarzun et al., 1998).

Characterization of isolates

RFLP fingerprints were obtained for all isolates from both collections using the moderately repetitive probe RG57 (Goodwin et al., 1992b). Two µg of DNA from each isolate were digested with *EcoRI* for 24 h, then underwent electrophoresis on 0.7 or 0.8% agarose gels (56 V, 20 mA) for 24-45 h in TBE 1X. Hybridization and detection were done using the nonradioactive kit ECL^{TM5} according to the manufacturer's instructions.

Mitochondrial DNA (mtDNA) haplotypes were determined for the 1995 collection by amplification of DNA of each isolate using primers designed for specific regions of the mitochondrial genome of *P. infestans* (Griffith and Shaw, 1998). Digestion of the amplified regions with *CfoI*, *MspI* and *EcoRI* restriction enzymes yielded band patterns by which the isolates could be classified into four haplotypes: Ia, Ib, IIa, and IIb (Carter et al., 1990; Griffith and Shaw, 1998).

Isozyme electrophoresis for the enzymes glucose-6-phosphate isomerase (Gpi) and peptidase (Pep) was done on cellulose acetate (Goodwin et al., 1995b) for the 1997-98 collection and on polyacrylamide gels for the 1995 collection. Polyacrylamide gel electrophoresis (PAGE) was done using 1 mm thick 7.5% gels with 25 mM Tris-0.19 M glycine, pH 8.8 as separating gel and electrode buffer. Bands were clearer when a 1 cm stacking gel (2.5% acrylamide 0.06 M Tris-HCl, pH 6.7) was used (Davis, 1964). PAGE gels were run with a constant current of 5 mA for 1 h, then increased to 10 mA. Voltage rose continuously throughout, from about 50 to 280 V. Electrophoresis was terminated when the bromophenol blue dye reached the bottom of the gel, about 16 cm. Allozyme genotypes for both cellulose acetate and PAGE were scored as described in Spielman (1991), which represent the relative mobilities of the enzyme alleles to

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an allele designated as 100. Isolates with known alleles from the collection of W. E. Fry were used for comparison.

The isolates collected in 1997-98 were tested for resistance to 5 and 100 mg/ml metalaxyl in 10% unclarified V8 medium and classified as resistant, intermediate, or sensitive. Conditions of the test and criteria for classification were described previously (Forbes et al., 1997).

Two pathogenicity tests were done to compare aggressiveness of a subset of isolates from 1995 on potato and tomato. In the first, five potato and five tomato isolates were inoculated on three potato cultivars (Yungay, Cruza-148, and Chata Blanca) and three tomato cultivars (Caribe, Flora Dade, and FMX-193). The second test involved six isolates from the 1995 collection. Four were repeated from the first test (two from each host) and two (one from potato and one from tomato) were assessed for the first time. The same potato and tomato cultivars were used in the second test. Experimental design, statistical models, and evaluation procedures were as reported previously (Oyarzun et al., 1998).

Results

All isolates collected in 1995 and 1997-98 belonged to the US-1 clonal lineage, based on RFLP fingerprint (Figure 1), Pep phenotype of 92/100, and, for the 1995 collection, an mtDNA haplotype of IB (Goodwin et al., 1994; Griffith and Shaw, 1998). All isolates from potato had the US-1 Gpi phenotype 86/100 (Goodwin et al., 1994), which is typical of US-1, but all tomato isolates were 100/100 at this locus (Figure 2).

A high level of metalaxyl resistance was found among the isolates collected from potato in 1997-98 (those collected in 1995 were not assessed). The overall percentage of resistant isolates was 73%, with 86% in Kenya and 59% in Uganda. Metalaxyl resistance was found in all districts

