CASSAVA AND TROPICAL FRUIT PATHOLOGY

Activity 1. Detection of a phytoplasma associated with cassava Frogskin Disease (FSD) in Colombia

Objective

To confirm the presence of a phytoplasma associated with cassava frogskin disease (FSD).

Introduction

Frogskin disease (FSD) is an important disease affecting cassava roots, whose causal agent remained unknown for many years. FSD has been reported with increasing frequency in Colombia, Brazil, and Venezuela. In Colombia, for example, incidences of up to 70% have been recorded in commercial fields in the production areas of Valle del Cauca, Cauca, Meta, and the North Coast. Disease symptoms consist of small, longitudinal fissures distributed throughout the root. As the roots increase in diameter, the fissures tend to heal, giving the injuries a lip form. Root cortex or epidermis presents a cork-like appearance that is peels off easily. Depending on the severity of symptoms, the depth and number of lesions increase until the root becomes deformed.

This study evidences the existence of an association between FSD and phytoplasma. By applying molecular tools and microscopy, phytoplasma was successfully detected in FSD-infected cassava roots, leaf midribs, petioles, and peduncles.

Methodology

Plant tissue. Roots, stems, petioles, and leaf midribs of both FSD-infected and healthy cassava plants, grown in the field and greenhouse, were processed.

Microscopic analysis. Small pieces of tissue, about 1 mm × 2 mm, were excised and then fixed in 2%-3% glutaraldehyde/0.1M phosphate buffer. The samples for electron microscopy were prepared by ultra thin section (60-90 nm) and viewed with a transmission electron microscope.

DNA extraction. Total DNA was extracted as described by Gilbertson *et al,*1991.

Nested PCR analysis. The primer pairs P1/P7 or R16mF2/R16mR1 were used for the first amplification, with an annealing temperature of 55°C. For the nested PCR, diluted (1:30) PCR products were used for amplification, with the primer pair R16F2n/R16R2 at an annealing temperature of 50°C. PCR products were analyzed by electrophoresis on 1.5% agarose gel.

RFLP analyses. The amplified PCR products were digested with the restriction endonucleases *Taq* I, *Rsa* I, and *Alu* I. The restriction products were analyzed by electrophoresis on 5% polyacrylamide gel.

Cloning and DNA sequencing. Purified PCR products were ligated in pGEM-T Easy vector, which was introduced into the *Escherichia coli* strain DH5-a 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. 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.

Grafting. Cassava cuttings from the highly susceptible genotype Secundina were grafted on cassava infected plants.

Results: The presence of phytoplasma in different plant tissues of affected plants was confirmed by electron microscopy **(Figure 1)**. The specific primers R16mF2/R16mR1 and R16F2n/R16R2 were used in a nested PCR assay to detect phytoplasma. Nested PCR revealed 1.3 kb fragments in root, stem, and leaf samples from symptomatic plants **(Figure 2)**. No fragments were obtained from healthy plants.

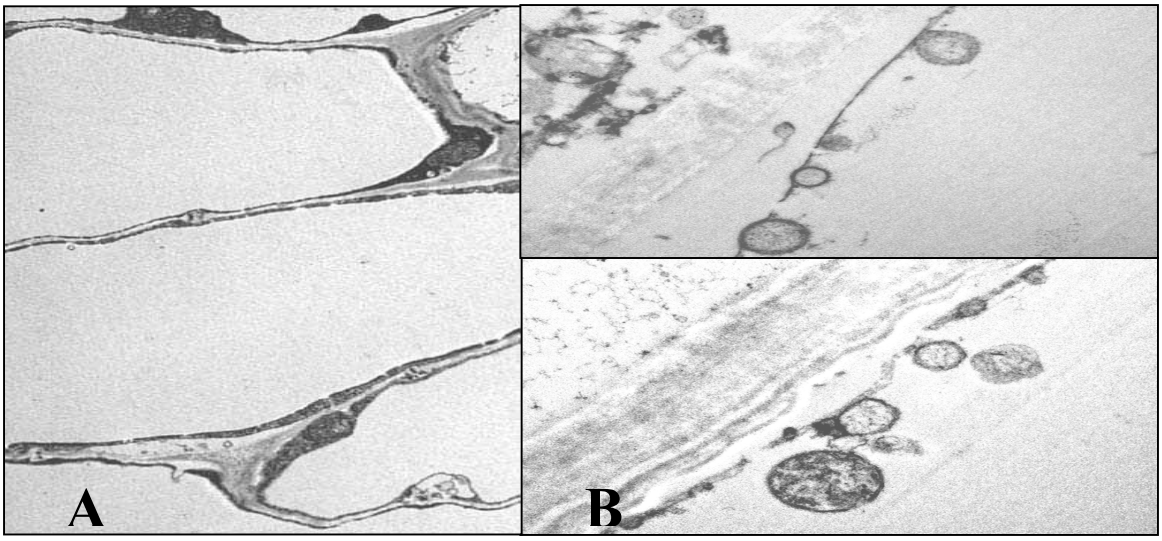


Figure 1. Electron microscopy of healthy (A) and infected (B) cassava tissue.

Phytoplasma was also detected by PCR in the leaves of grafted stem fragments on infected plants under greenhouse conditions, indicating successful transmission of the pathogen. Sequence analysis of a cloned fragment 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 **(Figure 3)**.

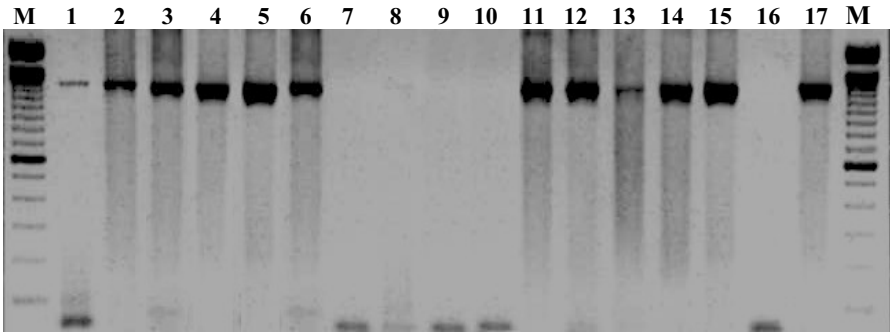


Figure 2. **A 1.3-kb fragment was amplified from diseased samples by. Lanes 1-2, infected stems; 3-4, infected petioles; 5-6, infected leaf midribs; 7, healthy roots; 8, healthy stems; 9, healthy petioles; 10, healthy leaf midribs; 11-12, infected roots; 13-14, infected stems; 15 and 17, periwinkle (*Catharanthus roseus*); lane 16, negative control; and lane M = 100 pb DNA marker.**

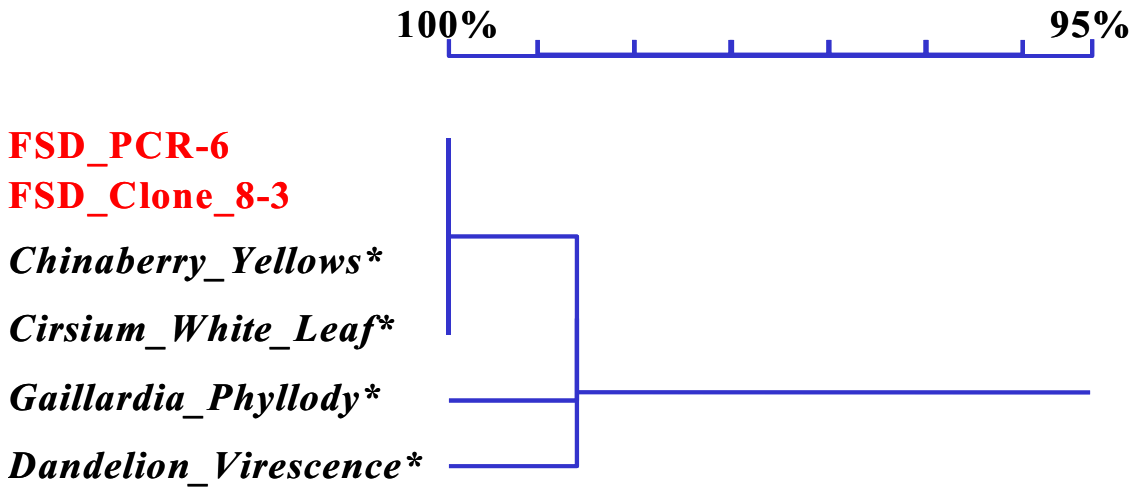


Figure 3. **Homology tree of 16S rRNA sequences from 6 phytoplasmas, including the sequences from cloned and direct PCR fragments obtained from cassava. * = GenBank accession.**

Digestion with *Taq* I, *Rsa* I, and *Alu* I of amplified products of different samples showed similar restriction patterns **(Figure 4)**.

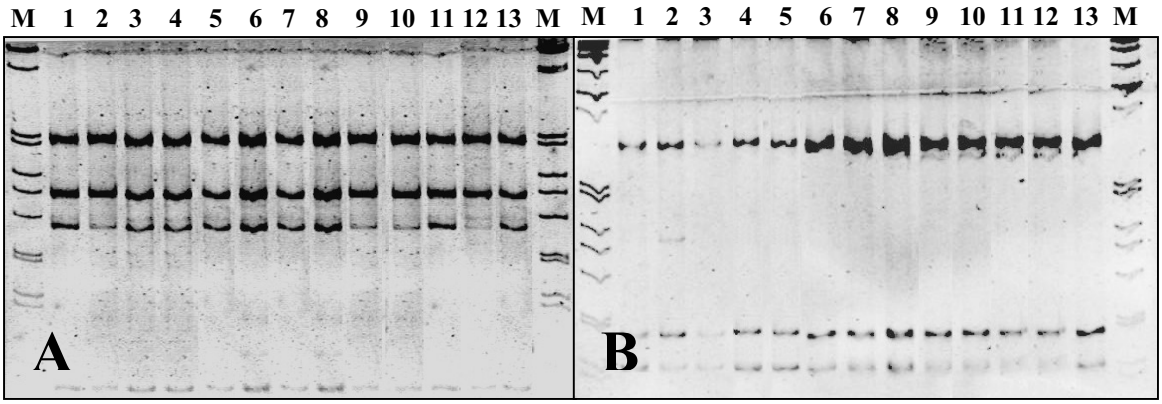


Figure 4. Restriction enzyme analysis of 16S rDNA after PCR amplification with primer pair R16F2n/R2, using the endonucleases *Rsa* I (A) and *Alu* I (B). Lane M = 1-Kb DNA marker.

Conclusions: Phytoplasma was successfully detected in all FSD-infected tissues by electron microscopy, and nested PCR techniques. Among the methods used in this study, PCR was the most sensitive for detecting, identifying, and classifying phytoplasma**.** Sequence homology from a cloned fragment, obtained from an infected cassava plant, was 100% similar to the Chinaberry yellows phytoplasma and 99% similar to that of Cirsium white leaf. This is the first report of a phytoplasma being associated with FSD in cassava. These results allow us to infer the possible role played by the phytoplasma in this disease. Future research will involve the evaluation of additional samples with other groups of enzymes as well as sequence analysis to classify the phytoplasmas. Experiments are underway to achieve remission of symptoms with the antibiotic oxychlortetracycline. Other research topics will include the development of specific primers for pathogen detection, vector identification, and classification of phytoplasmas associated with FSD.

