PROJECT PE-1

IPM for a Safer Environment: Integrated Pest Management in Major Agroecosystems in the Americas

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PE-1: IPM for a Safer Environment: Integrated Pest Management in Major Agroecosystems in the Americas

Objective: To develop and transfer improved pest and disease management components for major agricultural production systems and reduce environmental damage due to excessive pesticide use.

Outputs: Improved pest and disease management components and implementation strategy developed on CGIAR commodity crops; improved crop management components relevant to IPM strategies in the Americas developed; NARS capacity to design and execute integrated crop management research and development projects strengthened.

Gains: Increased crop yields and reduced environmental damage; natural enemies of major pests and diseases evaluated. IPM tested and verified on farms. Knowledge on biology, ecology behavior and damage of pests, and diseases. Molecular characterization of major pathogens and diagnostic kits available. Whitefly biodiversity characterized.

Milestones:

1998 Whitefly biodiversity determined and natural enemies identified; cassava mite predators and entomopathogens evaluated and released in LA and Africa; CVMV yield loses determined.

1999 Natural enemies of whiteflies, mites, burrowing bug released in LA and Africa; Molecular markers tagging resistance to CBB and Phytophthora root rot identified; resistance to cassava frog skin virus identified and Elisa diagnostic kit developed.

2000 Biological control implemented for several arthropod pests and root rot pathogens; Cassava geminiviruses characterized. Farmers and extensionists trained in utilization of diagnostic kits.

Users: Biodiversity of agroecosystems determined and available to researchers. NARS scientists, extensionists and farmers trained in IPM methodologies. Crop yields for small producers increased and stable production systems identified.

Collaborators: EMBRAPA, CORPOICA, IITA, ICIPE, CATIE, NARO, NRI, Universities of Florida and Wisconsin, John Innes Center, ETH, OSTROM/CIRAD; and others. NARS, including EMBRAPA, CORPOICA, INIAP, INIVIT, NGO's and biological control industries (COINBIOL).

CG System Linkages: Increasing Productivity 30%; Biodiversity - 20%; Protect Environment - 40%; Strengthening NARS - 10%. Manages Whitefly and Participatory Methods Projects in Systemwide IPM Program.

CIAT Project Linkages: Collaborates with breeding projects (IP1, IP2, IP3, IP4, IP5) in host plant resistance; provides biocontrol agents to proejct PE5; uses inputs from PE4, SB2, and SN3.

Financing Plan: 20% unrestricted core, 80% special projects.

PE-1. IPM for a Safer Environment: Integrated Pest Management in Major Agroecosystems

Project Objective

To develop and transfer improved pest and disease management components for major agricultural production systems and reduce environmental damage due to excessive pesticide use

	I	II	III	IV
Outputs	**Improved pest and disease management components and implementation strategy developed on CIAT commodity crops**	**Improved crop management Components relevant to IPM strategies developed in the Americas**	**NARS capacity to design and execute integrated crop management research and development projects strengthened**	**Global whitefly research network to reduce crop losses initiated**
Activities (Subprojects)	• Identification and quantification of major arthropod complexes in selected agroecosystems; development of effective control measures. • Identification and characterization of root rot and superelongation pathogens and CBB races; development of rapid detection methods; development of effective control measures. • Identification and characterization of major viruses; development of rapid detection methods; development of effective control measures.	• Determination of crop management practices that effect pest and diseases populations and dynamics. • Development and evaluation of cultural control practices such as crop rotation, intercropping, etc. for selected pests and diseases. • Evaluation of alternate safer chemical control agents.	• Development of methods for farmer's participatory diagnosis and research in IPM and ISCM. • Ex-post adoption and impact studies on IPM costs and crop productivity. • Integrated management technologies for cassava planting material. • Assessment of impact of IPM measures and policy recommendations. • Dissemination of IPM methodologies through training, workshops, etc. • Assessment of impact of farmer participatory methodologies including participation of women in IPM projects.	• Formation of an international whitefly network. • Diagnosis and characterization of whitefly problem and target areas. • Improvement of understanding of pest and disease dynamics. • Preliminary development and testing of management strategy and tactics through participatory research.

CIAT -Project: PE1. INTEGRATED PEST MANAGEMENT IN MAJOR AGROECOSYSTEMS

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Critical Assumptions beyond control of Project Team
Program Goal: To increase crop yields and reduce environmental contamination through the effective management of major pests and diseases.	-Increased cassava yields -Reduction in environmental degradation due to adoption of improved technology -Reduced losses due to several major diseases	-Production statistics -Adoption and impact studies	-National policies favorable to adoption of IPM strategies (i.e. increased support to extension, reduction of subsidies to pesticides). -National programs are active and strong in key countries
Project Purpose: To develop and transfer improved pest management components for sustainable productivity and rational use of pesticides.	-Adoption of germplasm with resistance to biological constraints -Establishment of release natural enemies -Use of environmentally friendly control strategies -Improved understanding major biotic constraints	-End of project reports -Refereed publications -Adoption and impact studies	-Financial resources are mobilized -Active collaboration with NARs -Active collaborations with other IARCs and Developed countries research organizations -Active collaboration with advanced research institutions
Outputs:		All areas: -Project reports and refereed publications, book chapters, etc.	
• Improved understanding of pest and disease management components.	-Pests, diseases, natural enemies and vectors characterized -Host/pest/natural enemy/vector interactions analyzed -Better diagnostic tools available -Biological control agents established	-Reports with maps, economic damage, biological information. -Analysis of experiments -Transfer of tools to seed health facilities	-NARs have the needed resources -Adequate interaction with other disciplinary scientists -Successful experiments -Continued development of new varieties that are commercially acceptable -Farmers have adequate access to extension agents, credits and other factors that impact on adoption
• Development and integration of pest and crop management components relevant to IPM strategies.	-Testing of components for effectiveness. -Control strategies recommendations clearly identified and crop management practices determined -Farmer testing of components -Guides on IPM strategies published	-Analysis of experiments -Guidelines for IPM -Reports on field effectiveness and probability of adoption of components -Field oriented brochures	
• NARS capacity to design and execute integrated crop management research and development project strengthened.	-Training especially in FPR -Development of projects with NARs -Training materials developed	-Reports on training courses -Concept notes and projects prepared with partners	
• Globalization of IPM research activities on pest and crops initiated.	-Network of researchers established -Preparation of web pages and databases with relevant IPM information	-Electronically published web pages and databases	
Inputs: **Core** **Non-core** Senior staff: 160,420 Support staff: 116,847 Operations: 75,642 Total: 352,909 707,160	Senior Staff: 3.3 Support Staff: 5.0 Secretaries: 0.5 Field Workers: 4.3	-Accounting of budgets -Project reports -Donor reports	-Administration commitment to stable core support -Ability to attract continued donor support -Project office support

Highlights:

- Phytoseiidae reference collection, held at CIAT, systematized, inventory made and database organized.
- Taxonomic key for Phytoseiidae identified on cassava being developed.
- Early instars of thrips and whiteflies identified as alternative food sources for Phytoseiidae.
- Relationship between phytophagous mite egg density and Phytoseiidae fecundity determined for six species of predator mites.
- Effect of relative humidity on survival of 6 species of Phytoseiidae determined.
- A virus disease of cassava mites, *M. tanajoa* and *M. caribbeanae* found to cause high mortality.
- *Aenasius vexans* verified as specialist parasite on the mealybug *Phenacoccus herreni; Acerophagus coccois* confirmed as generalist parasitoid.
- Project to identify whitefly parasitoid complex on cassava and other crops initiated.
- Resistant cassava clones may effect the behavior and development of parasitoids of whiteflies.
- Isolates of two entomopathogens *Metarhizium anisopliae* and *Beauveria* sp. evaluated as pathogenic to the cassava hornworm, *Erinnys ello.*
- Project to identify major pests of asparagus initiated with group of asparagus growers.
- Methodology for Electronic Monitoring Whitefly feeding behavior was established including:
  Technique for wiring up whiteflies with a thin (6.5 um) gold wire.
  EMS Calibration and establishment of proper voltage and frequency.
  Computer acquisition and display of electronic monitoring data.
  Identification of preliminary waveforms on susceptible cassava CMC-40
  Training of CIAT personnel to operate the Electronic Monitoring System (EMS).
- With the participation of local partners and cassava growers, an exploratory survey of *Phytophthora* root rot was conducted and on-farm trials established in the Departments of Cauca and Vaupés, Colombia.
- A simple, rapid, and accurate PCR method was developed to detect the causal agent of *Phytophthora* root rot in infected cassava sprouts.
- Extremely high genetic diversity was found among 122 *Phytophthora* isolates using restriction analysis of amplified ribosomal DNA and RAPD analysis.
- Several potential agents for the biocontrol of two highly pathogenic *Phytophthora* isolates were successfully tested in the greenhouse.
- Significant differences in heat sensitivity were found among different *Phytophthora* species and isolates of *Xanthomonas axonopodis* pv. *manihotis*, the causal agents of root rots and cassava bacterial blight, respectively. A heat treatment, tested in vitro and in the greenhouse, in which cassava stakes were heated for 30 min at 47 °C, eliminated the pathogens but did not affected the stakes' germination rate and vigor.
- A cryopreservation method was developed to store the entire *Phytophthora* collection.
- A fragment of a viral RNA dependent RNA polymerase gene has been cloned and sequenced.

- Progress towards the output of the identification of CFSD resistant cassava is being made.
- *P. herreni* is mainly a phloemophagous insect with piercing-sucking mouthparts, present in females throughout their life cycle and in males only till the second nymphal stage after which they do not need to feed to complete their development.
- Cassavas in water deficit conditions induced a reduction in the duration of female development, and an increase in fecundity, intrinsic natural growth rate (Rm) and in weight.
- Leaf aminoacid composition showed a significant increase in the percentage of free serine, asparagine, glutamine and arginine of leaves from water stressed plants.
- By utilizing an artificial diet, it was shown that asparagine and arginine played an important role during the development of females of *P. herreni*. In fact, their absence does not allow nymphs to reach the adult stage, but insect aminoacid composition wasn't affected.
- Parasitized mealybug did not significantly change their feeding behavior compared with non-parasitized mealybugs, indicating that they feed mainly on phloem sap until the appearance of mummy.
- Evaluation and identification of varieties resistant to cassava root rot continued through farmer participatory trials in the States of Paraiba and Bahia. Six genotypes were selected as promising genotypes for further testing and selection
- Adoption of important cultural practices for control of cassava root rot continued to be tested through farmer participatory trials in the States of Bahia and Pernambuco. The use of ridges as planting system is showing promising results in comparison with traditional flat planting.
- Molecular diversity of Sphaceloma manihoticola (Super-elongation disease-SED) was low, thus facilitating the task of selecting cassava germplasm resistant to SED.
- Cassava varieties resistant to SED identified.
- To address the need critical mass of trained technicians both in research and extension at the state level in the northeast of Brazil, a proposal training is considered a priority by CNPMF.
- Started during the UNDP-IPM project, 25 CIALs are functioning and many are running their third consecutive experiment.
- The training master plan is composed of three modules: diagnosis, planning and experimentation and evaluation. After two cycles, the trainees have the basic skills to facilitate collective problem solving.
- COPALs are making their own decisions using FPR.
- Decisions on the best varieties for the community and the use of fertilizers are examples of the products of the COPALs research.
- The farmer's criteria for selecting the best varieties are more than just yield. Shape of roots and color of flour are examples of characteristic that were identified as important by farmers.
- Evaluation and identification of resistant varieties to cassava green mite continued through farmer participatory trials with two COPALs in Bahia.

- Through coordination of the research efforts especially in the standardization of protocols, the information that is being collected in the Americas can be directly compared with the data generated from Africa.
- Significant progress has been made in determining the distribution of whitefly pests in four countries.
- The carboxy oxidase subunit I gene is proving useful in studies of the evolutionary relatedness of whiteflies. This gene has two highly variable regions and is widely used in evolutionary studies.

OUTPUT I. IMPROVED PEST AND DISEASE MANAGEMENT COMPONENTS DEVELOPED

Output 1. Identification and Quantification of Major Arthropod Complexes: Development of control measures.

Sub-output 1.1. Biological Control and Plant Interactions of Cassava Mealybugs.

Although several species of mealybug will feed on cassava only *Phenacoccus herreni* and *Phenacoccus manihoti* are reported causing yield losses. Both species are of neotropical origin but *P. manihoti* only causes severe crop losses in Africa. It has become a well known example of classical biological control by the introduction of natural enemies, especially the parasite *Apoanagynus lopezi* collected in Paraguay and Brazil.

Although *P. herreni* is distributed throughout northern South America it has – from the 1970's onward – caused serious losses, but primarily in NE Brazil. Surveys in the 1980's found few parasitoids in the region suggesting that *P. herreni* may be an exotic pest, probably coming from northern South America, which is geographically separated from NE Brazil by the Amazon basin. Surveys conducted in Colombia and Venezuela identified three encyrtid parasitoids [*Apoanagyrus* (Epidinocarsis) *diversicornis, Aenasius vexans* and *Acerophagus coccois*] as effective parasitoids of *P. herreni*. The three parasitoids were imported from CIAT and released in NE Brazil in 1994-95, where they have become established. *A. diversicornis* and *A. coccois* have dispersed readily and have been recovered in high numbers hundreds of kilometers from their release sites. *A. vexans*, despite being consistently recaptured at its release site has not dispersed.

In coordination with these field releases, considerable research is being carried out to determine the effect of plant volatiles on parasitoid behavior. Previous studies (See Annual Reports 1995-1997; and Bertschy, et. al. 1997) show that all three parasitoids were attracted to cassava mealybug infestations.

Activity 1.1.1. Ecological cultivation of cassava: To render natural enemies of sucking pests more effective and reliable.

Reduction of the cassava mealybug *Phenacoccus herreni* in Latin America relies on biological control with natural enemies as germplasm with adequate resistance is not available. The two encyrtid parasitoids *Aenasius vexans* and *Acerophagus coccois* have been mass-reared and released. The quality of released parasitoids is a key factor for successful biological control. In mass rearing facilities natural enemies may loose their behavioral traits related with successful host searching and location. It has been shown that volatile chemicals emitted by herbivore infested plants are an important source of information for natural enemies during host location and that they are capable to learn these foraging cues. Enhancement of this behavioral trait by exposing parasitoids in the pre-release phase to host related cues may be one possibility to render biological control organisms more effective and reliable. Ecology and behavior of natural enemies varies depending on their degree of dietary specialization. It is suggested that learning of odour cues is more important in species with a broad host range (generalists) than in species with a narrow host range (specialists) as they deal with greater host and environmental variation. Generalists are expected to learn stimuli of host and environmental characteristics where they successfully encountered host before. Specialists are supposed to already show high innate preference towards specific host-derived stimuli. For them learning is expected to be of minor importance. At present the specificity level of the two parasitoids has not been investigated in depth. Available date suggests that *A. vexans* is more specialized than *A. coccois*. The two parasitoids have been shown to respond to and being attracted from mealybug induced synomones from cassava. *A. coccois* responded to non-infested plant odour while *A. vexans* responded more specifically to odours associated with mealybug infestation (Bertschy *et al.*, 1997). Our objective was to verify the above mentioned assumption on the degree of dietary specialization of *A. vexans* and *A. coccois* as a basis for studying the learning capability of the two species.

In a greenhouse study a comparative quantification of the physiological host range of the two parasitoids was carried out. Seven different mealybug species were collected at CIAT, Colombia, in 1998, from cassava, tropical forage legumes and fern.

Each mealybug species was exposed to parasitism by *A. coccois* and *A. vexans* in a no-choice bioassay. A first evaluation of the results, based on emergence of the second generation of parasitoids is presented in the following table:

Table 1.1.1.1. Host specificity of the two encyrtid parasitoids *Aenasius vexans* and *Acerophagus coccois* on seven different mealybug species. First column indicates successful parasitism, the second column represents a new parasitoid generation.

Mealybug species	*Phenacoccus herreni*		*Phenacoccus madeirensis*		*Ferrisia virgata*		*Ferrisia virgata*		*Pseudococcus jackbeardsleyi*		*Planococcus citri*		*Pseudococcus importatus*		*Pseudococcus longispinus*	
Plant	Cassava		Cassava		Cassava		Bean		Cassava		Bean		Bean		Fern	
A. vexans	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. coccois	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

+ successful parasitism
- parasitism failed

The assumption on the specificity level of the two parasitoids could be proven. *A. vexans* only parasitized and developed successfully in *P. herreni*, whereas *A. coccois* was able to parasitize and develop in *P. herreni*, *P. madeirensis* and *F. virgata*. *A. vexans* therefore can be regarded as a specialist and *A. coccois* as a generalist parasitoid. This information will be used in the forthcoming studies for pre-release conditioning of both species.

Sub-output 1.2. Biological Control and Species Diversity of Cassava Whiteflies.

Whiteflies (Homoptera: Aleyrodidae) cause yield losses on numerous agricultural crops throughout the tropical and subtropical regions of the world. They damage crops through their direct feeding and are vectors of numerous plant viruses. As direct-feeding pests and vectors of plant viruses, whiteflies constitute a major problem in cassava production in Africa, the Neotropics and, to a lesser degree Asia.

Species Complex. The largest complex of whiteflies is in the Neotropics where, recent studies indicate, there are 11 species, *Aleurotrachelus socialis, Trialeurodes variabilis, Bemisia tuberculata, Aleurothrixis aepim, B. tabaci, B. argentifolii, Trialeurodes abutileneus, Aleurodicus dispersus, Paraleyrodes* sp., *Aleuronudus* sp. and *Tetraleurodes* sp. *A. socialis* is the predominant species in northern South America, but is also found in Brazil and other areas. *A. aepim* is the predominant species in Brazil; *B. tuberculata* and *T. variabilis* are reported in low populations from Brazil, Colombia and several other countries. The spiraling whitefly *A. dispersus* is reported feeding on cassava in Nigeria and *Bemisia afer* in Kenya and the Ivory Coast. *B. tabaci* has a pantropical distribution, feeding on cassava throughout Africa and several countries in Asia, including India.

Since the early 1900s, a new biotype (B) of *B. tabaci* (The silverleaf whitefly), considered by some to be a separate species (*B. argentifolii*), has been reported feeding on cassava in the Neotropics (USA, Dominican Republic, Ecuador, Brazil, Colombia, and Puerto Rico). Africa Cassava Mosaic Disease (ACMD) which causes heavy crop losses in Africa is vectored by *B. tabaci*. Now that the B biotype of *B. tabaci* has been found feeding on cassava in the America, ACMD poses a serious threat to cassava production as most traditional varieties are highly susceptible to the disease. Thus a continued strong research emphasis on whiteflies in cassava is justified. (For additional information on whiteflies, see Output IV of this report).

Biological control agents, e.g. parasitoids, predators and entomopathogens, are increasingly accepted as a means of pest control which reduce contamination and other disadvantage that arise from the excessive use of chemical pesticides. Although knowledge of whitefly parasitoids is growing, many gaps exist in the successful implementation of biological control. It is still not clear which of the potential biocontrol agents are most common in the field, and what level of efficacy they exert.

A project was initiated during 1998, in collaboration with the University of Florida (USA), to determine the efficiency of indigenous parasitoids from the Andean Region of South America (Colombia, Venezuela, and Ecuador), against whiteflies on cassava and beans, in order to select the best potential parasitoids for continued research. It is hoped that some of the general lack of awareness of the potential use of biocontrol agents against whiteflies may be overcome through this project.

Activity 1.2.1. Exploration for whiteflies and natural enemies on cassava and other crops.

The purpose of this activity is to determine the efficiency of indigenous South American parasitoids on cassava, beans and other crops, in order to select the best potential parasitoids for continued research, and to compare efficiency of indigenous species to that of exotic whitefly parasitoid species being recommended for introduction into the region.

This is a USAID funded collaborative project between CIAT and the University of Florida "Biological Control of Whiteflies by Indigenous Natural Enemies for Major Food Crops in the Neotropics." This collaboration will allow training, and improved in-country capacity for research, production, delivery and management of biological control agents. The project will focus principally on CIAT's traditional food commodity crops, beans and cassava, but will also involve other crops. This project also enters into the CGIAR System Wide Whitefly IPM Project (see this report).

The University of Florida will provide expertise and input on parasitoid taxonomy, biology, behavior, collecting, rearing, identification and data analysis. During 1998 two University of Florida researchers, Dr. Jorge Peña and R. Nguyen, visited CIA and collaborated in training CIAT personnel in the above areas. Additional future visits are planned. Whitefly parasitoids that are collected from field sampling are being sent to University of Florida taxonomist (G. Evans) for identification.

Sampling methodologies have been developed and project start-up was March, 1998. A description of preliminary activities follows.

Three geographic areas have been selected for exploration for whitefly species and their parasitoid natural enemies. These are the Department of Cauca, and the Atlantic Coast area of Colombia (especially the Department of Atlántico, Córdoba, Bolívar and Magdalena) and Manabí, Ecuador.

Sampling will be done on several crop hosts, including cassava, beans, tomato, and soybeans. Each zone will be characterized by taking data on m.a.s.l., rainfall, temperature range, vegetation type, latitude, geographic area, etc. From each collection site or field, 100 leaves will be random by collected; a one inch square will be selected to take a count of the different whitefly species. Numbers of whitefly nymphs and pupae will be recorded.

To determine the rate of parasitism, 40 leaves will be collected and one inch square of leaf area will be removed. Only one whitefly species will be allowed to remain on each leaf square and the emergence of parasitoids will be recorded for each species of whitefly. In this way we will be able to determine the exact parasitoid species associated with each whitefly species. Additionally we will obtain data on the levels of parasitism related to the density of whiteflies.

Exploration and sampling has already been initiated; the Atlantic Coast has been sampled once, and Cauca twice. A total of 21 samples have been collected that are in the process of being evaluated and analyzed. Preliminary results, from sampling on the Atlantic Coast show low to moderate levels of whitefly populations and parasitoids were found in all sampling sites except one (**Table 1.2.1.1**). In those sties where parasitoids were found, % parasitism ranged from 1.92 % to 54.9%. Parasites are being sent to collaborating specialists at the University of Florida and the British Museum for identification.

Table 1.2.1.1. Exploration and sampling for whiteflies and their natural enemies on the North Coast of Colombia.

Locality	Whitefly Population*	Parasites Collected	% Parasitism
Pivijay, Magdalena	697	80	11.5
Caracoli, Atlántico	334	39	11.7
Polonuevo, Atlántico	217	68	31.8
Repelón, Atlántico	441	12	2.7
Luruaco, Atlántico	191	36	18.8
Magdalena, Bolívar	61	9	14.7
San Jacinto, Bolívar	52	1	1.9
Córdoba, Bolívar	592	0	0
C. de Bolívar, Bolívar	63	18	28.6
San Pelayo, Córdoba	78	42	53.8
Berastegui, Córdoba	148	54	36.5
San Carlos, Córdoba	113	62	54.9
Cereté, Córdoba	257	68	26.5

* Nymphs, pupae and adults of Whiteflies emerging 40 one inch square leaf' samples.

Activity 1.2.2. Evaluation of whitefly parasites.

Numerous natural enemies of the whitefly complex associated with cassava have been recorded. Recent surveys in Colombia show that the most representative group is the microhymenopteran parasitoid complex, which includes the genera *Encarsia, Eretmocerus* and *Amitus* associated with *A. socialis, B. tuberculata* and *T. variabilis*. The predominant species were *Encarsia hispida, Amitus spiniferus,* and *Eretmocerus* sp. (undescribed). Based on these data a research effort was initiated to study the behavior and effectiveness of some of these parasitoids.

During 1997, after considerable effort, a colony of *Encarsia hispida* was established, and activities were initiated to study its biology and behavior.

The small, yellowish parasite is very agile and moves rapidly walking over a leaf, using its antennae to guide, recognize and explore a surface. Upon locating a nymph of *A. socialis*, the parasitoid circles it, examining it, and with its forelegs, begins to remove the waxy particles protecting the nymph. When parasitizing it places its abdomen with ovipositor directly over the nymph; it then initiates an up and down movement which can least from 15 to 45 minutes. Upon completing oviposition, the parasitoid spends the next 10 to 15 minutes cleaning its whole body with the use of its fore, mid and hind legs. Not all nymphs that are examined are parasitized; the parasites may feed from the wound it makes in the nymph with its ovipositor.

The female parasitoid oviposits an egg within the nymph; the egg measures an average of 0.0679mm width x 0.195mm length. Female *E. hispida* are 0.399mm in length and males are 0.479mm.

Studies to determine the whitefly instar most susceptible, or attractive to the parasitoid was done by placing infested cassava leaves (remaining attached to the plant) within a petri dish (150 x 25mm) and releasing 30 parasitoids into this experimental chamber. When the first adult whiteflies began to emerge, leaves were collected and placed in a plastic box and emerging parasites were collected over a 20 day period. Results show that the most parasitized instar was the third instar with 29.7% parasitism. The 1st, 3rd and 4th instars had parasitism levels of 0.4%, 12.6%, and 3.6% respectively. Parasitism begins soon after the nymphs are exposed to the parasitoids. Forty eight hours after exposure 35.7 third instar nymphs were parasitized; at 96 hours 45.9% were parasitized **(Figure 1.2.2.1).** These studies will continue with *E. hispida* and eventually include additional parasitoid species.

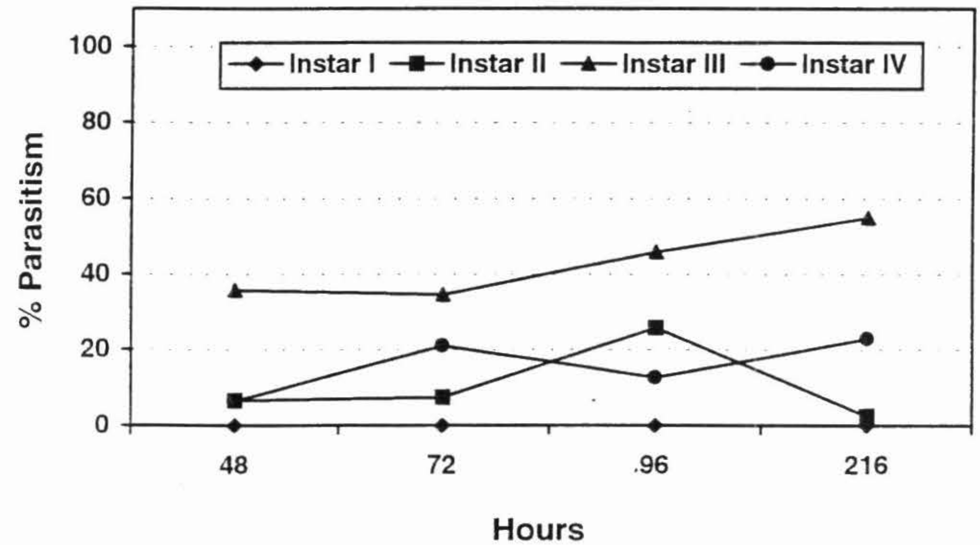

Figure 1.2.2.1. Parasitism of whitefly nymphs (*Aleurotrachelus socialis*) by *Encarsia hispida* 48 to 216 hours after exposure.

Activity 1.2.3. Tritrophic relationships: Determine effect of HPR on whitefly parasitism.

It has been observed that farmers will use pesticides to control high populations of cassava whiteflies. Pesticide applications will reduce the effectiveness of biological control as well as cause environmental contamination. Several years of research at CIAT has identified cultivars with varying levels of resistance to whiteflies, especially the species *A. socialis* (see Previous Annual Reports for Project IP3).

Present research is investigating the compatibility between host plant resistance and biological control. The combination of these two methods could reduce pest populations below economic injury levels or extend the usefulness of host plant resistance. In addition it is important to know the effect plant resistance, especially antibiosis, might have on parasitoid populations.

The compatibility of the parasitoid, *Encarsia hispida* was evaluated on whitefly immatures feeding on whitefly resistance genotypes of cassava. The resistant varieties tested were MEcu 72, and CG489-4; the susceptible check was CMC-40. Parasite development time, fecundity and survival were measured.

Plants, one month of age, of the above mentioned varieties were infested with *A. socialis* eggs by exposing the leaf undersurface to ovipositing adults for 36 hours. After 15 days, when whitefly immatures were in the second instar, they were exposed to *E. hispida* parasites. *E. hispida* was obtained by collecting cassava leaves with parasitized whitefly pupae in the field at CIAT, placing the leaves in blackened plastic traps and capturing emerging parasites. The results at this point are preliminary.

Results to date indicate that the female longevity of *E. hispida* was similar on varieties CMC-40 and CG489-34 (experiments 1 and 2) with values of 17.8, 12.2 and 13.8 days respectively **(Figure 1.2.3.1).** *E. hispida* longevity on MEcu 72 was greatly reduced, only 7.7 days, indicating that this variety has factors or characteristics that affect the longevity of the parasite. These factors could be the number of tricomes or a chemical component that is interfering with parasitoid feeding **(Table 1.2.3.1).**

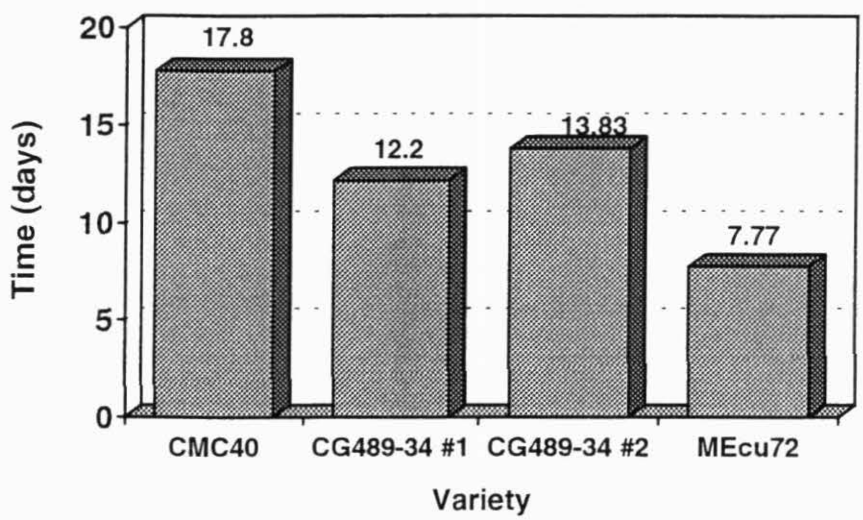

Figure 1.2.3.1. Longevity of the parasitoid *Encarsia hispida* on the whitefly *A. socialis* on 3 cassava varieties.

Table 1.2.3.1. Whitefly, (*Aleurothrachelus socialis*) survival, development time and fecundity on 4 cassava varieties.

Variety	Percentage Survival of Immatures	Development Time/Days	Number Eggs/Female	Tricomes/cm^2 on Second Leaf
MBra-12	75.0 A	32.18 C	107	4.653
CMC-40	66.0 AB	32.07 C	64	189
CG489-34	65.5 AB	33.13 B	46	15.290
MEcu-72	27.0 C	34.45 A	15	33.048

E. hispida emergence from *A. socialis* pupae was measured on the varieties CG489-34 and MEcu 72. Results show a very low rate of emergence of the parasitoid, 0.43 and 0.86 for CG489-34 and MEcu 72 respectively. These preliminary results indicate that host plant resistance in cassava may affect the development or emergence of parasitoids. Experiments using susceptible varieties (CMC-40) still need to be completed. Since *E. hispida* is the most frequently collected parasitoid collected in cassava fields a much higher emergence is expected.

The survival of *E. hispida* when associated with *A. socialis* on the cultivar MEcu 72 decreased rapidly, when compared to the other cultivars. One hundred percent mortality occurred within 19 days (**Figure 1.2.3.2**). Longest survival was on CMC-40 with individuals living for forty days. Variety CG 489-34 was intermediate; in both experiments with this cultivar individuals survived until 34 days. These data indicate that there may be varietal influence associated with *E. hispida* survival. Since MEcu 72 and CG 489-34 are resistant varieties, this resistance factor or mechanism may be affecting parasitoid survival.

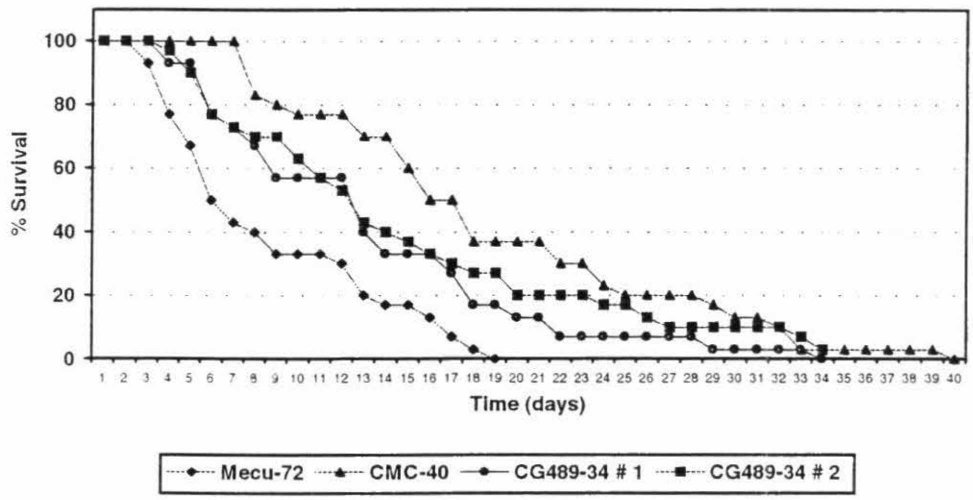

Figure 1.2.3.2. Survival of *Encarsia hispida*, parasitoid of *Aleurotrachelus socialis* on three varieties of cassava.

Activity 1.2.4. Feeding behavior of whiteflies on susceptible and resistant cassava clones.

Cassava genotypes with resistance to whiteflies have been identified at CIAT. Resistant clone MEcu 72 shows high mortality for both adult and immature whiteflies, which may suggest less feeding on this genotype under natural conditions. It is necessary to identify whitefly feeding behavior on susceptible genotypes and to make comparisons with MEcu 72 in order to better understand the mechanisms of resistance.

Electronic monitoring of insect feeding (EMIF) is a technique that permits the identification and quantification of the feeding behaviors of hemipteran insects. By passing an electrical signal to a test plant, and tethering an insect with a fine gold wire, modifications caused by stylet movements and feeding behaviors can be observed as waveforms. EMIF has extensively been used for the study of mechanisms of plant resistance to insects. CIAT presently owns two AC-

Electronic feeding monitors, and has access to a DC version of the system (EPG). The AC systems have been used with leafhoppers (*E. kraemeri*) on beans, and the DC systems with cassava mealybugs. Preliminary observations with both systems suggested that an easy protocol could be developed for monitoring the whitefly. In addition, CIAT technicians needed to be trained in wiring techniques, operation of the system, computer display and data acquisition.

Electronic Monitoring Methodology. The methodology devised for electronic monitoring of whitefly feeding behavior includes two major steps: a wiring technique to attach the thin wire to the mesonotum of an adult whitefly, and the proper settings (ground voltages, signal frequency) of the electronic monitoring system.

The "normal" gold wire used with leafhoppers (12.7 um) is too thick and stiff for the small whitefly adults. A thinner wire needed to be obtained. Since purchase of a thinner wire was very difficult in the short period of time allowed for this project, thinning of the existing gold wire was necessary. To do this a 10-20 cm piece of the thick wire is placed for 45 min in a solution of 3 mol Nitric acid and 9 mol Chlorhydric acid. This is a potent oxidizer that dissolves away part of the gold leaving a thinner wire while conserving its electricity conducting properties. Pieces of this thin gold wire are attached with silver paint to a copper stub.

Female whiteflies from a greenhouse colony are placed by mouth aspirator on a vacuum stand and held in place by a very gentle vacuum. Several individuals can be placed simultaneously. With the help of the copper stub, the thin gold wire is placed on the mesonotum of a female, and a very small drop of electrically conducting silver paint is used to attach the wire to the insect. Care needs to be taken for not getting silver paint on the whitefly's eyes or wings. This affects their behaviors even more than the tethered condition. New insects need to be used should silver paint get on their wings or eyes.

Tethered whiteflies can be acclimated to the wire by placing them on a cassava leaf for 1 h. Before the electronic monitoring session begins, whiteflies are starved for 30 min. The copper stub holding the tethered whitefly is attached to the alligator clip on one of the input electrodes of the electronic monitor.

A constant signal, 250 mV at 250 Hz was established as the best setting for *Aleurotrachelus socialis*. This signal is transmitted via an electrode inserted in the substrate of a potted plant (4-8 weeks old). Detached leaves placed on a container filled with water and sealed with the output electrode of the electronic monitor also worked and showed better conductivity than the potted plants. However, the effects of detached leaves vs. whole plants on whitefly feeding behavior needs to be evaluated further.

Feeding Behavior. During probing stylet movements and other feeding behaviors induce changes to the constant signal. These modifications, known as waveforms, are captured in a computer using an analog to digital converter board, displayed on the screen on a time scale, and stored for post-acquisition measurement and analysis. Once waveforms have been correlated with specific behaviors, quantification can be made on the frequency and duration of these behaviors.

Waveforms produced by probing *A. socialis* on CMC-40 are shown in **Figure 1.2.4.1**. Due to time limitations it is impossible to correlate waveforms with specific behaviors for this whitefly species. However, Walker (1998) reports that several species of whiteflies share very much stereotypical styles of feeding and that waveforms patterns are very similar among them. Since waveforms shown are similar in appearance to the waveforms reported in the literature, we will describe these. All probes observed started with salivation waveforms corresponding to the intercellular path that the stylets follow in their way to the phloem. These waveforms are shown in figs 1a and 1c as "intercellular stylet path". They have also been correlated with the deposition of a salivary sheath. In some instances the stylets come across treachery elements in the xylem and the whitefly ingests from them (Fig. 1c). This particular waveform has not been completely correlated with, but there is evidence of ingestion. After reaching the phloem, normally whiteflies go into continued ingestion, producing a flat waveform whose beginning is illustrated toward the end of the trace in Figs. 1b and 1c.

A new waveform that has not previously been identified or associated with any behavior is shown in fig. 1d. It would be expected that as more electronic monitoring of whitefly feeding more patterns would be identified and more correlational work would be needed. Now that a methodology exists for the study of whitefly feeding behavior at CIAT, comparisons can be made among resistant and susceptible genotypes.

Training. Two research assistants spent about 10 h per week training in electronic monitoring of insect feeding. They developed skills for whitefly wiring and for data display and acquisition. They also received extensive lecturing in the principles and applications of the technique, and in the basics of homopteran feeding behavior.

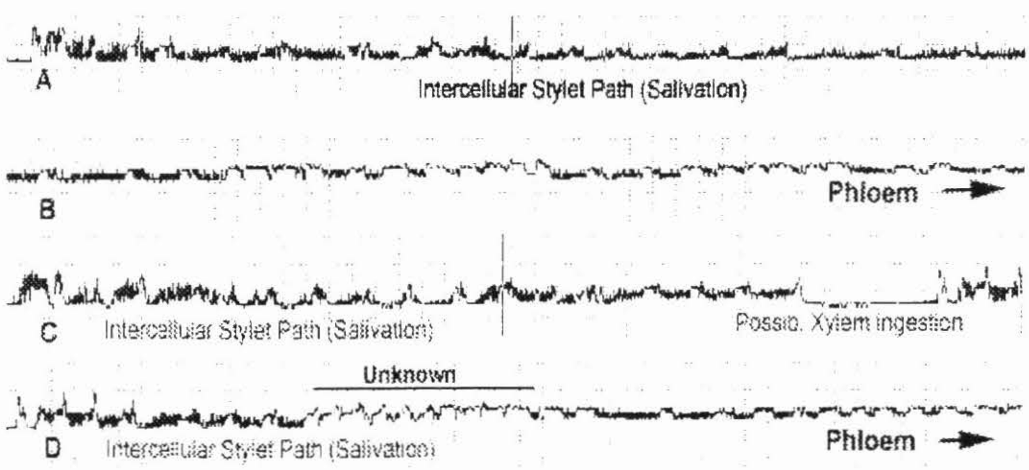

Figure 1.2.4.1. Waveforms produced by probing *Aleurotrachellus socialis* on Cassava cv. CMC-40. A. and B. Single probe (stylet penetration). B and C. Single probe forms a second whitefly. Note waveform identified as possible xylem ingestion and an unknown waveform that has yet to be correlated with a feeding behavior.

Sub-output 1.3. Biological Control of *Erinnyis ello*.

Activity 1.3.1. Pathogenicity of entomopathogenic fungi on the cassava hornworm, *Erinnyis ello*.

The cassava hornworm, *Erinnyis ello* (Lepidoptera: Sphingidae) is a serious pest of cassava in the Neotropics, with a broad geographic range extending from southern Brazil, Argentina and Paraguay to the Caribbean basin and the southern US. Sporatic attacks of the hornworm usually coincide with the rainy season and the mass migratory flight of *E. ello* account for the sudden "invasions" and subsequent increase in oviposition in cassava fields. Although a large complex of natural enemies exits, their effectiveness is greatly reduced because of the migratory behavior of the hornworm, adults. Effective control has been obtained through the use of granulosis virus (Baculoviridae), a technology that has been developed at CIAT.

In recent years outbreaks of hornworms have occurred in the Cauca Valley, including on the CIAT, Palmira station. During the course of 1997-1998 the opportunity presented itself to evaluate fungal pathogens, collected in cassava fields, on the cassava hornworm. Two students from the Universidad Nacional de Palmira, undertook this research as a Ing. Agr. thesis project.

The entomopathogens evaluated were of the genera *Beauveria* (isolates B.b. 9602 and 9601) and *Metarhizium* (isolate M.a. 9206 of *M. anisopliae*). Identifications were done by the Entomology Department of CENICAFE. Hornworm larvae were obtained from a small rearing colony at CIAT. Third instar larvae were inoculated using two methods, spraying on the larval surface and tarsal contact with a fungal infected leaf surface. Plasticified cardboard boxes were the experimental unit used to hold cassava leaves and hornworm larvae.

Preliminary experiments with the three aforementioned fungal isolates determined that isolates B.b. 9601 and M.a. 9206 were the most pathogenic, causing higher levels of mortality than B.b. 9602. Experiments were then carried out to evaluate these two isolates using the above described methodology. A third treatment where the fungal pathogen was sprayed on the larvae as well as the leaf surface was added. Ten third instar larvae were used in four repetitions fore each treatment.

Results show that both fungal pathogens isolates M.a. 9206 and B.b. 9601 can cause high levels of hornworm larval mortality (**Table 1.3.1.1**). In the first experiment M.a. 9206 caused 92.5% mortality when the fungus was applied to the leaf. Application of B.b. 9601 to the hornworm larvae or to the larvae/hoja also resulted in a 92.5% mortality. In the second experiment mortality caused by both pathogens was slightly low, 80% mortality of hornworm larvae (**Table 1.3.1.1**).

To determine the larval instar most to the isolate B.b. 9601, hornworm larvae of the second to fifth instar were inoculated with a concentration of 3.75×10^{10} conidia/ML^{-1} by spraying both larvae and leaf. The instar with the highest mortality (87.5%) was the third instar (**Figure 3.1.1**). Mortality of the 2^{nd} and 4^{th} instar was 57.5% and the least susceptible instar was the 5^{th} with only 27.5%. The difference were significantly different ($P < 0.05$; Duncan Multiple Range).

Table 1.3.1.1. Cassava hornworm (*E. ello*) mortality caused by two fungal pathogen isolates, M.a. 9206 and B.b. 9601 using three inoculation methods.

		Inoculation Method (Spraying)		
Experiment	**Isolate**	**Aspersion Larvae**	**Aspersion Leaf**	**Aspersion Larvae-Leaf**
	M.a. 9206	67.5	92.5	55
1	B.b. 9601	92.5	75	92.5
	Testigo	10	7	7.5
	M.a. 9206	80	45	67.5
2	B.b. 9601	72.5	57.5	80
	Testigo	2.5	0	5

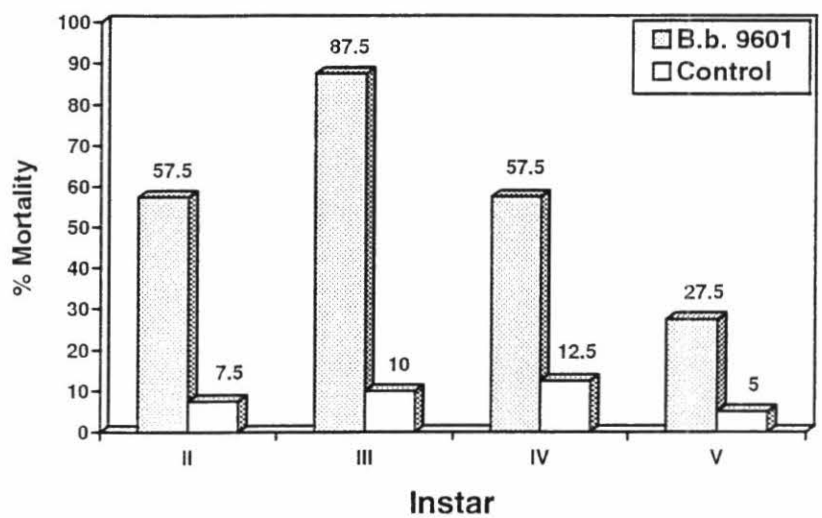

Figure 1.3.1.1. Mortality of *Erynnis ello* instars caused by the isolate B.b. 9601 of *Beauveria* sp.

As noted in the above experiment, larval mortality was lowest in the fifth instar (this is supported by similar data when pesticides or baculovirus are applied to fifth instars). An experiment was designed to determine the long range effect of the fungal isolate B.b. 9601 when applied to fifth instar. Application was the larvae/leaf method. In this trial, 40% of the 5th instar larvae died before population. The 60% that pupated also emerged as adults, showing no deformities, and oviposited normally. Egg viability was 97.8%. These results indicate that the fungal pathogen does not pass from the 5th instar to the pupal stage and has no effect on the behavior of the subsequent generation.

These results indicate that the fungal pathogen isolates evaluated have potential to be used in a hornworm pest management program but further research to evaluate these pathogens in the field is required.

Sub-output 1.4. Arthropod Complexes in Selected Agro-ecosystems.

Activity 1.4.1. Identification of pests associated with the asparagus in Cajibío, Cauca, Colombia.

Asparagus (*Asparagus officinalis*) is a member of the Liliacea, originally of the coastal zones of Europe and Asia. It is a perennial crop and will produce for 15 or more years once it becomes established. Although it is normally considered to be a crop from the Northern Hemisphere, in more recent years, production has been increasing in tropical regions, primarily for expert, due to the high market value of the crop.

World production is estimated at about 140,000 hectares; 40% of this is in Europe, 31% in North America, 14% in Asia, and the rest in South America and Africa. Peru is the largest producer in SA with more than 16,00 ha, followed by Chile and Argentina. It is estimated that about 95% of production in SA is exported to the USA and Europe. Export to the USA is almost 100% fresh product.

In recent years, Colombia has entered into this market. Most production takes place in the Departments of Cauca and Antioquia. The primary advantage to production in Colombia, especially in these Departments, is that asparagus harvest is a year-around activity. Asparagus production in these regions is, therefore, an all-year activity and being a labor intensive crop, employs a considerable amount of rural labor.

Asparagus, being a high value crop, and almost solely for export, requires considerable "quality control" in production process. Upon arrival (via air cargo) in ports of entry in the USA, it is subjected to a rigorous inspection for arthropod pests and/or insecticide residues. A rejection of the shipment, due to the aforementioned reasons, results in considerable loss in revenue.

CIAT, over the past few years, has been approached by asparagus producers to assists them in some of their arthropod problems. Since one of the asparagus pests, was also a pest of cassava (The Burrower Bug, *Cyrtomenus bergi*), we have occasionally responded to this request, and suggesting that a more permanent and formed arrangement be made. Around mid 1998 we entered into an agreement with "Compañía Agrícola de Espárragos, CAESA, to provide technical assistance. The first phase of this project is for 6 months and provides funding for personnel and operating expenses. It now appears that two additional asparagus companies will also enter into this agreement.

The objective of the initial phase is to determine what are the principal entomological problems affecting asparagus production and to identify which arthropods could be present on produce when entering into export markets. Once this is determined a second phase to the project will be negotiated.

Systematic surveys on three asparagus farms (totaling about 300 ha) have already been initiated and arthropod species are being collected, processed and sent for identification (USDA and British Museum). Insects from 8 different Orders have been collected, with the majority belonging to the Order, Hemiptera (….. bugs). In addition a characterization of the cropping and harvesting cycles of each plantation is being carried out.

This project officially started in August 1998, and only preliminary information is available at this time.

Activity 1.4.2. Determine white grub biodiversity in selected agro-ecosystems.

White grubs are a subterranean pest of cassava, beans, tropical pastures and numerous other crops in temperate and tropical agriculture. They are considered by some agricultural researchers, to be globally the second most important agricultural pest, after whiteflies. As part of the CGIAR, Systemwide IPM Program, the IPM of white grubs and other soil borne arthropods is now a systemwide project and a Task Force will be convened in early 1999. CIAT is the lead center for this project.

During 1998, the CIAT IPM project has been collaborating with local NGO's to identify existing white grub problems in the Cauca Department. The objective of this initial research is to collect and identify, at least to genus the principal root feeding white grubs in the region. This is a collaborative effort between CIAT, FIDAR (Fundación para la Investigación y Desarrollo Agrícola, a NGO) (Luis Carlos Pardo Locarno) and the Universidad Nacional, Jorge Andrés Victoria Taborda. CIAT's major contribution in this project, at this time, is to provide laboratory and greenhouse facilities and logistic and intellectual support.

