Activity 1. Detection of a phytoplasma associated with frogskin disease in cassava (*Manihot esculenta* Crantz) in Colombia

Introduction

Frogskin disease (FSD) was first reported in 1971, in the Department of Cauca, southern Colombia, apparently originating from the Amazon region of either Brazil or Colombia (Pineda and Lozano 1981). The disease has since spread throughout Colombia (Atlantic Coast, and Departments of Cauca, Valle de Cauca, Vaupés, and Putumayo), Venezuela (States of Amazonas, Aragua, Barinas, Cojedes, Monagas, and Portuguesa) (Chaparro-Martínez and Trujillo-Pinto 2001), and Brazil.

Frogskin disease directly affects root production, causing yield losses of 90% or more. Although symptoms vary according to temperature and genotype, the roots become thin and woody, and starch content is very low. The causal agent has not been identified, although research so far suggests that FSD may have a viral etiology and may be transmitted by an aerial vector.

Frogskin disease can be controlled by using tolerant varieties, healthy vegetative planting materials, and adequate plant health management.

Materials and methods

Plant tissue. Several molecular and microscopy staining techniques were applied to detect phytoplasmas in plant tissues from cassava (*Manihot esculenta*, 10 samples), periwinkle (*Catharanthus roseus,* 4 samples), and naranjilla or *lulo* (*Solanum quitoense,* 2 samples). Vegetative tissues from the following FSD-infected cassava varieties were used: CM 849-1, SM 1219-9, Parrita, and M Bra 383, all harvested at Jamundí, Valle de Cauca, Colombia. The plants used were about 12 months old. The roots were severely infected by FSD. The leaves and flowers did not show visible symptoms caused by phytoplasmas (such as witches' broom) or viruses. Samples of healthy 'Secundina', obtained by *in vitro* culture of meristem tips, were used as negative control. Infected plants from plots at CIAT (Palmira) were also included in the analysis.

Microscopy. Two staining methods were used: DAPI (4,6 diamidine 2-phenylindole), which stains the phloem (Sinclair et al. 1989); and Dienes' stain, which metabolizes and produces a blue color (Deeley et al. 1979).

DNA extraction. Total DNA was extracted as described by Gilbertson and Dellaporta (1983) from samples of each of the following tissues: roots, stems, petioles, leaf midribs, and flowers of FSD-infected and healthy cassava plants. DNA was also extracted from the leaves of naranjilla and periwinkle, infected by phytoplasmas. DNA was diluted in sterilized deionized water to a final concentration of 20 ng/µL.

Direct and nested PCR. DNA samples were amplified in a nested PCR. For the first amplification, we used the primer pairs P1/P7 or R16mF2/R16mR1 **(Table 1)** under the following conditions: 120 ng of diluted DNA, 1X buffer, 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.1 µM

of each primer, and 1U *Taq* polymerase. Thirty-five cycles were conducted in a PTC–100 thermocycler (Programmable Thermal Controller, MJ Research, Inc., Watertown, MA) as follows: 1 min (2 min for the first cycle) denaturation step at 94˚C, annealing for 2 min at 55˚C, and primer extension for 3 min (10 min in final cycle) at 72˚C. PCR products were diluted at 1:10 with sterilized deionized water. For the nested PCR, we used 2 µL of diluted PCR to amplify with the primer pair R16F2n/R16R2 as described above, but using an annealing temperature of 50˚C. PCR products were analyzed by electrophoresis on 1.2% agarose gels and photographed, using an Eagle Eye II I image analyzer (Stratagene, La Jolla, CA).

Table 1. Primers used for PCR amplification and sequencing of 16S rRNA genes of plant pathogenic phytoplasmas.

Primer	Sequence 5' - 3'	Reference
R16R2	TGACGGGCGGTGTGTACACCCG	Gundersen and Lee (1996)
R16mF2	CATGCAAGTCGAACGGA	Gundersen and Lee (1996)
R16mR1	CTTAACCCCAATCATCGAC	Gundersen and Lee (1996)
R16F2n	GAAACGGCGGTGTGTACAAACCCCG	Gundersen and Lee (1996)
P1	AAGAGTTTGATCCTGGCTCAGGATT	Deng and Hiruki (1991)
P7 (23S)	CGTCCTTCATCGGCTCTT	Smart et al. (1996)

RFLP analyses. The nested-PCR products of the controls, and the 16S rDNA sequences of cassava, periwinkle, and naranjilla were amplified with primer pair R16F2n/R2. A 5-µL aliquot of each PCR product (1.2 kb) was digested with each of the restriction endonucleases *Alu*I and *Rsa*I according to manufacturer's instructions (Promega, Madison, WI). The restriction products were then analyzed on a 2% agarose, visualized, and saved in a gel documentation system (Eagle Eye II, Strategene). The restricted-DNA patterns of infected cassava, periwinkle, and naranjilla were compared with the RFLP patterns produced by the control strains.

Cloning, transformation, and sequencing of DNA. Six PCR products were sequenced directly, using a DNA-sequencing kit from Applied Biosystems, with 3 µL water, 1 µL primer, 4 µL mix from kit, and 1 µL DNA. The PCR products were purified, using the QIAquick PCR Purification Kit (QIAGEN), ligated in pGEM-T Easy vector, which was introduced into the *Escherichia coli* strain DH5-α by electroporation at 2.4 kV/cm^2. Transformants were selected on blue/white color screening by plating on LB/ampicillin/IPTG/X-gal media. Plasmids were extracted with a Plasmid Miniprep System Kit (Gibco-BRL). Positive inserts were observed by plasmid restriction with *Eco*RI and electrophoresis in 1.5% agarose gel. Different-sized fragments were selected for sequencing by automated dideoxy sequencing (ABI Prism 377-96 DNA Sequencer), using a DNA-sequencing kit from Applied Biosystems, with 3 µL water, 1 µL primer, 4 µL mix from kit, and 1 µL DNA. Sequences were analyzed with Sequencer 4.1 software and matched by nucleotide, using the Blastn tool in GenBank (**www.ncbi.nlm.nih.gov**).

Results and discussion

CIAT's Cassava Pathology programmed a series of activities aimed at identifying the possible causal agent of FSD in cassava. The principal advances are summarized below.

For many crops, the causal agents of similar diseases were considered to be viruses. However, over the last 20 to 25 years, the causal agents were found to be phytoplasmas. For example, lethal yellowing disease in the coconut palm was reported by Nutman and Roberts (1955) as being viral, whereas Beakbane et al. (1972), Heinze et al. (1972), Plavsic-Banjac et al. (1972), and (Mariau et al. 2002) all identified the causal agent as being a phytoplasma.

In this study, we present evidence that FSD is associated by a phytoplasma and that, by applying molecular tools and microscopy, we successfully detected phytoplasmas in FSD-infected cassava roots, leaf midribs, petioles, and peduncles.

The specific primers R16mF2/R16mR1 and R16F2n/R16R2 were successfully used in a nested-PCR assay to detect and confirm that phytoplasmas were associated with FSD.

To detect and subsequently classify the phytoplasmas, two pairs of universal primers (P1/P7 and R16F2n/R2) were used to amplify the 16S rDNA gene. A 1.2-kb fragment was amplified from all samples, including infected roots **(Figure 1)**. This fragment was present only in samples collected from plants showing visible external symptoms in the roots. Direct PCR, using the primers R16mF2 and R16mR1 also detected phytoplasmas.

The presence of phytoplasmas in roots, stems, petioles, leaf midribs, and flowers was confirmed by DAPI and Dienes' stain by microscopy **(Figures 2** and **3)**.

Sequence analysis of the cloned fragment **(Figure 4)** revealed that the cassava phytoplasma was similar to the chinaberry yellows phytoplasma (GenBank acc. no. AF495657, 16SrXIII Mexican periwinkle virescence group) and cirsium white leaf phytoplasma (GenBank acc. no. AF373106, 16SrIII X-disease group), both with a sequence homology of 100% and 99% in two partial fragments with a total of 1.01 kb **(Table 2)**. The sequence length was 1202 bp **(Table 3)**.

According to the RFLP patterns with *Rsa*I, the cassava phytoplasma was similar to that for naranjilla, whereas that for periwinkle was different again. *Alu*I did not highlight differences among the samples **(Figure 5)**. Future research will involve evaluation with another group of enzymes, and sequence analysis will be carried out to classify the phytoplasmas.

We have already started studies on the transmission of the causal agent of FSD. Remission experiments, using chlortetracycline, with cassava, periwinkle, and poinsettia are being conducted, and we will need to determine the role of phytoplasmas in this destructive disease.

This is the first report of phytoplasmas being associated with FSD in cassava. Future research topics will include the development of molecular detection methods, vector identification, and classification of phytoplasmas associated with FSD. The design of novel approaches to achieve effective control will remain a constant goal.

Table 2. Homology found between DNA obtained from cassava infected by FSD by nested PCR and phytoplasma sequences reported in GenBank.

Matching in GenBank	GenBank number	Sense	Homology score (bits)	Probability of higher homology	Homologued fragment (bp)	Identities	
						Absolute	(%)
Cirsium white leaf phytoplasma rRNA operon B	AF373106.1	5'- 3'	1084	0.04	546	546	100.0
		3'- 5'	856	0.0	465	459	98.7
Chinaberry yellows phytoplasma 16S rRNA gene	AF495657.1	5'- 3'	1084	0.0	546	546	100.0
		3'- 5'	872	0.0	465	461	99.1
Chayote witches' broom phytoplasma ChWBIII strain 16S rRNA gene, 16S–23S rRNA intergenic	AF147707	5'- 3'	1076	0.0	546	545	99.8
		3'- 5'	856	0.0	465	459	98.7
Poinsettia branch-inducing phytoplasma rRNA operon B	AF190223	5'- 3'	1068	0.0	546	544	99.6
		3'- 5'	856	0.0	465	459	98.7
Gaillardia phyllody phytoplasma 16S rRNA gene	AY049029	5'- 3'	1060	0.0	542	540	99.6
		3'- 5'	872	0.0	465	461	99.1
Dandelion virescence phytoplasma rRNA operon B	AF370120.1	5'- 3'	1045	0.0	546	541	99.1
		3'- 5'	864	0.0	465	460	98.9

Table 3. Sequences of a phytoplasma obtained from cassava infected by frogskin disease.

Identification	Size (bases)	Sense	Sequence
PCR-6RF Phytoplasma	546	Forward	TTGAAGGTATGCTTAAGGAGGGGCTTGCGACACATTAGTTAGTTGGCAGGGTAAAGGCCTACCAA GACTATGATGTGTAGCTGGACTGAGAGGTTGAACAGCCACATTGGGACTGAGACACGGCCCAAAC TCCTACGGGAGGCAGCAGTAGGGAATTTTCGGCAATGGAGGAAACTCTGACCGAGCAACGCCGCG TGAACGATGAAGTACCTCGGTATGTAAAGTTCTTTTATTAAGGAAGAAAAAAGAGTGGAAAAACT CCCTTGACGGTACTTAATGAATAAGCCCCGGCTAATTATGTGCCAGCAGCCGCGGTAATACATAAG GGGCGAGCGTTATCCGGAATTATTGGGCGTAAAGGGTGCGTAGGCGGTTTAATAAGTCTATAGTTT AATTTCAGTGCTTAACGCTGTTGTGCTATAGAAACTGTTTTACTAGAGTGAGTTAGAGGCAAGCGG AATTCCATGTGTAGCGGTAAAATGCGTAAATATATGGAGGAACACCAGAGGCGTAGGCGGCTTGC TGGGACTTTACTGACGCTGAGGC
PCR-6RR2 Phytoplasma	593	Reverse	CGAAATGCTGATTCGCGATTACTAGCGATTCCAACTTCATGAAGTCGAGTTGCAGACTTCAATCCG AACTGAGATTGATTTTGTGAGATTGGCTAAGAACTCGCGTTTCAGCTACTCTTTGTATCAACCATTG TATCACGTTTGTAGCCCAGATCATAAGGGGCATGATGATTTGACGTAATCCCCACCTTCCTCCAAT TTTTCATTGGCAGTCTCGTTAAAGTCCCCATCATTACATGCTGGCAATTAACGACAAGGGTTGCGC TCGTTTTAGGACTTAACCTAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTTTTC CTGATAACCTCCATTATATTTCTATAACTTCGCAAGAAAATGTCAAGACCTGGTAAGGKTTTTCGT GTATTCTTCGAAATTAAACAACATGGATCCACCGCTTGTGCGGAGTCCCGTCAATTCCTTTAAGTTT CATACCTTGCGTAACGGNACTACTCAGGCGGGAGGACTTAATGGTGTTAAACTTTCAANAAACCG GGGTTTACCCGGAACACYTTAANTACCTCAATTCGGTTTACGGGNGGTKGGGACCTACCCAGGG

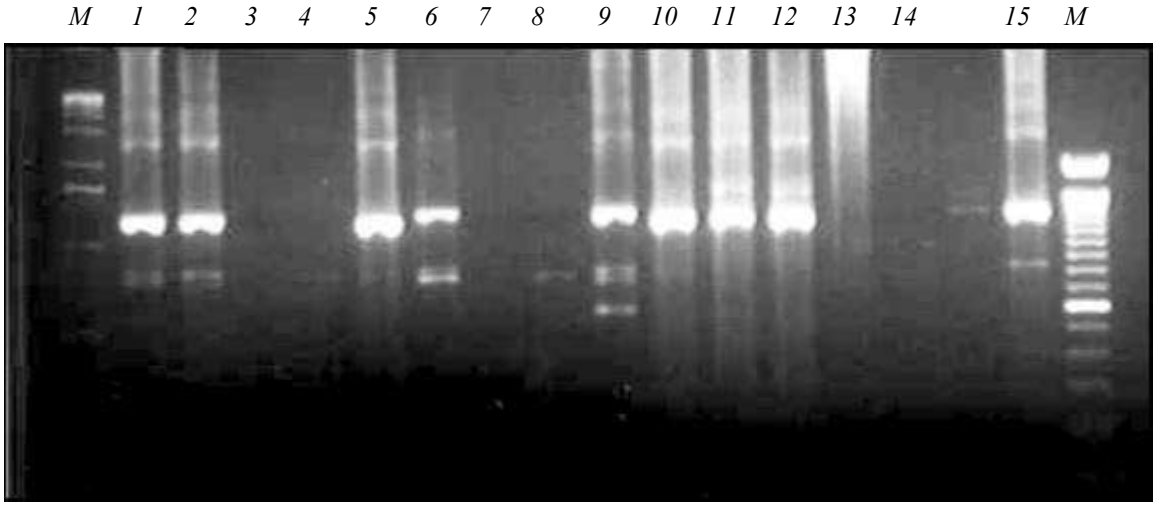

Figure 1. Nested PCR of infected and healthy plant tissues, using primers R16MF2, R16MR1/R16F2N, R16FR2. Lanes 1 and 2 = infected cassava roots (pulp); lanes 3 and 4 = leaves and shoot from healthy cassava plants; lane 5 = stem tissue from an infected cassava plant; lane 6 = petiole from an infected cassava plant; lanes 7 and 8 = peel from infected cassava roots; lanes 9 and 10 = infected cassava roots; lanes 11 and 12 = leaf tissue from naranjilla and periwinkle, respectively; lane 13 = degraded DNA from periwinkle; lane 14 = negative control without DNA; lane 15 = positive control; lane M = bp ladder.

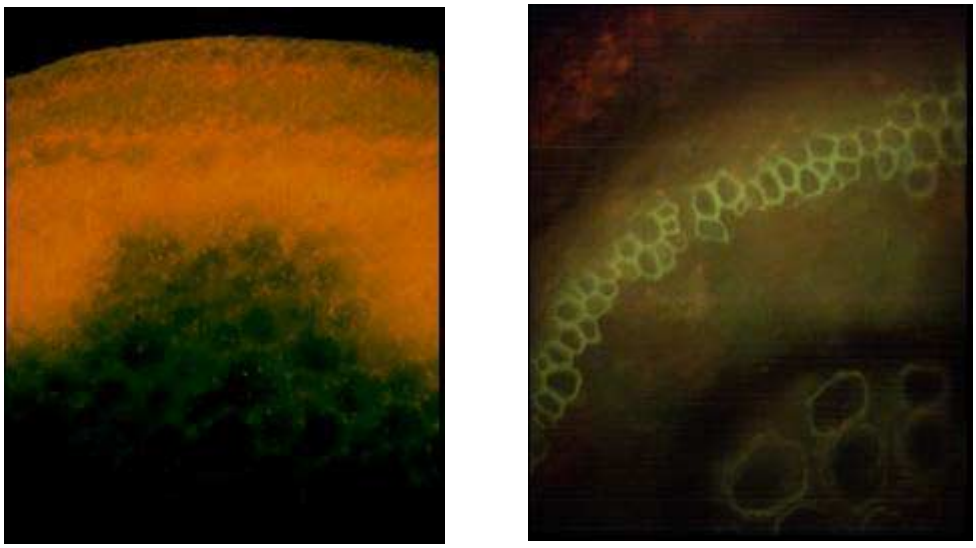

Figure 2. DAPI stain of healthy (A) and infected (B) cassava leaf tissue.

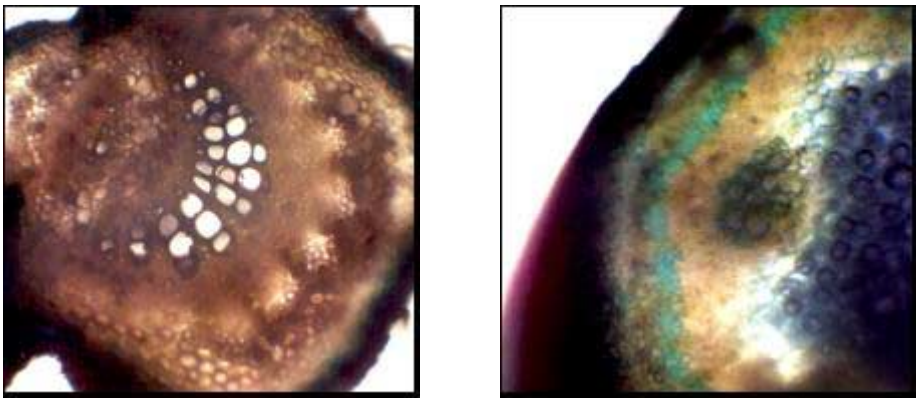

Figure 3. Dienes' stain of healthy (A) and infected (B) cassava leaf tissue.

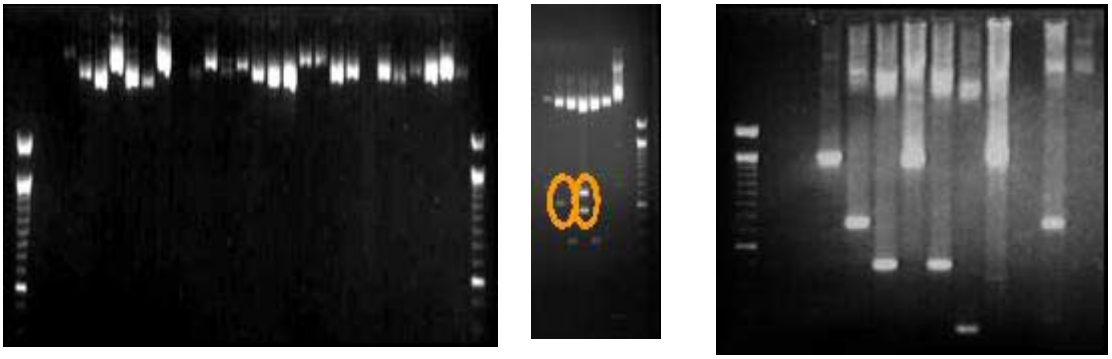

Figure 4. Cloning and restriction with *Eco*RI: (A) plasmids; (B) their restriction; and (C) PCR to confirm presence of inserts in the plasmids.

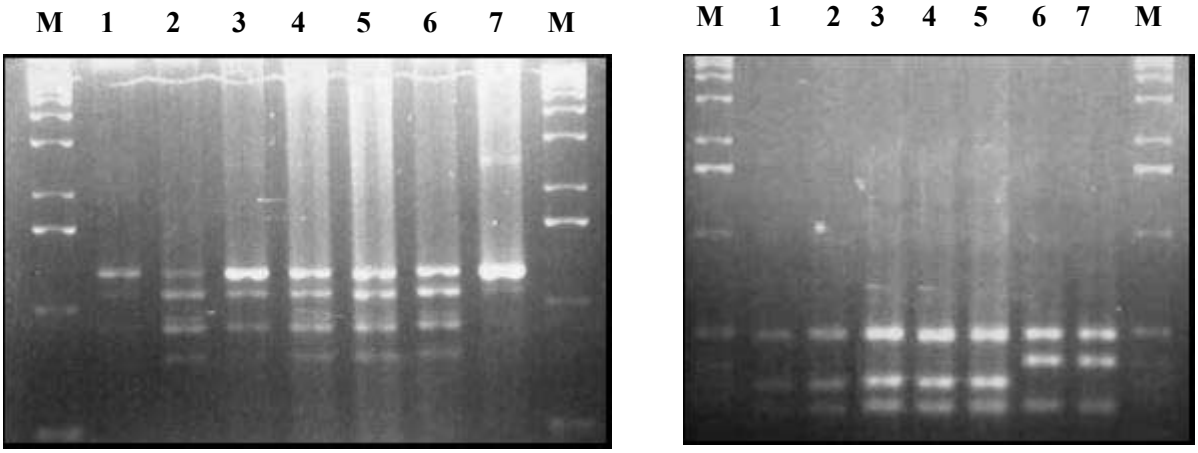

Figure 5. RFLP patterns obtained: (A) *Alu*I; (B) *Rsa*I. Lanes 1-3 = cassava; lanes 4 and 5 = naranjilla; lanes 6 and 7 = periwinkle; lane M = bp ladder.

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Activity 2. Characterization of *Xanthomonas axonopodis* pv. *manihotis*, causal agent of Cassava Bacterial Blight

Introduction

Bacterial blight, caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), provokes heavy losses in cassava production. By using a technique with restriction fragment length polymorphism (RFLP) with *pth*B, 26 haplotypes of the bacterium was found to exist in Colombia, concentrating mainly in the Atlantic Coast and Eastern Plains (Restrepo and Verdier 1997). The pathogen's population in Colombia was thus shown to be highly heterogeneous. Restrepo et al. (1999), used a technique with amplified fragment length polymorphism (AFLP) as described by Voss et al. (1995) to detect polymorphism in bacterial populations. This extremely useful technique is highly reproducible, and can be used to study genetic diversity, particularly in populations where analyses with RFLP/*pth*B present low levels of diversity.

In our study, by using AFLP, we characterized 100 *Xam* isolates from different regions of Colombia, Venezuela, Brazil, and Cuba to analyze genetic variability in the bacterium.

Materials and Methods

Isolates. We used 100 isolates of *Xam* **(Table 1)**, collected from different cassava varieties in Colombia, Brazil, Venezuela and Cuba. Most of the isolates were from the CIO collection held by the Biotechnology Unit at CIAT and ORSTOM, while others were obtained from the Cassava Pathology Laboratory at CIAT.

Isolating DNA. *Xam* isolates, conserved in 60% glycerol at –80°C, were cultured onto YDCA medium (5 g yeast extract, 15 g agar, 5 g dextrose, and 10 g sodium carbonate) and left to grow overnight. DNA was later extracted, following the protocol by Boucher et al. (1985). The DNA was then dissolved in 100 µL of TE buffer (Tris-EDTA pH 8.0), and its concentration determined in a spectrophotometer. DNA quality was checked in 0.8% agarose gel.

AFLP. A 1000-ng sample of DNA was digested with two restriction enzymes (*Eco*RI and *Mse*I). The digested fragments were ligated with their respective adapters. Later, 5 µL of the restriction-ligation reaction were amplified, followed by a selective amplification of those fragments that had the nucleotide sequence annexed during ligation. For such amplification, eight combinations of primers were evaluated with five isolates, of which EC/MA as selected Gibco-BRL, AFLP Analysis System for Microorganisms).

Table 1. Description of isolates of *Xanthomonas axonopodis* pv. *manihotis*, causal agent of cassava bacterial blight, used in this study.