References

Deeley, J., W.A. Stevens, and R.T.V. Fox. 1979. Use of Dienes' stain to detect plant diseases induced by MLOs. Phytopathology 69**:**1169-1171.

Dyer, A.T. and W.A. Sinclair. 1991. Root necrosis and histological changes in surviving roots of white ash infected with mycoplasma-like organisms. Plant Dis. 75:814-819.

Gilbertson, R.L., M.R. Rojas, L.D. Russel, and D.P. Maxwell. 1991. The use of the asymetric polymerase chain reaction and DNA sequencing to determine genetic variability among isolates of bean golden mosaic geminivirus in the Dominican Republic. J. Gen. Virol. 72:2843-2848.

Activity 2. Transmission of a phythoplasma affecting cassava seedlings and identification of indicator plants.

FSD symptoms recede after chlortetracycline treatment

Objective

To determine the causal agent of FSD in the cassava crop.

Experiment 1:

Materials and Methods: Plantlets from 10 Catumare- and 10 Manzana-affected field plants (Rozo, Palmira, Valle del Cauca, Colombia), and 20 disease-free plants of the same varieties (Montenegro, Quindío, Colombia) were treated by chlortetracycline.

For all experiments, the following precautions were included. Stakes were selected at harvest time to ensure FSD was present. CIAT virologists indicated that roots were affected by FSD according to symptoms. The stakes were planted in pasteurized soil, free of FSD, in plastic pots (10") or bags placed in isolated glass- or screenhouses at CIAT-Palmira (except Experiment no. 3). All plants were maintained in anti-aphid cages, and healthy Secundina plants were included. This was to monitor the presence of vectors. These plants did not show any symptoms during the experiments. Plants and cages were fumigated perodically, rotating the following products: Vertimec® 1.8% CE (0.5 cc/l of commercial product, abamectin), Malathion® (malathion, 1 cc/l of commercial product), Sistemin® (dimethoate, 3 cc/l of commercial product), and foliar fertilizers. All results presented in this report were checked by a CIAT Virology Specialist to make sure symptoms were caused by FSD.

Stem cuttings, with the medulla previously perforated with a drill, were immersed in a solution of 1500 ppm of tetracycline (750 ppm prepared based on a liquid solution and 750 ppm on capsules) during 10 min. on planting day. Plants of each treatment were planted at CIAT-Palmira in a glasshouse (temperature and RH: minimum 19 °C and 31%, maximum 28 °C and 98%), and in a screenhouse (temperature and RH: minimum 20 °C and 26%, maximum 39°C and 98%). All stem cuttings were maintained in different cages and other precautions were taken to avoid infection among plants. During 3 months, the soil was watered monthly with the same tetracycline solution (200 ml/plant). Plantlets were treated twice a week with 1500 ppm of tetracycline by foliar applications. After 3 months, the dose was reduced to 1000 ppm. Twenty plantlets (Manzana and Catumare) were also included from plants affected with FSD, and 20 plantlets from healthy plants, without FSD.

The plants were evaluated periodically to detect symptoms on leaves. After 4 months, stems of germinated plants were grafted with Secundina. Grafts were made directly on plants or through rootstock cuts, maintained in deionized water.

Results: Table 1 shows results obtained after grafting with Secundina.

Table 1. Effect of applications of tetracycline on phytoplasm of FSD-infected cassava Plants.

Variety	Origin of plant material	Place	Applications with chlortetracycline	No. of FSD-affected plants by grafting with Secundina[a]
Catumare		Screenhouse		3 (3)
Manzana		Screenhouse		3 (3)
Catumare		Glasshouse	Yes	5 (5)
Manzana	FSD-affected plants	Glasshouse		5 (5)
Catumare		Screenhouse		2 (2)
Manzana		Screenhouse		3 (3)
Catumare		Glasshouse		4 (5)
Manzana		Glasshouse		4 (5)
Catumare		Screenhouse		0 (4)
Manzana	FSD-free plants	Screenhouse		0 (3)
Catumare		Glasshouse	No	0 (5)
Manzana		Glasshouse		0 (5)

[a]No. of plants analyzed in parentheses.

All plantlets from affected plants in the field showed foliar symptoms in the Secundina grafts. No plantlet from the field of healthy plants showed symptoms in Secundina grafts on Catumare or Manzana. The effectiveness of obtaining grafts in the screenhouse was less than in the glasshouse. However, foliar symptoms were observed in both. Foliar applications of tetracycline do not reduce the incidence or severity of FSD.

Phytoplasm transmission to cassava (Secundina) plants susceptible to FSD, produced *in vitro*, and free of disease was successful through grafting.

Experiment 2:

Materials and Methods: Two foliar applications of chlortetracycline (1000 ppm, liquid form] were made weekly during 6 weeks to plants of SM 1219-9 and La Reina that showed leaf symptoms indicating FSD infection. Plants were located in a glasshouse (temperature and RH: minimum 19 °C and 31%, maximum 28 °C and 98%) at CIAT-Palmira. The stakes of these plants were obtained from FSD-affected plants (Jamundí, Valle del Cauca, Colombia).

Results and Conclusions: We observed that the affected leaves (curling and mosaic) remained affected through the applications (six plants). The new leaves also showed a severity similar to the affected leaves of plants (five) untreated with chlortetracycline. It is concluded that foliar applications with a high dosage of chlortetracycline do not inhibit leaf symptoms related to FSD.

Experiment 3:

Materials and Methods: Cuttings of Secundina genotypes, M Bra 383 and La Reina, from affected plants from the glasshouse (cuttings with FSD leaf symptoms) or from the screenhouse (cuttings without FSD leaf symptoms) were taken from plants in plastic pots. After cutting the true leaves of the cuttings, they were rooted in deionized water at different doses of chlortetracycline (injectable form, capsules can cause greater levels of intoxication of the plant, and are less effective against FSD). The cuttings were incubated in a laboratory with a controlled

temperature system (min. 20 °C, max. 25 °C), and 12-h alternate periods of light and darkness. The high humidity (66%-98%) was achieved through the use of closed boxes for the rooting of the cuttings.

Results and Conclusions: Table 2 presents the treatments used and results obtained.

Table 2. Effect of tetracycline on cassava frogskin disease (FSD)-affected cuttings.

Genotype	Source	Dose of chlor-tetracycline (ppm)	No. cuttings affected by FSD and total no. cuttings analyzed[a]
Secundina	Screenhouse, asymptomatic plants (foliar); these were previously in a glasshouse and expressed leaf symptoms	0	4 (4)
		2.5	2 (3)
		5	4 (4)
		10	4 (4)
		25	3 (6)
		50	0 (3)
	Glasshouse, plants affected with leaf symptoms	0	5 (5)
		2.5	5 (5)
		5	5 (5)
		10	5 (5)
		25	1 (5)
		50	0 (5)
M Bra 383	Glasshouse, plants affected with leaf symptoms from field plants with FSD-affected roots	0	5 (5)
		2.5	5 (5)
		5	5 (5)
		10	5 (5)
		25	3 (5)
		50	2 (5)[b]

[a]Evaluated 26, 32, and 40 days after initiation of chemical treatment. No. of plants analyzed given in parentheses.
[b]Two cuttings without FSD, one affected cutting, two cuttings with no leaf formation.

The inhibition of leaf symptoms caused by FSD was successful in two experiments using a dosage of 50 ppm chlortetracycline. The leaves of affected plants treated with 0 ppm of tetracycline showed presence of phytoplasm through nested PCR. Similar tests should be carried out with a greater number of cuttings. The cuttings of the variety La Reina did not show FSD foliar symptomology despite being infected and showing foliar symptoms in a glasshouse where cuttings were obtained to establish the experiment.

Cuttings treated with gentamicin (50 ppm) did not form leaves to adequately evaluate the antibiotic's effect on FSD. A treatment with 10 g/l of sugar to reduce the effect of phytotoxicity of chlortetracycline on the formation of leaves and roots did not function because it increased rotting of the stems by microorganisms.

Cuttings place in continuously oxygenated deionized water (by means of an air pump) with (50 ppm) and without chlortetracycline did not reduce the effect of phytotoxicity on the plants. Plants without the antibiotic rapidly (3 weeks) formed roots and leaves. Plants with the chemical treatments formed leaf primordials and rooting callus, but because of the phytotoxicity, no true leaves were formed.

In preliminary trials treating cuttings with 50 ppm of gentamicin, inhibition of FSD foliar symptoms was not observed.

Experiment 4:

Stakes with one, two, or three leaf buds from FSD-affected plants (Jamundí) of the variety SM 1219-9 were treated during 15 min. with 0, 25, and 50 ppm of tetracycline or gentamicin respectively, and planted in sterile sand under screenhouse conditions at CIAT-Palmira.

The substratum is humidified daily with the antibiotics (renewed every 3 days). This trial is in progress. Transplanting is scheduled to containers with sand, and applying nutritive solutions, constantly adding the antibiotics to obtain plants with roots developed sufficiently large to observe the effect of chlortetracycline on FSD. The treatments with 50 ppm of chlortetracycline and gentamicin inhibit germination of the leaf buds; 25 ppm permits germination, and adequate plant development.

Acknowledgments

- Robert Zeigler, Kansas State University
- Jaime Eduardo Muñoz, Universidad Nacional de Colombia, Palmira
- CIAT-Virology Unit facilitated the Secundina grafting, and Tulio Rodríguez performed the indexing.
- Agrovelez S.A. (Jamundí, Valle).

Activity 3. Identification of cultural practices and strategy to control frogskin disease in cassava.

Objective

To develop a methodology that includes a process for disinfecting cassava stakes of FSD.

Experiment 1:

Before planting, infected cassava stakes (M Bra 383, harvested at Jamundí, Valle del Cauca) were treated with thermotherapy. Temperature in the glasshouse ranged from 19 °C to 28 °C (although the temperature sporadically rose above 25 °C in the anti-aphid cage), and RH from 31% to 98%. Plants were evaluated periodically to detect FSD symptoms in leaves. Table 1 presents results.

Table 1. Effect of four hot water treatments on germination of stem cuttings and frogskin disease (FSD).

Pretreatment	Main treatment	Germination of stem cuttings (no. of cuttings)[a]	Disease incidence of FSD according to foliar symptoms (%)
None	None	5	100
54 °C during 5 min	54 °C during 10 min	4	50
56 °C during 5 min	56 °C during 10 min	2	50
58 °C during 5 min	58 °C during 10 min	3	0
60 °C during 5 min	60 °C during 10 min	1	0

[a]For each treatment six cuttings were used.

Stakes of the variety M Bra 383, treated with hot water for 15 min at 60 °C, reduced germination severely. Germination rates after 54 °C was highly acceptable. Plants treated at 58 °C or 60 °C apparently were clean of FSD, therefore the following experiment was designed with the main objective to improve plant vigor of treated plants to realize analysis for presence of FSD.