Systematic collecting of white grub specimens is being done in 3 municipalities of North Cauca, Caldono, Buenos Aires and Santander de Quilichao. Collecting is being done at night using light traps that are place din all 3 zones. Collecting during the day is often done by excavating 30 x 30 x 30cm areas in the soil rhizosphere in fields where crop plants show white grub damage symptoms. Biological control agents that might also appear in the samples are also collected, isolated and identified.

White grub larvae that are collected in the field is brought into the laboratory or greenhouse, placed in plastic boxes and reared to adult for identification. Only preliminary results are available at this time.

Specimens collected at this time belong primarily to 3 genera although about 15 different genera have been identified. The three principal genera are *Phyllophaga*, *Ciclocephala* and *Anomala*.

Sub-output 1.5. Acarology: Biological Control of Cassava Green Mites in Africa and Latin America.

The cassava green mite (CGM), *Mononychellus tanajoa*, is probably native to NE Brazil, where it was first reported in 1938. The natives had long known the damage symptoms, which provided the name "tanajoa." It attacks young leaves and meristems of cassava in the neotropics but it is normally a serious problem only in regions where there is a prolonged dry period (3 to 6 months). The mite first appeared in Africa (Uganda) in 1971 and by 1985 it had spread across most of the African cassava belt, occurring in 27 countries. Today it is one of the principal pests of cassava in Africa and in certain areas of the neotropics, causing estimated root yield losses up to 80%.

The principal objective of present research is to identify natural enemies of CGM, evaluate their potential as biological control agents, and send them to EMBRAPA in Brazil and IITA in Benin, Africa for release. Research to date has concentrated primarily on mite predators of the family phytoseiidae.

Activity 1.5.1. Inventory of phytoseiidae reference collection presently held at CIAT.

Arthropod collections are important for storing reference materials that are needed to verify species identification. This is most applicable for ecological research, especially in biological control programs where it is common to find similar or the same species at different sites at different times.

The present predator mite reference collection being held at CIAT was initiated 12 years ago and conserves, primarily those mite predators related to phytophagous mites found on cassava. Collections were made mostly in Latin American and Caribbean countries. Collection zones were usually chosen because of their similitude to ecological homologues in Africa and Brazil. Eighty seven species have been collected and stored; 25 of these are new or unrecorded species. Seventy six percent of the samples were taken from cassava.

During the past years this collection was systematized and inventory of existing slides was compared to the information available in the database. This will allow for a more rapid access for materials which are often requested for collaborators.

The collection contains nearly 10,000 microscope slides with specimens. Slides are organized by genus, species and sample number. There are 2362 samples in the data base and 1874 registrations of species collected. The majority of the samples were made on cassava as expected, although specimens from several other crops were collected (**Figure 1.51.1**).

Explorations were made in 24 countries (**Figure 1.5.1.2**): sampling was done primarily in Colombia, Ecuador, Venezuela, Cuba, and Brazil. Extensive exploration and collecting was done in Brazil by our Brazilian colleagues as part of the UNDP sponsored PROFISMA/ESCaPP, Cassava IPM roject. A phytoseiid collection and data base is held in Brazil.

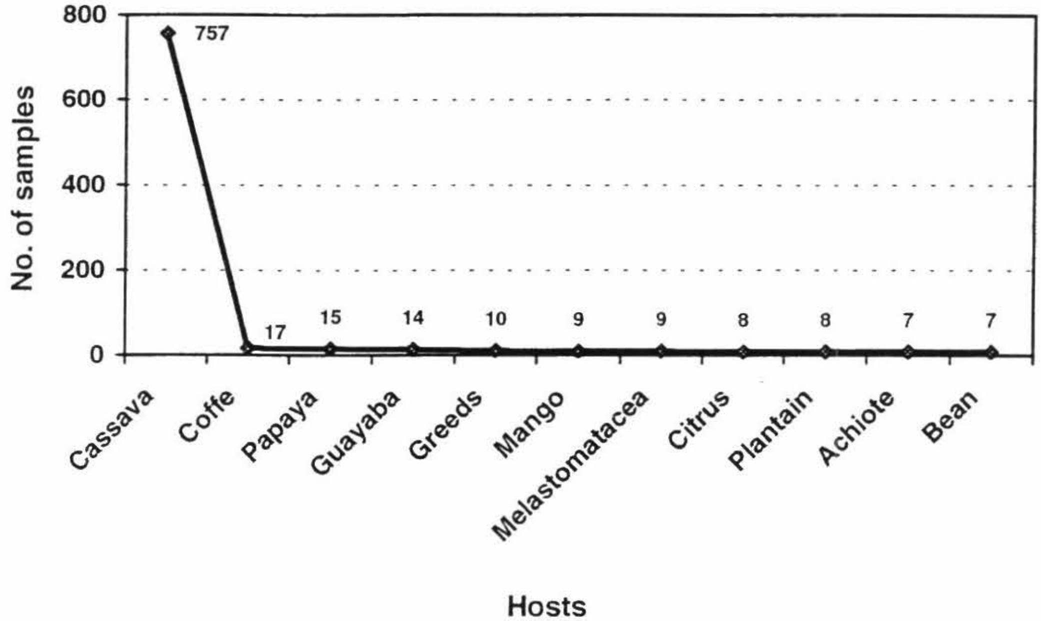

Figure 1.5.1.1. Host sampling of Phytoseiidae (predatory mites) in CIAT Reference Collection (1998).

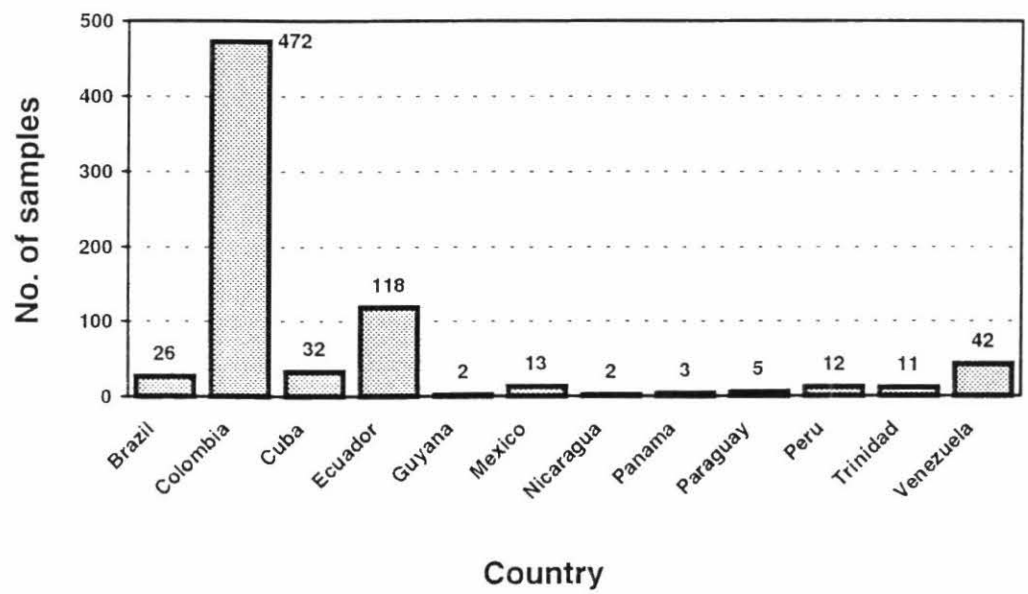

Figure 1.5.1.2. Samples of Phytoseiidae collected from cassava in the CIAT Reference Collection (1998).

Although 87 species of Phytoseiidae have been collected, 13 species are frequently collected **(Figure 1.5.1.3)**. *Typhlodromalus manihoti* is the species most frequently recorded, followed by *Neoseiulus idaeus* and *T. aripo*. The latter two species have been most important and successful for the control of *M. tanajoa* in Africa.

This collection is now organized into a true reference collection and can be readily used for species description or redescription, where types and paratypes may be found and species comparisons can be done.

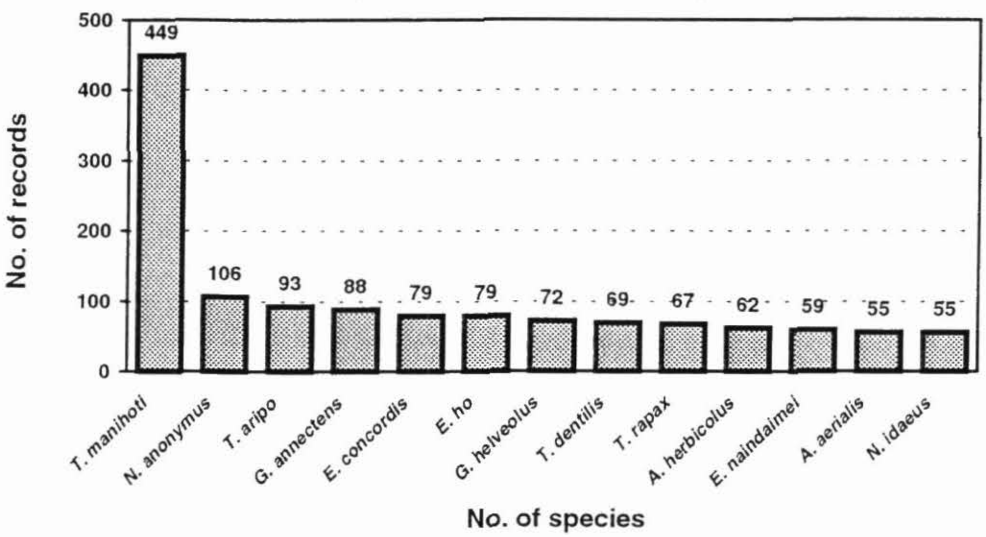

Figure 1.5.1.3. Phytoseiidae species frequency in CIAT Reference Collection (1998).

Activity 1.5.2. Development of a taxonomic key for phytoseiidae found on Cassava.

Taxonomy is often the starting point in most all basic and applied arthropod-plant research. This is especially true in continuing biological control programs where the correct identification of the pest and its natural enemies is usually key to success. Errors in identification often imply lost time and money. The misidentification of the mealybug attacking cassava in Africa (4 year delay) and the mealybug attacking coffee in Kenya (12 years delay) are examples.

The phytoseiid mite most widely distributed in Latin America on cassava was identified as *Typhlodromalus limonicus*; the same species was originally described in California (USA) on other crops and feeding on a range of phytophagous mite species. The fact that it was not found on the same crops in Latin America led to a more detailed research, which eventually resulted in this being identified as a separate species, *T. manihoti*.

Based on these types of problems it was concluded that there is a need to develop a taxonomic key to identify those species of Phytoseiidae found on cassava. It was decided that this key should be comprised of those species most frequently found in Colombia, Ecuador and Venezuela. The key contains morphological description; central and dorsal characteristics, and important structures for identification. Drawings will accompany each species. This work is being done in collaboration with Dr. Gilberto J. de Moraes, a world authority Phytoseiidae taxonomist from the University of Piracicaba in Sao Paulo, Brazil, who has collaborated with CIAT and IITA for more than 10 years on cassava mite biological control projects.

The classification system being used is dividing the Order Parasitiformes, which contains the Family Phytoseiidae, into 2 subfamilies: Amblyseiinae and Phytoseiinae, and including 11 general and the 28 most important species from the region (**Table 1.5.2.1**).

The publication of this key is of utmost importance for our continued research in biological control and demonstrates our interest in taxonomic studies of Phytoseiidae. It is hoped that in the future other crops besides cassava will also be included.

Table 1.5.2.1. Taxonomic classification of most important phytoseiidae species found on cassava and distributed in Colombia, Venezuela and Ecuador.

Order	Subfamily	Genus	Species
Parasitiformes	*Amblyseiinae*	*Proprioseiopsis*	*mexicanus*
			neotropicus
			cannaensis
		Neoseiulus	*neotunus*
			californicus
			idaeus
			anonymus
		Euseius	*alatus*
			concordis
			ho
		Amblyseius	*largoensis*
			herbicolus
			aerialis
			chiapensis
		Typhlodromalus	*tenuiscutus*
			rapax
			limonicus
			manihoti
			peregrinus
		Typhlodromips	*dentilis*
			mangleae
			bellotti
		Phytoseiulus	*macropilis*
		Iphiseiodis	*zuluagai*
	Phytoseiinae	*Galendromus*	*annectens*
			helveolus
		Phytoseius	*purseglovei*
		Clavidromus	*transvaalensis*

Activity 1.5.3. Preliminary studies of crosses between populations of the phytoseiid predator *Typhlodromalus manihoti*.

T. manihoti is the predominant species of Phytoseiidae found in most cassava growing regions of South America. Of the 1261 cassava fields surveyed over a several year period, *T. manihoti* was found in 50% of these (see CIAT Cassava Program Annual Report 1989 and 1991). The species is distributed from Mexico to Paraguay and in Colombia at temperatures from 15 to 35°C. Given this wide range of adaptation, it was determined to do hybridization studies to confirm that this is one species and to determine the presence of different races. Often these races, varieties or subspecies cannot be distinguished morphologically, but biologically they are distinct. Therefore within a species you can have populations that are adapted to different host species or different habitats. These populations may be able to cross with others and often it is difficult to determine their exact taxonomic position. It is important to know that these populations exist and from the point of biological control, these populations can be as important as distinct species. Unpredictable effects of climate or other ecological factors in the "new home" or place of introduction of exotic natural enemies, can often counteract some characteristics that manage the efficiency of the species in its new habitat.

Crosses were made by taking females from the *T. manihoti* colony maintained in the laboratory at CIAT (25°C and 70±RH). Populations collected in Cruz das Almas, Brazil and two populations collected in Colombia (Cauca-Cajibío and Guajira-Villanueva) were crossed with the laboratory colony. Molecular studies were done on these stocks by Dr. M. Hoy of the University of Florida; she found molecular differences that allowed her to group the populations in the following manner: One from Brazil (Cruz das Almas), three from Colombia (Cauca-Cajibío; Antioquia-Copacabana; Guajira-Villanueva). The colony from Brazil was different from the other three populations and the one from the Guajira was different from all the others. The crossing scheme can be seen in **Table 1.5.3.1**. An ANOVA analysis of variance was used for total oviposition data.

Results show that crosses made between the populations from Guajira and Cruz das Almas always resulted in both males and fertile females. The average time for preovipositon was one day. This was different for all the crosses, being greatest in the homogamic cross of Guajira and least for the backcross of the F1 female from Brazil x Guajira when crossed with males from Brazil.

There were no significant differences for total oviposition with any of the crosses, not even the backcrosses. There was an average of 25 eggs during 10 days of ovipositon (**Table 1.5.3.2**). For crosses between populations from Cruz das Almas and Cajibío the average preoviposition time was 1.6 days (**Table 1.5.3.3**). The homogamic crosses (controls) had the shortest preoviposition time; the greatest preoviposition time was with the backcross of Female progeny (CxB) x Male/Brazil, the shortest was Female progeny (CxB) x Male/Cajibío. Total oviposition resulted in an average of 16 eggs per cross, fewer than in the Cruz das Almas (Brazil) by Guajira cross, and more variability. Many females did not oviposit although they were observed copulating with males. The controls and backcrosses had the highest oviposition while the heterogamic crosses had the fewest (**Table 1.5.3.3**).

Table 1.5.3.1. Crossing scheme of populations of *T. manihoti*.

Populations	Crosses
Cruz das Almas (B) x Guajira Gu)	B x B
	Gu x Gu
	*f (B) x m (Gu)
	f (Gu) x m (Cd)
	Female offspring (BxGu) x m B
	Female offspring (BxGu) x m Gu
	Female offspring x Male offspring (B x Gu)
	Female offspring (Gu x B) x m B
	Female offspring (Gu x B) x m Gu
	Female offspring x Male offspring (Gu x B)
Cruz das Almas (B) x Cajibío Cj	B x B
	Cj x Cj
	*f (B) x m (Cj)
	f (Cj) x m (B)
	Female offspring (BxCj) x m B
	Female offspring (BxCj) x m Cj
	Female offspring x Male offspring (BxCj)
	Female offspring (CjxB) x m B
	Female offspring (CjxB) x m B
	Female offspring x Male offspring (CjxB)

*f = female, m = male

(Crosses between the populations from Guajira and Cajibío still need to be done)

The highest percentage of non-egg hatch was 16% for the progeny of Guajira x Brazil were crossed (Males x Females) (**Table 1.5.3.2**) followed by the females of the same cross, backcrossed with the Guajira male (15.2% non hatch). The Guajira x Brazil cross had the lowest hatch (only 1%). This same cross also had the lowest mortality at only 2.1%. In general egg hatch was higher in the population crosses of Brazil x Guajira than it was for the Brazil x Cajibío crosses (**Table 1.5.3.2** and **1.5.3.3**).

Progeny mortality was considerably higher for the B x G crosses, especially for the backcrosses, than it was for the B x C crosses **(Tables 1.5.3.2 and 1.5.3.3).**

Normal sex ratio is 70:30 (females/males): in the majority of the crosses this ratio did not vary too much. However in the control from Brazil and the Brazil x Guajira the proportion of females was only 0.4, much lower than normal **(Tables 1.5.3.2 and 1.5.3.3).**

Table 1.5.3.2. Effect of population crosses of *T. manihoti* (Cruz das Almas, Brazil, (B) and Guajira, Colombia (G) on fecundity and progeny development.

Cross: Female	Cross: Male	Non Eclosion Egg Hatch: n	Non Eclosion Egg Hatch: %	Mortality %	Preoviposition Time (Days)		Total Oviposition (10 Days)		•Proportion of Females
Control Cruz das Almas		109	6.4	17.4	0.8	CD*	22.4	A*	0.4
Control Guajira		97	5.2	7.2	2.0	A	20.0	A	0.7
Brazil x Guajira		105	5.7	9.5	1.3	ABC	25.0	A	0.4
Guajira x Brazil		96	1.0	2.1	1.7	AB	23.1	A	0.7
Female offspring (BxG) x Male Brazil		94	13.8	18.1	0.4	D	26.0	A	0.6
Female offspring (BxG) x Male Guajira		104	4.8	13.5	0.9	CD	27.0	A	0.7
Offspring/Female x Male (BxG)		96	10.4	16.7	0.5	D	28.1	A	0.7
Female offspring (GxB) x Male Guajira		105	15.2	25.7	0.4	D	26.3	A	0.8
Female offspring (GxB) x Male Brazil		102	8.8	16.7	1.1	CD	27.0	A	0.8
Offspring/Female x Male (GXB)		94	16.0	24.5	0.7	CD	23.0	A	0.7

* Averages followed by the same letter are not significantly different (Ryan Welsh Multi Range Test = 0.05).

• Sex relationship calculated on individuals often completing development of egg to adult (Female/Female + Male).

Table 1.5.3.3. Effect of population crosses *Typhlodromalus manihoti* (Cruz das Almas, Brazil (B) x Cajibío, Colombia (C)) on fecundity and progeny development.

Cross		Non Eclosion Egg Hatch		Mortality	Preoviposition Time (Days)		Total Ovipositon (10 Days)		• Proportion of Females
Female	Male	n	%	%					
Control Cruz das Almas		92	1.1	3.3	0.5	CD*	21.3	A*	0.6
Control Cajibío		90	3.3	4.4	0.6	CD	26.2	A	0.7
Brazil x Cajibío		88	2.3	6.8	4.3	BC	1.8	C	0.6
Cajibío x Brazil		51	9.8	9.8	6.1	AB	0.9	C	0.6
Female offspring (BxC) x Male Brazil		85	2.4	5.9	1.5	CD	19.6	AB	0.6
Female offspring (BxC) x Male Cajibío		90	3.3	7.8	1.7	CD	10.9	B	0.7
Offspring Female x Male (BxC)		96	3.1	7.3	0.0	D	19.6	AB	0.7
Female offspring (CxB) x Male Cajibío		101	1.0	4.0	0.2	D	21.5	A	0.7
Female offspring (CxB) x Male Brazil		65	7.7	7.7	8.2	A	17.0	AB	0.8
Offspring/Female x Male (CxB)		90	0.0	5.6	0.8	CD	22.8	A	0.8

* Average followed by the same letter are not significantly different (Ryan Welsch Multi Range Test = 0.05).

• Sex relationship calculated on individuals after completing development of egg to adult (Female/Female x Male).

Development time varied significantly among the different crosses. The longest development time was for the Guajira control and the shortest for the backcrosses of Brazil females x Guajira males (**Figure 1.5.3.1**). Differences in development time were less in the Brazil x Cajibío crosses than the Brazil x Guajira (**Figures 1.5.3.1** and **1.5.3.2**).

Future studies will involve crosses from populations from Cajibío x Guajira as well as other populations suggested by M. Hoy at the University of Florida. Results at this point, indicate that some reproductive isolation exists between these populations but this is not yet defined.

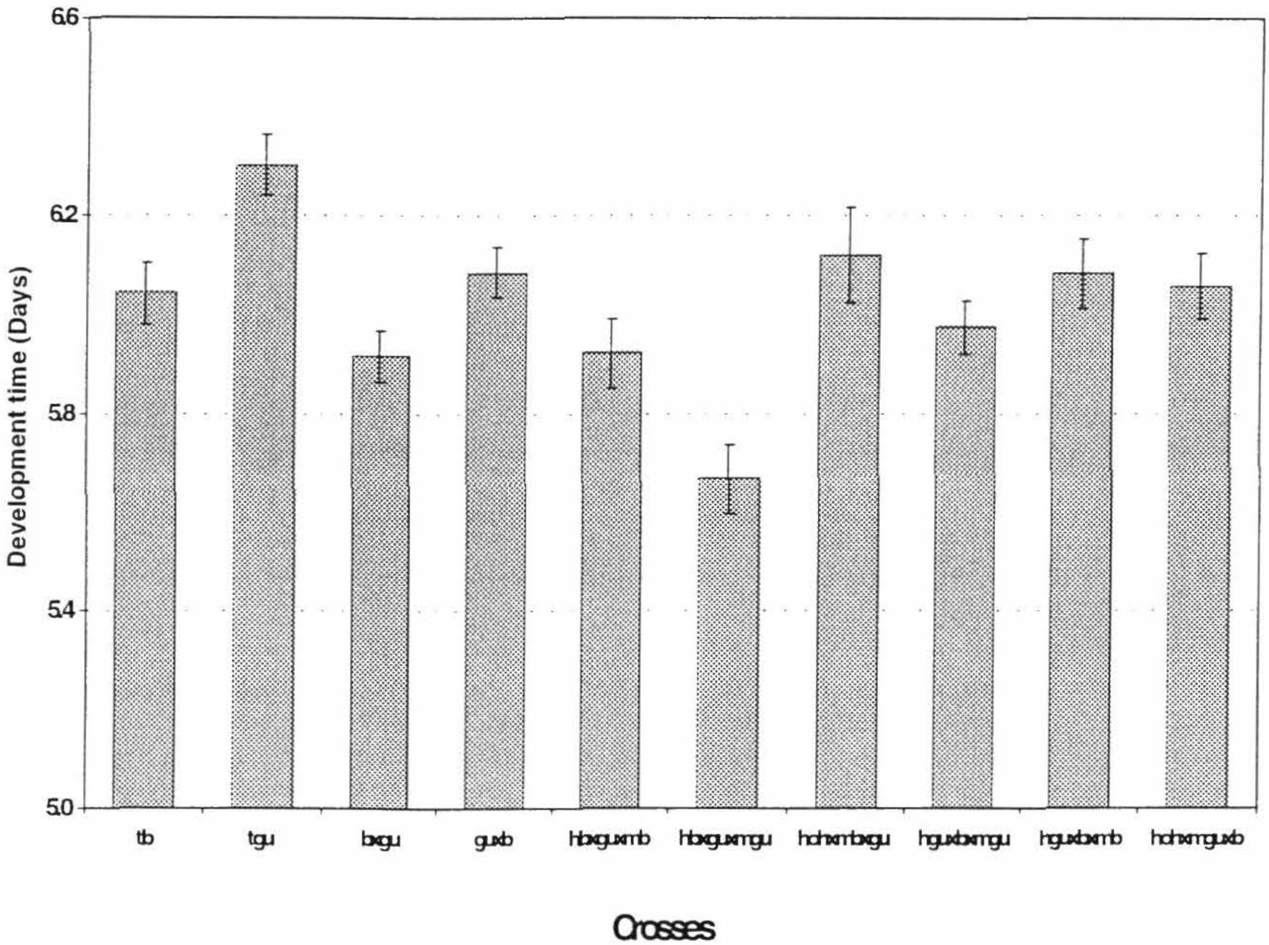

Figure 1.5.3.1. Development time for populations crosses of *Typhlodromalus manihoti* (Cruz das Almas Brazil (b) x Guajira (gu)) - t=control, h=female, m=male, ho=offspring.

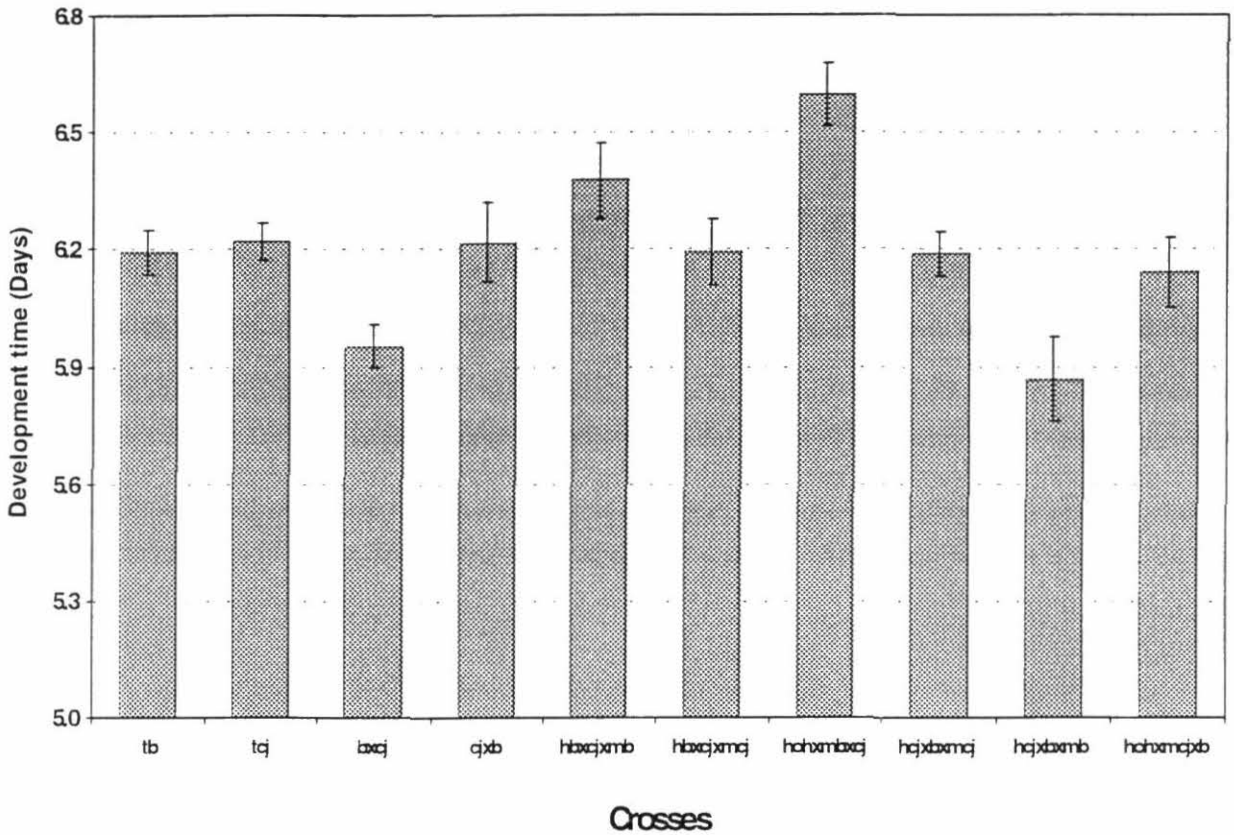

Figure 1.5.3.2. **Development time for populations crosses of *Typhlodromalus manihoti* (Cruz das Almas Brazil (b) x Cajibío (cj)) - t=control, h=female, m=male, ho=offspring.**

Activity 1.5.4. The effect of alternative food sources on the fecundity and longevity of three phytoseiid populations

The phytoseiid species *Typhlodromalus aripo,* collected in Brazil, and introduced into Africa, has been successful in reducing CGM populations in 14 African countries. This species prefers the growing tips of cassava plants where it can usually find populations of the CGM. During specific times of the year, especially during the wet season, CGM populations will decrease considerably. This suggests that the predator is capable of finding alternative food sources during periods of low CGM populations.

The objective of this study was to determine the effect of alternative food sources on the fecundity and longevity of two populations of *T. aripo* (one from Cruz das Almas, Bahia, Brazil, and the other from Pivijay, Colombia) and the species *Neoseiulus anonymus* from Armenia, Colombia. *N. anonymus* was selected for this study because it occupies a very different niche, principally the lower leaves of the cassava plant.

The food sources offered were of three types; cassava phytophagous mite species (*M. caribbeanae*, and *Tetranychus urticae*), insect species feeding on cassava (1st and 2nd instar nymphs of the whitefly *Aleurotrachellus socialis*, the mealybug *P. herreni*, and the thrips *Scirtothrips manihoti*), and food of vegetable origin (fresh pollen of *Ricinus communis*, and exudate from cassava leaves).

Virgin females and a male of each phytoseiid species were place individually in an experimental arena, which contained one of the aforementioned food source. Every 48 hours the predator species were transferred to a new arena and the number of eggs oviposited was noted daily. Female death was recorded to determine longevity. There were 50 repetitions of each food source for each predator species.

Fecundity: Results show that the predator populations would oviposit when feeding on phytophagous mites, thrips, whitefly and pollen, and did not produce eggs when feeding on mealybug nor cassava exudate (Fig.). *T. aripo*/Cruz das Almas oviposited when feeding on all five aforementioned food sources. *T. aripo*/Pivijay oviposited when feeding on *M. caribbeanae*, thrips, whitefly and pollen, but did not oviposit when fed *T. urticae*. *N. anonymus* oviposited only when fed *T. urticae* and *M. caribbeanae*, but on no other food source.

T. aripo (both populations) had highest oviposition when feeding on *M. caribbeanae* and thrips **(Figure 1.5.4.1)** and very low oviposition when feeding on whiteflies and pollen. *T. aripo*/Pivijay had difficulty, feeding on *T. urticae* because of the webbing characteristic of this species. *T. aripo*/Cruz das Almas was able to feed on *T. urticae;* it is known that *M. tanajoa* in Brazil can produce webbing. Therefore the *T. aripo* from Cruz das Almas may be more adapted to the webbing and able to feed on *T. urticae*. Although *N. anonymus* only oviposited when feeding on the mite species, oviposition was high and especially superior when feeding on *T. urticae*. *T. urticae* is most often found on the lower leaves of cassava, where *N. anonymus* also predominates.

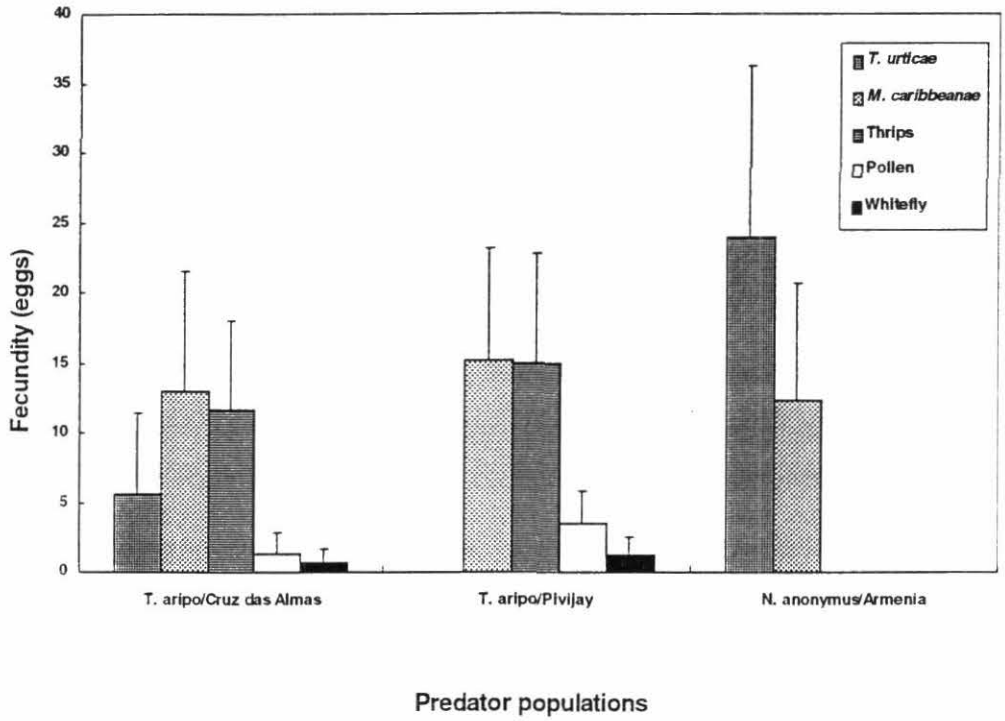

Figure 1.5.4.1. The effect of different food sources on the longevity of three populations of Phytoseiidae.

Longevity: Females from all three phytoseiid populations were able to survive on the food sources offered (results from the experiment on thrips on not yet complete) (**Figure 1.5.4.2**). Longevity of *N. anonymus* was greatest when it fed on *T. urticae* and comparatively lower on other food sources. *T. aripo*/Cruz das Almas showed good survival on all food sources while *T. aripo*/Pivijay survived well on all food sources except *T. urticae*.

These results indicate although not all food sources are good protein sources for oviposition, they are important for survival when populations of *M. tanajoa* are low.

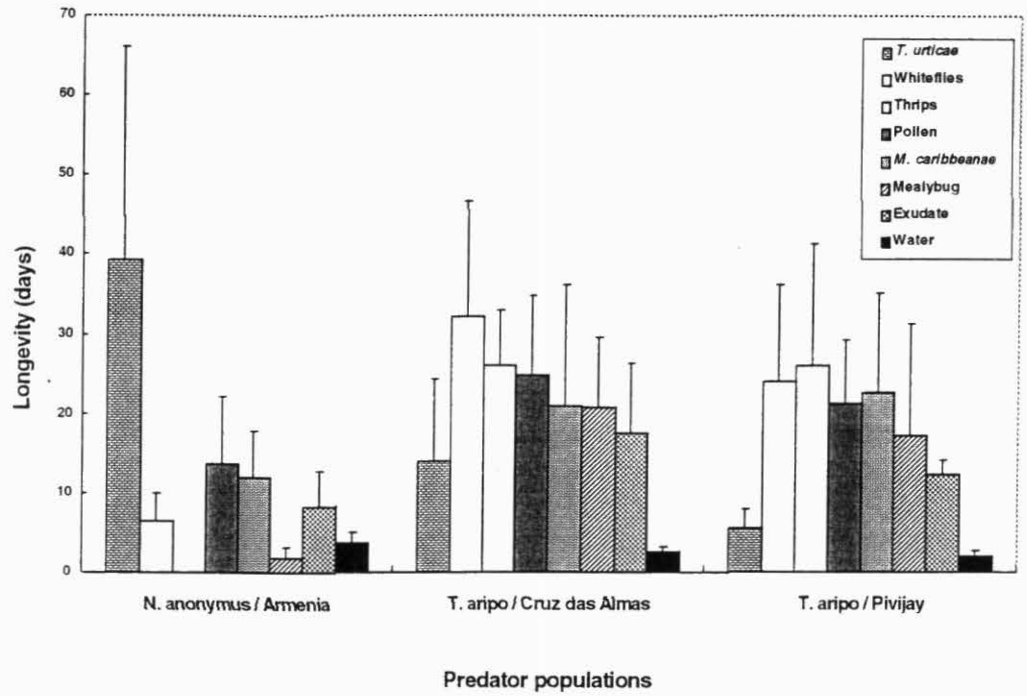

Figure 1.5.4.2. The effect of different food sources on the fecundity of three populations of Phytoseiidae.

Activity 1.5.5. The effect of *Mononychellus tanajoa* egg densities on oviposition efficiency of six species of phytoseiidae.

Ovipositon of female phytoseiids is influenced by the quantity of food available during the reproductive stage. Whereas tetranichids produce large numbers of smaller eggs, phytoseiids invest more biomass in producing larger eggs. A phytoseiid female, in one day, can produce a biomass of eggs equal to her own biomass. However we also know that greater consumption of prey is not directly related to increased ovipositon; there is a buffer related to transformation efficiency.

The objectives of this study were: 1) to quantify predator consumption at different egg densities, and 2) determine the effect of egg consumption on predator ovipositon.

The six predator species evaluated in this study were *Euseius ho* (Ecuador), *Galendromus annectens* (Ecuador), *Typhlodromalus tenuiscutus* (Ecuador), *Neoseiulus californicus* (Ecuador), *Typhlodromalus manihoti* (Colombia), and *Neoseiulus idaeus* (Colombia). Egg densities offered were 105 and 30 (based on previous studies: See Annual Report 1995), and additional densities of 15, 7, 3 and 1. Females were placed on clean cassava leaf lobes, which were placed in McMurtry trays. Each lobe was bordered with wet kleenex so that each female ovipositon was concentrated in a given area. Females in their early reproductive period (2 to 3 days) were used as they are more voracious during this period. Evaluations were made every 24 hours during a four day period, registering the number of eggs consumed and the number oviposited. The first days data was registered but not included in the analysis as oviposition was influenced by the previous days feeding. There were 15 repetitions per treatment.

Results show that the *M. tanajoa* egg consumption by all six phytoseiid species increased as egg density increased **(Figure 1.5.5.1)**. At egg densities of 1 to 30 consumption by the six phytoseiid species was nearly equal. However at the maximum density, 105 eggs, there was considerable variability in consumption according to phytoseiid species. *T. manihoti* can consume nearly all eggs offered (S.D. ± 25.6) while *G. annectens* consumed the lowest average but is also capable of high consumption (S.D. ± 46.6).

Euseuis ho, as other species in this genus, is a generalist, and whether feeding on *M. tanajoa* or pollen, has an adequate reproductive capacity (Annual Report 1997). That *E. ho* was second only to *T. manihoti* in consumption (at 105 egg density) is unexpected as both *T. tenuiscutus* and *N. idaeus* are considered very voracious species.

Figures 1.5.5.2 and **1.5.5.3** show the relationship between the number of eggs consumed and daily ovipositon by the phytoseiid species. As can be seen ovipositon increases as the number of eggs consumed increases. It is also possible that this study should be repeated using a higher egg density for the species.

For the species *N. idaeus*, *N. californicus* and *G. annecteus* less than 40 *M. tanajoa* eggs are require to get maximum oviposition **(Figure 1.5.5.3)**. Higher consumption does not necessarily result in higher overposition. They consume less eggs (*N. idaeus* = 33.1, *N. californicus* = 26.5, and *G. annectens* = 45.4), yet oviposit the same or greater number of eggs. These results indicate that *N. idaeus*, *N. californicus* and *G. annecteus*, have a greater efficiency in transforming prey into progeny.

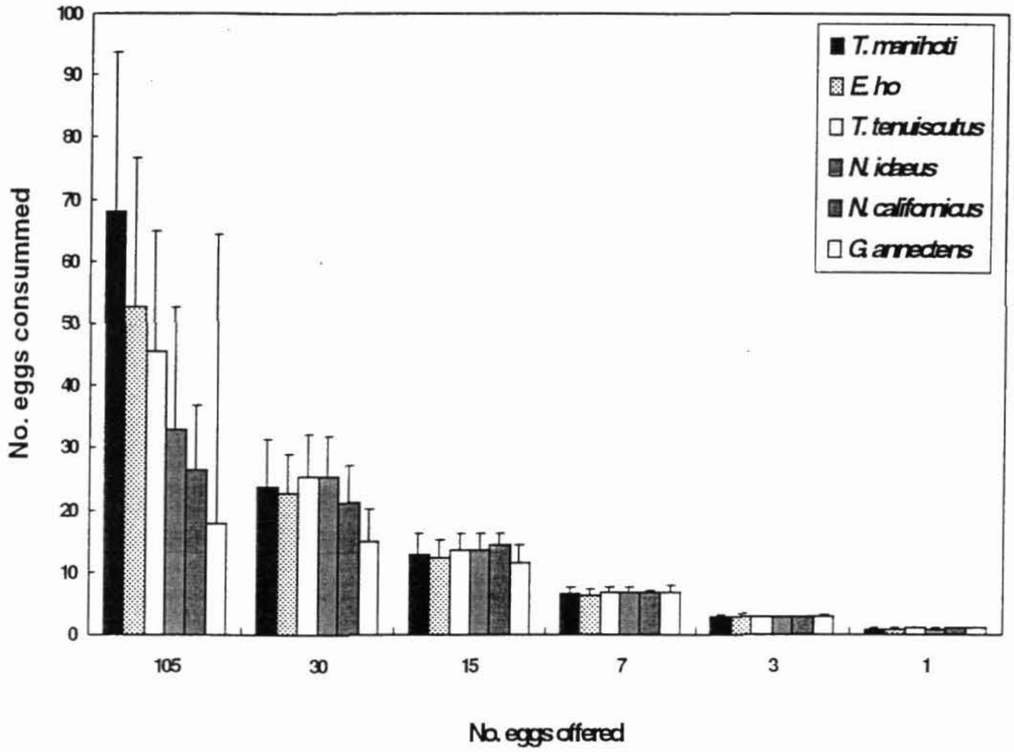

Figure 1.5.5.1. **Average cosumpiton of *Mononychellus tanajoa* by six phytoseiid species in three days in the laboratory.**

Activity 1.5.6. The effect of different levels of relative humidity on survival of phytoseiidae populations

Abiotic factors, such as climate variations, can have an affect on predator establishment. Relative humidity is one of the factors that can most influence phytoseiid survival. Phytoseiid eggs are the most susceptible stage to low humidities, probably because they are immobile and not able to escape to more favorable sites. The majority of phytoseiid species display very low egg hatch below 60 to 70% RH. Tetranichids, on the other hand, increase their reproductive rates in low humidity and high temperature conditions.

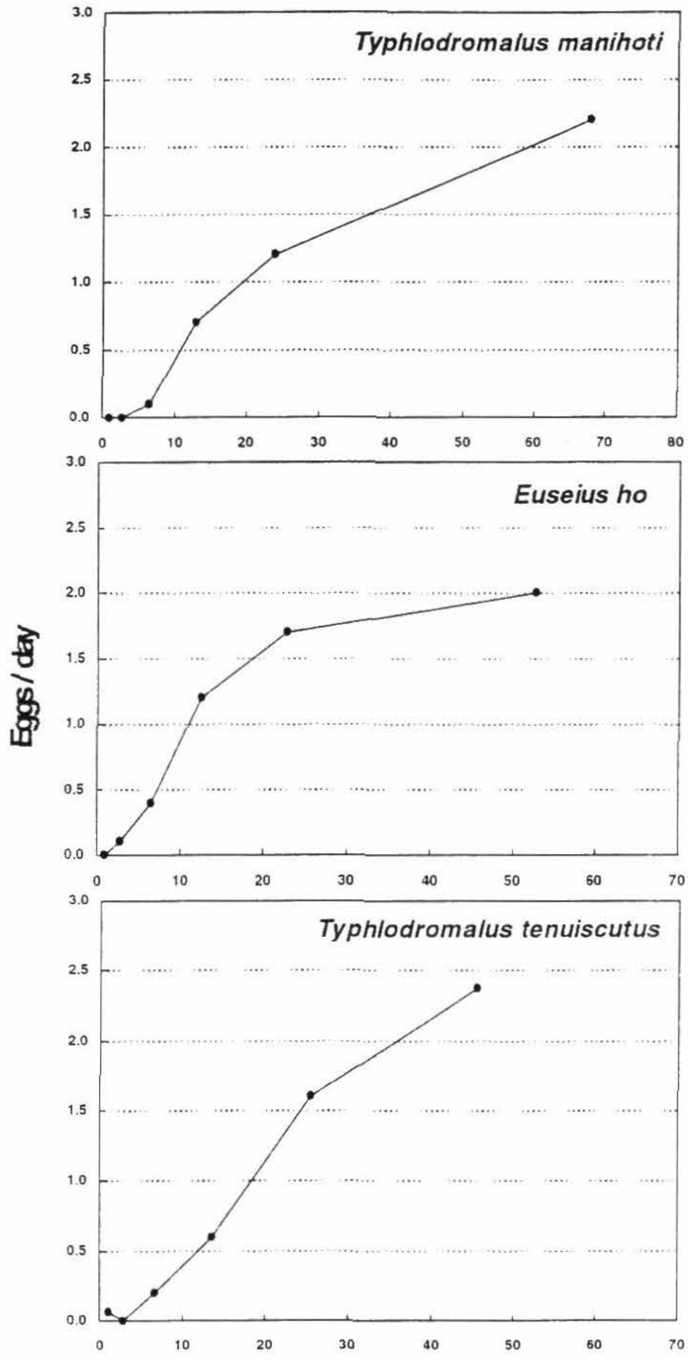

Figure 1.5.5.2. The effect of *M. tanajoa* egg consumption on oviposition of three phytoseiid predator species.

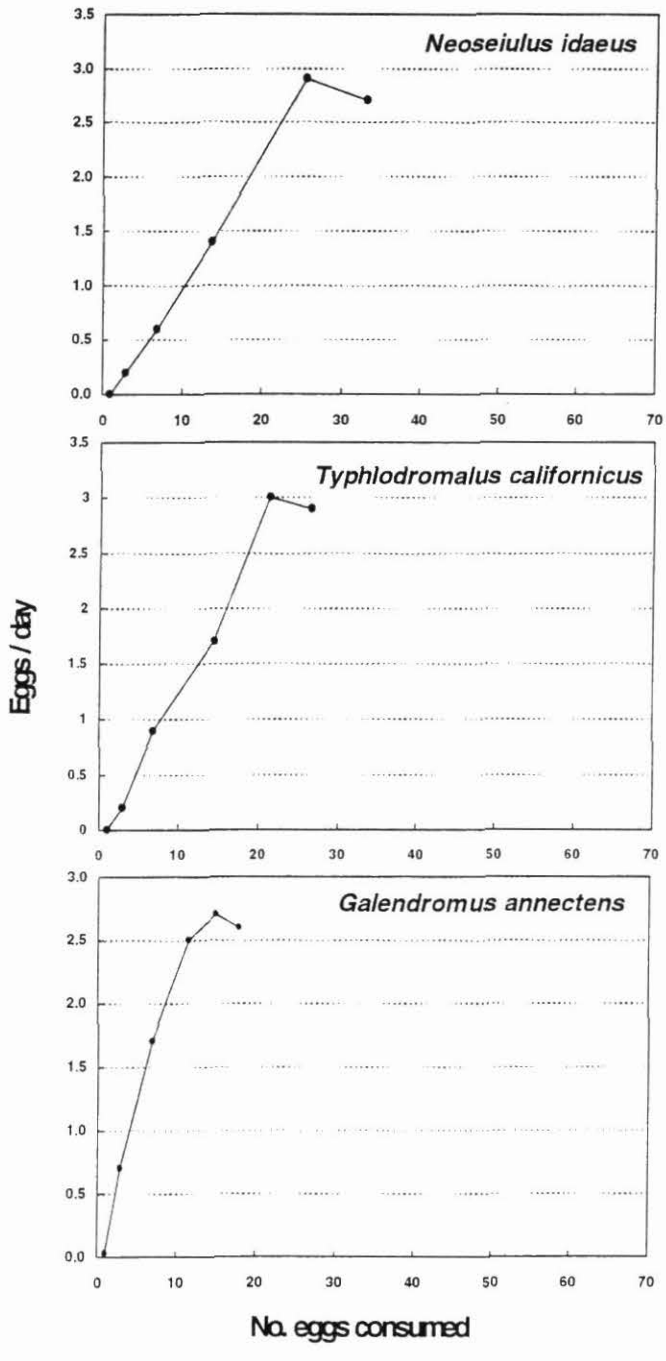

Figure 1.5.5.3. The effect of *M. tanajoa* egg consumption on oviposition of three phytoseiid predator species.

Thirteen populations of six phytoseiid species were maintained in the laboratory using the Mesa and Bellotti rearing method (**Table 1.5.6.1**), on cassava leaves with *M. tanajoa*.

Table 1.5.6.1. Phytoseiidae populations evaluated for sensibility to relative humidity.

Species	Locality
Typhlodromalus tenuiscutus	Portoviejo, Ecuador
" "	Puerto Cayo, Ecuador
Typhlodromalus aripo	CIAT, Palmira, Colombia
" "	Pivijay, Colombia
" "	Cruz das Almas, Brazil
Euseius ho	Rocafuerte, Ecuador
Galendromus annectens	Crucita, Ecuador
" "	Fonseca, Colombia
G. helveolus	Puerto Cayo, Ecuador
Neoseiulus idaeus	Fonseca, Colombia
" "	Carretalito, Colombia
" "	La Paz, Colombia
" "	Rocafuerte, Ecuador

Different humidity levels were established by using super saturated salt solutions (Winston and Bates Method, 1960) (**Table 1.5.6.2**) in sealed plastic boxes (19 x 14 x 16cm). Boxes were held in a room with a constant temperature of 25°C. Humidity was measured using a Cole-Parmer Hygrothermograph. Twenty four hours old phytoseiid eggs were placed on microscope slides which were placed a wire mesh screen about three cm above the liquid surface. Three repetitions of 30 eggs each were evaluated in each treatment and egg eclosion was measured every 3 days.

