PROJECT PE-1

INTEGRATED PEST AND DISEASE MANAGEMENT IN MAJOR AGROECOSYSTEMS

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Project PE-1: Integrated Pest and Disease Management in Major Tropical Agroecosystems

Objective: To develop and transfer knowledge systems and pest and disease management components for sustainable productivity and healthier environment.

Outputs:

1. Pest and disease complexes described and analyzed.
2. Pest and disease management components and IPM strategies and tactics developed.
3. NARS capacity to design and execute IPM research and implementation strengthened.
4. Global IPM networks and knowledge systems developed.

Gains: Increased crop yields and reduced environmental damage. Natural enemies of major pests and diseases evaluated. IPM developed, and tested and verified on-farm. Increased knowledge of biology and ecology behavior of pests and diseases and the damage they cause. Molecular characterization of major pathogens and diagnostic kits available. Whitefly biodiversity characterized. FPR methods for IPM developed and implemented. Biological control agents established in new regions.

Milestones:

2000 Biological control implemented for selected arthropod pests and root rot pathogens. Cassava geminivirus and additional whitefly parasites characterized. IPM strategies and tactics initiated for selected crops. Diagnostic surveys for whitefly, cassava root rots completed and initiated in NR agroecosystems. Diffusion of diagnostic techniques through training. Marker aided selection for phytophthora used to screen germplasm. Molecular markers tagging resistance to CBB identified.

2001 Whitefly parasites evaluated and selected species released in cassava fields. IPM strategies and tactics developed for specified crops. Diagnostic surveys in NR ecosystems continued and recommendations made. Biological and thermotherapy control implemented for cassava virus and root rot diseases. Marker aided selection expanded to CBB and other problems. IPM control of fruit and other crops initiated. Use of heterologous genes applied to the identification of resistant germplasm to Phytophthora Root Rot.

2002 A global network and website for information on tropical agroecosystems developed. Evaluation and dissemination of biological control agents of major pests of targeted crops. IPM projects developed for NR agroecosystems. Components of integrated pest management package for global whitefly project ready for diffusion. First crop viruses identified and diagnostic tools developed. Whitefly resistance mechanisms in cassava identified. IPM for cassava viruses and root rot diseases implemented. Resistant cassava germplasm to CBB identified by the use of molecular markers.

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

Collaborators: IARCs (IITA, ICIPE, CIP). Advanced research institutes (e.g., CATIE, NRI, universities of Florida, Wisconsin, and São Paulo, John Innes Center, ETH/ORSTOM/CIRAD, Boyce Thompson Institute), NARS (e.g., EMBRAPA, CORPOICA, INIAP, INIVIT, NARO), NGOs, private industries (CENIPALMA, Compañía Agrícola de Espárragos).

CGIAR system linkages: Increasing Productivity (30%); Saving Biodiversity (20%); Protecting the Environment (40%); Strengthening NARS (10%). Manages Whitefly and Participatory Methods Projects in Systemwide IPM Program.

CIAT project linkages: Collaborates with breeding projects (IP-1, IP-2, IP-3, IP-4, and IP-5) in host-plant resistance. Provides biocontrol agents to project PE-5.Uses inputs from PE-4, SB-2, and SN-3.

Table of Contents

CIAT - Project: PE1. INTEGRATED PEST AND DISEASE 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 -Project reports	-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 knowledge systems and pest and disease management components for sustainable productivity and healthier environment.	-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, book chapters -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.	
1. Pest and disease complexes described and analyzed.	-Pests, diseases, natural enemies and vectors characterized -Host/pest/natural enemy/vector interactions analyzed -Better diagnostic tools available -Biological control agents established -Better understanding of the influence of drought in hot/pest interactions -Identification of cassava with tolerance to diseases -Pest and disease distribution (maps) determined	-Reports with maps, economic damage, biological information. -Analysis of experiments -Transfer of tools to seed health facilities -Analysis of experiments -Guidelines for IPM -Reports on field effectiveness and probability of adoption of components -Field oriented brochures	-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 -Collaborative with NARS possible -Evaluation, screening, exploration sites accessible
2. Pest and disease management components and IPM strategies and tactics developed.	-Testing of components for effectiveness. -Control strategies recommendations clearly identified and crop management practices determined -Farmer testing of components -Guides on IPM strategies published -Disease detection methods available -Web site published	-Reports on training courses -Concept notes and projects prepared with partners -Electronically published web pages and databases	
3. NARS capacity to design and execute IPM research and implementation strengthened.	-Training especially in FPR -Development of projects with NARs -Training materials developed		
4. Global IPM networks and knowledge systems developed.	-Network of researchers established -Preparation of web pages and databases with relevant IPM information		
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

PROJECT SUMMARY

The development of low cost technologies, that form the basis of successful integrated pest and disease management projects in the developing world, depend on high quality and relevant research. It will be seen in the accompanying report, during 1998-1999 the CIAT Integrated Pest and Disease Management Project (IPDM) has achieved important advances in acquiring the basic knowledge needed to develop these technologies. Project PE1 expanded into Africa with two African bean scientist, Dr. Robin Buruchara and Dr. Kwasi Ampofo, joining the project.

In addition to research on CIAT's traditional commodities, research on pests and diseases in novel agroecosystems was advanced and expanded to include asparagus, oilpalm, cut flowers and fruits. The CIAT IPDM project was invited to participate in a workshop on new invasive pests in the Caribbean Region and make a presentation on CIAT's present and potential role in dealing with this threat. This opens up new opportunities for CIAT scientists, and to further utilize CIAT's research and networking capabilities.

The first phase of the CGIAR Systemwide Whitefly IPM project, that CIAT convenes is nearly complete, providing the scientific community with a considerable amount of critical information that will allow us to build for a successful second phase of this global project. The USDA (United States Department of Agriculture) and ACIAR (the Australian Center for International Agricultural Research) have joined the team of participants and donors in this pan-tropical project.

Collaboration with I.R.D. (Institut de Recherche pour le Developpment) was further solidified and a new project on the feeding behavior of whiteflies on cassava was initiated with a French scientist, Paul Calatayud, during the latter part of 1999.

The CIAT IPDM project prepared several documents in response to the CGIAR's IAEG (Impact Assessment and Evaluation Group) 1999 Impact Report on Integrated Pest Management. These documents describe CIAT's IPDM activities, funding and impact over the past 25 years, but concentrate on the last decade.

HIGHLIGHTS

OUTPUT I

- Influence of cassava plants under water deficiency on the cassava mealybug *Phenacoccus herreni* and on its parasitoids used in biological control analyzed.
- Cassava leaves under water deficiency exhibited an increase in the concentration of some compounds such as free amino acids, carbohydrates (sucrose) and organic acids (malic acid, succinic acid) contributing to decrease the osmotic potential of the tissues in order to protect cellular structures.
- These changes in the trophic quality of cassava favor the cassava mealybug growth and reproduction because plant nutrients are either more concentrated in sucrose and free amino acids and better balanced (ratio "sucrose/free amino acids").
- Cassava under water deficit enhances the cassava mealybug immune`s system against parasitoid egg or larva.
- Cassava in drought conditions has a negative effect on parasitoids affecting the parasitism success and, depending on parasitoid species, disfavoring the parasitoid development.
- A new phenolic compound synthetized by the cassava mealybug *Phenacoccus herreni* discovered.
- *Jatropha* sp. (Euphobiaceae): sound to be a toxic plant to the cassava mealybug ***Phenacoccus herreni.***
- Mealybug bioassay studies with the parasitoids *Aenasius vexans* and *Acerophagus coccois* show that parasitism is higher with experienced females than with naïve ones.
- A large complex of parasitoids have been collected from cassava whiteflies during surveys of selected cassava growing regions of Colombia: several are unrecorded species and are being identified by taxonomists.
- Several parasitoids of the genera *Amitus, Encarsia, Entonocerus* and *Metaphycus* have been collected from whiteflies on cotton, eggplant, beans, cucumber, snapbeans and tomatoes in surveys in Colombia.
- In laboratory studies with the parasitoid *Encarsia hispida*, 45% of the cassava whitefly, *Aleurothrachelus socialis*, pupa were parasitized.
- Whitefly survey data from farmers fields show that, in general, parasitoid population were higher in fields where pesticides were not applied, regardless of whitefly species. Results also show that populations of *Encarsia* are less infected by pesticide application than *Amitus*.
- Whitefly *Encarsia* parasitoid efficacy and emergence was significantly depressed when parasitizing *A. socialis* immature feeding on the resistant cassava cultivar MEcu 72.
- No type of reproductive isolation was observed in crosses, between 3 geographically distinct populations of the phytoseiids mite predator *Typhlodromulus manihoti*.
- Phytoseiid mite predators could play an important role as biological control agent for *Thrips palmi* a major pest of numerous food and horticultural crops.
- Two Phytoseiidae mite species, *Typhlodromulus aripo* and *Neoseiulus cucumeris* were found to feed readily on the important crop pest, *Thrips palmi*.