Experiment 2:

Fourteen cassava genotypes (M Chn 2, HMC 1, M Arg 2, M Bra 325, M Bra 829, M Bra 839, M Bra 856, M Bra 882, M Bra 886, M Col 634, M Col 1178, M Col 1468, M Cub 74, and M Per 16), infected by FSD, were harvested at CIAT-Santander de Quilichao. Thirty-two hot water stake treatments in combination with Agrodyne®SL (13.20 g/l iodine polietoxi-polipropoxi-poloetoxi–ethanol complex, 1.59 g/l iodic acid, Electroquímica West S.A., Medellín, Colombia) were tested for their effect on germination of stem cuttings and presence of FSD, applying two methods of detection. All stem cuttings were planted in plastic bags, and maintained isolated in an anti-aphid cage outdoors.

Germination of treated stem cuttings was better outside the glasshouse (temperature and RH: minimum 20 °C and 30%, maximum 38 °C and 96%) than inside, because stakes are less affected by saprophytic fungi. Stems of germinated plants (about 3 months old) were grafted onto Secundina. Table 2 presents results.

Table 2. Effect of hot water treatment and iodine on germination rates of stakes from cassava infected with frogskin disease (FSD).

Pretreatment		Main treatment		No. ungermi-nated stakes	No. germi-nated stakes	Germin-ation of stakes (%)	No. of plants with positive response to phytoplasm (PCR) or FSD (indexing), and no. of plants analyzed	
Without Agrodyne	With Agrodyne	Without Agrodyne	With Agrodyne				Nested PCR	Grafting with Secundina
-	-	-	25°C/5 m [c]	0	6	100.0	2 (2)	1 (2)
-	-	-	25°C/1 h [c]	0	12	100.0	1 (2)	1 (1)
No treatment	-	No treatment	-	1	13	92.9	0 (1)	
-	-	-	25 °C/5 m [d]	1	5	83.3	0 (2)	
49 °C/1 h	-	60 °C/10 m	-	6	16	72.7	1 (2)	0 (2)
-	-	60 °C/5 m	-	3	8	72.7	0 (2)	
55 °C/10 m	-	60 °C/10 m[b]	-	2	4	66.7	0 (1)	
-	60°C/5 m[c]	-	60 °C/10 m [c]	1	2	66.7		
-	-	-	49 °C/1 h [c]	6	4	40.0	1 (2)	
-	-	60 °C/10 m	-	6	3	33.3	0 (1)	1 (1)
60 °C/5 m	-	60 °C/10 m	-	24	11	31.4	1 (2)	
55 °C/10 m	-	60 °C/10 m	-	9	3	25.0	1 (1)	
-	-	-	60 °C/5 m [c]	3	1	25.0	1 (1)	
60 °C/5 m	-	60 °C/12.5 m	-	7	2	22.2	1 (2)	1 (1)
55 °C/10 m	-	60 °C/12.5 m[b]	-	4	1	20.0	0 (1)	
-	-	60 °C/20 m	-	6	1	14.3	0 (1)	0 (1)
60 °C/5 m	-	60 °C/15 m	-	9	1	10.0	0 (1)	
55 °C/10 m	-	60 °C/12.5 m	-	9	1	10.0		
60 °C/5 m	-	60 °C/10 m[b]	-	6	0	0.0		
60 °C/5 m	-	60 °C/12.5 m[b]	-	6	0	0.0		
55 °C/10 m	-	60 °C/15 m	-	8	0	0.0		
55 °C/10 m	-	60 °C/15 m[b]	-	5	0	0.0		
60 °C/5 m	-	60 °C/15 m[b]	-	5	0	0.0		
55 °C/10 m	-	60 °C/17.5 m	-	7	0	0.0		
55 °C/10 m	-	60 °C/17.5 m[b]	-	4	0	0.0		
60 °C/5 m	-	60 °C/17.5 m	-	8	0	0.0		
60 °C/5 m	-	60 °C/17.5 m[b]	-	5	0	0.0		
49 °C/1 h	-	60 °C/20 m	-	10	0	0.0		
60 °C/5 m	-	60 °C/20 m	-	10	0	0.0		
60 °C/5 m	-	60 °C/30 m	-	10	0	0.0		
-	-	60 °C/30 m	-	2	0	0.0		
49 °C/1 h	-	60 °C/30 m	-	10	0	0.0		

[a]Agrodyne®SL (13.20 g/l iodine complex polietoxi-polipropoxi-poloetoxi–ethanol, 1.59 g/l iodic acid, Electroquímica West S.A., Medellín, Colombia), 1 ml/l Inex A. [b]6 hours after pretreatment. [c]1.5 ml/l Agrodyne. [d]3 ml/l Agrodyne.

The treatment of stakes with Agrodyne without thermotherapy does not affect germination. The use of hot water at 60 °C up to 10 min reduces germination, but the plants obtained are possibly

healthier. At this temperature, the use of iodine can be included in the hot water treatment. It is unclear whether phytoplasm can be definitely inactivated through thermotherapy. A trend was observed that treatment is more important during a relatively long period, rather than at an extremely high temperature.

The most effective treatment is 49 °C during 1 hour (pretreatment) followed by a main treatment at 60 °C for 10 min, without using Agrodyne. The germination achieved with this treatment was most acceptable (72.7%, 17 stakes germinated). Cleaning was demonstrated as effective through indexing with Secundina.

Experiments 3 and 4:

Stakes with one, two, or three leaf buds of the SM 1219-9 genotype affected by FSD (Agrovelez, Jamundí), were treated at 60 °C during 5 min (pretreatment), and the following day at 60 °C during 10 min (main), and planted in sterile sand in a screenhouse (temperature and RH: minimum 20 °C and 26%, maximum 39 °C y 98%) at CIAT-Palmira. This treatment was carried out with 1 ml of Agrodyne/l in hot water. The treatment was too strong, and affected germination of leaf buds. No plant germinated, although callus was present in the stakes. In another experiment, without Agrodyne, the temperature for the pretreatment was lowered to 55 °C (treatment 1) and to 50 °C (treatment 2), and for the main treatment to 60 °C (treatment 1) and 55 °C (treatment 2), but without promising results because no stake germinated.

Stakes of a leaf bud without heat treatment did not germinate either; stakes with two or three leaf buds germinated satisfactorily. Experiments are being scheduled with a treatment of leaf buds at 49 °C, with a duration between 30 and 60 min.

Acknowledgements

We thank CIAT-Virology for facilitating the Secundina grafts, and Tulio Rodríguez for carrying out the indexing. We also thank Agrovelez S.A. (Jamundí, Valle) for access to infected field materials, and James George (Central Tuber Crops Research Institute, Kerala, India) for suggestions concerning use of short stem cuttings.

Activity 4. Evaluation of the influence of the soil as a source of FSD vectors.

Objectives

1. To evaluate the soil as a possible source of microorganism vectors of FSD.
2. To evaluate whether the presence of aerial vectors is related with dissemination of the disease.

Materials and Methods: In the municipality of Sincelejo (Sucre), Chochó basin, the possible influence that the soil may have as a source of FSD vectors is being evaluated. In the region, the disease occurs most frequently in lots where the presence of FSD has been reported previously, unlike nearby lots that remain disease free. For the trial, the variety M Tai 8 was chosen as presenting high susceptibility to FSD, and being one of the commercial genotypes most cultivated in the region, facilitating the obtaining of seed from lots where the disease has never occurred. Treatments were:

- Healthy plants in the screenhouse,
- Diseased plants in the screenhouse,
- Healthy plants outside the screenhouse, and
- Diseased plants outside the screenhouse.

Two muslin cages were constructed, 1.80 m in height, one for healthy plants (10.5 m long x 5 m wide), and one for affected plants (4.5 m long x 5 m wide). The rest of the trial was planted outside the screenhouse.

An experiment design was used of divided plots with three repetitions. The experiment unit for healthy plants inside the screenhouse, consisted of a plot of nine plants, distributed in three furrows of three plants each planted at 1 m x 1 m. For the diseased plants, three plants were planted for each repetition.

Outside the cage, the same experiment design was kept—three plots of healthy and diseased plants, with two furrows for 11 healthy plants and eight diseased.

For the seed cut, the machete was disinfected in a solution of 1% sodium hypochlorite. As part of the management, insecticide was applied, 1 week Sistemin® (dimethoate, 3 cc/l of commercial product), and the following week Malathion® (Malathion, 1 cc/l of commercial product). Application was only made within cages, and to half the outside plots, so that of the three repetitions outside, half received insecticide, and the other half was conserved without application. A barrier of the commercial genotype M Ven 25 was planted between the two to also evaluate the effect of aerial vectors, or whether the transmission is carried out through some agent of the soil.

Evaluations of the treatments will be made at time of harvest, observing incidence and severity in the roots.

Activity 5. Isolation and characterization of *Agrobacterium tumefaciens* from soil and cassava roots.

Objectives

1. To isolate *Agrobacterium tumefaciens* from soil samples and cassava roots.
2. To evaluate the pathogenicity of strains isolated from several cassava varieties.
3. To characterize isolates of *A. tumefaciens* pathogenic on carrot disks using PCR with specific primers.

Materials and Methods

***Isolation of* Agrobacterium tumefaciens.** To isolate *A. tumefaciens*, samples were initially taken of both healthy and diseased cassava roots and of the soil where diseased plants were sown.

Of each soil sample, 100 grams were weighed and dissolved in 100 ml sterile distilled water (SDW), agitated for 30 minutes, and then left to settle for another 30 minutes. Four serial dilutions in 9 mL of 0.75% NaCl were performed based on this first mixture (base solution) and 0.1 mL of each was planted on DIM (D1) media, which is selective and differential for *Agrobacterium* sp. isolates. The Petri dishes were incubated at 30 ºC for 24 hours. Colony-forming units (CFUs) growing on D1 were counted and those of yellowish-orange color were selected and planted per isolate on the same media for purification.

The healthy and diseased roots collected were washed with deionized water and processed by separating the plant tissue in 4 layers depending on their distance to the phloem (layer 1 = external, layer 4 = near the phloem). The portion obtained from each layer was washed with deionized water for 15 minutes, disinfected in 50% alcohol and then excess alcohol was eliminated with SDW. Smaller portions of each sample were cut and placed on D1 medium and then incubated at 30ºC for 48 hours. *Agrobacterium* colonies of characteristic color (yellowish orange) in the D1 medium were selected and purified on this media.

Pathogenicity test. Bacterial colonies isolated from the soil and cassava roots **(Table 1)** were planted on nutritive agar with sucrose (5%) and incubated at 30ºC for 48 hours.

To evaluate pathogenicity on carrot disks, fresh tubers were washed in deionized water, then submerged in sodium hypochlorite (5%) for 10 minutes and subsequently washed with SDW. Tubers were finally dried with sterile towels and small carrot disks were cut and placed in sterile Petri dishes.

The colonies obtained were inoculated by puncturing around the vascular cambium of the carrot disks and incubated for 3 weeks at 25ºC in moist chambers. Each strain was inoculated by duplicate and gall formation around the vascular cambium was observed on a daily basis.

Carrot disks inoculated with SDW were included as negative check, and *A. tumefaciens* isolate 1182 as positive check.