Results show that populations of *N. idaeus* present the greatest tolerance to low relative humidity levels (**Figure 1.5.6.1**). Three of the four *N. idaeus* populations display over 85% survival a the lowest RH level, 43%. The two populations of *T. tenuiscutus* were the least tolerant or most susceptible, as neither survived at RH below 83%. *E. ho* from Rocafuerte, Ecuador, and *G. annecteus* of Fonseca, Colombia had about a 50% survival at 63% RH. The eggs of *G. heveolus* and *G. annecteus* (Crucita, Ecuador) are able to hatch at 75% RH. *T. aripo* needs RH above 75% to survive.

This research has been done on several occasions and results are consistent. We have not found other phytoseiid species populations that can survive low humidities equal to *N. idaeus*. The question therefore becomes, should we continue to search for species that can survive at very low humidities to release into these very dry areas?

Table 1.5.6.2. Relative humidity (%) values for six salt solutions.

Solution	Relative Humidity (%)
H_2O	95.0
KCl	82.5
NaCl	75.0
NH_4NO_3	62.5
$Ca(NO_3)_2.4H_2O^c$	50.5
K_2CO_3	43.0

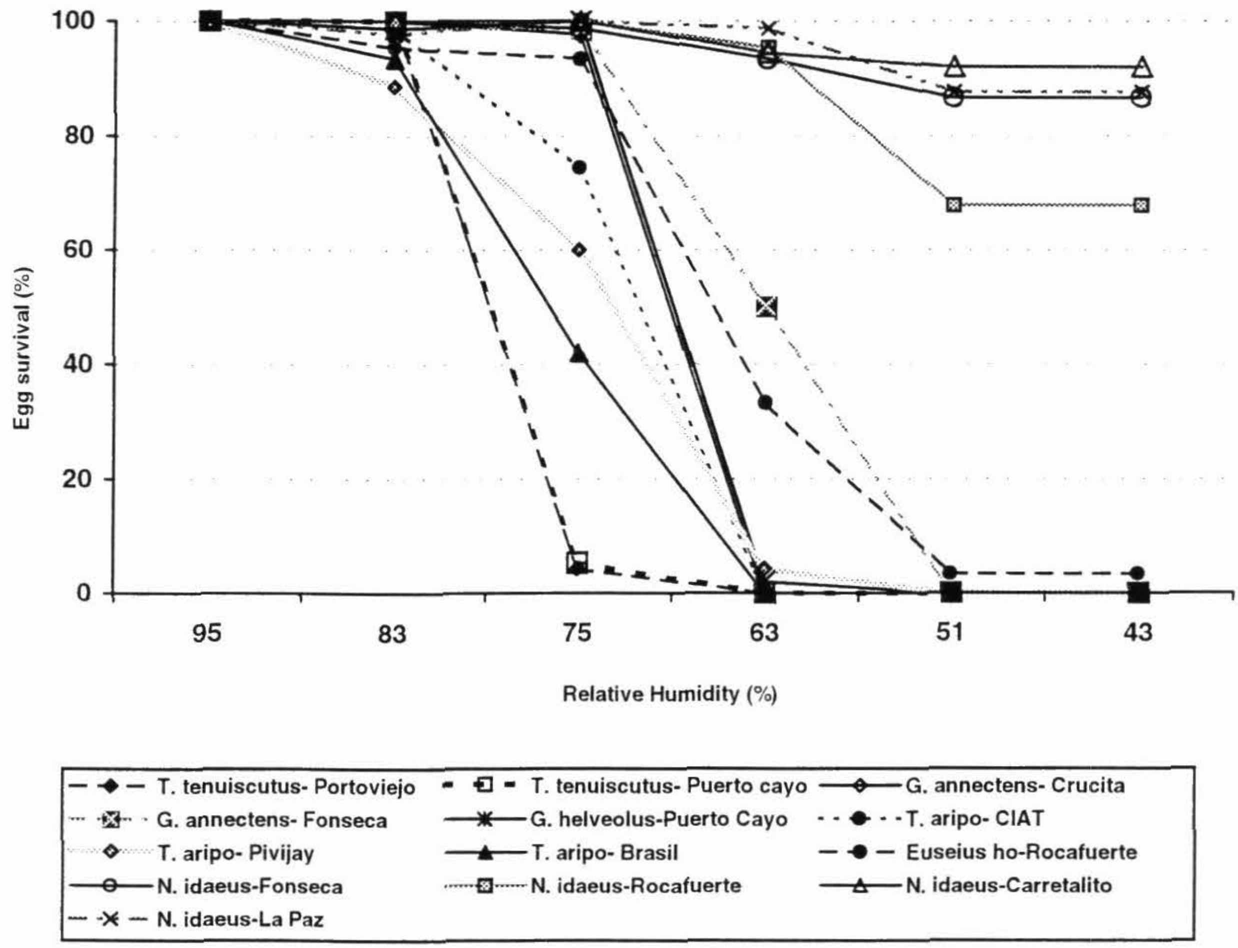

Figure 1.5.6.1. The effect of relative humidity on the survival of Phytoseiidae eggs.

Activity 1.5.7. Molecular characterization of selected species of phytoseiidae.

The complex of phytoseiidae found on cassava in the Neotropics has its greater genetic diversity in Colombia where 40 species have been identified. Approximately 20 species have been sent to Africa for the biological control of *M. tanajoa*. Three of these have become established, *Neoseiulus idaeus, Typhlodromalus manihoti* and *T. aripo*. It has been found that isolates of *N. idaeus* do not show any variability at the isoenzymatic level, perhaps owing to its wide distribution in different ecosystems. *T. manihoti* is the predominant species in most of the cassava growing areas of South America. Isoenzymatic studies done at CIAT show that *T. manihoti* has considerable diversity in its banding pattern. Because of this hybridization studies have been carried out between populations of this species to determine if reproductive differences exist or if there is an isolation of this group.

In the present research, molecular studies using AFLP's, is being done in populations of the different species found in hot semiarid and high altitude regions of the neotropics that correspond to similar regions of Africa (the Sahara and the East African plateau. The objective of this work is to identify their phylogenic relationships and contribute to the selection and release of the most promising species for biological control of *M. tanajoa* in Africa.

Eight phytoseiid species, including numerous populations collected in several countries were analyzed with AFLP's **(Table 1.5.7.1)**. Due to the problems that have occurred in the reproducibility of banding patterns of AFLP's with arthropods in general, special extraction techniques have been developed, which have given good results. The methodology of Vos et al. 1996, with Operon Technologiest adaptors and Primers was used. Non degenerated DNA, at good concentrations, between 10 and 25ng/ul, was obtained.

Of the species indicated in **Table 1.5.7.1**, diverse combinations have been analyzed and at this time results have been obtained for the combinations PEIC/PMIC for *N. idaeus* and *T. manihoti*. Using a NTSYS program, with UPGMA methods of classification realized with a matrix of similarity calculated with the DICE index, the following dendograms were obtained (**Figure 1.5.7.1 and 1.5.7.2**).

Populations of *N. idaeus* showed a very high similarity index of 0.96 in the case of populations coming from different geographic zones such as Brazil and Venezuela and up to 1.00 in the case of populations from the same geographic zone. These similarity indexes, being so high, show no significant difference and we can say that they are monomorphic populations.

Populations of *T. manihoti* display indexes of 0.2 to 0.7, tending to be more similar when from the same geographic zones, as in the case of Villanueva (Colombia) and Yaracuy (Venezuela) of 0.7. These populations come from the north coast of both countries, geographically close and with similar climates. It can also be concluded that the populations of *T. manihoti* analyzed show a high polymorphism. The analysis of the remaining populations is continuing.

Table 1.5.71. Phytoseiidae species analyzed with AFLP's.

Species	Country	Locality
T. manihoti	Colombia	Cajibío, Cauca
		Chinchiná, Caldas
		Barbosa, Antioquia
		Armenia, Quindío
		Copacabana, Antioquia
		Sta. Rosa Cabal, Risaralda
		Bijagual, Santander
		Bucaramanga, Santander
		Villanueva, Guajira
		Pivijay, Magdalena
	Venezuela	Marín, Yaracuy
	Ecuador	Calderón, Manabí
	Brazil	Cruz das Almas, Bahia
T. aripo	Colombia	Palmira, Valle
		Pivijay, Magdalena
	Brazil	Cruz das Almas, Bahia
	Benin	Ab-Calavi, Benin Station
T. tenuiscutus	Colombia	Los Córdobas, Córdoba
	Ecuador	Chone, Manabí
		Cantagallo, Portocayo, Manabí
		Portoviejo, Manabí
		Guayaquil, Guayas
N. idaeus	Colombia	Armenia, Quindío
		Fonseca, Guajira
		Carretalito, Guajira
		La Paz, Cesar
	Ecuador	Danzarín, Manabí
	Brazil	Petrolina, Pernambuco
		Crató, Ceará
		Capim Grosso, Bahia
		Piritiba, Bahia
	Venezuela	Carora, Lara
G. annectens	Ecuador	Crucita, Portoviejo, Manabí
		Calderón, Manabí
		Charapoto, Manabí
		Rocafuerte, Danzarín, Manabí
	Colombia	Fonseca, Guajira
G. helveolus	Ecuador	Santa Ana, Manabí
		El Rodeo, Portoviejo, Manabí
		Puertocayo, Cantagallo, Manabí
Euseius ho	Ecuador	Santa Ana, La Teodomira, Manabí
		Santa Ana, Santa Ana, Manabí
		Rocafuerte, Danazarín, Manabí
		Puertocayo, Cantagallo, Manabí
N. californicus	Ecuador	Portoviejo, Manabí
		Chone, Manabí
		Machala

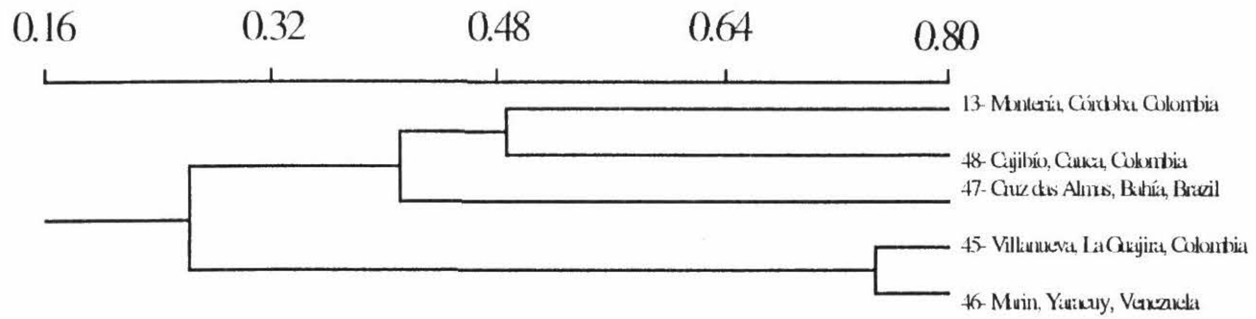

Figure 1.5.7.1. **Similarity index of five populations of *T. manihoti* with the PE1C combination.**

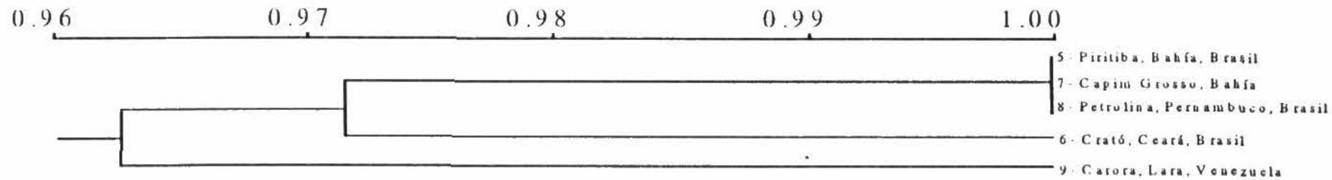

Figure 1.5.7.2. **Similarity index of five populations of *N. idaeus* with the PE1C/PM1C combination.**

Activity 1.5.8. Biological control by the Neozygites fungus.

Molecular characterization of the entomopathogenic fungus, *Neozygites* sp, pathogen of phytophagous mites

Neozygites cf. *floridana*, a fungal pathogen (Zygomycetes: Entomophthorales), cause irregular or periodic mortalities on mite populations in Colombia and the NE Brazil. The pathogen has been found on cassava mites throughout many cassava growing regions of the neotropics. This fungus shows considerable promise for biological control of the CGM and is being studied in Brazil and Africa, as well as at CIAT.

Due to taxonomic problems with this type of fungus, it has been difficult to determine exact species identification. There is interest to introduce the neotropical strain of the fungus into Africa to control CGM, but before this can be done, it is necessary to determine certain characteristics of the fungus, such as the existence of different strains or species.

Molecular techniques are presently being used to determine taxonomic identification of entomopathogenic fungi, such as AFLP's. However due to the difficulties in rearing this fungus *in vitro*, it is difficult to obtain sufficient quantities of DNA. We have improved on the method developed by Luis Leite and Donald Roberts (Boyce Thompson Institute, Ithaca, New York) (see 1997 Annual Report). Work by Delalibera (CNPMF/EMBRAPA, Cruz das Almas, Brazil) has also contributed significantly to the in vitro culture of this fungus.

DNA extraction was done by modifying the methodology of DNA MiniCTAB (developed by Zeller at Purdue University) developed for the *Pyricularia* fungus. The extraction of DNA is made from fungal isolates that have been growing in a liquid media, TNMFH, for 23 days. This is then lyophilized to reduce humidity and facilitate maceration. For AFLP's, the methodology developed by Vos et. al. (1995), with some modifications was used. Two kits were tried, the Small Genome of Gibco BRL Technologists, and the kit that contains the primers and adaptors of Operon Technologists.

It was possible to obtain (Zeller technique) DNA of good quality and concentrations of 5 to 150 ng, depending on the quantity of tissue obtained from the in vitro culture. Strains from several different sources were used in the analysis **(Table 1.5.8.1)**. In some cases (Mt Benin) we were unable to obtain sufficient DNA to observe bands in the gels, perhaps due to the insufficient amount of hiphal bodies produced in the in vitro culture.

Table 1.5.8.1. Strains of Neozygites used for DNA analysis collected from several sources.

Mite Host	Locality where Collected	Name
Tetranychus urticae	CIAT, Palmira, Valle (Colombia)	TuCIAT 1
T. urticae	CIAT, Palmira, Valle (Colombia)	TuCIAT 2
T. urticae	Avakpa, Benin	TuBenin
Mononychellus tanajoa	CIAT, Palmira, Valle (Colombia)	MtCIAT 1
M. tanajoa	CIAT, Palmira, Valle (Colombia)	MtCIAT 2
M. tanajoa	CIAT, Palmira, Valle (Colombia)	MtCIAT 3
M. tanajoa	Media Luna, Magdalena (Colombia)	MtML
M. tanajoa	Santander de Quilichao, Cauca (Colombia)	MtSQ
M. tanajoa	Avakpa, Benin	MtBenin
M. tanajoa	Cruz das Almas, Bahia (Brazil)	MtCDA 1
M. tanajoa	Cruz das Almas, Bahia (Brazil)	MtCDA 2
M. tanajoa	Caruaru, Pernambuco (Brazil)	MtCar
M. tanajoa	Piritiba, Bahia (Brazil)	MtPir

With the small Genome kit it was possible to obtain good banding patterns, although the numbers are small (10 to 20) and insufficient to make a true characterization. With the Operon kit, it was possible to evaluate more primer combinations (**Table 1.5.8.2**). The combinations with the greatest numbers of bands were +1/+3 and the analysis has focused on these types of combinations. At this time we only have partial characterization that indicate marked differences between the fungal strains. However these analysis need to be repeated to confirm these results and the statistical analysis is being done.

Table 1.5.8.2. Strains of Neozygites and primer combination for AFLP analysis.

No. Strains of *Neozygites* sp.	Type of Combination	Combination	No. of Bands
6	+2 / +3	PE1AA/PM1A	32
8	+2 / +3	PE1AA/PM1B	44
8	+2 / +3	PE1AA/PM1C	39
5	+2 / +3	PE1AA/PM1E	24
5	+2 / +3	PE1AA/PM1F	40
3	+ 1 / + 3	PE1A/PM1B	44
3	+ 1 / + 3	PE1A/PM1C	40
3	+ 1 / + 3	PE1A/PM1D	34
3	+ 1 / + 3	PE1A/PM1F	57
3	+ 1 / + 3	PE1A/PM1G	70
3	+ 1 / + 3	PE1A/PM1H	64
3	+ 1 / + 3	PE1A/PM1I	67

Activity 1.5.9. Studies on the viral pathogens found in *Mononychellus tanajoa* and *M. caribbeanae*

Previously we have reported at CIAT, epizootics causing high mortality in field and greenhouse populations of *M. tanajoa* and *M. caribbeanae*. The pathogen, analyzed under the electron microscope appears to be of viral origin. The particles are approximately 60 nm in size. Extractions of DNA have been made from the infected mite populations for molecular analysis. In agarose gels that displayed complete DNA, the presence of six different sized molecular bands was observed. This confirmed the presence viral particles. It has also been observed that these viral epizootics occur normally during wet or humid periods.

Reports in the literature on viral diseases of mites are few and little is known about its biology, mode of infection, symptoms, transmission, mite stages attacked, etc. In addition it is not known if it will attack beneficial mite predators. The present study is aimed at understanding and describing the signs and symptoms, oviposition of diseased females, mortality, viral presence in all mite stages and transoval transmission.

Four cassava leaves containing diseased *M. tanajoa* mites, and four leaves with diseased *M. caribbeanae* mites were used. From each leaf ten repetitions of each mite stage (eggs, larvae, nymphs, and adults), that showed symptoms of viral infection were removed. Infested leaves with healthy mites were used as a control. Observations with the election microscope were used to insure that mites were infected with the virus.

The biology, mortality and oviposition of mite stages were observed for 12 days. Each infected development stage was placed on a clean cassava leaf disc in a petri dish with moist tissue and in a 23 to 25°C chamber.

Viral disease symptoms are expressed by a clear amber discoloration of mite eggs, larvae and nymphs, and dark amber to brown colored adults. Upon death, hemocyle can be observed coming out of the anal pore resulting in a completely dry cadaver. Mortality is probably produced by the virus as virus particles have been found in the cadavers, of all stages of both species of mites. Mortality is highest in eggs and adults which could be due to the delicacy of the eggs and the age of the adults and the time of infection of the virus (**Figure 1.5.9.1**). After 12 days, individuals in the control treatments showed no evidence of viral infection and those examined under the electron microscope showed no virus particles.

Oviposition by diseased females was greatly reduced when compared to healthy females (**Figure 1.5.9.2**). When the F2 generation was evaluated mortality for all the stages of *M. caribbeanae* was 25.3% and for *M. tanajoa* 29.8% due to the virus. These results indicate that there is tranoval transmission of the virus. The F2 generation of the control mites did not show these symptoms.

Preliminary results with this virus disease indicate a good potential for biological control of cassava mites. Considerable research, however, still needs to be accomplished before this disease can be of practical use in an IPM program for control of cassava green mite.

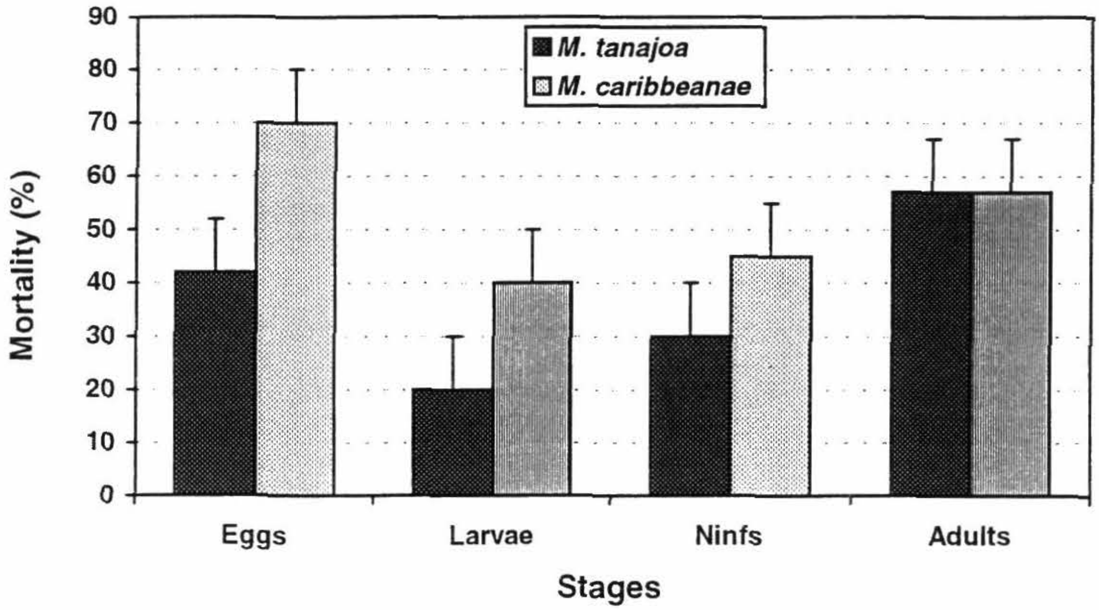

Figure 1.5.9.1. Percent Mortality of For Stages of *Mononychellus tanajoa* and *M. caribbeanae* due to Virus Infection.

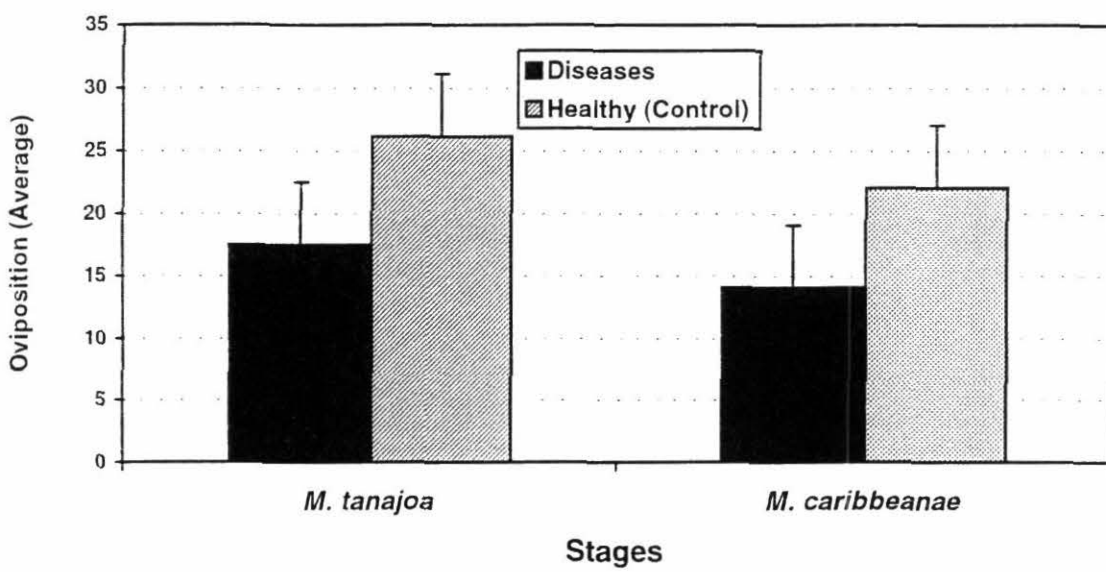

Figure 1.5.9.2. Oviposition of *M. tanajoa* and *M. caribbeanae* females infected with a virus disease.

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Output 2. An Integrated Control Method for Cassava Rot Diseases in Colombia.

Introduction

In 1998, *Phytophthora* root rots affected 23.5 hectares in 16 village districts (or *veredas*) of Santander de Quilichao (Department of Cauca). Other Colombian departments also reported losses caused by this destructive disease. After farmers asked for technical assistance, the Cassava Pathology section in collaboration with ICA, UMATAs, Universidad Nacional, and FIDAR, began a diagnostic survey in three pilot regions. To quantify losses caused by the disease and to obtain detailed information about the effects of different farming systems on the incidence of root and stem rot diseases, 130 farmers were interviewed. The pathogen was identified, and two field trials, to be conducted over 3 years, were established. COLCIENCIAS and PRONATTA provided funds, and a bulletin, describing effective control practices was disseminated.

Root rot pathogens are soil-borne, and difficult to control. Research has yet to generate efficient, easy-to-use, practices for control, especially for cassava farmers of few resources. Work on thermotherapy for cassava cuttings was therefore continued, and *Trichoderma*, a potential biocontrol agent, was successfully tested in the greenhouse.

Highly useful information on the genetic structure of *Phytophthora* spp. was obtained through characterizing their DNA by RAPD analysis. The entire *Phytophthora* collection, isolated from cassava, was also successfully cryopreserved, thus facilitating further evaluation of cassava germplasm for resistance to known *Phytophthora* groups.

Sub-output 2.1. Development of an Integrated Control Strategy for Cassava Rots and Its Evaluation in Pilot Regions in Colombia.

Activity 2.1.1. Exploratory survey.

Areas affected by rots and suffering crop losses were assessed in three pilot regions in Colombia where rots are endemic: Santander de Quilichao (Cauca), Buenos Aires (Cauca), and Mitú (Vaupés).

With the participation of cassava growers and technicians from the Ministery of Agriculture, UMATAs, and FIDAR, we conducted disease diagnostic surveys and described farming systems.

Participatory farm surveys.

Mitú (Vaupés). To study how the disease was being managed, we conducted a diagnostic survey, interviewing 31 farmers: 29 indigenous women from eight communities around Mitú and two settlers who lived on the road between Mitú and Monfort (also Vaupés).

The Indian women practice a system of subsistence agriculture that consists of slashing and burning between 1 and 1.5 ha of forest. This area is called *chagra*, and is planted for 2 or 3 years with the high-cyanide cassava varieties generally known as 'Yuca Brava'. Planted as the principal crop, cassava is associated with such crops as pineapple, plantain, sugarcane, yam, sweetpotato, hot pepper, and naranjilla.

The principal problems described by the women are shown in **Table 2.1.1.1.** In half of the *chagras* visited, symptoms of root rots were observed. Crop losses caused by root rots can involve as much as 0.5 ha, according to the women.

Table 2.1.1.1. Farmers[a] in the Departments of Cauca and Vaupés, Colombia, regard root rots as the major problem in cassava cultivation. Figures for the Colombian Eastern Plains are given for comparative purposes.

Problems	Cauca	Vaupés	Plains
Root rots and wilt	39	65	27
Ants	15	10	27
White grubs	14	0	0
Whitefly	8	0	0
Stem borers	7	3	13
Leaf-eating			
Lepidoptera spp.	0	3	20
Termites	0	3	13

a. Of the farmers interviewed, percentages are given of those who mentioned the problem as a major constraint to cassava production.

Table 2.1.1.2 summarizes the application of control practices for root rots by farmers. Some sites are easy to flood, but farmers are not accustomed to draining the soil to control rots. The soil is maintained fallow for several years, except along the Mitú-Monfort road, which is left to regenerate for only 3 years, because of the high population pressure on this land.

Almost all women (97%) were interested in planting new 'Yuca Brava' genotypes, even though they preferred to grow low-cyanide cultivars because they are easier to market in the town of Mitú. They much preferred tall varieties with yellow pulp and few branches, because these produce more seeds.

By analyzing samples of cassava roots and stems, we identified *Phytophthora* spp. as the causal agent of root rots at Mitú. We also observed symptoms of superelongation disease (SED) and cassava bacterial blight (CBB), and damage by termites and ants.

The soils of Mitú and Monfort are predominantly sandy, with the top 20 to 30 cm being a clay loam. pH is about 3.8, organic matter 3.4%, and phosphorous 3.6 ppm. Soil content of bases is very low. The extremely low soil fertility is compensated by ash, produced by burning the forest. The ash is extremely alkaloid (pH = 10), rich in organic matter (11.5%), and has a high content of calcium (8.3

meq/100 g), magnesium (7.5 meq/100 g), potassium (25 meq/100 g), and phosphorous (66 ppm). The crop can therefore grow.

Table 2.1.1.2. Diagnosis of farmer[a] management of root rot diseases affecting cassava, Departments of Cauca, Vaupés and Colombian Eastern Plains (Meta and Casanare).

Recommended practice to reduce root rots	Cauca	Vaupés	Meta and Casanare
Select healthy planting material	27	0	87
Before planting, treat stakes with pesticides	25	0	7
Plant different cassava varieties in the same field	30	100	67
Change variety	18	0	7
Cultivate on ridges or hills	66	0	20
Collect affected plants	43	0	20
Destroy affected plants (e.g., by burning)	12	0	13
Collect affected crop residues	35	0	53
Destroy crop residues	7	0	20
Crop rotation	21	0	0
Fallow for 3 to 4 years	26	94	27
Change the crop	17	0	27

a. Percentage of farmers who apply the control practice.

Department of Cauca. We interviewed 120 cassava growers in the municipalities of Santander de Quilichao (79 interviews), Buenos Aires (26), Caldono (14), and Piendamó (1). The farmers indicated that the disease is most common when the crop is between 3 and 8 months, although damage has been observed in younger and older crops (**Table 2.1.1.2**).

The farmers consider the most susceptible varieties to root rots are Batata (23.3% of farmers interviewed), Algodona (17.5%), and Barranqueña (11.6%), whereas the most tolerant variety is ICA Cebucán (30.8%). Most farmers (51.6%) are interested in planting another variety. 74% prefer varieties for starch extraction, and most prefer varieties with an intermediate plant height, few brances, and purple roots with white pulp.

Colombian Eastern Plains. We interviewed 15 farmers in the municipalities of Villavicencio, Puerto López, Restrepo, Guamal, San Martín, and Fuente de Oro (Department of Meta); Paratebueno (Cundinamarca); and near Villavicencio and Villanueva (Casanare).

Table 2.1.1.3. Frequency of cassava varieties used by farmers in the Department of Cauca and the Colombian Eastern Plains (Meta and Casanare).[a]

Variety	Cauca	Meta and Casanare
Algodona	63	0
ICA Catumare	24	0
Llanera	16	20
Batata	13	0
Chirosa	7	40
Brasileira	0	47

a. At Mitú, the women farmers use local varieties.

'Brasileira' is the most common variety, being planted in areas that averaged about 0.5 ha in size, although at Fuente de Oro, one farmer planted 180 ha. Root rot is a secondary problem, being found only in soils that are readily flooded (**Table 2.1.1.1**). At four farms at Puerto López, Restrepo, and Villanueva some root rot was found, with 'Chirosa' being the most affected variety, and 'Brasileira' the least affected.

Activity 2.1.2. Establishing on-farm trials to select different components in the control of root rots.

To evaluate tolerant cassava genotypes identified in the CIAT greenhouse and local varieties, seven on-farm trials were established in May and September 1998, in various regions where root rots are endemic:

Location	Varieties planted (no.)
Mitú (Vaupés)	
Seima Cachivera	7
Seima Central	7
Puerto Paloma	7
Cucura	7
Model farm, Secretaria de Agricultura	9
Cauca	
Santander de Quilichao	16
Buenos Aires	3

Currently, we are evaluating, at Mondomo and Buenos Aires, the potential of the *Trichoderma* isolate 14PDA-4 to control *Phytophthora* root rot, and the isolate's effect on cassava plant development and root yield. Before planting, stakes of the susceptible local variety Algodona were inoculated by immersion in a suspension of about 1×10^6 conidia/ml of *Trichoderma* for 30 min. After planting, 30 ml of the same inoculum was applied around each planted stake. The isolate used has been a highly efficient control agent for the same disease in other experiments. Isolation and symptoms confirmed presence of *Phytophthora*. Three controls were used: stakes treated with Ridomil (metalaxyl, 3 g/l) and Captan (captafol, 3 g/l); stakes treated with Sistemin (dimetoate, 3 cc/l); and stakes immersed in water. All field plots, carrying 16 treated plants each, were surrounded by the local susceptible variety to obtain a high and uniform disease pressure. Results obtained so far indicate that cassava germination rate and growth were not affected by the treatments, but the evaluations are still continuing.

The experiment at Buenos Aires consisted of a two factor randomized complete block design with 8 plants/plot. One factor comprised of a tolerant variety (MBRA 383) and a highly susceptible variety (Algodona), that had been selected previously by inoculating stems of young plants in the greenhouse. The second factor consisted of several control practices: two fertilization treatments (organic minerals and chicken manure), thermotherapy of stake material for 49 min at 49 °C, and a biological control agent (isolate 14PDA-4).

Activity 2.1.3. Study of rot-causing pathogens.

Isolation and conservation. Storage of *Phytophthora* isolates in solid medium or water is costly or not effective. Therefore, a methodology from Dr. Michael Coffey (University of California Riverside) was successfully adopted to conserve a group of *Phytophthora* isolates in liquid nitrogen. Seven day old fungal plugs approximately 5 mm in diameter, of cultures grown on oatmeal agar (agar, 20 g; oatmeal, 20 g; 1 L distillated water) were placed in 1.8 ml cryotubes, previously filled with 0.7 ml of a 15% solution of sterile dimethyl sulfoxyde (DMSO). Prefreezing was done for half an hour at 5 °C and then stepwise by the Cryomed system, until the temperature decreased to -196 °C. One week after conservation the isolates were retrieved by warming at 37 °C for 4 minutes, dried on filter paper and cultured on oatmeal agar medium. All isolates were successfully retrieved, without contamination or loss in pathogenicity.

Identification, pathogenicity and virulence testing. From one hundred and ninety three cassava samples naturally infected by *Phytophthora* species, a total of one hundred twenty two *Phytophthora* isolates were obtained using a direct plating method and a baiting technique in which fragments of cassava platelets propagated *in vitro*, were used.

Most isolates could not be clearly differentiated on potato dextrose agar, V8-agar and oatmeal agar by colony type or growth. Identification of the isolates by morphology was difficult because not all isolates produce fungal structures. Amplification of the ITS (Internal Transcribed Spacer) region of the rDNA was done using DNA extracted from 122 isolates. The amplified product for the ITS region of all isolates was approximately 900 bp. Restriction analysis of the amplified ITS region, using the enzymes *Alu*I, *Msp*I and *Hind*I, revealed different patterns, corresponding to the different species tested. Analysis of genomic DNA using 10 RAPD primers (Operon Technologies, INC) that gave reproducible bands, revealed polymorphism's which clearly discriminated between the different *Phytophthora* species. (**Figure 2.1.3.1 and Figure 2.1.3.2**). Isolates clustered into 12 groups showing that the Colombian population of *Phytophthora* is very diverse. The DNA hybridization probe (Lee *et al.* 1993)[1], was a more sensitive method than the ELISA test for identifying the pathogen (**Figure 2.1.3.3**).

---

1. Lee, S. B., White, J. and J. W. Taylor. 1993. Detection of *Phytophthora* Species by Oligonucleotide Hybridization of Amplified Ribosomal DNA Spacers. Phytopathology 83: 177-181.

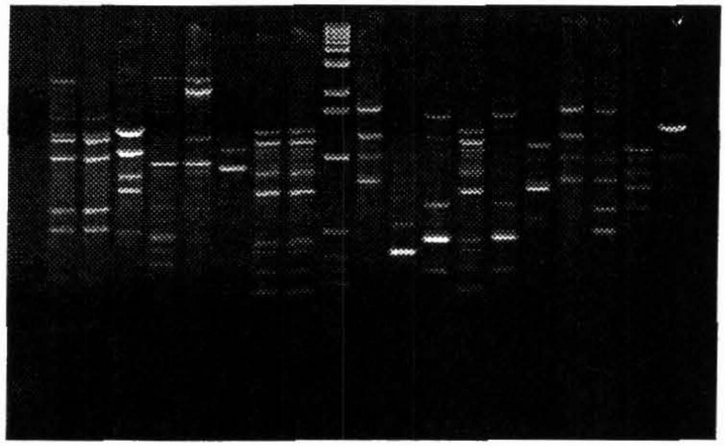

Figure 2.1.3.1. Variation among isolates of *Phytophthora* spp. detected with primer OPH-04. Lane M=1Kb Marker. Each lane corresponds to a *Phytophthora* spp. isolate.

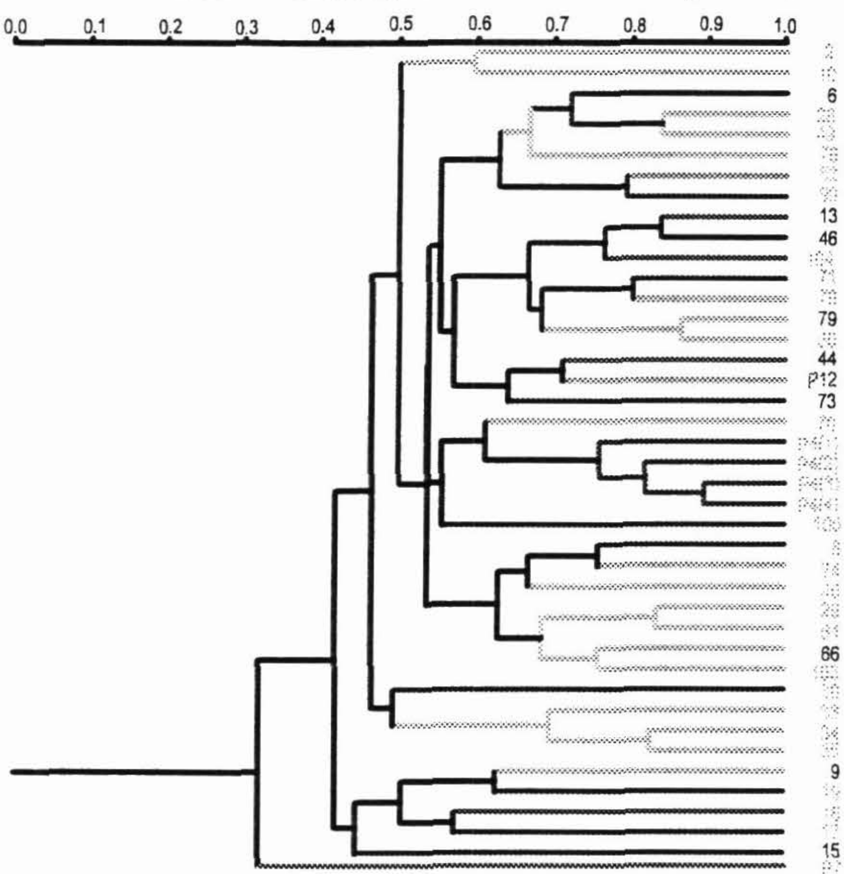

Figure 2.1.3.2. Phenogram from hierarchical cluster analysis of RAPD data. Clusters were formed using the unpaired group mean average (UPGMA). The similarity scale shown corresponds to de average similarity at which clusters fuse.

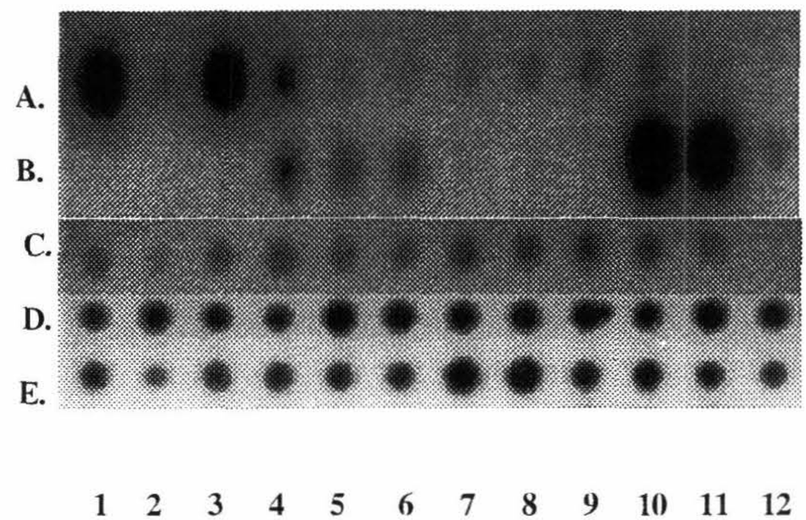

Figure 2.1.3.3. DNA-DNA hybridization of the ITS region of the rDNA with the genus specific probe. Each dot represents a positive reaction. C12= negative reaction.

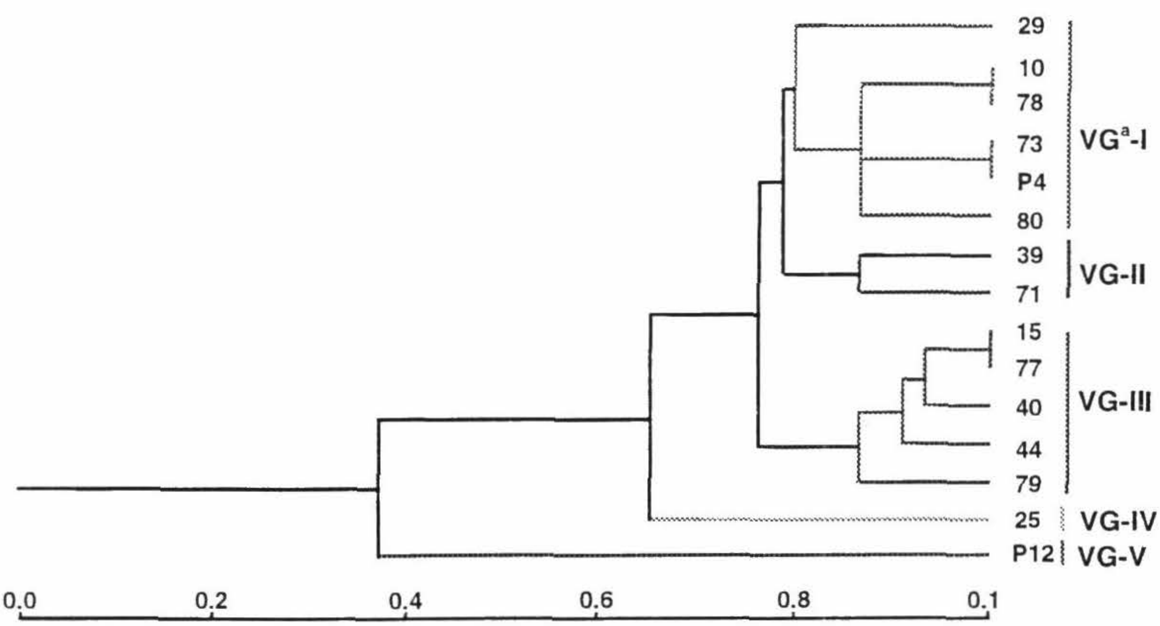

Figure 2.1.3.4. Phenogram of *Phytophthora* isolates based on virulence on 15 cassava genotypes using the Unweighted Pair Group Arithmetic Mean (UPGMA) program of NTSYS-pc. [a]VG, virulence group.

In collaboration with the University of California Riverside and the Scottish Crop Research Institute the identification of representative isolates, through sequencing of the ITS region of the rDNA, is in progress.

Other root rot causing pathogens, *Fusarium oxysporum*, *Fusarium solani* and *Diplodia manihotis*, were tested for their pathogenicity on cassava platelets and swollen roots but had low aggressiveness compared to *Phytophthora*.

After inoculation of cassava plantlets of different genotypes in the greenhouse with genetically different *Phytophthora* isolates, a differential set was selected. This set allows the identification of virulence types and host-pathogen interactions (**Table 2.1.3.1**), and is considered to be of crucial importance to understanding the pathogenic variability of different *Phytophthora* species that were found. Using cluster analysis the 15 isolates tested were separated into five main groups (*P*=96%, **Figure 2.1.3.4**). Analysis of possible correlation between genetic diversity using molecular markers and virulence is ongoing.

Table 2.1.3.1. Identification of virulence phenotypes of 15 isolates of *Phytophthora* spp. using 15 cassava germplasm accessions in the greenhouse.

	Differential genotypes[a]															Geographic origin		
Isolate	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**Department**	**Municipality**	**Country**
29	a[b]	b														Quíndio	Quimbaya	Colombia
10	a		C		e											Valle	B/ventura	Colombia
78	a		C		e											Atlántico	Barranquilla	Colombia
73	a	b	C		e										o	Quíndio	Montenegro	Colombia
P4	a	b	C		e										o			Colombia
80		b	C		e											Atlántico	Barranquilla	Colombia
71	a	b	C		e					j			m			Valle	B/ventura	Colombia
39	a	b	C		e					j	K					Quíndio	Cordoba	Colombia
15	a	b	C		e					j				n	o	Quíndio	Montenegro	Colombia
77	a	b	C		e					j				n	o	Atlántico	Caracolí	Colombia
40	a	b	C		e									n	o	Valle	Caicedonia	Colombia
44	a	b	C		e			h		j				n	o	Quíndio	Barcelona	Colombia
79		b	C		e									n	o	Atlántico	Barranquilla	Colombia
25														n	o	Quíndio	Barcelona	Colombia
P12	a	b	C	D	e	f	g	h	i	j	K	l		n				Brazil

[a] ifferential genotypes: a, MTAI 1; b, MTAI 8; c, MARG 6; d, MCR 45; e, MCR 81; f, MCOL 1505; g, MCOL 2265; h, MBRA 12; i, MBRA 71; j, MBRA 191; k, MBRA 222; l, MBRA 532; m, MBRA 1045; n, MNGA 2; o, CM 2177-2 [b]Lowercase letters a to o indicate susceptibility of that respective differential cassava genotype to the specific isolate of *Phytophthora* spp.

Activity 2.1.4. Didactic material, training and field days.

A field day on management of cassava root rot diseases was organized for farmers and technicians at Santander de Quilichao. Several meetings have been realized with indigenous people and technicians working in Vaupés, to discuss the progress of the project activities.

A technical handout on the management of Phytophthora Root Rot was prepared, which will be distributed to farmers in Colombia. The preparation of a pictographic manual about cassava production and root rot control is in progress. This will be useful to the indigenous people from Vaupés and other regions with similar production systems.

A workshop was organized to train farmers, and technicians of UMATA, ICA, FIDAR and the Comité de Cafeteros technicians to recognize *Phytophthora* disease symptoms in the field, and to introduce control strategies. A training course about *Phytophthora* was organized, with participants from different backgrounds. Conferences were given to students from six Colombian Universities.

Sub-output 2.2. Development of a PCR Method to Detect Phytophthora spp. on Vegetative Tissue.

Activity 2.2.1. Phytophthora root rot pathogen detected on cassava sprouts.

An effort was made to develop a simple, rapid and effective method to detect the pathogen on sprouts of young cassava plants.

A quick DNA extraction procedure was adopted following the protocol described by Ristaino *et al.* (1997)[2]. Polymerase Chain Reaction (PCR) amplification of the ITS and 5.8S rDNA was achieved using procedures developed for *Phytophthora* species by Lee and Taylor (1990)[3]. PCR inhibition was observed from all the samples tested. To eliminate the problem the following conditions were evaluated: 1, addition of proteinase K (0.1 μg) and 2, addition of bovine serum adjuvant (0.2 μl/sample at 20 μg/μl) to the extraction buffer; and 3, boiling the samples for 10 minutes.

Bovine serum adjuvant (BSA) had the greatest effect in reducing PCR inhibition, compared with boiling the samples or adding proteinase K. BSA was successfully applied to the amplification of *Phytophthora* DNA directly from the infected sprout tissue.

The simplicity of the PCR method and the accuracy of its predictions, makes it acceptable for detection of *Phytophthora* from sprouts and stem cuttings. These results will be used for further development of a seed health test for this pathogen.

DNA hybridization was performed following the procedure described by Lee (Lee *et al.* 1993) using a *Phytophthora* - specific probe. All samples that were positive using PCR amplification and ELISA, were also positive using DNA hybridization with *Phytophthora*-specific probe.

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2. Ristaino, J.B., Madritch M. Trout, C. L. and Parra, G. 1998. PCR Amplification of Ribosomal DNA for Species Identification in the Plant Pathogen Genus *Phytophthora*. Appl. Environ. Microbiol. 64:948-954.

3. Lee, S. B. and J. W. Taylor. 1990. Isolation of DNA from Fungal Mycelia and Single Spores. PCR Protocols pp. 282-287.

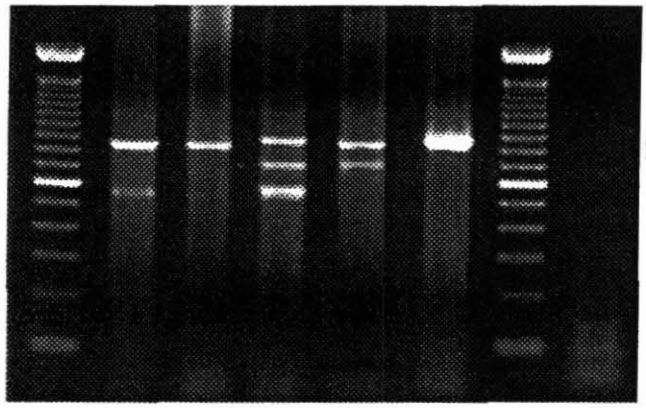

Figure 2.2.1.1. **Detection of *Phytophthora* spp. in affected cassava sprouts was achieved by the amplification of the ITS region with primers ITS1 and ITS4. Lanes 1 to 4 = amplified DNA from infected sprout samples. Lane 5 = *Phytophthora* DNA from a pure culture. Lane M= 100 pb Marker. C=control.**

Sub-output 2.3. Potential of Heat Treated Plant Propagation Material for Controlling Diseases in Cassava.

Activity 2.3.1. **Heat sensitivity of the causal agent of *Phytophthora* root rot disease of cassava determined.**

The sensitivity of seven *Phytophthora* isolates to heat was determined in 50-ml Pyrex tubes filled with clarified V8 juice as culture medium (**Table 2.3.1.1**). A heat treatment of 47 °C or higher was lethal for all seven isolates tested.