- The biological development of two phytoseiid mite species, *T. aripo* and *N. cucumeris* equally positive results when feeding on the prey *Thrips palmi*, when compared to phytophagous mite prey.
- Methodologies are being developed for collecting, conserving, rearing and evaluating entomopathogens for use in arthropod pest management projects.
- A diagnostic evaluation of the arthropod pest complex associated with the asparagus crop was done on three plantations in Cauca, Colombia.
- Surveys of three asparagus plantations resulted in 1440 specimens collected, representing 8 insect orders and numerous families; 72 species, including beneficials, were selected as of major importance for identification purposes. Several appear to be unrecorded species.
- The AFLP (amplified fragment length polymorphism) technique was used to characterize the genetic diversity of *Phytophthora* species in cassava. This is the first time that *P. vignae*, a cassava pathogen, was studied, using this technique.
- *Phytophthora vignae*, *P. citricola*, and *P. nicotianae*, isolated from cassava, were identified through the ITS (internal transcribed spacer) region sequence analysis of ribosomal DNA. This is the first time that *P. vignae* and *P. citricola* are reported as causing root rots in cassava.
- A simple zoospore production method was developed for *Phytophthora* isolates from cassava and oil palm. An *in vitro* test was developed to ensure purity of *Phytophthora* cultures.
- Fertilization with potassium chloride at a rate of 50 kg/ha significantly reduced severity of the disease caused by *Phytophthora* in the greenhouse.
- A rapid and sensitive assay, using PCR analysis, was developed for detecting *Sphaerotheca pannosa* var. *rosae*, the causal agent of powdery mildew in rose.
- High genetic variation was detected among *S. pannosa* var. *rosae* isolates by using the RAPD (random amplified DNA polymorphism) technique.
- A simple and effective inoculation method was developed to test the pathogenicity of *S. pannosa* var. *rosae* isolates.
- Through the sequencing analysis, two *Ceratocystis* species were determined: *C. paradoxa sensu stricto* and a new species still to be described. Both species are causal agents of bud rot in oil palm.
- A cloned fragment with homology to a viral RNA dependent RNA polymerase gene is specific and associated with some isolates of cassava frogskin disease.
- The search for germplasm that is tolerant to cassava frogskin disease is progressing and the next phase is multilocational trials.
- A strategy to reduce the incidence of CFSD is being implement on the CIAT campus. This can be a model for other rapid multiplication activities.
- Cassava vein mosaic virus does not appear to be incorporated into the genome of cassava making the PCR assay a suitable diagnostic tool.
- Baseline molecular data on major tropical whitefly species is showing that there are only two principal *B. tabaci* biotypes in Northern Latin America, Central America, and the Caribbean zones.
- A regional map of whiteflies was made. The use of RAPD proved an effective method to map the distribution of *B. tabaci* biotypes A & B.

- Isolates (6) of the angular leaf spot pathogen Phaeoisariopsis griseola, from Madagascar were characterized for the first time using virulence method and showed occurrence of the Mesoamerican pathogen group on the island. DNA extraction of P. griseola was initiated with 60 isolates using modified procedures adapted to standard laboratory facilities and conditions.

OUTPUT II

- A participatory research approach successfully developed an effective disease management system to control cassava root rots in the Colombian departments of Vaupés, Cauca, Valle del Cauca, and Quindío.
- Severity of Pythium root rot was shown to be a good phenotypic measure of resistance. Percentage reduction in root and shoot weights of infected plants were closely associated with severity being high in susceptible or less tolerant and little or no reduction in more resistant entries implying that these parameter may be used in conjunction with severity in characterizing resistance. Root length was less useful.
- A very small proportion of evaluated germplasm in different nurseries (Core Collection, IBN-96, SOH and BILFA) was tolerant to Pythium root rot. Out of a total of 612 entries, 1 was resistant, 28 (4.5%) gave intermediate reactions while more than 95 % were susceptible.
- In Africa chemical seed dressing reduced infestation by bean stem maggots and increased yields by about 50% above the traditional practices, but the combination of half the recommended rates of FYM and DAP doubled yields over the traditional practices. Farmers found this strategy particularly suited to climbing bean production as they could treble productivity of their small holdings.
- In farmer participatory IPM in northern Tanzania, farmers developed technologies to control the bean foliage beetle (*Ootheca* spp.) that includes crop rotation, post harvest tillage, and the application of botanical repellents.
- Host plant resistant research for the bean stem maggot identified the lines TBF-151 and G5625 as best performers when compared to the control varieties.

OUTPUT III

- A course on DNA extraction and molecular characterization of key bean pathogens was conducted for 8 scientists from east, central and southern Africa using modified procedures adapted to relatively unsophisticated facilities which helped build confidence in the use of molecular tools. DNA extraction was meant to be a first step in the application of molecular tools and in allowing division of responsibility between NPs in Africa and advanced labs in the characterization of pathogen diversity. It also facilitates across country movement of non-pathogenic forms of pathogens that can be characterized by molecular methods.
- In collaboration with ISAR, fifty-three government and NGO extension staff and 23 representatives of farmer associations in Rwanda were trained on different aspects of climbing bean and root rot management technologies. The trainees are expected to be involved in further training and participation in dissemination of the technologies in Rwanda.

OUTPUT IV

- CIAT's impact a IPDM analyzed and documents prepared for CGIAR's, IAEG, 1999 Impact Report on Integrated Pest management.
- CIAT's invited participation in the University of Florida organized workshops on new invasive pests in the Caribbean Region opens up new possibilities for CIAT IPDM scientists.

OUTPUT I. PEST AND DISEASE COMPLEXES DESCRIBED AND ANALYZED

Sub-output 1. Identification, Quantification and Analysis of Major Arthropod Complexes.

Activity 1. Biological control of whiteflies by indigenous natural enemies for major food crops in the neotropics

Introduction

Whiteflies, as direct feeding pests and virus vectors, constitute a major problem in cassava and associated crops in Central and South America and the Caribbean region. There is a large complex in the neotropics, where 11 species are reported on cassava alone (Bellotti et al. 1999). In the northern region of South America (Colombia, Venezuela and Ecuador) the major species is *Aleurotrachelus socialis*. Two additional species, although of lesser importance, but frequently found on cassava, are *Bemisia tuberculata* and *Trialeurodes variabilis*. High populations of *A. socialis*, frequently observed in the region, can cause serious yield reductions in cassava. There is a correlation between duration of attack and yield loss: Infestations of 1, 6, and 11 months resulted in a 5, 42, and 79% yield reduction respectively.

Until recently, the *Bemisia tabaci* biotypes found in the America did not feed on cassava. It has been speculated that the absence of ACMD in the Americas may be related to the inability of its vector, *B. tabaci*, to colonize cassava. Since the early 1990's a new biotype (B) of *B. tabaci*, considered by some to be a separate species (*B. argentifolii*) has been found feeding on cassava in the neotropics. It is considered that ACMD now poses a more serious threat to cassava production, as most traditional varieties in the neotropics are highly susceptible to the disease. In addition the B biotype of *B. tabaci*, as a virus vector, causes heavy crop losses on numerous other crops in the neotropics, and these are often grown in association with cassava, or in the same area. The possibility of viruses diseases moving between these crops, or the appearance of previously unrecorded viruses has become a potential threat.

Host plant resistance and biological control agents (e.g. parasitoids, predators, and entomopathogens) are increasingly accepted as a means of pests control which reduces environmental contamination and other disadvantages that arise from the excessive use of chemical pesticides. Although there is considerable data available of whitefly parasitoids. It is still not clear which of the potential biocontrol agents are most common in the field, and what level of efficacy they exert.

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

This is a USAID funded collaborative project between CIAT and the University of Florida. This collaboration will provide training, and improved in-country capacity for research, production, delivery and management of biological control agents. The University of Florida will provide expertise and input on parasitoid taxonomy, biology, behavior, collecting, rearing, identification and data analysis. Since 1998, University of Florida Researchers (Dr. Jorge Peña and R. Nguyen), visited CIAT and collaborated in training CIAT personnel in some of the above areas. Whitefly parasitoids that are collected from field sampling are sent to University of Florida taxonomists (G. Evans, M. Rose, A. Hammonds) for identification.

Methods

Three geographic areas were selected for exploration for whitefly species and their parasitoid natural enemies. These are the Caribbean Coast (the Departments of Atlántico, Córdoba, Bolívar and Magdalena), the mid altitude central highlands (Departments of Cauca, Valle del Cauca, Caldas, Quindío and Risaralda) of Colombia, and Manabí, Ecuador. The Colombian sites have been surveyed and the Ecuador site will be surveyed during late 1999.

The Colombian departments surveyed represent two distinct ecological zones. The Caribbean coast is hot and fairly dry; temperatures ranged from about 27° to 36°C and the average relation humidity is 25 to 70%. The Andean sites are much cooler with temperatures ranging from 22 to 33° and relative humidity ranging, from 7 to 100%. The Caribbean coast is also characterized by a 4 to 6 month dry seasons. Dry periods in the Andean region are usually 2 to 3 months. Altitude, for the collection sites on the Caribbean Coast was 0 to 200 meters and in the Andean region it was 25 to 1750 meters.

Sampling is being done on several crop hosts, including cassava, beans, tomato, eggplant, cotton, cucumber and snap bean. Each zone is characterized by taking data on m.a.s.l., rainfall, temperature range, vegetation type, latitude, geographic area, etc. From each collection site, 100 leaves were randomly collected; a one square inch leaf area was examined to determine the whitefly species present and the number of nymphs and pupae is recorded.

The rate of parasitism was determined by collecting 40 leaves randomly from the field and removing a one inch square leaf sample. Only one whitefly species was allowed to remain on each leaf square and the emergence of parasitoids is recorded for each whitefly species. This methodology allows us to accurately determine the parasitoid species associated with each whitefly species. Identifications are still pending for some whitefly and parasitoid species.

Results: Cassava

Cassava results are separated into three geographic zones of Colombia, the Atlantic Coast, and the departments of Cauca and Valle del Cauca.