Two tissue-culture grown varieties Secundina and M Bra 383 were used to test the pathogenicity of strains isolated from cassava. The in vitro plants were planted on sterile soil, kept in cages to avoid the presence of insects as possible disease vectors, and fertilized weekly by intercalating the following three NPK fertilizers: 15-15-15, Coljap Producción 5-15-30, and Coljap Desarrollo 30-7-6, produced by Industria Agroquímica S.A., Bogotá, Colombia. Inoculation was carried out 1 month afterwards. Strains selected for a pathogenicity test in cassava were isolated from cassava roots (isolates 23, 24, and 27) with typical symptoms of frogskin disease and from soil samples of infected cassava crops (isolates 28, 29, 30, 33, and 35B).

For inoculation, a bacterial suspension of 1x108 CFU/mL (0.5 absorbancy) of the strains was prepared to inoculate the soil, roots, or base of corona. The soil was inoculated by adding the bacterial suspension to the surface. To inoculate the roots, plants were removed from the soil, their roots washed and tips cut, after which they were submerged in bacterial suspension for 30 minutes. Plants were then re-planted and placed within the cage. To inoculate the base of the corona, a puncture was made diagonally in this part of the plant using a 1-mL syringe to inject the inoculum. Once the inoculation was finished, all plants were placed in the cages and kept in a closed growth chamber, with 12 hours daylight, at a constant temperature of 20-25ºC and 80% relative humidity, thus favoring the colonization of the pathogen. Each month the roots were evaluated under the stereoscope to determine any changes in morphology.

Molecular characterization of isolates. The DNA was initially extracted from 43 strains isolated from soil and roots that resulted positive in pathogenicity tests on carrot disks and the check strain of *A. tumefaciens* (1182). Extraction was performed following the Boucher *et al.* (1985) protocol and the concentration of all strains was adjusted to 20 ng/µL to be amplified with the specific primers VCR/VCF paired with the Vir C region of the Ti plasmid of *A. tumefaciens* (Sawada, 1995). The Vir C region forms part of the Ti plasmid virulence genes that measured the transfer of plasmid T-DNA to the plant where an excessive proliferation of cells occurs, causing one or more galls to form. Each PCR reaction was carried out in 25-µL final volume, with final concentrations of 0.2 mM dATP, dCTP, dGTP, and dTTP; 1.5 mM $MgCl_2$; 1.5 U *Taq* polymerase; 0.1 µM of each primer; 1X *Taq* polymerase buffer; and 100 ng template DNA. For the reaction of the negative check, the DNA was replaced by SDW. The PCR was performed in a MSJ-Research PTC-100 thermal cycler with the following amplification program: 2.5 min at 95°C; 40 cycles of denaturation for 1 min at 95°C, pairing for 1 min at 55°C, and extension for 2 min at 72°C; and a final extension for 7 min at 72°C.

The amplification product was visualized in 2.0% agarose gel with TBE 0.5X buffer dyed with ethidium bromide (1 µL disolution at 10 mg/ml for 100 ml agarose gel). A 100-bp molecular weight marker was used to estimate the size of the amplified fragments.

Results

Pathogenicity test. Fifty-five strains with typical *A. tumefaciens* morphology were initially isolated from soil and cassava roots in D1 media. All were submitted to pathogenicity tests on carrot disks, where gall formation was observed 2 weeks after inoculation **(Figure 1)** with 43 of the isolated strains **(Table 1)**.

The pathogenicity test in cassava has not yet shown changes in root morphology. Inoculated plants have been periodically examined and no apparent changes have been observed in the roots. To promote plant development and root enlargement, all inoculated plants were transplanted to large pots and will be monitored monthly until roots reach a size in which any morphological change resulting from disease caused by the inoculated strains can be observed by the naked eye.

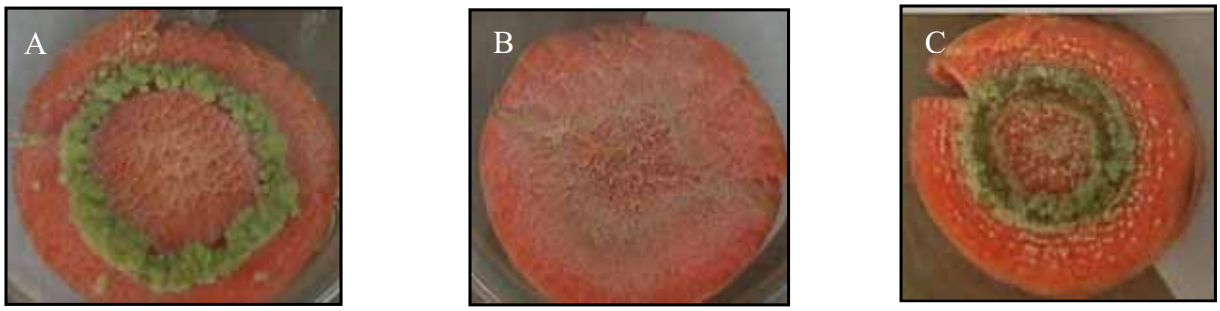


Figure 1. Gall formation on a carrot disk. A. Positive control (*A. tumefaciens* strain 1182). B. Negative control (sterile distilled water). C. Isolate 23 (root, genotype CM 2772-3).

Once the pathogenicity test was performed, the DNA of positive strains was extracted and quality visualized. The concentration of all strains was adjusted in 20 ng/µL and was amplified with the specific primers VCR/VCF. A 730-bp band was observed in the check strain 1182 and in most of the amplified strains **(Figure 2)**.

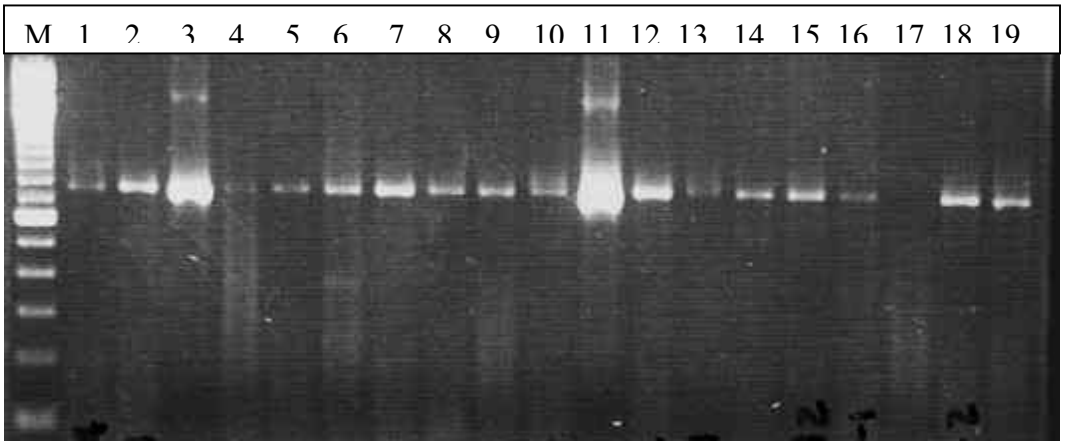


Figure 2. Amplification of DNA of *A. tumefaciens* strains obtained with the specific primers VCR/VCF from samples of infected cassava roots and soil. Lane M = 100-bp marker; lanes 1-19 = *Ralstonia solanacearum* (18, 23, 24, 26B, 27, 28, 30, 32, 33, 40, 1182, 21, 10, 11, 15, 19, 1, 6); lane 17 = negative control.

Table 1 presents the results obtained in the pathogenicity test and the amplification of strains with VCR/VCF primers.

Table 1. Pathogenicity of *A. tumefaciens* isolates and DNA amplification with specific primers (Vir C).

Isolate no.	Source	Origin	Identification	Pathogenicity 1	Pathogenicity 2	Specific DNA amplification
1	Root 1	Growth chamber	Petri dish 1	-	+	+
2	Root 1	Growth chamber	Petri dish 2	+	-	+
3	Root 2	Growth chamber	Petri dish 1	+	+	+
4	Root 2	Growth chamber	Petri dish 2	+	+	+
5	Root 2	Growth chamber	Petri dish 3	+	+	+
6	Root 4	Growth chamber	Petri dish 2	+	+	+
7	Root 4	Growth chamber	Petri dish 3	+	+/-	+
8	Root 1, diseased	Santander de Quilichao	Layer 1	+	+	+
9	Root 1, diseased	Santander de Quilichao	Layer 2	+	+	+
10	Root 1, diseased	Santander de Quilichao	Layer 3	+	+	+
11	Root 1, diseased	Santander de Quilichao	Layer 4	+	+	+
12	Root 2, diseased	Santander de Quilichao	Layer 1	+	+	+
13	Root 2, diseased	Santander de Quilichao	Layer 2	+	+	-
14	Root 2, diseased	Santander de Quilichao	Layer 3	+	+	+
15	Root 2, diseased	Santander de Quilichao	Layer 4	+	+	+
16	Sterilized soil	Quindío	Dil 10^1	-	+/-	+
17	Soil of a healthy crop (Catumare)	Quindío	Dil 10^2, 2nd camping	+/-	+/-	+
18	Root (Manzana)		Layer 2	+/-	+/-	+
19	Root (GM 309-7)		Layer 1	+	+	+
20	Root (GM 309-7)		Layer 2	+/-	+/-	+
21	Root (GM 309-7)		Layer 4, colony 1	+	+	+
22	Root (GM 309-7)		Layer 4, colony 2	+	+	+
23	Diseased root (CM2772-3)	CIAT	Layer 4	+	+	+
24	Diseased root (MBRA 383)	Palmira	Layer 1	+	+	+
25	Diseased root (Catumare)	Palmira	Layer 4	+	+	+
26A	Diseased root (Manzana)	Palmira	Layer 1	+	+	
26B	Diseased root (Manzana)	Palmira	Layer 2	+	+	+
27	Diseased root (Venezolana)	Sincelejo, Sucre	Layer 4	+	+	+
28	Soil of a diseased crop	Jamundí, Valle	No. 2, colony 2	+	+	+
29	Soil of a diseased crop	Jamundí	No. 3, colony 2	+	+	-
30	Soil of a diseased crop	Jamundí	No. 4, colony 1	+	+	+
31	Soil of a diseased crop	Jamundí	No. 5, colony 1	+	+	
32	Soil of a diseased crop	Santander de Quilichao	No. 1, colony 2	+	+	+
33	Soil of a diseased crop	Sincelejo	No. 1, colony 1	+	+	+
34A	Soil of a diseased crop	Sincelejo	No. 2, colony 1	+	+	+
34B	Soil of a diseased crop	Sincelejo	No. 2, colony 2	+	+	+
35A	Soil of a diseased crop	Sincelejo	No. 3, colony 1	+	+	-
35B	Soil of a diseased crop	Sincelejo	No. 3, colony 2	+	+	+
36	Soil of a non-diseased crop (Catumare)	Quindío	Colony 1	+	+	+
37	Soil of a non-diseased crop	Quindío	Colony 2	+	+	+

Isolate no.	Source	Origin	Identification	Pathogenicity 1	Pathogenicity 2	Specific DNA amplification
	(Catumare)					
38	Soil of a non-diseased crop (Catumare)	Quindío	Colony 3	+	+	+
39	Soil of a non-diseased crop (Catumare)	Quindío	Colony 4	+	+	-
40	Soil of a non-diseased crop (Manzana)	Quindío	Colony 1	+	+	+
41	Soil of a non-diseased crop (Manzana)	Quindío	Colony 2	+	+	
42	Soil of a non-diseased crop (Manzana)	Quindío	Colony 3	+	+	+
43	Soil of a non-diseased crop (Manzana)	Quindío	Colony 4	+	+	+
1182	Agrobacterium tumefaciens	Biotechnology Project-CIAT		+	+	+
Ti plasmid	Agrobacterium tumefaciens	Miniprep				+

References

Boucher C; Barberis P; Trigalet A; Demery D. 1985. Transposon mutagenesis of *Pseudomonas solanacearum:* isolation of Tn5-induced avirulent mutants. J Gen Microbiol 131:2449-2457.