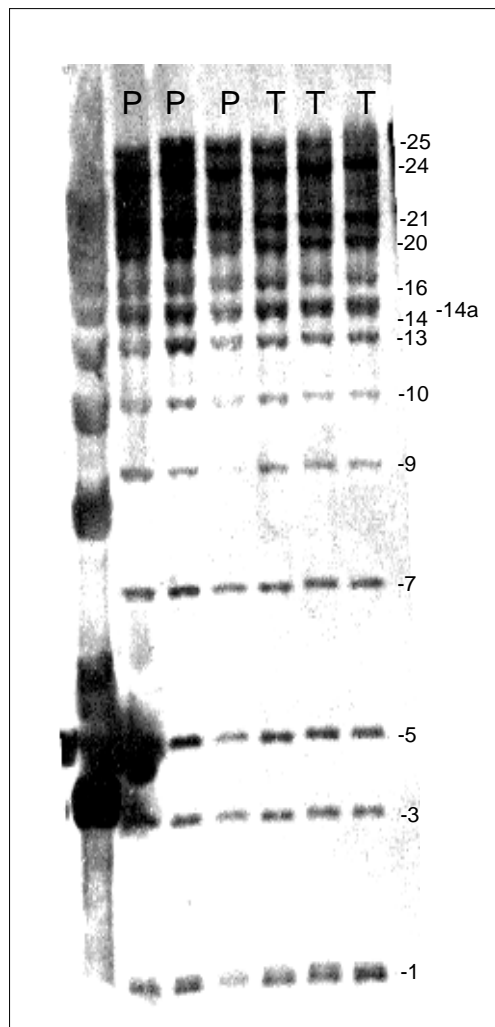


Figure 1. RFLP fingerprints of three potato (P) and three tomato (T) isolates collected in Kenya and Uganda in 1995. These are the same isolates shown in Figure 2. All represent a typical US-1 fingerprint. Numbers on the right represent conventional numbering of bands (Goodwin et al., 1992).

sampled. There were no pronounced differences between these areas. We have no quantitative information on the use of metalaxyl on potato in the two countries, but workers in the region believe that metalaxyl is seldom used on either crop (P. Ewell, CIP, Nairobi, pers. comm.).

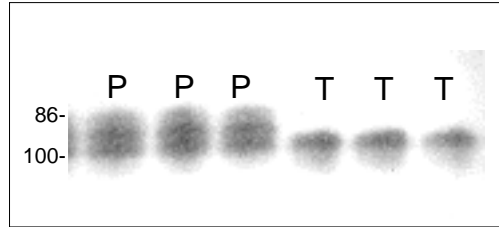


Figure 2. Glucose-6-phosphate isomerase banding pattern for three potato and three tomato isolates collected in Kenya and Uganda in 1995. These are the same isolates shown in Figure 1. The three potato isolates have an 86/100 phenotype; the tomato isolates are 100/100. Electrophoresis was done with cellulose acetate.

The 1995 sample consisted of A1 isolates only. The isolates from the 1997-98 sample were tested for mating type at Cornell University and also found to be A1. Earlier they were paired with an A1 tester in Kenya, and the number of oospores was low (1-10/plate) in 20% (13/66) of the pairings. Various oospore counts were also found in 31% (18/58) of the 1997-98 isolates of single cultures. A few isolates produced hundreds or even thousands of oospores in single culture. However, selfing and mating results were not strictly repeatable.

The interaction between the host and the source of the pathogen (potato or tomato) was evident by visual examination of plotted means of lesion length (Figure 3). This interaction was highly significant for both tests at $P < 0.0001$ (Table 1). Lesions on both hosts produced by isolates from potato were always accompanied by noticeable necrosis (Figure 3). Isolates from tomato produced no necrosis on tomato leaves during the 7 days after infection, even though abundant sporulation could be seen. On the other hand, when inoculated onto potato, these same isolates induced necrosis. We conclude that host specificity is quantitative rather than qualitative, because isolates were more aggressive on their primary hosts. This is similar to a situation recently described in Ecuador

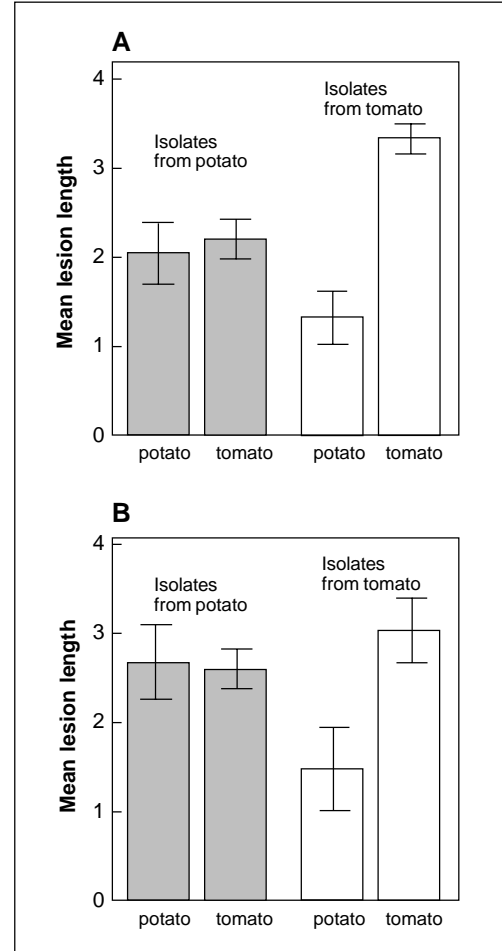


Figure 3. Histograms of mean lesion length on potato and tomato plants caused by isolates of *P. infestans* collected from potato in Kenya and Uganda in 1995. (A) Data from seven isolates each from tomato and potato. (B) For the second test, four isolates were repeated (three from each host), and two new isolates were assessed (one from each host). Error bars represent 95% confidence intervals.

(Oyarzun et al., 1998) for isolates of different clonal lineages attacking potato and tomato.