The effect of three methods of heat treatment on the variety CM 3306-4 was evaluated in the greenhouse: 1. immersion of cassava stakes in hot water; 2. in a steam chamber; and 3. a dry oven. Stakes were treated according to the following combinations of time and temperature: 27, 40, 45, 50, 52, 55, 60 °C for 10, 20, 30, 40, 50, 60, 120 and 240 min. Using a regression analysis it was calculated that immersion of stakes in water at 47 °C for 30 minutes inactivates or kills the pathogen, without damaging host tissue or affecting germination and formation of sprouts and plant growth.

A method for inoculating stem cuttings with *Phytophthora* spp. was tested. Symptoms did not develop when stakes were inoculated by puncturing the xylem with an iron punch (diameter of 3 mm, 5 cm long) and placing a fungal plug in the stake.
Greenhouse trials to test the effect of heat (47° C for 30 minutes) for inactivating or eradicating *Phytophthora* in cassava stem cuttings were conducted using 20 varieties. No differences were observed for germination rate, vigor and growth between treated and untreated stakes.

Table 2.3.1.1. ***In vitro, Phytophthora*** **isolates were successfully inactivated by a heat treatment at 47° C for 30 minutes.**

Temperature (°C)	Time (min)	*Phytophthora* isolate						
		P12	P4	5	7	Stef-B	120	39A
26	15, 30, 45, 60, 120	+[a]	+	+	+	+	+	+
45	15, 30	+	+	+	+	+	+	+
	45	+	+	-[b]	+	-	-	-
	60, 120	-	-	-	-	-	-	-
46	15	+	+	-	-	-	+	+
	30	-	+	-	-	-	-	+
	45, 60, 120	-	-	-	-	-	-	-
47	15	+	+	-	+	+	+	+
	30, 45, 60, 120	-	-	-	-	-	-	-
48, 49, 50	15, 30, 45, 60, 120	-	-	-	-	-	-	-

[a] Growth of *Phytophthora* 7 and 14 days after the heat treatment.

[b] Absence of growth of *Phytophthora* after heat treatment.

Activity 2.3.2. Heat sensitivity of *Xanthomonas axonopodis* pv *manihotis*, causal agent of Cassava Bacterial Blight determined.

Losses can reach 80% in only three crop cycles after incidence have started. These losses correlate with the number of infected cuttings used during planting. In this study the thermal sensitivity of five pathogenic *Xam* isolates, collected in Colombia and Africa, was established (**Table 2.3.2.1**). The method used has been described above. The effect of the treatment on the viability of the isolates was evaluated by counting the number of colony forming units 48 hours after the heat treatment which consisted of two factors: time (15, 30, 45, 60 and 120 minutes) and temperature (47, 48, 49, 50, 51, 52, 53 °C). The results show significant differences between the thermal sensitivity of the isolates tested. The average temperature and duration to deactivate *Xam* is far to destructive for cassava to treat planting material.

Table 2.3.2.1. Critical temperature and time for treatment of the studied isolates of *Xanthomonas axonopodis* pv *manihotis*, established by regression analysis.

Xam isolate	Temperature (°C)	Time (minutes)
X-27	49.0	86
CIO-12	49.7	48
CIO-81	49.6	81
CIO-46	52.0	79
CIO-188	52.0	237
Average	50.5	106

Sub-output 2.4. Potential of *Trichoderma* spp. for controlling *Phytophthora* spp. in Cassava.

Activity 2.4.1. Effectiveness of *Trichoderma* isolates evaluated in the greenhouse.

In three experiments 37 *Trichoderma* strains were compared under greenhouse conditions for their possible efficiency in the control of *Phytophthora drechsleri* (isolate no. P12 collected in Brazil) and isolate no. 44 (Colombia), representing the most pathogenic species affecting cassava. **Table 2.4.1.1,** summarize the details and results of the experiments. Before planting, stakes from susceptible varieties widely used in Colombia, were inoculated with a suspension of approximately $1X10^6$ conidia/ml of *Trichoderma*. for 30 minutes. After planting 30 ml of the same inoculum was applied around the planted stake. 25 to 30 days later, cassava plants were inoculated with *Phytophthora* by placing a small mycelial fragment of each isolate on a small wound previously made in the stem. Following inoculation, the plants were maintained in the greenhouse under humid conditions. Each week lesion area was measured and area under the disease progress curve was calculated. The isolate 14PDA-1, significantly reduced the severity of lesions in stems caused by both pathogenic isolates (**Table 2.4.1.1**).

Table 2.4.1.1. **Efficiency of selected *Trichoderma* isolates in controlling two species of *Phytophthora* in the greenhouse following inoculation of young cassava plants.**

			Disease control		
Exp. Number[a]	No. of cassava varieties inoculated	No. of Trichoderma isolates evaluated	Pathogen	Variety	Trichoderma isolate
1	3	30	P12	CG 1-37	19TSM-3A
			P12	CG 1-37	20TSM-2
			P12	MCOL 1468	14PDA-4
			P12	MCOL 1468	18TSM-3
			44	CG 1-37	41TSM-4
2	14	7[c]	P12 and 44	-	-
3	9	5	P12	MCOL 2066	9-1B
			44	MCOL 2066	14PDA-4

[a] Four replications, each treatment consisted of 2 plants per replicate.
[b] Significant differences between plants inoculated with *Trichoderma* and control without *Trichoderma* ($P=5\%$).
[c] Best isolates of experiments no. 1 and 2.

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Output 3. Identification and Characterization of Major Viruses.

Sub-output 3.1 Cassava frogskin disease is increasing its range.

The range of cassava frogskin disease (CFSD) is increasing in Costa Rica, Venezuela and Brazil. A similar disorder is reported from Cuba. The increasing incidence of CFSD appears part of a long term tend and the principal mode of spread is infected stem cuttings. *Bemisia tuberculata* is the insect vector of the disease. The zones that are reporting increased incidence of CFSD are also reporting problems because of the introduction of the silverleaf whitefly (*B. tabaci* biotype B or *B. argentifolia*). In the St. Victoria region of Bahia Brazil, the only whiteflies collected on CFSD infected cassava were *B. tabaci* biotype B. Because of the small sample size, the absence of *B. tuberculata* cannot be eliminated. The hypothesis that the B biotype is a vector of CFSV is being tested. If it proves to be a vector, CFSD can be expected to continue to rapidly increase in range.

Activity 3.1.1. Molecular characterization of CSFV

A fragment of a viral RNA dependent RNA polymerase gene has been cloned and sequenced.

The attempts to produce CFSV cDNA clones from dsRNA isolated from CFSD infected plants continue. The dsRNA was denatured using 90% DMSO at 65C. A tail of poly A was added to the RNA. The reverse transcriptase reaction to produce cDNA was primed using an oligo dT. The cDNA was amplified by PCR using various 10 base oligo nucleotides. The PCR products were cloned into pCR-script SK(+) 10 (Stratagene). The ends of the clones were sequenced and compared using the BLAST programs in the GeneBank of the US National Center for Biotechnology Information (NCBI). One clone of 876 nucleotides (FSDPOL) had 43% identity and 63% similarity at the amino-acid level with the RNA dependent RNA polymerase gene of the *Saccharomyces cerevisiae* virus L-A (totiviridae: totivirus). There was also homology with other RNA dependent RNA polymerase genes of viruses including *Leismania* RNA virus (totiviridae: leshmaniavirus), *Trichomona vaginalis* virus (totiviridae: giardiavirus), cucurbit yellows-associated (potyviridae), and *Helminthosproium victoriae* virus 190S (totiviridae: totivirus). The greatest percent of homology was with the viruses that are in the family totiviridae. These are isometric viruses that consist of 1 species of dsRNA. The degree of homology leaves no doubt that this cDNA clones represents an RNA dependent RNA polymerase of a virus. There was 30% identity and 49% similarity with the RNA dependent RNA polymerase of the cucurbit yellows-associated virus. This is a potyvirus that is transmitted by the whitefly *Trialeurodes vaporariorum*.

To determine if this clone is associated with CFSD, specific oligonucleotide primers based on the sequence of the FSDPOL were designed. The some of the healthy materials were grown from *in vitro* plantlets in controlled conditions. Reverse transciptase PCR (rtPCR) is used to amplify RNA. Total RNA was extracted from healthy and CFSD infected plants. Using FSDPOL specific primers in rtPCR, some of the healthy materials and the CFSD infected materials had PCR products of the expected size (ca. 450 nucleotides). The PCR products were transferred to membranes. The membrane was hybridized with a ^{32}P labeled FSDPOL probe. The probe hybridized with the four sizes of PCR products from healthy and infected materials.

Using the same set of test plants, DNA was extracted. Since the clone FSDPOL is homologous with an RNA dependent RNA polymerase, a DNA product of the virus was not expected. The extracted DNA was amplified using the FSDPOL specific primers. There were generally four PCR products. The sizes of the products were ca. 450, 500, 900, and 1200 nucleotides. When hybridized with the FSDPOL probe, these PCR products appear to be specific.

The results of these experiments raise many questions. There is little doubt that the FSDPOL clone represents a viral RNA dependent RNA. Since this clone has the highest degree of homology with the totiviridae, the possibility that this is a virus infecting fungi is being explored. Even if this is a virus that infects a fungus (for example *Oidium* spp. or an endophytic yeast), this would not explain the result the direct PCR experiments. Why are multiple viral specific PCR products amplified from a DNA extraction? A viral specific product could come from a DNA virus, an RNA virus with a DNA intermediate, or the viral genome is incorporated into the genome of cassava or an endophytic fungus. The totiviridae are dsRNA viruses and there are no reports of DNA intermediates. Also the simplest explanation for the multiple bands is that this virus sequence in incorporated into the genome of its host. Banana streak virus (BStV) is a plant pararetrovirus and it is reported to be incorporated into the genome of banana. When BStV became incorporated into the genome, there were rearrangements and there are multiple copies in the banana genome. The experiments to determine if there is incorporation into the genome of cassava are in progress. These experiments will also help to determine if there is an association with the virus encoding the RNA dependent RNA polymerase and CFSD.

Activity 3.1.2. Identification of cassava germplasm that is resistance to CFSD

Progress towards the output of the identification of CFSD resistant cassava is being made.

This is the third year of study to identify cassava that is resistant to CFSD. During the first two years approximately 460 accessions of the cassava core collection inoculated with the same source of CFSD by grafting to infected stem cuttings of the cassava accession CM5460-10. The entry of materials into the experiment took two years. This was due to availability of accessions from the core collection and the desire to use only one source of inoculum for the entire experiment. Two stem cuttings of each accession that were inoculated CFSD by grafting with infected CM5460-10 stem cuttings. After the graft as removed, the materials were planted in the field. During the second and third cycles, ten-twelve stem cuttings of each accession were planted. The plants were grown for one year and evaluated for root symptoms, vigor of plants, and the weight of the roots. Since the accessions were from the cassava core collection and many are poorly adapted to the Cauca valley, the root yield and vigor are secondary factors that will be used in later selections of CFSD resistant germplasm with good agronomic characters. The long cycle of cassava and the erratic symptom expression of CFSD are factors that make the evaluation for resistance a time consuming task.

The root symptoms are classified as absent, mild, moderate or severe, and the rating is the primary selection characteristic. The 213 accessions still in the field study were rated for the CFSD symptoms. The plants with moderate or severe symptoms are eliminated from the study. After three years, there is a trend in the reaction of the germplasm and the country of origin. There are 76 accessions with mild symptoms. Nearly all of these are from Brazil or Colombia. There are 105 accessions the are still symptomless. Some of these were inoculated in the second cycle of this experiment and additional time may be needed for the full expression of CSFD symptoms. Twenty-eight of accessions without symptoms are from Peru, 28 are from Paraguay, 18 are from Mexico, 8 from Malaysia and 8 from Guatemala. All of these countries are very over represented in the category of plants showing no symptoms. There are no accessions from either Brazil or Colombia with any symptoms.

There were 76 accessions that were showing mild root symptoms. These included 46 Brazilian accessions and 24 Colombian accession. There are accessions that have had mild symptoms for three consecutive years and this may indicate that these accessions are tolerant of infection with CFSD.

The 105 accessions with no symptoms and the 76 accessions with mild symptoms were planted for another field cycle. Now that there is a more manageable number of accessions, a more intensive monitoring needs to be done. The accessions without symptoms will be assayed for the presence of CFSV. Consideration must be given to multilocational testing. CFSD symptoms are generally more severe in cool climates and a testing site that is at higher elevation may change the symptom expression. Inoculation with a second isolate of CFSD may also increase the reliability in selecting resistant germplasm. These accession were all graft inoculated and they need to be tested for field resistance.

Sub-output 3.2. Development of a PCR Detection Method for Potexviruses.

There are three potexviruses that are known to infect cassava. Cassava X virus (CsXV) and cassava Colombian symptomless virus (CsCSV) cause no symptoms. Cassava common mosaic virus is a significant pathogen that produces mosaic symptoms on the leaves of cassava. The potexvirus group is fairly diverse. Unlike some groups of viruses, the coat protein does not contain core regions that share a high degree of homology. While sufficient antisera exist for the detection of CsCMV and CsXV, there is no good source of antisera for CsCSV. Sequence information exists for many potexviruses, but CsCMV is the only sequenced potexvirus infecting cassava. Using a comparison of CsCMV and other potexviruses a set of degenerate primers were designed. Conserved motifs in the RNA dependent RNA polymerase were used as the basis of the primers. In testing, the primers did amplify CsCMV but did not amplify CsXV or CsCSV. The process of selecting other conserved regions such as the helicase motif, designing, ordering and testing other sets of primers is in train.

OUTPUT II. IMPROVED CROP MANAGEMENT COMPONENTS RELEVANT TO IPM STRATEGIES

Sub-output 1. Determine the influence of cassava in water deficiency on the development of the cassava mealybug *Phenacoccus herreni*

In South America, the cassava mealybug *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae) is an important pest of cassava, *Manihot esculenta* Crantz (Euphorbiaceae), especially during drought, when insect population's increase (Bellotti *et al.*, 1983; Noronha, 1990). This phenomenon might be a response to biochemical changes in cassava leaves induced by water deficiency, which enhances insect development. Most plants react to water deficiency by an increase in the concentrations of some compounds such as amino acids, carbohydrates and organic acids contributing, by their accumulation, to decrease the osmotic potential in plant cells (Lamaze *et al.*, 1996). Such increasing of these nutrients should favor insect growth and reproduction (Mattson & Haack, 1987). In order to verify this phenomenon, our research is focused on the importance of drought tolerance mechanisms of cassava, and the changes they might trigger in plant physiology and biochemistry, and what changes this might trigger in mealybug development.

Activity 1.1. Determination of the feeding behavior of male and female of *Phenacoccus herreni* on cassava

In order to identify the biochemical changes of cassava leaves induced by water deficit associated with the nutritional needs of *P. herreni*, it was first necessary to study the feeding behavior of the insect. Observation of the mouthparts by scanning electron microscopy and by fase contrast microscopy were carried out and the feeding activity was studied by electropenetrography (EPG, DC-system). The results showed that *P. herreni* is mainly a phloemophagous insect with piercing-sucking mouthparts, present in females throughout their life cycle and in males only till the second nymphal stage after which they do not need to feed to complete their development. Females feed mainly on phloem sap of cassava throughout their life cycle, approximately 43 days, while the males during 13 days (all data not shown). These results suggest that the females are more responsible for plant damage than males and therefore subsequent studies were done on females.

Activity 1.2. Influence of cassava in water deficit conditions on the development and the fecundity of females of *P. herreni*

The cassava varieties used are CMC-40 and CM 507-37 known to be less and more drought tolerant, respectively. In glasshouse conditions, one month old plants were used. Drought stress was imposed by decreasing the irrigation volume from 500 ml (three times a week per plant, control) to 50 ml (two times a week per plant, stress). After 30 days of water deficiency, shoot development was affected **(Table 1.2.1).** The stems stopped growing from the onset of the period of water limitation. The area of the mature leaves was reduced under water limitation. These modifications were more pronounced with CM 507-37 and could be due to the fact that it's more drought tolerant. Furthermore, for both varieties the stomatal resistance was very high under water limitation. These modifications of plant growth and stomatal resistance demonstrate that our experimental conditions mimic water stress and thus allow study of the development of *P. herreni* on water stressed plants.

Table 1.2.1. Shoot characteristics of two varieties of cassava grown with high or low water availability for 30 days. The area of mature leaves expanded during the treatment, the height of plant stems and the stomatal resistance of young leaves were determined. At the beginning of the experiment, plant height was *ca* 23 cm for both varieties. Data are means* ± SE and results of 2-way ANOVA are given (variety, treatment).

Variety	Treatment**	Foliar Area (cm2)	Height of Plant Stem (cm)	Stomatal Resistance (s/cm)
CMC 40	NS	63.7 ± 6.4 a	51.6 ± 7.8 a	6.0 ± 0.3 a
	S	52.3 ± 2.1 a	34.0 ± 2.0 a	30 ± 3.4 b
CM 507-37	NS	107.4 ± 6.9 b	39.8 ± 3.0 b	6.0 ± 0.4 a
	S	74.8 ± 11.1 a	29.0 ± 1.7 a	21.1 ± 6.2 b
Factors of ANOVA				
Variety (A)		0.0002	0.0791	0.1571
Treatment (B)		0.0070	0.0055	0.0001
A x B		0.1590	0.4621	0.1647

* Means followed by the same letter are not different at 5 % level (Fisher's PLSD test following ANOVA). a, b: for each variety, column comparison (treatment factor).

** NS: control plants and S: water stressed plants.

In order to limit the influence of parental trophic feeding, four distinct populations of mealybug, reared for 4 generations on the studied host plants well-watered or unwell-watered, were used for determining the development and the fecundity of females of *P. herreni* on CMC 40 and CM 507-37 under the two water status.

For both varieties, plants in water deficit conditions induced a reduction in the duration of female development, and an increase in fecundity, intrinsic natural growth rate (Rm) and in weight **(Table 1.2.2 and Figure 1.2.1).**

Table 1.2.2. Development and fecundity of female of *P. herreni* reared on two varieties of cassava grown with high or low water availability. Duration of development, fecundity, intrinsic natural growth rate (Rm) and weight of female were determined. Newly hatched larvae obtained from four distinct populations of mealybug, reared on the two varieties well-watered or unwell-watered, were used. Data are means* ± SE and results of 2-way ANOVA are given (variety, treatment).

Variety	Treatment**	Duration of development (days)	Fecundity (No. of egg per female)	Rm	Weight of female (mg)
CMC 40	NS	23.4 ± 1.4 b	394.1 ± 34.9 a	0.285 a	1.13 ± 0.04 a
	S	22.4 ± 0.5 a	532.5 ± 39.5 b	0.304 b	1.35 ± 0.03 b
CM 507-37	NS	23.2 ± 0.4 a	420.1 ± 40.0 a	0.286 a	1.29 ± 0.04 a
	S	22.7 ± 1.0 a	496.5 ± 27.9 a	0.299 b	1.45 ± 0.04 b
Factors of ANOVA					
Variety (A)		0.7423	0.9005	0.6103	0.0004
Treatment (B)		0.0032	0.0095	0.0001	0.0001
A x B		0.3942	0.4335	0.4098	0.5

* Means followed by the same letter are not different at 5 % level (Fisher's PLSD test following ANOVA). a, b: for each variety, column comparison (treatment factor).

** NS: control plants and S: water stressed plants.

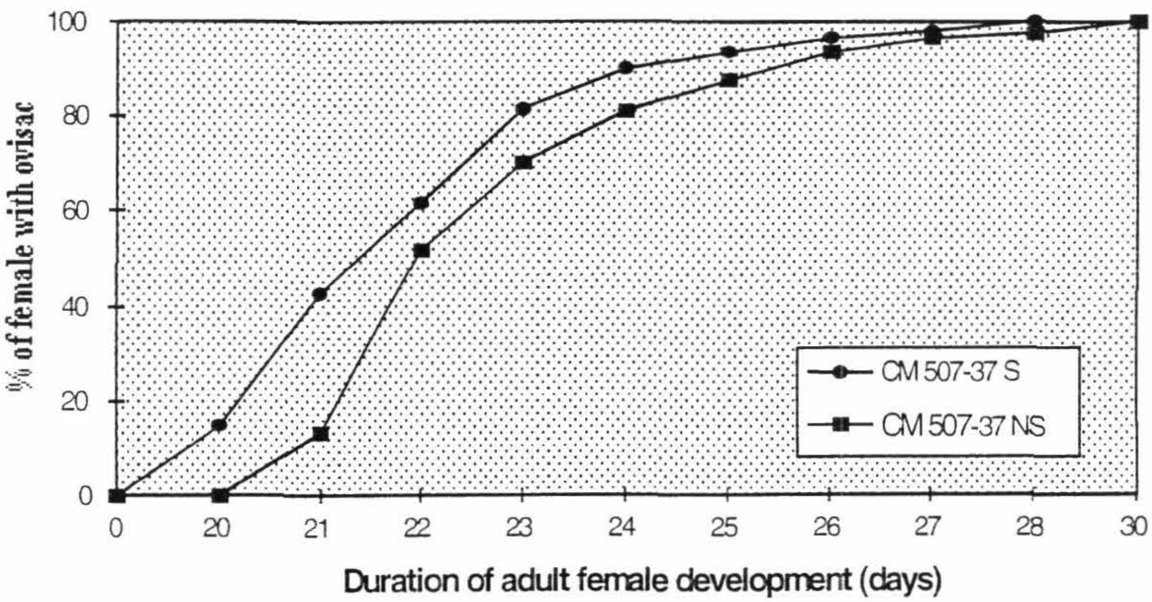

Figure 1.2.1. Percentage accumulated of mature female (*i.e.* beginning to form ovisac) of *P. herreni* reared on two varieties of cassava (CMC 40 and CM 507-37) grown with high (NS) or low (S) water availability.

An analysis of amino acids of cassava leaves infested by mealybug during the experiment mentioned above were realized. Leaf aminoacid composition showed a significant increase in the percentage of free serine, asparagine, glutamine and arginine of leaves from water stressed plants. This result was obtained for the both varieties used and only one amino acid composition is presented in **Figure 1.2.2.**

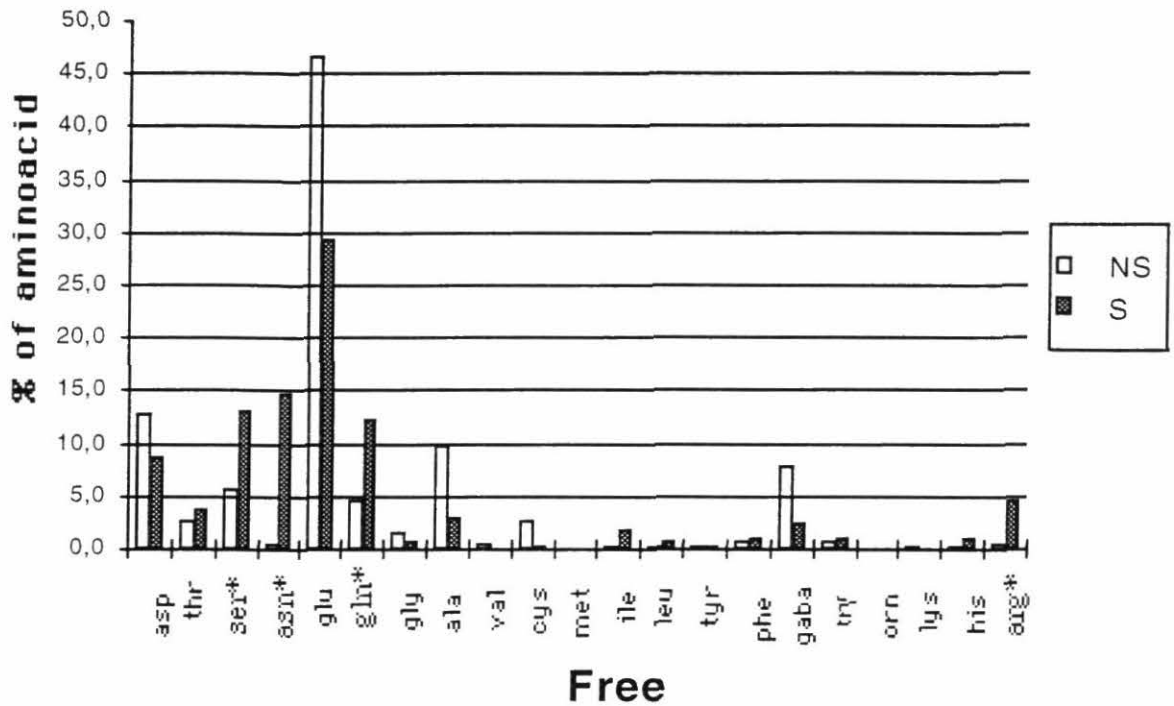

Figure 1.2.2. Composition of free aminoacids of cassava leaves infested by *P. herreni* from control plant (NS) and water stressed plants (S). * Aminoacids which their percentage increased significantly at 5 % level (Fisher test).

By utilizing the artificial diet from Calatayud *et al.* (1998), it was shown that asparagine and arginine played an important role during the development of female of *P. herreni*. In fact, their absence does not allow nymphs to reach the adult stage **(Table 1.2.3)**, but insect aminoacid composition wasn't affected (data not shown), suggesting that the incomplete development is more an "energetic problem" and that mealybugs are physiologically capable of balancing their diet by themselves or by the aid of symbiotic bacteria, which were observed by light microscopy. The effect of the absence of glutamine on the development of females of *P. herreni* reared on an artificial diet is presently in course.

In preliminary conclusion, we can suggest that asparagine and arginine are necessary to the female of *P. herreni* to complete their development and therefore an increase in the concentration of these aminoacids in the diet should favor female's development. This working hypothesis is being verified.

Table 1.2.3. Biological performance of female of *P. herreni* on different diets. Weight and developmental stage of females reared on different diets during 35 days were determined. Data are means* ± SE.

Diet	Weight (mg)	Developmental Stage
Control	0.15 ± 0.02 b	Adult
Without serine	0.20 ± 0.02 b	Adult
Without asparagine	0.04 ± 0.005 a	Third instar
Without arginine	0.05 ± 0.007 a	Third instar

* Means followed by the same letter are not different at 5 % level (Fisher's PLSD test following ANOVA). a, b: column comparison (diet factor).

Sub-output 2. Determine the influence of changes of the nutrition of the cassava mealybug *Phenacoccus herreni* on its parasitoids development *Acerophagus coccois*, *Aenasius vexans* and *Apoanagyrus diversicornis*

The natural enemies of phytophagous insects function and develop in a multitrophic context (Price *et al.*, 1980). Thus their behavior and physiology, which determine their fitness, are influenced by many factors and stimuli derived from the plant (first trophic level) and the phytophagous host (second trophic level)(Vinson, 1976; Takabayashi *et al.*, 1991). A great number of studies have focused on the interactions between plants and pests (Maxwell & Jennings, 1980) and between pests and parasitoids (Waage & Hassell, 1982). In recent years, one common theme of integrated control has been to combine the selection of plant variety with biological control, and therefore, it has also been necessary to study the interactions between herbivorous insect pests and their parasitoids (Price *et al.*, 1980; Van Emden, 1987). In the field, while the parasitoids of the cassava mealybug are present, it frequently registered an increase in the population of the pest on plants during long dry seasons. This result was reported with the mealybug species in Africa, *P. manihoti* by Le Rü *et al.* (1991) and also with *P. herreni* in Brazil (A.C. Bellotti, *pers. comm.*). Such increasing of mealybug population's could be due to biochemical changes in cassava leaves induced by water deficiency, which enhances pest development but not parasitoid development. In order to verify this phenomenon, our research is focused on the importance of drought tolerance mechanisms of cassava, and the changes they might trigger in plant physiology and biochemistry, and what changes this might trigger in mealybug and in parasitoid development. The species of parasitoids used in this study were: *Acerophagus coccois* Smith, *Aenasius vexans* Kerrich and *Apoanagyrus diversicornis* Howard (Hymenoptera: Encyrtidae).

Activity 2.1. Determination of the feeding behavior of parasitized mealybug

In order to identify how some changes of the nutrition of *P. herreni* could influence parasitoid developments, it was necessary to first study the feeding behavior of parasitized mealybugs. The feeding activity was studied by electropenetrography (EPG, DC-system). The parasitoids used in this study prefer to parasitize female of *P. herreni*, and for technical reasons we used female of third instar. Before doing EPG recording, we evaluated the time between parasitism and appearance of mummies for each species of parasitoid studied. According to the results obtained in the **Table 2.1.1**, we decided to realize EPG recording two days before appearance of mummies (*i.e.* at day 6 for *A. coccois* and at day 8 for *A. vexans* and *A. diversicornis*). EPG's registrations showed that the parasitized mealybug did not significantly change their feeding behavior compared with non-parasitized mealybugs, indicating that they feed mainly on phloem sap (data not shown). This result was confirmed by the production of honeydew containing aminoacids until the appearance of mummy.

Table 2.1.1. Time between parasitism and appearance of mummies for three species of parasitoids. Data are means* ± SE.

Parasitoid species	Time (Days)
A. coccois	8.5 ± 0.2 a
A. vexans	10.3 ± 0.2 b
A. diversicornis	10.9 ± 0.3 c

* Means followed by the same letter are not different at 5 % level (Fisher's PLSD test following ANOVA). a, b, c : column comparison (parasitoid species factor).

Activity 2.2. Influence of cassava in water deficit conditions on the parasitism success and on the development of *A. coccois*, *A. vexans* and *A. diversicornis*

In order to study the influence of cassava in water deficit conditions on the parasitism success and development of *A. coccois*, *A. vexans* and *A. diversicornis*, it was necessary to first know which developmental stage of female *P. herreni* are more preferred by the parasitoids. Due to the fact that the youngest developmental stage of *P. herreni*, like first and second instars, are incompatible for the parasitism success of *A. vexans* and *A. diversicornis* due to size incompatibilities, only third instars and adults of *P. herreni* were used. It appeared that the use of adults for parasitism studies was more interesting because all parasitoid species showed an increase in percentage of appearance of mummies and a decrease in percentage of individual deaths (caused by direct feeding or by ovipositor insertion) (**Table 2.2.1**). The percentage of mealybug deaths could be directly related to size incompatibilities. According to the results of the Table 2.2.1, the next studies were done on adult females of *P. herreni*.

Table 2.2.1. Parasitism characteristics of three species of parasitoids according to the developmental stage (third instar and adult) of female of *P. herreni*. The percentages of parasitism (*i.e.* appearance of mummies), of encapsulation, of alive and dead mealybugs were determined.

Parasitoid Species	Developmental Stage of *P. herreni*	% Mummy	% Encapsulation	% Alive Mealybugs*	% Dead Mealybugs **
A. coccois	L3	58.5 a	0 a	23.2 a	18.3 b
	Adult	80.9 b	1.5 a	14.7 a	2.9 a
A. vexans	L3	69.0 a	12.7 b	12.7 b	5.6 a
	Adult	90.9 b	1.1 a	3.5 a	4.5 a
A. diversicornis	L3	50 a	8.4 a	6.9 a	34.7 b
	Adult	64.6 b	17.1 a	9.8 a	8.5 a

For each parasitoid species, the percentages were compared between third instar and adult. Percentages followed by the same letter are not different at 5 % level (Fisher test).

* Alive mealybugs after the parasitism.

** Dead mealybugs caused by direct feeding or ovipositor insertion.

The percentage of parasitism and encapsulation as well as the sex ratio and the tibia longitude of the parasitoids were determined as parameters to evaluate the parasitism success and parasitoid development from mealybugs reared on cassava in water deficit conditions. We determined a percentage of parasitism less important and a percentage of encapsulation more important on mealybugs reared on water stressed plants. The sex ratio and the tibia longitude were not affected by the water status (all data not shown). These results suggest that the mealybugs feeding on cassava in water deficit conditions do not affect the development of the parasitoid but could disturb their attractive behavior. Nevertheless, the low number of replicates do not allow definitive conclusion and this experiment is being confirmed.

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OUTPUT III. NARS CAPACITY TO DESIGN AND EXECUTE INTEGRATED CROP MANAGEMENT RESEARCH AND DEVELOPMENT PROJECTS STRENGTHENED.

The purpose of Output III is to improve the capacity of farmer groups and technical personnel from research and extension agencies in Northeast Brazil to implement technology generation and transfer activities based on the use of farmer participatory methods. This capacity building process has been implemented since the inception of the PROFISMA project and the period covered by this report (Jan. to Sept. 1998) represented the last participation of a CIAT staff member directly in charge of their activity within the PROFISMA project.

Sub-output 1. Implementation of Farmer Participatory Research Activities with Researchers Extensionists and Farmer Groups.

Activity 1.1. Participatory evaluation of experiment at the CIAL Colonia Agrícola Roberto Santos (Bahia).

The adaptation of participatory methods for evaluating trials established and administered by the CIAL's continued successfully during 1998. During the harvest of the 1996-98 trial at the CIAL Colonia Agrícola Roberto Santos six cassava varieties were evaluated using the opened interview methodology. This evaluations included the participation of 16 farmers and gave very useful information related to the criteria which is important for cassava farmers in the region when they make choices about cassava varieties. **Table 1.1.1.** presents the content of the open-ended evaluation interviews. This analysis is based on the number of times that each criteria is spontaneously mentioned by farmers. It can be observed that farmers gave the highest importance to **root form and size** (peeling the roots before processing is a labor that demands a lot of time), **height of the plant** (they prefer simple branching varieties that facilitate hand weeding) **root yield** (amount of roots), **processing yield** (starch and dry matter content) and **pulp colour** (white cassava flour is easy to commercialize). Criteria with some importance for farmers were top part yield, and pest and disease tolerance/resistance. Criteria considered not very important for farmers were flour flavour, precocity and harvest period.

Boxes 1 to 6 present the opinions expressed by farmers in relations to each one of the six cassava varities included in the trial. The top part of the Box represents the comments made by farmers, using their own words. The lower part of the box refers to the same criteria, as it is known by researchers and extensionists.

Another form of interpreting this information is by means of the accumulated frequency method in which the evaluation made by farmers is combined with an analysis of ranking preferences. This method gives information about the probability that a given variety or technology has of being selected by farmers as their 1^{st}, 2^{nd}, etc. option. **Tables 1.1.2 to 1.1.7** and **Figures 1.1.1** to **1.1.6** present the cummulative probability of preference for the six cassava varieties evaluated by the CIAL Colonia Agrícola Roberto Santos.

Table 1.1.1. Analysis of content of open-ended evaluation interviews with farmers of the CIAL Colonia Agrícola Roberto Santos (Bahia). Six cassava varieties. N = 16 farmers.

Farmers' Criteria	Frequency Mentioned [1]	
	N	%
Root form and size [2]	76	79
Height of the plant [3] (branching characteristics)	70	773
Root yield	69	72
Processing yield [4]	65	68
Pulp colour [5]	54	56
Top part yield [6]	39	41
Resistance to pest and disease [7]	18	19
Flour flavour	5	5
Flour quality	4	4

1 Based on 96 (16 farmers and six varieties)
2 Cassava roots long, thick, big, are preferred by farmers because they facilitate the peeling operation during the processing into cassava flour
3 Farmers do not appreciate cassava plants with low branching characteristics. They make more difficult the hand-weeding activities. They also occupy more space
4 Farmers due to its low processing yield do not prefer cassava roots with high water contents. Root content of dry matter and starch are very important parameters for farmers0
5 Cassava varieties with yellow pulp colour give yellow colour flavour, which is not good for commercialisation
6 Cassava varieties with good top part development are preferred by farmers for its use in animal feeding
7 According to farmers' criteria, cassava varieties with low branching characteristics are more susceptible to pest attack

Box 1.

CIAL: COLONIA AGRICOLA ROBERTO SANTOS

EXPERIMENTAL CYCLE 1996-97

OPEN-ENDED EVALUATION OF SIX CASSAVA VARIETIES

Identification of the Variety: *Platina*

Agricultor: 16 farmers

FARMERS COMMENTS	*# of farmers*
A. " Rende bem na farinha. boa na massa, amido alto, bom rendimento"	16
B. " É boa de raspar, tem a pele fragil, raízes grossas, de bom tamanho"	15
C. " Rende bem, é carregadeira"	12
D. "Estirada, suspende bem, facil de selar, boa de capinar, manivas não esgalham	10
E. "Dá farinha de cor alvo"	8
F. "Rende na rama"	6
G. "Farinha de sabor bom, gostoso"	5
H. "Fácil de colher"	2
I. "Fraca para pragas, dá muita doença nas folhas"	2

Codes for comments

Criteria	Positive Aspects	Negative aspects
A. Processing yield	+ good (good root to flour conversion factor)	
B. Root form and size	+ good (facilitates root processing)	
C. Root yield	+ good (good root yield)	
D. Branching height	+ good (facilitate hand weeding)	
E. Pulp colour	+ good (white flour)	
F. Top part yield	+ good	
G. Flour flavour	+ good (flour with good flavour)	
H. Easy to harvest	+ good (easy to harvest)	
I. Resistance to pest and disease		- bad (very susceptible to pest and disease attack in the leaves)

Note: The letter in front of each farmer comment corresponds to the same criteria in the lower part of the Box

Box 2.

CIAL: COLONIA AGRICOLA ROBERTO SANTOS

EXPERIMENTAL CYCLE 1996-97

OPEN-ENDED EVALUATION OF SIX CASSAVA VARIETIES

Identification of the Variety: 194-16

Agricultor: 16 farmers

FARMERS COMMENTS	*# of farmers*
A. " É boa de raspar, é mais amena, fácil de descascar, boa forma de raízes, grandes	16
B. " Bom rendimento em farinha, é enxuta, boa na massa, farinha pesada, matéria seca muito boa, boa de peso	14
C. " Parte aérea é boa para trato cultural, boa de capina, boa de selar porque não é esgalhadeira, é linheira, sobe só uma galha	11
D. " Bom rendimento de raíz "	11
E. " Boa produção de parte aérea para maniva semente e ração animal"	11
F. " Farinha de muito boa qualidade, cor alvo, polpa branca, gostossa "	10
G. " Muito resistente a pragas e doenças "	4
H. " Qualquer epoca é boa de colher, aguenta na roça, pode colher com até três anos	3
I. " Não apodrece "	1
J. " É uma variedade mais cedera, cresce mais rápido e mais cedo "	1

Codes for comments

Criteria	Positive Aspects	Negative aspects
A. Root form and size	+ good (facilitates root processing)	
B. Processing yield, dry matter content	+ good (good root to flour conversion factor)	
C. Branching height	+ good (facilitate hand weeding)	
D. Root yield	+ good (good root yield)	
E. Top part yield	+ good (good yield for seed and animal feeding)	
F. Pulp colour, flour colour	+ good (white flour)	
G. Resistance to pest and disease	+ good	
H. Harvest period	+ good (could be harvested at different periods)	
I. Resistance to root rot	+ good	
J. Precocity, earlyness	+ good (grows fast)	

Note: The letter in front of each farmer comment corresponds to the same criteria in the lower part of the Box.

Box 3.
CIAL: COLONIA AGRICOLA ROBERTO SANTOS
EXPERIMENTAL CYCLE 1996-97
OPEN-ENDED EVALUATION OF SIX CASSAVA VARIETIES
Identification of the Variety: Voadeira
Agricultor: 16 farmers

FARMERS COMMENTS	*# of farmers*
A. " É boa de raspar, é mais amena, fácil de descascar, raíz estirada , boa forma , grandes "	16
B. " Parte aérea é boa para trato cultural, boa de capina, boa de selar porque não é esgalhadeira, não empata	12
C. " Bom rendimento de raíz "	11
D. " Farinha de muito boa qualidade, cor alvo, polpa branca, gostossa "	10
E. " Boa produção de parte aérea para maniva semente e ração animal"	9
F. " Muito molhada, frouxa de peso "	8
G. " Resistente a pragas e doenças "	3
H. " Anda rápido, cresce cedo "	1

Codes for comments

Criteria	Positive Aspects	Negative aspects
A. Root form and size	+ good (facilitates root processing)	
B. Branching height	+ good (facilitate hand weeding)	
C. Root yield	+ good (good root yield)	
D. Pulp colour, flour colour	+ good (white flour)	
E. Top part yield	+ good (good top part yield for seed and animal feeding)	
F. Processing yield, dry matter content, starch content		- bad (low root to flour conversion factor)
G. Resistance to pest and disease	+ good	
H. Precocity, earliness	+ good (grows fast)	

Note: The letter in front of each farmer comment corresponds to the same criteria in the lower part of the Box.

Box 4.
CIAL: COLONIA AGRICOLA ROBERTO SANTOS
EXPERIMENTAL CYCLE 1996-97
OPEN-ENDED EVALUATION OF SIX CASSAVA VARIETIES
Identification of the Variety: 128-08
Agricultor: 16 farmers

FARMERS COMMENTS	*# of farmers*
A. " Não progressa de raízes, pouca quantidade de raízes, é uma mandioca que não carrega, não tem rendimento	14
B. " Não presta para capina, parte aérea é *muito esgalhadeira*, porte baixo, difícil de limpa, ruim de selar, abre muito e os velhos já não temos espinhaço para capinar"	12
C. " Difícil de raspar, as raízes são entrecortadas, tortas e a casca é muito grudada"	12
D. " Muito molhada e isso não ajuda, ruim de amido "	8
E. " Muito susceptível a doenças, parte aérea muito apetecida pelo tanajoá, cria mais doença quando a parte aérea abre muito e o cultivo fica fechado, muito doentía, não resiste a praga "	7
F. " Produção de parte aérea é fraca, não gosoto da maniva, a parte aérea é ruím, a parte aérea não aguenta tempo, seca logo, *ruím para tirar semente*	7
G. " Boa qualidade da farinha, farinha de cor amarela, a cor é boa, boa aceitação no mercado, a polpa é amarela	6
H. " A cor amarela da farinha não é boa para o mercado "	3
H. " *Farinha taluda, cheia de fibras*, comprida, não redondinha "	2

Codes for comments

Critéria	Positive Aspects	Negative aspects
A. Root yield		- poor (low root yield)
B. Branching height		- bad (difficult for hand weeding)
C. Root form and size		- bad (difficult for peeling)
D. Processing yield, dry matter content, starch content		- bad (low root to flour conversion factor)
E. Resistance to pest and disease		- poor (susceptible to pest and disease attack)
F. Top part yield		- poor (low top part yield)
G. H. Pulp colour, flour colour	+ good (white flour) (6)	- bad (yellow colour is not good) (3)
H. Flour quality		- bad (too much fibre)

Note: The letter in front of each farmer comment corresponds to the same criteria in the lower part of the Box.

Box 5.

CIAL: COLONIA AGRICOLA ROBERTO SANTOS

EXPERIMENTAL CYCLE 1996-97

OPEN-ENDED EVALUATION OF SIX CASSAVA VARIETIES

Identification of the Variety: 47-19

Agricultor: 16 farmers

FARMERS COMMENTS	*# of farmers*
A. " Difícil de raspar, não tem espaço para a faca, não rende, raíz curta e pequena, acanhada"	15
B. " Não gosto dela porque tem mais parte aérea do que raiz, é ruim em rendimento de raíz "	12
C. " Parte aérea é boa, tem bastante folha, se a formiga atacar, fica folha, boa de capina, boa de selar porque não é esgalhadeira, tem tamanho médio, é linheira e isso facilita colheita	10
D. " Farinha muito molhada, rende menos na massa "	10
E. " A cor da polpa é amarela e da farinha muito ruím, não é boa para o mercado"	8
F. " Boa quantidade de maniva "	4
G. " Farinha taluda, cheia de fibras, comprida, não redondinha "	2

Codes for comments

Criteria	Positive Aspects	Negative aspects
A. Root form and size		- bad (difficult for peeling)
B. Root yield		- poor (low root yield)
C. Branching height	+ good (low branching	
D. Processing yield, dry matter content, starch content		- bad (low root to flour conversion factor)
E. Pulp colour, flour colour		- bad (yellow colour is not good)
F. Top part yield	+ good top part yield for seed and animal feeding)	
G. Flour quality		- bad (too much fibre)

Note: The letter in front of each farmer comment corresponds to the same criteria in the lower part of the Box.

Box 6.
CIAL: COLONIA AGRICOLA ROBERTO SANTOS
EXPERIMENTAL CYCLE 1996-97
OPEN-ENDED EVALUATION OF SIX CASSAVA VARIETIES

Identification of the Variety: 189-11

Agricultor: 16 farmers

	FARMERS COMMENTS	*# of farmers*
A	" Parte aérea é muito esgalhadeira e dificulta a capina, ruím de selar porque e baixa, abre muito e precisa mais espaço para plantar, ajuda a doenças, abre no méio, não desenvolve "	15
B	" Não gostei da cor da polpa amarela "	9
C	" Raiz muito molhada, rende menos na massa, fraca em farinha "	9
D	" Facil de raspar, casca lisa, descasca fácil, raíz estirada "	7
E	" Carrega pouco, não tem rendimento"	6
F	" Parte aérea boa para ração animal "	2
G	" A parte aérea é muito atacada por ácaro "	2

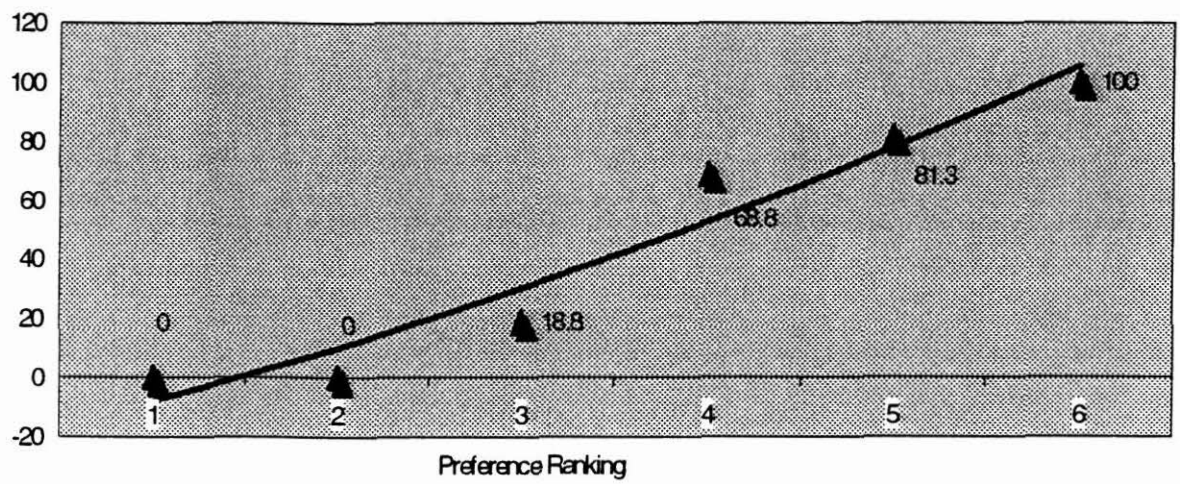
Codes for comments

Criteria	Positive Aspects	Negative aspects
A. Branching height		- bad (difficult for hand weeding)
B. Pulp colour, flour colour		- bad (yellow colour is not good)
C Processing yield, dry matter content, starch content		- bad (low root to flour conversion factor)
D. Root form and size	+ good (easy for peeling)	
E. Root yield		- poor (low root yield)
F Top part yield	+ good (good top part yield for animal feeding)	
G. Resistance to pest and disease		- poor (susceptible to pest and disease attack)

Note: The letter in front of each farmer comment corresponds to the same criteria in the lower part of the Box

Table 1.1.2. Comparative analysis of preferences / acceptance for the Variety: Platina (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	0	0	0
2	0	0	0
3	3	18,8	18,8
4	8	50	68,8
5	2	12.5	81,3
6	3	18,8	100
Totals	**16**	**100**	

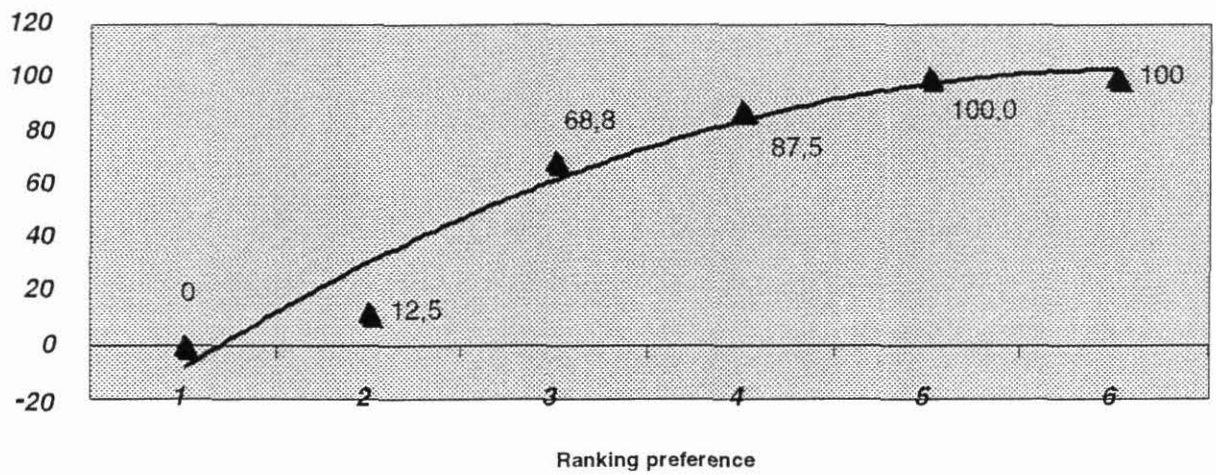


COPAL: Colonia Agrícola Roberto Santos, Experimental cycle 1996-97.