Four species of whiteflies were confirmed as feeding on cassava, *Aleurotrachelus socialis, Bemisia tuberculata, Trialeurodes* sp. (probably *T. variabilis*) and *Tetraleurodes* sp. (**Table 1.1**). *A. socialis* was the predominant species found in all three areas, however its population was

considerably higher in Valle del Cauca and lowest in Cauca **(Figure 1.1)**. All four species were collected from the Atlantic Coast, whereas *Tetraleurodes* sp. was not collected in either Cauca or Valle del Cauca.

Pupal populations of *A. socialis* ranged from an average of about 55 per inch square on leaf samples from Valle del Cauca to about 5 on the Atlantic Coast and 2 in Cauca **(Figure 1.1)**. The data also show that whenever there are high whitefly densities or populations, *A. socialis* is the predominant species regardless of geographic area. However when densities are low at sampling sites other species may predominate **(Figure 1.2)**. On the Atlantic Coast, under low population densities *B. tuberculata* and *A. socialis* had similar populations whereas in Valle del Cauca, *B. tuberculata* predominated. In Cauca, *Trialeurodes* had the highest population.

Numerous whitefly parasitoids were collected from the three areas (**Table 1.1**). These parasitoids belong to the genera *Encarsia, Eretmocerus, Mataphycus* and *Euderomphale*, indications are that the collection contains several unrecorded species. The hyperparasitoid, *Signiphora aleyrodis* was collected only in Cauca. *Encarsia* was the genera most frequently collected in Cauca and Valle del Cauca **(Figure 1.3)**, with the highest percentage in the latter. On the Atlantic Coast, *Eretmocerus* was the most frequent genus collected. *Metaphycus* was only collected on the Atlantic Coast and *Euderomphale* only from Cauca.

The association between whitefly species and parasitoid species was also evaluated. Results indicate a sizable parasitoid species complex associated with each whitefly species and that this can be influenced by geographic area. For example, with *A. socialis*, the predominant parasitoid genera in the Atlantic Coast was *Eretmocerus*, while in Cauca and Valle del Cauca it was *Encarsia* **(Figure 1.4)**. 99.6% of the parasitism of *A. socialis* in Valle del Cauca was by *Encarsia* and 0.4% by *Eretmocerus*. The most numerous complex of parasitoids was found associated with *B. tuberculata* (**Table 1.1**). *Eretmocerus* and *Metaphycus* were the predominant genera on the Atlantic Coast and *Eretmocerus* in Cauca. The hyperparasite, *S. aleyrodis*, was collected from both *A. socialis* and *B. tuberculata* pupae.

Trialeurodes sp. is most frequently parasitized by *Encarsia*. Two species of *Encarsia* were identified, *E. hispida* and *E. pergandiella*, and a third species is yet to be identified.

Encarsia sp. and *Eretmocerus* spp. are frequently collected from both high and low whitefly populations. *Metaphycus* sp. and *Euderomphale* sp. are only observed when whitefly populations are low. Parasitoids of the genera *Encarsia* and *Eretmocerus* were collected from sea level to about 2400 m.a.s.l. *E. pergandiella* appears to be especially adaptable being collected in high populations at all levels.

Once we have the complete species identification we will be able to better define this complex. However these results do indicate a rich parasitoid species complex associate with cassava whiteflies. It is expected that continued surveys, especially in Ecuador, will contribute additional information.

Results: Diverse Crops

Whitefly and parasitoids surveys and collections were done in two regions of Colombia using the methodology described above. These regions which include the departments of Atlántico, Córdoba, Quindío, Risaralda and Valle del Cauca, range from about 20 to 1500 m.a.s.l. with average temperatures of 19°C (Caldas and Quindío) to 33°C (Atlántico).

The whitefly species complex associated with cotton, legume and vegetable crops is distinct from that described on cassava. Two whitefly species were collected, *Bemisia tabaci* and *Trialeurodes vaporariorum*. During the period of field collections (Jan. to June, 1999) *B. tabaci* was the only species collected on the Atlantic Coast (Depts. Of Atlántico and Córdoba) while in the Andean Region (Depts. of Caldas, Quindío, Risaralda and Valle del Cauca, *T. vaporariorum*, was the only species collected and the highest populations observed were in Valle del Cauca and Caldas.

B. tabaci was collected from cotton, eggplant and tomato, while *T. vaporariorum* was collected from beans, snap beans, cucumber and tomato. Populations of *T. vaporariorum*, in general, were much higher than *B. tabaci* and this was especially the case on beans and snap beans **(Figure 1.5)**. These results indicate that *B. tabaci* may be more adapted to the warmer temperatures of the coastal region whereas *T. vaporariorum* prefers the cooler temperatures of the Andean region.

Parasitoids from five genera, *Encarsia* and *Eretmocerus* (Aphelinidae), *Amitus* (Platygastridae), *Metaphycus* (Encirtidae) and *Signiphora* (Signiphoridae) were collected. The latter is reported as a hyperparasite. These collections have been sent to taxonomists and species identifications are pending. Therefore, in this report, they are referred to only by genera.

Encarsia was collected for all of the departments except Risaralda (where only two sites were surveyed), but *Amitus* presented the highest populations, especially in Valle del Cauca and Caldas **(Figure 1.6)**. This corresponded to the two departments with the highest whitefly populations. *Eretmocerus* was the predominant species in Córdoba. Parasitoid populations were highest in Valle del Cauca and lowest in Risaralda and Quindío. Only one specimen of *Metaphycus* and *Signiphora* were collected across the sites.

Encarsia parasites were collected from all of the plant hosts and had the overall highest populations of parasitoids **(Figure 1.7)**. *Amitus*, although not collected from all hosts also presented high populations, especially on beans and cucumber. *Eretmocerus* was associated almost exclusively with cotton (only one individual was collected from tomato).

Encarsia was collected from both whitefly species, with higher populations on *T. vaporariorum* **(Figure 1.8)**. *Amitus* was collected only from *T. vaporariorum* while *Eretmocerus, Metaphycus* and *Signiphora* were collected only from *B. tabaci*.

During surveys, data on pesticide applications was recorded during farmer interviews. In general parasitoid populations were higher in fields where pesticides were not applied regardless of the whitefly species. Results show that populations of *Encarsia* are less affected by pesticide

applications than populations of *Amitus,* indicating that *Encarsia* may have acquired a degree of resistance to some of the pesticides applied.

Laboratory Studies on Encarsia hispida

Periodic field surveys, carried out over several years, have shown that the parasitoid *Encarsia hispida* is frequently found parasitizing cassava whiteflies. A colony of this species was established on *A. socialis* on cassava in the greenhouse and activities were initiated to study the biology and behavior of *E. hispida.*

Rearing Methodology for Encarsia hispida

The successful rearing of whitefly parasitoids requires the availability of a constant supply of whitefly nymphs. A colony of the whitefly *Aleurotrachelus socialis* is maintained on cassava (var. CMC-40) in the greenhouse. Five week old, potted cassava plants are infested weekly by exposing them to *A. socialis* adults for oviposition for 48 hours. Consequently the four whitefly nymphal stages, as well as adults, are constantly available for studies with natural enemies or for host plant resistance. This colony is periodically replenished with whitefly populations from the field.

The parasitoid colony is initiated by collecting cassava leaves with parasitized *A. socialis* pupae from the field. These are placed in a black plastic box (emergence chambers) with a clear glass bottle attached to an opening in the lid where emerging parasites are drawn to the light and collected. The parasitoids are removed from the bottle with an aspirator and identified. Species of three genera have been collected from CIAT fields, *Eretmocerus, Amitus* and *Encarsia hispida*, as well as the hyperparasite *Signiphora* sp. With experience and practice, species identification becomes easier.

The desired parasitoid species (*E. hispida*) is collected in sufficient numbers and released into nylon mesh cages (50 x 50 x 90 cm) in the greenhouse (25 to 32°C; 60-80% RH). Each cage contains the potted cassava plants infested with 2nd to 3rd instar *A. socialis* nymphs; 16 to 18 days after release, adult parasitoids will begin to emerge.

Once emergence is first detected, cassava leaves (now with parasitized pupae) are collected and placed in the "emergence chamber" where parasitoids are collected and species identification is confirmed. Parasitoids can be collected on a daily or hourly basis so the exact age (date of emergence) can be recorded. These parasitoids can be used to maintain the parasite colony or employed in the various studies that are described in this report.

Colony maintenance requires a constant (daily) care to avoid contamination by other parasitoid species, predators or hyperparasites. A well functioning, healthy, and vigorous colony will insure a constant supply of parasitoids.

Parasitoid description

E. encarsia, a small yellowish parasitoid, is very agile, moving rapidly as it walks 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 last from 15 to 45 minutes. Upon completing the oviposition, the parasitoid spends 10 to 15 minutes cleaning its whole body with use of its fore, mid and hind legs. Not all nymphs that are examined are parasitized; the parasites may feed from the wound that it makes in the nymph with its ovipositor.

The female parasitoid oviposits an egg within the nymph; the egg measures an average of 0.068 mm width x 0.195 mm length. Female *E. hispida* are 0.399 mm in length and males are 0.479 mm.

In greenhouse studies at CIAT (27.28°C:80-90% RH), female parasitoid development time from egg to adult emergence is between 16 to 25 days. Females oviposit only one egg in each host. Reproduction is by telitoquia parthenogenesis often resulting in a population high in females or totally female.