Isolate No.	Identification	Genotype	Source	Location	Isolate No.	Identification	Genotype	Source	Location
1	CIO 6	*Manihot* sp.		Brazil	44	CIO 353	SRT 1319	Leaf	Brazil
2	CIO 7	*M. esculenta*	Stem	Brazil	45	CIO 356	IAC 576-70	Leaf	Brazil
3	CIO 8	*M. esculenta*	Stem	Brazil	46	CIO 367	SRT 1363	Leaf	Brazil
4	CIO 29	M Pse-004 Sylv	Stem	Meta-Colombia	47	CIO 465	AM 244-17	Leaf	Atlántico-Col.
5	CIO 34	SM 593-8	Stem	Meta-Colombia	48	CIO 469	M Col 2215	Leaf	Atlántico-Col
6	CIO 63	Mona Blanca	Stem	Sincelejo-Col	49	CIO 485	Black -Stick	Leaf	San Andrés-Col
7	CIO 66	M Col 1505	Stem	Sincelejo-Col.	50	CIO 500	Cod 88	Exudate	Meta-Col
8	CIO 68	M Col 2215	Stem	Bolívar-Col.	51	CIO 507	Cod 702	Stem	Meta-Col
9	CIO 71	M Col 2215	Leaf	Bolívar-Col.	52	CIO 725		Leaf	Latipilla-Cuba
10	CIO 148	CM 7274-1	Exudate	Meta-Col.	53	CIO 762	Brava-nativa	Leaf	Vaupés-Col
11	CIO 184	Var. Paraguai	Stem	Brazil	54	CIO 764	Brava-nativa	Leaf	Vaupés-Col
12	CIO 187	M. Joliana	Stem	Brazil	55	CIO 806	M Col 2215	Leaf	Magdalena-Col
13	CIO 191	Cacho Toro	Stem	Venezuela	56	CIO 833	M Ven 25	Leaf	Magdalena-Col
14	CIO 192	Tres Brincos	Stem	Venezuela	57	CIO 841	SM 1791-40	Stem	Córdoba-Col
15	CIO 210	M Ven 77	Stem	Venezuela	58	CIO 849	CM 6119-5	Leaf	Córdoba-Col
16	CIO 212	Lancetilla Negra	Stem	Venezuela	59	CIO 854	SM 1411-5	Leaf	Córdoba-Col
17	CIO 214	SG 104-57	Stem	Venezuela	60	CIO 856	CG 1141-1	Leaf	Atlántico-Col
18	CIO 241	Caribe Medio	Stem	Venezuela	61	CIO 901	M Nga 2	Exudate	Brazil
19	CIO 243	Bonifacio	Stem	Venezuela	62	CIO 905	K45	Exudate	Brazil
20	CIO 249		Stem	Venezuela	63	CIO 909	K140	Exudate	Brazil
21	CIO 259	Paigua Negra		Venezuela	64	CIO 910	K9 P4	Stem	Cauca-Col
22	CIO 276	Tres Brincos		Venezuela	65	CIO 911	K9 P4 Parc 483 Pt 2	Leaf	Cauca-Col
23	CIO 277	Tres Brincos		Venezuela	66	CIO 954	K33	Leaf	Meta-Col
24	CIO 278	Tres Brincos		Venezuela	67	CIO 961		Leaf	Brazil
25	CIO 280	Tres Brincos		Venezuela	68	CIO 964	Yuca dulce	Leaf	Amazonas- Col
26	CIO 281	Paigua Negra		Venezuela	69	CIO 974	Parc 25 Pt 4 MCOL 2215	Leaf	Magdalena-Col
27	CIO 282	Paigua Negra		Venezuela	70	CIO 988	T8	Leaf	Magdalena-Col
28	CIO 283	Paigua Negra		Venezuela	71	CIO 1017		Leaf	Brazil
29	CIO 286	IAC 114-80	Leaf	Brazil	72	CIO 1070	M Bra 383	Stem	Valle-Col

Isolate No.	Identification	Genotype	Source	Location	Isolate No.	Identification	Genotype	Source	Location
30	CIO 288	IAC 44-82		Brazil	73	CIO 1072	CM 8491	Leaf	Valle-Col
31	CIO 289	IAC 105-88		Brazil	74	CIO 1074	CM 8491	Leaf	Valle-Col
32	CIO 330	Xingu	Leaf	Brazil	75	CIO 1238	SM 1794-2	Leaf	Meta-Col
33	CIO 333	Quro do Vale	Leaf	Brazil	76	CIO 1279	M Bra 383	Leaf	Quindío-Col
34	CIO 335	Mico	Leaf	Brazil	77	XAMJV VII	CM 6740-7	Leaf	Valle-Col
35	CIO 336	Fibra	Leaf	Brazil	78	XAMJV VIII	M Bra 383	Leaf	Valle-Col
36	CIO 337	IAC 44-82	Leaf	Brazil	79	XAMVM 1O	SM 1855-9	Leaf	Meta-Col
37	CIO 338	Fibra	Leaf	Brazil	80	XAMVM 1Y	SM 1642-13	Leaf	Meta-Col
38	CIO 339	IAC 12-829	Leaf	Brazil	81	XAMS-1	SM 1624-2	Leaf	Sincelejo-Col
39	CIO 340	IAC 89-87	Leaf	Brazil	82	XAMS-1B	SM 1624-2	Leaf	Sincelejo-Col
40	CIO 342	IAC 12-829	Leaf	Brazil	83	XAMVM 6	GM 221-38	Leaf	Meta-Col
41	CIO 343	Fibra	Leaf	Brazil	84	XAMVM 7	GM 223-70	Leaf	Meta-Col
42	CIO 346	IAC 144-86	Leaf	Brazil	85	XAMVM 8	GM 220-52	Leaf	Meta-Col
43	CIO 348	Taquari	Leaf	Brazil					

The amplified products were denatured at 95°C and separated in polyacrylamide gels at 4% (w/v) in 0.5X TBE buffer for electrophoresis **(Figure 1 and 2)**.

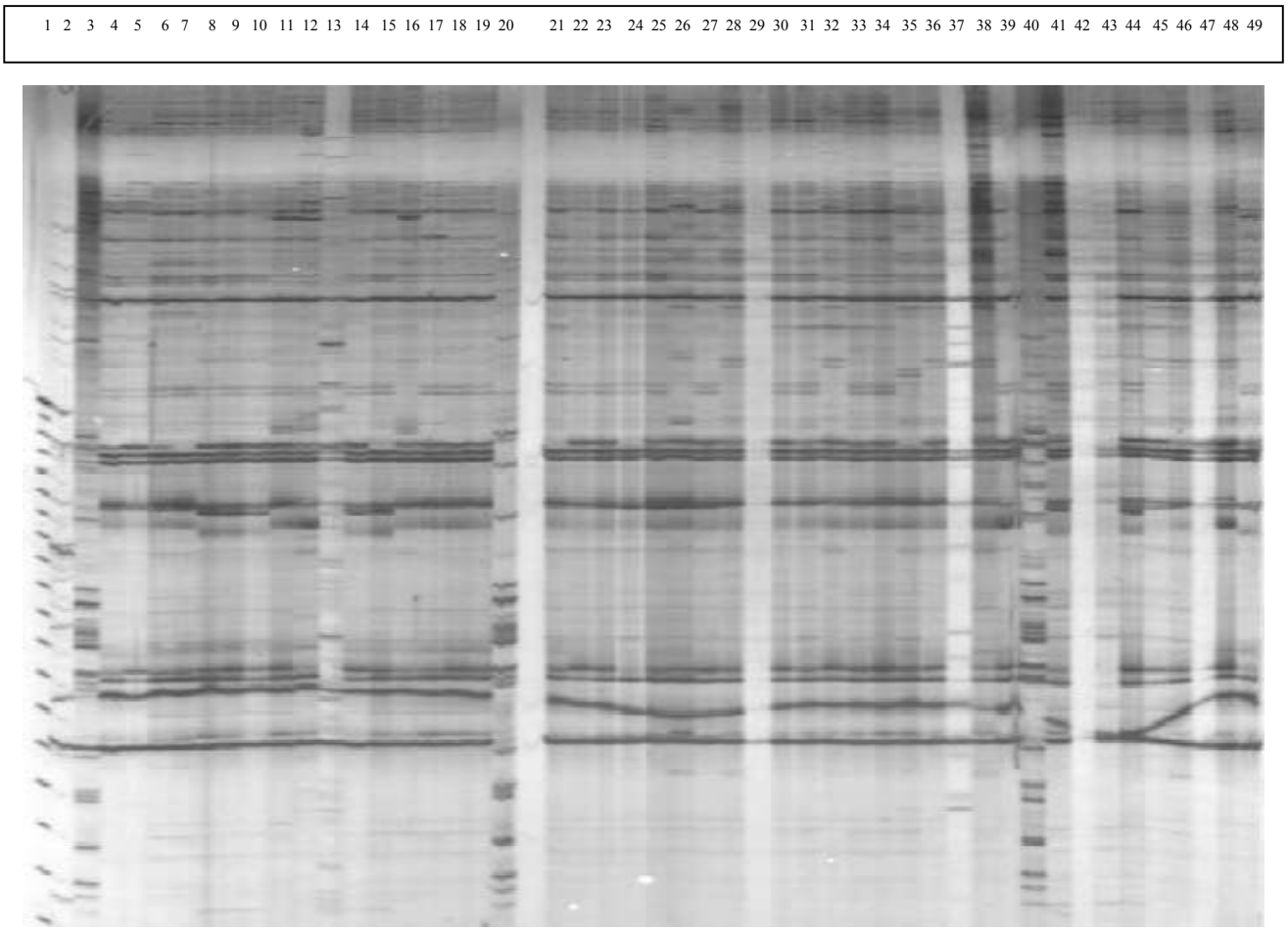

Figure 1. AFLP patterns of thirty eight isolates of *Xanthomonas axonopodis* using EC/MA primer combination (Lines 2-49), line 1 is a ladder 30-330.

Data analysis. To determine genetic variability among isolates, a phylogenetic tree was constructed, using the SAHN method and the tree option of NTSYS-pc 2.02 (FJ Rohlf, Exeter Software, New York).

Results and Discussion

On analyzing the genetic variability of 85 isolates of *Xam* through the AFLP technique, three groups could be distinguished **(Figure 3)**. The first group clustered at a similarity level of 0.6, and is formed of isolates from different localities in Colombia. The second group clustered at 0.7, and comprises 81% of the Venezuelan isolates included in this study, and 4 Brazilian isolates. The third group clustered at 0.4, and is formed by most of the Brazilian isolates, 3 isolates from Venezuela, 1 from Cuba, and 3 from Colombia. In this group, clustering below the 0.4 similarity level also occurred, indicating great genetic variability within the Brazilian locations, possibly related to the also high level of genetic diversity observed for the host plant

(Roa et al. 1997; Sánchez et al. 1999). When new pathogen strains are introduced into a given area, the genetic diversity already found within the pathogen population is increased, thereby favoring the development of new pathotypes (Restrepo 1999).

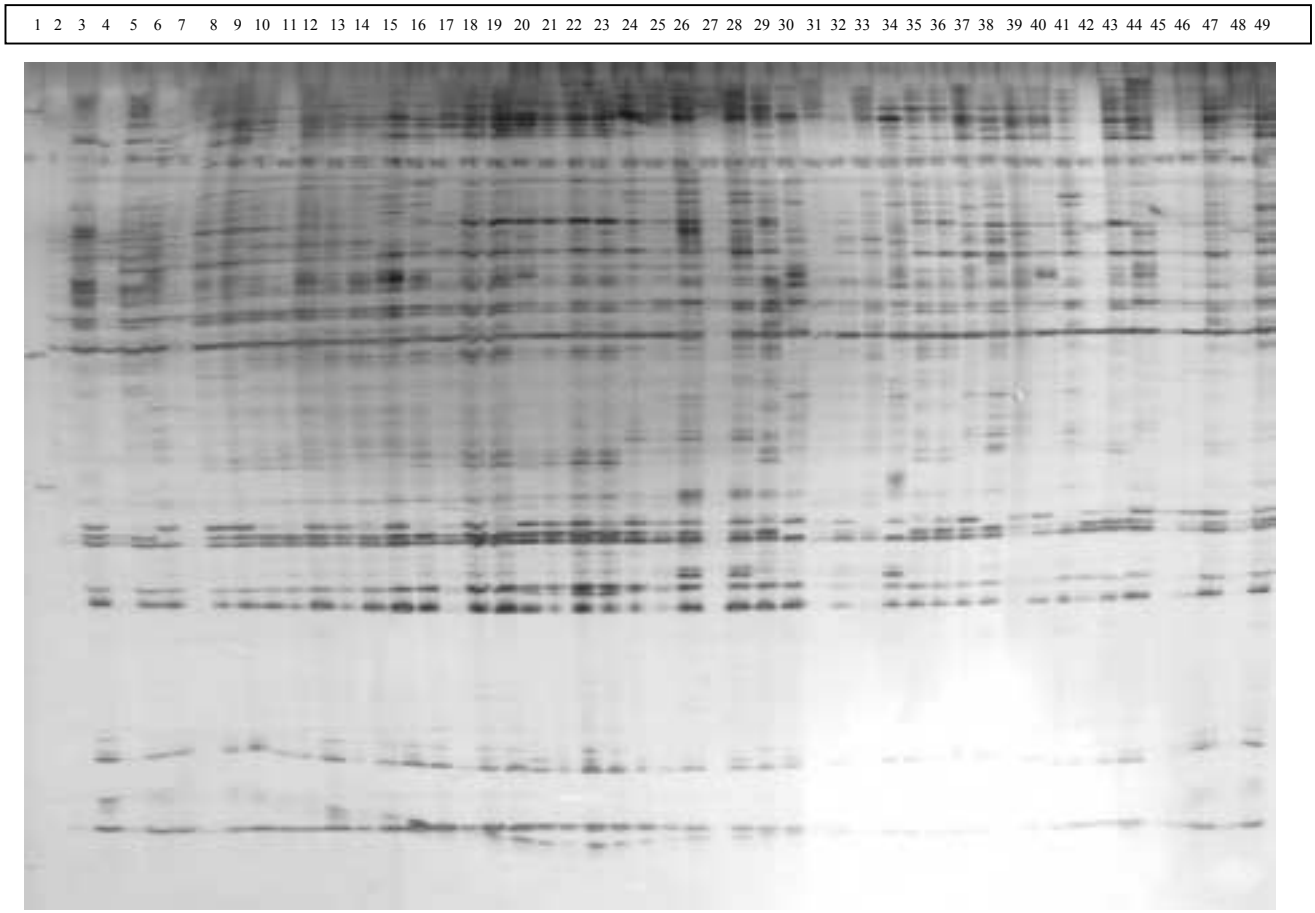

Figure 2. AFLP patterns of forty eight isolates of *Xanthomonas axonopodis* using EC/MA primer combination (Lines 2-49), line 1 is a ladder 30-330.

Cluster analysis led to the formation of three groups of isolates that could be separated by country (i.e., Brazil, Colombia, and Venezuela). Within these large groups, subgroups can be found, based on different areas within the countries. The cluster of these *Xam* populations showed high variability—a significant finding, because a population with high genetic variability can adapt faster to antibiotics and resistant hosts. Several evolutionary factors can affect a population's genetic structure, including size and number of individuals, gene flow, and host selection (Mejía 2002).

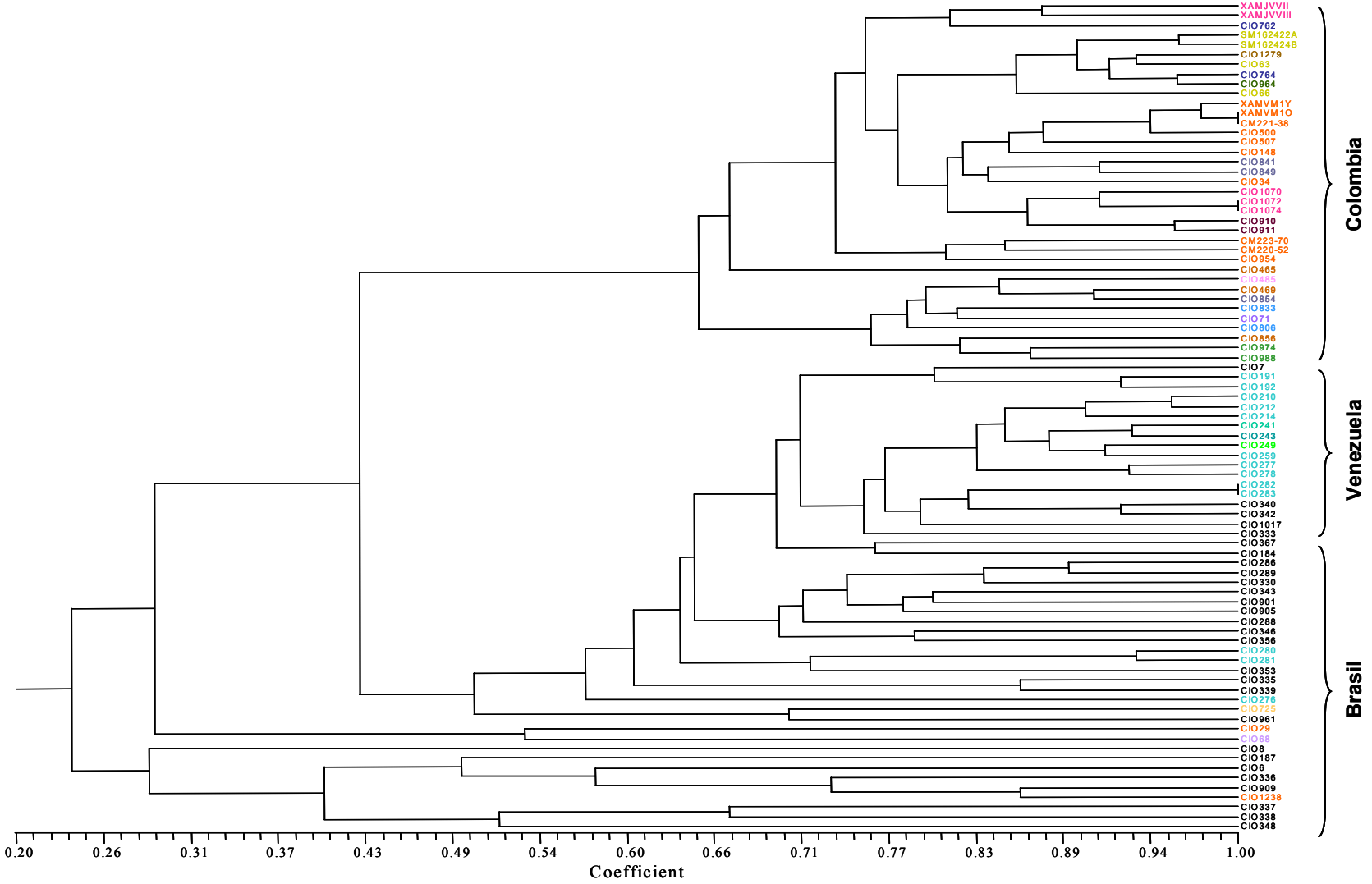

Figure 3. Similarity dendrogram of 85 isolates of *Xanthomonas axonopodis* pv. *manihotis*, based on AFLP analysis, using the unweighted pair-group method with arithmetic averaging (UPGMA) program of NTSYS-pc 2.02 (FJ Rohlf, Exeter Software, New York).

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Vos P; Hogers R; Bleeker M; Reijans M; van der Lee T; Hornes M; Frijters A; Pot J; Peleman J; Kuiper M; Zabeau M. 1995. AFLP: A new technique for DNA fingerprinting. Nuc. Ac. Res. 23:4407-4414.

Activity 3. DNA sequence analysis of the ITS region of *Sphaceloma manihoticola* and *Phytphthora* spp. obtained from cassava, heliconia, cacao and calathea

Introduction

The identification of *Phytophthora* and *Sphaceloma* (*Elsinoe*) species by taxonomy is difficult because intra specific variability is high. In this study, we present a ITS – based phylogenetic analysis of the two fungus genera. The ITS information will strengthen and extend current PCR – based diagnostic, detection and identification of *Sphaceloma* and *Phytophthora* species.

Materials And Methods

Nuclear ribosomal DNA (rDNA) was obtained from isolates of *Sphaceloma*, *Phytophthora* and *Pythium*. DNA was amplified using PCR and products were sequenced directly with the universal primers ITS 4 and ITS 5. Amplification products were purified by QIA quick PCR purification kit and the automated dideoxy sequencing (Sanger, 1975).

Reactions were set up, using the Applied Biosystems (Foster City, CA) Prism Big Dye terminator-cycle-sequencing kit and Qiagen *Taq* DNA polymerase. Electrophoresis was carried out on an Applied Biosystems Prism 377 DNA sequencer. Each sequence was aligned, using the Sequencer Program, and comparisons were made against *Elsinoe* spp. and *Phytophthora* spp. database managed by the National Center for Biotechnology Information (NCBI; Internet address is http://www.ncbi.nlm.nih.gov). To obtain high quality sequences, PCR products were also amplified using primers ITS 1- ITS2 and ITS4 - ITS5 for *Phytophthora* and *Sphaceloma* isolates respectively.

Results

Elsinoe (Sphaceloma). Computer assisted sequence alignments revealed that the ITS sequences from *Sphaceloma* isolates were similar, but not identically, to related species in the database of GenBank **(Tables 1-3)**. The isolates of *Sphaceloma* obtained from *Manihot esculenta* and *Euphorbia heterophylla* (Amendoin Bravo) in Brazil have similar sequences, and both are close related to *Elsinoe banksiae* with a homology of 94% (NCBI). The isolate obtained from *E. heterophylla* in Brazil indicate that this specie hosts *Sphaceloma*, which is relevant for the management of Super Elongation Disease.

Table 1. Homology found between DNA obtained from isolate no. 2 (*Elsinoe* sp.) of cassava affected by Super Elongation Disease in Brazil and sequences of *Elsinoe* spp. from different crops reported in GenBank.

Matching in GenBank	GenBank Number	Homology Score (bits)	Probability of Higher Homology	Homologued Fragment (bp)	Identities	Identities (%)
Elsinoe banksiae, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF097572	887	0.0	592	558	94.0
Elsinoe fawcettii, internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF097577	745	0.0	480	456	96.0
Elsinoe banksiae, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF227197	723	0.0	478	451	94.0
Sphaceloma protearum strain STE-U 2036, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	AF131083	709	0.0	474	444	93.0

Table 2. Homology found between DNA obtained from isolate no. 47 (*Elsinoe* sp.) of cassava affected by Super Elongation Disease in Colombia and sequences of *Elsinoe* spp. from different crops reported in GenBank.

Matching in GenBank	GenBank Number	Homology Score (bits)	Probability of Higher Homology	Homologued Fragment (bp)	Identities	Identities (%)
Elsinoe banksiae, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF097572	907	0.0	592	563	95.0
Elsinoe fawcettii, internal transcribed spacer 1, partial sequence;5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF097577	772	0.0	483	464	96.0
Elsinoe banksiae, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF227197	750	0.0	481	459	95.0
Sphaceloma protearum strain STE-U 2036, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	AF131083	744	0.0	477	453	94.0
Sphaceloma protearum strain STE-U 2037, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	AF131084	726	0.0	477	452	94.0

Table 3. Homology found between DNA obtained from isolate no. 1 (*Elsinoe* sp.) obtained from *Euphorbia heterophylla* in Brazil and sequences of *Elsinoe* spp. from different crops reported in GenBank.

Matching in GenBank	GenBank Number	Homology Score (bits)	Probability of Higher Homology	Homologued Fragment (bp)	Identities	Identities (%)
Elsinoe banksiae, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF097572	797	0.0	531	504	94.0
Elsinoe fawcettii, internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF097577	678	0.0	413	398	96.0
Sphaceloma protearum strain STE-U 2036, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	AF131083	656	0.0	409	393	96.0
Elsinoe proteae, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF097578	652	0.0	413	398	96.0
Sphaceloma protearum strain STE-U 2034, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	AF131081	646	0.0	409	393	96.0

Phytophthora. The following ITS sequence was obtained from a *Phytophthora* isolate from cacao fruits (*Theobroma cacao*) from Finca La Margarita at Palestina (Caldas, Colombia) **(Table 4)**:

TGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAA
AACTTTCCACGTGAACCGTATCAAAACTTAGTTGGGGGTCTCTTTCGGCGGCGGCTGCTGGCTTCAT
TGCTGGCGGCTGCTGTTGGGAGAGCTCTATCATGGCGAGCGTTTGGGCTTCGGTCTGAACTAGTAG
CTTTTTTAAACCCATTCTTTATAACTGATTATACTGTAGGGACGAAAGTCTCTGCTTTTAACTAGAT
AGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGT
AATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTC
CTGGGAGTATGCCTGTATCAGTGTCCGTACATCAAACTTGGTTTTCTTCCTTCCGTGTAGTCGGTGG
TGGATGTGCCAGATGTGAAGTGTCTTGCGGCTGGTCTTCGGATCGGCTGTGAGTCCTTTGAAATGTA
CTGAACTGTACTTCTCTTTGCTCCAAAAGCGTGGCCGTTGCTGATTGTGGAGGCTGCTTGCGTAGCC
AGTCTGGCGACCAGTTTGTCTGCTGTGGCGTTAATGGAGGAGTGTTCGATTCGCGGTATGGTTGGCT
TCGGCTGACCAGACGCTTATTGAATATTTCTTCAGCTGTGGTGGTATGATTGGTGAACCGTAGCTAT
GTGAGCTTGGCTTTTGAATTGGCTTTGCTGTTGCGAAGTAGAGTGGCGGCTTCGGCTGT

The ITS sequence from another *Phytophthora* isolate obtained from cacao, Granja Experimental Casa Luker (Palestina) **(Table 4)**:

TGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAA
AACTTTCCACGTGAACCGTATCAAAACTTAGTTGGGGGTCTCTTTCGGCGGCGGCTGCTGGCTTCAT
TGCTGGCGGCTGCTGTTGGGAGAGCTCTATCATGGCGAGCGTTTGGGCTTCGGTCTGAACTAGTAG
CTTTTTTAAACCCATTCTTTATAACTGATTATACTGTAGGGACGAAAGTCTCTGCTTTTAACTAGAT
AGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGT
AATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTC
CTGGGAGTATGCCTGTATCAGTGTCCGTACATCAAACTTGGTTTTCTTCCTTCCGTGTAGTCGGTGG
TGGATGTGCCAGATGTGAAGTGTCTTGCGGCTGGTCTTCGGATCGGCTGTGAGTCCTTTGAAATGTA
CTGAACTGTACTTCTCTTTGCTCCAAAAGCGTGGCGTTGCTGATTGTGGAGGCTGCTTGCGTAGCCA
GTCTGGCGACCAGTTTGTCTGCTGTGGCGTTAATGGAGGAGTGTTCGATTCGCGGTATGGTTGGCTT
CGGCTGAACAGACGCTTATTGAATATTTCTTCAGCTGTGGTGGTATGATTGGTGAACCGTAGCTATG
TGAGCTTGGCTTTTGAATTGGCTTTGCTGTTGCGAAGTAGAGTGGCGGCTTCGGCTGTCGAGG

According to sequence analysis, both isolates correspond to *Phytophthora palmivora* **(Table 4)**.

