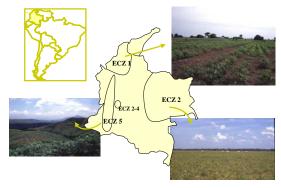
# Population dynamics of *Xanthomonas axonopodis* pv. *manihotis* populations in Colombia from 1996 to 1999

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# INTRODUCTION

Cassava Bacterial Blight (CBB), caused by Xanthomonas axonopodis pv. Manihotis (Xam) is a major disease of cassava, (Manihot esculenta Crantz). The most realistic way to control the disease is the use and deployment of resistant cultivars. The change in the pathogen population structure may result in the overcoming of the deployed resistance as it was recently observed in Africa. The spatial and temporal context in which plant-pathogen associations occur is very important in determining the evolution of host-pathogen interactions. RFLP analysis was used to assess the population structure and genetic diversity of 643 strains of X. a. pv. manihotis collected between 1996 and 1999 in Colombia. Temporal changes and population dynamics were addressed by comparing samples from four years in six locations spanning four different edaphoclimatic zones (ECZs).



Spatial distribution of the Xam populations in the different ECZs in Colombia

#### **RESULTS AND DISCUSSION**

⊗ Over the 5-year period, 42 different haplotypes were found in 6 different sites. The total genetic diversity was 0.7 in Colombia. A very large part of the total variation was within populations showing a high level of polymorphism at the field level. A large part of variation was also ascribed to differences among ECZs confirming the geographical differentiation of the diversity found in 1995-1996.

Source of variation	Variance component	%of total	
Among ECZs	1.29	29.3	
Among sites/within ECZs	0.53	12	
Within sites	2.57	58.7	

③ Genetic diversity in Xam was being maintained from year to year. AMOVA analysis ascribed most of the total variation to differences among the years that differences within a year.

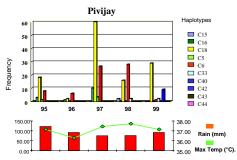
	%	Variation ascrib	ed to Year of	f collection			
(among/within)							
Cajibio	Mondomo	Villavicencio	Carimagua	Pivijay	Stdr Quilichao		
ECZ5	ECZ5	ECZ2	ECZ2	ECZ1	ECZ2-4		
0/100	36/64	20/80	3/97	17/83	40/60		

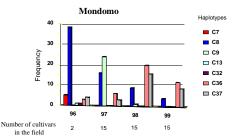
③ Chi-square tests showed that differences in haplotype frequencies over the 5year were statistically significant, except for Cajibio, indicating that the populations of *Xam* at the site level were unstable.

Site	ECZ	Year	H <sub>Year</sub>	H <sub>SITE</sub>	$X^2$
Pivijay	1	1995	0.58		
		1996	0.56	0.69	118.8 (0.001) <sup>2</sup>
		1997	0.56	0.09	118.8 (0.001)
		1998	0.52		
		1999	0.46		
Carimagua	2	1995	0.83	0.87	113.5 (0.001)
		1996	0.83	0.87	115.5 (0.001)
		1997	0.78		
Villavicencio	2	1995	0.81		
		1996	0.91	0.80	201.1 (0.001)
		1997	0.64		
		1998	0.62		
Mondomo	5	1996	0.46		
		1997	0.65	0.73	120.1 (0.001)
		1998	0.69		
		1999	0.69		
Cajibio	5	1995	0.26	0.22	2.7 (0.446)
		1996	0.15		
Stder Quilichao	2-4	1996	0.30	0.58	66 (0.001)
		1997	0.26	0.58	00 (0.001)
		1998	0		

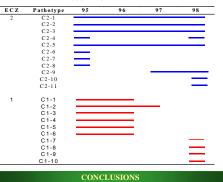
a The chi-square value was significant at the 5% level.

<sup>®</sup> Several evolutionary forces interact to structure the populations of plant pathogens. Migration of strains between fields located in the same ECZ has been a very important factor to maintain the high levels of Xam diversity withinthe ECZs. Genetic drift might play a role in Pivijay (ECZ1) after the El Niño phenomenon in 1997. During 1997, the environmental conditions were not conducive for CBB development. Our results suggested that the host also played a role in causing pathogen differentiation. In Mondomo, population change and diversity was higher with the introduction of new cultivars.





③ Different pathotypes were defined in ECZs 2, 1 and 5 (11, 10 and 1 respectively) from 1995 to 1998. Some pathotypes were found in all years. New pathotypes appeared in 1997 in ECZ2 after the introduction of new cultivars and in 1998 in ECZI after El Niño.



③ The Xam population has shown a high degree of genetic diversity and the role of different evolutionary forces in structuring pathogen population was assessed.

The next step is to determine the prevalence and distribution of virulence characteristics and to use the knowledge on the dynamics within the bacterial population to predict the incidence of the disease and diversity of the pathogen using GIS and geostatistics.

## REFERENCES

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Symptoms induced by Xanthomonas axonopodis pv. manihotis

## MATERIALS AND METHODS

#### Xam isolates.

643 strains strains were isolated from cassava stem or leaf samples collected between 1995 and 1999 in six sites that were located throughout four ECZs: Villavicencio and Carimagua (ECZ2), Pivijay (ECZ1), Mondomo and Cajibio (ECZ5), and Santander de Quilichao (ECZ2-4).

#### RFLP using pthB as probe.

RFLP markers were performed as previously described (Restrepo and Verdier, 1997). The probe, designated as *pthB*, harbors a pathogenicity gene related to the *avr/pth* gene family.

#### Pathogenicity tests

Virulence patterns of strains representing the different RFLP haplotypes described in Colombia from 1995 to 1999 were determined. Strains were inoculated as previously described by stem puncture on a set of differential cultivars previously selected (Restrepo *et al.*, 2000).

#### Statistical analysis.

Banding patterns of hybridization were used to characterize the genetic diversity of *Xam* populations. Each distinct RFLP banding pattern, obtained after hybridization with probe *pthB*, was regarded as a **haplotype**. The haplotype diversity was estimated using the Nei and Tajima index (Nei and Tajima, 1981)

## $H = [n/(n-1)](1 - X_i^2)$

where  $X_i$  is the proportion of the *i*th distinct *pth*B haplotype, and *n* is the number of strains.

Significance testing for differences in haplotype frequencies over the years in each site or ECZ was performed by a chi-square analysis. Analysis of molecular variance (AMOVA) was used to estimate variance

Analysis of molecular variance (AMOVA) was used to estimate variance components for RFLP haplotypes, partitioning the variation among individuals within and among sites, ECZs and years. The analysis were undertaken with the ARLEQUIN program (Excoffier *et al.*, 1992).