Results: Parasitism Studies

Three methodologies were evaluated to determine parasitoid efficiency:

1. Four nylon meshed cages (50 x 50 x 90 cm) containing two potted cassava plants infested with whitefly (*A. socialis*) nymphs. 60 parasitoids were released into each cage with four repetitions for each instar.

2. Four whitefly infested cassava plants; four infested leaves were placed in a petri dish (100 x 15mm) and 30 parasitoids were released into each petri dish and 16 repetitions for each instar.

3. A muslin "bag" is placed over an entire cassava leaf and sealed at the petiole. Each leaf is infested with 80 nymphs and 20 parasitoids are released into each bag with four repetitions per instar.

Parasitism results using the first two methodologies were low. The highest rate of parasitism with methodology one was 9.0% in the third instar. This improved with the second methodology to 30.5% and 26.0% in the 2nd and 3rd instar respectively.

The employment of muslin bags, as described in the third methodology gave the best results. In the first of two experiments using this methodology, parasitism rates reached 75.3% in the third instar and 15.6, 44.7 and 43.1% in the 1st, 2nd and 4th instars respectively **(Figure 1.9)**. Results of a second experiment were similar with 75.5% parasitism in the third instar and 18.6, 61.4 and 25.0% in the 1s^{t}, 2nd and 4th instars respectively **(Figure 1.9)**. The average parasitism rate for

these two experiments were 44.7 and 45.1 respectively, whereas average parasitism rates using methodologies 1 and 2 were only 5.6 and 20.3%.

These results also show that the third whitefly instar is preferred for parasitism by *E. hispida*. An average of all four experiments resulted in percent parasitism rates of 21.1, 35.2, 46.4 and 21.9 for the first through 4th instar respectively. However the average of the two experiments using methodology 3 (muslin bags) is 17.1, 53.1, 75.4 and 34.1 percent for the 1st through 4th instar respectively. The highest parasitism rate is in the third instar, followed by the second, fourth and first instar respectively.

The time period for optimal parasitoid activity was evaluated using the third methodology described. Percent parasitism evaluations were made at 48, 72, 96, and 216 hours after parasitoid release. Peak parasitism activity occurred between 72 and 96 hours 34.7 and 32.7% parasitism respectively **(Figure 1.10)**. However even at 216 hours, parasitism rates still remained relatively high, nearly 29%, indicating a relatively lengthy parasitoid activity and the need to do further evaluations at time periods between 96 and 216 hours.

The high rates of parasitism, especially using the muslin cage methodology, indicates that *E. hispida* could be an effective parasite in a biological control program for *A. socialis*. However, whitefly populations, especially of *A. socialis*, have been extremely high at CIAT for the past four or five years. During this period, *E. hispida* is the most frequently observed parasite. These previously described experiments, were carried out under controlled conditions where the parasitoid had easy access to the whitefly prey and the parasitoid is probably not adversely influenced by environmental factors. Under natural field conditions, parasitoid activity may not be as efficient as indicated by these greenhouse studies.

Observations of pupal populations in the field at CIAT and other selected site (i.e. Tolima) have shown that a high percentage of pupae are parasitized. However whitefly populations remain high and cause considerable plant damage and yield loss, indicating that the actual activity of parasitoids is not sufficient to reduce whitefly populations below economic injury level that reduce cassava yields.

In future studies additional parasites, such as *Eretmocerus* and *Amitus* species will be evaluated in similar studies as those with *E. hispida*. Continued survey activities to identify additional natural enemies are also underway. During this phase of exploration, more emphasis will be given to regions or fields where there are low whitefly populations with the hope of identifying key parasitoids, predators or entomopathogens that are preventing eruptions of whitefly populations. For this reason explorations are planned for Ecuador, where *A. socialis* feeds on cassava but is seldom reported in high populations nor causing yield losses.

Table 1.1. Whitefly species collected from cassava and their associated parasitoid complex collected from these geographical regions of Colombia.

Area	Whitefly species	Parasitoid species
Atlantic Coast	*Aleurotrachellus socialis*	*Encarsia sp.* *Eretmocerus sp.*
	Bemisia tuberculata	*Encarsia sp.* *Eretmocerus sp.* *Metaphycus sp.*
	Trialeurodes sp.	*Encarsia sp.* *Eretmocerus sp.*
	Tetraleurodes sp.	
Valle del Cauca	*Aleurotrachellus socialis*	*Encarsia sp.* *Eretmocerus sp.*
	Bemisia tuberculata	
Cauca	*Aleurotrachellus socialis*	*Encarsia bellottii* *Eretmocerus sp.* *Signiphora aleyrodis*
	Bemisia tuberculata	*Encarsia pergandiella* *Eretmocerus sp.* *Euderomphale sp.* *Signiphora aleyrodis*
	Trialeurodes sp.	*Encarsia hispida* *Encarsia pergandiella* *Eretmocerus sp.*

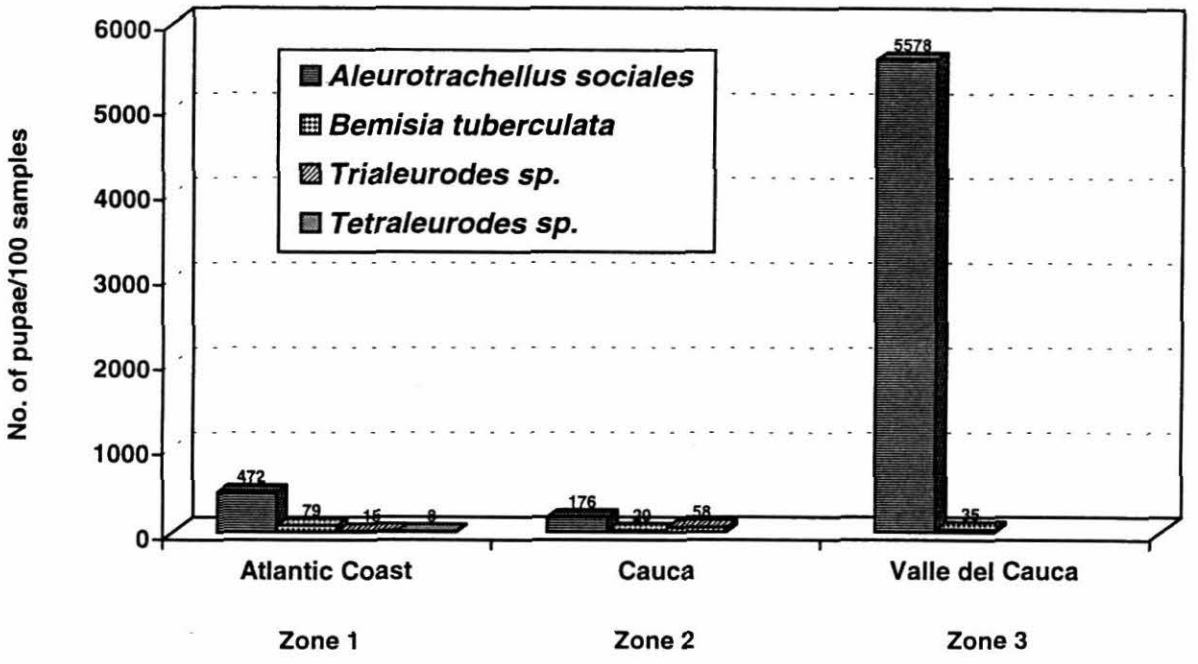

Figure 1.1. Whitefly population densities on cassava in three geographic zones of Colombia.

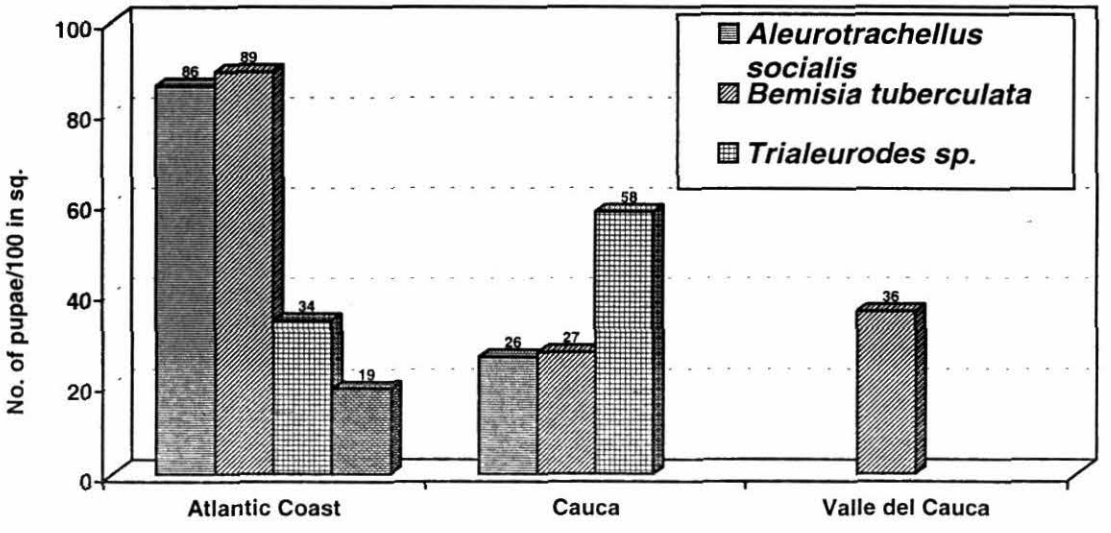

Figure 1.2. The whitefly species complex at collecting sites with low population densities in three areas of Colombia.