From the ornamental plant calathea, a *Phytophthora* isolate was obtained (The Netherlands, Crop Protection Service) and identified as *P. cinnamomi* by sequence analysis **(Table 4)**:

AAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAAAA
TCTTTCCACGTGAACCGTATCAACCCCCTTCGTTGGGGGCCTGCTCTGGGCGGCGGCTGTCGATGTC
AAAGTCGGCGGCTGCTGCTGCGTGGCGGGCCCTATCACTGGCGAGCGTTTGGGTCCCTCTCGGGGG
AACTGAGCTAGTAGCCCCTATTTTAAACCCATTCTGTAATACTGAACATACTGTGGGGACGAAAGT
CTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACG
CTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATAT
TGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTATCAGTGTCCGTACATCAAACTTGGCTCTCTTC
CTTCCGTGTAGTCGGTGGATGGAGGCGCCAGACGTGAGGTGTCTTGCGGGCGGCCTTCGGGCTGTC
TGTGAGTCCCTTGAAATGTACTGAACTGTACTTCTCTTTGCTCGAAAAGCGTGACGTTGCTGGTTGT
GGAGGCTGCCTGTATGGCCAGTCGGCGACCGGTTTGTCTGCTGCGGCGTTTAATGGAGGAGTGTTC
GATTCGCGGTATGGTTGGCTTCGGCTGAACAAAGCGCTTATTGGATGTTCTTCCTGCTGTGGCGGTA
CGGATCGGTGAACCGTAGCTGTGCAAGGCTTGGCGTTTGAACCGGCGGTGTTGTGGCGAAGTAGAG
TGGCGGCTTCGGCTGTCGAGGGTCGATCCATTTGGGAACTCTGTGTCTTTTCTTGCGGCGCACGTTT
GTGTAGTTGCAAGGGATGGCA

From the ornamental plant heliconia (Palmira, Valle, Colombia) *Phytophthora cryptogea* was isolated **(Table 4)**:

TGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAA
AAACTTTCCACGTGAACCGTATCAACCTTTTTAAATTGGGGGCTCCCGTCTGGCCGGCCGGTTCTCG
GCTGGCTGGGTGGCGGCTCTATCATGGCGACCGCTTGGGCCTCGGCCTGGGCTAGTAGCGTATTTTT
TAAACCCATTCCTAATTACTGAATATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAA
CTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGC
GAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGG
AGTATGCCTGTATCAGTGTCCGTACACTAAACTTGGCTCCCTTCCTTCCGTGTAGTCGGTGGATGGG
GACGCGCAGATGTGAAGTGTCTTGCGGCTGGTCTTCGGTCCGGCTGCGAGTCCTTTGAAATGTACT
ACACTGTACTTCTCTTTGCTCGAAAAGCGTGACGTTGCTGGTTGTGGAGGCTGCCTGTGTGGCATGT
CGGCGACCGGTTTGTCTGCTGCGGCGTTTAATGGAGGAGTGTTCGATTCGCGGTATGATTGGCTTCG
GCTGAACAGACGCTTATTGGGTGCTTTTCCTGCTGTGGCTGGATGGACTGGTGAACCGTAGCTGTG
CTAGGCTTGGCGTTTGAACCGGCGGTGTGGTGCGAAGTAGGGTGTCTGTTCCGGCGCAAGCTGGGG
TGGACGAGGGTCGATCCATTTGGGAAACGTTGTRWGCGCAACGGCGCGCATSKCAAWTCAACAA

The isolate number 71 obtained form cassava at Barcelona, Quindío (Colombia) was identified as *Phytophthora tropicalis* **(Table 4)**:

ACCTAAAARACTTTCCACGTGAACCGTATCAACCCTTTTAGTTGGGGGTCTTGTACCCTATCATGGC
GAATGTTTGGACTTCGGTCTGGACGAGTAGCCTTTTGTTTTAAACCCATTTCTCAATACTGATTATA
CTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGC
ACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCG
AAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTATCAGTGTCCGTACAT
CAAACTTGGCTTTCTTCCTTCCGTGTAGTCGGCGGAGGATGTGCCAGATGTGAAGTGTCTTGCGGTT
TGTCCTTCGGGTCGTCTGCGAGTCCTTTTAAATGTACTGAACTGTACTTCTCTTTGCTCGAAAAGCG
TGGTGTTGCTGGTTGTGGAGGCTGCCTGCGTGGCCAGTCGGCGACCGGTTTGTCTGCTGCGGCGTTT
AATGGAGGAGTGTTCGATTCGCGGTATGGTTGGCTTCGGCTGAACAGGCGCTTATTGTATGCTTTTC
CTGCTGTGGCGTGATGGGCTGGTGAACCGTAGCTGTGTGTGGCTTGGCTTTTGAATCGGCTTTGCTG
TTGCGAAGTAGAGTGGCGGCTTCGGCTGTCGAGGGTCGATCCATTTTGGGAAGTTTGTRTGCACCT
CGGTGCGCATSKCAA

The isolate MTR4 from cassava obtained at Mitú (Vaupés, Colombia) was identified as *Pythium chamaehyphon* **(Table 4)**:

TTCCCACCTAAAAACCCCTTCCCGTGAACCGTTTTGTTTTGCTTTTCGAGTGCTTTGTTGCGCTCGG
AGCATGTTTGGGCTTCGCTGCTGGCGCTTGATTGTGCTGGCGGCTCGAGGCCATCAAGTGGCGTTT
TGAGTGTGCCTTGCGCAATTAAAACGTCCAAACCTTTTAAACCCATTTGATTGAAAACTGAAGTAT
ACTGTGGGGACGAAAGTCCTCGCTTTGAAACTAGATAACAACTTTCAGCAGTGGATGTCTAGGCTC
GCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGAGTCAT
CGAACTTTTGAACGCATATTGCACTTTCGGGTTACGCCTGGAAGTATGTCTGTATCAGTGTCCGTA
CACTAAACTTGCGTCTCTTCCGTCGTGTAGTCGTCGGTTGTTTGATTGCAGATGTGAGGTTGTCTCG
CGATGTACCATCTTTTTTTGTTGGATAGGTTGCGAGTCCCTTTAAAGTCGGACGCGTGTTTTTCCGT
TTTGTGCTTGATGGGGTGCGGCTGCGGCCGTGTCTGCTGGCGGGTCCGGTGACCTTTGGCGATGGC
ATGAGAGTGGATTGCTCGATTTGCGGTATGTTAGGCTTCGGCTTTGACAATGCAGCTTATTGTGTA
TGTTCGCTTGGCTGTTGCTGTATGGGTGAGCTGGATGGTCGGTGGATGCGTTTGTTGCGTGTCGTTT
TCATTGAGTGCGTTGCGGTTGTMGTCGCCATWTGGGAATTTAAT

Reference

Sanger F; Coulson AR. 1975. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol 94:441.

Table 4. Homology found between *Phytophthora* spp. and *Pythium* sp. DNA sequences from different crops and sequences reported in GenBank.

Source Crop	Matching in GenBank	GenBank Number	Homology Score (bits)	Probability of Higher Homology	Homologued Fragment (bp)	Identities (%)
Cacao, *Theobroma cacao*	*Phytophthora palmivora* (isolate P80) 18S ribosomal RNA (18S rRNA), 5.8S ribosomal RNA (5.8S rRNA), and 28S ribosomal RNA (28S rRNA) genes	L 41384	1479	0	766	99.3
Calathea sp.	*Phytophthora cinnamomi* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	CI 457835	1501	0	821	98.1
Heliconia sp.	*Phytophthora cryptogea* strain PcrG2 (KACC40189) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	AF 087475	1576	0	818	98.8
Cassava, *Manihot esculenta* Isolate P71	*Phytophthora tropicalis* 18S rRNA gene (partial), 5.8S rRNA gene, 28S rRNA gene (partial), internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), isolate H 778-1	AJ 299734.1	1464	0	748	99.3
Cassava, *Manihot esculenta* Isolate MTR4	*Pythium chamaehyphon* ITS1, 5.8S rRNA gene and ITS2, strain MS6-10-8V	AJ 233440	812 [a]	0	458	97.8
			496 [a]	e -137	275	97.5

[a] Blast (GenBank), reported two alignments in different regions.

Activity 4. Management of Phytophthora Root Rot of cassava in the field

Department of Cauca

To evaluate the effect of five control practices on *Phytophthora* fungi, which induce root rot in cassava, experimental plots were established on a farm in the Municipality of Pescador (Cabuyal Village District), Department of Cauca, Colombia, in April 2002. The farmer is indigenous and belongs to the Interinstitutional Consortium for Sustainable Agriculture in Hillsides (CIPASLA, its Spanish acronym).

Treatments

Planting stakes were grouped for use in five treatments, which were then evaluated for their effect on the incidence and severity of root rots in the harvested roots of each group. The types of control were:

1. *Biological control*: Strain 14PDA-4 of the fungus *Trichoderma* sp., which attacks root rot fungi (*Phytophthora* spp.), was used to make a suspension of 1×10^6 conidia/mL. Planting stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil near the base of each plant. The effectiveness of the *Trichoderma* strain in controlling *Phytophthora tropicalis* was also evaluated in *in vitro* tests and the greenhouse.

2. *No treatment:* Traditional farmer's practice.

3. *Selection of quality planting materials*: Stakes were selected for their health and from middle parts of stems.

4. *Chemical control*: Planting stakes were immersed for 5 min in Ridomil® (metalaxyl) at 3 g/L of water.

5. *Thermotherapy*: Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.

For all treatments, stakes of the regional cassava variety Algodona (M Col 1522) were used, and chicken manure was incorporated into the soil at 2.5 t/ha.

The experimental design was randomized complete blocks, with two replicates and 44 plants per treatment.

As checks, other cassava genotypes that had previously given high yields in field experiments were planted in the same plot, at 38 plants per genotype. These genotypes were CM 7438-14, M Bra 383, SM 1053-23, SM 1058-13, and SM 1937-1. All plots were planted in association with beans.

Results

In the trial, the heat treatment did not affect germination **(Table 1)**.

Table 1. Effect of different practices of root-rot management on germination, Pescador, Department of Cauca, Colombia.

Treatment	Germination (%)[a]
Algodona (M Col 1522)	
Stake selection	96.6
Thermotherapy	93.3
Traditional farmer's practice	95.3
Trichoderma strain 14PDA-4	88.6
Chemical control	98.9
Check varieties	
CM 7438-14	100.0
M Bra 383	100.0
SM 1053-23	94.6
SM 1058-13	100.0
SM 1937-1	97.4
Average	96.5

[a] 30 days after planting.

Department of Quindío

Different control practices for *Phytophthora* spp. were evaluated for disease incidence and severity, and for yield in two field trials at "La Elena" Farm, Municipality of Montenegro.

Treatments for the first trial

The first trial was planted in June 2001 with the local variety Manzana. Treatments were as follows:

1. *Thermotherapy*: Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.

2. *Chemical control*: Planting stakes were immersed for 5 min in a mixture of Orthocide® (captan) at 4 g/L and Ridomil® at 3 g/L of water.

3. *Biological control*: Strain 14PDA-4 of *Trichoderma* sp. was used to make a suspension of 1×10^4 conidia/mL. Planting stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil around the stake, using 100 mL/plant.

4. *No treatment*: Traditional farmer's practice.

All the plots received fertilizers 45 days after planting, that is, 500 kg/ha of the fertilizer mix

Nitrax®, DAP, and KCl was applied at a rate of 1:2:2.

The experimental design was randomized complete blocks, with three replicates and 55-60 plants per treatment.

Treatments for the second trial

The second trial was planted in August 2001:

1. *Thermotherapy*: Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.

2. *Chemical control*: Planting stakes were immersed for 5 min in a mixture of Orthocide® at 4 g/L and Ridomil® at 3 g/L of water.

3. *Biological control*: Strain 14PDA-4 of *Trichoderma* sp. was used to make a suspension of 1×10^4 conidia/mL. Planting stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil around the stake, using 100 mL/plant.

All the plots received fertilizers 45 days after planting, that is, 500 kg/ha of the fertilizer mix Nitrax, DAP, and KCl was applied at a rate of 1:2:2. Four months after planting, 1% of each of Kelatex-Mn® and Kelatex-Zn® were applied to the foliage and 55 kg/ha of the same products to the soil.

The experimental design was the same as for the first experiment.

Results

The applied biological control agent *Trichoderma* strain 14PDA-4 reduced root rot strongly in two experiments conducted at Quindío **(Table 2 and 3)**. Traditional farmer's practice resulted in consistently higher levels of root rot. However, the resulting yields were not good, and germination and plant development following treatment were generally low. Thermotherapy showed similar results. It can be concluded that *Trichoderma* or heat affected germination and crop development of stem cuttings obtained from immature cassava plants.

For the cassava variety Chiroza, the biological (*Trichoderma*) and chemical (Orthocide® and Ridomil®) treatments reduced the severity of root rot caused by *Phytophthora* sp. **(Table 3)**, but did not affect disease incidence. The manganese and zinc applications, in contrast, did reduce incidence (data not presented). Yields were fairly high, considering the field had been cropped for 5 cycles and the pressure of Phytophthora root rot was high. Thermotherapy and the zinc and manganese applications gave the highest yields, whereas the chemical and *Trichoderma* treatments had the lowest.

To take advantage of the fairly good control by *Trichoderma*, it is suggested to apply several control practices and improve crop fertilization in future experiments.

Clone HMC-1 showed acceptable levels of root rot infection; incidence was much lower than other varieties or treatments. The variety Reina (Cm 6740-7) will be included in future trials at Quindío.

Table 2. Effect of stake treatments, thermotherapy, biological, chemical control and fertilizers on the development of cassava variety Manzana and root rot disease, Department of Quindío, Colombia.

Parameter	Control practice				
	Thermo-therapy[a]	*Tricho-derma*[b]	Chemical	Traditional farmer's	Average
5 months after planting					
Germination (%)	66.7	41.7	94.0	88.1	72.6
Height (cm)	62.3	56.3	72.3	55.0	61.5
12 months after planting					
No. stakes/plant	10.6	13.5	16.8	10.1	12.7
Harvested plants (%)	71.7	46.7	87.2	75.0	70.1
Yield (t/ha)					
Commercial roots	1.9	1.3	5.9	4.0	3.3
Noncommercial roots	2.0	2.6	3.8	2.7	2.8
Disease incidence (%)					
Plants with root rot	75.6	90.0	70.0	73.2	77.2
Severity (%)					
Rot (by no. of roots)	44.8	41.3	39.3	40.6	41.5
Rot (by weight)	65.9	44.4	51.0	49.8	52.8
Root rot (t/ha)					
Commercial roots	3.0	2.1	5.9	3.4	3.6
Noncommercial roots	4.1	1.8	2.9	3.0	3.0

[a] Roots immersed for 49 min in hot water (47ºC-49ºC) in an oil drum on a wood fire.
[b] Strain 14PDA-4.

Table 3. Effect of stake treatments thermotherapy, biological (*Trichoderma* sp.), chemical control and fertilizers (Mn + Zn) on the development of the cassava variety Chiroza and Phytophthora root rot, Department of Quindío, Colombia.

Treatment	Root yield (t/ha)		DM[a] content (%)	Infected roots			
				%		T/ha	
	Commer.	Non-commer.		No.	Weight	Commer.	Non-commer.
Three replicates per treatment							
Mn + Zn	19.3	5.3	34.4	55.1	40.4	8.1	1.8
Thermotherapy[b]	19.1	6.9	35.8	34.5	24.8	5.4	1.0
Traditional farmer's practice	18.5	5.4	40.7	35.9	29.5	6.2	0.8
Chemical control	17.9	7.0	35.0	27.5	18.3	3.5	1.0
Trichoderma strain 14PDA-4	16.7	5.5	35.5	31.5	26.0	4.6	1.2
One replicate per treatment							
Catumare	3.5	3.9	41.6	46.2	54.2	3.3	0.8
HMC-1	9.6	8.2	38.2	13.4	21.4	3.5	0.3
Chiroza	3.0	2.3	24.3	61.8	70.3	2.6	1.1
La Reina (Cm 6740-7)	17.2	7.0	38.1	16.0	16.9	4.0	0.1

[a] DM = dry matter.
[b] Roots immersed for 49 min in hot water (47ºC-49ºC) in an oil drum on a wood fire.

Activity 5. Genetics of resistance to root rot caused by *Phytophthora tropicalis* in two segregating populations of cassava

Introduction

In Brazil, of the *Phytophthora* spp., *P. drechsleri* Tucker most severely attacks cassava (Albuquerque and Figueiredo 1968). This species has been identified in Colombia (Oliveros et al. 1974), together with *P. nicotianae* var. *nicotianae* (Sánchez 1998; Lozano and Loke 1994; Soto et al. 1988). Other species reported as cassava pathogens in different countries are *P. erythroseptica* (Fassi 1957), *P. cryptogea* (CIAT 1991), *P. meadii* and *P. arecae* (Alvarez et al. 1997; Barragán et al. 1998), and *P. tropicalis* (which is similar to *P. capsici*).

The development of *Phytophthora* spp. is favored by use of inadequate agronomic practices and ineffective fungicides, transport of material from affected areas to those free of the pathogen, and by planting in compact or very clayey soils (Takatsu and Fukuda 1990).

Currently, CIAT selects for resistance to *Phytophthora* spp. under greenhouse conditions, inoculating shoots and roots with isolates that were previously identified by sequencing the ITS region in the rDNA.

Molecular techniques are increasingly being used to decipher the genetic base of complex agronomic traits. Genetic improvement for disease resistance can be achieved more quickly and effectively by using molecular markers.

To better understand the genetics of resistance to *Phytophthora* spp., this study evaluates individuals from the cassava populations K family and CM 9582 for their reaction to root rot caused by *P. tropicalis*.

Materials and Methods

Plant materials. In 2000 and 2001, 1-year-old roots of 69 cassava genotypes belonging to the K family grown at CIAT's experiment station at Santander de Quilichao (Department of Cauca) were inoculated and evaluated. In July-August 2001, 1-year-old roots of 43 cassava genotypes belonging to the CM 9582 population were harvested at the Centro de Investigación de la Caña de Azúcar de Colombia (CENICAÑA, Florida, Department of Valle) and evaluated. Also included in the study were four genotypes from CIAT's Quilichao station: one resistant (M Bra 1045) and three susceptible (M Col 2066, CM 2177-2, and M Nga 2) to *Phytophthora tropicalis* (*Pt*):

Parent	Origin	Reaction to Pt[a]
K Family		
M Nga 2	Nigeria	Susceptible
CM 2177-2	Hybrid, CIAT	Susceptible
CM 9582		
M CR 81	Costa Rica	Susceptible
M Bra 1045	Brazil	Resistant

[a] *Phytophthora tropicalis*.

For the QTL analysis, roots of 92 genotypes of the K family were harvested at CIAT in 2000. The roots were then washed with drinking water and detergent, and disinfected, first with 1% hypochlorite, then with 30% ethanol, each for 10 min. The roots were then dried with sterilized paper towels. The material that was disinfected but not inoculated the same day was stored (for a maximum of 24 h) in a cold room at 4°C until inoculated.

The pathogen. As inoculum, isolate 71 was used. It was identified, through sequencing the ITS region of ribosomal DNA, as *P. tropicalis* (which is similar to *P. capsici*). This isolate was found in cassava infected with root rot in Barcelona (Department of Quindío). The inoculum was cultured in medium prepared with oat agar (2% Quaker® oats, 2% agar) and antibiotics (penicillin at 900 mg/mL; rifampicin, 0.2 g/mL; and ampicillin, 750 mg/mL). Incubation was carried out at temperatures between 20°C and 26°C for 4 to 11 days for the K family and 6 to 7 days for the CM 9582 population.

Inoculation. Within an isolation chamber, in front of a burner, a piece of cassava root, about 15 mm long, was extracted with a punch, 7 mm in diameter. At the bottom of the perforation left behind, a piece of the fungus, also extracted with a punch, 5 mm in diameter, was deposited. The extracted piece of cassava root was replaced and secured with masking tape. Each genotype was also inoculated with a negative control, that is, the medium of oat agar and three antibiotics, but no *P. tropicalis.* Once inoculated, the treated cassava roots were deposited in plastic bags, containing moist, sterilized, paper towels. The closed bags were then placed in plastic trays, and left at 22°C in darkness for 7 (K family) or 5 days (CM 9582).

Evaluation. From each cassava root, a cross section was taken at the point where the inoculum was desposited. The height and width of both wound and entire cross section were measured, together with root length, and depth of inoculum in the root. The type of rot was also evaluated: 1 = soft/moist; 2 = dry; 3 = soft/dry; 4 = soft/moist and dry. These data were recorded and processed through *Excel*'s calculation program.

Data analysis. The experimental unit was the root. For the K family, the following were taken into account: (1) genotypes with fewer than five (2000) or six (2001) roots were excluded; (2) roots with an average diameter of less than 3 cm were discarded; (3) slices of root with wounds wider than 7 (2001) or 8 cm (2000) were considered as having 100% of their area infected, and values for roots wider than 7 (2001) or 8 cm (2000) were converted for 7 or 8 cm, respectively.

For the CM 9582 population, the following were taken into account: (1) genotypes with fewer than four replicates were excluded; (2) roots with an average diameter of less than 3 cm were eliminated; (3) slices of root with wounds wider than 6 cm were considered as having 100% of their area infected, and values for roots wider than 6 cm were converted for 6 cm.

QTL analysis. One framework map was used for QTL analysis, based on the segregation of molecular markers in a population from a cross between two heterozygous parents: M Nga 2 (female) and CM 2177-2 (male). The female-derived map was based on the segregation of female alleles, corresponding to 192 markers that compromised RFLP, random amplified polymorphic DNA (RAPD), isoenzymes, microsatellites, expressed sequence tags (ESTs) and known genes (Fregene 2002, in preparation).

A significant association between a DNA marker and *Phytophthora* resistance was declared if the probability was more than 0.005 to minimize the detection of false positives. The degree of phenotypic variance explained by each marker was obtained from the regression coefficient (r^2 values). Total r^2 values from each QTL were computed as:

(sum of squares for each marker)/(total sum of squares)

All data were analyzed with Q-Gene on McIntosh.

Results

The roots of 69 individuals of the K family (M Nga 2 × CM 2177-2) and of 43 individuals of the CM 9582 population (M Bra 1045 × M CR 81) were inoculated with *P. tropicalis* to determine the genetic base of these populations' resistance to Phytophthora root rots (PRR).

The K family genotypes evaluated in 2000 and 2001 showed 30%-70% of areas continuously infected **(Figure 1)**. Some genotypes that, in 2000, had intermediate resistance to *P. tropicalis* tended to become susceptible in 2001 and vice versa.

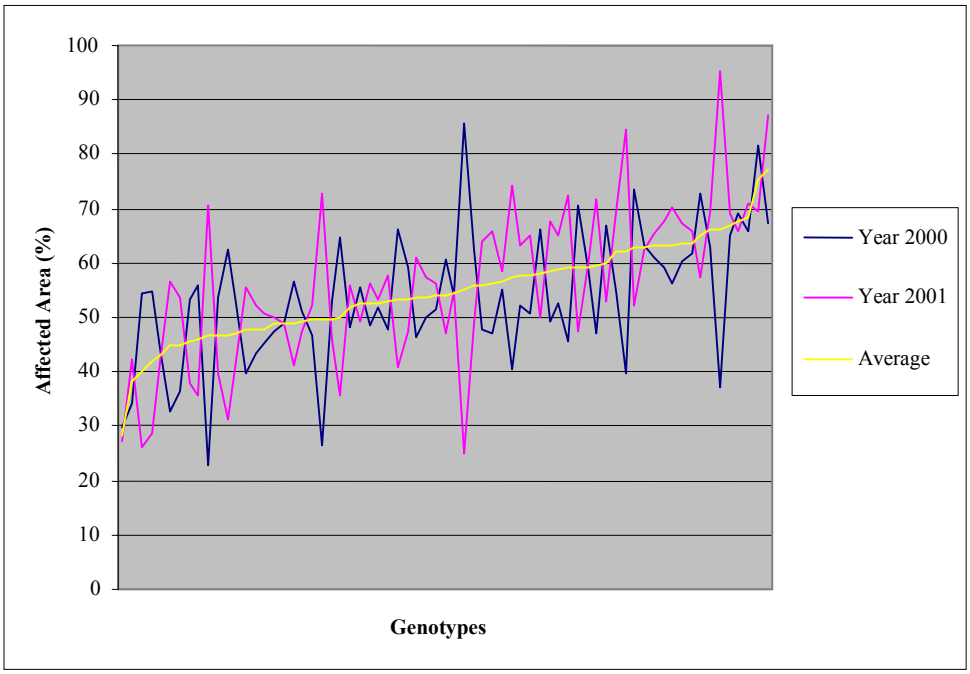

Figure 1. Percentage of root area affected by the fungus *Phytophthora tropicalis* across genotypes of the cassava K family for years 2000 and 2001.

Certain genotypes with intermediate resistance in 2000 continued presenting intermediate resistance in 2001, showing 30%-60% of area infected. Genotypes from the CM 9582 population (2001) maintained 70%-90% of areas continuously infected. Few genotypes had intermediate resistance (**Figure 2)**. Figures 3 and 4 show the distribution of individuals by group, according to the degree of resistance to the pathogen. Resistant materials were not detected.