Figure 1.1.1. Probability of acceptance / preference of the variety: Platina (%).

Table 1.1.3. Comparative analysis of preferences/acceptance of the variety: 47-19.

Preference Ranking	Frequency	Probability	Cumulative Probability
1	0	0	0
2	2	12,5	12,5
3	9	56,3	68,8
4	3	18,8	87,5
5	2	12,5	100
6	0	0	100
Totals	**16**	**100**	



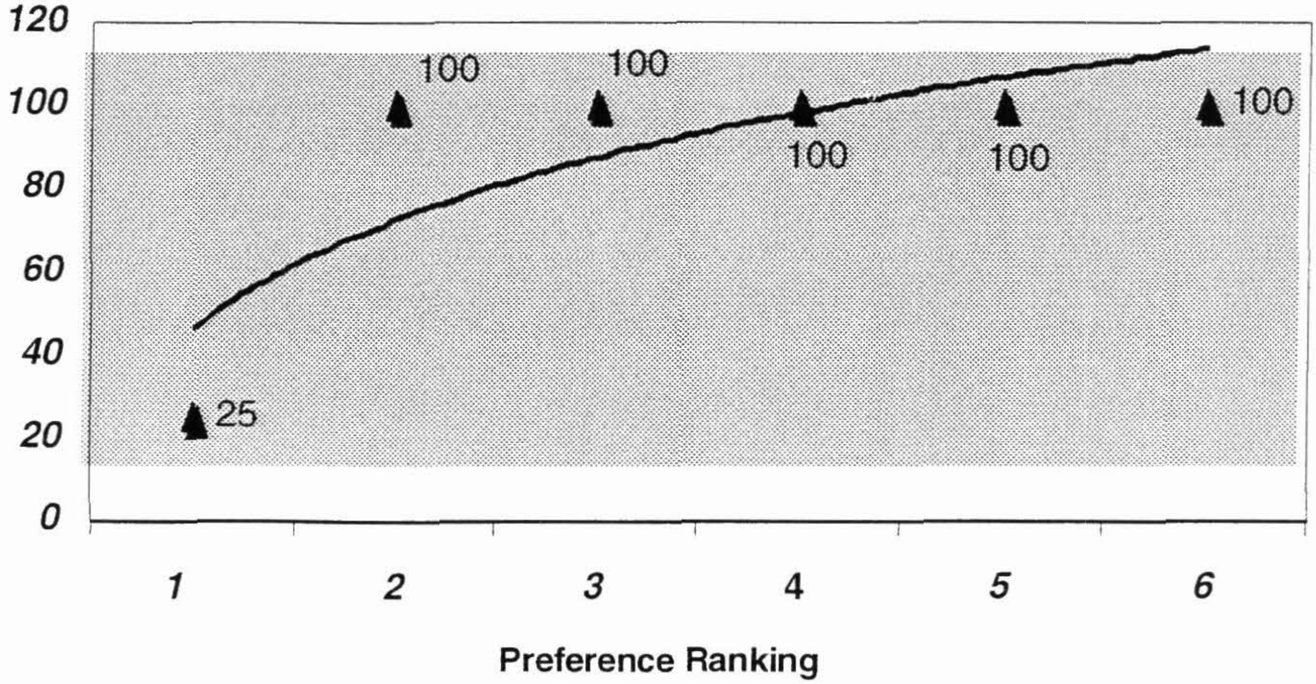
COPAL: Colonia Agrícola Roberto Santos, Experimental cycle 1996-97)

COPAL: Colonia Agrícola Roberto Santos, Experimental Cycle 1996-97.

Figure 1.1.2. Probability of acceptance / preference of the variety 47-19 (%).

Table 1.1.4. Comparative analysis of preference / acceptance of the variety 194-6 (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	4	25	25
2	12	75	100
3	0	0	100
4	0	0	100
5	0	0	100
6	0	0	100
Totals	**16**	**100**	

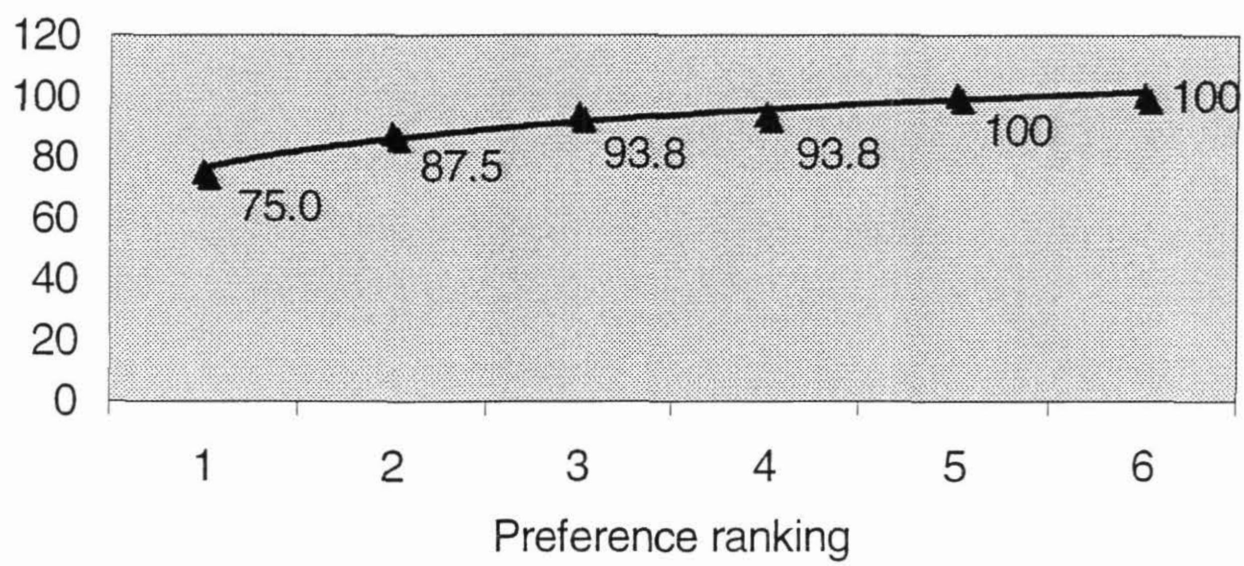


COPAL: Colonia Agrícola Roberto Santos. Experimental cycle 1996-97.

Figure 1.1.3. Cumulative probability of preference/acceptance of the variety: 194-6 (%).

Table 1.1.5. Comparative analysis of preference / acceptance of the variety: Voadeira (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	12	75	75
2	2	12,5	87,5
3	1	6,3	93,8
4	0	0	93,8
5	1	6,3	100
6	0	0	100
Totals	16	100	

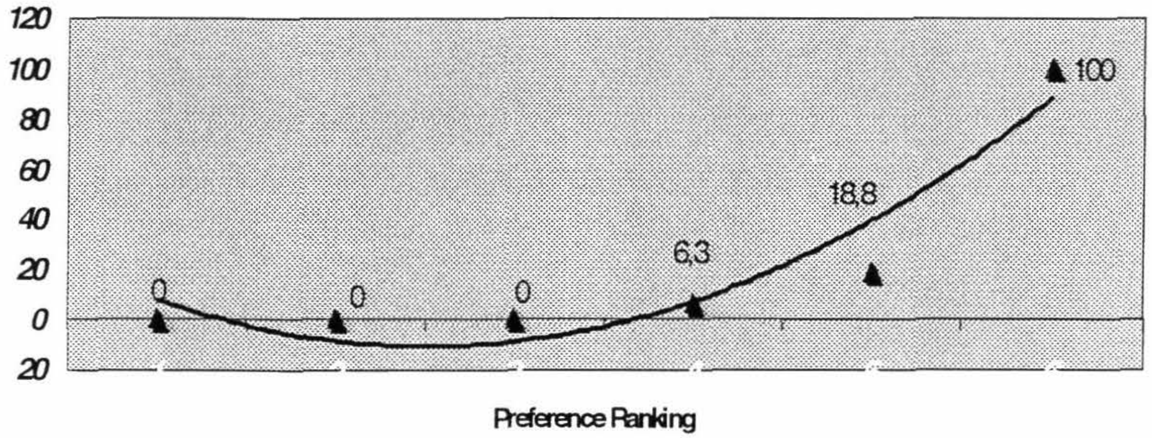


COPAL: Colonia Agrícola Roberto Santos, Experimental cycle 1996-97).

Figure 1.1.4. Cumulative probability of preference / acceptance of the variety: Voadeira (%).

Table 1.1.6. Comparative analysis of preference / acceptance of the variety: 128-08.

Preference Ranking	Frequency	Probability	Cumulative Probability
1	0	0	0
2	0	0	0
3	0	0	0
4	1	6,3	6,3
5	2	12,5	18,8
6	13	81,3	100
Totals	**16**	**100**	

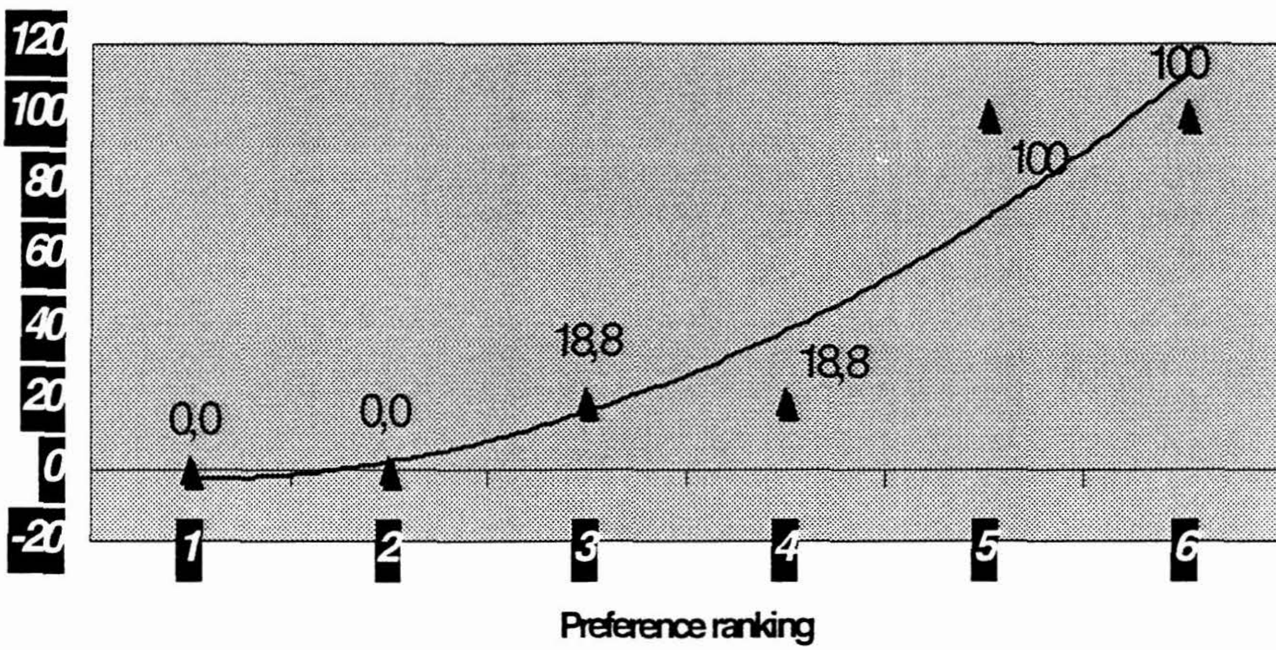


COPAL: Colonia Agrícola Roberto Santos. Experimental cycle 1996-97

Figure 1.1.5. Cumulative probability of preference/acceptance of the variety: 128-08 (%).

Table 1.1.7. Comparative analysis of preference / acceptance of the variety 189-11.

Preference Ranking	Frequency	Probability	Cumulative Probability
1	0	0	0
2	0	0	0
3	3	18,8	18,8
4	3	18,8	37,6
5	10	62,5	100
6	0	0	100
Totals	**16**	**100**	



COPAL: Colonia Agrícola Roberto Santos. Experimental cycle 1996-97.

Figure 1.1.6. Cumulative probability of preference/acceptance of the variety: 189-11 (%).

Activity 1.2. Participatory evaluation of experiments at CIAL Buril (Bahia).

The CIAL established at the community Buril (Bahia) harvested its second experiment in April. The objective of this experiment was to observe the resistance/tolerance of six cassava varieties to cassava green mite damage. Four of the varieties were introduced from EMBRAPA/CNPMF and the other two were obtained locally. The experiment was harvested with 21 months and the results obtained, for most of the varieties, were rather low. Farmers argued that low yields were caused by heavy rains during the last month prior to harvest (starch from the roots is used to produce new top part) and late harvest because the soil was too dry and the experiment could not be harveste at the right time. The results are presented in **Figure 1.2.1.**

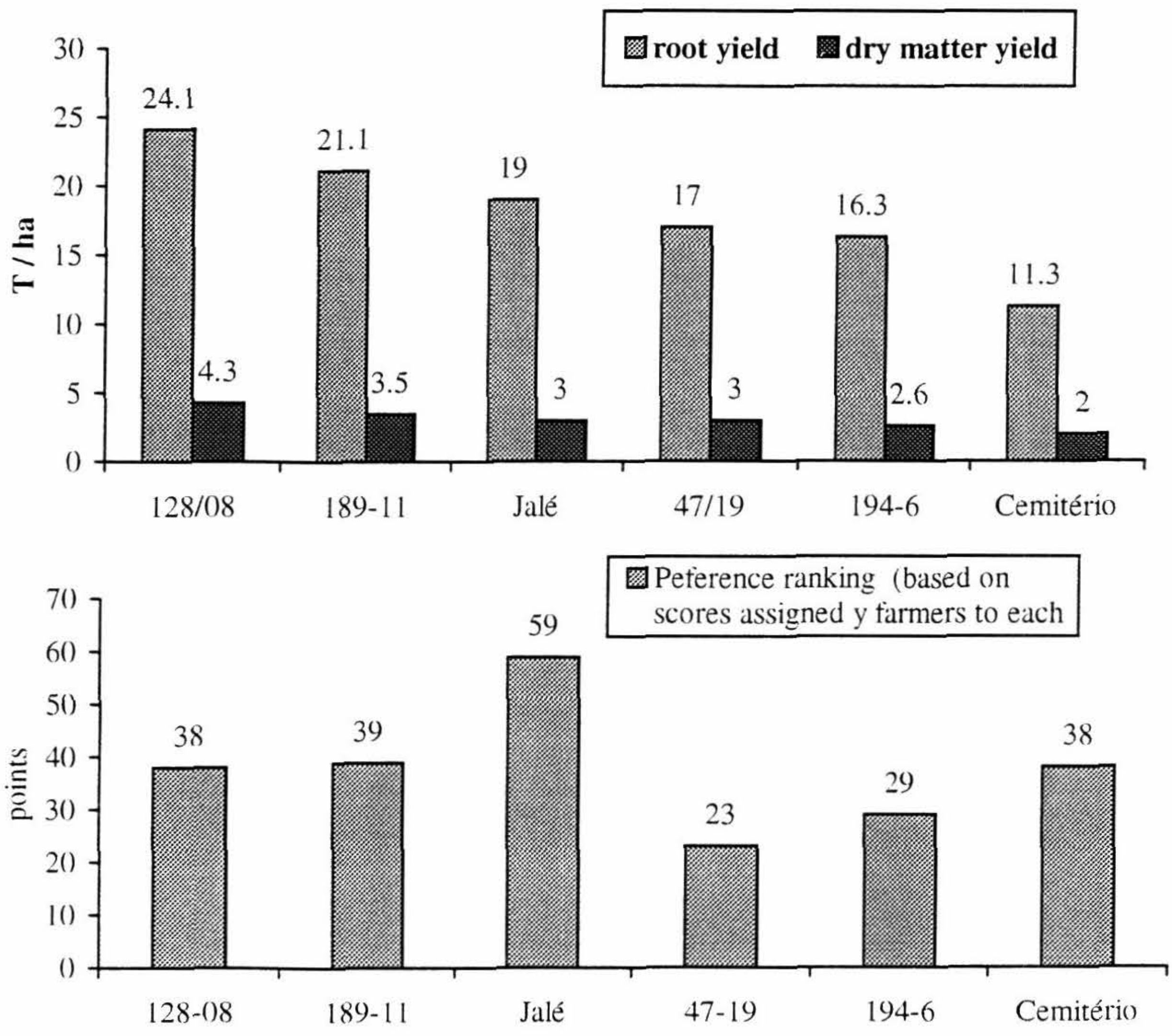
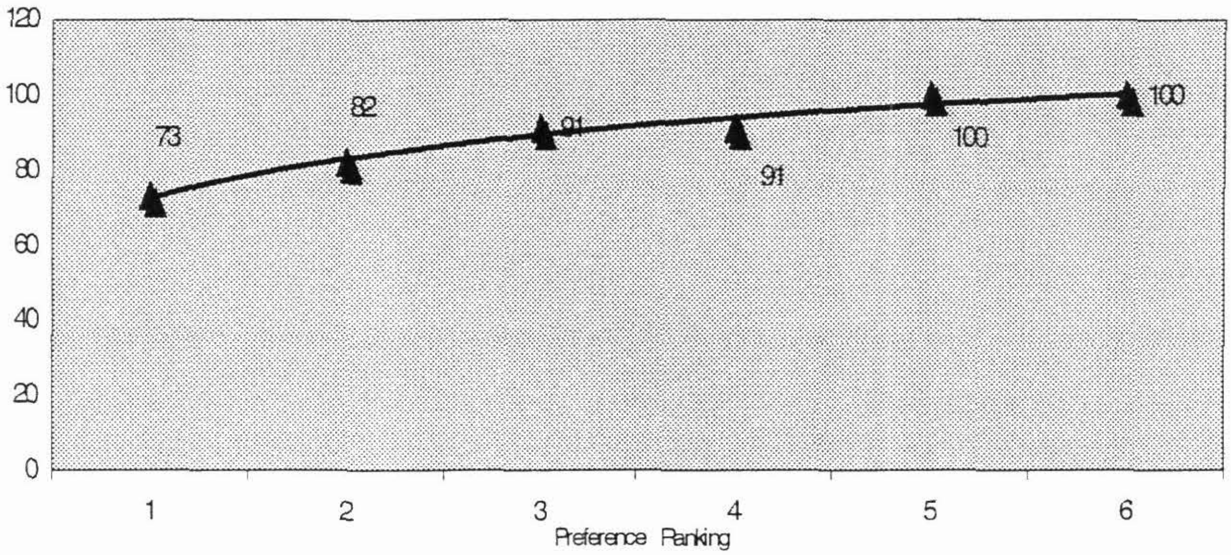


Figure 1.2.1. Evaluation of six varieties of cassava for its tolerance / resistance to cassava green mite Agronomic and preference ranking evaluation COPAL: Buril, Crisópolis, Bahia. Agronomic cycle 1996-97.

This information could also be interpreted using the cummulative frequency method. **Tables 1.2.1 to 1.2.6** present the results of the evaluation made by 11 farmers of this CIAL of six cassava varieties. This information will be very useful for cassava breeders at EMBRAPA/ CNPMF who are currently working on the selection of elite cassava varieties for this region of NE Brazil.

Table 1.2.1. Comparative analysis of preference / acceptance for the Variety: Jalé (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	8	0,73	73
2	1	0,09	82
3	1	0,09	91
4	0	0	91
5	1	0,09	100
6	0	0	100
Totals	**11**	**100**	

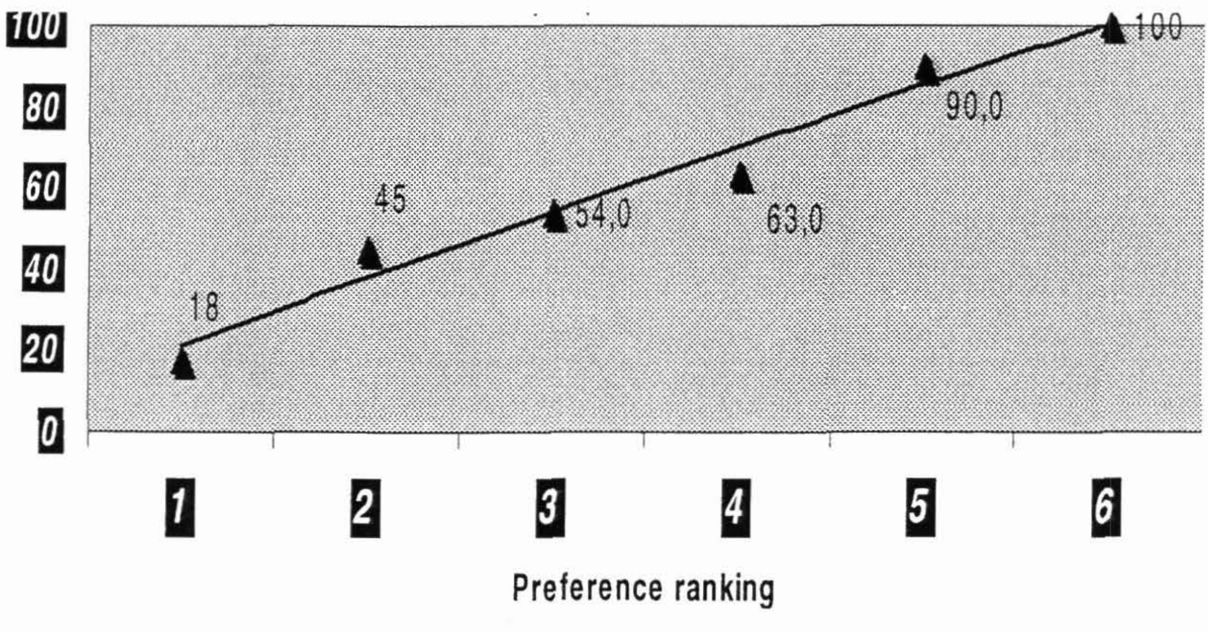


COPAL: Buril, Experiment cycle 1996-97.

Figure 1.2.1. Probability of acceptance of the variety: Jalé (%).

Table 1.2.2. Comparative analysis of preference / acceptance for the Variety: 189-11 (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	2	0,18	18
2	3	0,27	45
3	1	0,09	54
4	1	0,09	63
5	3	0,27	90
6	1	0,09	100
Totals	**11**	**100**	

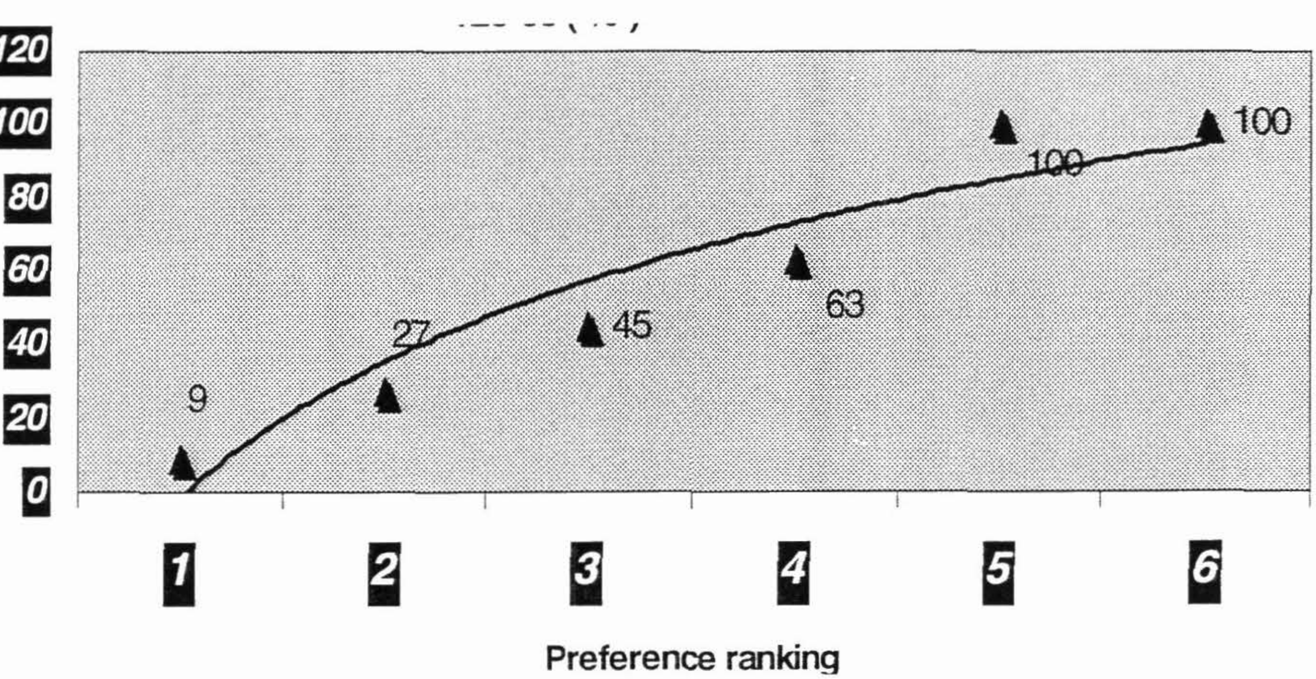


COPAL: Buril, experimental cycle 1996-97.

Figure 1.2.2. Probability of acceptance of the variety: 189-11 (%).

Table 1.2.3. Comparative analysis of preference / acceptance for the Variety: 128-08 (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	1	0,09	9
2	2	0,18	27
3	2	0,18	45
4	2	0,18	63
5	4	0,36	100
6	0	0	100
Totals	**11**	**100**	

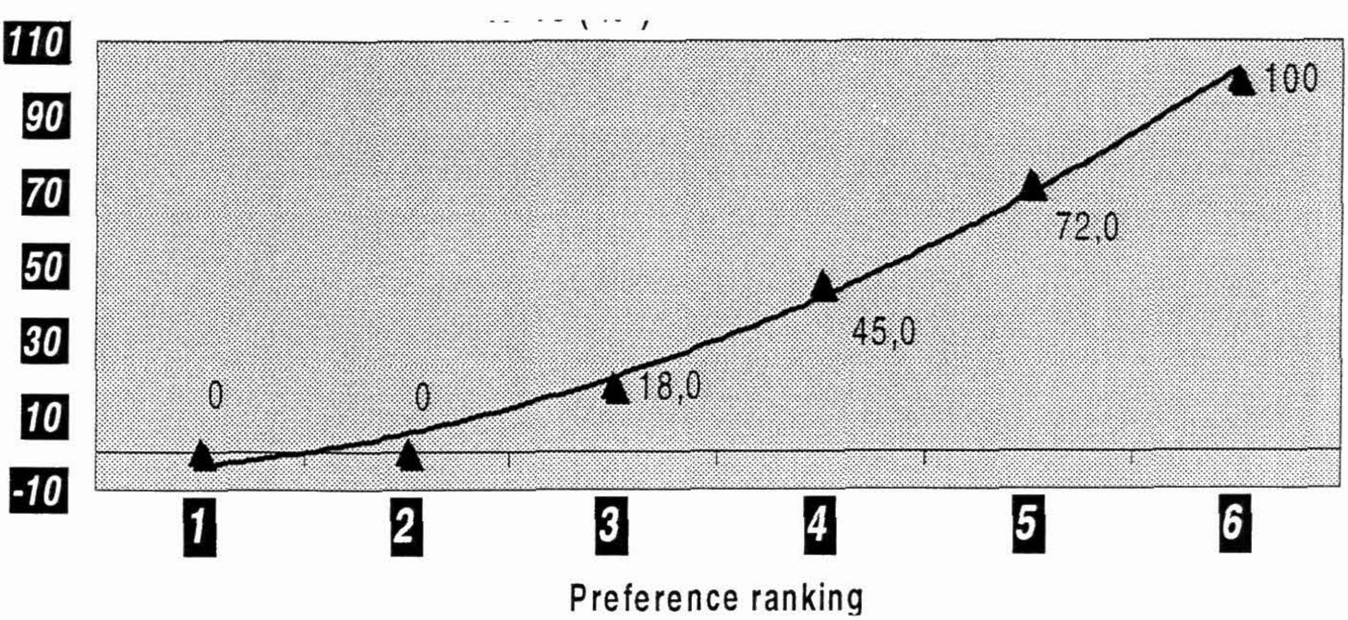


COPAL: Buril, experimental cycle 1996-97.

Figure 1.2.3. Probability of acceptance of the variety: 128-08 (%).

Table 1.2.4. Comparative analysis of preference / acceptance for the Variety: 47-19 (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	0	0	0
2	0	0	0
3	2	0,18	18
4	3	0,27	45
5	3	0,27	72
6	3	0,27	100
Totals	**11**	**100**	

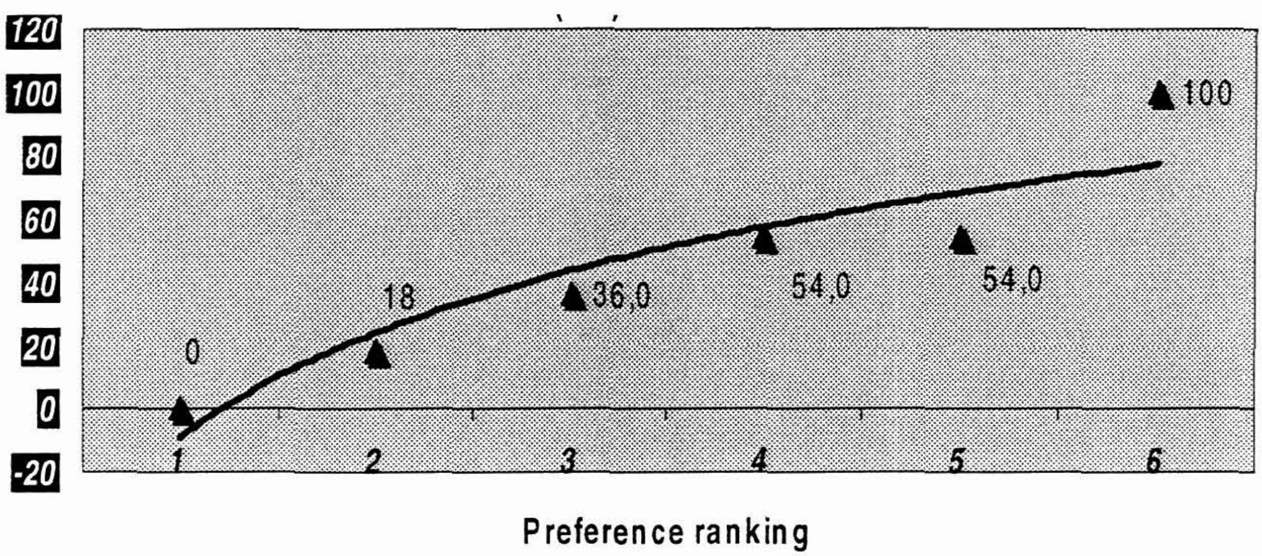


COPAL: Buril, experimental cycle 1996-97.

Figure 1.2.4. Probability of acceptance of the variety: 47-19 (%).

Table 1.2.5. Comparative analysis of preference / acceptance for the Variety: 194-16 (%).

Preference Ranking	Frequency	Probability	*Cumulative* Probability
1	0	0	0
2	2	0,18	18
3	2	0,18	36
4	2	0,18	54
5	0	0	54
6	5	0,45	100
Totals	**11**	**100**	

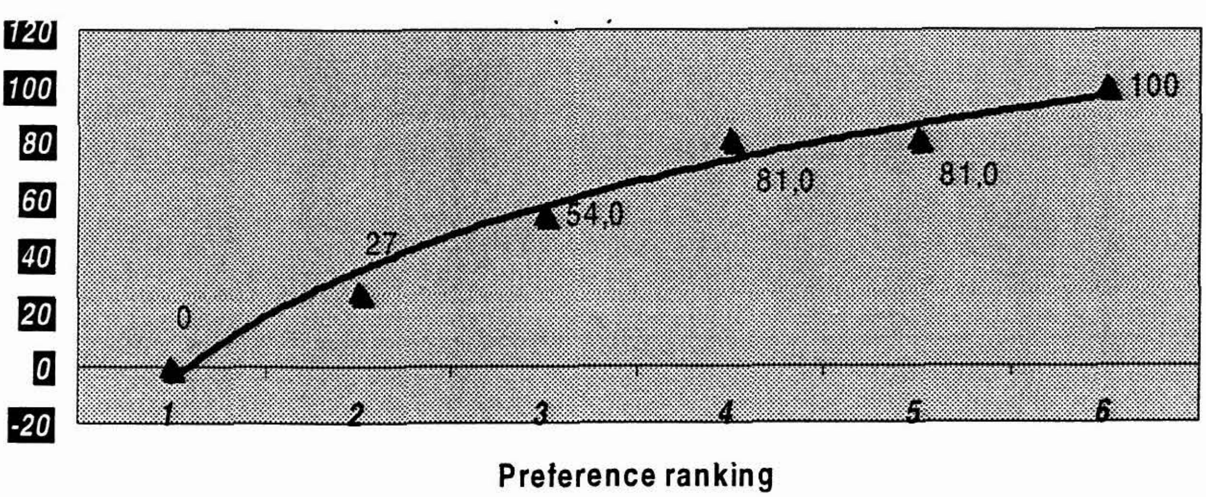


COPAL: Buril, experimental cycle 1996-97.

Figure 1.2.5. Probability of acceptance of the variety: 194-16 (%).

Table 1.2.6. Comparative analysis of preference / acceptance for the Variety: Cemitério (%).

Preference Ranking	Frequency	Probability	Cumulative Probability
1	0	0	0
2	3	0,27	27
3	3	0,27	54
4	3	0,27	81
5	0	0	81
6	2	0,18	100
Totals	**11**	**100**	



COPAL: Buril, experimental cycle 1996-97.

Figure 1.2.6. Probability of acceptance of the variety: Cemitério (%).

Activity 1.3 Participatory evaluation of experiments at CIAL Cadete (Bahia).

Copal: Cadete, Cruz Das Almas, Bahia- Experiment 1996-97

After the first experiment (cycle 1995/96), the COPAL at the community Cadete decided to decided to test five cassava varieties introduced from EMBRAPA/CNPMF and to compare them with their preferred local variety. The COPAL decided to plan the experiment without any fertiliser to observe the behaviour of the varieties under extreme conditions. The six varieties were planted with spacing 1.0 x 1.0, with three repetitions and randomised blocks. The experiment was harvested with 19 months. As expected, root yields were very low. Besides the lack of fertiliser, the experiment was not well taken care of, especially in relation to weed control. Lack of land is a striking characteristic in this community and the COPAL was forced to plant the experiment in a farmer's field, which is not visited by some members of the COPAL due to personal conflicts. The farmer that owns the field did not give enough attention to the experiment and this contributed to low yields. Nonetheless, two of the clones introduced from EMBRAPA/CNPMF, *189-11 and 192-13,* gave the best yields with 11,4 and 10,4 t per ha, respectively. Two local varieties, *Arrebentaburro and Cigana,* although with lower yields (9,8 and 8,1 t per ha, respectively), presented higher dry matter content than the two introduced clones. Farmers made a selection of the best three varieties and chose the two clones and *Arrebentaburro* as the three varieties with which they would like to continue the research work. Additionally, they concluded that the local variety Cigana, under the conditions of the community, is not worth to plant without using fertilisers. **Figure 1.3.1** presents these results.

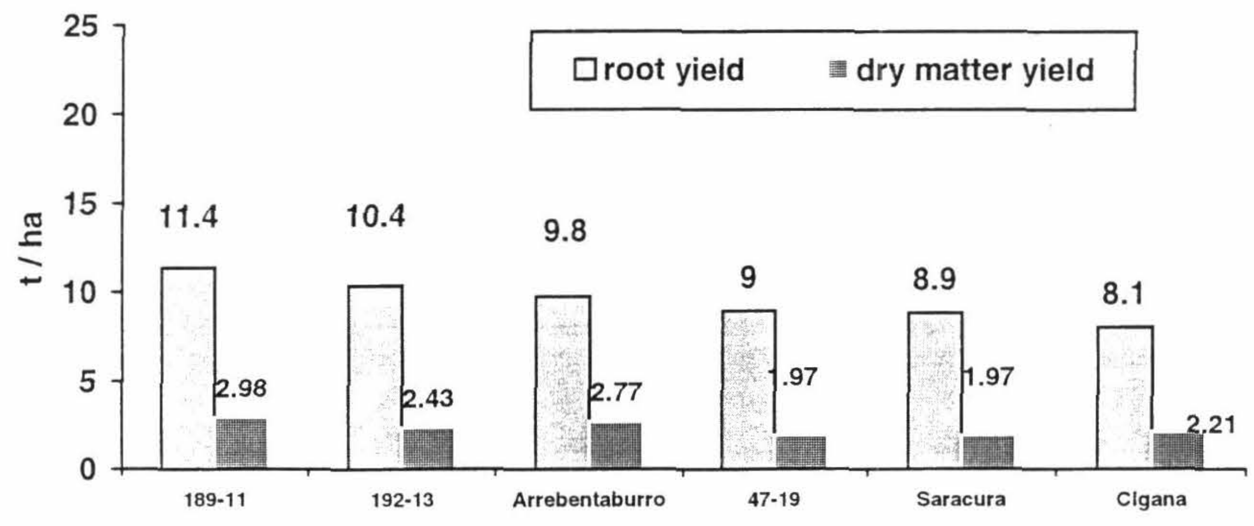


Figure 1.3.1. Evaluation of six varieties of cassava under stress conditions (no fertilisers) COPAL: CADETE, Cruz das Almas, Bahia. Growing cycle 1996-97.

Activity 1.4. Participatory evaluation of experiments at CIAL Campina Nora (Pernambuco).

Copal: Campina Nova, Vitoria De Santo Antão, Pernambuco Experiment 1995-96

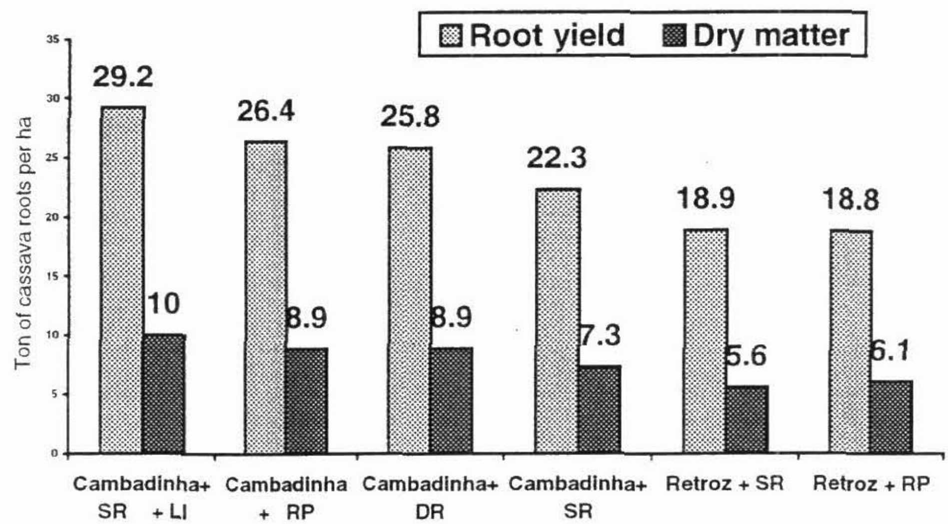
Planting Date: 21/06/95, Harvest Date: 30/09/96; 15 Months

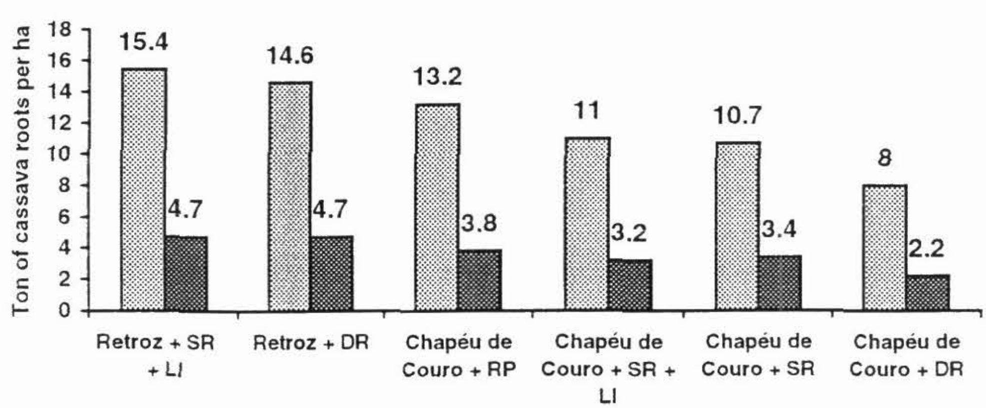
In the community Campina Nova, the cassava crop is planted since many years ago. During the last 5 years, cassava root rot has become one of the main constraints forcing many farmers to stop planting the crop. The COPAL selected root rot as their main problem. With technical support from IPA (Instituto de Pesquisa Agricola) and Emater-PE (Empresa de Assistencia Técnica e Extensão Rural), farmers designed an experiment to test some technologies with potential to control this disease. These technologies have not yet been tested yet in the community. Planning meetings with participation of the COPAL members, other farmers, researchers and extension workers defined 12 treatments to be included in the experiment. These treatments included: 3 cassava varieties, one local (*Retroz*) and two introduced from IPA germplasm bank (*Cambadinha* e *Chapeu de Couro*). These three varieties were planted using different planting arrangements and cultural practices. The experiment was planted in three different sites located at short distances between them. Harvest was conducted when the experiment completed 15 months. Figures 18 and 19 present the results obtained in the agronomic evaluation and also in the preference ranking exercise. A preliminary discussion of these results allowed the following conclusions:

a) The variety *Cambadinha*, introduced in the community for the first time, obtained the best results for all the treatments included in the experiment (29.2 ton/ha).
b) The use of lime in the traditional planting system gave higher yields only for the variety *Cambadinha*.
c) Planting in double rows system gave higher yields for the variety *Cambadinha*, when compared with the traditional system (single row). On the contrary, the other two varieties, *Retroz e Chapéu de Couro* presented lower yields when planted in double rows.
d) The ridges planting system presented higher yields for the varieties *Cambadinha and Chapéu de Couro* in comparison with the traditional system. The variety *Retroz* did not show any yield difference using the ridges treatment.
e) The introduced variety *Chapéu de Couro* presented lower yields for all the treatments of the experiment showing a very poor adaptation to specific growing conditions in the community

To stimulate farmer's participation in the evaluation of the experiment, a preference ranking methodology was used. Results obtained indicated the degree of difficulty of using this methodology when the experiment includes a very large number of treatments. In these conditions, it results almost impossible to obtain an objective evaluation of the criteria that farmers use to rank their best preferences. The results obtained did allow farmers to classify the treatments in two main groups: a) the more attractive to them and b) those they rejected. Within the first group, they selected four treatments and the other eight treatments received a low preference by them. The best four treatments were those that included the variety *Cambadinha*, a point that confirmed the excellent results obtained with this variety during the agronomic evaluation. This variety was planted in the community for the first time during this experiment.

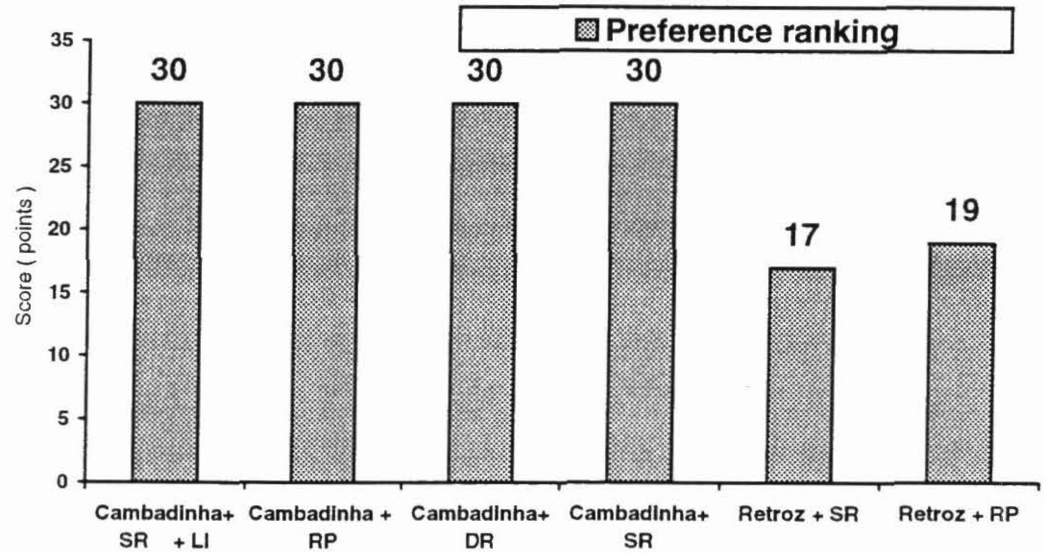
and based on the results obtained, the COPAL decided to continue working with it in the second experiment planned for the growing period 1996-97. The second experiment was planted in July 1996. The design included only one variety, *Cambadinha,* three planting systems: ridges, single row and double row, and the use of Lime. The COPAL learnt its lesson passing from a very complex first experiment with 3 varieties and 12 treatments to a second experiment, simpler, with just one variety and six treatments.

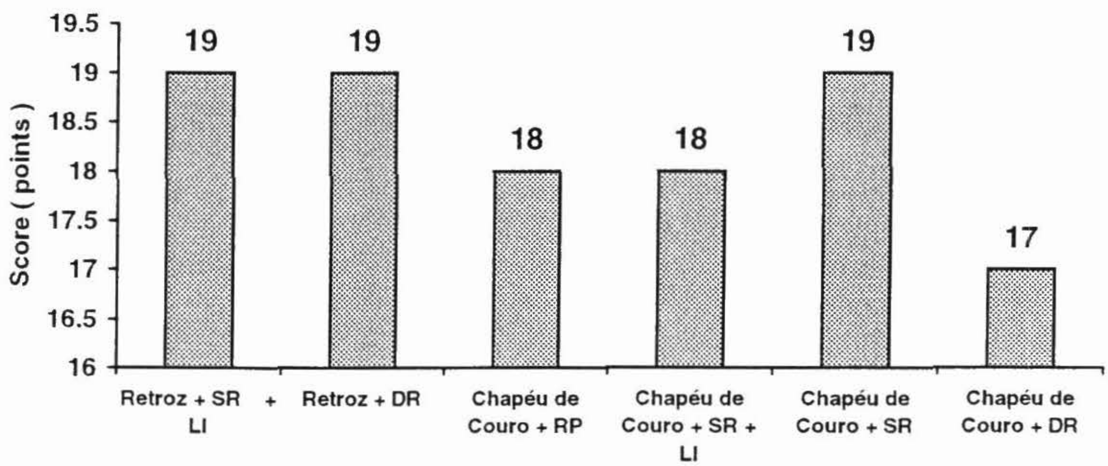




SR = Single row planting;. DR = double row planting ; RP = ridges planting LI = Lime

Figure 1.4.1. Evaluation of varietal resistance/tolerance, cultural practices and planting system for cassava root rot control . Agronomic evaluation - COPAL: Campina Nova, Vitória de Santa Antão, Pernambuco, Experiment 1995-96.





SR = Single row planting;, DR = double row planting ; RP = ridges planting LI = Lime

Figure 1.4.2. Evaluation of varietal resistance/tolerance, cultural practices and planting system for cassava root rot control . Preference Ranking – COPAL: Campina Nova, Vitória de Santa Antão, Pernambuco, Experiment 1995-96.

Activity 1.5. Participatory evaluation of experiments at CIAL Gameleira (Pernambuco).