Sawada H; Ieki H; Matsuda I. 1995. PCR detection of Ti and Ri plasmids from phytopathogenic Agrobacterium strains. Applied and Environmental Microbiology. February, p. 828-831.

Activity 6. Identification of RAMS markers to evaluate genetic diversity of selected isolates of *Ralstonia solanacearum* obtained from plantain.

Objective

To detect and characterize *Ralstonia solanacearum* isolates by PCR and RAM analyses.

Methodology

Isolates. A total of 107 *R. solanacearum* isolates were obtained from diseased plants, soil and water samples from different regions of Colombia by isolation on TZC medium (Kelman 1954). Only Gram-negative isolates, positive to oxidase and KOH, were used for PCR.

DNA extraction and PCR analysis. The DNA extraction of 87 isolates was performed as described by Boucher *et al.* (1985). The other 20 isolates were evaluated using whole cells by boiling a colony resuspended in 100 µL sterile distilled water (Seal *et al.*, 1993). The OLI1 and Y2 primers used by Seal *et al.* (1993) were used to amplify DNA from all isolates. Each PCR reaction was performed in 25-µL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 µL 10X *Taq* polymerase buffer; 1.5 mM $MgCl_2$; 0.5 U *Taq* polymerase; 0.5µM primer; and 100-150 ng template DNA or 5 µL boiled culture. Amplification was carried out in a MSJ-Research PTC-100 thermal cycler programmed for 2 min at 96°C; 50 cycles of denaturation for 20 s at 94°C, annealing for 20 s at 68°C, and extension for 30 s at 72°C; and final extension for 10 min at 72°C.

RAM analysis. A total of 107 isolates were evaluated with seven RAM primers (ACA, CCA, CGA, AG, CT, GT and TG). ACA, CCA, and CGA were useful to evidence polymorphisms between isolates. Each PCR-RAM reaction was performed in 12.5-µL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 µl 10X *Taq* polymerase buffer; 1.5 mM $MgCl_2$; 0.5 U *Taq* polymerase; 0.5 µM primer; and 50-100 ng template DNA. Amplification was performed in a MSJ-Research PTC-100 thermal cycler programmed for 5 min at 95°C, 37 cycles of denaturation for 30 s at 95°C, annealing for 50 s at 49°C (ACA primer), 55°C (CCA primer) or 62°C (CGA primer), and extension for 2 min at 72°C; and final extension for 7 min at 72°C.

Results: Results showed that, by specific OLI1-Y2 PCR amplification, 36 of the 107 isolates **(Table 1)** evaluated can be classified as *R. solanacearum*, confirmed by the presence of the 288-bp band similar to the check isolates **(Figure 1)** included in the evaluation.

High genetic polymorphism was observed among the *R. solanacearum* isolates from different localities, using the primers ACA, CCA, and CGA. Despite the fact that the primers ACA and CGA were useful to detect polymorphisms among strains, the CCA primer amplified DNA of all isolates and the products obtained showed clear bands **(Figure 2)**. Groups formed by strains from the same habitat (plant tissue, soil, or water) were observed.

The dendogram evidences high genetic polymorphisms among isolates.

In addition, two different groups were formed. Isolates R22 and R18 (obtained from water) were grouped with isolates g175, g214, g216, which had previously identified by other scientists as *R. solanacearum.* Both groups consist of isolates obtained from soil and plants.

Table 1. List of *Ralstonia solanacearum* isolates obtained from different sources by PCR amplification.

Isolate no.	Source
1, 2, 9, 10, 11, 79	Pseudostem, plantain affected by Moko
18	Water, source of a stream
22	Water
48, 53, 56	Soil, 0-30 cm
47	Soil, 30-60 cm
59	Soil, 5 m below focus, 0-30 cm
50, 54, 62, 63	Soil, 10 m below focus, 0-30 cm
68, 69	Soil, focus treated a year ago, on the surface
70, 81	*Emilia sanchifolia* (weed)
73	Black nightshade (*Solanum nigra*)
78	Pulp with borer, near focus
Dapa 2, Dapa 4	Soil of Heliconia plants affected by Moko
Heliconia 2, 11, 12	Heliconia plant, affected by Moko
G176, g214, g216, g217, g218	Check strains from collection

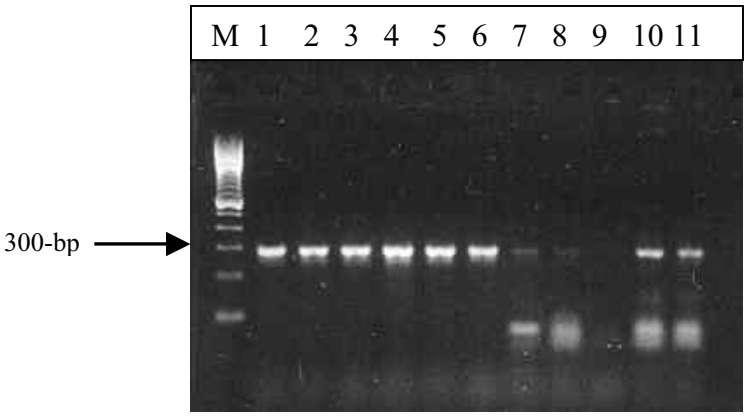


Figure 1. PCR patterns from genomic DNA of different strains, using OLI1 and Y2. Lane M = 100-bp marker; lanes 1-8 and 10–11 = *Ralstonia solanacearum* isolates; lane 9 = negative control (without DNA).

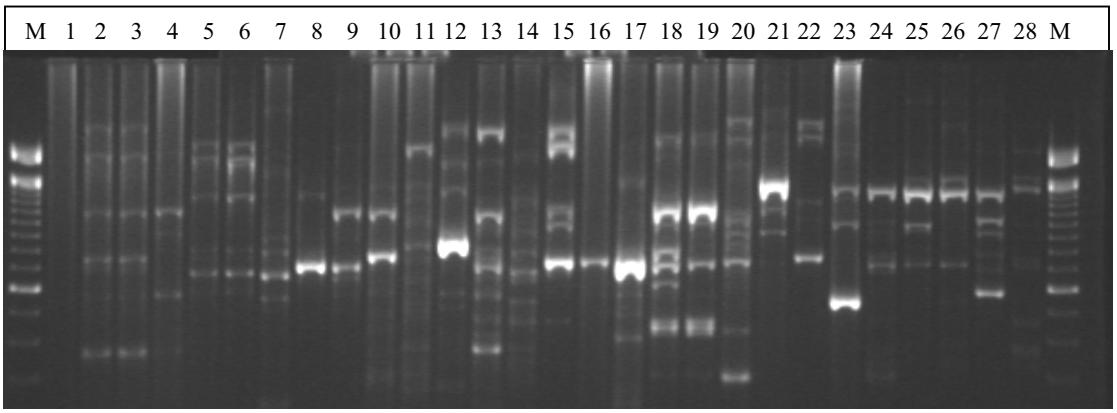


Figure 2. **RAM patterns from genomic DNA of different strains of *Ralstonia solanacearum* with the CCA primer. Lanes M = 100-bp marker; lane 1 = negative control; lanes 2-28 = *Ralstonia solanacearum* (1, 2, 3, 9, 10, 11, 18, 22, 47, 48, 50, 53, 54, 56, 59, 62, 63, 68, 69, 70, 73, 78, g175, g214, g216, g217, g218).**

Activity 7. Evaluation of the effect of hot water treatment of stem cuttings, biocontrol agents, selection of healthy stem cuttings and fertilizers on *Phytophthora* Root Rot under field conditions in Cauca and Quindío. Application of fungicides will be included as control treatments.

Cauca

Methodology: To evaluate the effect of five control practices on *Phytophthora*, which induce root rot in cassava, experimental plots were established on a farm in the Municipality of Caldono (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).

At 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 are described in Table 1. 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, with 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 this trial, the heat treatment did not affect stake germination **(Table 1)**. No root and stem rot was observed. In spite of fertilization with chicken manure the root yield was low. *Trichoderma* enhanced yield dramatically, 65% compared to tradicional farmer's practice. By training farmers to use this technology and by analysis of soil samples, yields in this part of Cauca will increase.

Quindío

Methodology: Different control practices for *Phytophthora* spp. were evaluated for disease incidence and severity, and for yield in one field trial at "La Elena" Farm, Municipality of Montenegro. The trial was planted in November 2002 with the local variety Chiroza (M Col 2066). Treatments were as follows:

1. Integration of the following practices:
   a. Selection of high quality stem cuttings, including root yield per plant harvested.
   b. Thermotherapy: Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.
   c. Biological control: Strain 14 PDA-4 of *Trichoderma* spp. was used to make a suspension of 1×10^4 conidia/mL Planting stakes 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.

2. Traditional farmer's practice, including 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.

Table 1. Effect of different control practices to manage root-rot on germination, Caldono, Department of Cauca, Colombia.

Treatment	Germination (%)[a]	Yield (T/ha)[b]
Algodona (M Col 1522)		
Stake selection[c]	96.6	4.0
Thermotherapy[d]	93.3	5.7
Traditional farmer's practice	95.3	5.1
Trichoderma strain 14PDA-4[e]	88.6	8.4
Chemical control[f]	98.9	6.0
Check varieties		
CM 7438-14	100.0	
M Bra 383	100.0	Will be harvested at the end of Octubre 2003.
SM 1053-23	94.6	
SM 1058-13	100.0	
SM 1937-1	97.4	
Average	96.5	5.8

a. 30 days after planting.
b. 16 months after planting.
c. Stakes were selected for their stake quality.
d. Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.
e. Strain 14 PDA-4 of the fungus *Trichoderma* sp. which attacks root rot fungi (*Phytophthora* spp.), was used to make a suspension of 1 x 10^6 conidia/mL. Planting stakes were then inoculated by immersing in the suspension for 10 min. The suspension was also applied to the soil near the base of each plant.
f. Planting stakes were immersed for 5 min in Ridomil® (metalaxyl) at 3 g/L of water.

All plots were fertilized 45 days after planting with 500 kg/ha of the fertilizer mix Nitrax®, DAP, and KCl, applied at a rate of 1:2:2. A randomized complete block experiment design was used with three replicates and 55-60 plants per treatment.