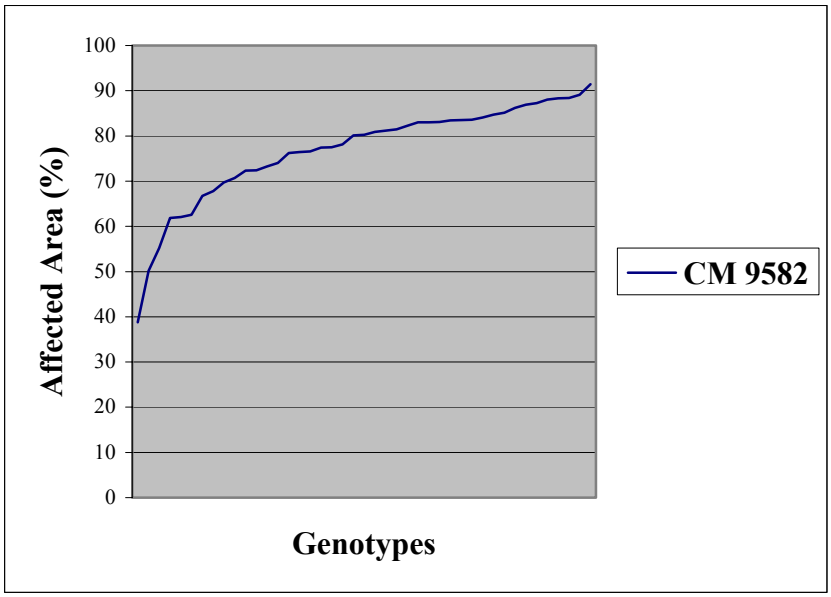

Figure 2. Percentage of area affected by the root rot fungus *Phytophthora tropicalis* across genotypes of the cassava CM 9582 population for year 2001.

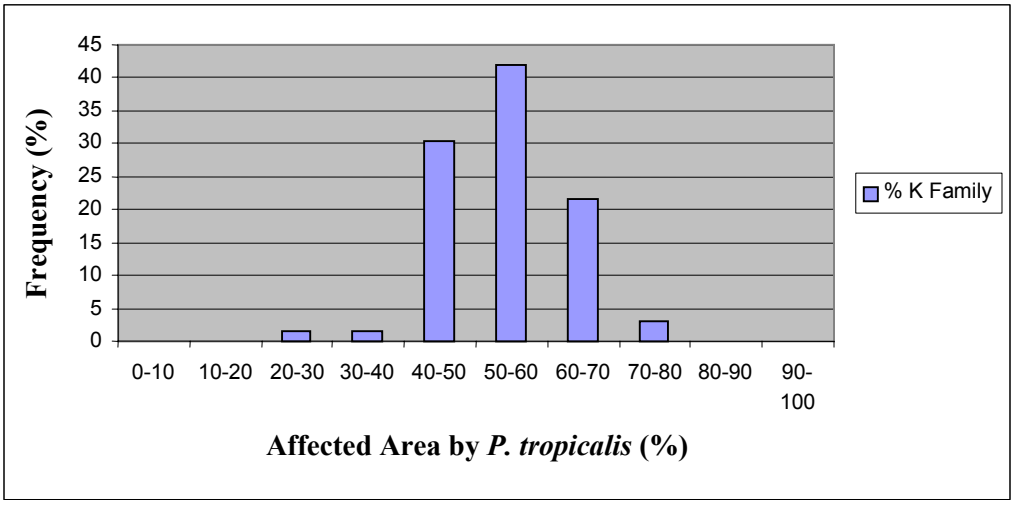

Figure 3. Distribution of the frequency of genotypes from the cassava K family according to the percentage of area affected by the root rot fungus *Phytophthora tropicalis*.

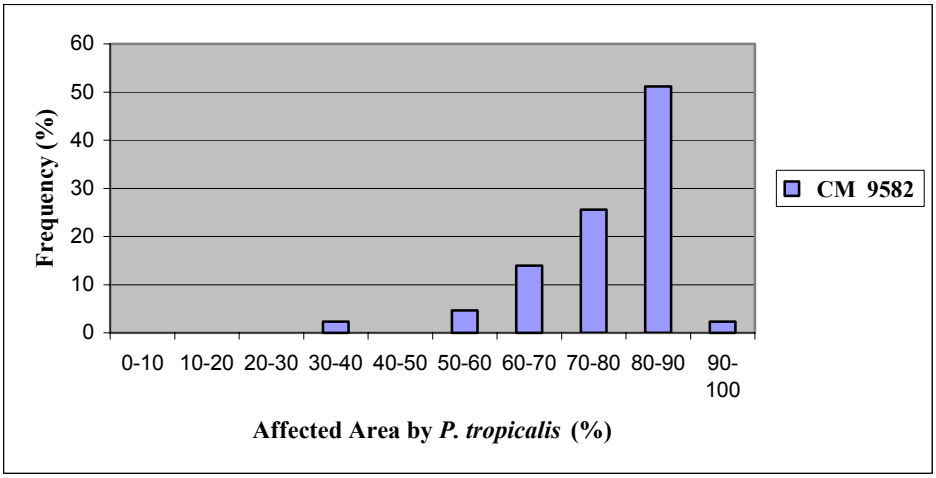

Figure 4. Distribution of the frequency of genotypes from the cassava CM 9582 population according to the percentage of area affected by the root rot fungus *Phytophthora tropicalis*.

For the K family across 2000 and 2001, the 10 genotypes with the highest intermediate resistance to *P. tropicalis* had values that ranged between 28% and 47%; and the 10 most susceptible genotypes averaged between 63% and 77%. For the CM 9582 population, the figures were, respectively, 35% and 69% and 84% and 88% **(Table 1)**.

Of the 10 intermediately resistant genotypes from the K family, six (K19, K88, K98, K69, K66, and K30) presented very low intermediate resistance during 2000, increasing toward the end of the year. The other four genotypes (K81, K79, K110, and K114) had higher intermediate resistance in 2000 than in 2001.

Of the 10 K family genotypes showing susceptibility in 2001, seven (K9, K57, K148, K39, K35, K122, and K64) were less susceptible in 2000 than in 2001, with the other three being more susceptible.

On average, the 10 intermediately resistant genotypes from the K family were more resistant than the 10 intermediately resistant genotypes from the CM 9582 population. Likewise, on average, the 10 most susceptible genotypes of the CM 9582 population were more susceptible than the 10 susceptible genotypes of the K family.

Table 1. Phenotypic evaluation of two cassava populations segregating for resistance to root rot (RR) caused by the fungus *Phytophthora tropicalis*.

Population or Genotype	Reaction to RR			Population or Genotype	Reaction to RR	
	Year				Year	
	2000	2001	Average		2001	CV (%)
Family K				**CM 9582**		
Intermediate (I) to resistant (R):						
K 19	29.7	27.1	28.4	136	35.2	21.3
K 110	34.2	42.1	38.2	148	49.6	57.3
K 88	54.4	26.0	40.2	150	54.9	25.6
K 98	54.8	28.8	41.8	133	60.3	20.5
K 69	44.2	41.8	43.0	151	61.2	21.0
K 114	32.8	56.6	44.7	121	61.6	16.5
K 79	36.3	53.6	44.9	71	66.7	6.9
K 66	53.2	37.8	45.5	115	67.8	6.2
K 30	55.9	35.8	45.9	140	68.9	13.3
K 81	22.8	70.5	46.7	47	69.1	14.7
Average	41.8	42.0	41.9		59.5	20.3
Correlation between years			-0.67			
Susceptible (S):						
K 9	60.2	67.3	63.8	42	84.4	5.4
K 57	61.6	65.9	63.8	68	84.7	8.4
K 92	72.7	57.3	65.0	172	85.2	7.5
K 148	62.7	69.4	66.0	153	85.4	13.5
K 39	37.1	95.2	66.1	62	85.9	8.9
K 35	64.9	69.0	66.9	45	86.2	4.4
K 6	69.2	65.9	67.5	52	86.2	9.7
K 122	65.9	71.0	68.5	163	87.5	6.5
K 145	81.6	69.4	75.5	78	87.9	13.2
K 64	67.3	87.2	77.3	91	88.0	5.3
Average	64.3	71.7	68.0		86.3	8.3
Correlation			-0.66			
General						
Average	53.6	56.0	54.8		76.0	
Correlation			-0.15			
Parents						
M Nga 2	66.3	56.7	61.5	M CR 81	--	
CM 2177-2	69.6	83.9	76.7	M Bra 1045	46.1	19.6
Checks						
M Bra 1045 (R)	11.6	51.5	31.5	M Col 2066 (S)	70.9	11.6
M Col 2066 (S)	70.5	86.2	78.3	M Nga 2 (S)	55.4	19.1
				CM 2177-2 (S)	68.3	7.4

The coefficient of variation calculated for the CM 9582 population was 12.6%, indicating that the study was reliable with a margin of relatively low experimental error.

For genotypes from the K family with intermediate resistance, the distribution of frequency of genotypes against area infected by *P. tropicalis* presented a curve similar to that of a normal distribution **(Figure 3)**. In contrast, for the most susceptible genotypes from the CM 9582 population, the curve was rising **(Figure 4)**, with M Bra 1045 showing 56.3% of area infected.

The correlation between root length and area infected was –0.30 for the CM 9582 population, indicating that the longer the root, the less disease found.

Table 2 shows the results of the single-marker regression analysis of percentage of infected area in roots inoculated in the laboratory. Markers defined eight QTLs located on linkage groups C, H, J, N, Q, and V **(Table 2)**. The QTLs explain between 1.3 and 9% of the variance, the most significant QTL being no. 7, located in linkage group V (chromosome no. 22) of the female-derived framework map.

Table 2. QTLs explaining the highest values of variance for resistance in cassava, as described by the percentage of root area infected. Values in bold are significant at $P = 0.05$.

Linkage Group (Female Map)	Markers (Position in cM)[a]	F[b]	V[c] (%)	P[d]	QTL no.
C (3)	RGY172	0.029	5.4	<0.0500	1
H (8)	SSRY178	0.315	1.3	<0.0500	2
J (10)	CDY76	0.163	4.0	<0.0500	3
	K2a	0.040	8.6	<0.0500	4
N (14)	SSRY13	0.078	4.2	<0.0500	5
Q (17)	SSRY911	0.047	5.7	<0.0500	6
V (22)	NS911	0.007	9.0	0.0070	7
	GY153	0.049	4.5	<0.0500	8

[a] Distance from the first marker noted (o).
[b] *F* statistics from analysis of variance.
[c] Percentage of variance explained (from r^2 coefficient of regression).
[d] Probability of *F* statistic.

Discussion and Conclusions

No reports exist on the genetic basis of resistance to root rot caused by *P. tropicalis* in cassava. Hence, this resistance was evaluated phenotypically in two populations: K family and CM 9582.

K family. Some of the genotypes evaluated from the K family expressed intermediate resistance to *P. tropicalis*, with some presenting intermediate resistance in 2000 but susceptibility in 2001. The opposite also occurred, where some susceptible genotypes in 2000 presented intermediate resistance in 2001.

Such changes may have been triggered by changes in the soil, environmental conditions, or use of chemical products (e.g., fertilizers). These factors indirectly affect partial resistance to pathogens—as corroborated by a study on the partial resistance of maize to *Puccinia sorghi*—and affect QTL expression (Lübberstedt et al. 1998).

Other factors may include the long vegetative cycle, vegetative propagation without quality control of planting stakes, and changes in populations of microorganisms (either beneficial or detrimental) in the rhizosphere and roots. Variability in resistance across years may indicate a polygenic nature of the K family, although the environment usually influences phenotypical expression, generating variation. It is important to note that certain genotypes of the K family with intermediate resistance in 2000 continued expressing it in 2001.

Although both parents of the K family are susceptible to *P. tropicalis*, a group of genotypes from this family showed intermediate resistance. This indicates that the parents are heterozygotes (Fregene et al. 1997) and that they both have resistance genes.

CM 9582 population. The CM 9582 population is obtained by crossing M Bra 1045 with M CR 81. In previous studies, M Bra 1045 has shown resistance to *P. tropicalis*, but in this study, it is susceptible, probably because of changes in environmental factors, as explained above. The genetic base of M Bra 1045 can be assumed to be polygenic, and to have epistasis in this crossing.

The two populations. On comparing the intermediate resistance presented by the K family and CM 9582 population, we found that the CM 9582 population had few genotypes with intermediate resistance to *P. tropicalis*. That is, the 10 most resistant genotypes of the K family had a higher degree of resistance than did the 10 most resistant genotypes of the CM 9582 population. The differences probably lie in the genetic crossings between the parents, which differ for the two populations.

Although the populations differed in their genetic base of resistance to *Phytophthora*, the levels of resistance observed were not sufficiently high to warrant use in genetic improvement programs. Hence, identifying new parents and developing new populations are desirable.

QTLs. Results show that resistance to *Phytophthora* root rot is polygenic in the K family. Results also suggest that the parameters measured for resistance are different and may represent different components of resistance. The occurrence of individuals more resistant than the two parents and the detection of QTLs associated with molecular markers from the female-derived map show that resistance alleles coming from both parents contribute to resistance in the progenies (transgressive segregation). Such characteristics are well known in heterozygous species and are useful for combining resistance genetic factors in the same cultivar (Jorge et al. 2001).

Genotypes classified as resistant in 2000 and susceptible in 2001, and vice versa, can be explained by the effect of environmental factors on the biochemical composition of inoculated cassava roots. Such a hypothesis, however, has to be proved.

Future research, ideally, should include:

- Inoculation of each root with a negative control and the pathogen, thereby reducing the probability of evaluating false positives.
- Use of roots without frogskin disease and with diameters measuring 4 to 7 cm.
- Study of factors influencing the expression of resistance.
- Evaluation of roots from different localities, such as Quindío and Cauca.
- Study of *Phytophthora* pathogenesis in cassava roots and resistance mechanisms.

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Activity 6. Super Elongation Disease: Development of hot water treatment of cassava cuttings in the greenhouse at CIAT and in the field on the Colombian North Coast and Llanos Orientales

Greenhouse CIAT

As series of control practices for Super Elongation Disease (SED, causal agent *Sphaceloma manihoticola)* were evaluated in a greenhouse at CIAT. All treatments were applied to two varieties 'Brasilera' (M Col 2737) and 'La Reina' (CM 6740-7). The treatments were:

1. Thermotherapy, stem cuttings immersed in a water bath at 49°C during 49 min.
2. Stem cuttings immersed for 5 min in Score® (difenoconazole, 2.5 cc/L of the commercial product)
3. Stakes immersed for 5 min in Kocide® (copper hydroxide, 5g/L of the commercial product)
4. Stakes immersed for 5 min in water
5. Untreated stakes of the variety M Tai 8 was used as a susceptible control.

The experimental design was a randomized complete block design, with seven replicates and 20 plants per treatment for each variety. Each plant was protected with a plastic cover to avoid contamination by spores between the treatments.

The highest infections (average AUDPC of 22,3) occurred in the varieties M Col 2737 and Cm 6740-7 treated with water **(Table 1)**. This confirms that the stem cuttings, obtained in the field, were highly infected by SED. The highly susceptible variety M Tai 8 did not show any disease symptoms, which indicates that cross contamination did not occur.

Kocide®, a protectant in the case of stem cuttings, provided the best control with an average AUDPC of 6,3. We suggest that foliar applications during the periods when inoculum pressure is great could provide better control. The thermotherapy The AUDPC of 10,5 in thermotherapy treatments was similar to that of water only. Hence we suggest that higher temperatures and longer exposures be tested.

Stem cuttings treated with Score®, which is known to be systemic, had an AUDPC of 19,6. This is a low level of control and for future experiments the immersion time will be prolonged and foliar applications will be made. At the moment in the Atlantic Coast (Sincelejo, Sucre) stem treatment and foliar applications of Score® (2.5 cc/L) and Kocide® (5 g/L) are being tested on susceptible varieties. Stem cuttings were also treated before planting using the same dosis of Score. Score® was more effective than Kocide. Foliar applications appear to be more effective then treatment of stem cuttings. Leave rapidly absorb the product, which affects the subcuticular growth the hyphae of *Sphaceloma manihoticola*. Although the mode of action of the Score® is both has protective and curative, applications should be initiated when the first symptoms of the disease appears (label information, Score®).

Table 1. Effect of stake treatments on Super Elongation Disease.

Treatment	SED	
	Germination[a]	AUDPC[b]
CM 6740-7		
Thermotherapy	80	7.0
Score®	95	15.1
Kocide®	85	4.4
Water	85	24.5
M Tai 8	95	0
M Col 2737		
Thermotherapy	100	11.1
Score®	100	19.6
Kocide®	85	7.6
Water	100	20.1
M Tai 8	100	0
M Col 2737 and CM 6740-7		
Thermotherapy	90	10.5 c[c]
Score®	97	18.3 b
Kocide®	85	6.3 d
Water	92	22.3 a
M Tai 8	97	0 e

[a] Percentage of germination, number of germinated plants.
[b] Average of the Area Under the Disease Progress Curve.
[c] Duncan's multiple range test, alpha <= 0.05.

Score®® and Kocide® do not persist for long on the leaves, whereas other products like Daconil (chlorothalonil) which have adherents persist for longer periods and can be applied less frequently then Score®® and Kocide®. This product and others will be tested to optimize disease control.

Field evaluations at the Colombian North Coast and Llanos Orientales

Field tolerance to SED, Cassava Bacterial Blight (CBB) and other disease control practices were evaluated in field trials in the municipalities of Sincelejo (Sucre) and Villavicencio (Meta). .

The incidence and severity of SED under various treatments **(Tables 2** and **3)** are currently being evaluate in Sucre with the variety 'Venezolana' (M Ven 25) and Meta with 'Brasilera' (M Col 2737). The regional variety 'Venezolana' was planted with vegetative seed obtained from a farm at Sincelejo, where SED was present. Stem cuttings of the variety 'Brasilera' were collected from a CBB affected crop. The experimental design was a randomized complete block design, with three replicates and 45 plants (Sucre) or 50 plants (Meta) per treatment. Fertilized plots at Meta consisted 80 plants per treatment.

The following genotypes were planted at Sucre to evaluate resistance to SED and CBB (120 plants/genotype): SM 6758-1, SM 1665-2, CM 6119-5, SM1565-17, CM 4843-1, CM 6754-8, CM 4919-1, SM 1438-2, M Tai 8 and the local variety Venezolana (M Ven 25). At Meta the genotypes, 'La Reina' (Cm 6740-7), 'Brasilera' (M Col 2737), and 'Vergara' (Cm 6438-14), were evaluated for their reaction to SED and CBB (500 plants/genotype). Control measures were not applied.

Table 2. Evaluation of the germination of control practices on SED at the Colombian North Coast and Los Llanos Orientales.

Treatment	Germination (%)	
	Sincelejo (Sucre)	Villavicencio (Meta)
Fertilized[a]		
Stake selection	98	56
Thermotherapy[b]	100	-
Kocide®, Sistemin®[c]	99	27
Score®, Sistemin®[d]	100	-
Control, traditional farmer's practice	100	45
Without fertilizer		
Stake selection	100	59
Thermotherapy[b]	100	-
Kocide®, Sistemin®[c]	100	23
Score®, Sistemin®[d]	100	-
Control, traditional farmer's practice	100	60

[a] Percentage of germination, number of germinated plants. [a]Sucre: 15-15-15 (NPK) 300 kg/ha; 10-20-20 (NPK), 300 kg/ha.

[b] Stakes immersed in water heated over a wood fire to 49°C for 49 min.

[c] Stakes immersed for 5 min. in Kocide® (copper hydroxide, 5 g/L) and Sistemin® (dimethoato, 3cc/L).

[d] Stakes immersed for 5 min in Score® (difenoconazol, 2.7 cc/L) and Sistemin®. Score® was applied four months after planting.

Table 3. Evaluation of the germination of 10 different cassava genotypes planted at Sincelejo (Sucre) and Villavicencio (Meta).

Genotype	Germination (%)
Sincelejo (Sucre)	
SM 1565-17	99
SM 1438-2	97
CM 4919-1	87
CM 6119-5	86
SM 1665-2	77
CM 6754-8	98
CM 4843-1	74
CM 6758-1	92
M Ven 25	100
M Tai 8	92
Villavicencio (Meta)	
La Reina (Cm 6740-7)	90
Brasilera (M Col 2737)	95
Vergara (Cm 6438-14)	85

Activity 7. Organic matter and ash amendments as strategies to manage cassava root rots and improve soil fertility

Specific Objectives

To evaluate the effect of amendments of ash and organic matter on root rot incidence, soil fertility and cassava yield

Methodology

Ash (200 g/plant), organic matter (200 g/plant) from dead leaves taken from forest, and an ash:organic matter mixture at a 1:1 ratio were evaluated, for their effects on the yield of two native cassava varieties, using farmer participatory research methodologies. Cassava was grown in *chagras*, which are small plots of slash-and-burn agriculture in rain forest, by women from a Tukano indigenous community in Mitú (Vaupés). Cuttings per plant, yield, root rot percentage and soil analyses were recorded.

Results

Incorporation of ash mixed with organic matter into soil increased cassava yields to 10,058 Kg/ha and 6,153 Kg/ha for yellow and white varieties respectively, while traditional management produced 710 Kg/ha and 1694 Kg/ha respectively. Ash alone produced 3195 Kg/ha and 7277 Kg/ha , while yield by organic matter incorporation reached 5680 Kg/ha and 1553 Kg/ha, for yellow and white varieties respectively Cutting selection without amendments had superior behavior than traditional management (**Table 1**). Root rot incidence was lower when ash was incorporated in the soil. Traditional management, without amendments nor cutting selection, had the lowest root yield and higher root rot. P and Ca were increased by amendments of ash and mix of ash and dead leaves at planting increased levels of P end Ca (**Table 2)**.

Table 1. Effect of organic matter, ash and cutting selection on cassava yield and root rot incidence in two varieties in Mitú (Vaupés, Colombia).

Treatment	Variety	Cuttings/Plant	Yield (Kg/ha)	% Root Rot
Ash	Yellow	4.9	3195	0.0
Organic matter	Yellow	4.2	5680	0.0
Ash + organic matter	Yellow	6.8	10058	0.0
Cutting selection	Yellow	9.6	2536	0.0
Traditional management	Yellow	1.0	710	0.0
Ash	White	5.6	7277	2.1
Organic matter	White	4.3	1553	12.5
Ash + organic matter	White	4.9	6153	0.0
Cutting selection	White	7.9	8289	3.7
Traditional management	White	2.7	1694	36.3

Table 2. Effect of organic matter from, ash and, cutting selection on soil fertility in an indigenous community in Mitú (Vaupés, Colombia).

Treatment	pH	OM[a]	Al	Mg	Ca	Na	K	P
Ash	4.5	4.0	0.16[b]	0.22	1.39	0.25	0.08	12.5
Ash + organic matter	4.1	5.1	0.26	0.27	1.55	0.25	0.07	17.5
Organic matter	3.9	5.1	0.37	0.23	0.98	0.20	0.06	7.3
Traditional management	3.9	5.1	0.33	0.19	0.96	0.21	0.07	9.7

[a] Organic matter.
[b] Parts per million.

Activity 8. Evaluating thermotherapy and biological products for controlling Frogskin Disease in cassava

Objectives

Frogskin disease (FSD) in cassava is transmitted by planting contaminated stakes. No effective method exists for disinfecting stakes and preventing the disease's dissemination. In the following experiments we aim to develop a methodology that includes products and processes for disinfecting cassava stakes of FSD.

Methodology

Experiment 1

Stakes of the cassava variety Parrita—a Chiroza type for the fresh market—were collected from a highly infected commercial cassava crop at Jamundí (Department of Valle, Colombia). Stakes were selected at harvest time to ensure FSD was present. About 80% of the plants presented symptoms, many very severely. The stakes were planted into pasteurized soil, free of FSD, in pots placed in an isolated greenhouse at CIAT.

Before planting, the cassava stakes were treated with one of the following: thermotherapy; immersion in fresh, whole, cow's milk; and immersion in gliricidia (*Gliricidia sepium*) leaf extract. The methodology for thermotherapy, involving hot water, was based on that developed to control *Phytophthora* species (sp. *tropicalis* and others) and *Xanthomonas axonopodis* pv. *manihotis*. Cow's milk has been reported to denaturalize tomato virus and gliricidia is well known in organic agriculture as a viricide.

Treatments:

1. No treatment
2. *Trichoderma* sp., strain 14PDA-4 (1×10^6 conidia/mL); stakes immersed for 30 min and solution applied to soil at planting time
3. Tachigaren® (hymexazol, granular presentation), 0.75 g/L; stakes immersed for 30 min and solution applied to soil at planting time
4. Whole, pure, fresh, cow's milk; stakes immersed and not rinsed
5. Gliricidia (leaf extract, 100 g/L in 50% alcohol, blended, 1 night fermentation), stakes immersed and solution applied to soil at planting time

6 to 20. Hot water therapy:

- immersion in hot water for 49 min at 49°C (no pretreatment)
- pretreatment for 20 min at 49°C followed by 1, 2, or 3 h at each of 49°C, 51°C, 53°C, 55°C, 57°C

Experiment 2

Three genotypes from Jamundi were used: M Bra 383, CM 849-1, and SM 1219-9. Incidence of FSD was between 10% and 70%, according to genotype. Experimental conditions and characteristics were similar to those of Experiment 1.