COPAL: Gameleira, Gloria De Goita, Pernambuco Experiment 1995-96

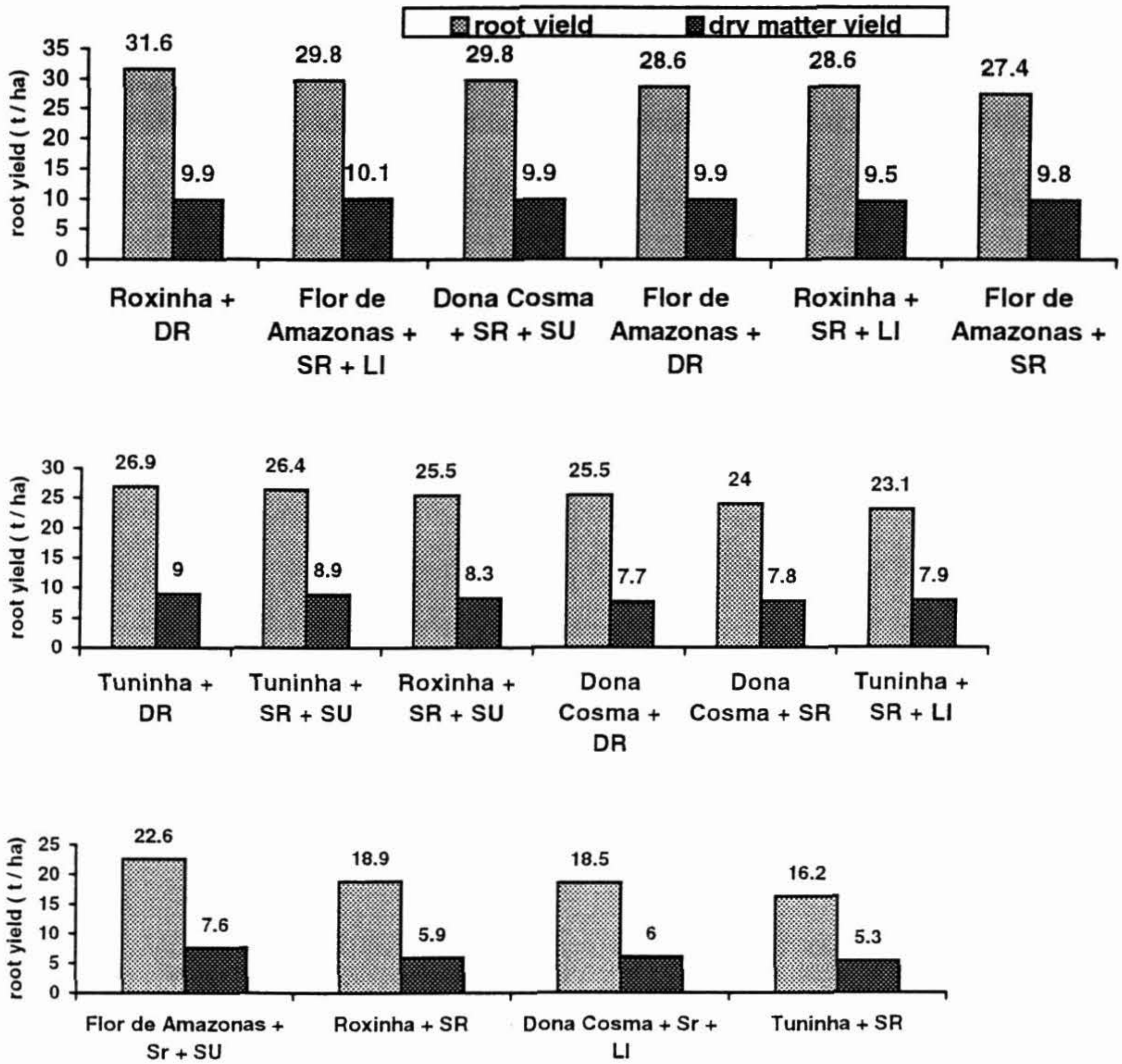
Planting date: 03/08/95 ; harvest date: 02/12/96 (16 months)

Gameleira, is another community of the State of Pernambuco that is participating in Profisma, in which the cassava crop plays a very important role as a commercial and economic activity. Cassava cultivation in the region has been reduced drastically during the last five years due to problems with cassava root rot disease. The COPAL established in this community selected this problem as the topic for its experiment and with technical support from IPA and EMATER designed it including some technologies that have shown some potential to reduce/control this disease and that have not been tested before in the community. Group discussions with participation of farmers, extensionists and researchers allowed the identification of 16 treatments to be included in the experiment. These treatments included four varieties, two widely used in the community, *Tuninha and Roxinha, and* two introduced from IPA, *Flor de Amazonas and Dona Cosma*. The experiment was planted in three different fields within the community. Management of this experiment resulted very difficult due to the excessive number of treatments but farmers did not accept to eliminate any of the 16 treatments, some of which have been suggested by them. The experiment was harvested with 16 months and farmers participated in two types of evaluation: agronomic and preference ranking. The results obtained in the evaluation are presented in Figures 20 and 21. Discussion about the results of the experiment with the farmers group allowed the following preliminary conclusions:

a) The variety *Roxinha*, a local material, planted in double row, gave the highest yield in the whole experiment.
b) In the three localities in which the experiment was planted, the variety *Flor do Amazonas*, an introduced clone, presented good yields and very low incidence of the cassava root rot disease
c) The use of Lime presented higher yields for three of the varieties, when compared with traditional, single row planting. The only exception was the variety *Dona Cosma*
d) Double row planting gave higher yield for all four varieties when compared with traditional, single row planting. In the local varieties, the difference in yield between the two planting systems was higher than with the introduced varieties.
e) The use of Sulphur, presented higher yields, when compared with traditional single planting, for all varieties with the exception of *Flor do Amazonas*
f) The variety *Flor de Amazonas*, planted with the traditional system (single row), gave higher yields than the other three varieties with the same planting system

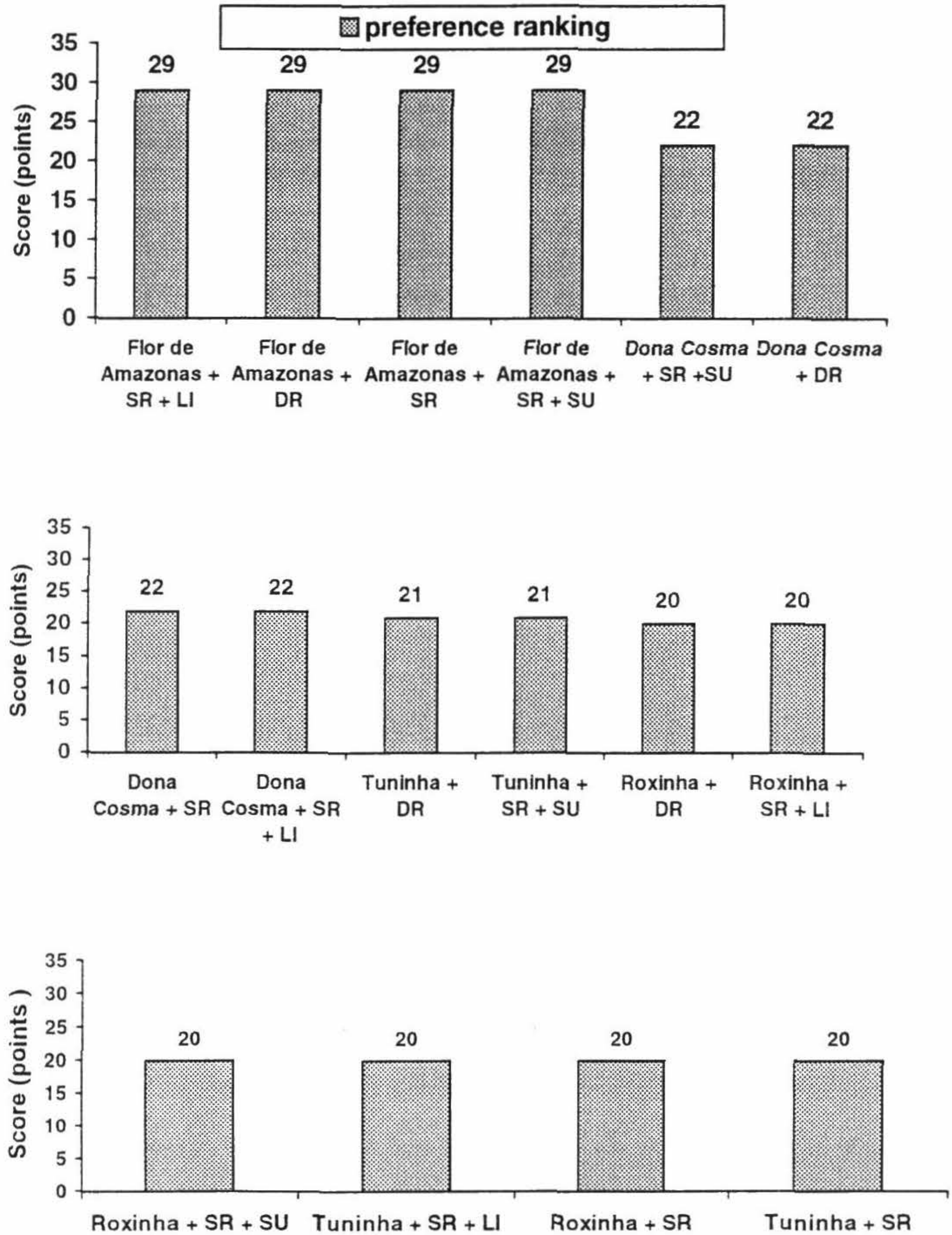
The farmers group conducted a preference ranking evaluation exercise. It was confirmed how difficult it is to realise an objective evaluation of the criteria that farmers use to select or reject technologies, when there are a large number of technologies to be selected. The method used in this experiment allowed farmers to classify the 16 treatments in those more attractive and those not attractive. In the first group, four of the treatments were selected and in the second another 12 treatments. The four treatments selected by farmers as their best options included the variety Flor de Amazonas. Based on these results, the COPAL decided to continue the research work with this variety. The second experiment was planted in July 1997, including just one variety

(*Flor de Amazonas),* two planting systems (single and double row), and two cultural practices (Lime and Sulphur).



(SR = single row planting ; DR = double row planting ; SU = Sulphur ; LI = Lime)

Figure 1.5.1. Evaluation of four cassava varieties, cultural practices and planting systems for resistance / tolerance to cassava root rot - COPAL: Gameleira, Gloria de Goitá, Pernambuco. Experimental cycle 1995-96.



(SR = single row; DR = double row; SU = Sulfur ; LI = Lime)

Figure 1.5.2. Preference ranking evaluation - COPAL: Gameleira, Gloria de Goitá, Pernambuco. Experimental cycle 1995-96.

Activity 1.6. Participatory evaluations of experiments at CIAL Tatú (Pernambuco).

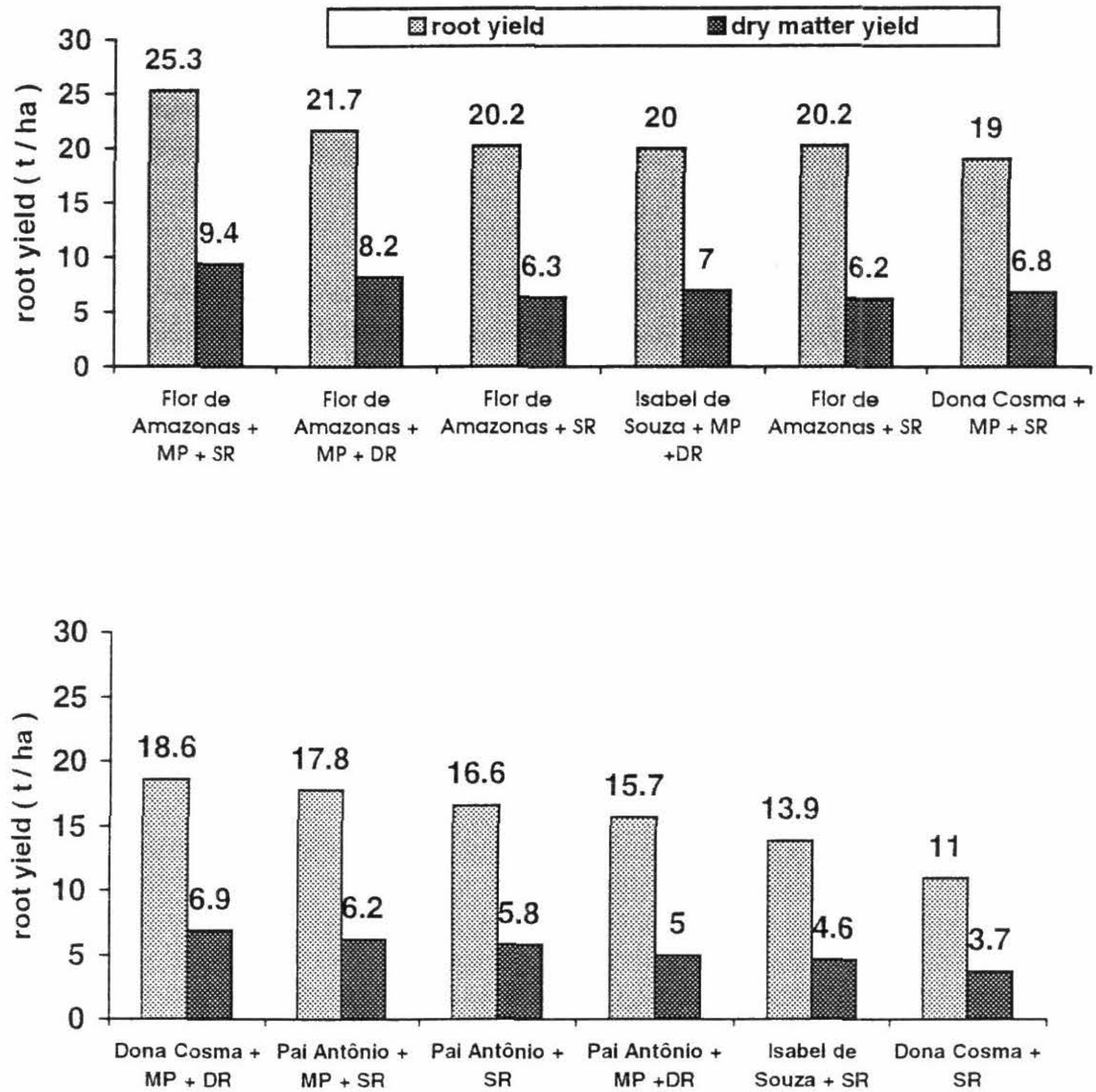
Copal: Tatu, São Bento De Una, Pernambuco Experiment 1995-96

Planting date: 06/06/95 ; harvest date: 17/12/96 (18 months)

In the community Tatú, the principal cassava varieties are: Isabel de Souza and Pai Antônio. Cassava flour processing houses with low scale of operation and rudimentary type of technology are very common in the region. The cassava crop represents the main source of cash, income and food for this community The participatory diagnostic conducted in the community indicated that the main problem for farmers was the very low productivity in the agricultural system within the community and at regional level. Farmers are not use to apply fertilisation in the crops and are forced to plant in the same plots year after year. The CIAL, in collaboration with IPA and extension workers from EMATER-PE, and with collaboration from other farmers of the community, designed an experiment to evaluate some technologies with potential to increase cassava productivity in the community and in the region. The design included two local varieties: Pai *Antônio e Isabel de Souza*, and two other varieties introduced from IPA: *Flor de Amazonas e Dona Cosma*. The experiment was planted in three different fields and included a total of 12 treatments. Other treatments included in the experiment were two planting systems: single row (traditional) and double row. The experiment also include one treatment with *Mucuna Preta* as a source of green manure. At harvest time, farmers participated in two types of evaluation: agronomic and preference ranking. Figures 22 and 23 present this information. The results obtained with this experiment allowed the following initial conclusions:

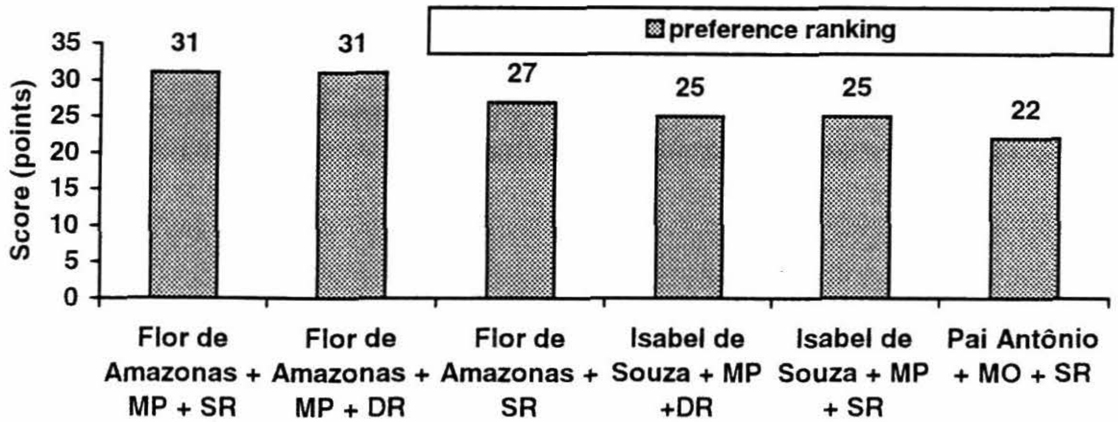
a) The introduzed variety *Flor de Amazonas* presented the best yields , for all the treatments, with a difference of up to 30% in comparison with the other varieties. With the traditional system, the yield of this variety was higher than the other varieties in up to 40 %.
b) The best treatment was a combination of *Mucuna Preta,* incorporated into the soil and the variety *Flor de Amazonas,* using the traditional system.
c) For all four varieties included in the experiment, the use of *Mucuna Preta* incorporated into the soil increased yields in up to 50% in comparison with the traditional system..
d) The use of the double row planting system did not present significative yield differences in comparison with the traditional system.

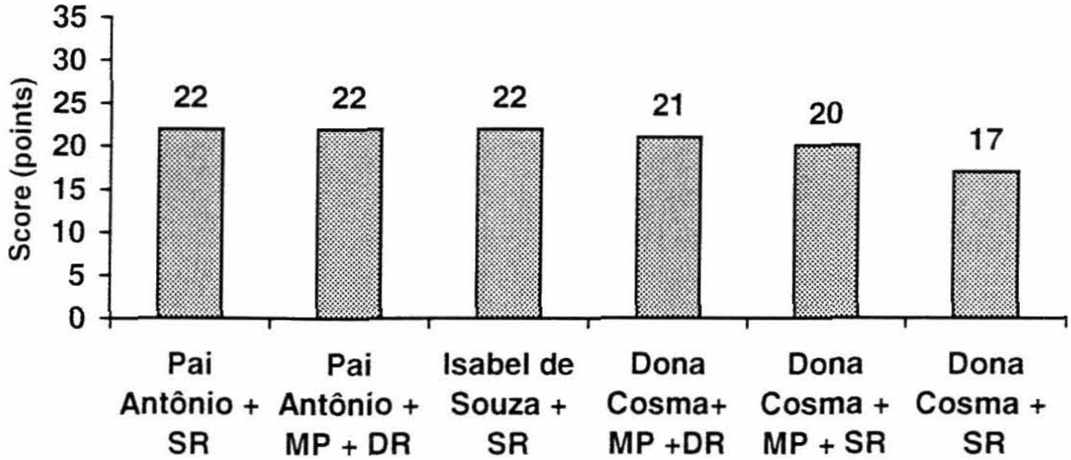
The preference ranking evaluation exercise was difficult to apply due to the large number of treatments. However, the farmer group was able to evaluate all the treatments in the three fields. Results obtained with this evaluation allowed farmers to classify the treatments in two main groups: the first group, with three treatments, were those options approved by them, and the second group, with nine treatments were those options rejected by them.. The first group of three approved treatments included the variety *Flor de Amazonas*, in single row and double row planting and with Mucuna Preta in both cases. This evaluation was consistent with the agronomic evaluation in which these two treatments have occupied also the first two places. Based on this results, the CIAL planned the second experiment that was planted in June 1996 and included just one variety, *Flor de Amazonas*, planted in single and double row, with and without Mucuna Preta incorporated before planting.



(SR = single row; DR = double row; MP = Mucuna Preta)

Figure 1.6.1. Evaluation of four cassava varieties, cultural practices and planting systems for its effect on cassava yields. Agronomic evaluation - COPAL: Tatú, São Bento do Una, Pernambuco. Experimental cycle 1995-96.





(SR = single row; DR = double row; MP = Mucuna Preta)

Figure 1.6.2. Evaluation of four cassava varieties, cultural practices and planting systems for its effect on cassava yields. Preference ranking evaluation - COPAL: Tatú, São Bento do Una, Pernambuco. Experimental cycle 1995-96.

Activity 1.7. Participatory evaluation of experiments at CIAL Nova Veneza (Ceará).

COPAL: Nova Veneza, Ubajara, Cearà Experiment 1996-97

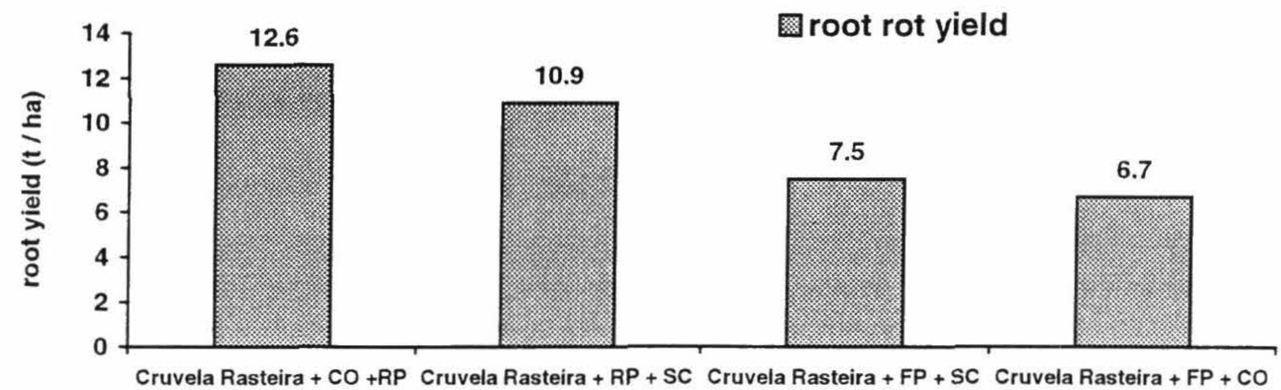
Planting date: 08/04/96; harvest date: 18/07/97 (15 months)

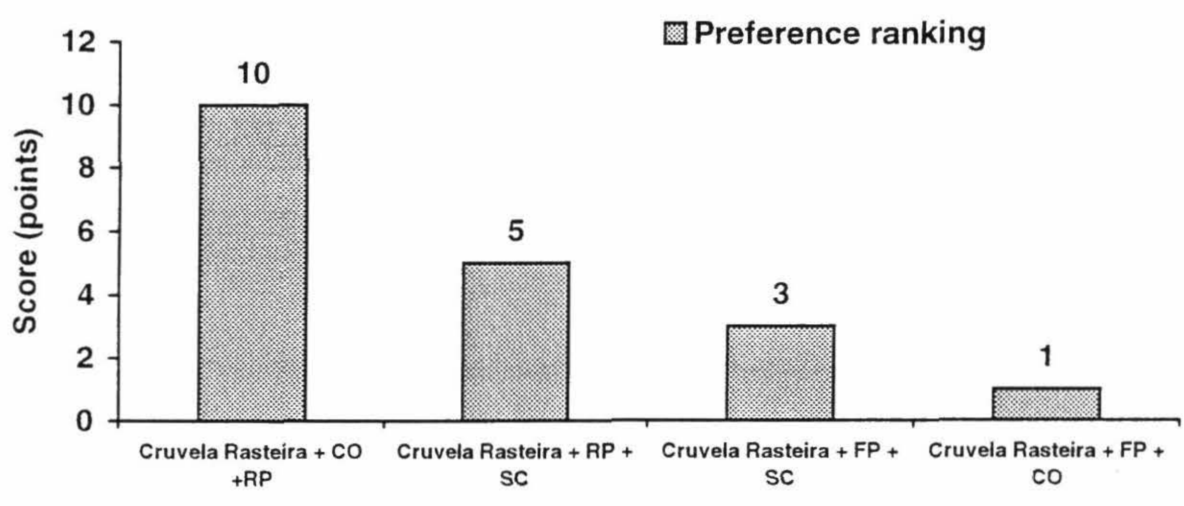
The community Nova Veneza is located in the region known as Serra de Ubajara which is characterised by good edaphoclimatic characteristics that allow production of several crops such us cassava, corn, beans, sugar cane and vegetables. In 1996, the CIAL planted its second experiment based on the use of two planting systems (ridges and flat, traditional planting) and also the use of compost. *Cruvela Rasteira*, a local variety was used for this experiment. Planting was done in double row system with 12820 plants per hectare and four repetitions. A severe incidence of root rot was present causing total lost in two plots and considerable reduction in the number of harvested plants in the rest of the plots. At harvest time, farmers participated in two types of evaluation: agronomic and preference ranking. Figure 24 presents this information. The results obtained with this experiment allowed the following initial conclusions:

a) The effect of using compost with the introduced planting system (ridges), gave the best yield of the experiment with a 25% increase in comparison with the traditional system without compost and 30% difference in comparison with ridges planting system without compost.
b) The use of compost in the traditional system (flat planting) had a negative effect with lower yields when compared with the same system without compost. These results could be explained by the fact that in all the treatments that included compost, the incidence of cassava root rot was more severe, with lower number of harvested plants.
c) The local variety *Cruvela Rasteira,* although very popular in the community, is not showing a very good performance and the CIAL will try to test new varieties in future experiments.
d) The use of ridges planting system gave higher yields than the traditional system, almost 50% more in both cases, with and without compost.

The preference ranking evaluation performed by the farmers group was in agreement with the results obtained in the agronomic evaluation with the treatment ridges planting + compost selected as the first option, and the traditional planting system (flat) without compost selected as the last option. Figure 24 presents this information.

This CIAL planted their third experiment in March 1997. In this experiment, for the first time they designed an experiment that included cassava, intercropped with passion fruit and beans, two other very important crops in the region. The experiment includes two varieties, Buja and Cruvela and two planting systems: ridges and flat planting. Harvest will be conducted during May-June 1998.





CO = Compost ; RP = Ridges planting ; SC = without compost ; FP = flat planting

Figure 1.7.1. Evaluation of cultural practices and planting systems for its effect on cassava yields . Agronomic and preference ranking evaluation - COPAL: Nova Veneza, Ubajara, Ceará. Experimental cycle 1996-97.

Sub-output 2. Participatory Planning and Installation of Technology Evaluation Trials at CIAL's.

During the period covered by the present report some of the CIAL's established with support from the PROFISMA project continued their assimilation of the CIAL methodology. This year corresponded to the third consecutive experimental cycle since the inception of the project and the fact that some CIAL's have planted their third consecutive experiment could be interpreted as the best symptom that the CIAL methodology is being adopted and that some of the CIAL's established in Northeast Brazil.

Activity 2.1. Establishment of participatory technology evaluation trials in 3 CIAL's of the state of Bahia.

The three CIAL's established in the state of Bahia (Colonia Agrícola Roberto Santos, Buril and Chapade) planted their third experiment during 1998. In all three cases, the CIAL acted more autonomously requesting support from researchers at EMBRAPA/CNPMF only during the planning phase. The presence of researchers during harvest and planning of experiments is not as necessary now as it was before, however the contribution of EMBRAPA with improved germplasm continues to be very important. In the region of Alagoinhas, Bahia, where these three CIAL operate, there are already three cassava varieties produced by EMBRAPA/CNPMF that have shown very good potential and that could be used as the basis for a propagation/multiplication program aimed at making good quality planting material available for farmer groups. These three varieties are: 194-16 (Valenca) 189-11 (Catalina) and 128-08 (Bibiana).

Activity 2.2. Establishment of participatory technology evaluation trials in CIAL's of the states of Pernambuco and Ceará.

The three CIAL's established in the state of Pernambuco planted their third consecutive experiment this year.

The CIAL's Nora Veneza and Valparaiso located in the state of Ceará planted their third experiment in 1998.

Sub-output 3. Preliminary Analysis of the Development of the CIAL Methodology in Northeast Brazil

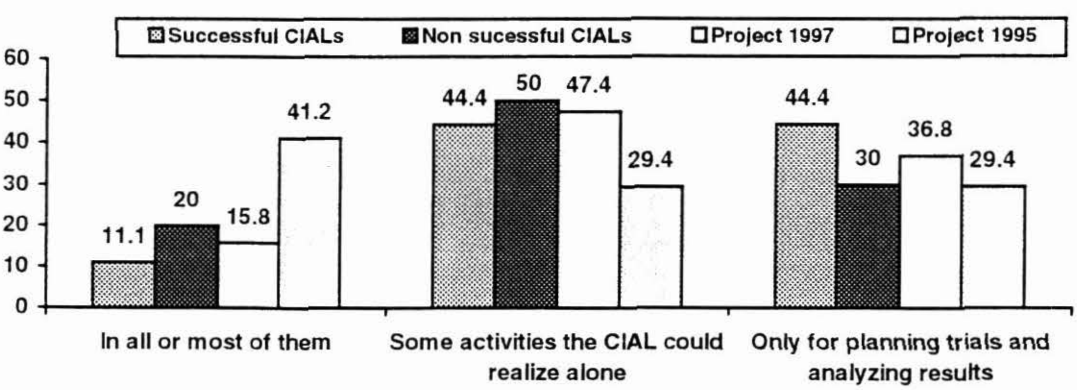
The process of assimilation of the CIAL methodology and its incorporation into their normal forms of operation by counterpart institutions and farmer groups is generally slow and may take several years to be fully achieved. In this sense, it is important to develop mechanisms that allow the identification of constraints and limitations that could be affecting this process. In the case of assimilation of the CIAL methodology by farmer groups and communities, the IPRA project has developed a semi-structured that is applied to each CIAL in two different moments to assess its consolidation and progress. This questionnaire is usually applied during the first year and applied again after two or three experimental seasons. The questionnaire includes topics related to group consolidation, self-autonomy, farmer participatory research, technology comprehension, the use of training materials and devolution of information. The methodology for its application is based in conducting group meetings with the CIAL and other collaborating farmers to discuss each topic. For each of the topics, some questions are asked to the group and a unique answer is reached by consensus of the group. After a period working with participatory research methods, a given CIAL or community could present increases or decreases in its understanding and assimilation of the main principles of the methodology. By applying the same questionnaire in two different moments, this analytical tool tries to identify these changes and to analyze the reasons behind them.

Activity 3.1. Follow-up analysis of CIAL methodology development in Northeast Brazil.

With the aim of evaluating the development of the CIAL methodology among the CIALs established in Northeast Brazil during the PROFISMA project, a follow-up questionnaire was applied to 18 CIALs in 1995 and applied again in 1997. The sample in 1997 included the same 18 CIALs plus another one recently established. To facilitate the analysis of the results, the CIALs interviewed in 1997 were divided into two groups according to their performance since the inception of the project. The main criteria used for this division was the number of technology trials that each CIAL has planted and harvested. Those CIALs that had harvested at least two experiments and that had already planted a third one were considered **"successful CIALs"**. Conversely, the CIALs with two or less experiments planted were considered **"non-successful CIALs"**. Annex 1 presents this classification. The following section presents the results obtained. This section tries to look at the degree of consolidation of the CIAL, the dependence they feel of the presence of the technician and the decision-making process that the CIAL follows to resolve their day-to-day questions. To addresses these aspects the questionnaire included four questions as follows:

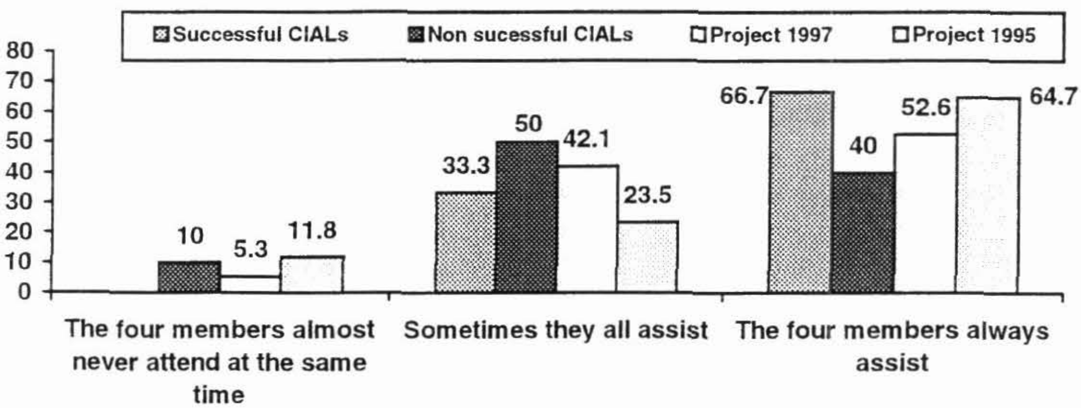
A. Group consolidation. This section tries to look at the degree of consolidation of the CIAL, the dependence they feel of the presence of the technician and the decision-making process that the CIAL follows to resolve their day-to-day questions. To addresses these aspects the questionnaire included four questions as follows:

A.1 In which activities the technician should accompany the CIAL?



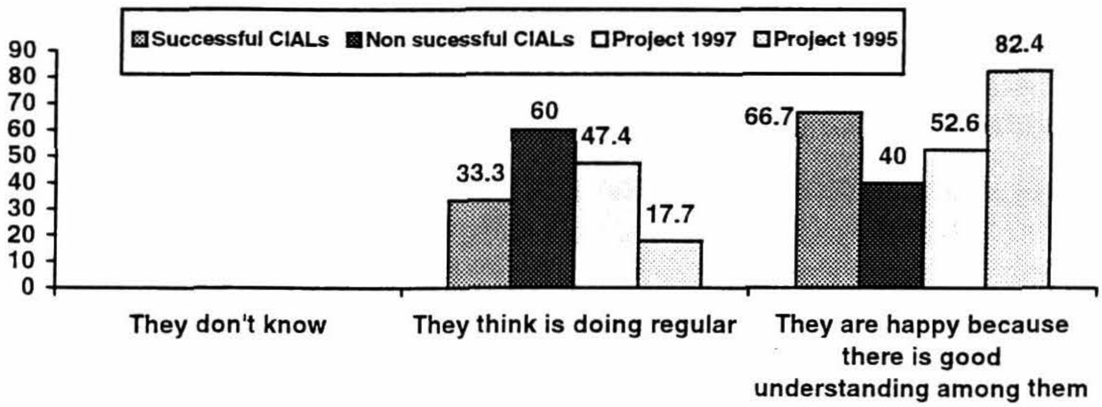
The results indicate that near 90% of the CIALs in the group of the successful ones considers that the presence of the technician is no longer needed with the same intensity as the first year. They consider this presence important only in specific moments of the process (planning, analysis of results). In the group of non-successful CIALs , 20% of them are still demanding the presence of the technician and about one third of them are in agreement with its presence only in specific moments. Looking at the results of the entire sample for the two periods, it can be observed that in 1997, after 3 years of work, near 85% of the CIALs are expressing a degree of consolidation indicated by their recognition that they can perform most of the activities involved in the process, in contrast with 1995 where only 58% of the CIALs expressed the same opinion. Additionally, the number of CIALs expressing dependency on the presence of the technician dropped from 41% in 1995 to 16% in 1997.

A.2 Assistance. How has been the assistance of the CIAL members to the activities conducted so far?



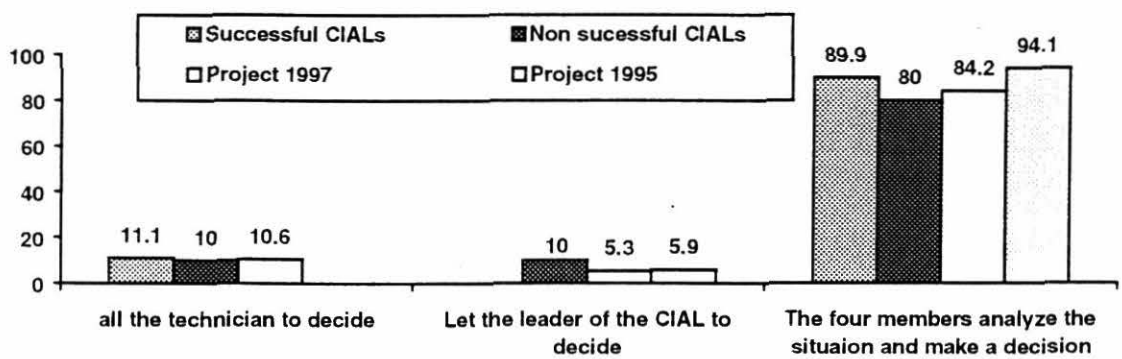
The results indicate that among the successful CIALs, the attendance of their four members to all or most of the activities is perhaps one of the main reasons behind their success. In CIALs that are not functioning well, the absence of some of their members in some activities could be considered one of the factors that is affecting the consolidation of the group. In fact, some of these CIALs have been forced to change some of the members that were initially appointed. Considering the whole sample of CIALs in the two periods, it could be observed that the number of CIALs whose members always attend the activities dropped from 66.7% in 1995 to 52.6% in 1997. This changes could be explained by the "enthusiasm factor" that the establishment of the CIALS produces during the first year but later on when problems such us drought, experimental failures, lack of technical assistance, etc, star to appear, some of the farmers loose motivation and the normal functioning of the CIAL is affected. Additionally, the number of CIALs whose members never attend the activities at the same time dropped from 11% in 1995 to 5.3% in 1997. This could be an indication that although in some regions the normal functioning of the CIALs has been affected by various reasons, the idea of having a CIAL is still seen by these communities as an important strategy.

A.3 The group environment. How is the CIAL doing?



The results are rather self-explaining. In a period of three years, the number of CIALs that manifested their "happiness" with the functioning of the CIAL dropped from 82.4% to 52.6%. Among the CIALs that are running well in 1997, only one third of them think the CIAL is doing regular whereas among the non-successful CIALs this figure is 60%. This tendency could be attributed to the lack of activities of some CIALs after the first year. Planning, planting and harvesting a new experiment is a very important activity that leads to the consolidation of the CIAL and in several cases among the group of non-successful CIALs, this process has completely stopped. This could be seen by looking at the number of CIAL's that think they are doing regular which grows from 17.7% in 1995 to almost 50% in 1997.

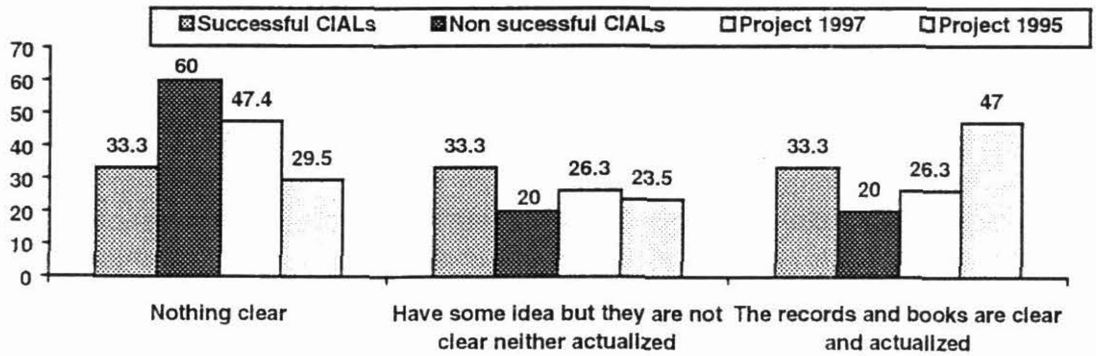
A.4 Decision making. What does the group do when it is difficult to reach an agreement?



The data obtained indicates that in general, the CIALs, with few exceptions, have managed to develop internal decision making processes and that their dependency for the technician to decide in cases when they cannot reach agreement has become minimal. During the first year, none of the CIALs indicated that they needed the presence of the technician to decide upon difficult matters. Three years later, when the decisions become more complicated, some of the CIALs are still expressing that in some cases, when they have difficulties reaching agreement among them, they prefer the technician to step in and decide for them.

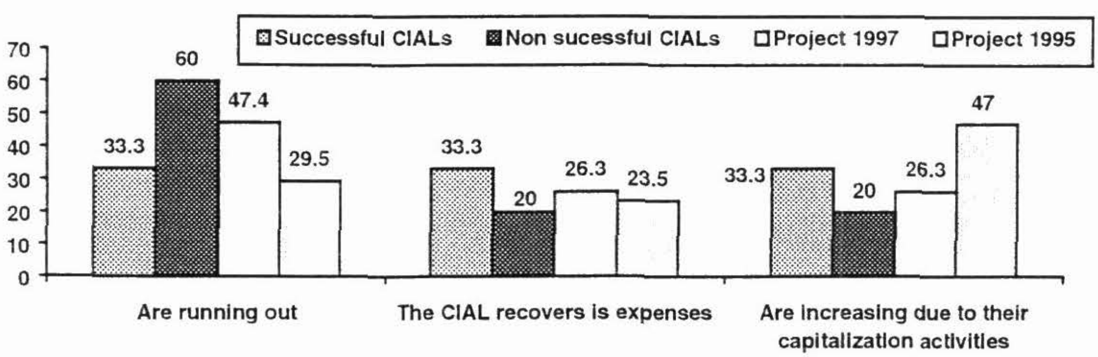
B. Group self-autonomy. This section refers to the development of the CIAL in relation with keeping books and records, administering funds and building skills for their relation with institutions. Three questions were included to deal with these aspects as follows:

B.1 Records. In which stage are the books and records of the CIAL?



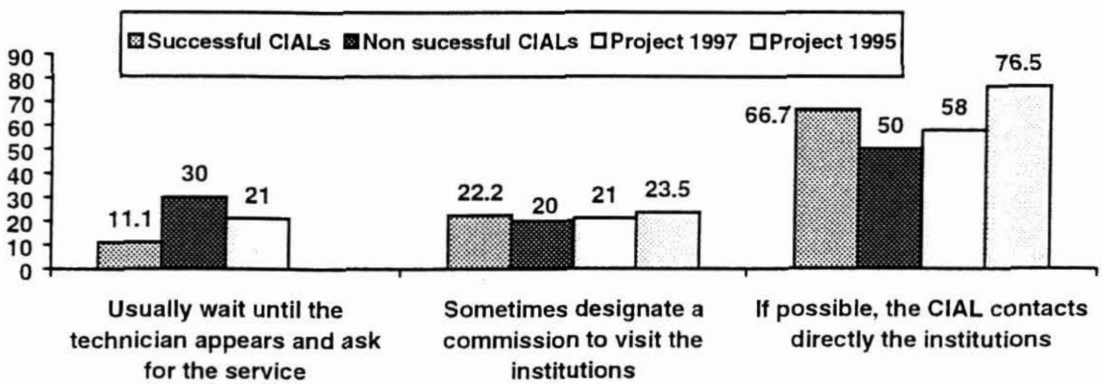
The results obtained show that two thirds of the CIALs that are running well are maintaining their records and accounts well kept in contrast with the group of the non successful CIALs in which only 40% are doing so. It can also be observed a drop in the number of CIALs that are maintaining their books and record actualized which in 1995 was almost half of them and in 1997 came down to only one fourth. It should considered as a very preoccupying indicator the fact that 60% of the CIALs that are functioning with problems and 50% of all the CIALs operating in 1997 are virtually not keeping any kind of books and records. Additionally, in one third of the CIALs that could be considered as well established and developed, bookkeeping and maintaining records are activities that need to be reinforced

B.2 Capitalization How are the funds that the CIAL received for their research activities?



As it can be seen from the data obtained, the seed money granted by the project to each CIAL has been maintained in almost 70% of the successful CIALs despite the fact that in Northeast Brazil, experiments based on cassava usually take a long period before harvest.. In the case of the CIALs that are not functioning very well, 60% of them indicate that the seed money is already spent. It is interesting to notice that one third of the successful CIALs indicate that their funds are increasing. Considering the total number of CIALs it is observed than from 1995 to 1997, the number of CIALs running out of seed money increase from one third to near half of them. In most of the cases, planting and taking care of the experiment until harvest time does not imply expenses for the CIAL because they participate voluntarily in the activities. Thus, the funds are kept in interest-bearing accounts and are very seldom used. In some cases these funds were used to pay farmers work and due to failure in the experiment the funds were not recovered. In general, it can be argued that the concept of a seed type financial support for the CIAL is a sound strategy, that the CIALs are making good use of this support and that in the case of CIALs dealing with the cassava crop this support should be split in two consecutive seasons to avoid running out of money if the first experiment fails.

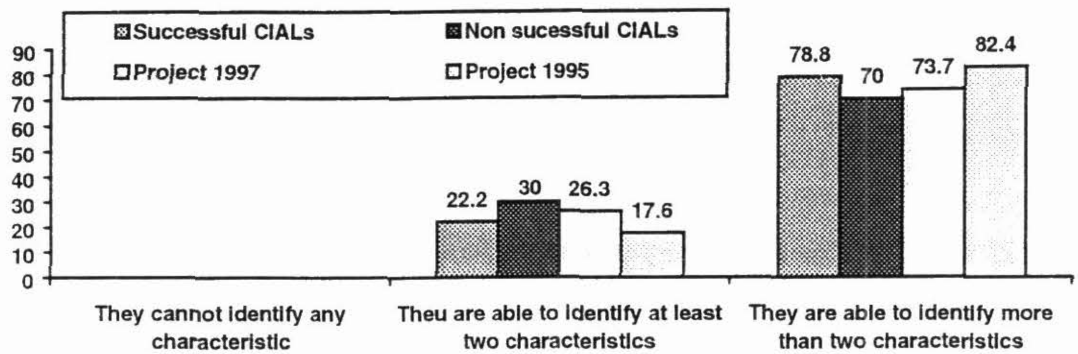
B.3 Capacity to interact with institutions. What does the CIAL do when they need some service from one institution?



Data obtained indicates a decrease in the number of CIALs that have developed enough organizational skills to establish contacts on their own with the institutions. In 1995, almost three fourths of the CIALs indicated that would contact directly the institutions whereas in 1997 only 58% manifested this capacity. In 1995 none of the CIALs expressed that they will wait for the technician to show up and then ask for the service. In 1997, near 20% of all the CIALs expressed this opinion. Among the non-successful CIALs roughly one third of them manifested this dependency on the presence of the technician. Comparing the groups of successful and non-successful CIALs it can be observed that two thirds of the CIALs in the former group express their confidence and capacity to interact directly with institutions whereas in the group of non-successful CIALs only half of them agreed with this opinion. The importance of this indicator lies in the fact that establishing self-autonomous farmer groups is one of the basic principles of the CIAL methodology so that these empowered CIALs could become reliable and accountable partners of the institutions. It is to be expected that after two or three years of work with participatory research activities, a CIAL and its surrounding community should have develop enough skills to be able to identify their main problems, prioritize them and communicate them to the institutions collaborating with them.

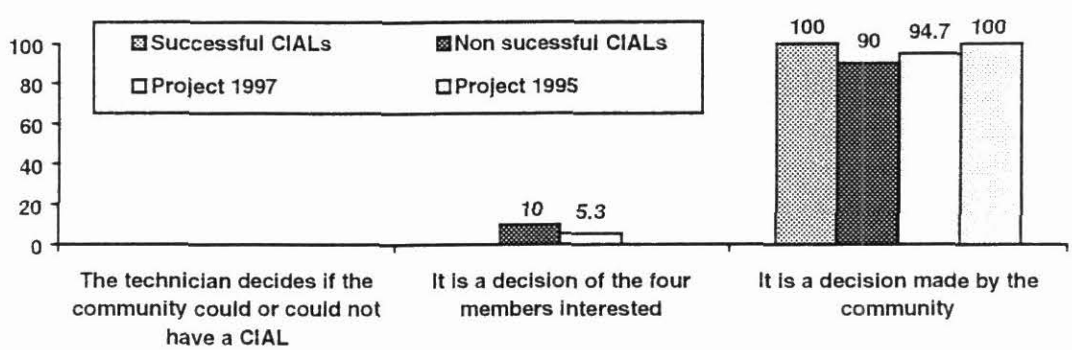
C. Farmer participatory research. This section of the questionnaire tries to assess the level of understanding reached by the community in relation with the CIAL methodology, the role of the CIAL and the principal concepts involved in participatory research activities. Three questions were included as follows:

C.1 Profile of CIAL members. How should be the members of the CIAL?



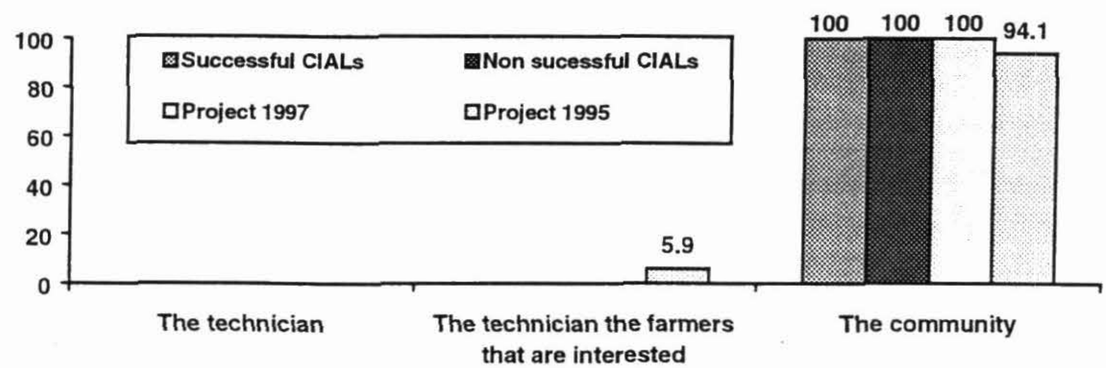
Analysis of the data obtained does not indicate great differences. In general it could be said that among the 18 communities that are working with CIALs in Northeast Brazil, there is a good understanding of the principal characteristics that a farmer must have to become a good CIAL member.

C .2 Compromise. How does a community decide to establish a CIAL?



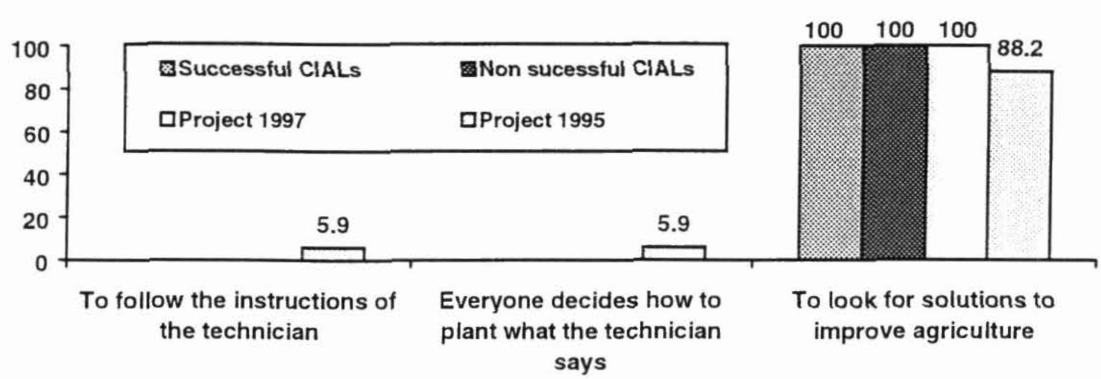
According to the data obtained, there remains only one community in which the members of the CIAL have not yet been able to attract other farmers of the community to their activities and all the decision making process is dominated by them.. As a consequence, the CIAL remains divorced from the rest of the community. None of the 18 CIALs established reported interference of the technician to any extent in decision-making processes.