Results: No differences in germination of stem cuttings were observed. *Phytophthora* sp. was successfully isolated from infected roots of trial plants. Chiroza can be considered as highly susceptible to Phytophthora root rot. The field experiment will be harvested in 2004. This year more than 2,500 Colombian farmers and researchers have visited this experiment to learn, first-hand, how to grow cassava and manage root rot diseases affecting this crop.

Acknowledgements
- Silverio González, Special, La Tebaida (Quindío).
- Rosy Santacruz, Universidad de Quindío, Armenia (Quindío) Biogreen, Palmira.
- Bolívar Muñoz and Adelmar Chucue, Interinstitutional Consortium for Sustainable Agriculture in Hillsides (CIPASLA), Pescador and Caldono (Cauca) respectively SENA and ICA, Armenia.

Activity 8. Use of organic matter and ash amendments as a strategy to manage cassava root rots and improve soil fertility in Tukanoan chagras in Mitú, Vaupés.

Objectives

To evaluate the effect of amendments of ash and organic matter, cutting selection and association with cowpea, on root rot incidence, and cassava yield.

Materials and Methods: The effects of applying ashes (200 g/plant), organic matter (200 g/plant) obtained from dead leaves taken from forest soil surface, and an ash:organic matter mixture at a 1:1 ratio on the yield of a native cassava crop were evaluated using a farmer participatory research approach. The selection of cuttings and the associated cropping of cassava with cowpea (*Vigna unguiculata*) were also compared with traditional management practices. The test site was a Tukano indigenous community located in Mitú (Vaupés, Colombia), where women grow cassava as a second-cycle crop in small plots of slash-and-burn agriculture with decreased soil fertility, called *chagras* in the northern Amazon rainforests. Cassava yields and root rot percentage were recorded.

Results: The incorporation of ashes mixed with organic matter, organic matter alone, and ashes alone into the soil increased cassava yields to 10, 8.41, and 8.38 t/ha, respectively, while the selection of cuttings produced cassava yields of 5.13 t/ha and the associated cropping of cassava/cowpea, without soil amendment, cassava yields of 4.99 t/ha. Crops under traditional management practices yielded 5.57 t/ha **(Table 1)**.

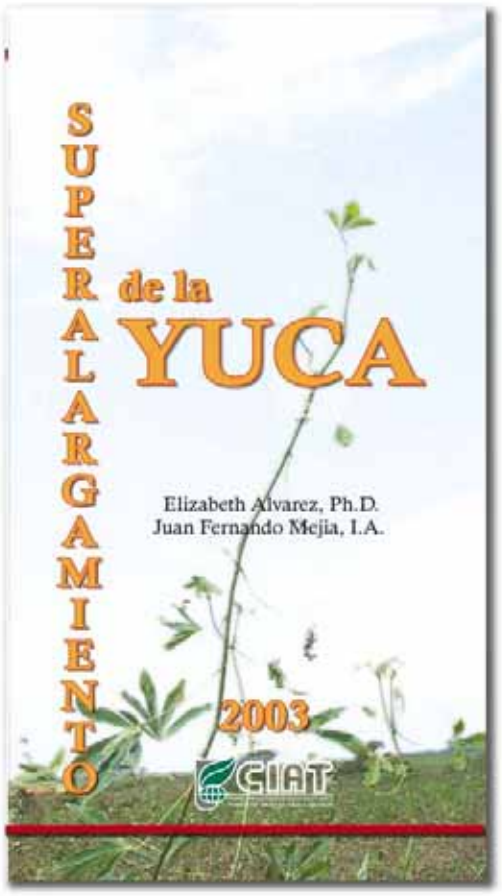
Table 1 shows root rot reduction from 21.97% to zero, when ash mixed with organic matter was incorporated into the soil, and cowpea was associated with cassava.

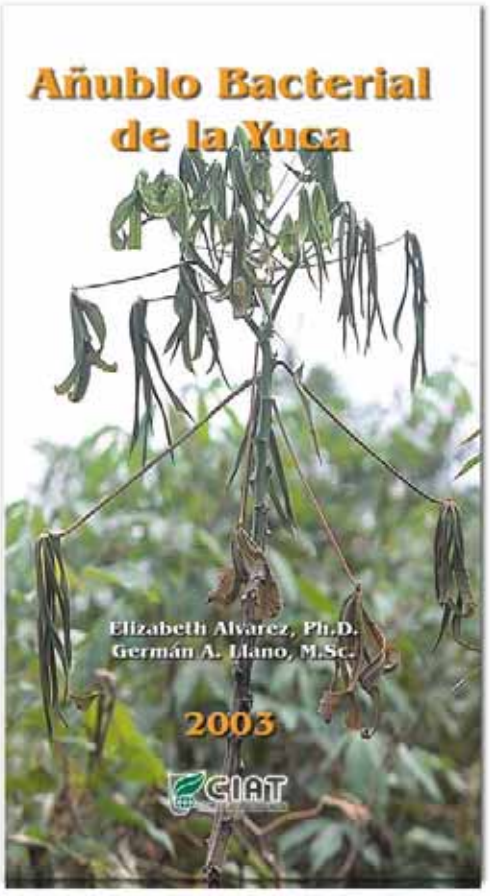
Table 1. Effect of the organic matter, ash, cutting selection and association with cowpea, on cassava yield and root rot severity in Mitú (Vaupés, Colombia).

Treatment	Yield (T/ha)	% Root Rot
Ash	8.38	1.89
Organic matter	8.41	7.81
Ash + organic matter	10.00	0.00
Cutting selection	5.13	3.10
Association with cowpea	4.99	0.00
Traditional management	5.57	21.97

Activity 9. Preparation of a brochure on disease control for farmers.

The following informative brochures were designed to help train farmers how to prevent and manage superelongation disease (SED) and bacteriosis (CBB), two diseases that seriously affect cassava production.





Activity 10. On-farm evaluations of the effects of cover crops, green manure, organic compost, and plant extracts on *Ralstonia solanacearum* in Quindío.

In close collaboration with plantain producers of Quindío, Colombia, field experiments were designed to validate the efficiency of velvet bean (*Mucuna pruriens*) and crotalaria (*Crotalaria juncea* L.) as control of *R. solanacearum* (Kelaniyangoda 1997). The incorporation of these cover crops into the soil aims to disinfect hotspots of plantain plants affected by Moko or banana bacterial wilt. In addition, Calfos (10% assimilable phosphorus, 48% calcium, 1% magnesium, and 1% trace elements), lime extract (residual product of CICOLSA, Armenia, Quindío) and plantain lixiviate are being tested because these products demonstrated good control of *R. solanacearun* in vitro (see CIAT annual report 2002).

During 2003, farmers and researchers planted velvet beans and crotalaria to obtain seed for future trials on Moko-free farms. As a result, this material can be multiplied to satisfy the high demand for this type of seed of farmers interested in evaluating the efficiency of both crops in controlling Moko.

The practices to be studied, as well as different combinations, were determined during field days held with farmers, representatives of the private sector, and staff of official entities. The advances achieved during these events resulted in the establishment of eight trials.

- Field trial (no. 1) established on a disease-free farm in Montenegro, Quindío, to train farmers.
- A complete block design was used. A farmer established another trial (no. 2) in the region, on his own initiative.
- A field trial (no. 3), arranged in three complete blocks, was established in a Moko hotspot (Armenia).

These trials involved the following treatments:

Trial no.	Treatment
1	No treatment (farmer-managed).
2	ICA's recommended Moko control methodology (with several modifications), which includes use of ditches, application of Roundup (glyphosate) and Basamid (dazomet), application of formol to the soil, and coverage with a piece of clear plastic.
3	ICA's methodology, but without the application of formol nor coverage with plastic. Included use of ditches; planting of crotalaria and velvet beans (intercropped); application of Roundup, plantain lixiviate, lemon juice, orange juice, and Calfos. Several products were periodically applied, alternating different products or practices. When velvet beans and crotalaria reach flowering, their leaves and stems were incorporated into the soil and then both cover crops were replanted.

Two more trials (no. 4 and 5) were established on another farm in La Tebaida (Quindío). These aimed to disinfect the Moko-contaminated soil. On each farm, 10 plots (7.5 m^2 per plot) were marked in form of a circle (radius = 5 m) around a production unit initially infected with Moko. The procedure was as follows:

- Prepare a ditch the width (30 cm) and depth (50 cm) of a garden spade, on the outside edge of the hotspot.
- Build a filtration well the size of a barrel (55 gallons).

- Keep the land out of the ditch.
- Apply Roundup to all lots.
- Eliminate three healthy plants around Moko-diseased plants by injecting a solution of Roundup at 20% (3 injections per plant), according to ICA's recommended methodology.

Each plot received a specific treatment, which included cost analysis of materials (Quindío, 2003):

1. Crotalaria: Planted at 20 cm between furrows, in a continuous line within the furrows and parallel to the exterior row of the circle.
2. Velvet beans: Planted, one seed per hole, at 50 cm between plants. A machete is used to keep the growth of velvet bean within plot limits. Cut plant material is incorporated into the soil.
3. Calfos: 5 tons/ha applied to the soil; reapplied at 4-month intervals (this schedule may vary among farmers). Cost = US$5/100-$m^2$ hotspot.
4. Farmer check: No treatment.
5. Formol: Holes 0.4 m deep made with a garden spade (or hole digger), leaving 0.5 m between holes; 750 ml of a formol:water (1:5) solution applied per hole. The entire plot was then covered with a piece of plastic. Cost = US$70/100-$m^2$ hotspot. Although CIAT has continuously recommended to not apply formol because of its high toxicity for humans, both ICA and INIBAP recommend its use.
6. Fresh coffee pulp: A 20-cm layer was applied. Once the pulp has dried in the field, another application is made. Cost = US$30/100 m^2.
7. Plantain lixiviate: 20 L applied per plot with a water can; three applications per year at 4-month intervals. Cost = US$10/100-$m^2$ hotspot.
8. Basamid: A dose of 0.2 kg incorporated per plot (250 kg/ha) and immediately covered with a piece of transparent plastic. Cost = US$60/100-$m^2$ hotspot.
9. Solid Fulvan, an organic fertilizer based on the fermentation of cardboard residues, press cake, and other wastes produced by sugar mills (Biogreen, Palmira, Colombia): 4 kg incorporated into the plot (5 t/ha); three applications per year, at 4-month intervals. Cost = US$5/100-$m^2$ hotspot.
10. Combination: Calfos, velvet beans, crotalaria, and solid Fulvan (see above). Direct contact of Calfos (or Huila phosphorous) with the seed should be avoided. Calfos and solid Fulvan should be applied three times per year, at 4-month intervals.

Two field trials (no. 6 and 7) were established nearby trials no. 4 and 5 in La Tebaida and Montenegro as negative checks.

The youngest stems of 30 production units were tagged and the following treatments applied:

- Manual weeding of the production unit.
- Application of a mixture of 1.8 kg Calfos and 2 kg solid Fulvan around the production unit.
- Planting of crotalaria around the production unit, 1 furrow in line, using a planter.
- Elimination of the terminal part of the raceme or acorn and hermetical bagging of inflorescence (closed, white plastic bag).

The check treatment involved 30 production units to which nothing was applied. Bags were left open.