Treatments:

A. Stakes planted vertically:

1. Gliricidia (50% ethanol, no filtering), stakes immersed, applications to soil
2. Milk (pure), stakes immersed and not rinsed
3. Stakes immersed in water
4. No treatment

B. Stakes planted horizontally:

1. Control

2 to 17. Hot water therapy:

- immersion in hot water for 49 min at 49°C (no pretreatment)
- pretreatment for 20 min at 49°C followed by 1, 2, or 3 h at each of 49°C, 51°C, 53°C, 55°C, 57°C

Experiment 3

See Experiment 2 for the genotypes and experimental design used. Cassava stakes were prepared either with or without a longitudinal perforation that extended from the top of the stake to the center of the medula, using a small drill. After treatment, the top of the stakes were covered with paraffin.

Treatments:

1 and 2. Chlortetracycline (100 ppm or 1000 ppm), stakes immersed and applications (at 1000 ppm) to foliage on a weekly basis

3 and 4. Chloramphenicol (100 ppm or 1000 ppm), stakes immersed and applications (at 1000 ppm) to foliage on a weekly basis

5. Chlortetracycline (1000 ppm), stakes immersed, and alternating weekly foliar applications with either chloramphenicol or chlortetracycline at 1000 ppm

Results and Discussion

Although the experiments are still continuing, we found that germination rates of cassava after the stakes were treated with milk and gliricidia improved by rinsing the stakes (milk) or filtering the extract (gliricidia).

Stakes of the variety Parrita treated with hot water for 3 h at 55°C and for any time at 57°C did not germinate **(Figure 1)**. Germination rates after the other temperatures (49°C, 51°C, and 53°C) were highly acceptable. Germination rates after 1 or 2 h at 55°C were 88% and 78%, respectively. These treatments included a pretreatment of 20 min at 49°C, 24 h before the main treatment.

Hot water treatment of stakes from M Bra 383, CM 849-1, and SM 1219-9 at 53°C (including pretreatment) improved germination (average of 80.6%), compared with no treatment (75.6%). Germination after pretreatment for 10, 20, or 30 min and followed by a main treatment at 53°C for 1 h was, respectively, 91.7%, 77.8%, and 86.1%. Where no pretreatment was given, germination was 66.7%, thus highlighting the importance of applying a pretreatment to improve germination.

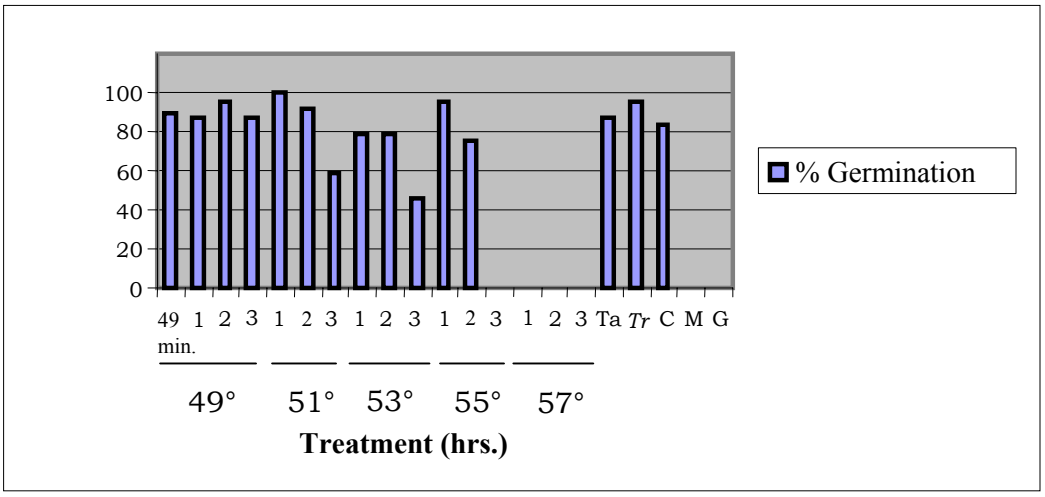

Figure 1. Effect of hot water treatment, Tachigaren® (Ta), *Trichoderma* (Tr) isolate 14PDA-4, milk (M), and gliricidia (G) on germination rates of stakes from the cassava variety Parrita infected with FSD.

Activity 9. Evaluation of organic and no-contaminant products on cassava production

Specific Objectives

- To evaluate the effect of liquid compost, on cassava yield.
- To evaluate the effect on cassava yield of different no-contaminant biostimulant and disinfectant products for cuttings treatment.

Methodology

Cassava cuttings were treated by immersion for 10 min in a composting solution by earthworms at 35%, 50% and 70% and plantain liquid compost at 35%, 50% and 70% compared with chemical treatment with Captan (1.5 gr/Lt) Benomyl (1.5 gr/Lt) and Dimetoato (1.2 cc/Lt and water as control. Cuttings were planted in Ginebra (Valle) and watered after planting with the same suspension for each treatment. Applications each 15 days during first 4 months, were done, to evaluate the effect on cassava yield.

In other assay, cassava cuttings were immersed for 10 min in Agrispon, Agromil, Ecolife, Citroemulsión and Pursue solutions; the first two products are recommended as biostimulants and the others as disinfectants. The cuttings were planted in the same field in Ginebra and watered with the same suspension of each treatment. Plant germination and cassava yield were reported.

Results

In the first assay, the highest yield (35.7 T/ha) was observed after treating CM 523-7 cuttings with plantain liquid compost 50%, while control produced 34.4 T/ha. The average yield for three genotypes treated with plantain liquid compost 70% and 50%, was 26.2 T/ha and 23.8 T/ha, respectively **(Figure 1)**.

In the second trial, no significant differences were observed for germination among treatments, except Ecolife, which had the lowest value (88.4%). The highest yield was obtained in a water treatment, although no significant differences were observed with Ecolife and Pursue (**Figure 2)**.

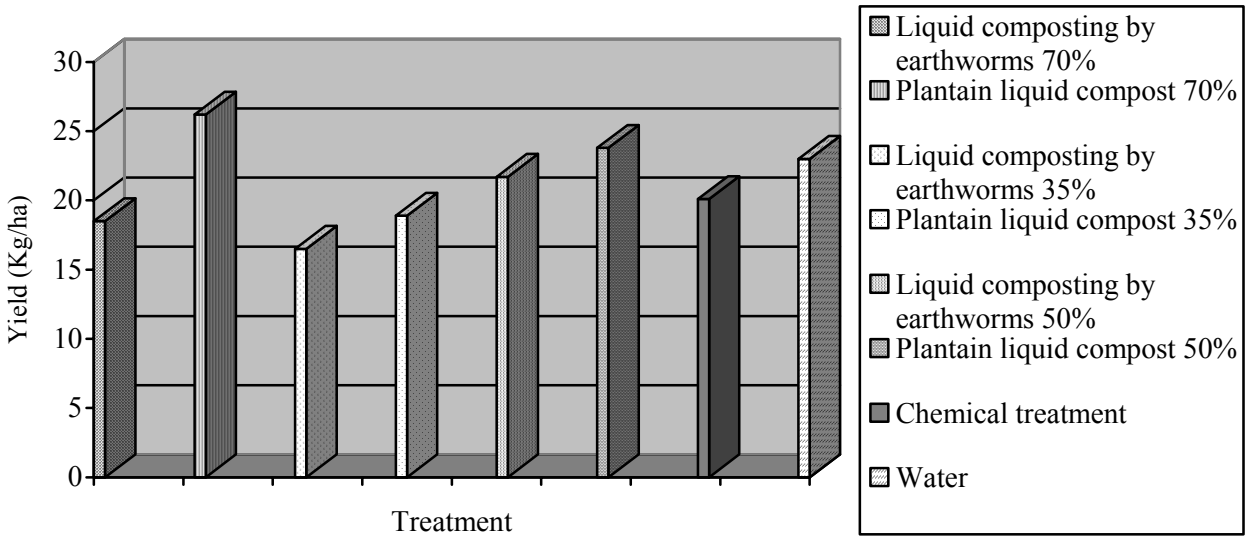

Figure 1. Effect of liquid compost from two sources, and chemical treatment, on cassava yield.

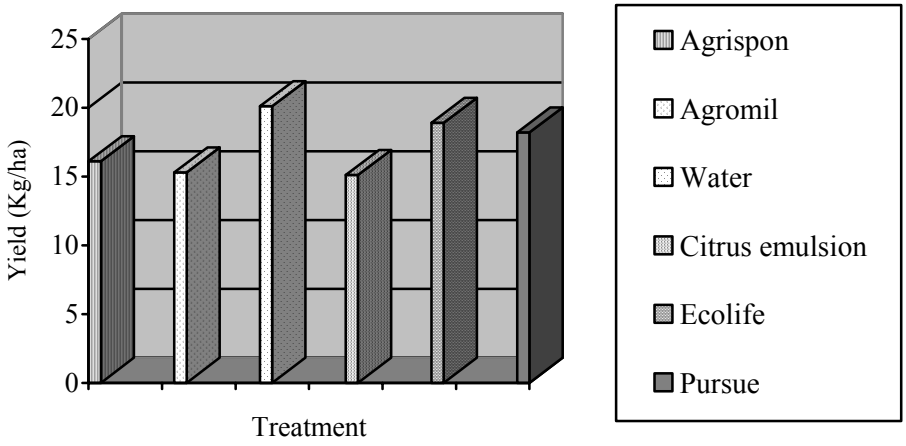

Figure 2. Effect of biostimulants and disinfectants for cuttings treatment, on cassava yield.

Activity 10. Validating a biofungicide and foliar fertilizers as alternative controls of Powdery Mildew (*Sphaerotheca pannosa* var. *rosae*) in rose (*Rosa* sp.) crops

Introduction

Floriculture plays a very important role in the Colombian economy because of growing demand for its products in international markets such as Europe, USA, and Asia. According to the National Administrative Department of Statistics (DANE), the value of Colombian flower exports in 1996 was US$803 million, surpassing products such as coal, banana, petroleum derivatives (except crude oil), and sugar.

The rose crop has brought significant economic and social benefits to several sectors of the Colombian population, and has been a major source of work for female family heads.

Powdery mildew (PM) is caused by the fungus *Sphaerotheca pannosa* (Wallr., Fr.) Lév. var. *rosae* Woronichin. It is widespread and currently affects the quality of *Rosa* varieties cultivated in both the greenhouse and open field, causing serious economic losses. In Colombia, losses are due as much to the costs of chemical control and cultural practices for combating this pathogen as to problems of toxicity arising from regular applications of agrochemicals.

The chemical fungicides used to control powdery mildew are usually sulfates of copper or iron. Inhibitors of sterol biosynthesis are also applied. Many strains of *Sphaerotheca*, resistant to these controls, can survive for several years, thus creating a high risk of strengthening an already resistant population through repeated applications of ineffective fungicides.

Demand for alternative methods of controlling diseases that attack flower crops is growing for two reasons: the society continually demands reduced levels of pesticides in greenhouse drainage water, and few varieties of commercially acceptable roses are also resistant to PM (Reuveni et al. 1995).

Results of previous studies indicate that the foliar fertilizer monoacid phosphate of potassium (K_2HPO_4) induces resistance in plants to the fungus and reduces incidence of the disease by 75%. The studies also established that applications of swinglea (*Swinglea glutinosa*) extract at 100 g/L reduce PM by 85%.

Kettlewell et al. (2000) indicate that PM symptoms in wheat are reduced with applications of potassium chloride (KCl), which creates an osmotic effect in spore germination. Another study found that, in certain cases, the stock for grafted plants induces resistance to some diseases caused by viruses and soil pathogens (Smirnov 1997; Sule 1999).

We discuss below results obtained for commercial rose crops from a combined analysis of six trials that permitted the evaluation and comparison of the effectiveness of several alternatives for controlling *S. pannosa*, such as swinglea extract and certain foliar fertilizers.

Materials and Methods

Powdery mildew. Each experiment began with heavily infecting trial beds with the causal agent of PM. High inoculum pressure made artificial inoculation of plants unnecessary for these trials. While the trials were being carried out, farm personnel suspended disease control for the selected rose beds.

Applying the products. The trials were conducted on three farms located in the Municipalities of Gachancipá ("El Ciprés"), Madrid ("Megaflor"), and Chía ("La Valvanera") in the Department of Cundinamarca, Colombia. At "El Ciprés", applications were carried out on all rose stems in the selected plots, using a motorized sprayer (Maruyama) with two nozzles at a pressure of 25 to 30 psi. The foliage was thoroughly moistened with the product through misting. The treatments were applied every 7 days.

At "Megaflor" and "La Valvanera", applications were made with a 1-L pump, the pressure of which was achieved manually. Only those stems that were selected and marked for evaluation were sprayed. Each application required 1 to 2 liters per plot. On both farms, the treatments were applied every 4 days **(Table 1)**.

Table 1. Treatments applied in the trials at the "Megaflor" (Madrid) and "La Valvanera" Farms (Chía), Department of Cundinamarca, Colombia.

Treatment (Management Type)	Date of Evaluation and Application in Year 2001					
	12 June (day 1)	16 June (day 5)	20 June (day 9)	24 June (day 13)	28 June (day 17)	2 July (day 21)
Traditional control (chemical control)	Meltafun® and Stroby®	Rubigan®	Meltafun®	Rubigan®	Meltafun® and Stroby®	Rubigan®
Swinglea leaf extract in ethanol (SEE) (biological control)	SEE	SEE	SEE	SEE	SEE	SEE
Fertilizers and water (cultural control)	Monobasic phosphate of potassium (8 g/L)	Phosphoric acid	Nutriphite®	Washing with water	Monobasic phosphate of potassium (8 g/L)	Phosphoric acid
Chemical, biological, and cultural control: (integrated control, expensive option)	Meltafun® and Stroby®	SEE	Monobasic phosphate of potassium (8 g/L)	Meltafun®	SEE	Monobasic phosphate of potassium (8 g/L)
Chemical, biological, and cultural control: (integrated control, economic option)	Elosal®	SEE	Monobasic phosphate of potassium (1.5 g/L)	Washing with water	Elosal®	SEE

Preparing the extract at "El Ciprés". Swinglea extract was prepared with leaves from shrubs established at CIAT's Palmira experiment station, Department of Valle. A 100-g sample of clean leaves of various ages and no stems attached was liquefied in each liter of drinking water and filtered through six layers of gauze. Before each application, Inex-A® was added at 1 mL to

1 L of solution to serve as dispersant and adherent. The original extract was conserved in a cold room at 4°C, either on the farm or at CIAT.

Preparing the extract at "Megaflor" and "La Valvanera". Again, leaves from swinglea shrubs established at CIAT were used. The base solution of the extract was prepared by taking 180 g of clean leaves of various ages and no stems attached for each liter of 50% ethanol (industrial grade) and liquefied, using a scythe, in drinking water held in a 55-gallon drum. The mixture of water, ethanol, and submerged leaves was left for 24 h at 18°C to 30°C. Before being bottled, the mixture was filtered through two layers of gauze. Before application, the extract was diluted with drinking water to a concentration of 30 g of leaves per liter of solution, which then contained 8.8% of 50% ethanol. Inex-A® was also added at 1 mL per liter of solution to serve as dispersant and adherent. Between applications, the base solution was stored in a cold room at 4°C, either on the farms or at CIAT.

Resistance inducers. At "El Ciprés", two fertilizers of industrial grade were tested: monobasic phosphate of potassium (KH_2PO_4 at 13.6 g/L) and bibasic phosphate of potassium (K_2HPO_4 at 17.4 g/L). Phytotoxicity was observed and the rates accordingly reduced to 8 and 7 g/L, respectively (trials 3 and 4).

At the farms in Madrid and Chía, two rates of monobasic phosphate of potassium were tested: 8 g/L and 1.5 g/L **(Tables 1** and **2)**; together with phosphoric acid (H_3PO_4 at 1 mL/L) and Nutriphite® (2 mL/L). The last compound, made by Biagro Western Sales Inc., Visalia, USA, contained assimilable phosphorus at 434.0 g/L and soluble potassium at 403.0 g/L.

Fungicides. The following treatments were applied: Elosal® (80% sulfur element at 1 mL/L), Rubigan® (fenarimol) at 0.6 mL/L (no Inex-A®), Stroby® (kresoxim-methyl at 0.25 mL/L), Meltafun® (or Meltatox®, both have as active ingredient dodemorph acetate) at 2.5 mL/L, and a mixture of Meltafun® and Rubigan® (equal dosage for either, no Inex-A®). Those treatments not containing Rubigan® received 1 mL/L of Inex-A®. Table 2 presents data from the evaluations of these fungicidal chemicals and other products applied.

Cultural practices. The efficiency of washing the plants with abundant water was evaluated.

Table 2. Chemical fungicides and other products evaluated for their effectiveness in controlling powdery mildew on roses.

Fungicide	Manufacturer	Chemical Group	Active Ingredient	Toxicological Category[a]	Cost/application (US$/L)	Dose per Solution Liter
Stroby®	BASF	Strobilurins	Kresoxim-methyl	I	0.056[f]	0.25 mL
Meltafun® or Meltatox®	BASF	Morpholine	Dodemorph acetate	II	0.080[g]	2.5 mL
Rubigan®	DOW	Triazole	Fenarimol	II		0.6 mL
Elosal®	Aventis	Sulfurates	Sulfur	IV	0.004[h]	1.0 mL
SEE[b]					0.050[i]	30 g
SEW[c]					0.071[j]	100 g
KH_2PO_4[d]					0.005[k]	1.5 g
KH_2PO_4					0.028[k]	8 g
KH_2PO_4					0.048[k]	13.6 g
K_2HPO_4[e]					0.035[l]	7 g
K_2HPO_4					0.086[l]	17.4 g

[a] Categories I to IV, where I is the most toxic (*Farm Chemicals Handbook*. 2001. Meisterpro Information Resources).
[b] Swinglea leaf extract prepared in ethanol.
[c] Swinglea leaf extract prepared in water.
[d] KH_2PO_4 = monobasic phosphate of potassium.
[e] K_2HPO_4 = bibasic phosphate of potassium.
[f] US$55.94/250 mL.
[g] US$31.79/L.
[h] US$3.54/L.
[i] Labor US$5.30 + ethanol US$7.07 + various US$2.48; total = US$14.85 per 300 L of extract.
[j] Labor US$17.68 + various US$3.54; total = US$21.22 per 300 L of extract.
[k] US$3.54/kg.
[l] US$4.95/kg.

Evaluation. Twenty, previously selected, stems per plot were evaluated at "El Ciprés", 9 at "Megaflor", and 9 at "La Valvanera". At "El Ciprés", the stems with the most number of infected leaves were selected. At "Megaflor" and "La Valvanera", in contrast, those stems that were free of powdery mildew at the beginning of the experiment were marked with tape and not harvested until the end of the trial. At "El Ciprés", those stems located at the extremes of each plot, or close to streets, were not evaluated. Each stem was evaluated for severity of PM according to a scale of 1 to 5, as follows:

1 = healthy stem
2 = 1 to 2 leaves per stem infected, but with few symptoms; lesions 4 to 6 mm in diameter (disease begins)
3 = stem moderately infected; lesions larger than 7 mm in more than 2 leaves so that they superimpose a white hue on the foliage
4 = most leaves on the stem are infected; the area affected in each leaf is relatively large (often as much as half of the surface area); the disease is seen on more than three leaves and on the stem itself

5 = many leaves are infected; the leaves are totally colonized; small fungal colonies are seen on more than five leaves per stem

Those leaves presenting tiny fungal colonies were recorded as infected. Data were analyzed through the Excel calculation program. At the end of each experiment, the following variables were also evaluated: incidence of disease, number of infected leaves per stem, and degree of fungal sporulation (on a scale of 1 to 3, where 1 = no sporulation and 3 = abundant sporulation).

Experimental design. The design of the trials on the three farms in the Sabana de Bogotá, Cundinamarca, was randomized complete blocks. Each trial had three blocks or replicates. One treatment per block was assigned at random.

Analysis of observations. The SAS statistical package was used to conduct an analysis of variance of the six trials combined, and significant differences established with Duncan's test (α = 5%).

The incidence and severity of the disease were corrected according to the damage done by the disease at the beginning of each trial.

Results and Discussion

In 2001, six trials were carried out on three farms in Cundinamarca to validate the efficiency of different practices for controlling powdery mildew of roses. At "El Ciprés", four trials were carried out with the cultivars Aalsmeer Gold and Charlotte. These practices were evaluated individually and the results presented by Alvarez et al. (2000b). The other two trials were conducted on the other two farms to evaluate different methods or combinations of methods that control PM in cultivar Aalsmeer Gold. Both cultivars were evaluated under conditions of very high inoculum pressure **(Table 3)**. Table 4 shows the results of the combination analysis of the six trials. Results were:

Table 3. Extent of damage caused by powdery mildew (PM) found on rose cultivars at the beginning of each of six trials held in Cundinamarca, Colombia.

			Area Under the Disease Progress Curve (AUDPC)	
Farm (Municipality)	**Cultivar**	**Trial**	**For Incidence (%)**	**For Severity[a]**
"El Ciprés"	Aalsmeer Gold	1	0	1.0
(Gachancipá)	Charlotte	2	0	1.0
	Aalsmeer Gold	3	12.5	1.9
	Charlotte	4	21.9	2.0
"Megaflor" (Madrid)	Aalsmeer Gold	5	54.0	1.9
"La Valvanera" (Chía)	Aalsmeer Gold	6	76.3	2.0
Average			27.5	1.6

On a scale of 1 to 5, where 1 = the evaluated stems do not show PM, and 5 = plants have many leaves infected by PM, with the leaves being totally invaded; small colonies found on more than five leaves per stem.

- *Swinglea extract prepared in ethanol* (SEE, biological control treatment) is the only one to significantly reduce both the rate of increase in the incidence (percentage of infected stems per plot) and severity of PM (degree of disease per stem). The importance of ethanol in preparing the extract must be emphasized, because the ethanol:

- Breaks up the cells of the swinglea leaves, thus liberating a chemical containing the active ingredient that controls PM;

- Helps control insects, bacteria, and fungi present in the plant sample and in the extract preparation; and

- Reduces the rate of decomposition of the plant extract, thus permitting conservation over more time.

The great difference observed between the first four trials and the last two is a result of reducing the strength of the swinglea extract. Similar results were obtained in the greenhouse at CIAT (Palmira, Valle) with another plant extract: lixiviate of plantain. Disease control effectively increases even when relatively low doses of the extract are applied. Likewise, experiments at CIAT showed that (1) ethanol (to as much as 50% in the extract) does not cause phytotoxicity in rose; and (2) ethanol alone does not control powdery mildew.

- *Integrated (and expensive) control* and *traditional control (chemical)* also reduce the rate of increase in stems infected by PM and the severity of disease, but not as significantly as swinglea extract prepared in ethanol.

- *The integrated (and economic) control* reduced incidence of the disease on "La Valvanera" Farm. This treatment comprised two applications of swinglea extract prepared in ethanol, one application of monobasic phosphate of potassium at 1.5 g/L), and one washing of the plants with water. Two applications of Elosal® was also used, although this product was no more effective in the trials on this farm than in those of the others.

None of the treatments succeeded in reducing incidence of PM, merely "restraining" the rate of increase in the number of infected stems. It should be pointed out that, at the beginning of each experiment, the rose beds were very heavily infected with PM: in the six trials, disease incidence was 27.5% and the severity score 1.6. This last value indicates that infection by *S. pannosa* var. *rosae* was in its early stages **(Table 3)**.

Better control can be achieved and greater differences among the treatments detected if disease incidence and severity were lower. Consequently, constant monitoring of the disease and its timely control are most important.

Inducers. No clear differences were found in the response of cultivars to resistance inducers.

Severity and incidence. Figures 1 and 2 indicate that the treatments "chemical control" and "biological control" (swinglea leaf extract in ethanol) were more efficient in disease control than were the other treatments. However, all the control packages significantly reduced severity in terms of numbers of lesions and foliar area infected.

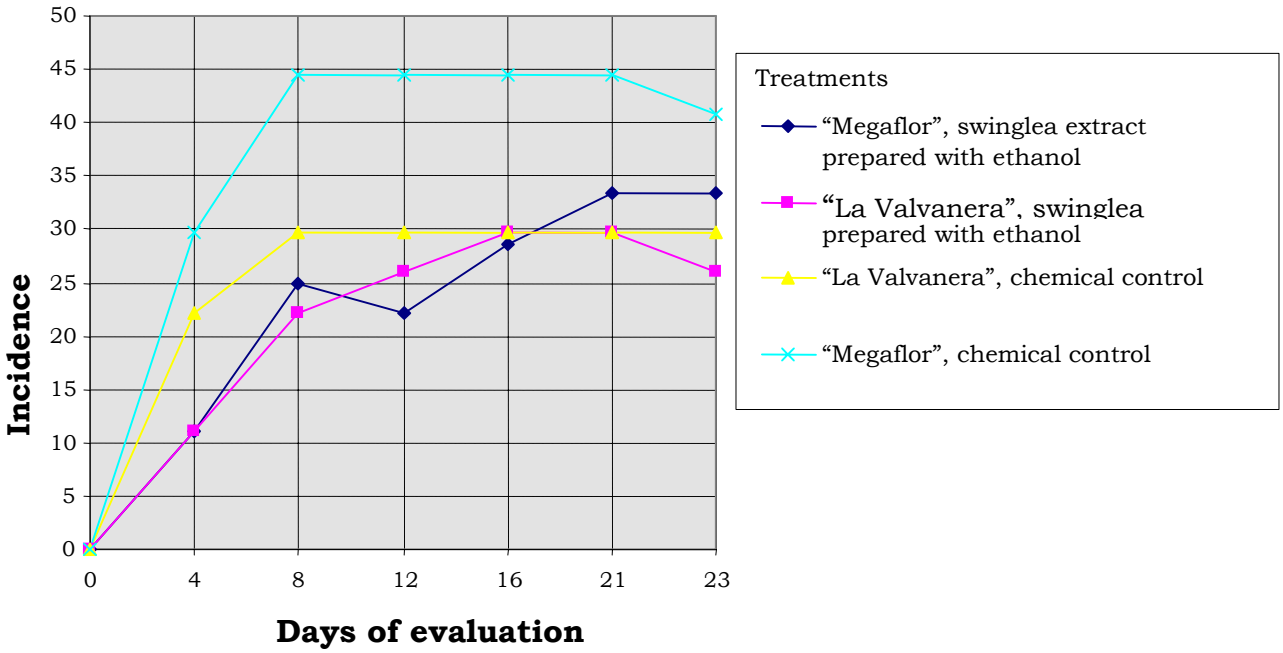

Figure 1. Effect of swinglea leaf extract prepared with ethanol on a commercial rose crop on the farms "Megaflor" and "La Valvanera", Department of Cundinamarca, Colombia. Averages were corrected, based on *incidence of disease* observed on day 0. The check is traditional chemical control, where the fungicides Meltafun®, Stroby®, and Rubigan® are used.