C..3 Representation of the community. Who selects the members of the CIAL?



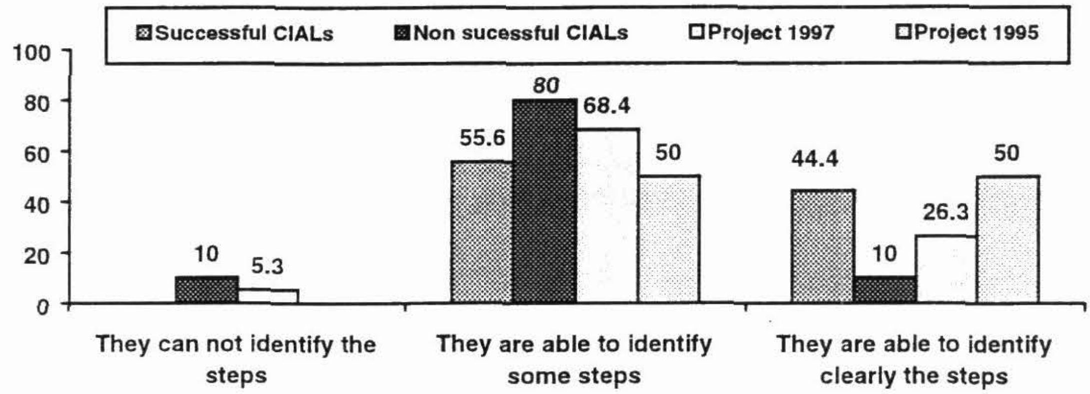
The information obtained confirms that the decision to establish a CIAL has been a process fully validated and accepted by each of the communities involved in the project. In 1995, one of the communities had only few members participating. In 1997, all the communities claim ownership and autonomy in the decision process.

C.4 Understanding of the concept of doing research. What is it to investigate?



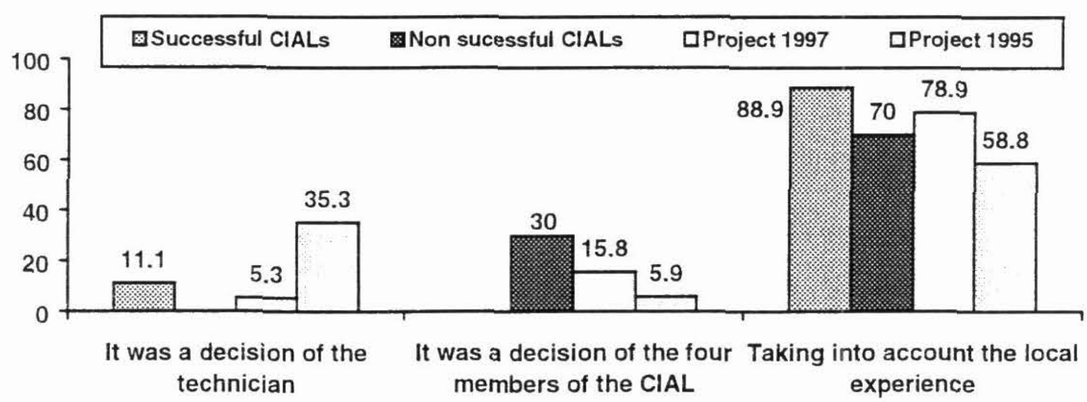
According to the data obtained it can be seen that in 1997, after three years of work, all the CIALs have reached a good understanding of the concept of doing research and are able to relate these activities to a community effort to improve their local agriculture.

C.5 Understanding of the concept of doing research. What are the steps to be followed in participatory research?



Understanding the different steps that have to be followed sequentially in the application of the CIAL methodology, has become, according to the data obtained, one of the main limitations in the development of the CIALs and the consolidation of the methodology itself. It can be observed that even among the CIALs that are functioning well, more than half of them still need to improve their understanding of the steps involved in the application of the methodology. In the group of the non-successful CIALs the situation is even worse since only 10% of them are able to identify all the steps correctly and another 10% are not able to identify any step at all.

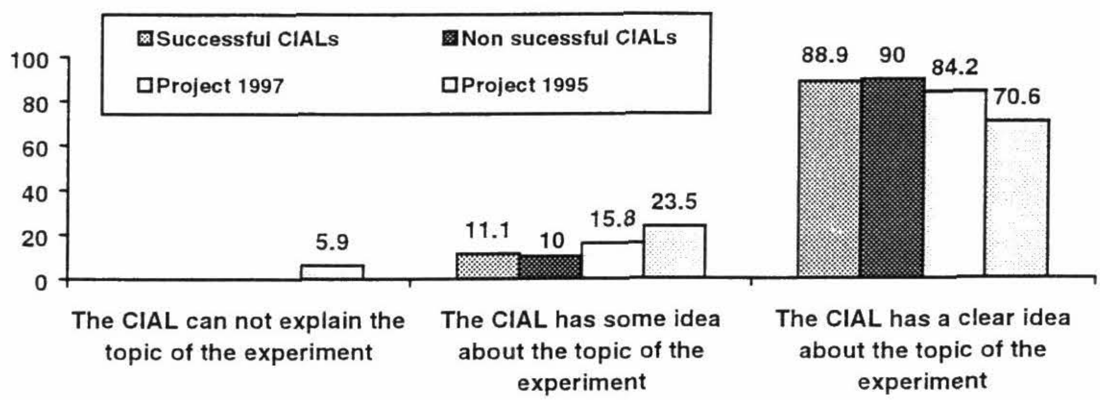
C..6 Consultation of local experience. How did they decide the topic of the research?



This question was meant to determine the degree of participation of the community in the selection of the research topic. It can be observed that compared with the situation in the first year of the project, the interference of the technician in this decision has been reduced considerably thus giving more opportunities to farmers to express their ideas and to contribute with their experiences. Among the group of the successful CIALs there still remains one community in which this decision is not being taken with participation of the members of the CIAL and the rest of the community. In the case of the non-successful CIALs, still 30% of them are presenting a concentration of this decision-making process in the hands of the CIAL without participation of other farmers. This factor could become crucial in the consolidation of the CIAL as an organizational instrument of the community to work towards improving the agriculture in a given region.

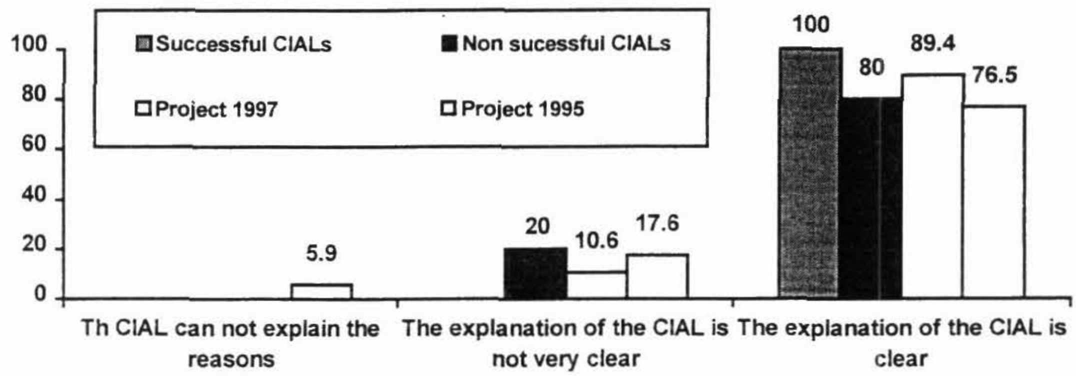
D. Understanding the concepts involved in technology generation work. This section of the questionnaire was meant to determine the degree of understanding of the CIAL of aspects related to the experimental work that each CIAL realizes. This is one of the most controversial issues in the academic debate as to whether farmers are able to understand concepts related to scientific experimentation. In the CIAL methodology, emphasis is put to help farmers understand the reasons behind the selection of a specific topic to be investigated, the interpretation of the experimental design, the concepts of check, repetition and randomization and the ability of the farmer to anticipate possible results of the experiment. To address these issues, nine questions were included in the questionnaire as follows:

D.1 Topic of the experiment. What is the topic about which the CIAL is doing the experiment?



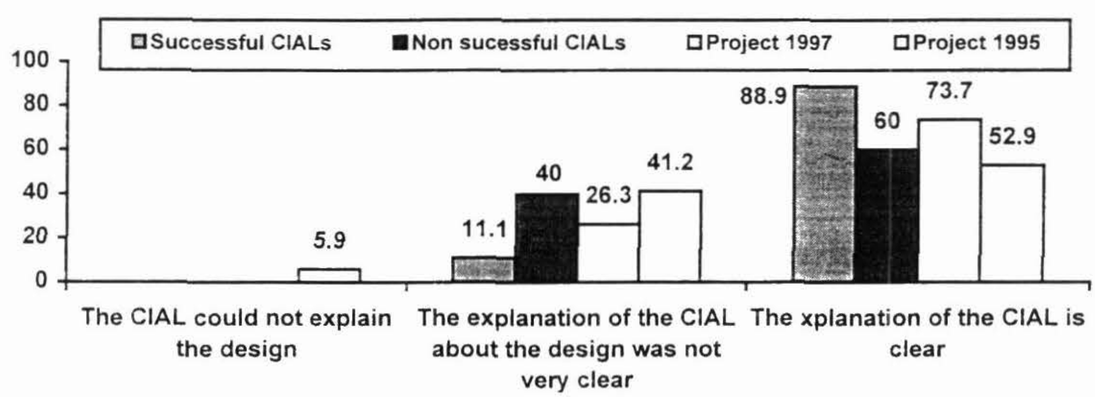
In general, it can be observed that after three years of work, there has been an evolution of the CIALs in this aspect. In 1997, none of the groups is not able to explain the experiment and almost 90% of all the CIALs in both groups, the successful and the non-successful, are able to explain the topic of the experiment clearly.

D.2 Justification of the topic of the experiment. Why is the CIAL conducting this experiment?



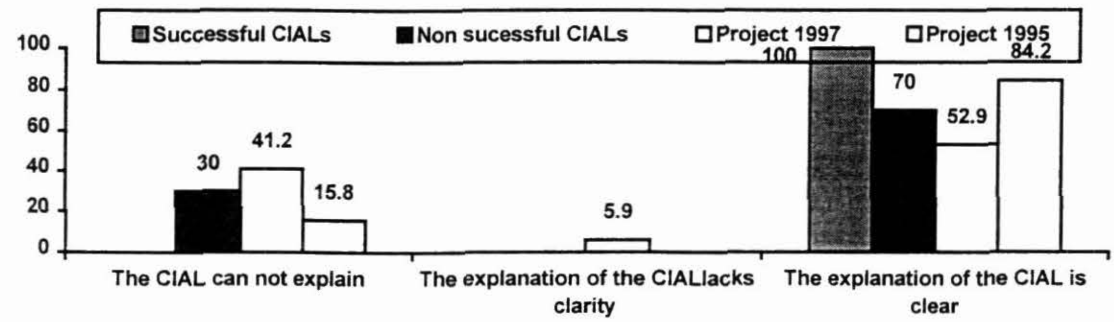
As it was to be expected, the answer to this question by the group of successful CIALs was more positive since they have had more experiments planted and harvested and consequently more opportunities to consolidate their understanding of the methodology. In the group of non-successful CIALs, still 20% of them are not able to explain clearly the reasons for conducting the experiments. Looking at the total number of CIALs there is a decrease in the number of CIALs with explanation not very clear from near 20% in 1995 to only 10% in 1997.

D.3 Understanding of the experiment in design and in the field. Explanation of the experimental design.



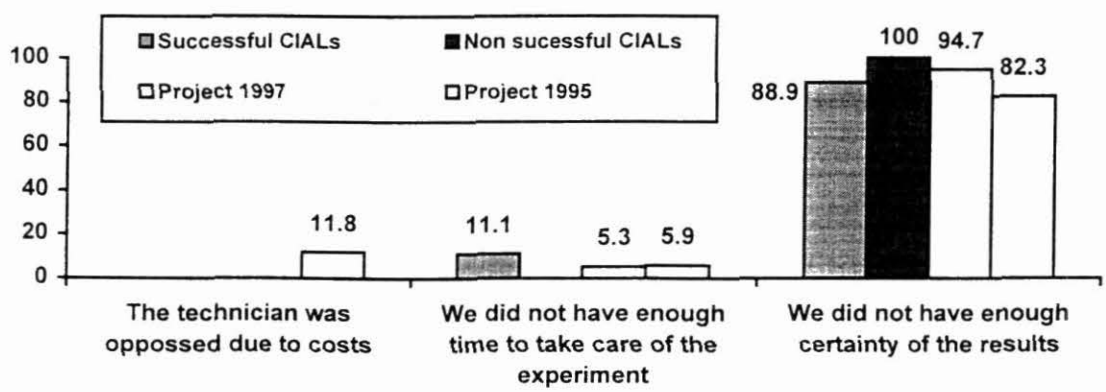
According to the data obtained, it can be seen that in 1997 all the CIALs are able to explain the design of the experiment, a positive indicator especially for those that are skeptical about farmers' understanding of the concepts of modern science. However, the fact that 40% of the CIALs among the group of non-successful is still lacking clarity in their explanation of the experimental design reinforces the need to maintain the support and technical assistance to these groups. A basic principle in the CIAL methodology is that " not to experiment is a mistake" and since several of these CIALs stopped their work after the first or second cycle, their development has had some limitations. Among the group of successful CIALs, only 10% of them still need to reinforce this ability to interpret and explain experimental designs.

D.4 The concept of check. Why the need to include a check in this experiment?



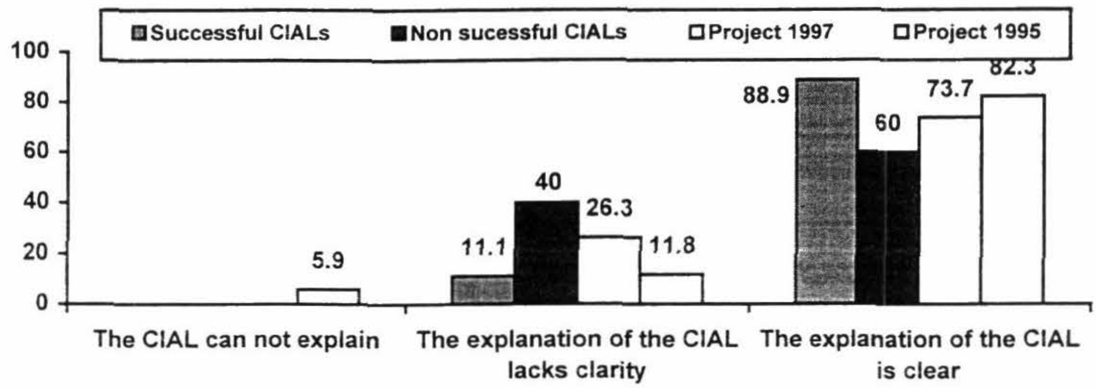
This question was meant to determine the degree of participation of the community in the selection of the research topic. It can be observed that compared with the situation in the first year of the project, the interference of the technician in this decision has been reduced considerably thus giving more opportunities to farmers to express their ideas and to contribute with their experiences. Among the group of the successful CIALs there still remains one community in which this decision is not being taken with participation of the members of the CIAL and the rest of the community. In the case of the non-successful CIALs, still 30% of them are presenting a concentration of this decision-making process in the hands of the CIAL without participation of other farmers. This factor could become crucial in the consolidation of the CIAL as an organizational instrument of the community to work towards improving the agriculture in a given region.

D.5 Understanding of the risk involved in research. Why the CIAL did not plant larger experiments?



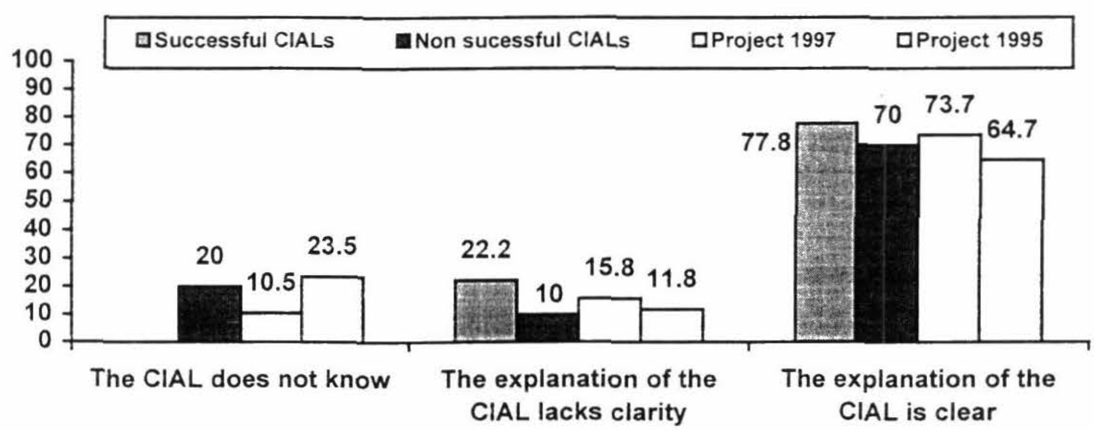
The results obtained indicated a very good assimilation of this concept by all the CIALs. In 1997, none of them was reporting any interference of the technician in the decision process related to the size of the experiments. In the group of the non-successful CIALs, all of them did show a good explanation for the size of their experiments. In the group of the successful ones, only 11% of them related the choice of the size to lack of time.

D.6 Understanding the treatments. Which are the treatments in this experiment?



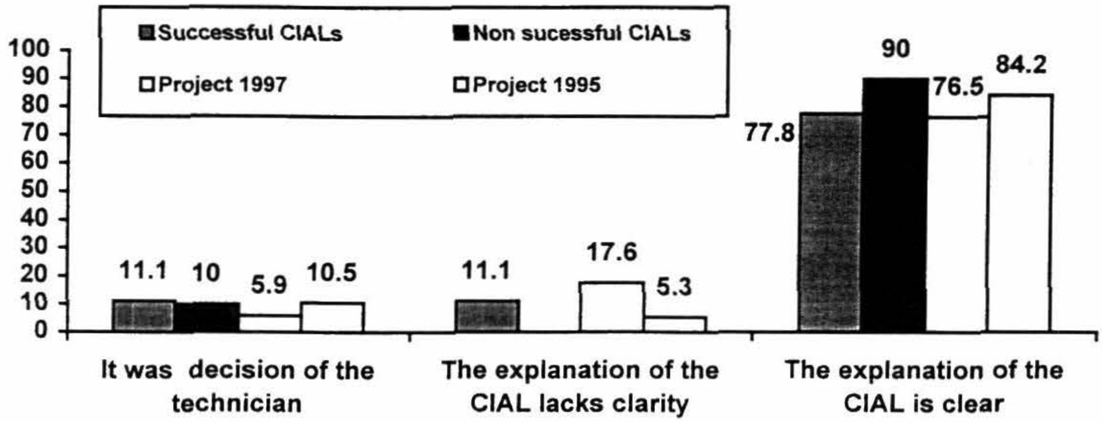
As it can be observed from the data, 40% of the CIALs in the group of the non-successful still needs to reinforce their abilities to explain the different treatments in the experiment. A positive factor is that in 1997 all the CIALs are able to explain the treatments. In the group of the successful CIALs, 90% of them are able to explain correctly the different treatments that were included in their experiments.

D.7. Understanding the repetition concept. Why did you plant the same experiment several times?



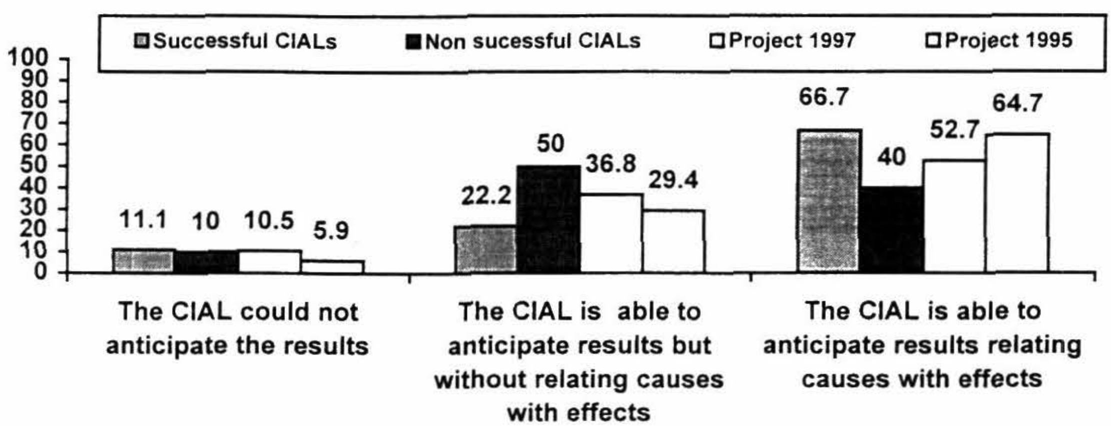
The data obtained indicates that although the number of CIALs that does not know this concept reduced by 50% during the period of three years, among the non-successful CIALs there are still 20% of them that must be worked in this aspect. Among the successful CIALs, near 80% of them are showing a good understanding of this concept and the other 20% are able to partially explain it. Hopefully, as they plant and harvest additional experiments, their understanding will improve.

D.8. Understanding about the selection of the local for installing the experiment. Why did you plant the experiment in this place?



It could be observed that in about 10% of both successful and non-successful CIALs, there is still some technician interference. The number of CIALs with this problem reduced by half in the period of three years. It is interesting to observe that among the non-successful CIALs 90% of them are able to explain with clarity the decision about the selection of the local whereas among the successful CIALs near 80% of them could do so.

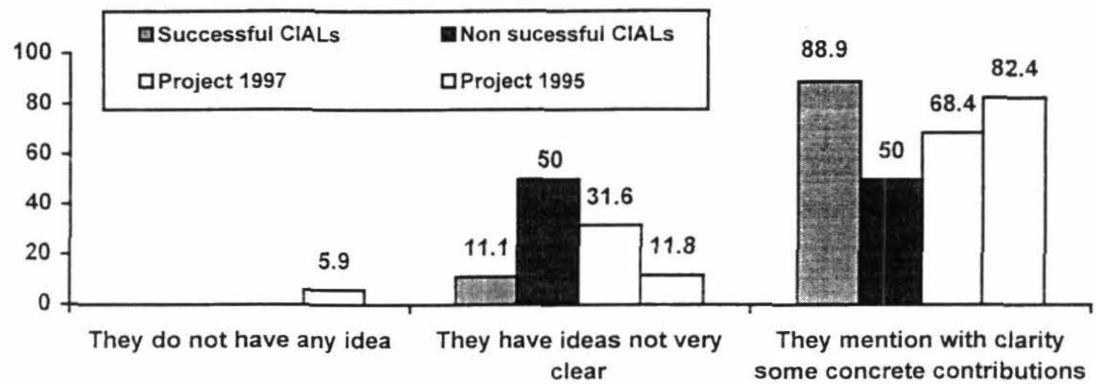
D.9. Capacity to relate causes and effects. Could you anticipate the results of this experiment?



Data obtained indicates that the number of CIALs that are not able to anticipate results from the experiments has doubled in the last three years suggesting that this aspect of the methodology could be one of the more difficult to grasp by farmers. Among the successful CIALS only two thirds of them are able to anticipate results relating causes with effect whereas among the non-successful CIALs only 40% of them fulfill this criteria.

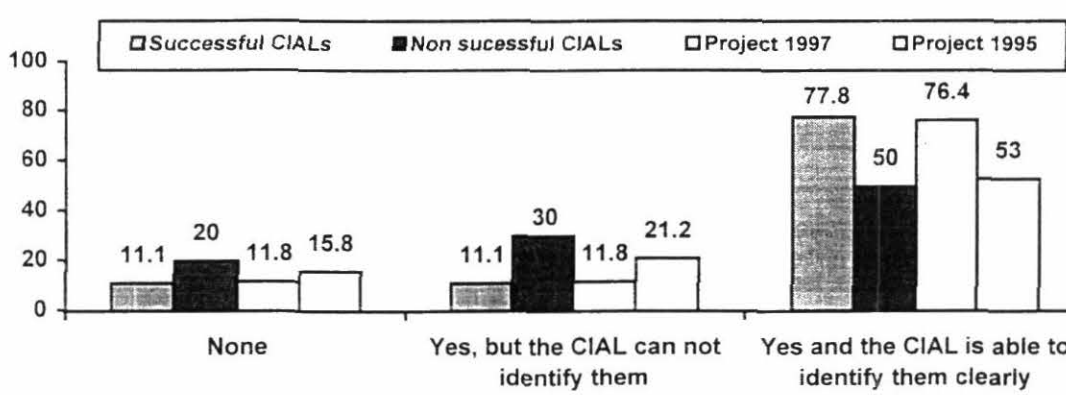
E. Devolution of information to the institutions. This section tries to identify the impact that working with the CIAL methodology could have in institutions, the technicians and also at the community level. To address it, questions were asked to farmers about the learning process for the institutions, the perception of the farmers about changes in the behavior of technicians and institutions and also the sharing of information obtained by the CIAL with the rest of the community. The questions included were as follows:

E.1 Devolution of information to the institutions. What does the CIAL think that the institutions learned with this work?



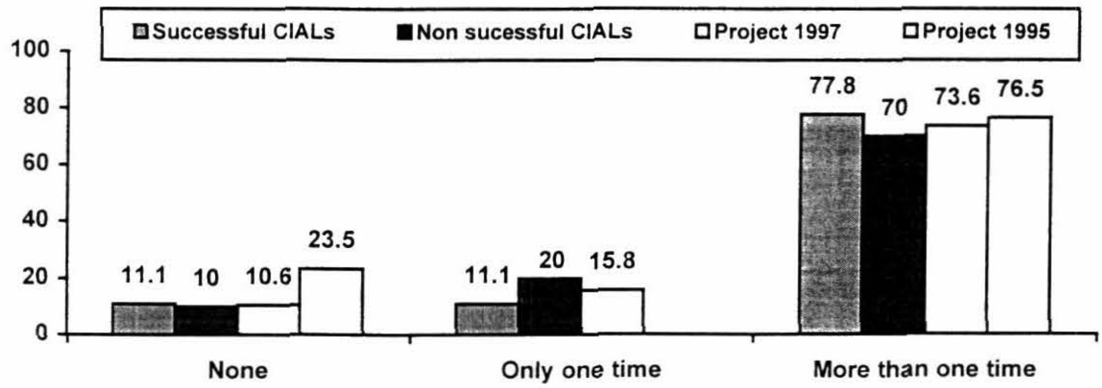
From the data obtained, a striking difference could be observed among successful and non-successful CIALs in this aspect. Although *all* CIALs *in both groups are able* to mention some contributions, 50% of the non-successful CIALs have ideas not very clear whereas in the group of the successful CIALs 90% of them are able to identify concrete contributions of the work to the institutions. It can also be observed that after three years of work, the number of CIALs without a clear understanding of this aspect increased almost three times. This could be explained by the fact that the technical assistance service that supported the work of the CIALs, which during the first year has a good coverage and presence, by the third year, has almost stopped in some regions.

E. 2 Devolution of informations to the technicians. Has the CIAL observed changes in the technicians as a consequence of this work?



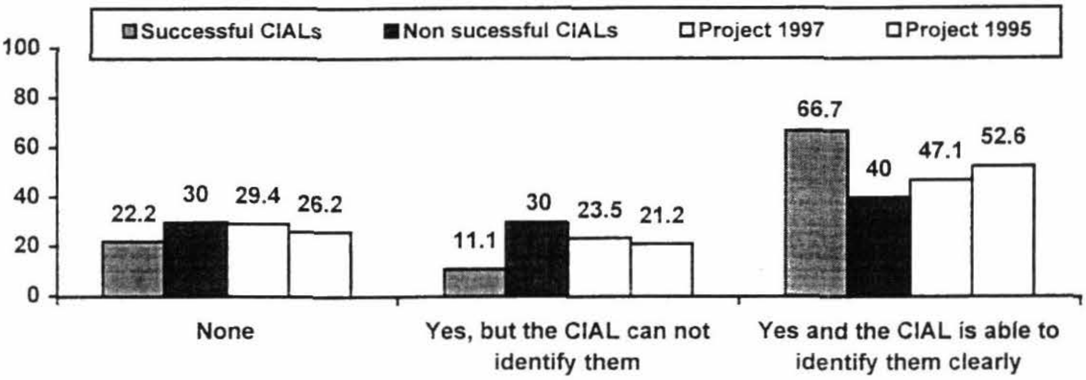
The results in this part of the follow-up questionnaire indicate that among the group of non-successful CIALs, only half of them are able to identify changes in the work of the technicians that give technical assistance to them and 20% of them can not mention any contribution. . Among the group of successful CIALS, almost 80% of them mention concrete contributions. This data suggests that the more intense contact and presence of the technicians that support these groups could be a factor that facilitates farmers' understanding of the effect that working with the CIAL methodology has in the work of the technicians. Looking at the data for the total number of CIALs, it can be observed that during the period of three year, there is a slight reduction in the number of CIALs that can not mention any contribution from 16% in 1995 to 12% in 1997.

E.3 Devolution of informations to the community. How many times the CIAL called meetings with the community in relation with this work?



The CIAL is established in each community as a legitimate organization that works for and is responsible before the community. In this sense, it is very important that the CIAL develops a good communication and collaboration with the rest of the community. The data indicates there are still around 10% of the total number of CIALs that have not given any feedback of their work to the community within which they operate. This means that they are working isolated from their larger group of farmers and the overall benefits to the community are limited. Although this number of CIALs dropped from 23% in 1995 to 10% in 1997, still represents a critical factoir that needs to be revised and corrected. Without a clear link and strong communication channels between the CIAL and the community the risk of failure of the CIAL increases and its work and results end up without an audience beyond the four members of thew CIAL.

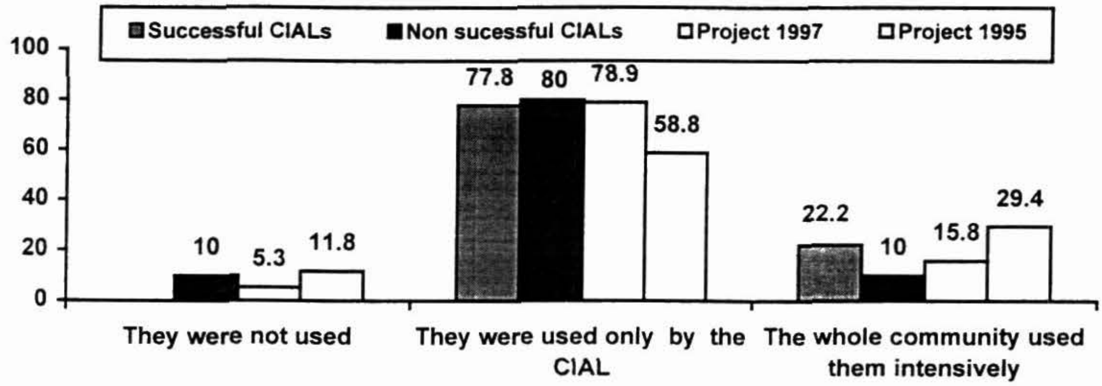
E.4 Devolution of informations to the community. Has the CIAL observed changes in the community as a consequence of this work?



Another aspect related to the degree of communication and integration that a CIAL is able to develop with its surrounding community is the perception that the members of the CIAL have of changes in the behaviour of the community as a consequence of the work of the CIAL. This question tried to address this aspect and data obtained indicates that only 40% of the non-successful CIALs are able to identify and to explain clearly some changes in the community whereas among the group of successful CIALs this numbers is almost 70%. This could be explained by the fact that doing research with cassava in Northeast Brazil is a rather slow activity in which the results are only seen every 16 to 18 months. Then, those CIALs that have planted and harvested more experiments are able to produce more effects in the community. The fact that 30% of CIALs in the group of the non-successful and 22% in the group of the successful are not able to explain any change in the community as a consequence of their activities, indicates that there is a need for work in this area aimed at validating the work of the CIALs before the community. This way, its benefits could be reached by and spread among the other farmers of the community.

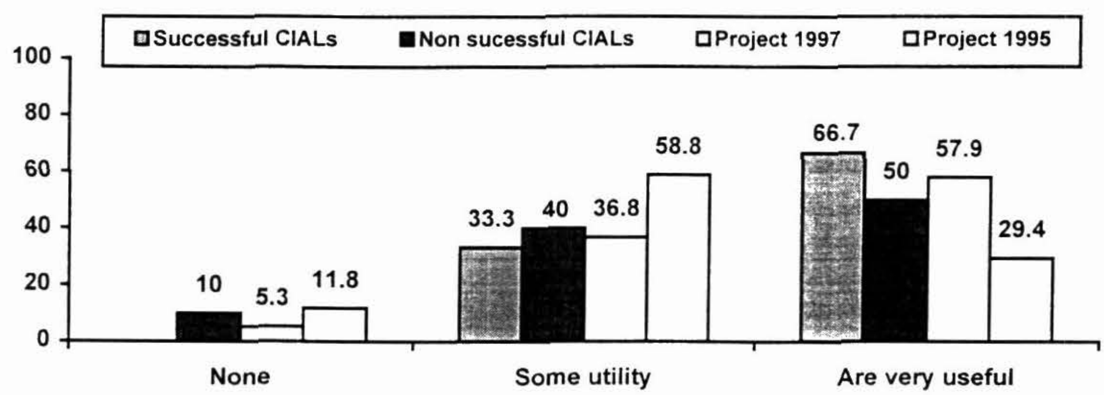
F. Training materials. To reinforce the understanding of the CIAL methodology by farmers groups, some training materials have been designed that facilitate this learning process. This section looks at the use that farmers make of these materials and its utility. The questions included were as follows:

F.1 Use of Cartilhas about CIAL methodology. Has the CIAL used the Cartilhas?



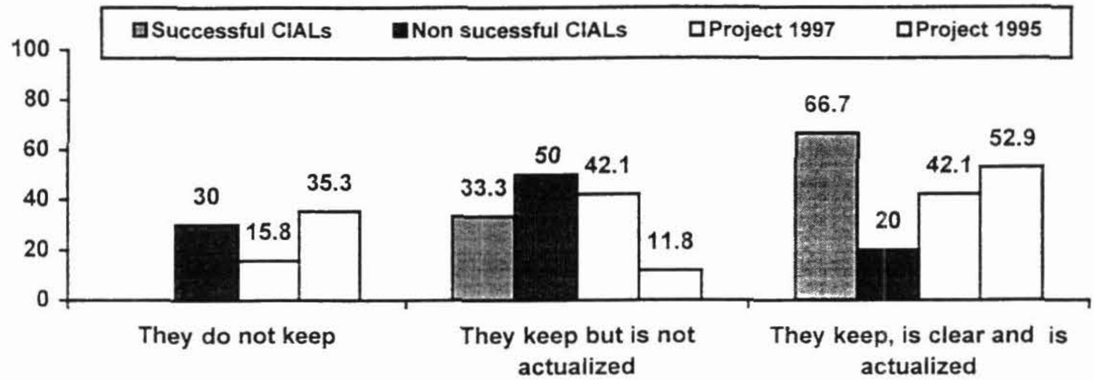
It can be seen from the data obtained that the use of the Cartilhas has been limited mainly to the members of the CIALs although among the group of the successful CIALs, 22% of them have also managed to motivate the community to use them. Among the group of the non successful CIALs, 10% of them have not made any use of the Cartilhas, a factor that indeed contributes to their low performance. Another interesting observation is that the use of the Cartilhas by the whole community which in 1995 ocurred in 30% of the CIALs drops to 15% in 1997.

F.2 Use of Cartilhas about CIAL methodology. Has the CIAL used the Cartilhas?



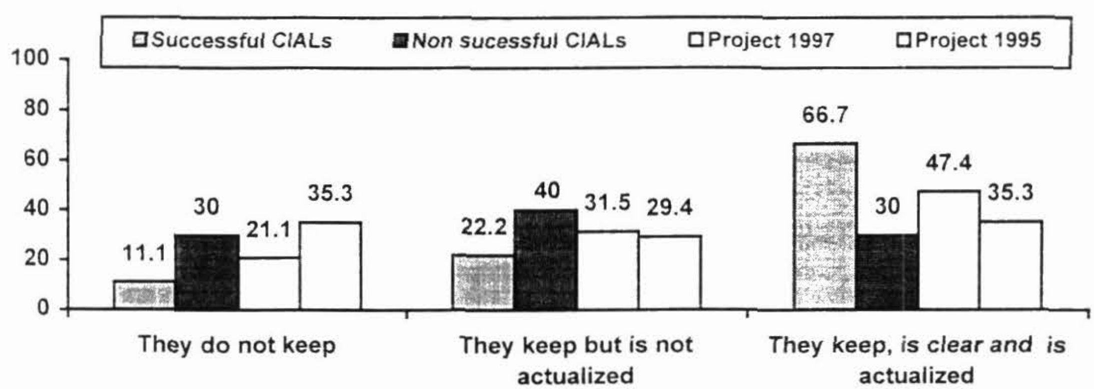
Almost two thirds of the successful CIAL are finding the Cartilhas very useful whereas in the group of the non-successful CIAL only 50% share this affirmation. Additionally, 10% of the CIALs in this later group are finding that the Cartilhas do not have any utility. The number of CIALs that find very useful the Cartilhas increased from 30% in 1995 to almost 60% in 1997 an indication that these materials were used intensively. One aspect that could be contributing to these results is the fact that the Cartilhas, although developed in a friendly, easy to handle way, are based in drawings and illustrations developed by CIAT's IPRA project and may not always be appropriate to northeast Brazil context. Additionally, another factor that has to be taken into account is the high illiteracy rate that is commonly found in Northeast Brazil rural areas.

F.3 Registers of the CIAL. Does the CIAL keep a Register of Activities?



Another instrument that helps a CIAL to develop internal organization and cohesion is keeping a systematic record of all the activities realized. This becomes the memory of the CIAL and helps communicating their findings to the community and to the institutions. This part of the follow-up questionnaire tried to identify to what extent the CIALs is using this instrument. Data obtained indicates that among the successful CIALs, two thirds of them are maintaining their records actualized and the other third is maintaining them although to a lesser extent. In contrast, among the non-successful CIALs, only 20% of them maintain their records actualized and 30% of them do not keep any record at all. Under these conditions it becomes very difficult to establish and maintaing good relations and communication with the rest of the community and with the institutions. Considering all the CIALs operating in 1997, it could be observed a decrease in the number of those that do not keep any record from 35% in 1995 to 15% in 1997.

F.4 Registers of the COPAL. Does the CIAL keep a Diary of the Experiment?



The importance of keeping a record or diary of the experimental work performed by the CIAL is that they can show more easily to the community and to the institutions the more important findings of their work. They could also benefit from this information when they have to plan a new experiment. Looking at the data obtained, it could be observed that again, among the group of the successful CIALs, there is a very good use of this instrument with almost 70% of the CIALs keeping it clear and actualized. In the group of the non-successful CIALs only 30% of them maintain the Diary actualized and other 30% do not keep it at all. This is another area in which there is a need to implement a remedial action that helps farmers understand the importance of this instrument.

ANNEX 1.

To facilitate the analysis of the follow-up questionnaire, the CIALs were distributed into two groups as follows:

Successful Cials	Non-Successful Cials
Serra da Boa Vista (Pernambuco)	Umbuzeiro (Bahia)
Tatu (Pernambuco)	Sumaré (Bahia)
Gameleira (Pernambuco)	Caldeirao (Bahia)
Chapada (Bahia)	Quiteria (Paraiba)
Valparaiso (Ceara)	Sitio Souza (Paraiba)
Colonia Agricola Roberto Santos (Bahia)	Vila Moura (Ceara)
Buril (Bahia)	Barra (Bahia)
Vitoria de Santo Antao (Pernambuco)	Cadete (Bahia)
Nova Veneza (Ceara)	Lagoa Grande (Ceara)
	Gamileira (Paraiba

OUTPUT IV. GLOBAL WHITEFLY RESEARCH NETWORK TO REDUCE CROP LOSSES INITIATED.

Activity 1. Formation of an International Whitefly Network.

In March of 1996, the Danish International Development Agency *(Danida) invited* CIAT to submit a proposal for the start-up phase (Phase 1) of the Whitefly IPM Project. Phase 1 focuses on the formation of a network of professionals working on whiteflies, whitefly-transmitted viruses and crop management for these pests in the Tropics; and the establishment of a collaborative research agenda by which whitefly problems in the Tropics can be better characterized, corresponding to Outputs 1 and 2 in the work breakdown structure for the CGIAR Whitefly IPM Project (**Figure 1**). The objective of the Danida-funded Phase 1 Project is to gather and analyze the data which already exist, and to generate a limited, additional, critical database, in a standardized fashion, such that qualitative and quantitative analyses will indicate where further whitefly/virus research should be carried out, and what research should be prioritized.

Geographically, the Danida Project is carrying out work in 12 Latin American and 10 African countries (**Figure 2**), to better characterize:

1. Whiteflies as pests in the tropical highlands of Latin America,

2. Whiteflies as vectors of viruses in legumes and mixed cropping systems in the tropical lowlands of Central America, Mexico and the Caribbean,

3. Whiteflies as vectors of viruses in vegetable and legume mixed cropping systems in Eastern and Southern Africa, and

4. Whiteflies as vectors of viruses in cassava and sweet potato in sub-Saharan Africa.

The Project funds, totaling US$1,200,000, were received by CIAT in March of 1997. During March and April 1997, the Project and Sub-project Coordinators revised the Coordination and Sub-project budgets. From April to August, 1997, Memoranda of Understanding were written and signed between the collaborating CGIAR centers, and NARS, to formalize the working relations of this Project. Project funds were transferred to IITA and ICIPE in late August 1997, and subsequently to NARS of all sub-projects. Project operations began in October - November 1997.

Additional donor support and linkages. In response to concerns regarding Project sustainability, The Coordination Team of the Whitefly IPM Project, and the Management Team of the Coordinating Center have been successfully pursuing other linkages with the objective of constructing a constellation of donors to support the global Whitefly IPM Project **(Figure 3)**. ACIAR has approved approximately US$200,000 (1999-2001) to begin a Phase 1-type characterization for whiteflies as pests and vectors in 8 Asian countries. This makes the CGIAR Whitefly IPM Project, truly pantropical.

Fig. 1. Work breakdown structure for CGIAR Whitefly IPM Project, indicating Danida-funded activities.

Sustainable integrated management of whiteflies as pests and vectors of plant viruses in the Tropics

Program Goal

To improve living conditions of rural families through the effective management of whiteflies, resulting in increased crop production and a safer environment

Project Purpose

To reduce crop losses due to whitefly feeding damage and whitefly transmitted viruses, and reduce environmental degradation due to excessive pesticide use, leading to more sustainable and environmentally sound agricultural systems

Outputs / *Activities*

Formation of international whitefly network

- Develop linkages between regional whitefly networks
- Develop an electronic communication network for CG Centers and collaborators in the IPM Whitefly Project
- Standardize methodologies for identification and diagnosis, experimental research, on-farm participatory research and impact assessment
- Publish bibliography of the grey, and other relevant, literature on whiteflies and whitefly-transmitted viruses
- Disseminate research results through CG System electronic and printed media, and publications

Diagnosis and characterization of whitefly problems and target areas

- **Identify whitefly and disease affected areas**
- **Survey farmers' perception of whitefly problem and their crop protection practices, using farmer participatory methods**
- **Collect baseline data on yield loss, pesticide use and resistance levels, and production costs**
- **Identify whitefly species and biotypes and virus strains encountered**
- **Assess prevalence of natural control agents, including entomopathogens**
- **Characterize cropping systems, cropping patterns and cropping intensity in target areas**
- **Characterize agro-ecology, including agrometeorology, and socio-economy of target region**
- **Identify agricultural policies and policy environment in target areas**
- **Survey whitefly populations, disease incidence and production practices**

Improved understanding of pest and disease dynamics

- **Conduct GIS analysis of target eco-regions**
- **Develop analytical tools, including mathematical models, for analysis of pest and disease dynamics**
- Conduct necessary research on the biology, behavior and ecology of pests, vectors, viruses, host plants and beneficials
- Conduct comparative studies on whiteflies and viruses to study the nature and extent of inter-population variation
- Analyze climatological parameters affecting whitefly numbers and virus spread
- Collect and analyze data on whitefly population dynamics
- Develop pest and disease predictive models
- Develop and standardize culturing methodologies for whiteflies and natural control agents
- Map geminivirus plant resistance genes
- Determine components of pest and disease resistance and their epidemiological implications

Management strategies and tactics developed and tested through participatory methods

- **Conduct epidemiological analysis of most efficient intervention strategies**
- Conduct economic analysis, in eco-regional target areas, of feasible intervention tactics
- **Develop and evaluate phytosanitary measures**
- **Develop and evaluate whitefly and virus resistant germplasm**
- Develop and evaluate cultural control tactics
- Develop and evaluate biological control agents and tactics
- Evaluate alternate, safer chemical control agents
- Formulate, implement and validate IPM systems in eco-regional target areas, using farmer participatory methods
- Verify and validate pest and disease forecasters in eco-regional target areas, using farmer participatory methods

Strengthened NARS capacity in IPM research, policy formulation and implementation through information exchange and training

- Produce training and extension materials
- Provide policy recommendations on IPM implementation for whiteflies as pests and vectors
- Train NARS researchers in experimental methodologies
- Train NARS extension personnel and farmers in IPM methodologies and on-farm participatory research methodologies
- Disseminate IPM methodologies at national level through training courses, workshops and field days

Assessment of project impact

- Develop impact assessment methodologies
- Measure rates of adoption or adaptation of IPM systems by farmers, and evaluate constraints
- Assess impact of farmer participatory methodologies
- Assess impact of IPM measures and policy recommendations, specifically on yield loss, production costs and pesticide use

Fig. 2. Subprojects of Danida Phase 1 Whitefly Project.

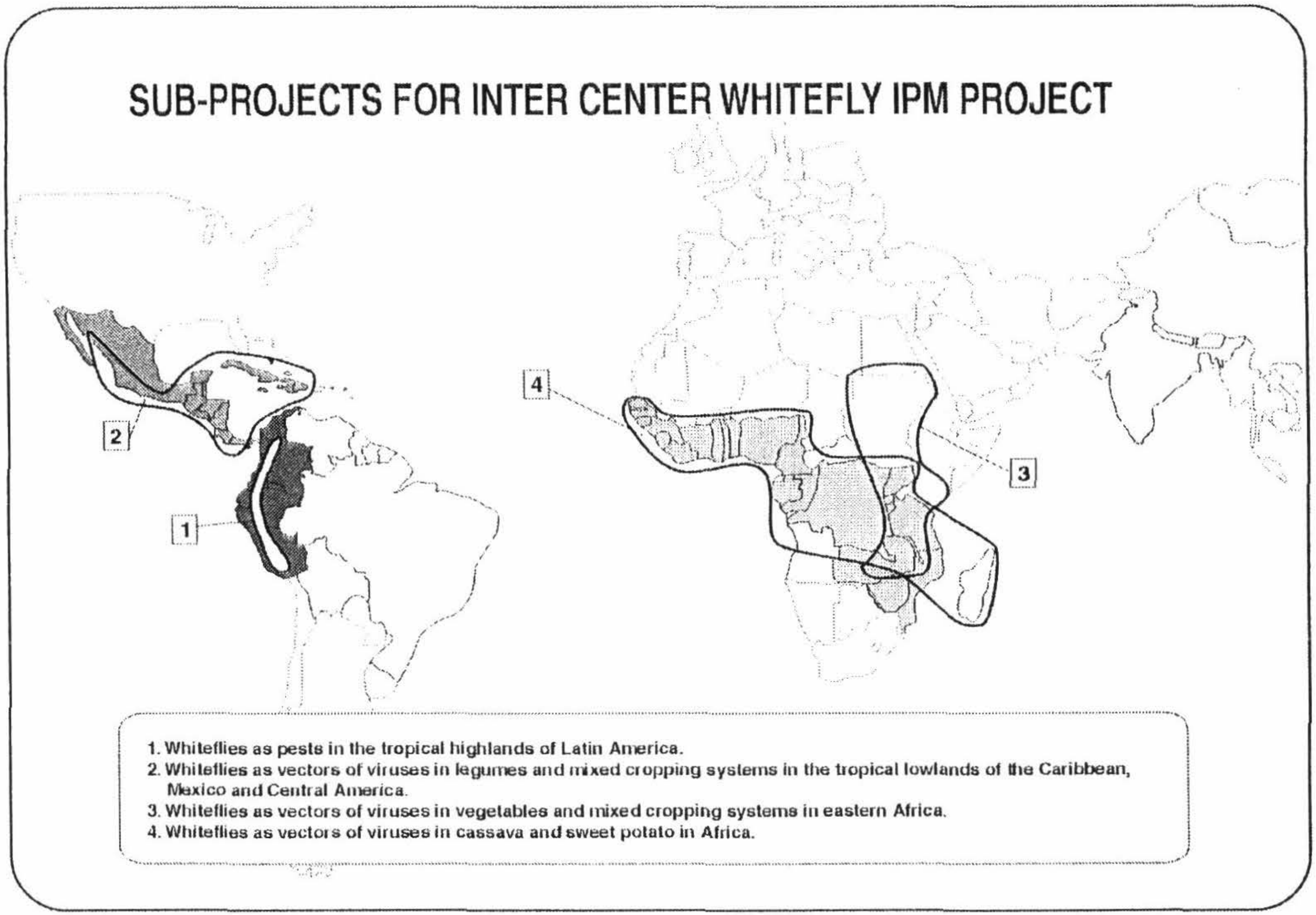


Fig. 3. Work Breakdown Structure: Activities currently receiving direct or indirect support

Sustainable Integrated Management of Whiteflies as Pests and Vectors of Plant Viruses in the Tropics

Network	Diagnosis	Basic	IPM	Training	Impact
Linkages	Yield loss	WF biology	Germplasm	Recommend.	Methodology
Methodology	Pesticide	WF dynamics	Sanitation	Materials	Collaboration
Bibliography	Species	Epidemiology	Biocontrol	IPM tactics	IPM
Directory	Biotypes	GIS	Cultural	FPR	FPR
Publications	Viruses	Biocontrol	Chemical	Implementation	Policies
WEB	Farmer percep.	Resistance	Packages		

DANIDA ACIAR USAID MFAT BMZ

The USAID Office of Foreign Disaster Assistance (OFDA) has granted US$150,000 (1999-2000) to IITA for the "Emergency Programme to Combat the Cassava Mosaic Disease Pandemic in East Africa". The objective of this disaster assistance is to boost production of cassava in Uganda, Kenya and Tanzania and enhance both short and longer term food security, through the implementation of an emergency program to multiply and disseminate mosaic resistant cassava.