Trial results are currently being evaluated. Several farmers who participated in the project have adopted the following practices:

- Use of Calfos or Huila phosphate.
- Use of iodine to disinfect tools, for example Agrodyne®SL (13.20 g/L iodine polyethoxi-polypropoxi-poloethoxi–ethanol complex, 1.59 g/L hydriodic acid, produced by Electroquímica West S.A., Medellín, Colombia) at 20% (manufacturer's dose was lower).
- Planting of Crotalaria.
- Application of fresh coffee pulp to Moko-diseased soil.

Farmers can prevent Moko occurring in their plantain crops by applying phosphorus, as evidenced in the comparison of different disease hotspots with disease-free lots. Tissue and soil analyses are needed, however, before this practice can be recommended as a disease management practice.

In Dapa (municipality of Yumbo, Valle del Cauca), the advance of Moko in a Heliconia crop for export was detained in a farmer-managed trial (no. 8), with CIAT's technical assistance. After a previous diagnosis in which *R. solanacearum* was isolated in TZC medium, the following measures were applied, in chronological order:

- Use of iodine (for example Agrodyne at 20%) to disinfect working tool, boots, and hands.
- Installation of a tray with Calfos, covered with a thin sheet of zinc, to disinfect boots or shoes before entering and leaving the diseased plot.
- Injection of both diseased and healthy plants within the diseased plots with Roundup at a concentration of 20%. Roundup was also applied to eliminate weeds.
- Fencing off of the three Moko-diseased plots with barbed wire.
- Digging of ditches around the diseased plots.
- Application of formol to the soil and to diseased plants, according to the above methodology.
- Coverage of Roundup-treated plants and ditches around the plots with a piece of transparent plastic.

Plants in production near the treated plots remained healthy.

Activity 11. Developing a heat treatment and establishing a plantain nursery.

An experiment was carried out at CIAT, with the participation of a plantain farmer of Valle del Cauca, with Moko-diseased suckers (Dominico Harton) to determine the optimal temperature for inactivating *R. solanacearum.* **Table 1** summarizes the treatments used.

Table 1. Temperature and duration of the test treatments.

Status of plants at the beginning of the experiment, temperature, and duration of heat treatment	Total no. of plants	Percentage plants[1]			
		Good growth, healthy	Retarded growth, but healthy	Severely affected by Moko	Dead
Moko-diseased suckers					
No treatment	10	0	10	0	90[2]
45°C/30 min	12	33	17	17	33[3]
47°C/30 min	12	42	0	17	42[3]
53°C/30 min	11	9	0	9	82[3]
Healthy suckers					
No treatment	5	80	20	0	0

1. Two months after heat treatment and planting.
2. Possibly by Moko.
3. Possibly by heat therapy.

In tandem the farmer carried out a series of experiments on his farm. After experimenting with a temperature of 51°C (30 minutes), the temperature was lowered to 49°C and the water:sucker ratio was changed from 2:1 to 3:1 (140 L water and 2 sacks of suckers, weighing 400 g on average) in a 50-gallon barrel heated with firewood instead of a gas burner. The temperature in the middle of the suckers reached 45°C. Losses in the commercially managed nursery, covered with saran, were minimal (10%). The farmer is planning to treat 4000 suckers.

Another farmer established a nursery in La Tebaida (Quindío), with a quarterly production of 5000 Moko-free Dominico Harton suckers, treated at 49°C for 30 to 60 minutes, as phytosanitary practices. All treated suckers germinated. Propagation material obtained in this nursery will be used to establish nurseries in other departments, such as Caquetá, Cauca, and the Amazon region.

Activity 12. Evaluating three doses of tetracycline to control the growth of bacteria isolated from oil palm.

Materials and Methods

Isolation of bacteria. The UNIPALMA plantation sent four small oil palms. Shoot, meristem, and root tissues were washed with deionized water for 15 minutes, then disinfected in 50% alcohol, excess alcohol being removed with SDW. Small pieces of each tissue were cut and placed on nutritive agar with sucrose (5%) and incubated at 30ºC for 24 hours. All possible colonies presenting different morphology and color were selected. Each isolate was cultured on the same medium for subsequent purification.

Antibiogram. Selected colonies were exposed to 3 doses of tetracycline (100, 500, and 1000 ppm) to select the concentration of antibiotic that inhibits microbial growth. Selected colonies were then mass planted in nutritive agar and 3 small filter paper disks, each moistened with a different dose of tetracycline, were placed on the surface of the Petri dish. Colonies were then incubated for 24-48 hours at 30ºC. The doses of antibiotic required to inhibit each isolate were determined.

Pathogenicity test. Each isolate was dyed using gram stain. The meristems of 3-month-old oil palms were inoculated with those isolates observed under the microscope as small gram (-) coccobacillus. The reaction of each was evaluated on a monthly basis.

Results: A total of 24 different bacterial colonies were isolated from oil palm. The results of the antibiogram and the pathogenicity test are indicated in **Table 1**. The bacteria evaluated were not pathogenic in oil palm.

Table 1. Growth inhibition depending on the dose of chlortetracycline applied.

					1st evaluation[1]			2nd evaluation[1]			
Isolate no.	Tissue	Palm no.	Colony no.	Gram	100	500	1000	100	500	1000	Patho-genicity
1	Root		1 (yellow)	-	+	++	++	+	++	++	-
2	Root		2 (white)	-	-	-	-	-	-	-	-
3	Shoot	3	1	+	+	++	++	+	++	++	-
4	Shoot	3	2	+	++	++	++	++	++	++	-
5	Shoot	3	3	-	+	++	++	+	++	++	-
6	Shoot	3	4	+	-	-	-	-	-	-	-
7	Shoot	3	5	-	-	-	-	-	-	-	-
8	Meristem	3	1	+	+	++	++	+	++	++	-
9	Meristem	3	2	-	+	+	+	+	+	+	-
10	Meristem	3	3	-	+	+	+	+	+	+	-
11	Meristem	2	1	-	+	+	+	+	+	+	-
12	Root		1 (white)	-	+	++	++	+	++	++	-
13	Shoot	4	1	-	-	-	-	-	-	-	-
14	Shoot	4	2	-	+	++	+++	+	++	+++	-
15	Root	3	1	-	+	++	++	+	++	++	-
16	Root	3	2	-	-	-	-	-	-	-	-
17	Root	3	3	+	+	++	++	+	++	++	-
18	Root	3	4	-	-	-	-	-	-	-	-

Isolate no.	Tissue	Palm no.	Colony no.	Gram	1st evaluation[1]			2nd evaluation[1]			Patho-genicity
					100	500	1000	100	500	1000	
19	Root	3	5	+	+	++	++	+	++	++	-
20	Root	3	6	+	+	++	++	+	++	++	-
21	Root	1	1	-	-	-	-	-	-	-	-
22	Root	1	2	-	-	-	-	-	-	-	-
23	Root	3	7	-	++	+	++	++	+	++	-
24	Root		3 (red)	-	-	-	-	-	-	-	-

1. Inhibition reaction on a scale of "-" to "+++": "-" = without inhibition halo; "+++" = large inhibition halo.

Activity 13. Diagnosing plant diseases and technical assistance.

Bacteriological and fungal diagnoses were performed on different samples obtained from farmers and institutions (see **Table 1**).

Table 1. Bacteria and fungi isolated from different crops and identified at the CIAT's Cassava Pathology Laboratory (Palmira, Colombia).

Location	Host plant	Disease	Detection method	Microorganism identified
Pereira	Onion	Nematodes	Isolation and direct observation under a light microscope	Nematodes
Ciat	Vanilla	Root and stem rot	Isolation and direct observation under a light microscope	*Pythium* sp.
Cajibío and La Cumbre	*Solanum quitoense*	Stem rot	Direct observation under a light microscope	*Phytophthora* sp.
Ciat	Cassava	Phytophthora root rot	Isolation and direct observation under a light microscope	*Phytophthora* sp.
San Pablo (Bolívar)	Cassava	Root rot	Isolation and selective media	Pythiaceae
Atlántico and CIAT	Cassava	Dry stem rot	Isolation and direct observation under a light microscope	*Diplodia manihotis*
San Pablo (Bolívar)	Cassava	Dry stem rot	Isolation and direct observation under a light microscope	*Botryodiplodia theobromae*
Dapa	*Heliconia* var. *estricta*	Moko	Isolation and selective media	*Ralstonia solanacearum*
Cali and Bitaco	Marjoram	Rust	Direct observation under a light microscope	*Puccinia rubsaameni*
Palmira	*Dendrobium*		Isolation and direct observation under a light microscope	*Colletotrichum*, *Alternaria*, and *Fusarium*

An *in vitro* bioassay to evaluate the effect of fungicides on *Colletotrichum* sp. isolates from blackberry showed that Molto EC (NA)® (Propiconazol + Prochloraz) was the most effective among the nine products evaluated.

At a farm in Sevilla (Valle del Cauca), a *Phytophthora*-resistance inducing agent was injected in avocado stems at doses of 3 cc/injection of 50% commercial product (Nutriphyte®, phosphorous acid: 43.4% P_20_5, potassium hydroxide, and potassium citrate: 40.3% K_2O).

Farmers say that disease incidence has been reduced with the application of the inductive agent, among several integrated management practices, such as disinfection after pruning and prevention of lesions during weeding.

Activity 14. Training farmers, technicians, and extension agents in participatory research, cassava and other crops management, and disease control strategies.

Training

- September 2002. Alejandro Corredor, Universidad de Caldas, Manizales. Taxonomy of *Phytophthora palmivora*.
- September – December 2002. Students from Universidad del Valle. Diana López y Adriana Arenas. Pathogenicity of *Agrobacterium tumefasciens* in carrot and "Coralito".
- October 3 2002. 15 students from Universidad San Buenaventura. Molecular tools for plant disease research.
- Ocober 28, and November 18 2002. 62 students from Universidad de Nariño. Molecular characterization of *Sphaerotheca pannosa*, *Xanthomonas axonopodis* and *Sphaceloma manihoticola*; integrated disease management; molecular tools for plant disease research.
- November 26, 2002. Universidad Nacional, Medellín. Three M.S students of Biotechnology. Molecular tools used in cassava pathology program.
- December 2002. Two research assistants from CENICAFE working on phytoplasma and biocontrol agents.
- March 14, 2003. Advisory for cassava assessment in Guainía, participatory research with indigenous and settlers.
- April 1, 2003. Pedro Molina, Miembro de la Comisión Evaluadora del Programa Redes de Asociación Cooperativa, Ministerio de Ciencia y Tecnología, Venezuela. Integrated cassava disease management.
- April 2, 2003. Universidad del Cauca. Professor Andrés Torres. Information on cassava pathology.
- April 4, 2003. Information on cassava diseases incidence in Valle del Cauca. Carlos Enrique Gómez and Greicy Andrea Sarria. ICA Palmira.
- April 22-May 2, 2003. Carlos Alberto Galvis. Cenicafé. Training in detection of Phytoplasma in coffee.
- May 8, 2003. Ten students from Universidad San Buenaventura. Diseases management and molecular techniques.
- May 20, 2003. Seven breeders form Cuba, Uganda, Thailand, and India. Cassava resistance to pathogens.
- June – September 2003. Technical assistance to Arvey Benavides, for *Phytophthora* management in lulo (*Solanum quitoense*).
- July 29–31, 2003. Gira tecnológica en cultivos de yuca con productores asociados y el equipo de trabajo de la Compañía Agroindustrial Yuquera. San Pablo (South of Bolívar Department).
- August 12, 2003. Rebecca Lee and Santiago Fonseca. Ceniflores (Asocolflores). Ecological practices for flowers disease management.
- August 15, 2003. Mario Pareja. Fundación Chemonics. Cassava disease management.
- July-Sepember, 2003. Hugo Martínez, Fundación Universitaria de Popayán (Ecology). Advisory for production of *Pleurotus ostreatus* y *Pleurotus sajor caju*
- January-September, 2003. Sandra Patricia Fajardo Daza and Rosa Judith Aranda Muelas, Universidad Nacional de Colombia, Ingenieria Agroindustrial, Palmira. Advisory for the production of *Pleurotus ostreatus*.