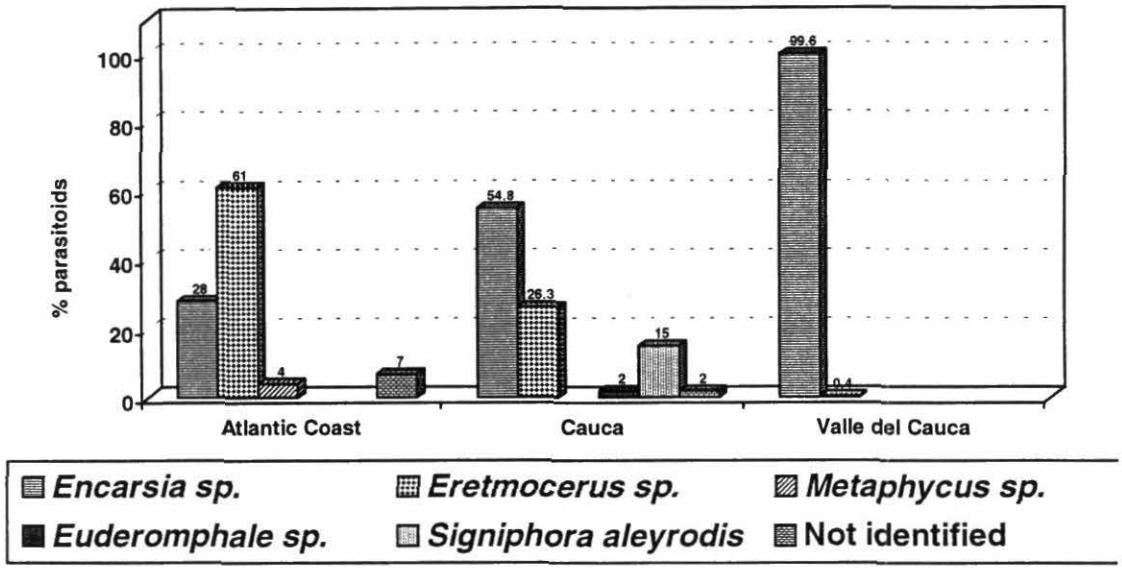

Figure 1.3. Parasitoid species (in %) collected from whiteflies on cassava in three geographic zones of Colombia.

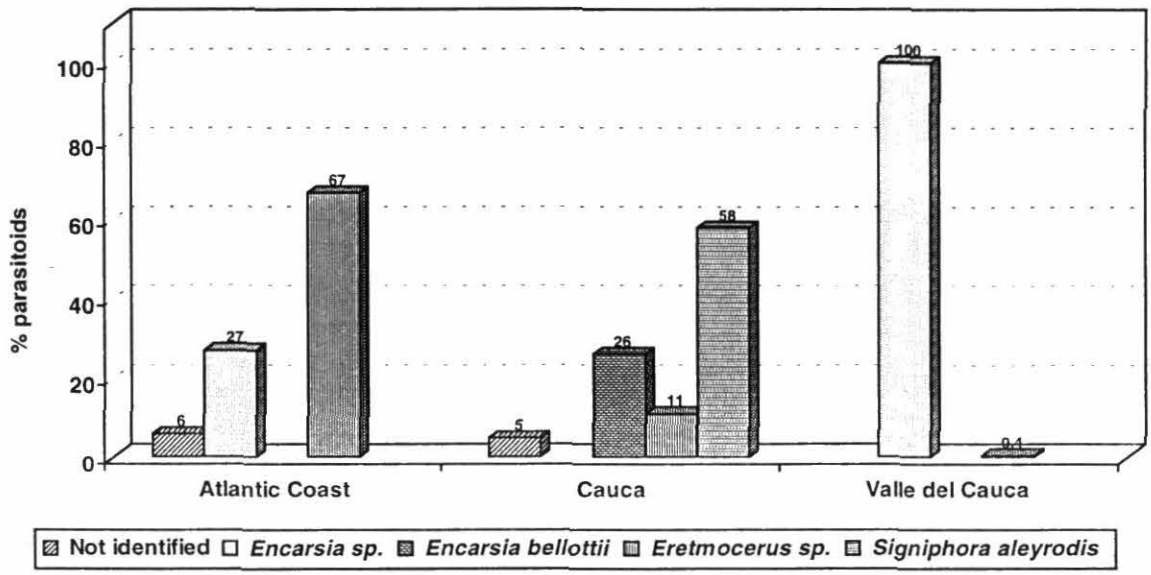

Figure 1.4. Parasitoid species collected from the whitefly *Aleurotrachellus socialis* in these geographic zones of Colombia.

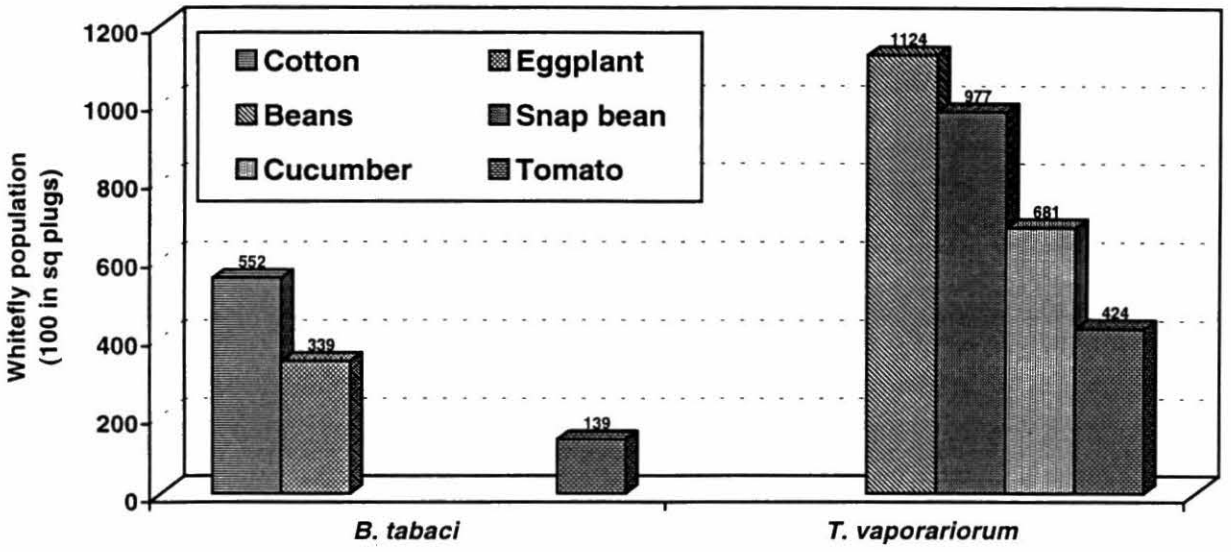

Figure 1.5. Whitefly nymph pupae populations on several crops in six Colombia departments.

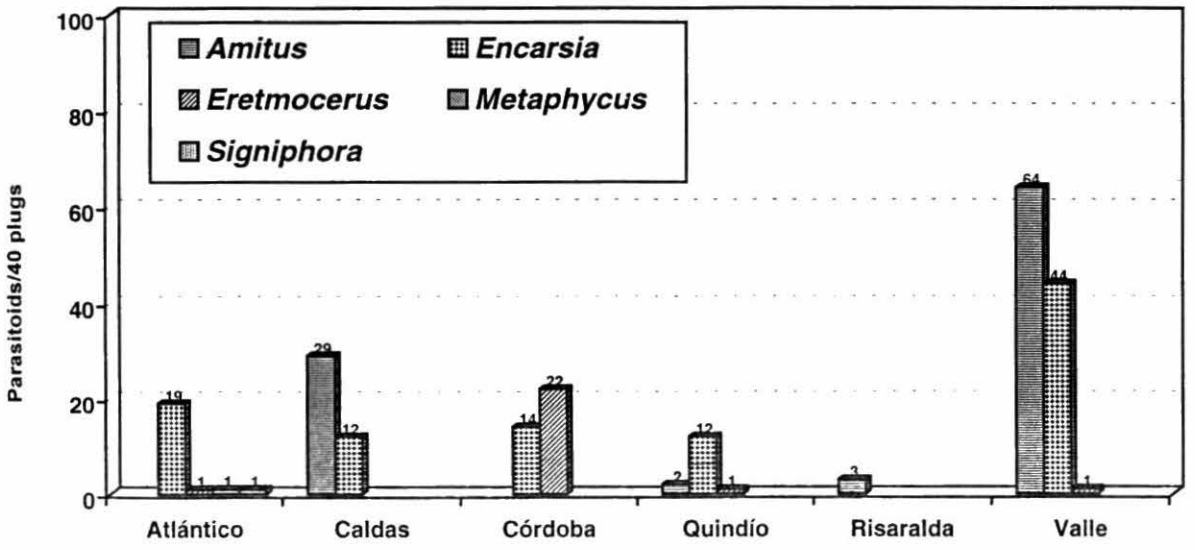

Figure 1.6. Whitefly parasitoids collected from several crops in six Colombia departments.