Discussion

Our data clearly show that the pathogen populations attacking potato and tomato in Kenya and Uganda are different from each

Table 1. Analyses of variance from two experiments that test effects of origin (potato or tomato) of isolates of *Phytophthora infestans* and inoculated host species (potato or tomato) on diameter of lesions (cm) in a detached-leaf inoculation assay involving isolates collected in Kenya and Uganda in 1995.

Source	Degrees of freedom	Mean square	F Value	Pr > F
First experiment				
Isolate origin (O)	1	3.71	NT ^a	
Host species (H)	1	17.23	NT	
O * H	1	21.19	58.78	0.0001
Isolate embedded in Origin (I ₀)	8	3.37	9.36	0.0001
Plant embedded in host (P _H)	4	14.43	40.00	0.0001
I ₀ * P _H	43	0.36	NT	
Residual error ^b	60	0.25		
Second experiment				
Isolate origin (O)	1	1.52	NT	
Host species (H)	1	42.9	NT	
O * H	1	30.6	63.52	0.0001
Isolate embedded in Origin (I ₀)	4	5.43	11.27	0.0001
Plant embedded in host (P _H)	4	3.27	6.79	0.0001
I ₀ * P _H	24	0.48	NT	
Residual error ^b	108	0.38		

^a NT = not tested. Main effects of isolate origin and host species were not tested because of their highly significant interaction, O * H. This interaction was tested using the mean square for the interaction between individual isolates and plants, Isolate * Plant (O*H). This interaction was also used to test Isolate (O), isolate embedded in origin, and Plant (H), plant genotype embedded in plant species.

^b Based on variance among petridishes, which are pseudo replications of the experiment.

other. Although all isolates had the US-1 RFLP fingerprint, isolates from tomato and potato could be separated based on Gpi genotype, aggressiveness on the two hosts, symptoms on tomato, and even isolation characteristics. Isolates from potato were easily trapped on potato tuber slices; isolates from tomato were most easily isolated with selective medium.

Specialization of *P. infestans* on potato and tomato has been detected at other sites (Brommonschenkel, 1998; Fry et al., 1991; Goodwin et al., 1992a; Koh et al., 1994; Oyarzun et al., 1998). In those cases the two populations belonged to different clonal lineages, or were different genotypes from areas where sexual recombination occurs. Sub-Saharan Africa seems to be the only area studied to date where two

coexisting, host-specific populations belong to the same clonal lineage.

Although our research demonstrates a common origin for these populations, it does not prove that they developed sympatrically. The population attacking tomato could have been an introduced population that evolved independently of potato LB. We believe, however, that it is more likely that the tomato population in Kenya and Uganda evolved from the potato population and was not introduced. The principal reason for this is the apparent scarcity of transport mechanisms by which it would have been introduced. We are unaware that tomato plantlets or fruits are imported into this region, nor is there evidence that *P. infestans* can be transported with tomato seed.

Potato tubers are generally considered to be the primary long-distance transport mechanism for the LB pathogen (Fry et al., 1993). Reduced aggressiveness of the tomato population on potato foliage and tubers argues against this type of transport. It is more likely that *P. infestans* was introduced into the region on potato and then evolved aggressiveness on tomato. This view is consistent with that of the authors of a recent study in the USA (Legard et al., 1995), who proposed that tomato aggressiveness develops within potato populations. Our data, however, indicate that aggressiveness on tomato in Kenya and Uganda is associated with a loss of aggressiveness on potato. Our isolates from tomato were less aggressive on potato than those isolated from the potato host. Our tomato isolates also did not grow well in potato tubers, as evidenced by the difficulty in isolating them with tuber slices.

The time at which the mutation of the Gpi locus occurred remains unclear, as well as factors which may have led to the apparent universality of 100/100 Gpi phenotype within the tomato population. Mutation from 86/100 to 100/100 within the US-1 clonal lineage has been reported previously (Forbes et al., 1998; Goodwin et al., 1994). There is no evidence, nor logic we know of, that would suggest a genetic linkage between tomato aggressiveness and the Gpi genotype 100/100. In South America, the 86/100 Gpi genotype occurs in the US-1 clonal lineage attacking tomato (Brommonschenkel, 1988; Oyarzun et al., 1998).

Our data also demonstrate that the populations of *P. infestans* in this region are not substructured geographically. In none of the characters we assessed was there any detectable polymorphism that could be associated with a geographical region. The area that was sampled in Uganda is in the southwestern part of the country, the principal potato-growing region. It is contiguous with the potato-growing region of Rwanda, so our results can probably be extrapolated to Rwanda.

The high level of resistance to metalaxyl is noteworthy because it demonstrates the degree to which this characteristic can develop within the US-1 lineage. Metalaxyl resistance has frequently been associated with other lineages or sexual populations that have occurred as a result of recent migrations of the pathogen (Fry et al., 1993; Goodwin, 1997).

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