The USAID Collaborative Research Grants Program has granted US$63,000 (1998-1999) for studies on biological control of whiteflies pests, by indigenous natural enemies, for major food crops in the Neotropics. The principal objective of this project is to continue exploration of indigenous parasitoids and determine the efficiency of indigenous South American parasitoids against whiteflies pests on cassava.

The New Zealand Ministry of Foreign Affairs and Trade (MFAT) has granted US$300,000 (1998-2000) to CIAT (core substitution funds) for the project Sustainable Integrated Management of Whiteflies through Host Plant Resistance, to be carried out in conjunction with New Zealand Crop and Food Research. From previous research at CIAT, a cassava variety (Ecuador 72), which is highly resistant to cassava whiteflies has been identified. The objective of the MFAT-funded project is to study the mechanism and genetics of this resistance, to map the genes for whitefly resistance in cassava, and develop molecular markers for subsequent use in the improvement of African, Latin American and Asian cassava germplasm.

A project proposal to BMZ is pending. CIAT has submitted a Special Project to BMZ (US$900,000, 1999-2001) on Alternative Management Strategies for Whitefly-transmitted Geminiviruses Affecting Horticultural Crops in Central America, Mexico and the Caribbean. The objective of the BMZ project, if funded, will be to configure and evaluate epidemiologically-effective IPM packages of alternative whitefly management tactics, in a set of pilot zones, utilizing participatory research/technology transfer methodologies.

The information in this progress report represents the efforts of the past year. Understandably, due to factors such as time of cropping seasons and human resources availability, different activities are in different states-of-progress. Much of the field survey information is still being processed and analyzed. Nonetheless, significant progress has already been made in a short period of time.

Networking/Informatics. A document entitled "Standardization of Methods for Whitefly IPM Project Activities" was prepared by the Coordination Team, in consultation with other whitefly and geminivirus experts. The first draft of the Methodology Guide was completed in April of 1997, pilot-tested in Colombia and Uganda and modified accordingly. The Methodology Guide has been translated into Spanish, French and Portuguese and circulated to project partners. It is also being used by colleagues (e.g. Brazil) at present, not formally involved in the Whitefly IPM Project.

A World Wide Web homepage for the CGIAR Whitefly IPM Project, is being developed. The homepage will serve to inform the broader scientific community as to the progress and information being generated by the Project and link the Whitefly IPM Project homepage and to other WWW whitefly homepages. Specifically, the Web page will provide information on: the

Whitefly IPM Task Force, the research projects contributing directly to the Project Work plan; a listing of the formal collaborators in the Project; the Standardization of Methods for Whitefly IPM Project activities, and the documentation project.

The documentation project consists of a searchable database of the grey literature and a directory of professionals working on whiteflies and whitefly-transmitted viruses, in the Tropics. A keyword list, compatible with the CABI Thesaurus, has been drawn up. Over 1100 citations of nonconventional literature (the grey literature) on whiteflies and whitefly-transmitted viruses in Latin America and Africa have been identified and obtained for introduction into the searchable database. To promote and facilitate communication among colleagues, a directory of professionals actively working on whiteflies and whitefly-transmitted viruses in the tropics is being compiled, to accompany the citations in the searchable database. At present, 375 professionals have been entered into the directory, based on information provided in the grey literature documents and workshop lists. Profiles are being mailed to colleagues to verify and update the information.

Activity 2. Diagnosis and Characterization of Whitefly Problem and Target Areas.

Field survey work in Colombia and Ecuador has been completed. Producer interviews are being analyzed. Preliminary findings point to several important conclusions. The dogma has been that *Bemisia* was found 0 to 1000 m and *Trialeurodes* above 1000 m, with numerous reports that *Bemisia tabaci* was expanding its range upwards. Survey work along altitudinal transects, from sea level to over 2500 m, showed that *Bemisia tabaci* is found from 0 to 920m, and *Trialeurodes vaporariorum* is found from 730m to 2740m. Many of the agricultural valleys in Colombia are found at the mid-altitudinal levels (700-900 m) where mixed populations would be present. Based on these findings, altitudinal transects are also being carried out in Costa Rica and Tanzania.

Previously, the focus of research in the highlands has been the common bean (*Phaseolus vulgaris* L.). Field work surveying a broader range of crops verified that in the highlands common bean is the most important host of *Trialeurodes vaporariorum*, followed by tomatoes, and potatoes to a much lesser degree. Thus, a pest management focus of *Trialeurodes vaporariorum* on *Phaseolus vulgaris* for the Andean highland region is justified.

Survey for Pesticide use against Whiteflies and Whitefly-Transmitted Viruses in Affected Zones.

Preliminary analysis of 83 data sets show that 80% of the farmers surveyed know whiteflies as pests and that 41% regard them as key pests on several different crops. In Colombia and Ecuador, 89% use insecticides to control whiteflies. Up to 43% of the applications are made with organophosphate insecticides (OPs) alone; however, OPs are also used in mixtures with carbamates and pyrethroids (**Figure 4**).

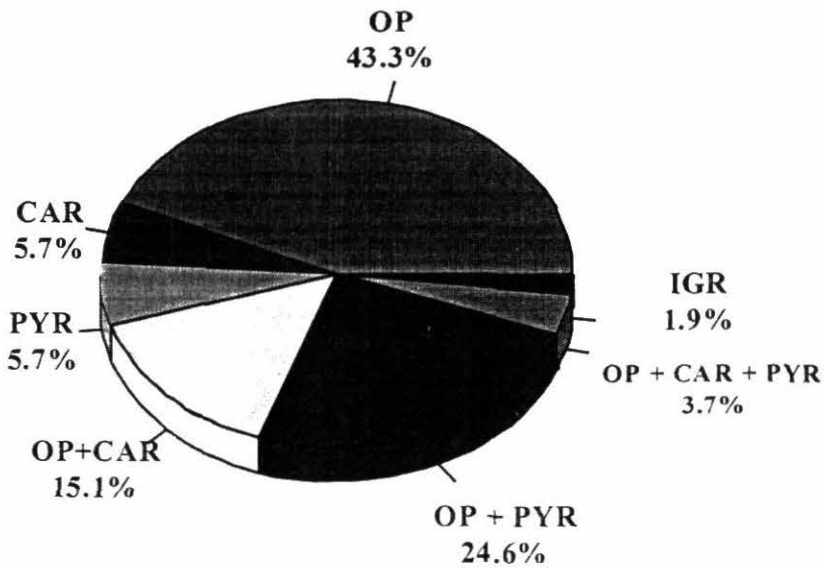


Figure 4. Insecticide use for whitefly control in the Andean zone.

Most insecticides used are highly toxic (30% category I, 44% category II). The frequency of insecticidal applications on tomatoes and snap beans can be as high as three times a week. The number of applications per season varies with regions, but it is interesting to note that 30% of the farmers surveyed make 10 or more applications per cropping season (**Table 1**). Most farmers (69%) use very low, ineffective dosages (0.2 cc/l) or extremely high (4.8 cc/l) dosages. Up to 80% of them take their own decisions on timing of applications. More than half (52%) spray on a calendar basis, regardless of infestation levels; only 30% of those surveyed receive some kind of technical assistance. Many (41%) are influenced by pesticide salesmen. Less than 10% take precautions (use of masks, gloves) when using highly toxic insecticides. Up to 35% of farmers claim that crops have been abandoned as a result of WF attack or WF-induced problems.

Table 1. Patterns of insecticide use for whitefly control in selected areas of the Andean zone.

No. of Insecticide Applications per Cropping Season[1]	% Farmers
1-3	34.0
4-6	21.4
7-9	14.3
10	8.9
>10	21.4

[1] 89% of farmers use insecticides for whitefly control; however, just 9% of the applications have only whiteflies as target insects; 59% of applications are made to control whiteflies and other insects; 21% are made to control other insects.

Baseline data for insecticide resistance levels in *T. vaporariorum* (**Table 2**) and *B. tabaci* (**Table 3**) were obtained using mass rearings maintained at CIAT for several years. For each species the LC_{50} values were calculated for an organophosphate (methamidophos), a carbamate (methomyl) and a pyrethroid (cypermethrin). With the baseline data, dosages for percentage mortalities ranging from 5 to 95% were used to calculate mortalities at diagnostic concentrations (**Table 4**). The diagnostic concentrations were then used to monitor insecticide resistance levels in the field using the vial technique.

To date, the analyses of 29 tests suggest that resistance of *T. vaporariorum* to organophosphates is widespread in the Andean regions of Ecuador and Colombia. Low levels of resistance of *T. vaporariorum* to cypermethrin occur in specific areas of the Andean zone. No significant resistance to methomyl was detected. Biotype B of *B. tabaci* present on the Atlantic Coast of Colombia showed high levels of resistance to carbamates (methomyl) (**Figure 5**) and to OPs (methamidophos) (**Figure 6**), with less resistance to pyrethroids (cypermethrin). Intensive survey of insecticide resistance levels should continue.

Table 2. Toxicological responses of laboratory strains of *T. vaporariorum* to three insecticides, using insecticide coated glass vials.

Insecticide	n	Slope ± SEM	LC_{50} (95% FL)[1]
Methomyl	457	2.2 ± 0.5	0.25 (0.15 -2.6)
Methamidophos	600	2.0 ± 0.5	5.3 (2.5 – 7.6)
Cypermethrin	480	1.2 ± 0.2	37.0 (22.0 - 55.7)

1 µg/vial

Table 3. Toxicological responses of laboratory strains of *B. tabaci* to three insecticides, using insecticide coated glass vials.

Insecticide	n	Slope ± SEM	LC_{50} (95% FL)[1]
Methomyl	500	1.7 ± 0.2	1.7 (1.1 – 2.3)
Methamidophos	517	1.9 ± 0.5	1.4 (0.9- 1.6)
Cypermethrin	502	1.1 + 0.1	14.4 (5.8 – 27.2)

1 µg/vial

Table 4. Response (% mortality) of *T. vaporariorum* and *B. tabaci* adults to three insecticides (diagnostic dosages were tested using insecticide coated glass vials).

Whitefly species	methomyl (2.5 µg/vial)	methamidophos (32 µg/vial)	cypermethrin (500 µg/vial)
T. vaporariorum	97	99	88
B. tabaci	99	100	98

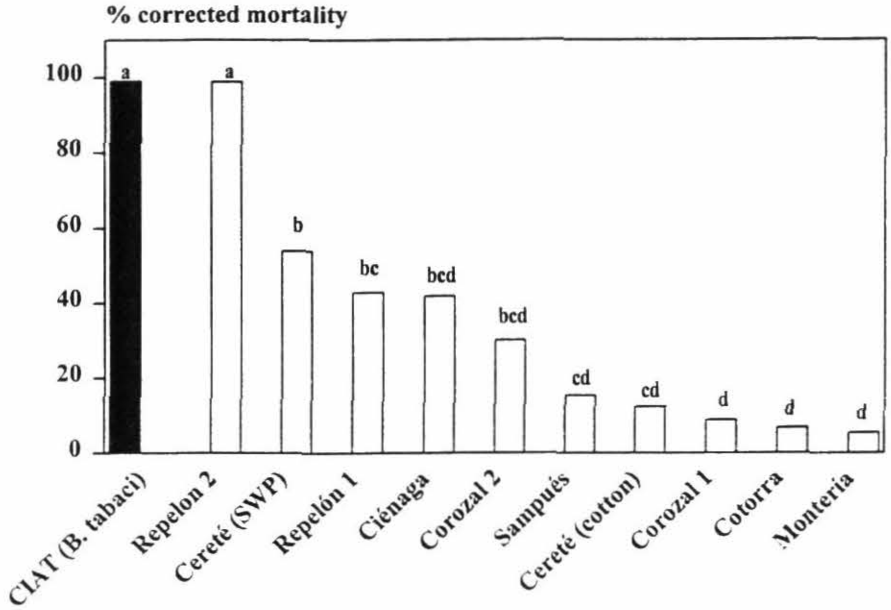


Figure 5. Responses of *B. tabaci*, biotype B, populations to methomyl on the Atlantic Coast of Colombia.

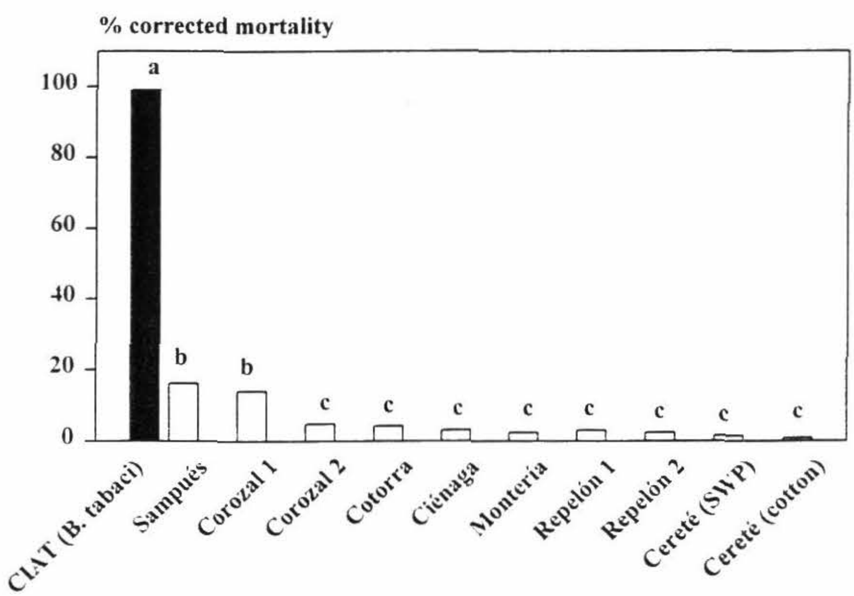


Figure 6. Responses of *B. tabaci*, biotype B, populations to methamidophos on the Atlantic Coast of Colombia.

Identify Whitefly Crop Reproductive Hosts.

The following crop plants have been identified as reproductive hosts of *T. vaporariorum* in the Andean region of Colombia and Ecuador: beans, tomatoes, potatoes, snap beans and Cucurbita spp. *B. tabaci* biotype A has been recorded breeding on soybean, tomato and eggplant. *B. argentifolii* has been found reproducing on cabbage, egg plant, lima beans, tomato, squash, pepper, *Brassica* spp., melon, dry beans, cotton and watermelon. This activity will continue. Identification of some wild hosts is pending.

A greenhouse-laboratory study on the comparative biology of biotypes A and B of *B. tabaci* on 20 cultivated host plants was initiated. Results obtained so far (10 host plants) can be summarized as follows: (a) There were no significant differences between biotypes in terms of total oviposition per plant. Ovipositional behavior did not differ either; (b) Biotype B bred faster on cabbage and cauliflower than biotype A; and (c) Percent emergence of biotype B was significantly higher on 9 out of 10 host plants studied (**Figure** 7).

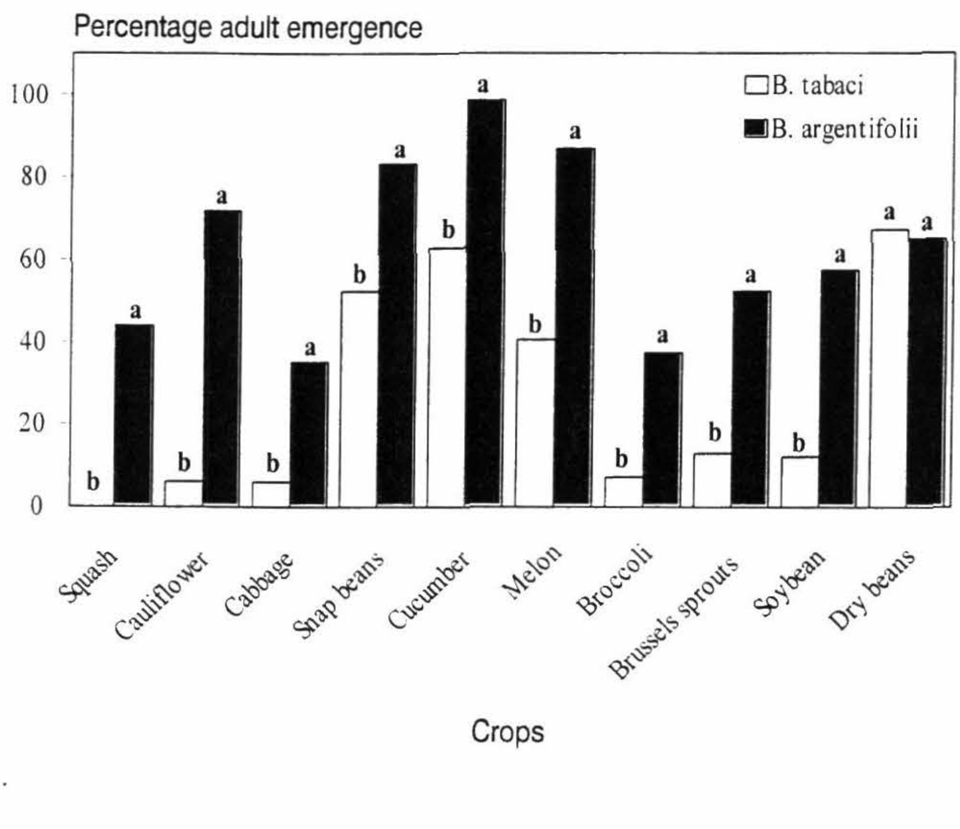


Figure 7. Percent emergence of two whiteflies species on different host plants.

Virus and Vector Characterization in Latin America.

From literature analysis it is clear that there are at least 3 bean-infecting geminiviruses, 17 tomato-infecting geminiviruses, 3 pepper-infecting viruses and geminivirus detections in melons, and cotton. Preliminary results from the field surveys indicate that over 90% of the bean samples collected are infected by geminiviruses, but the detection rate for geminiviruses in horticultural crops is lower: only 75% and 35% of the samples collected for tomato and pepper, respectively are infected with geminiviruses. There are other plant viruses affecting horticultural crops, which the producers are confusing with geminiviruses. Conversely, new detections of geminivirus in tobacco and soybean are being made. The B biotype of *Bemisia tabaci* is prevalent throughout the region.

Distribution and Molecular Information on the Whiteflies that can be Compared at a Global Level.

Through coordination of the research efforts especially in the standardization of protocols, the information that is being collected in the Americas can be directly compared with the data generated from Africa.

The use of taxonomic characteristics does not distinguish between biotypes of *B. tabaci*. The first part of this activity was the standardization of protocols for the extraction of DNA and the development of RAPD markers. The use of mitochondrial markers for the cloning and sequencing of the carboxy oxidase subunit I gene is being used for the evolutionary relatedness of whiteflies of the same species. This was done in conjunction with Peter Markham and Gina Banks of the John Innes Institute. Our efforts to coordinate the methods and materials with our partners in the region and with our partners at the John Innes Institute will allow the results from this survey to become part of a global database.

Use of RAPDs for the analysis of whitefly populations.

Significant progress has been made in determining the distribution of whitefly pests in four countries.

The identity of whiteflies from five countries on 16 different crop plants and several weed species were determined using RAPDs. From last year, there is baseline data for the identification of *B. tabaci* biotypes A and B, *B. tuberculata*, *Trialeurodes vaporariorum*, *Trialeurodes variabilis* and *Aleurotrachelus socialis*. To date 181 samples have been analyzed from Guatemala. The most frequent whitefly in approximately 60% of the samples was *B. tabaci* B-biotype. In the Department Zacapa the dominant whitefly on okra, melon, watermelon and peppers is the B-biotype. This contrasts with the Department Baja-Verapaz where *T. vaporariorum* is dominant on tomato, peppers, beans, and weeds. In the Department Jalapa *B. tabaci* A-biotype, B-biotype and *T. vaporariorum* are all present. Here there appears to be host preference. The *B. tabaci* A-biotype were mostly found on papaya. Most of the *T. vaprorariorum* were found on watermelon in one area. The B-biotype was found on watermelon and was the dominant species on beans, peppers, chilies, melons, eggplant, and tobacco.

In the Dominican Republic and Cuba nearly all of the samples were the B-biotype. About 15% of the samples from the Dominican Republic were not definitively identified. The morphological data indicated that the whiteflies in these samples were *B. tabaci*. The banding pattern was closer to the B-biotype than the A-biotype. It is unclear if these are a distinct sub-population or a variation of the B-biotype. This will be determined by analysis of the carboxy oxidase subunit I gene.

Most of the samples in Colombia were from the north coastal region. In the Department Sucre the B-biotype appears to be dominant. In the Department Atlantico, the B-biotype is very common but *T. vaporariorum* was found on tomatoes and an unidentified whitefly with a RAPD pattern similar to *B. tuberculata* was found on eggplant. This is another region where the B-biotype appears to be co-existing with other species or biotypes of whiteflies. The trend needs to be followed in time to determine if this is the middle of the transition in which the B-biotypes excludes other whiteflies or if the humid tropical environments are more favorable to mixed populations of whiteflies.

Several samples of whiteflies were collected from cassava. On the north coastal regions of Colombia, *B. tabaci* B-biotype, *B. tuberculata*, and *A. socialis* were all found on cassava. Cassava is a host to the B-biotype and this confirms observations that were made previously in the Caribbean region and southern Florida.

Whitefly samples were collected in two sites in Brazil. One site was near Sta. Maria da Victoria in Bahia. In the last 3-4 years CFSD has become a problem in this region. Only a few whiteflies were found and these were the B-biotype of *B. tabaci*. This is the first association between *B. tabaci* B-biotype and CFSD. The suspected vector of CFSD is *B. tuberculata*. Studies are underway to determine if the B-biotype can transmit CFSD.

During 1998, scientists from Cuba, Dominican Republic and Guatemala were trained in using RAPDs for the identification of whitefly species.

Mitochondrial Markers for Analysis with Species of whiteflies.

The carboxy oxidase subunit I gene is proving useful in studies of the evolutionary relatedness of whiteflies. This gene has two highly variable regions and is widely used in evolutionary studies.

Last year the analysis of biodiversity of whiteflies was begun using an intergenic region of the mitochondrial 16S ribosomal subunit. The cloning and sequencing of this region was completed for *B. tabaci* biotypes A and B, *B. tuberculata*, *Trialeurodes variabilis*, *T. vaporariorum* and *Aleurotrachelus socialis*. This complemented a study of the morphology of adult whiteflies of these species. The most curious result is the large differences between the two species of *T. variabilis* and *T. vaporariorum*. Only about 60% of the nucleic acids are identical (see Table 1 for the similar results that were obtained from the sequence of the carboxy oxidase subunit 1 gene). Using the Phylip parsimony analysis programs, these *T. variabilis* and *T. vaporariorum* are most closely related to each other compare to the other species (dendrogram not shown).

In consultation with our partners in John Innes Institute and others, and based on several studies of biodiversity including a range of insects, the carboxy oxidase subunit I gene was chosen to replace the intergenic region of the 16S ribosome subunit in all further analysis. This is based on the number of base changes that are significant. Within the carboxy oxidase subunit I gene, two regions have been identified as being the most variable. Several sets of primers were tested for their ability to amplify both variable regions of the carboxy oxidase subunit I gene. At present 10 different clones of the six whiteflies in the study using the 16S ribosomal region have been sequenced. **Table 5** gives the percentages of identity between the various clones. The two clones of the A-biotype are 99% identical. Also the three clones of the B-biotype are 99% identical. Additional whiteflies collected from the other regions are in the process of being cloned and sequenced. Between the A and B-biotypes of *B. tabaci*, there is about 90% homology. This compares with only 79% homology between *B. tuberculata* and either the A or B biotype of *B. tabaci*. The two clones of *A. socialis* have 94.5% identity, and degree of variation may indicate diversity within this species.

Table 5. The percentage of nucleotide identity between a region of the carboxy oxidase subunit I gene of a several whiteflies species and biotypes.

Whitefly spp. Location Host	*B. tab.* A	*B. tab.* A	*B.tab.* BY	*B.tab.* BF	*B.tab.* B Suc	*B. tub.*	*A. soc.*	*A. soc.*	*T. vap.*	*T. var.*
B. tabaci A CIAT Beans	–	99.2	90.5	90.3	89.6	78.3	71.8	71.3	71.2	62.3
B. tabaci A CIAT Beans		–	90.2	90.0	89.4	79.3	71.6	71.5	71.8	60.1
B. tabaci B CIAT Cassava			–	99.6	99.2	79.9	73.0	72.2	70.7	63.3
B. tabaci B CIAT Beans				–	98.8	79.7	72.8	72.2	70.5	63.0
B. tabaci B Sucre Col					–	79.3	72.4	71.8	70.8	63.6
B. tuberculata CIAT Cassava						–	76.2	75.9	71.7	64.7
A. socialis CIAT Cassava							–	94.5	70.8	62.0
A. socialis Mondomo- cassava								–	70.0	62.3
T. vapor- ariorum CIAT-Beans									–	61.0
T. variabilis CIAT Cassava										–

Development of Addition Markers with Emphasis on Insecticide Resistance.

The development of molecular markers for whiteflies is part of the strategy to develop a better methods to detect insecticide resistance. There are various known mechanisms of insecticide resistance including the over expression of esterases and point mutations with the gene for acetylcholinesterase. A set of degenerate primers based on sequences of known acetylcholinesterase was designed. The acetylcholinesterase gene from whiteflies could not be detected after PCR amplification. This result was expected since it is difficult to amply single copy genes using degenerate primers.

It was decided to make a library of cDNA clones from the messenger RNA. This produces a library of expressed sequence tags (EST). Good quality RNA was extracted from the pupa of the whiteflies. The first strand reverse transcriptase synthesis was primed using oligo T. A round of PCR amplification was done either with the acetylcholinesterase degenerate primers or groups of 10 base oligonucleotides. A small library was produced and is in the process of being analyzed. There are only a few known sequences of insect acetylcholinesterase genes. This gene is expressed in nerve cells and is a relatively scarce mRNA. It is too early to determine if this novel cloning method will allow a part of this gene to be cloned from whiteflies. To date there is no sequence information on this gene and this reflects the difficult nature of this experiment. A useful library of ESTs is being produced. ESTs are useful as markers and in the genome mapping of species. This resource will be used in the effort to find markers of insecticide resistance.

The biotype data generated, to date, is presented in **Table 6.** The *B. tabaci* biotype B geographical range is rapidly expanding and this insect is now a problem in most countries of tropical America. Using the oligonucleotide primers, this biotype looks very similar to the biotype B found in Israel, Egypt and Florida. A portion of the 16S gene from several isolates of the biotype B were cloned and sequenced. Only minor differences were found. There is 98-100% identity between the biotypes found in Colombia, Egypt and Florida. *B. tuberculata* was collected from cassava in several different regions, and all appear to be quite similar. This type of characterization is being done with all the whitefly species and over a range of regions.

Table 6. Completed whitefly species and biotype identifications using RAPDs and H9, H16 and F12 primers (CIAT).

Species/Biotype	Colombia	Dom. Rep.	Cuba	Guatemala	TOTALS
B. tabaci A	0	2	0	13	15
B. tabaci B	32	89	54	117	
B. tuberculata	28	9	0	0	37
T. vaporariorum	8	0	0	63	71
A. socialis	1	0	0	0	1
Undetermined	18	6	0	3	27
TOTALS	87	106	54	196	443

A Geographic Information System for the region has been set up for the data from Mexico, Central America and the Caribbean. Base maps (to the municipal level), and climatic maps have been completed. Development of crop use maps is advancing, but is slow due to the general lack of information on the geographical distribution of horticultural crops in the region. A separate database for the biological data (geminiviruses, whitefly species and biotypes, host plants, natural enemies) has been established. Once data from the literature and field surveys has been entered, this will be fed into the GIS.

Field work indicates that the diversity of IPM responses by the producers is much greater than originally anticipated and much more localized than expected (and it appears that the localization is a function of the research interests and biases of the local scientists/ agronomists/extension agents). A major step forward can be taken by gathering the diverse IPM tactics, and after epidemiological analysis, re-formulating them into a series of improved IPM packages that can be adapted depending upon the local socio-economic constraints. This can be done while needed research moves forward and continues to feed into the process of improved IPM options.

Research in Eastern and Southern Africa.

All tomato farmers seem to use some chemical pesticides to control the whitefly/disease problems. One farmer in Kenya also used ash in addition to chemicals to manage the whitefly disease problems on his tomato farm. Farmers in Sudan, Kenya and Tanzania made an average of 10, 9 and 7 applications of pesticides per season, respectively, against whitefly/disease problems, while in Malawi an average of 6 applications were made (**Figure 8**). The majority of farmers apply insecticides on a calendar basis and as a prevention measure rather than based on threshold levels or when they observe whitefly or disease damage. Recommendations for control methods were received by the farmer from other producers, technicians, sales persons, family members and neighbors, among others, in varying frequencies in the four countries (**Figure 9**).

As whitefly research is incipient at ICIPE, it was necessary to establish basic facilities and human resources for the Project work. A laboratory has been dedicated to the whitefly research, and a research team has been formed. ICIPE has also taken leadership in promoting the formation of an African Whitefly Network.

Field surveys have been initiated in all four partner countries. However, El Nino flooding severely affected the ability to access many sites. Survey work will continue in 1998. Nonetheless, preliminary findings are interesting.

Based on 354 whitefly collections, *Bemisia tabaci* is the most common whitefly species, but *Bemisia afer*, *Bemisia hirta*, *Aleyrodes proletella*, *Trialeurodes vaporariorum* and *Trialeurodes recini* were also found. More species of whitefly have been found to occur in the region than are been reported from the literature. The B biotype of *Bemisia tabaci* has been identified from Sudan.

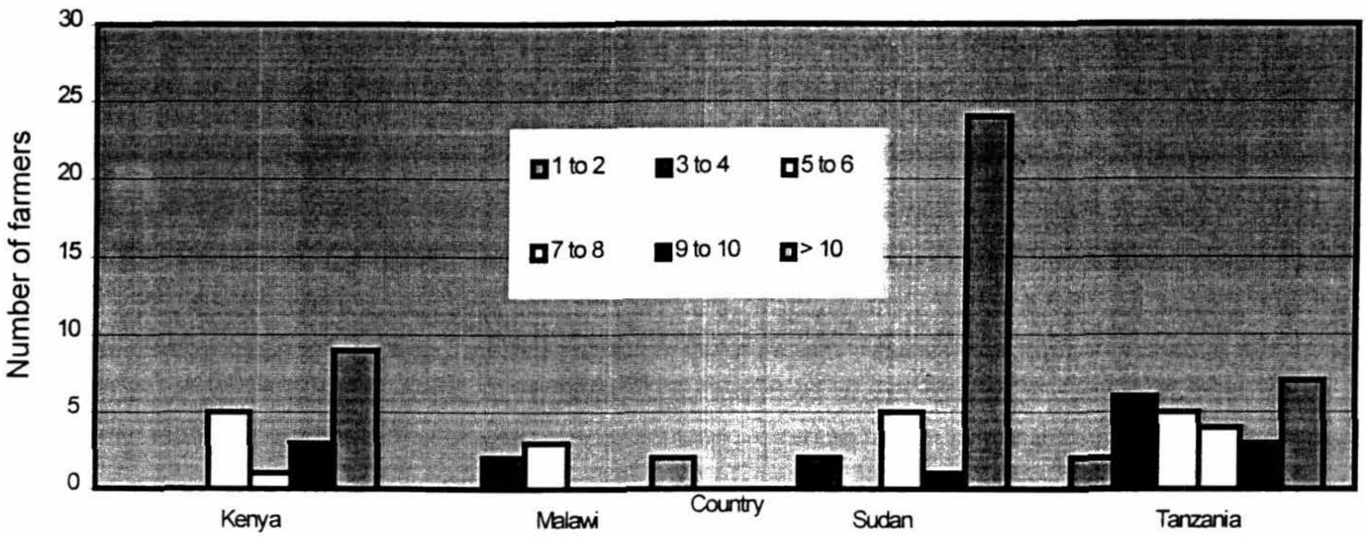


Figure 8. Frequency of insecticide application by farmers against whiteflies in one season.

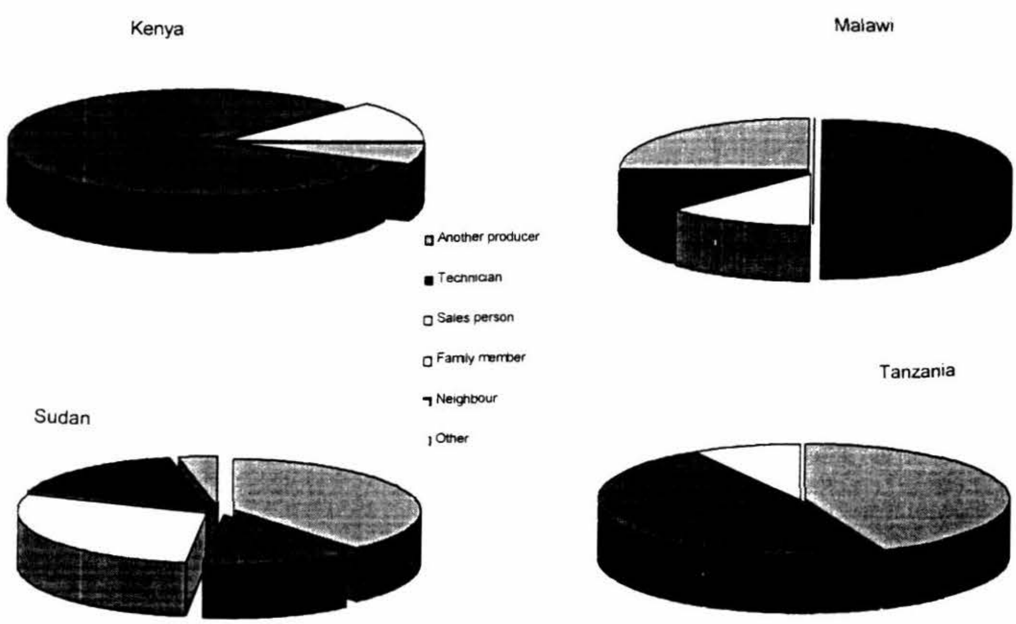


Figure 9. Who recommended the whitefly management practices that the farmers use.

Adults of Bemisia tabaci are commonly found on tomatoes, but do not reproduce on tomatoes, except in the Sudan. Identification of the most important reproductive hosts for *Bemisia tabaci* is a priority.

Bemisia-transmitted tomato yellow leaf curl virus (TYLCV) is found throughout the region. Perceived on-farm losses due to tomato geminiviruses were up to 40% in Malawi, 50% in Kenya, 75% in Tanzania and 100% in Sudan. Yield losses for tomatoes are high and often result in crop abandonment. Sudan suffers up to 75% crop loss, annually, in okra, watermelons/melons, peppers and *Vigna/Phaseolus* bean. Kenya and Tanzania are also finding problems on these crops, suggesting that the work should be expanded to include a mixed crop framework.

However, many of the virotic tomato samples collected have tested negative for TYLCV, suggesting that there may also be a complex of indigenous tomato-infecting geminiviruses in the region, similar to that found in Latin America. A scientific exchange between Project virologists is planned for early 1998 to share diagnostic techniques for general geminivirus detection. Other, interesting patterns are emerging, which need further exploration. TYLCV has not been identified from Western Kenya (ca. 1100 m). But at higher elevations (ca. 1500-1800 m), where it should be less likely to occur, the incidence of TYLCV is high.

Research in Sub-Saharan Africa.

At the outset of the Project, it was known that there was minimal if any usage of pesticides in cassava and sweet potato production systems in sub-Saharan Africa, and therefore agreed that it would be inappropriate to incorporate routine questions and protocols for insecticide use and the assay of insecticide resistance into the surveys of Subproject 4. It was suggested, however, that populations of *B. tabaci* from cassava and sweet potato in Africa might serve as good pesticide-sensitive controls for comparison with populations from the Americas.

Determine Insecticide Resistance Levels in Target Areas

Field surveys have been completed in 7 of the nine partner countries. Kenya and Tanzania will be completed in 1998. Interview data has been analyzed for Uganda. Western Africa and the rest of Eastern Africa data are currently being analyzed.

The disease front for the severe African cassava mosaic disease (ACMD) epidemic has been defined. Based on this knowledge, generated from the Phase 1 survey work, additional funding has been obtained to multiply and deploy the new elite lines of ACMD-resistant cassava. They will be planted ahead of the epidemic front, in an attempt to slow down and contain the epidemic, which is currently spreading at the rate of about 20 km per year, and now threatens Rwanda, Burundi, NW Tanzania and additional area in Western Kenya.

The new "Uganda variant" geminivirus seems to be pushing out the old ACMV virus. Mixed infections have been detected. But the distribution and the presence of separate African cassava mosaic geminiviruses, as well as mixed infections, needs better characterization.

With ACMD affecting cassava production, and declining banana production, sweet potato in becoming more important as a food security crop in the region. Phase 1 field survey work has shown that in addition to sweet potato weevils, sweet potato viral diseases are also a production constraint in southwestern Uganda and northwestern Tanzania, indicating the priority areas for deployment of resistant sweet potato varieties and screening work. Interestingly, in Northeastern Uganda, there is not much virus pressure, suggesting that these two areas would provide an instructive comparative study.

Based on over 200 whitefly samples, *Bemisia tabaci* is the predominate whitefly species on cassava and sweet potato. However, *Bemisia afer* is more prevalent in the southern area (e.g. Madagascar) than previously believed.

Parasitism is estimated at 30-60%, for *Bemisia tabaci* in cassava. *Encarsia transvena* is the most abundant parasitoid. *Encarsia formosa* and *Eretmocerus* spp. have also been found. However, parasitism has been difficult to evaluate in the extensive survey. Additional, intensive survey work targeting the biological control agents, is necessary as this may offer an important vector management component for this cropping system.

Phase 1 has been instrumental in defining the overall importance of the problem as well as the diagnostics, research and implementation needs. Phase 1 improved knowledge, gave a more reliable assessment of damage, IPM practices and what other IPM problems need to be studied, and the importance of the role for extension.

In less than one year, a coordination network has been developed, methodologies standardized, literature and field surveys conducted, biological samples diagnosed, regional epidemiology characterized and mapped, databases for literature and biological data developed, and a GIS established. All data collection will be finished in 1998, and analysis completed in early 1999. The publication of a book, to serve as the final report for Phase 1, is planned for mid-1999.

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Publications

Sánchez S. David. A.C. Bellotti. 1997. Patogenicidad de hongos hyphomycetes sobre *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) chinche subterráneo de la yuca. Revista Colombiana de Entomología. Vol. 23. No. 1 & 2. pp. 31-37.

Bellotti, A.C., L. Smith, S. Lapointe. Submitted. Recent advance in cassava pest management. Accepted: Jan. 1999. Annual Review of Entomology.

Evans, G.A. and J.A. Castillo. 1998. Parasites of *Aleurotrachelus socialis* (Homoptera: Aleyrodidae) from Colombia including descriptions of two new species (Hymenoptera: Aphelinidae: Platygasteridae). Florida Entomologist 81(2)171-178.

Calatayud, P.-A.; Rouland, C. & Le Rü B. 1997. Influence de la linamarine dans les relations manioc-cochenille. Acta Botanica Gallica 144(4): 427-432.

Calatayud, P.-A.; Delobel, B.; Guillaud, J. & Rahbé, Y. 1998. Rearing the cassava mealybug, *Phenacoccus manihoti*, on a defined diet. Entomologia Experimentalis et Applicata 86: 325 - 329.

Papers Submitted to Refereed Journals

Riis, L., B. Arias, A.C. Bellotti. Submitted. Bionomics and population growth statistics of the subterranean burrower bug, *Cyrtomenus bergi* on different host plants. Submitted to Entomologia Experimentalis et. Applicata.

Riis, L., A.C. Bellotti, M. Bonierbale, G. O'Brien. Submitted. Cyanogenic potential in cassava, its influence on a generalist insect herbivore. Submitted to Ecological Entomology.

Riis, L. A.C. Bellotti, O. Castaño. Submitted. In field damage on cassava clones of high and low cyanogenic potential due to a generalist insect herbivore. Submitted to International Journal of Pest Management.

Bento, J.M.S., A.C. Bellotti, J.A. Castillo, G.J. De Moraes, S.L. Lapointe, and J.F. Warumby. Submitted. Introduction of parasitoids for control of cassava mealybug in northeastern Brazil. Submitted. Bulletin of Entomological Research.

Alvarez E; Chacón MI; Grajales O; Loke JB; Gómez PL. Avances en la caracterización patogénica y genética de *Phytophthora* spp. agente causal del complejo pudrición de cogollo en palma africana *Elaeis guineensis* Jacq. Revista Palma.

Alvarez E; Chacón MI; Sánchez NJ; Loke JB. DNA Polymorphism and virulence variation of *Phytophthora* population isolated from Cassava *Manihot esculenta* Crantz. Plant Disease.

Alvarez E; Harrington TC; Chacón MI; Gómez P. L.; Ayala L;Villegas V; Loke JB; Llano G. Pathogenic and genetic characterization of *Thielaviopsis paradoxa* isolates from oil palm *Elaeis guineensis* Jacq. in Colombia, Ecuador and Brazil. Plant Disease.

Alvarez E; McGee DC; Harrington T. Molecular variation in strains of *Fusarium* spp. affecting corn seed quality. Plant Disease. (PD-8623).

Alvarez E; Ramírez J. Extracción rápida de ADN, detección por PCR y dot blot de *Phytophthora* spp. en material vegetal de yuca, *Manihot esculenta* Crantz. Fitopatología Colombiana.

Alvarez E; Ramírez J; Marmolejo F. Determinación *in vitro* de la sensibilidad térmica de cepas de *Xanthomonas axonopodis* pv *manihotis*, agente causal de la bacteriosis vascular de la yuca. Fitopatología Colombiana.

Molina ML; Alvarez E. Caracterización morfológica y molecular de aislamientos de *Sphaceloma manihoticola* Bitancourt & Jenkins, agente causal del superalargamiento en *Manihot esculenta* Crantz. Fitopatología Brasilera.

Molina ML; Alvarez E. Characterizing the *Sphaceloma* fungus, causal agent of superelongation in Cassava. Plant Disease. PD 8763.

Thesis

Múnera, D.F. and J. de los Ríos. 1998. Patogenicidad sobre *Erinnys ello* L. en condiciones de laboratorio de hongos entomopatógenos recolectados en cultivos comerciales de yuca *Manihot esculenta* Crantz en el Valle del Cauca, Colombia. Thesis de Grado. Universidad Nacional de Colombia, Palmira.

Riis, L. 1997. Behavior and populations growth of the burrower bug, *Cyrtomenus bergi* Froeschner; effects on host plants and abiotic factors. Thesis, Ph.D. Department of Ecology and Molecular Biology Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Bertschy, C. 1998. Diversified cassava agro ecosystems: Chemically mediated searching behaviour of parasitoids. Thesis, Ph.D. Swiss Federal Institute of Technology Zurich.

Sánchez NJ. Caracterización de *Phytophthora* spp. agente causal de pudrición en raíz de yuca (*Manihot esculenta* Crantz) utilizando pruebas de patogenicidad y técnicas moleculares.
B.S. Thesis. Universidad Nacional de Colombia (Santafé de Bogotá). High Honors.

Polanía, M.A. 1998. Influencia del déficit hídrico en la relación de la yuca (*Manihot esculenta)* y el pijo harinosos (*Phenacoccus herreni*): cambio en la composición foliar de aminoácidos y sus repercusión en el desarrollo del insecto. Thesis de Grado. Pontificia Universidad Javeriana, Facultad de Ciencias, Carrera de Biología, Bogotá, D.C.

Seligmann, C.D. 1998. Influencia de la nutrición del piojo harinoso *Phenacoccus herreni* de la yuca *Manihot esculenta* en el desarrollo de sus parasitoides *Acerophagus coccois*, *Aenasius vexans*, *Apoanagyrus diversicornis*. Universidad de los Andes, Facultad de Ciencias, Departamento de Ciencias Biológicas, Santafé de Bogotá, D.C.

Conferences and Posters

Anderson, P.K. 1997a. The CGIAR Whitefly IPM Project: A New Forum of Collaboration, presentation at the International Workshop on Geminivirus in the Caribbean, Havana, Cuba, November 28, 1997.

Anderson, P.K. 1998a. The CGIAR Whitefly IPM Project: A New Forum of Collaboration, presentation at the REDCAHOR 1st IPM planning meeting, Constanza, Dominican Republic, February 3, 1998.

Anderson, P. K. 1998b. The SP-IPM Project on Sustainable Integrated Management on Whiteflies as Pests and Vectors of Plant Viruses in the Tropics, symposium presentation at International Workshop on *Bemisia* and Geminiviruses, San Juan, Puerto Rico, June 7-12, 1998.

Anderson, P.K. and A.C. Bellotti. 1997. Sustainable Integrated Management of Whiteflies as Pests and Vectors in the Tropics. International Workshop on the Silverleaf Whitefly, *Bemisia argentifolii*. EMBRAPA/CENARGEN. Brasilia, Brazil, November 4-6, 1997.

Bellotti, A. C. 1998. Host Plant Resistance of Whiteflies in Colombia, symposium presentation at International Workshop on Bemisia and Geminiviruses, San Juan, Puerto Rico, June 7-12, 1998.

Smith, L., E.L. Melo, J.R. Escobar, M.E. Cuéllar and J.M. Guerrero. Dec. 1997. Foreign explorations for phytoseiid mites in Northern South America for classical biological control of the cassava green mite in Africa. Entomological Society of America National Conference, Louisville, KY.

Polanía, M.A., P.A. Calatayud, A.C. Bellotti. 1998. Estudio del comportamiento alimenticio de *Phenacoccus herreni* (Homoptera: Pseudococcidae). XXV Congreso de la Sociedad Colombiana de Entomología, Cali, Colombia. July 16-18, 1998.

Múnera, D.F., J. de los Ríos, A.C. Bellotti. 1998. Patogenicidad sobre *Erinnyis ello* (Lep.: Sphingidae) en condiciones del laboratorio de hongos entomopatógenos recolectados en cultivos comerciales de yuca, *Manihot esculenta* Crantz, en el Valle del Cauca, Colombia. XXV Congreso de la Sociedad Colombiana de Entomología, Cali, Colombia, July 16-18, 1998.

Seligmann, C.D.; Calatayud, P.-A. & Bellotti, A.C. 1998. Comportamiento alimenticio del piojo harinoso de la yuca *Phenacoccus herreni* parasitado por tres especies de parasitoides. II Seminario Taller Internacional "Aportes del Control Biologico en la Agricultura Sostenible" y I Congreso LatinoAmericano de la Seccion Regional Neotropical de la Organizacion International de Lucha Biologica. Lima, Perú, 12-22 Mayo 1998.

Calatayud, P.-A.; Delobel, B.; Guillaud, J. & Rahbé, Y. 1998. A new phenolic compound detected in the cassava mealybug *Phenacoccus manihoti*. Tenth International Symposium on Insect-Plant Relationships. Oxford, England, 4-11 July 1998.

Alvarez E. Use of molecular markers as tool in Cassava Pathology Laboratory. May 8, Universidad Nacional de Colombia- Palmira, Colombia.

Alvarez E; Barragán MI; Madriñán R. Pudrición radical y marchitez de la yuca. Boletín. FERIVA.

Alvarez E; Chacón MI; Sánchez NJ. DNA Polymorphism and virulence variation of *Phytophthora* population isolated from Cassava *Manihot esculenta* Crantz. 7 th International Congress of Plant Pathology. Volume 2, 1.8.30. ICPP98, Meeting, 9-16 August, Edinburg, Scotland.

Alvarez E; Harrington T; Gómez PL. Análisis genético del complejo *Ceratocystis paradoxa*. VIII Sala General de CENIPALMA, Congress on oil palm. June 3, Cali, Colombia.

Alvarez E; Loke JB; Gómez PL. *Phytophthora* spp. posible agente causal del complejo pudrición de cogollo. VIII Sala General de CENIPALMA, Congress on oil palm. June 3, Cali, Colombia.

Alvarez E; Sánchez Cusgüen NJ; Loke JB. Manejo de *Phytophthora* spp. en Laboratorio. Laboratory manual. Cassava Pathology. CIAT. July.

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