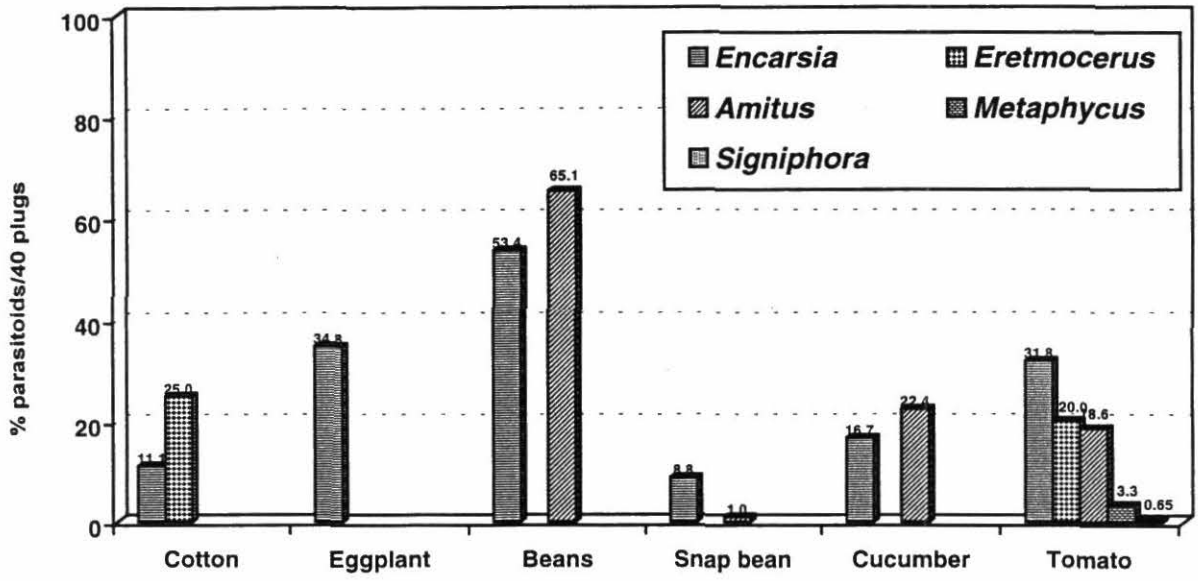

Figure 1.7. Whitefly parasitoid frequency collected from several crops in six *Colombia* departments.

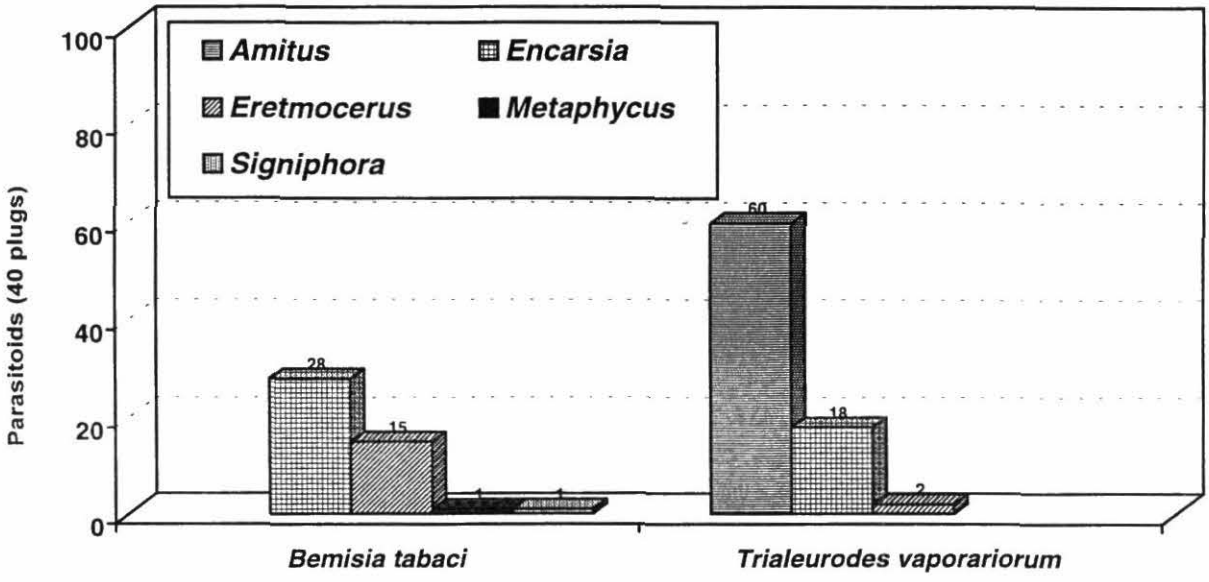

Figure 1.8. Relationship between parasitoid complex and whitefly species collected from several crops in six Colombian departments.

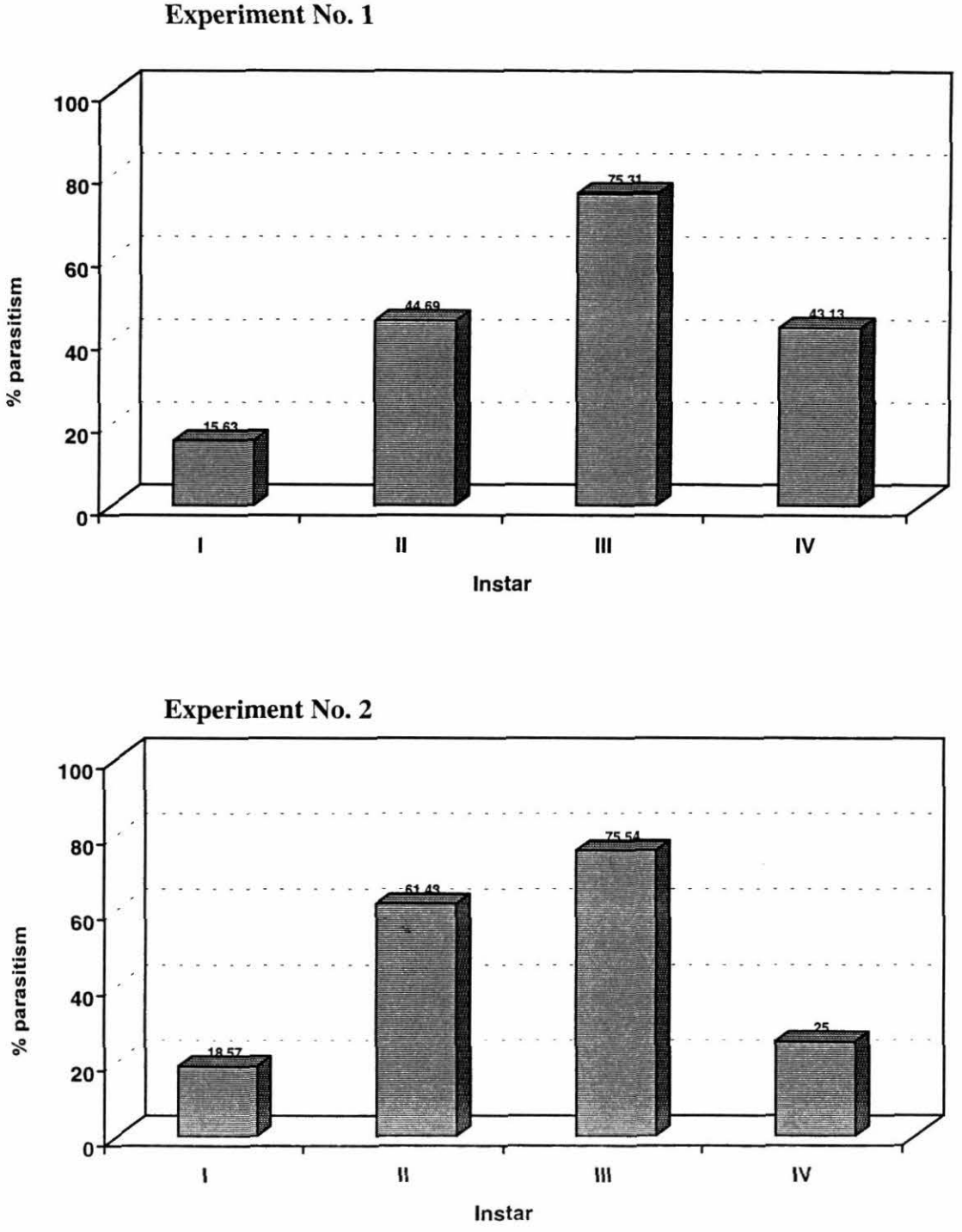

Figure 1.9. Cassava whitefly (*Alerotrachelus socialis*) instar preference by the parasitoid *Encarsia hispida* in greenhouse experiments.

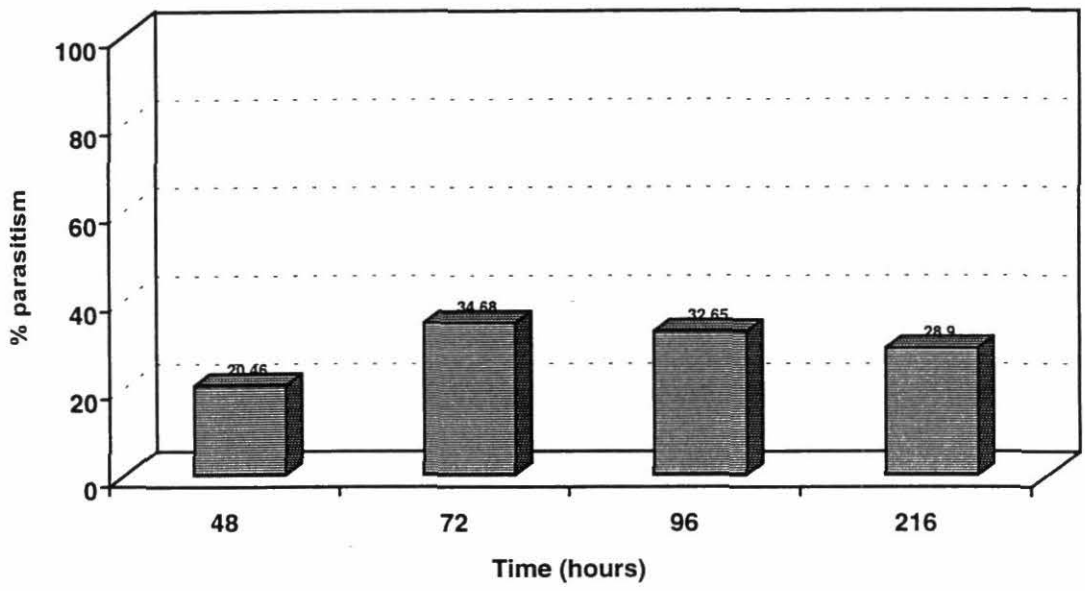

Figure 1.10. Parasitoid (*Encarsia hispida*) activity in relation to time of exposure to whitefly host, *Aleurotrachelus socialis*, on cassava in greenhouse studies.