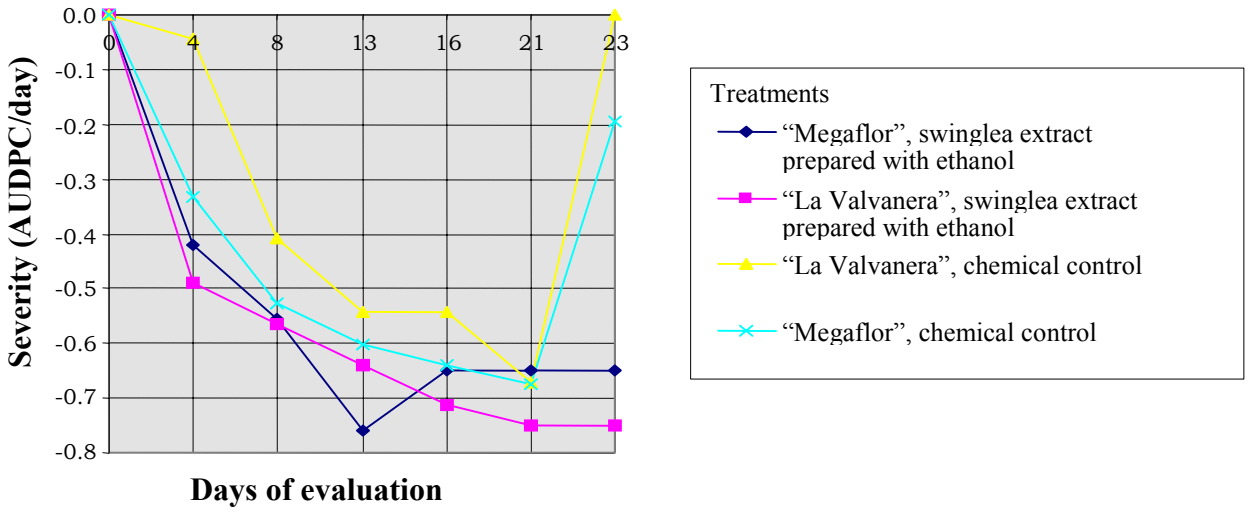

Figure 2. Effect of swinglea leaf extract prepared with ethanol on a commercial rose crop on the farms "Megaflor" and "La Valvanera", Department of Cundinamarca, Colombia. Averages were corrected, based on *severity of disease* observed on day 0. The check is traditional chemical control, where the fungicides Meltafun®, Stroby®, and Rubigan® are used.

Chemical fungicides. The products evaluated on the farms "Megaflor" and "La Valvanera" did not cause phytotoxicity nor left noticeable quantities of residue on the plants. The fungicides Stroby®, Rubigan®, and Elosal® did not control the disease. Initiating control when incidence of PM was still low was more effective. The number of farms on which the treatments were evaluated was small (only 3), not permitting adequate comparisons between farms. However, control of PM could be improved at "Megaflor" and "La Valvanera" (where disease incidence was higher) if applications of the products were more frequent, that is, at intervals of 3, not 7 days. The 7-day interval was acceptable at "El Ciprés", where the initial intensity of disease was low. This conclusion takes into account the short time between infection and sporulation that characterizes *S. pannosa*.

One interesting finding was that, in the experiments at CIAT, leaves of different rose cultivars were infected on both sides, whereas, in Cundinamarca, the leaves were colonized only on the lower side. Nevertheless, the practices controlling the disease at CIAT were also effective in Cundinamarca.

Flower quality. Table 5 lists the effect different control practices had on the quality of flowers harvested at the end of the trials.

Table 5. Effect of control practices for powdery mildew on flowering and stem thickening in rose plants.

Management	Flowering		Stem thickness		Average category[b]
	Value[a]	Category[b]	(mm)	Category[b]	
Cultural control	1.89	2	6.0	1	1.5
Integrated control (expensive option)	2.15	1	5.9	4	2.5
Biological control	1.82	4	6.0	2	3.0
Integrated control (economic option)	1.87	3	5.8	5	4.0
Chemical control	1.75	5	5.9	3	4.0

On a scale of 1 to 3, where 1 = closed floral button; 2 = floral button opening; 3 = floral button open.
On a scale of 1 to 5, where 1 = good, and 5 = deficient.

Control. An efficient and lasting control of PM is difficult to achieve because of rapid and continuous formation of new leaves, existence of different biotypes of the pathogen, the fungus's capacity to survive in both crop and soil over considerable time, and the absence of effective control practices.

When nonsystemic products are used, the continuous formation of new leaves in rose plants obliges increasing the frequency of applications. Should swinglea extract induce resistance (a question now being studied), then the time between applications could be increased. The trials did not show whether heavily infected beds could be made healthy. One alternative would be a heavy pruning, followed by applying an integrated management system that combines applications of swinglea leaf extract in ethanol, monobasic phosphate of potassium, and phosphoric acid, with washing plants with water.

Relatively small plots and variable initial damage levels were used in the treatments. These limitations could be drastically reduced by designing more blocks or replicates and using more stems per treatment on a larger number of farms.

An alternative practice. After a series of trials at CIAT and in the Sabana de Bogotá, we conclude that monobasic phosphate of potassium is an effective, low-cost, alternative practice of control, with very low levels of toxicity for humans. It is more efficient than the chemical fungicides Stroby® and Rubigan®, which are widely used in the Sabana de Bogotá and which, respectively, belong to toxicology categories I and II (i.e., highly poisonous).

Lyophilization. To optimize the effectiveness of swinglea leaf extract, experiments are being carried out to lyophilize the extract as prepared in ethanol. Lyophilization improves the product's durability, thus reducing transport costs. The freeze-dried product permits determination of the optimal dose for application according to its efficiency in disease control and minimizing the probability of causing phytotoxicity. Thus, a pure active ingredient does not have to be produced.

Biological control package. The biological control package discussed in this study consists only of swinglea leaf extract. A previous report demonstrated that lixiviate of plantain is also effective. Taking into account that packages are more effective than individual products, new experiments will be conducted with a so-called biological control package comprising swinglea leaf extract and lixiviate of plantain. The results of the combination analysis suggest including monobasic phosphate of potassium **(Table 4)**. To design a package, the order of the different control practices of each package should be based on the following criteria:

- The most efficient practices are applied first (efficiency as determined in experiments at CIAT); and

- Two products with a similar action on the disease are applied one after the other (e.g., monobasic phosphate of potassium and Nutriphite®, which both contain potassium).

Cultural control package. Including phosphoric acid in the "cultural control" package would be correct because, in preparing the fertilizer, this acid is obtained by dissolving phosphoric rock. Cultural control was initiated with an application of potassium phosphate because it contains different elements that are important for obtaining resistance to the disease.

Perhaps the most appropriate design of control packages or disease management is still unknown. Very efficient packages can be made if the mechanisms of action of each control practice and durability of effectiveness are known.

Table 4. Effect of different treatments on the incidence and severity of powdery mildew in three commercial rose crops, Department of Cundinamarca, Colombia.[a]

Treatment (Management Type)	Incidence		Severity		Average category
	Value[b]	Category[c]	Value[b]	Category[c]	
Swinglea leaf extract in ethanol (SEE) (biological control)[d]	21.9 a	1	-0.47 a	3	2.0
Chemical, biological, and cultural control: series of practices, expensive option[e] (integrated control)	30.6 abc	4	-0.53 a	2	3.0
Traditional control: series of practices[f] (chemical control)	31.8 abc	5	-0.53 a	1	3.0
Chemical, biological, and cultural control: series of practices, economic option[g] (integrated control)	24.8 ab	2	-0.39 a	5	3.5
Cultural control (series of practices)[h]	27.8 ab	3	-0.44 a	4	3.5
Monobasic phosphate of potassium[i]	34.8 abc	6	0.77 c	6	6.0
Bibasic phosphate of potassium[j]	39.5 bc	8	1.04 dc	7	7.5
Rubigan®[k]	39.0 bc	7	1.07 dc	9	8.0
Swinglea leaf extract in water (SEW)[l]	39.5 bc	9	1.05 b	8	8.5
Stroby®[m]	40.2 bc	10	1.19 d	11	10.5
Elosal®[m]	44.7 c	11	1.11 dc	10	10.5
Average	36.0		0.59		

[a] Although, at the beginning of each experiment, differences between treatments were small or nonexistent, the averages were corrected according to incidence and severity at day = 0 (beginning of the experiment). More detailed information on treatments is found under *Materials and Methods*.

[b] Values within a column having the same letter are statistically similar according to Duncan's test ($\alpha = 0.05$).

[c] Scale where 1 = good and 11 = deficient.

[d] Swinglea leaf (30 g/L), solution containing 8.8% of 50% ethanol.

[e] Meltafun® (2.5 mL/L) and Stroby® (0.25 mL/L), swinglea leaf extract in ethanol, monobasic phosphate of potassium (8 g/L), Meltafun®.

[f] Meltafun® and Stroby®, Rubigan® (0.6 mL/L), Meltafun®.

[g] Elosal® (1 mL/L), swinglea leaf extract in ethanol, monobasic phosphate of potassium at 1.5 g/L, washing with water.

[h] Monobasic phosphate of potassium (8 g/L), phosphoric acid (1 mL/L), Nutriphite® (2 mL/L), washing with water.

[i] Monobasic phosphate of potassium (13.6 g/L).

[j] Bibasic phosphate of potassium (17.4 g/L).

[k] Rubigan®.

[l] Swinglea leaf extract in water (100 g/L).

[m] Stroby®.

[n] Elosal®.

Conclusions and Recommendations

- Efficient and durable control of PM is difficult because of a lack of effective practices for controlling the disease, the rapid and continuous formation of new leaves in rose bushes, existence of different biotypes of the fungus causing PM (*S. pannosa* var. *rosae*), and the ease with which this fungus survives in crops and soil.

- The fungicides Stroby®, Rubigan®, and Elosal® do not effectively control the disease.

- Under high disease pressure, one of the experimented treatments that best controlled PM was swinglea leaf extract in ethanol (i.e., "biological control"). It was the only treatment to reduce the rate of increase in the incidence and severity of PM.

- To obtain an extract of swinglea leaves, the protocol developed during the project's execution is recommended (30 g of leaves per liter of dissolvent and 8.8% of 50% ethanol).

- The base solution of the extract can be stored in a cold room at 4°C for a maximum of 3 months.

- A prepared extract in ethanol can be optimized by lyophilization.

- Further study is recommended for the combined application of alternative products such as swinglea leaf extract with other plant extracts reported as effective.

- *Sphaerotheca pannosa* is more effectively controlled if practices are initiated while its **incidence** is still low.

- We recommend using management packages that comprise different practices or control products because their effectiveness is greater than that of individual products independently.

- Treatments (or management packages) of the "integrated control (expensive)" and "chemical (traditional) control" options reduced the rate of increase in the number of infected stems and severity of disease. Their efficiency, however, was less than that of the swinglea leaf extract in ethanol.

- We therefore recommend applying one of the following management packages, in the order of preference given:

    1. **Swinglea leaf extract prepared in ethanol** (biological control); frequency of application is every third day (i.e., 3 free days between applications).

    2. **Integrated control (expensive option)**, which integrates chemical, biological, and cultural controls, that is, Meltafun® and Stroby® (day 1), swinglea leaf extract in ethanol (day 5), monobasic phosphate of potassium at 8 g/L (day 9), Meltafun® (day 13), swinglea leaf extract in ethanol (day 17), and monobasic phosphate of potassium at 8 g/L (day 21).

    3. **Integrated control (economic option)**, which integrates chemical, biological, and cultural controls, that is, Elosal® (day 1), swinglea leaf extract in ethanol (day 5), monobasic phosphate of potassium at 1.5 g/L (day 9), washing plants with water (day 13), Elosal® (day 17), and swinglea leaf extract in ethanol (day 21).

We suggest using each product in the following doses:

- 8 g/L or 1.5 g/L of monobasic phosphate of potassium
- 7 g/L of bibasic phosphate of potassium
- 1 mL/L of phosphoric acid
- 2 mL/L of Nutriphite®
- 434.0 g/L of assimilable phosphorus
- 403.0 g/L of soluble potassium
- 1 mL/L of Elosal® (sulfur element at 80%)
- 0.6 mL/L of Rubigan® (no Inex-A®)
- 0.25 mL/L of Stroby® (kresoxim-methyl)
- 2.5 mL/L of Meltafun® (or Meltatox®, both have as a.i. dodemorph acetate)
- Meltafun® and Rubigan® (fenarimol) in equal doses (no Inex-A®)
- Inex-A® (1 mL/L) should be added to all treatments, except Rubigan® alone and the mixture of Meltafun® and Rubigan®.

The products mentioned above did not cause phytotoxicity to the rose plants in any great degree, nor left large quantities of residue on the plants.

Promising results have been obtained with lixiviate of plantain rachis in the greenhouse at CIAT's experiment station in Palmira. This product provides greater disease control at relatively lower doses.

A biological control package consisting of swinglea leaf extract and lixiviate of plantain should be tried out.
Lixiviates of plantain and fruits and vegetables effectively and constantly reduced sporulation levels of the fungus to almost zero.

Under greenhouse conditions at CIAT, the lixiviates succeeded in reducing the incidence and severity of PM to low levels.

Experiments should be carried out to determine the optimal concentration of lixiviate of freeze-dried plantain, accordingly adapt the system for producing lixiviates, calculate production costs, and determine their effectiveness on commercial crops.

Extracts of bay leaf, papunga (*Bidens pilosa*), and marigold have not demonstrated control of PM under greenhouse conditions at CIAT. Fermentation of these plant extracts in water alcohol for several days may improve their effectiveness.

If the initial attack of the disease is heavy, more frequent applications of these extracts (e.g., 3 days between applications instead of 7) will improve the effectiveness of these controls by shortening the time *S. pannosa* has between infection and sporulation.

Meltafun® is more effective than Elosal® and Rubigan®. Stroby® was not effective on any of the farms.

We conclude that swinglea leaf extract and monobasic phosphate of potassium are effective practices for controlling *S. pannosa*. They are also low cost, and of very low toxicity to man and the environment.

Larger scale studies should be carried out on the effects of the best treatments recommended for flower production and quality. The effectiveness of the different treatments tried here should also be proven against other diseases.

Practices that controlled PM at CIAT were also effective in Cundinamarca. Selecting under highly controlled experimental conditions at CIAT's Palmira station is therefore feasible for "best" treatments (e.g., plant extracts or resistance inducers), which can then be validated under greenhouse conditions in the Sabana de Bogotá for commercial management.

To take maximum advantage of the potential of the technology developed during the project's execution, we recommend analyzing the treatments' performance on commercial crops as presented in Table 6.

The effectiveness of swinglea leaf extract and lixiviate of plantain in controlling other diseases attacking rose and other crops should be determined.

Preliminary trials indicate that lixiviates obtained through composting prevent infection by PM. We therefore recommend that studies be conducted on the mechanisms of action of each practice in controlling the pathogen, the control's effect on the rose crop, and the time for which it is effective to thus obtain more efficient control packages.

We also recommend an analysis of the differential infection of the rose leaf (both sides versus lower side) according to trial site (CIAT versus Cundinamarca farms, respectively).

There were no signs of induced resistance to PM in a rose graft developing on a specific resistant stock.

Table 6. Treatments recommended for future evaluation in the control of powdery mildew of roses.

Treatment	Day of Application			
	0	3	7	10
Biological control	Swinglea leaf extract in ethanol (SEE), freeze-dried	Lixiviate of plantain leaves (LPL), freeze-dried	SEE, freeze-dried	LPL, freeze-dried
Control through integrated disease management	SEE, freeze-dried	Meltafun® and Stroby®	LPL, freeze-dried	Monobasic phosphate of potassium
Control through integrated disease management, but no fungicides of high toxicology	Mixture of freeze-dried SEE and monobasic phosphate of potassium	Mixture of monobasic phosphate of potassium and freeze-dried LPL	SEE, freeze-dried	LPL, freeze-dried
Current control (mostly fungicides)	Management characteristic to each farm			

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Activity 11. Controlling Powdery Mildew (*Sphaerotheca pannosa* var. *rosae*) of roses, using a lixivium of compost of plantain (*Musa* AAB) rachis

Abstract

Powdery mildew of roses, caused by the fungus *Sphaerotheca pannosa* (Wallr.; Fr.) Lév. var. *rosae* Woronichin, is an economically important disease of commercial rose crops. In search of an alternative controlling agent of the mildew, we evaluated under greenhouse conditions the effectiveness of a lixivium obtained from decomposed plantain rachis. The lixivium was applied periodically on young and diseased rose leaves. The severity of the disease was reduced to levels similar—and sometimes even superior—to those achieved after applications of conventional, but more expensive and toxic, chemical fungicides such as Rubigan*12EC® (Dow AgroSciences de Colombia S.A.), Meltafun® (BASF), and Stroby® (BASF). Results thus suggest that this lixivium is a viable biological alternative for controlling powdery mildew.

Introduction

Powdery mildew is an economically significant disease found in all rose-producing countries of the world, in both the greenhouse and field. To control or prevent the disease, expensive and toxic chemical fungicides are currently being used. When improperly handled, they represent a threat for workers using them and generate environmental problems such as contamination of drainage waters.

Lixivium from decomposed plantain (*Musa* AAB) rachis is produced on an artisanal basis **(Figure 1)** in some plantain plantations in Quindío, Colombia, to take advantage of an agroindustrial byproduct obtained after harvest. This biofungicide and others like it are being used to control diseases attacking flower crops, and are considered as an alternative to commercial fungicides.

A **B** **C**

Figure 1. Decomposing plantain rachis at a plantain plantation in Quindío, Colombia. (A) Collecting plantain rachis. (B) Arranging the rachis for decomposition. (C) Collecting the lixivium generated during decomposition.

Our research aimed to evaluate, in a series of trials, (1) the effect of lixivium on the disease and on the rose plant, and (2) the lixivium's effectiveness after lyophilizing (a dehydration process), which would facilitate its marketing.

Materials and Methods

During 2001 and 2002, three trials were carried out at CIAT's experiment station in Palmira, Valle, to validate the efficiency of lixivium obtained from decomposing plantain rachis (LPR) to control powdery mildew of roses. Trial 1 took 14 days and Trials 2 and 3, 17 days each.

Trial 1

We used 54 young and diseased rose plants of the variety Livia, distributing them randomly in nine blocks. For each block, six treatments of lixivium at different concentrations were applied, also distributed at random: 0.05%, 0.5%, 5%, and 50%. The negative checks were plants that did not receive applications, and the positive checks received applications of two chemical biofungicides: Meltafun® (or Meltatox®, which both have dodemorph acetate as the active ingredient, BASF) at 2.5 mL/L, and Rubigan*12EC® (fenarimol, Dow AgroSciences de Colombia S.A.) at 0.6 mL/L. All dilutions were made with tap water, and all treatments received Inex-A® (CosmoAgro) at 1 mL/L to ensure the products adhere to the leaves.

In studying the effect of the treatments, only the first six true leaves of each stem were evaluated, that is, the youngest and, thus, those with the greatest incidence of disease. The evaluations took into account the degree of sporulation per leaf foliole and the area infected per foliole, using a visual scale of 0 to 3 **(Table 1)**. Also evaluated were the variables of phytotoxicity (manifested as change in color or tissue necrosis) and product residue, expressed, respectively, as percentages of leaf area affected and of visible residue.

Table 1. Evaluation scale of the effect of lixivium of plantain rachis on powdery mildew of roses.

Scale	Degree of Sporulation	Area of Infected Foliage
0	None, disease absent	0% (no lesions)
1	Absent, dead fungus	0% to ≤12%
2	Low	12% to ≤25%
3	Abundant	25% to 100%

The trial was carried out within a glass greenhouse, the temperature of which fluctuated between 19°C and 31°C, and the relative humidity between 64% and 98%.

Applications were made by bundle of leaves and on the leaves' lower side, every 3 to 4 days in the morning, with a 700-cm^3 manual spray (Spray PlastiHogar). The pressure of this spray was achieved manually and with care to keep it constant. The nozzle was graduated to mist the solutions.

Four applications of all the treatments were carried out. In this trial, the chemical treatment was Rubigan® and Meltafun®, applied alternately.

Trial 2

We used 96 rose plants of the variety Livia that had a weak infection of powdery mildew. To strengthen the initial degree of infection, artificial inoculation was done with a portable

inoculation tower and several heavily infected leaves as inoculum. Artificial inoculations maintain a high level of inoculum, permitting greater precision when comparing treatments. The plants were distributed at random in 6 blocks of 16 plants each. Each block had two replicates of each treatment.

The lixivium treatments were also distributed at random within each block, and comprised the following concentrations: 25%, 5%, 1%, 0.2%, 0.04%, and 0.008%. Negative checks were plants with no applications, and positive checks were plants that received applications of Meltafun® at 2.5 mL/L and Stroby® (kresoxim-methyl, BASF) at 0.25 mL/L. All dilutions were made with tap water and all treatments received Inex-A® at 1 mL/L to ensure the products adhere to the leaves. The evaluations and applications were carried out under similar greenhouse conditions and taking into account the same parameters as for Trial 1.

Trial 3

This trial aimed to evaluate the effectiveness of LPR after being submitted to a dehydration process known as lyophilization.

We used 25 young and diseased rose plants of the variety Livia, distributing them at random in five blocks. The five treatments that were applied were also distributed at random. The treatments tested were lixivium freeze-dried to 5% (final concentration), 5% lixivium, Rubigan*12EC® at 0.6 mL/L as positive check, water, and plants receiving no applications (negative check). All dilutions were made with tap water and all treatments received Inex-A® at 1 mL/L to ensure the products adhere to the leaves.

To evaluate the effect of the treatments, only the first three true leaves of each stem were used. The evaluations and applications were carried out under the same greenhouse conditions and taking into account the same parameters as for Trial 1.

The dehydrated lixivium was obtained by lyophilizing liquid lixivium in a lyophilizer (Edwards Freeze Dryer Modulyd), the capacity of which was 240 kg. The lixivium yield was 4.9% (i.e., 4.9 g of lyophilized lixivium per 100 mL of liquid lixivium).

Results

The results of the three experiments are presented in Table 2 and Figures 2 to 4. The treatment with the best control over powdery mildew across the three experiments was lixivium concentrated at 5%, because it reduced fungal sporulation and the area of lesions **(Table 2)**. Satisfactory control was also obtained with the other concentrations of LPR, although the 50% lixivium was also phytotoxic.

Because inoculum pressure was high, the untreated plants showed increased sporulation of *S. pannosa* and increased leaf area infected by the fungus.

The fungicides Rubigan®, Meltafun®, and Stroby® significantly reduced fungal sporulation and infected leaf area. However, in two of the three experiments, 5% LPR was more effective in

controlling sporulation than were the chemical fungicides. For infected leaf area, both the 5% LPR and chemical fungicides achieved the same level of control.

LPR did not significantly alter its effectiveness after lyophilization, its positive effects being very similar to those of liquid LPR.