- September 8 2003. Most important cassava diseases. Ministry of Agriculture from Nigeria and eight attendants.

Workshops

October – November 2002. Four workshops with indigenous women from communities Macaquiño, Urania, Cachivera and Cucura communities, in order to create local agricultural research comitees. 87 participants.

June 9 – 13, 2003. Technicians and producers of plantain. Course: Integrated management of Moko disease in plantain. 23 participants.

Seminars

Detección de un Fitoplasma Asociado con la Marchitez Letal de Palma de Aceite (*Elaeis guineensis*) en Colombia. CIAT. Oct 30, 2002. E. Alvarez.

Development of ecological practices to manage *Phytophthora* root rot of cassava (*Manihot esculenta*) E. Alvarez, J. B. Loke, G. A. Llano. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 133.

Control of bud rot in oil palm, *Elaeis guineensis*, using resistance inducers. E. Alvarez, G. A. Llano, M. C. Feris, M. L. Hernández and S. M. Rodríguez. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 179.

Characterization and classification of *phytoplasmas* associated with oil palm (*Elaeis guineensis*). E. Alvarez and J. L. Claroz. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 284.

Detección de marcadores microsatélites asociados con la resistencia al Añublo Bacterial de la yuca (*Manihot esculenta* Crantz) en Colombia. P. X. Hurtado, E. Alvarez, M. Fregene and G. A. Llano. Presented at XXIV Congress of ASCOLFI. June 25 – 27, 2003. P. 25.

Caracterización genética y patogénica de *Colletotrichum* spp. agente causal de la antracnosis en guanábana (*Anona muricata*) en el Valle del Cauca. C.A Ospina and E. Alvarez. Presented at XXIV Congress of ASCOLFI. June 25 – 27, 2003. P. 6

Detecting the phytoplasm-frogskin disease association in cassava (*Manihot esculenta* Crantz) in Colombia. E. Alvarez, J.F Mejía, J.B Loke, L. Hernández, and G.A Llano. Phytopathology 93 (6): S4. Poster presented at APS annual meeting August 9 -13. Charlotte, NC, USA.

Detecting SSR markers associated with resistance to cassava bacterial blight (CBB) in Colombia. E. Alvarez, P. X. Hurtado, M. Fregene and G. A. Llano. Phytopathology 93 (6): S4. Poster presented at APS annual meeting August 9 -13. Charlotte, NC, USA.

Congress

8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand. Control of powdery mildew in roses by applying lixiviated plantain rachis compost. E. Alvarez, C. Grajales, J. Villegas and J. B. Loke. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 272.

XXIV Congreso Nacional de Fitopatología, held by ASCOLFI, Armenia. June 25-27. Enfermedades de yuca y perspectivas de manejo sostenible. E. Alvarez. Conference presented at Congreso Nacional de Fitopatología, held by ASCOLFI, Armenia. June 25, 2003

APS annual meeting. August 9 - 13. Charlotte, NC, USA.

Publications

Alvarez, E., Mejia, J.F., and Valle, T.L. 2003. Molecular and pathogenicity characterization of *Sphaceloma manihoticola* isolates from south- central Brazil. Plant Dis. 87: 1322-1328.

E. Alvarez, J. B. Loke, S. Rivera y G. A. Llano Genética de la resistencia a pudrición causada por *Phytophthora tropicalis* en dos poblaciones segregantes de yuca (*Manihot esculenta* Crantz).. Revista Fitopatología Colombiana Vol 26 (2): 61 - 66. Cali, Colombia.

Identificación de genes análogos de resistencia a enfermedades en yuca, *Manihot esculenta* Crantz y su relación con la resistencia a tres especies de *Phytophthora*. M.Sc. thesis. Palmira, Colombia: Universidad Nacional de Colombia. 119 pp. 2003

Two brochures on management strategies of Cassava Bacterial Blight and Superelongation Disease.

Submitted

Assessment of integrated management practices of cassava root rots, by participatory research with indigenous from Mitú, colombian noreast amazon. Agren. Agricultural research and extension network.

Hurtado, P.X., Alvarez, E., Fregene, M. Búsqueda de genes análogos de resistencia asociados con la resistencia al añublo bacterial de la yuca. Revista Fitopatología Colombiana.

Hurtado, P.X., Alvarez, E., Fregene, M. Llano, G.A. Detección de marcadores microsatélites asociados con la resistencia a *Xanthomonas axonopodis* pv. *manihotis* en una familia de yuca (BC1). Revista Fitopatología Colombiana.

Concept Notes and Proposals

- Identification of a phytoplasm associated to Lethal Wilt in oil palm. Palmar del Oriente, Palmas de Casanare and Palmeras Santana. US$29.500. **Approved**
- Caraterización molecular de un fitoplasma que esta afectando el cultivo del lulo en Colombia. ICA. US$4.000. **Approved**
- Detección y manejo de microorganismos asociados con cuero de sapo en yucca. Ministerio de Agricultura y Desarrollo-Colombia. Project cost: US$104.500. Funds requested: US$45.000. **Approved**
- Avance en el desarrollo de tecnologías de cultivo y postcosecha para una producción rentable y sostenible de guanábana (*Anona Muricata* Lynn) Fase 2: FONDO DE COOPERACION ESPAÑOLA. Funds requested US$27.000. **Approved.**
- The mechanisms behind disease resistance in cereals. Presented with Royal Veterinary and Agricultural University (KVL, Denmark) and CORPOICA (Colombia). Presented to the Cereals Comparative Genomic initiative. Cost: US$260.000. Funds requested: US$200.000.
- Generación de nueva tecnología para el manejo de Mildeo Velloso en rosa y la caracterización genética de su agente causal, *Peronospora sparsa*, en Colombia. ASOCOLFLORES/CENIFLORES. Project cost: US$238.000. Funds requested: US$119.000.
- Desarrollo de medidas de manejo del Moko (*Ralstonia solanacearum*), para evitar la destrucción de las plantaciones de plátano en Colombia. Presented with ICA and CORPOICA to ASOHOFRUCOL (Colombia). Project cost: US$188.200. Funds requested: US$117.200.

- Medidas Tendientes a Evitar que el Moko (*Ralstonia solanacearum*) Destruya las Plantaciones de Plátano y Banano en Colombia. Presented to the Ministerio de Agricultura y Desarrollo de Colombia. Funds requested: US$71.428.

Theses for Master of Sciences degree

Germán A. Llano. 2003: Identificación de genes análogos de resistencia a enfermedades en yuca, *Manihot esculenta* Crantz y su relación con la resistencia a tres especies de *Phytophthora*. M.Sc. thesis. Palmira, Colombia: Universidad Nacional de Colombia. 119 pp.

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.

Theses for Master of Sciences degree in progress

John B. Loke: Análisis genético de la resistencia de yuca (*Manihot esculenta* Crantz) a *Phytophthora tropicalis*, causante de pudrición radical. Universidad Nacional de Colombia—Palmira. For a Master of Plant Breeding.

Personnel – Staff: Elizabeth Alvarez, Germán A. Llano, John B. Loke, César A. Ospina, Juan Fernando Mejía, Juan Fernando Mejía, Lina María Tabares, Zulma Zamora, Tatiana Espítia, Herney Rengifo, Paula Hurtado.

Students and Technicians:

Universidad de los Andes—Bogotá:
Paula Ximena Hurtado
Universidad Católica de Manizales:
Samira Moreno
Colegio Bolívar: Cristina Londoño
Universidad del Valle: Sonia Ossa
Universidad de Caldas— Manizales:
Alejandro Corredor
Universidad de los Llanos—Villavicencio:
Sandra Milena Rodríguez
Técnico Agroforestal, Mitú, Vaupés:
Gabriel Paiva

Linkages with CIAT's Partner Institutions

ASOCOLFLORES
CLAYUCA
COLCIENCIAS
Corporación BIOTEC
Instituto de Investigaciones de Viandas Tropicales (INIVIT, Cuba)
IPRA (based at CIAT, Colombia)
Secretaría de Agricultura del Vaupés (at Mitú)
UMATAs (Mitú, Caicedonia, La Tebaida, and Montenegro)
Universidad Nacional de Colombia—Palmira (Valle del Cauca, Colombia)
Universidad de los Andes, Bogotá

Donors

Instituto Nacional de Investigación y tecnología Agraria y alimentacion INIA
Hacienda San José (Palmira)

ICA
Ministerio de Agricultura y Desarrollo Rural
Palmar del Oriente
Palmas de Casanare
Palmeras santana
PRONATTA

Collaborators-Local
CENICAÑA (Drs J. Victoria and F. Angel)
CLAYUCA (based at CIAT, Dr B. Ospina)
CORPOICA—Bogotá (Dr Jairo Osorio)
CORPOICA "La Libertad" (Villavicencio, Dr. A. Tapiero)
CORPOICA—Palmira (Dr G. Aya)
Corporación BIOTEC (Dr J. Cabra; B. Villegas)
Corporación para el Desarrollo Sostenible del Norte y Oriente Amazónico (CDA, Vaupés, Dr E. Polo and R. Peña)
CRIVA—Consejo Regional Indígena del Vaupés (Mitú)
ICA—Quindío and Valle (Drs E. Vargas, F. Varón, C. A. Montoya and C. Huertas)
Palmar del Oriente
Palmas de Casanare
Palmeras Santana
Secretaría de Agricultura del Vaupés (at Mitú, Dr G. Arbeláez)
Special (La Tebaida, Mr S. González)
UMATAs (Drs O. Holguín, L. Muñoz, and W. Ospina)
Unillanos—Villavicencio
Universidad Católica de Manizales
Universidad de Caldas—Manizales
Universidad de los Andes—Bogotá
Universidad del Valle (Cali)
Universidad Nacional de Colombia—Palmira

International
Cooperative Research Center for Tropical Plant Protection, University of Queensland, Brisbane, Australia (A. Drenth)
Iowa State University (Dr T. Harrington, Dr. T. Pepper)
Kansas State University (Drs S. H. Hulbert and R. Zeigler)
Michigan State University (Dr K. Lamour)
The Royal Veterinary and Agricultural University (Copenhagen, Denmark, Dr D. Collinge)