Activity 2. Tritophic interactions: Studies to determine the effect of HPR on whitefly parasitism

Biological control and host plant resistance can offer a low-cost sustainable solution to cassava losses from whitefly damage. Host plant resistance studies at CIAT, especially with the whitefly species *Aleurotrachellus socialis* are well advanced and several resistance sources have been identified (See Annual Report: Project IP-3).

In recent years we have increased our activities in biological control, surveying for natural enemies in several regions of Colombia and Venezuela. Numerous parasitoids have been collected from cassava whiteflies; these will be identified, studied and evaluated. The most frequently observed parasitoid species of *A. socialis* is *Encarsia hispida*. The genus *Encarsia* is recognized as possessing good searching ability, dispersion and adaptation (Castillo, 1996).

Present research is investigating the compatibility between host plant resistance in biological control, the two most important component in an integrated pest management system. Experiments were designed to determine the compatibility of the parasitoid *E. hispida* on the three cassava genotypes. This phase of the study had two major objectives:

1. Determine the preferred instar of *A. socialis* for *E. hispida* parasitism
2. Determine the effect of four cassava varieties, MEcu 72, CG489-4, MBra-12 and CMC-40, on the emergence and survival of *E. hispida* parasitizing *A. socialis*.

Materials and Methods

These four varieties were selected because of their resistance or susceptibility to *A. socialis*. MEcu 72 has consistently expressed a high level of resistance to *A. socialis;* CMC-40 is a highly susceptible variety (the cassava whitefly colony is maintained on CMC-40); MBra-12 is a tolerant (low levels of resistance) variety with good agronomic qualities; and CG489-34 is moderately resistant to A. *socialis* and the progeny of a MEcu 72 x MBra 12 cross.

Potted cassava plants of the above mentioned varieties are maintained in the screen house until 4 to 5 weeks of age. They are transferred to a whitefly infestation chamber in the greenhouse and subjected to *A. socialis* oviposition for approximately 36 hours. Infested varieties are maintained in a growth room for exposured to the parasitoid *E. hispida.*

The *E. hispida* colony was developed by collecting cassava leaves with whitefly parasitized pupae from the field. These leaves were placed in plastic boxes with a paper towel on the bottom and darkened with black cheesecloth. Clear glass gars were connected to an opening in the lid of the box, where parasitoids were drawn to the light and collected.

Studies on *A. socialis* instar preference by *E. hispida* were done on the variety CMC-40. Adult whiteflies were placed in small leaf cages on cassava leaves and allowed to oviposit for 8 hours. This was done periodically so that the cassava leaves eventually contained patches of immatures of instars I, II, III and IV of *A. socialis*. Infested leaves were isolated by placing a nylon mesh "bag" over each leaf. Twenty five (25) females collected from the field were released into each bag.

Studies on the biology of *E. hispida* were done on six week plants, and *A. socialis* infestations were done every second day with small leaf cages. When nymphal "patches" reached the third instar, one *E. hispida* parasitoid was introduced to each patch; there were 30 repetitions. The parasitoid was transferred to patches of the same instar three times each week until parasitoid death. The patches with parasitized nymphs remained on the plants until parasitoid emergence.

Results

Results of this experiments show that *E. hispida* prefers to parasitize third and forth instar nymphs **(Figure 2.1)**. There was no significant difference between the two instars. Parasitism of the instar was negligible, and very low in the second instar. A high number of nymphs had no parasitoid emergence. This phenomena occurred for all instars but was significantly higher in the forth instar **(Figure 2.2)**. These non emerged nymphs were either "non viable nymphs" or, as reported in the literature within the genus *Encarsia*, it is characteristic for the adult parasitoids to feed on its host. This host feeding characteristic can cause considerable nymphal mortality, especially in the early instars. The fact that the highest number or non-viable nymphs were in the Forth instar indicates a possible preference for parasitoid feeding on this instar.

The survival of *E. hispida* does not appear to be adversely affected by any of the four genotypes used in this experiment **(Figure 2.4)**. Female adult longevity was 27, 28, 32 and 35 days

respectively on the varieties MEcu 72, CMC-40, MBra-12 and CG 489-34 respectively. MEcu 72 and CMC-40, the highly resistant and susceptible verieties respectively gave very similar results related to longevity. Why longevity is about 25% longer on CG 489-34 is not known unless there are chemical factors in the leaf that are conducive to parasitoid longevity. MEcu-72 is a highly pubescent variety and CMC-40 is a non pubescent variety; MBra-12 and CG 489-34 are intermediate. It has been suggested that pubescence might play a role, perhaps a detrimental effect, to parasitoid longevity. These results do not support that hypothesis.

The emergence of *E. hispida* parasitoids from *A. socialis* pupae on the variety CMC-40, indicate that peak oviposition occurs about 3 days after parasitoid emergence, and tapers off rapidly with continued oviposition for about 23 days **(Figure 2.3)**. Results also dramatically indicate that genotype can have an effect on parasitoid development or emergence. Parasitoid emergence was considerably lower from *A. socialis* pupae feeding on MEcu-72 and CG 489-34 **(Figure 2.3)**. It was not possible to get results from MBra-12 as plant leaves dried up and dropped during the experiment. These results strongly indicate that whitefly resistant genotypes could have a detrimental effect on biological control agents, especially parasitoids.

It can generally be concluded, from these experiments, that *E. hispida* will parasitize all instars of *A. socialis* but is most successful on 3rd and 4th instars. There was no genotype effect on survival and longevity of *E. hispida* and that leaf tricomes do no alter these factors. However the low parasitoid emergence rate on the resistant cultivars MEcu-72 and CG489-34 indicate a possible negative effect of whitefly varietal resistance on biological control.

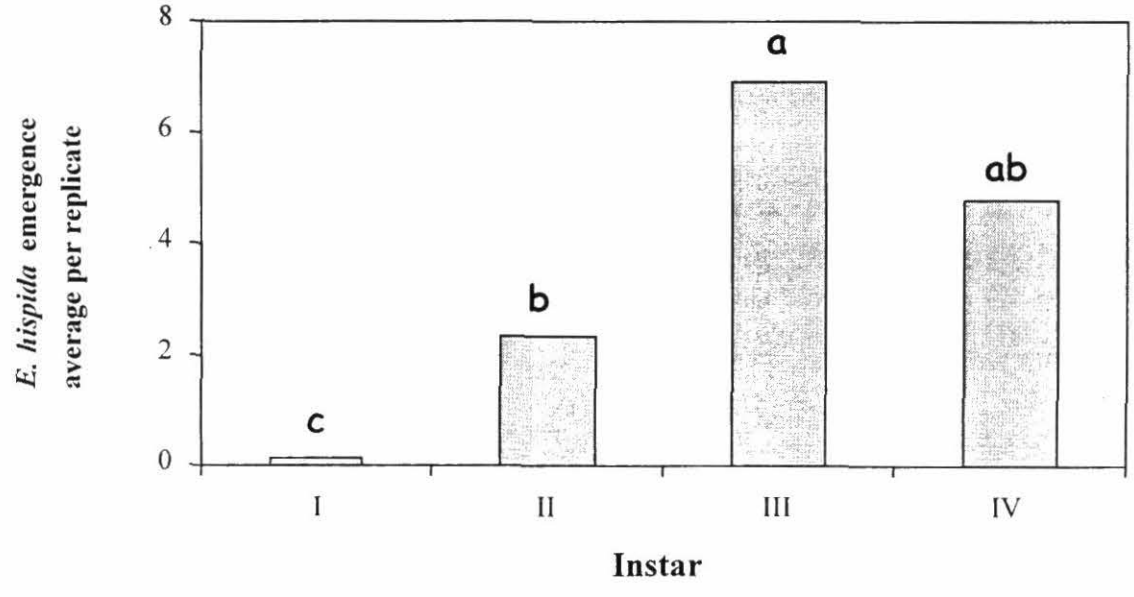

Figure 2.1. Emergence of the parasitoid *Encarsia hipida* from four instars of the cassava whitefly *Aleurotrachellus socialis*.