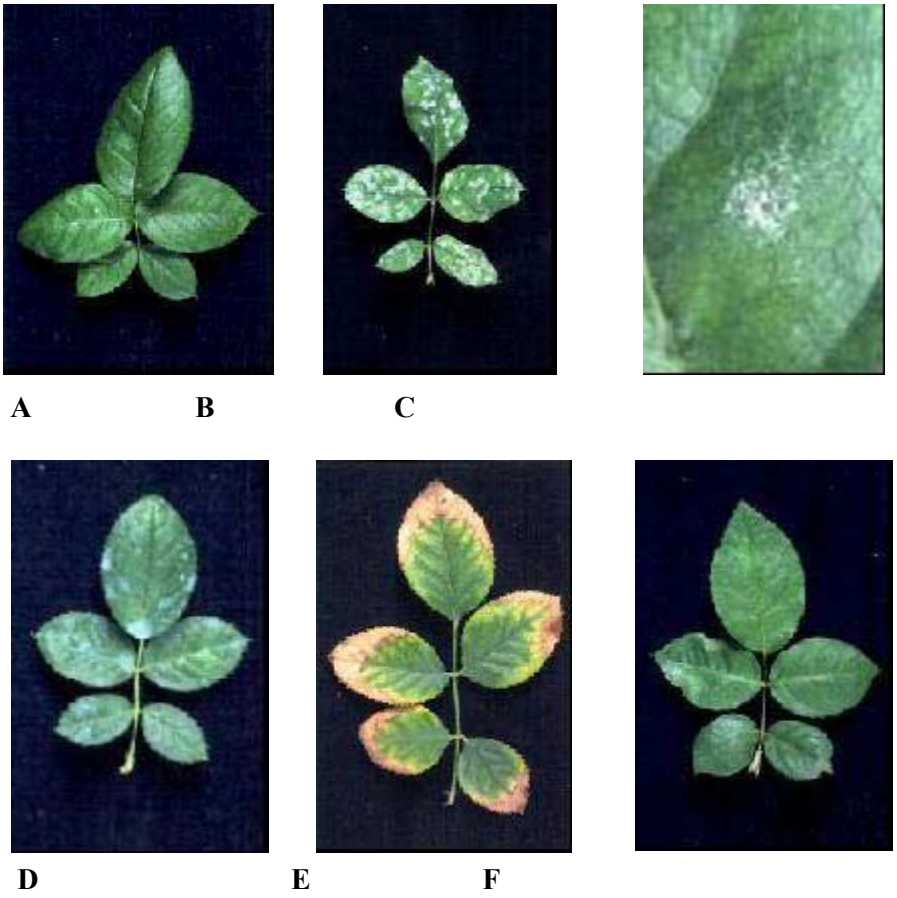
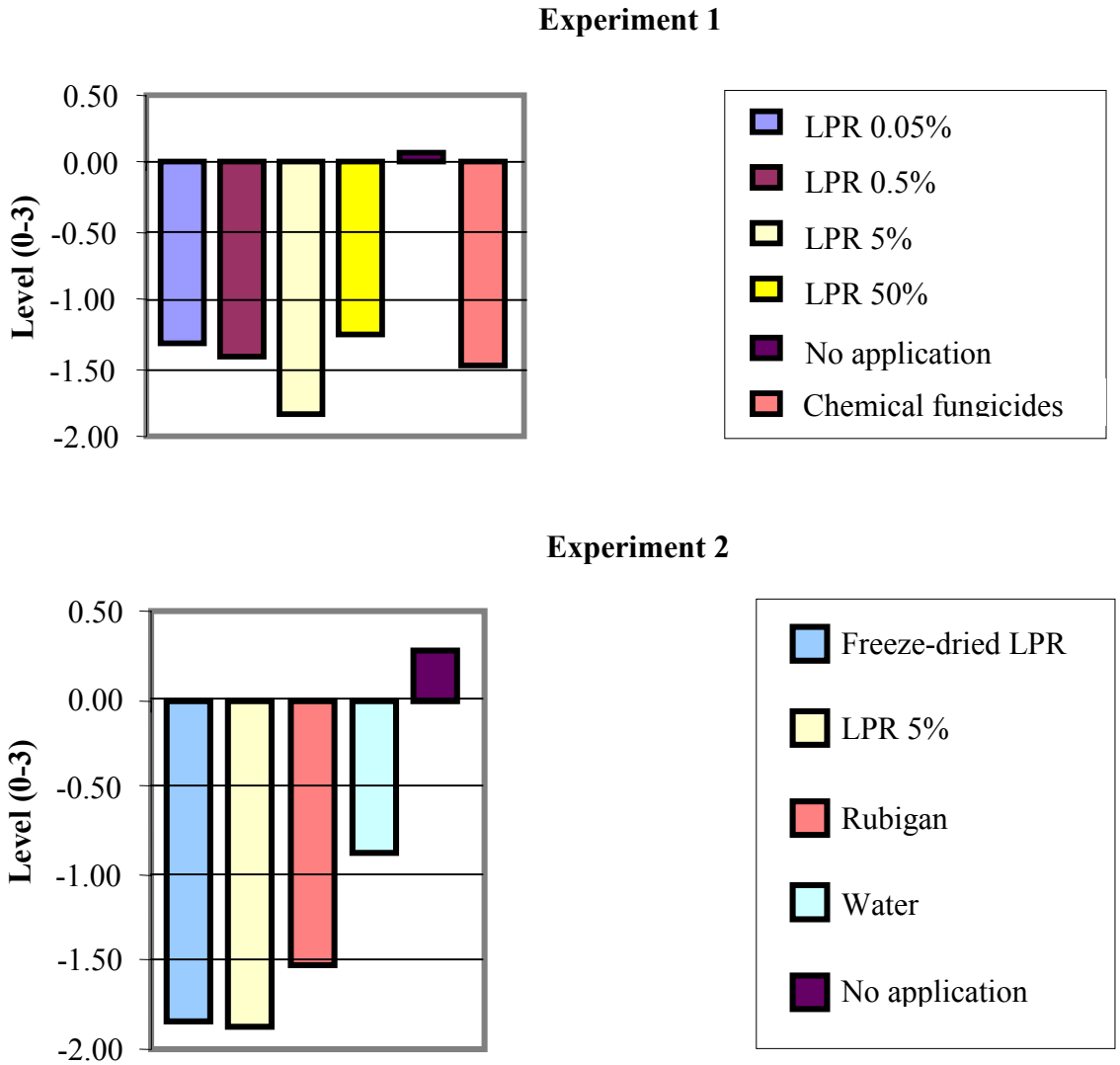


Figure 2. Sporulation of the powdery mildew fungus, infected leaf area, residues, phytotoxicity, and disease control. (A) Healthy leaf. (B) Severely infected leaf (grade 3 sporulation and grade 3 infected area). (C) Close up of an infected leaf (grade 3 sporulation and grade 1 infected area). (D) Residues from an application of chemical fungicide. (E) Phytotoxicity from a high (50%) concentration of lixivium. (F) Powdery mildew controlled by 5% lixivium.



Figures 3-4. **Effect of different concentrations of lixivium of plantain rachis (LPR) and of other treatments on the sporulation of the fungus causing powdery mildew of roses in two greenhouse experiments.**

Table 2. Effect of different concentrations of lixivium of plantain rachis (LPR) and of other treatments on the sporulation of the fungus causing powdery mildew of roses and on the degree of leaf area infected.

Treatment	Effect on:[a]					
	Sporulation[b]			Infected leaf area[c]		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
LPR concentrated to (%):						
0.008	-[d]	-1.08	-	-	1.75	-
0.04	-	-0.75	-	-	0.30	-
0.05	-1.32	-	-	-0.08	-	-
0.2	-	-0.53	-	-	0.31	-
0.5	-1.41	-	-	-0.12	-	-
1	-	-0.87	-	-	0.05	-
5	-1.83	-1.05	-1.86	0	-0.07	-0.26
25	-	-1.14	-	-	-1.89	-
50	-1.24	-	-	0.05	-	-
Freeze-dried LPR[e]	-	-	-1.83	-	-	-0.83
No application	0.07	-0.09	0.26	0.22	0.52	0
Chemical fungicides	-1.49	-1.20	-1.53	-0.19	0.10	-0.26
Water	-	-	-0.86	-	-	0.06

[a] Differences between final and initial evaluations. Negative values indicate reduction in disease.
[b] Scale of 0 to 3, where 0 = absence of disease and 3 = abundant sporulation.
[c] Scale of 0 to 3, where 0 = lesions are 0% and 3 = lesions occupy more than 25% of leaf area.
[d] Not determined.
[e] Equivalent to 5% liquid lixivium.

Discussion

Chemical fungicides (and other agrochemical products) are hazardous for the environment and their use is currently being restricted. However, the rational use of biological agents is being promoted, which are thus acquiring the importance that they merit, including lixiviums of plant tissues. For example, we demonstrated that LPR can control powdery mildew of roses at a level that is equal, and sometimes superior, to those achieved with conventional—but expensive and toxic—chemical products. This potential of LPR to control and prevent the disease will have great economic significance for Colombian rose producers.

Foliar applications of LPR reduced the severity of powdery mildew in rose plants. In other trials, Silverio González, a farmer from Quindío, found that the lixivium causes an inhibiting effect on black sigatoka disease (*Mycosphaerella fijiensis*), which attacks plantain crops.

Lixiviums have been used for years as topical sprays to control foliar diseases in plants (Stindt and Weltzein 1990; Weltzein 1992; Yohalem et al. 1994). Mechanisms suggested for explaining the action of lixiviums include induced resistance and direct inhibition of the pathogen (Weltzein 1992). At CIAT, analyses are being carried out *in vivo* and *in vitro*, using microscopy techniques **(Figure 5)**, to determine the type of control that a lixivium from decomposed plantain rachis exerts on powdery mildew of roses.

Through the collaboration of Flores del Cauca S.A. (Piendamó, Cauca), parallel trials of lixiviums on foliar diseases are being carried out on different flower crops, such as gerbera (*Gerbera* spp.), aster (*Aster* spp.), and chrysanthemum (*Chrysanthemum* spp.).

Adding ethanol as an inhibiting agent of microorganisms to the LPR and treating this lixivium to high temperatures did not significantly reduce the LPR's controlling effect (data not presented). This finding suggests that the LPR's effect results from a chemical agent that it contains rather than from microorganisms that it may carry.

Lixiviums contain unidentified biocontrol agents and chemical factors that play a role in the product's effectiveness (Cronin et al. 1996; Stindt and Weltzein 1990; Weltzein 1992; Yohalem et al. 1994). Chemical analyses performed with the LPR indicated high concentrations of potassium, an element that tends to associate with induced resistance to some diseases. We therefore suppose a systemic acquired resistance (SAR) effect—and induced resistance has great potential in disease control because of its preventive capacity. This would also permit, therefore, a more effective management of powdery mildew of roses.

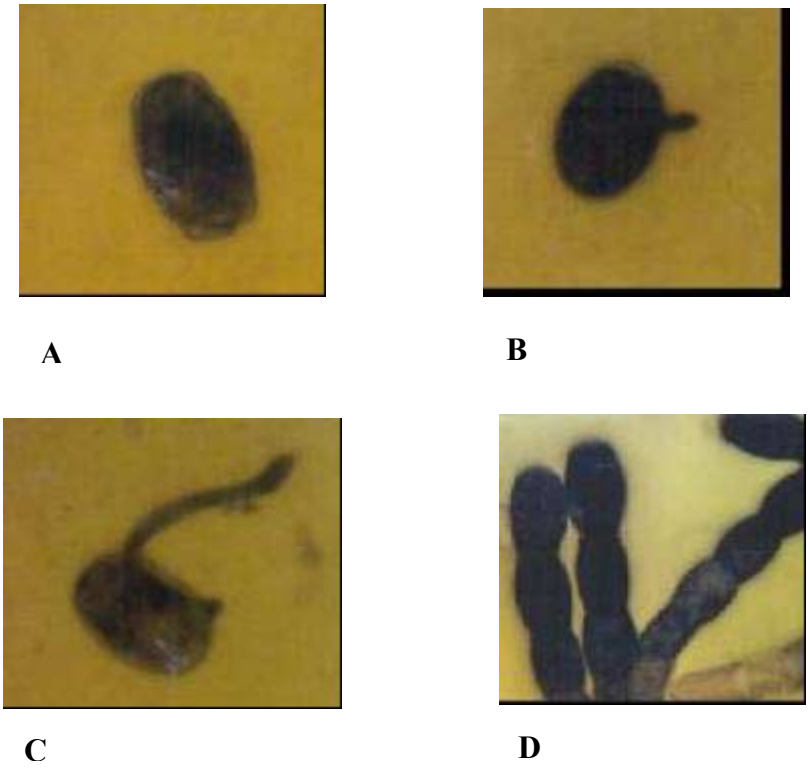


Figure 5. Surface stage of the development of *Sphaerotheca pannosa* var. *rosae* on young rose leaves inoculated *in vitro* in the Cassava Phytopathology Laboratory at CIAT (stained with Trypan Blue). (A) Ungerminated conidium (0-2 h after inoculation). (B) Germinated conidium (3-5 h after inoculation). (C) Conidium with germinal tube and anchorage hyphae (5-7 h after inoculation). (D) Conidium chain (6-7 h after inoculation).

Acknowledgements

To Silverio González (Especial, La Tebaida, Colombia), producer of plantain lixivium.

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Activity 12. Scanning electron microscopy shows that plantain lixivium controls *Sphaerotheca pannosa*, causal agent of Powdery Mildew of roses

To observe the effect of plantain lixivium on powdery mildew of roses, sections of leaflets from infected rose plants were viewed under a scanning electron microscope.

Materials and Methods

We used three sets of leaflets:

- Those collected from plants infected with *Sphaerotheca pannosa* and had not received treatment with plantain lixivium;
- Those that had received, under laboratory conditions, applications of lixivium 6 hours before inoculation; and
- Those that had been inoculated and had not received applications.

We used a scalpel to take samples of leaflets of about 1.0 × 0.5 cm in size, which were then fixed for observation under the scanning electron microscope according to the following protocol:

1. Fixation in phosphate buffer (25% glutaldehyde, phosphate buffer 0.1 M, pH = 7.2) for 24 h under refrigeration
2. Washing in phosphate buffer (0.1 M)
3. Post-fixation in osmium tetroxide (2% in distilled water)
4. Dehydration, using absolute alcohol for 20 min at each of five increasing values: 30% alcohol, 50%, 70%, 95%, and 100%, the last with three changes, also 20 min each
5. Drying to critical point
6. Placing the sample on a specimen mount, using a stereoscope
7. Gold filling (gold-plated palladium mount)
8. Observation under the scanning electron microscope

Results

A drastic effect on the mycelia of *S. pannosa* could be seen in the samples that received an application of lixivium 6 hours before inoculation. The effect was observed throughout the samples, thus corroborating the hypothesis that plantain lixivium exercises control over the disease **(Figures 1-3)**.

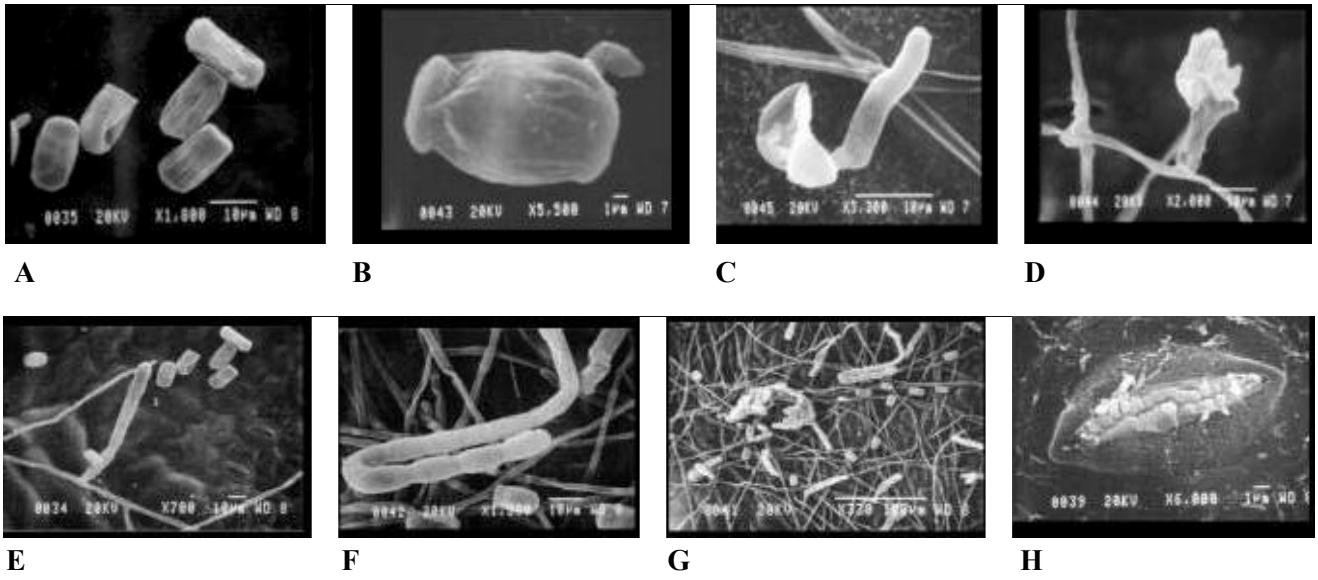

Figure 1. Development of *Sphaerotheca pannosa* on the surfaces of rose leaves as seen with a scanning electron microscope. (A) Conidia. (B) Conidium germinating. (C) Germinated conidium. (D) Conidiospore. (E) and (F) Conidia chains. (G) Mycelia. (H) Mycelia entering a stoma.

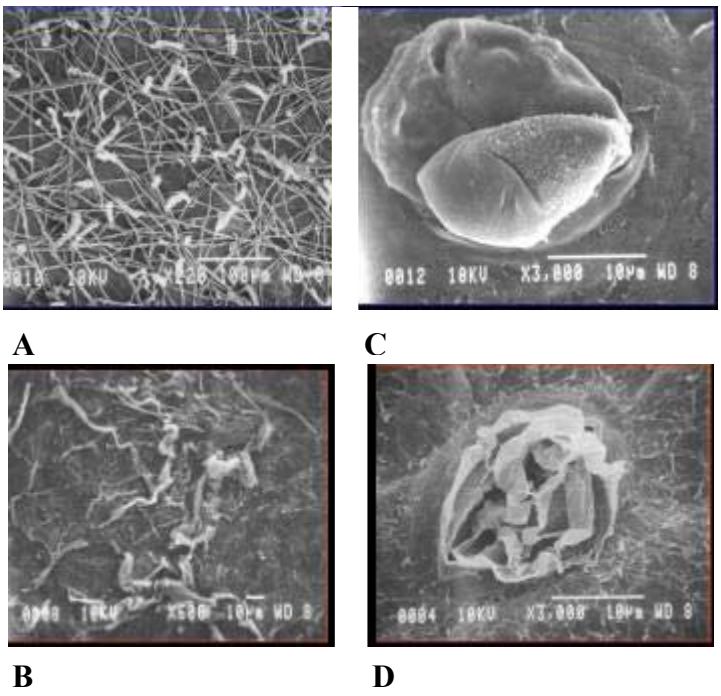

Figure 2. A scanning electron microscope shows the effect of plantain lixivium on mycelia of *Sphaerotheca pannosa*. (A) Mycelia in tissue that had not received lixivium. (B) Mycelia destroyed in tissue that received lixivium 6 hours before inoculation. (C) Conidium in tissue that had not received lixivium. (D) Conidium destroyed as it enters a stoma in tissue that had received lixivium 6 hours before inoculation.

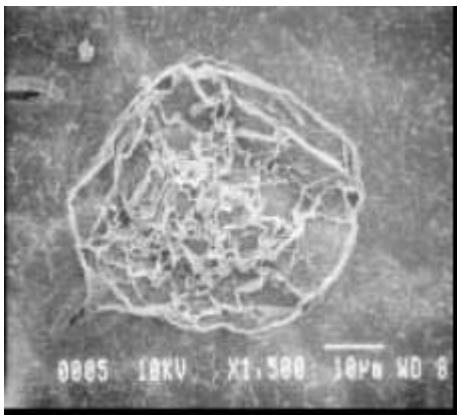

Figure 3. Lysis of conidia of *Sphaerotheca pannosa* by plantain lixivium at 5%.

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Contributor: José Alejandro Arroyave, Virology Unit at CIAT.

Activity 15. Characterization and classification of phytoplasmas associated with oil palm (*Elaeis guineensis*)

Marchitez letal, caused by an unidentified microorganism, is a very important disease of oil palm that has been noticed with increasing frequency in Colombia. Incidences of up to 30% have been recorded in some commercial fields in the production areas of Villanueva and Casanare. The specific primers R16MF2/R16MR1 and then R16F2n/R16FR2 were used in a nested PCR assay to detect and confirm that phytoplasmas were associated with Marchitez letal. To characterize and subsequently classify the phytoplasma, two pairs of universal primers (P1/P7 and R16F2n/R2) were used to amplify the 16S ribosomal DNA gene. A 1.5 kb fragment was amplified from all samples analyzed, revealing lack of size polymorphism in the phytoplasmas infecting oil palm in Colombia. This fragment was present only in samples collected from symptomatic plants. The fragment was cloned and sequenced. Sequence analysis revealed that the oil palm phytoplasma was similar to the aster yellows group, with a sequence homology of 98% to phytoplasma from oil rape seed (GenBank acc. no. U 89378). On the basis of DNA sequences, the oil palm phytoplasma was classified as a member of the 16 SrI in the aster yellows group. This is the first report of 16SrI group of phytoplasma in oil palm in Colombia.

Activity 16. Evaluation of fungicides and resistance inducers to control Bud Rot Disease of oil palm at Meta, Cundinamarca and Casanare (Colombia)

Specific Objectives

To evaluate the effect of a fungicide and a resistance inducer in the control of Bud Rot Disease

Methodology

Trials were carried out to evaluate the effect of induced resistance to bud rot using in 2.5 years old oil palm plant, applied in two ways: injecting 15 cm deep into the trunk below the meristem and absorption by the roots, cutting them 1.4 m from the palm and immersing them in a plastic bag with the inoculum solution. Phosphoric acid was used in concentrations of 25% and 40%, and the product NF (phosphorous acid: 434 g/L P_20_5, potassium hydroxide and potassium citrate: 403 g/L K_2O). Sterile water was used as control. Every 30 days, healthy palms and those moderately affected (2 to 3 according to scale) were treated with 20 cm^3 of each solution. Each month, during 13 months, evaluations were made using a scale from 1 to 4, where 1 represents asymptomatic plants, and 4, plants with all spears rotted, general yellowing in the crown and shortening of leaves.

Results

With the product NF, 38% disease reduction was obtained by means of injection to the trunk and 44% reduction by root absorption. Applications of 40% phosphoric acid or of NF, maintained the initially healthy palms with a low severity of disease (1.1 to 1.6 according to scale). With the application of 40% phosphoric acid, through root absorption, the production of racemes was increased by 37% in healthy plants and by 23% in diseased plants, although it was not as effective as NF in control of the disease. There were no significant differences between injection to the trunk and root absorption. It was concluded that bud rot could be controlled using potassium and phosphorus as induces inductors of resistance, for the integrated management of Bud rot of oil palm **(Figure 1)**.

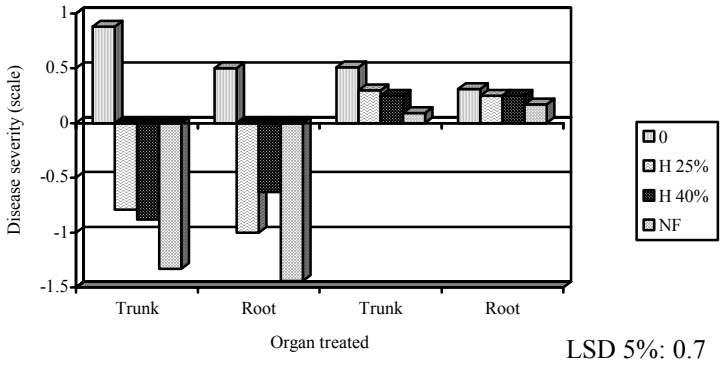

Figure 1. Bud Rot control in oil palm, through injection of a fungicide and a resistance inducer, at a field in Los Llanos Orientales in Colombia.

Activity 17. Evaluating the pathogenicity of *Fusarium oxysporum* f. sp. *vasinfectum* and its association with lethal wilt of oil palm

Introduction

In Casanare (Colombia), attention has recently been focused on a disease for which the causal agent has not yet been determined. Symptoms include yellowing, with reddish tones, of the lower leaves that then become a dark coffee color before drying up. The symptoms start in the apex of the leaf base and are accompanied by rot in the leaf shaft and racemes. The disease is lethal. In Africa, the causal agent of this major disease of oil palm has been reported as wilt by *Fusarium oxysporum*. Flood et al. (1989) have reported on the pathogenicity of Brazilian isolates in some oil-palm clones.

Objective

To determine the pathogenicity of seven *Fusarium* isolates in oil palm infected with lethal wilt.

Materials and Methods

Samples were obtained from plant tissue, leaf shafts, peduncles, and leaves of infected palms. The method of infiltrating petioles was used (Mepsted et al. 1995). Fungal disks, 5 mm in diameter and comprising mycelial growth of the isolates to be evaluated, were placed in Erlemeyer flasks containing a liquid medium with salts (Cooper and Word 1975). The flasks were left in constant agitation for 5 days at 25°C. The isolates were filtered with sterilized gauze, and the filtrate centrifuged for 5 min at 4000 rpm until a concentrate of conidia at 10^7/mL was obtained.

Fragments of petiole, measuring 2.5 × 4.0 cm, were inoculated by the infiltration technique under reduced pressure, achieved by connecting the hose of a vacuum pump to the petiole and the whole submerged in a suspension of conidia so that these infiltrated throughout the tissues. The inoculated fragments were incubated for 8 days at 20ºC-28ºC. Two controls were included: a moist control that was infiltrated with sterilized distilled water, and a dry control that did not receive any infiltration. Both controls were submitted to the same incubation conditions as for the petioles inoculated with *Fusarium*.

The petioles were opened up to evaluate the internal damage against a scale of 0 to 3, where 0 = healthy petiole, 1 = petiole with coffee-colored margins and healthy center, 2 = petiole with coffee-colored margins and the center slightly colored, and 3 = petiole completely coffee-colored.

The results were submitted to an analysis of variance and means separated by DMS (α = 5%), using the SAS statistical package (SAS Institute 1985).

Results and Discussion

Damage in the petioles was observed as changes in tissue staining. However, the change was more evident in the checks than in the inoculated petioles. Because both experiments were carried out under aseptic conditions and because the dry control was not submitted to infiltration, we concluded that such changes in staining are the product of oxidation of plant tissue. Oxidation becomes more pronounced after the petiole is opened and left exposed to oxygen in the ambience. Because these results were obtained with both moist and dry checks, we infer that any change in staining in petioles inoculated with *Fusarium* strains can be attributed to oxidation of plant tissue and is not a manifestation of disease.

The above discussion lead us to conclude that *Fusarium oxysporum* f. sp. *vasinfectum* and *Nectria gliocladiodes* do not cause the pattern of symptoms observed in plantations. Our findings corroborate those of other research on these same isolates but using different methods of inoculation whereby young and healthy palms were perforated to permit the introduction of *Fusarium* strains. This method did not permit reproduction of symptoms.

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Activity 18. Characterizing the Colombian population of *Colletotrichum acutatum*, causal agent of Citrus Anthracnose, using AFLP

Introduction

Citrus anthracnose, caused by *Colletotrichum acutatum*, is a disease that causes considerable losses in orchards because it affects principally flowers and floral buds, thus directly attacking the trees' production and yield.

Previous studies in the main producing area of western Colombia have confirmed the presence of a complex of two species of the pathogen, according to the ITS region of their rDNA and morphological characteristics of *in vitro* growth. One species has been designated as SGO (for "slow-growing orange" strain). It is similar to *C. acutatum* (according to the ITS region), and is considered as causing economic damage through attacking mainly floral organs. It is also tolerant of benomyl. The second species is denominated as the FGG (for "fast-growing gray" strain) group, and is related to *C. gloeosporioides* in that it is saprophytic, ubiquitous in orchards, and sensitive to benomyl.

The above problems encouraged us to carry out in-depth research on the *C. acutatum* population. We used AFLPs, a technique that best encompasses the complexity of the genome. The technique provides a series of comparable points related to phenotypic traits on which to standardize and group individuals, and search for populational subgroups that are specialized for specific conditions or factors such as area and/or host type.

Methodology

Isolates. For this analysis, CORPOICA-Regional 1 supplied us with isolates of *C. acutatum* from different regions of the country and cultured as monospores on paper **(Table 1)**.

DNA extraction. The isolates were cultured in liquid medium (PDA + lactic acid) to produce mycelia for extraction. After 15 to 20 days, mycelia were harvested, dried, macerated, and DNA extracted according to a modified version of the protocol by Dolli and Dolli. DNA quality was verified in 1% agarose gels and quantity calculated by fluorometry.

AFLP. For our situation, we modified and standardized the protocol given in the AFLP manual by Gibco after case. A 350-ng sample of DNA was digested with two restriction enzymes (*Eco*RI/*Mse*I). The digested fragments were ligated to their respective adapters. Then 5 µL of a dilution at 1:5 of the restriction-ligation reaction was amplified with the primers EO = 5'-AGACTGCGTACCAATTC-3' / MO 5'-GACGATGAGTCCTGAGTAA-3', using the thermocycler PTC100. In consecutive order, selective amplification was carried out on those fragments that presented one or two annexed nucleotides, starting with the 1:5 dilution of the previously amplified product (Gibco-BRL, AFLP Analysis System for Microorganisms).

Table 1. Isolates of the fungus *Colletotrichum acutatum* (causal agent of citrus anthracnose) and their origins, used for populational analysis (see also Figure 1).

Citric Line	Isolate	Area	Host Species as Source[a]
1	73c	Caicedonia	NV
2	552cd	Cundinamarca	NV
3	574cd	Cundinamarca	NV
4	648mg	Magdalena	LT
5	671mg	Magdalena	LT
6	653mg	Magdalena	LT
7	10mz	Manizales	LT
8	212c	Caicedonia	LT
9	264ad	Andalusia	Lp
10	8mz	Manizales	LT
11	18r	Armenia	LT
12	1000		
13	23ad	Andalusia	Lp
14	11c	Caicedonia	LT
15	55p	Pereira	LT
16	71mz	Manizales	NV
17	617mt	Meta	Tg
18	1001		
19	7a	Armenia	LT
20	599mt	Meta	Tg
21	621mt	Meta	Tg
22	560cd	Cundinamarca	NV
23	606mt	Meta	Tg
24	267ad	Andalusia	Lp
25	561cd	Cundinamarca	NV
26	14c	Caicedonia	LT
27	539cd	Cundinamarca	NV
28	570cd	Cundinamarca	NV
29	591mt	Meta	NV
30	567cd	Cundinamarca	NV
31	645mg	Magdalena	LT
32	569cd	Cundinamarca	NV
33	107p	Pereira	LT
34	13ar	Armenia	LT
35	201c	Caicedonia	LT
36	6mz	Manizales	LT
37	106p	Pereira	NV
38	132p	Pereira	NV
39	66ar	Armenia	NV
40	1002		
41	98mz	Manizales	NV
42	53p	Pereira	NV
43	1003		
44	398p	Pereira	NV
45	289p	Pereira	NV
46			Marker

[a] NV = Naranja Valencia ; LT = Lima Tahiti ; Lp = Limon Pajarito ; Tg = Tangelo.

The amplified products were denatured with 10 µL of loading buffer (xylene cyanol, bromophenol blue, and formamide) at 94°C for 3 min. They were then placed on ice and submitted to electrophoresis in polyacrylamide gel at 6% (w/v) in 0.5X TBE electrophoresis buffer. For this analysis, we used the EAC/MA combination (5'-AGACTGCGTACCAATTC/ AC-3' and 5'-GACGATGAGTCCTGAGTAA/A-3', respectively), which, in previous studies, showed polymorphism among a small number of isolates, according to differences in area and different citrus hosts.

Statistical analysis. Based on information resulting from the AFLP technique, we generated a binary matrix of data (presence or absence of bands), which was then analyzed with the NTSYS 2.0 statistical package, to generate a similarity dendrogram, using the Dice coefficient.

Results and discussion

A data matrix was obtained from 100 bands for 43 isolates based on the reading of band patterns for the set of analyzed individuals **(Figure 1)**. The similarity dendrogram generated **(Figure 2)** excluded those isolates that did not amplify, and showed a broad variability of the pathogen but no clear clustering of isolates by either area or citrus host.

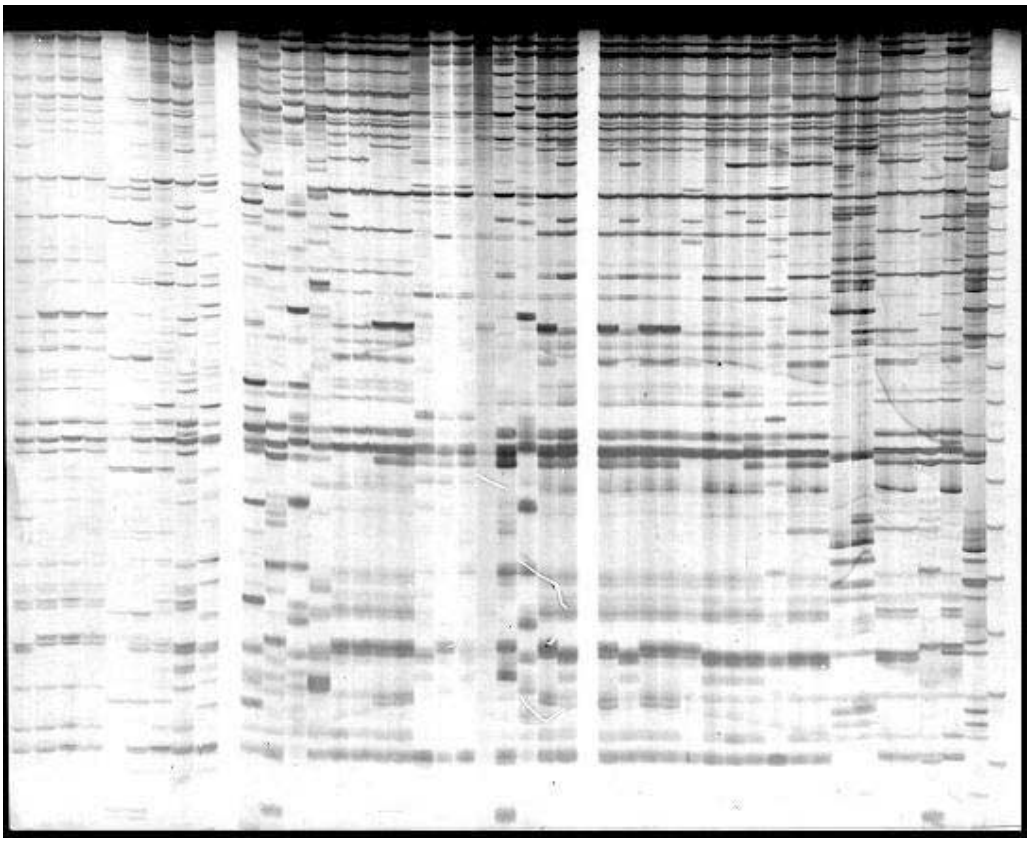

Figure 1. AFLP patterns of isolates of *Colletotrichum acutatum*. Lanes 1 to 45 = isolates of *Colletotrichum acutatum* of different origins; lane 46 = marker, 30-330 in weight.

The dendrogram showed two clusters separated by a similarity coefficient of 0.58 **(Figure 2**, clusters A and C), where A comprised isolates that came mostly from the coffee-growing region. Moreover, of all the isolates from oranges evaluated in the trial, 71% fell in this cluster; and of the isolates from limes, only 47% fell in this cluster. Two subclusters also separated out at the similarity coefficient of 0.8, notably a set of five isolates (subcluster B) from Valencia oranges.

In contrast, cluster C showed high variability, being comprised of isolates that, in their majority, clustered in groups of two isolates below the similarity coefficient of 0.73. About 29% of the isolates forming cluster C are from oranges and 53% from limes, including three isolates from 'Limón Pajarito', even though these show considerable variability among them. Isolate 671 from 'Lima Tahití', from Magdalena, is very different, clustering only at a similarity coefficient of 0.16.

The foregoing shows that, according to host species, isolates from oranges are less variable than those from limes. Consistent clustering by area is more difficult to see, requiring a larger number of isolates needs to be analyzed, including from another combination of primers for more data.

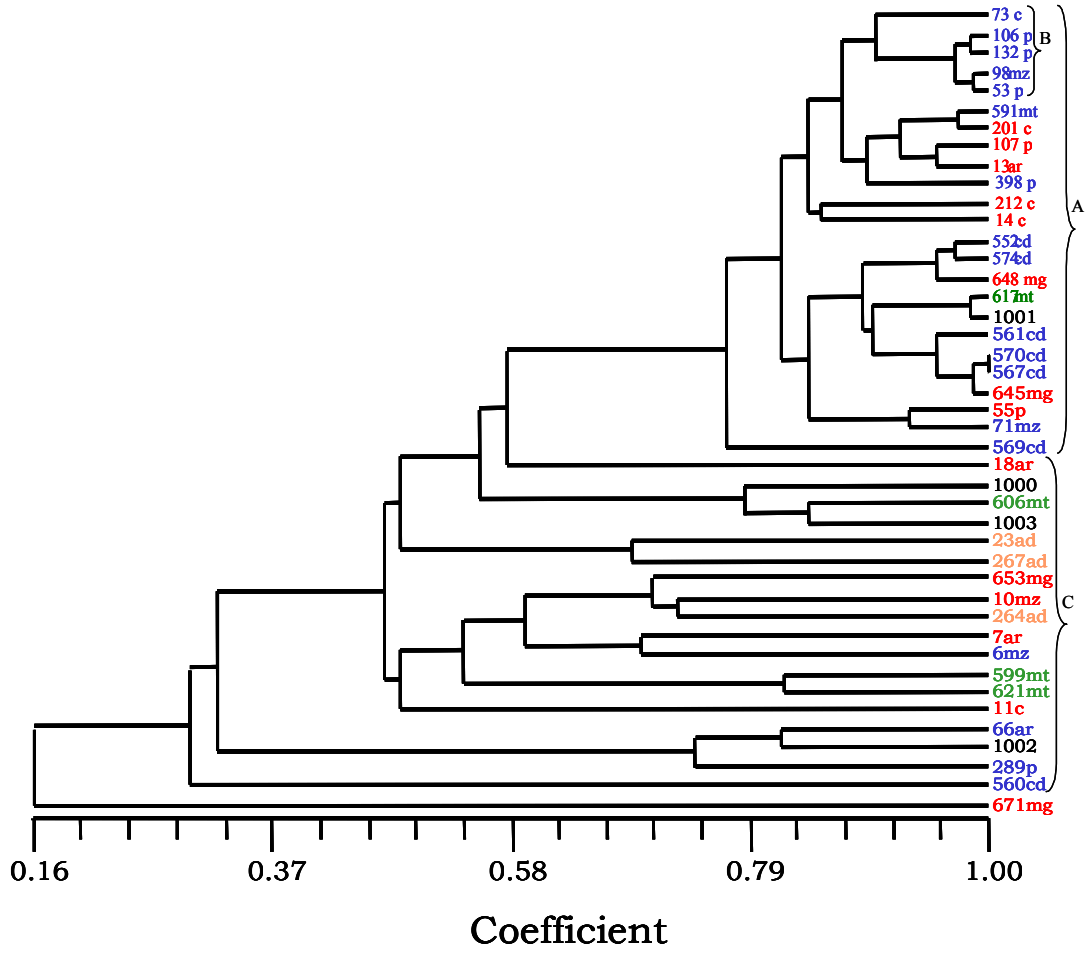

Figure 2. Similarity dendrogram, generated by NTSYS 2.0, for 43 isolates of *Colletotrichum acutatum*, causal agent of citrus anthracnose.

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Activity 19. Plant disease diagnosis

This year, we received samples for bacteriological and fungal diagnoses. Relevant data from bacteria and fungi that have been isolated and identified are presented in **Table 1**.

Table 1. Bacteria and fungi that have been isolated and identified at the Cassava Pathology Laboratory, CIAT, Palmira, Colombia.

Plant Host	Disease	Symptoms	Detection Method	Microorganism Identified
Mango	Anthracnose	Necrotic lesions in the fruit's epidermis, and sticky but dry substance on the upper side of each black leaf.	Isolation and direct observation under a light microscope.	*Colletotrichum gloeosporioides*
Palm		Systemic infection throughout vascular bundles, brown lesions, and necrosis on the inside of the cortex.	Isolation and direct observation under a light microscope.	*Colletotrichum* spp.
Aster chinensis		Irregular, coffee-colored, leaf spots.	Isolation, direct observation under a light microscope, and biochemical testing.	*Pseudomonas* spp., *Bacillus* spp., and *Epicoccum* spp.
Common stock (*Mathiola incana*)		Systemic infection in stalks, chlorosis of leaf veins, and small, moist, but non-necrotic spots.	Isolation, direct observation under a light microscope, and biochemical testing.	*Xanthomonas* spp. and *Bacillus* spp.
Heliconia spp.		Internal necrosis of stalks and crowns.	Isolation and direct observation under a light microscope.	*Phytophthora* spp.
Andean blackberry (*Rubus glaucus*)	Anthracnose	Exterior of stalks carry cankers, and dry rot found within the stalks.	Isolation and direct observation under a light microscope.	*Colletotrichum* spp.
Goldenberry (*Physalis peruviana*)		Whitish, translucent, leaf spots.	Leaves conserved in vials containing 0.05% sucrose, then left in humidity chamber to examine progress of lesions and fungus sporulation.	Obligated fungus (still under study)
Orchids (*Dendrobium* and *Oncidium* spp.)		Root rot: cortex falls away and roots become necrotic, turning coffee-colored or black. Also dry root rot.	Disinfection for 30 or 60 s in 50% alcohol, then cultured on agars AAV, PDA, V8A, AA, or AN, with or without antibiotics and fungicides.	*Fusarium* sp., *Pythium* sp., and *Phytophthora* sp.
Cacao *(Theobroma cacao*)			Isolation with carrot-trap technique and observation under a light stereoscope.	*Ceratocystis fimbriata* and *Phytophthora* sp.

Activity 20. Training farmers, technicians, and extension agents in participatory research, cassava management, oil-palm cultivation, and disease control strategies

Courses

- Fundamentals in molecular biology for plant pathologists, for ASCOLFI (Colombian Association of Plant Pathology and Related Sciences), September 2001.

- Modern systems of cassava production and processing in Colombia: cassava disease management, El Espinal, 20-22 November 2001.

- Cassava production: integrated disease management, three courses for 137 participants Venezuelan municipalities of El Tigre (Anzoátegui), San Carlos (Cojedes), and Maracaibo (Zulia), 4-15 May 2002.

- Modern production, processing, and utilization systems, for Technicians and farmers. CLAYUCA, 25-28 June 2002.

- Integrated management of cassava pests and diseases, for Master Science students from Escuela Politécnica del Ejército, ESPE, Ecuador, 10-12 September 2002

Seminars

- Management of major cassava diseases in the North Coast, with emphasis on Super Elongation Disease, Sincelejo, 19 November 2001.

- Molecular biology techniques applied to crop pathogen identification and characterization. Seminar given during a workshop for an international course on "Tropical hortifruticulture with emphasis on organic production and biological management," for 22 participants from Latin America, with two participants from the Cabildo de Guambía (Silvia, Cauca), CIAT, 15-16 November 2001.

- Advances in cassava pathology research, for 30 students from the Universidad de Caldas, Manizales, 20 December 2001.

- Cassava disease management, for Alejandro Larios, starch producer from Caicedonia (Valle), CIAT, 7 February 2002.

- Super elongation disease management in cassava. Seminar given during the I Regional Workshop on Fast Propagation (*In Vitro*) and Genetic Transformation, 32 participants from Brazil, Venezuela, Ecuador, and Colombia, CIAT, 26 February 2002.

- Advances in cassava pathology research, for 34 students from the Universidad de Caldas, Manizales, 18 April 2002.

- Cassava diseases: diagnosis and control, for 4 researchers from Haiti, 15 May 2002.

- Cassava pathogens, for 30 bacteriology students from the Universidad del Valle, Cali, July 2002.

- Integrated management of cassava diseases, for Manuel Naranjo and Jorge Peña, cassava agronomists from Casanare, 5 August 2002.

- Advances in the knowledge and management of rose mildews, for ASOCOLFLORES, Bogotá, 29 August 2002.

- Principal cassava pathogens. Seminar given during the III International Congress of the National College of Bacteriologists (CNB), Universidad del Valle, Cali, 1-4 November 2002.

- Advances in cassava pathology research, for 32 students from the Universidad de Caldas, Manizales, 26 September 2002.

Training

- Three courses on soil management and integrated pest and disease management, 242 indigenous women farmers of the communities of Cucura, Bocas del Yí, and Macaquiño, Colombia, October-December 2002.

- Establishing Local Agricultural Research Committees, 15 indigenous farmers and local technicians, April 2002.

- Isolating *Sphaceloma manihoticola* and understanding Super Elongation Disease, Juan Manuel López, Professor of Genetics, Universidad de Sucre, Colombia, April 2002.

- Isolation and inoculation of *Phytophthora* sp. in soybean, Ana Claudia Gordillo, CORPOICA "La Libertad", Villavicencio, April 2002.

- Isolation and inoculation of *Phytophthora* spp., *Pythium* sp., *Fusarium* spp., Alexandra Delgado, Hacienda San José, Palmira, April-September 2002.

- Soilborne pathogens in cassava and sugarcane, Mariela Becerra, Universidad Francisco de Paula Santander Facultad de Ciencias Agrarias y del Ambiente, Cúcuta, May-June 2002.

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue, soil, weeds, and water in crops affected by *moko,* Luz Piedad Estrada, ICA-Quindío, June 2002.

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue, soil, weeds, and water in crops affected by *moko,* Ana Lucía Gaviria and Yaneth Rivera, Universidad del Quindío, Armenia, June 2002.

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue, soil, weeds, and water in crops affected by *moko*, and management of *moko* by disinfection of soil and tools, Rosinelly Pérez, Especial, La Tebaida, Quindío, July 2002.

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue and soil in crops affected by *moko,* Carlos Aníbal Montoya, ICA-Palmira, 16 July 2002.

- Cassava disease management, Norman Pérez, Chemonics, Putumayo, 18 July 2002.

- Molecular and traditional characterization of *Ralstonia solanacearum,* Abraham Oleas, Ecuador, 16-17 September 2002.

- Biological controllers, César Cano, Perkins, July-August 2002

- *Phytophthora* spp. culture management, Alejandro Corredor, Universidad de Caldas, Manizales, 9 August 2002.

Publications

E Alvarez, JL Claroz, JB Loke, C Echeverri. 2001. Caracterización genética y patogénica en Colombia de *Sphaerotheca pannosa* var. *rosae*, agente causal del mildeo polvoso en rosa. Fitopatología Colombiana 25(1-2):7-14,

E Alvarez, CX Grajales, J Villegas, GA Llano, JB Loke. 2001. Control del mildeo polvoso en cultivos de rosa por aplicación de un lixiviado de compost de plátano. Poster presented at the XV Muestra Agroindustrial y Empresarial. Universidad La Gran Colombia, Armenia, Quindío, 1-3 November 2001.

E Alvarez, JB Loke, GA Llano. 2002. Desarrollo de prácticas ecológicas de manejo de pudrición radical (*Phytophthora* spp.) en yuca (*Manihot esculenta*). Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

E Alvarez, CX Grajales, J Villegas, JB Loke. 2002. Control de mildeo polvoso en rosa (*Sphaerotheca pannosa* var. *rosae*) por aplicación de lixiviado de compost de raquis de plátano. Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

GA Llano, E Alvarez, MC Feris, ML Hernández, SM Rodríguez. 2002. Evaluación de dos inductores de resistencia para el control de pudrición del cogollo en palma de aceite *Elaeis guineensis* en los Llanos Orientales de Colombia.Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

E Alvarez, JL Claroz. 2002. Caracterización molecular y clasificación de fitoplasmas asociados con la palma de aceite. Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

E Alvarez, GA Llano. 2002. Enfermedades del cultivo de la yuca y métodos de control. Chapter in: Cultivo de la yuca en el tercer milenio. Sistemas modernos de producción, procesamiento utilización y comercialización. Edited by B. Ospina and H. Ceballos. P. 131 – 147. CIAT, Cali, 2002.

E Alvarez, GA Llano. 2002. Guía práctica para el manejo de las enfermedades, las plagas y las deficiencias nutricionales de la yuca. Chapter in pocketbook. P. 19 – 40. CIAT, Cali, 2002.

GA Llano, E Alvarez, JB Loke, R Madriñán, JA Restrepo, JR Mora. 2002. Evaluación de la adaptación de variedades de yuca con resistencia a *Phytophthora* spp., mediante investigación participativa en comunidades indígenas de Mitú (Vaupés, Colombia). Revista Acta Agronómica Vol 51 (1/2): 31-39, 2001 - 2002. Universidad Nacional de Colombia.

GA Llano, E Alvarez. Selecting *Phytophthora*-resistant cassava, using participatory methodology, for conditions at Mitú, northeastern Amazon, Colombia. Agren (submitted).

Two brochures in process: Añublo bacterial de la yucca, Superalargamiento de la yuca

E Alvarez, CX Grajales, J Villegas, JB Loke. 2002. Control del mildeo polvoso (*Sphaerotheca pannosa* var. *rosae*) en rosa (*Rosa* sp.), usando un lixiviado de compost del raquis de plátano (*Musa* AAB). Revista ASOCOLFLORES # 62: 41 – 47. January –June, 2002.

E Alvarez, C Echeverri, JB Loke. Validación de un biofungicida y fertilizantes foliares como alternativa de control del mildeo polvoso en cultivos de rosa. Revista ASOCOLFLORES (in press).

Awards

- Second place, XV Muestra Agroindustrial y Empresarial, Universidad La Gran Colombia, Armenia, Quindío, 1-3 November 2001, for: E Alvarez, CX Grajales, J Villegas, GA Llano, JB Loke. Control del mildeo polvoso en cultivos de rosa por aplicación de un lixiviado de compost de plátano.

- Nomination for indigenous communities from Vaupés for the 2002 Equator Prize of UNDP for: Participatory research on the control of Phytophthora root rots in cassava, conservation of native cassava varieties, and agroecosystem sustainability. E Alvarez, GA Llano.

Meetings attended

XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

Perspectivas de la producción ecológica para productos hortifrutícolas, held by Fundación Centro de Investigiación Hortofrutícola de Colombia – CENIHF and Corporación Autónoma Regional del Cauca CVC. Roldanillo, Valle, 26-27 July 2002.

Bachelor theses presented

Juan Fernando Mejía. 2002. Caracterización molecular y patogénica de aislamientos de *Sphaceloma manihoticola* del sur y centro de Brasil. Universidad Nacional de Colombia-Palmira.

César Andrés Ospina. 2002. Caracterización poblacional de *Colletotrichum* spp., agente causal de la Antracnosis de cítricos en el núcleo productor de occidente. Universidad Nacional de Colombia-Palmira.

Claudia Ximena Grajales and Jimena Villegas. 2002. Control de *Sphaerotheca pannosa* var. *rosae* en rosas mediante la utilización de lixiviado de compost de raquis de plátano. Universidad de San Buenaventura-Cali.

Theses for Master of Sciences and Philosophy Doctor degrees in progress

John B. Loke. Identifying and isolating major genes conferring resistance to causal agents of the root rots *Phytophthora drechsleri*, *P. nicotianae*, and *P. cryptogea* in a segregating population of cassava (*Manihot esculenta* Crantz). Universidad Nacional de Colombia-Palmira.

Germán A. Llano. Evaluación de la asociación de sondas heterólogas y genes análogos con la resistencia de yuca a *Phytophthora* spp. For a Master of Agrarian Sciences in plant breeding at the Universidad Nacional de Colombia-Palmira.

Paula X. Hurtado. Evaluación de marcadores microsatélites y genes análogos, asociados a la resistencia de yuca a *Xanthomonas axonopodis* pv. *manihotis*. For a Master of Biology with emphasis in Plant Molecular Biology. Universidad de los Andes-Bogotá

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