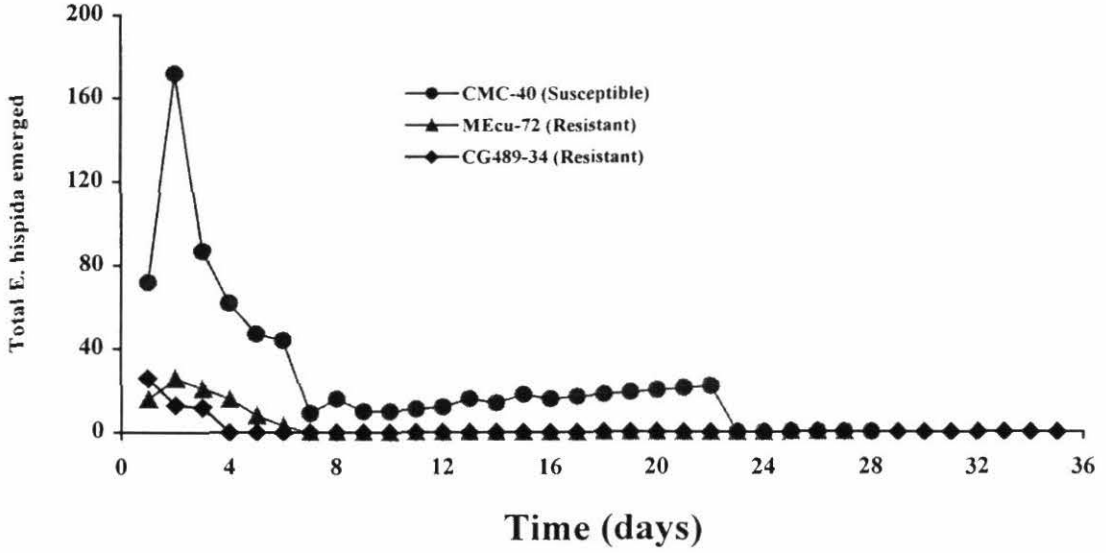

Figure 2.2. *Aleurotrachellus socialis* nymphs that were "non viable" in that no *Encarsia hispida* parasitoid emerged.

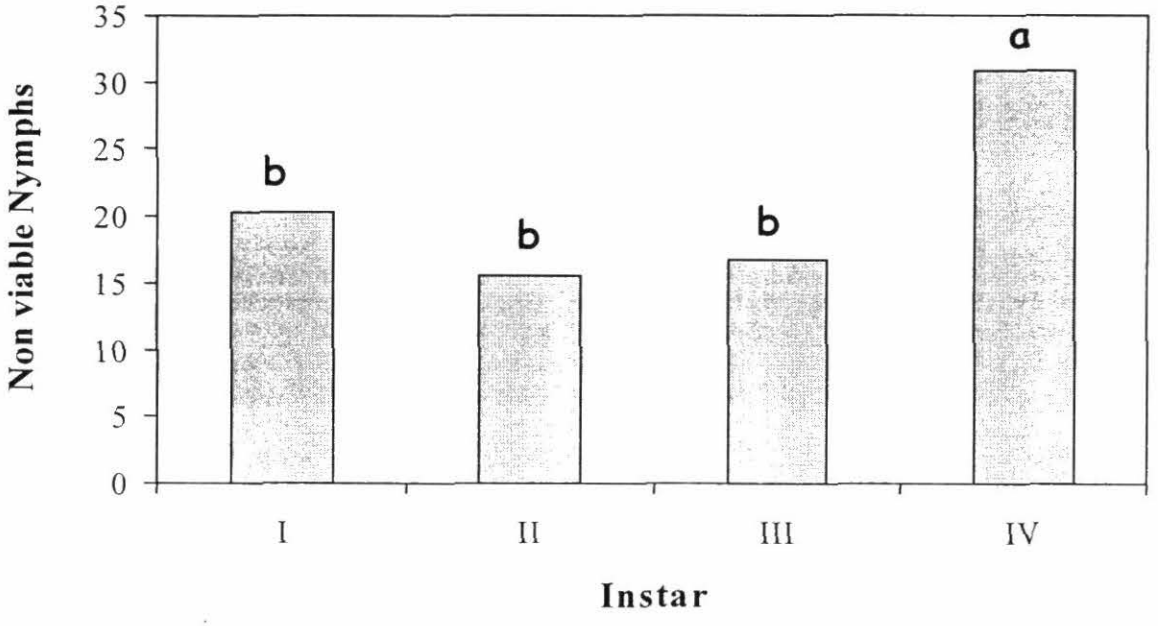

Figure 2.3. The effect of four cassava varieties on the survival of the parasitoid *Encarsia hispida* on the cassava whitefly *Aleurotrachellus socialis.*

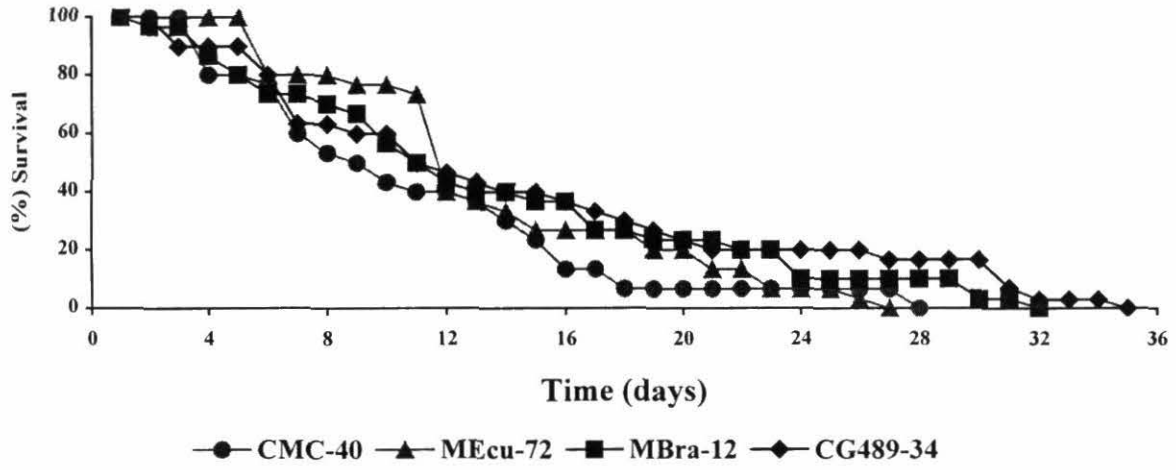

Figure 2.4. The effect of three cassava varieties on the emergence of whitefly parasitoid *Encarsia hispida* from parasitized *Aleurotrachellus socialis* pupae.

Activity 3. Recent studies of crosses between populations of the phytoseiid predator *Typhlodromalus manihoti*

Between 1983 and 1990, the cassava entomology/acarology project at CIAT carried out extensive surveys on cassava pests and their natural enemies. One of the major objectives, and successes, of these explorations was the identification of the species richness involving cassava mites and the phytoseiid predator complex associated with it. The species of phytoseiid most frequently collected was *Typhlodromalus manihoti*. It was found in more than 50% of the 1261 fields surveyed. Of those species most frequently observed, 96% of the collections correspond to *T. manihoti*. It is the most widely distributed species, being found from Mexico to Paraguay and in Colombia at temperatures of 15 to 35°C. It is generally associated with low populations of *Mononychellus tanajoa* (Braun *et al* 1993).

Behavioral differences between population of *T. manihoti* have been observed; for example, in multiplication colonies some populations increase at a more rapid rate than others. In addition differences in life table parameters and in ecological adaptation have also been noted (CIAT, 1990: Rogg & Yaninek, 1990: IITA 1991). For these reasons, it can be theorized that races or populations exist with biological or ecological characteristics that can be important in a biological control program; therefore the appropriate or correct selection of the most promising and effective species for natural control.

The terms subspecies and geographic race are used frequently and interchangeably by arthropod taxonomists. The nature of ecological races among animals is controversial, since no two

localities are exactly identical with respect to its environment, each subspecies is, at least theoretically also an ecological race. However some populations differ in their ecological requirements, without acquiring significant morphological differences (Mayr, 1969).

In biological control, the effectiveness or success of natural enemies is determined by their behavior. For example, those that are able to control host prey when at low populations, have aggressive tendencies, or those that can withstand adverse periods, may be the most effective as biocontrol agents. Therefore, it is advantageous to select the most effective race or strain of a natural enemy. The problem is to be able to distinguish these races. Field evaluation in different regions could identify the adaptive range of a species and molecular techniques can help distinguish differences between populations that upon being corroborated by crosses do not show reproductive incompatibility, but could involve factors that in certain cases could impede the genetic flow (Cuéllar 1992).

Therefore studies were designed to determine if the exists reproductive differences or some form of isolation between certain populations of *T. manihoti* and compare these to previous crosses that were made (Annual Report, PE-1, 1988).

Materials and methods

Three stocks of *T. manihoti* from Colombia (Cauca-Cajibío: Guajira-Villanueva: Antioquia-Copacabana) were used for the crosses. Females were removed from a laboratory colony of each and maintained at 25°C and 70+ RH.

These stocks were previously selected for molecular studies that were done by M. Hoy at the University of Florida. It was found that one population from Brazil (Cruz das Almas) was different from the three populations from Colombia (Cauca, Antioquia and Guajira) and the population from Guajira was different from all others. Crosses were made with the Brazil population (Annual Report PE-I, 1998).

To obtain same age eggs, females were allowed to oviposit in petri dishes and their development was monitored until the adult stage. These adults were used to make heterogamic crosses (the virgin female of one population is crossed with a male of another population), and oviposition was observed for 10 days.

With the eggs obtained from these crosses (F1), development time to the adult stage was recorded and the following back-crosses were made: females from each cross copulated with males of both populations and with males from the same cross (Sibblings) **(Table 3.1)**. For each cross and backcross, can control crosses were also made (homogamic crosses).

A descriptive statistical analysis was done. For data analysis of oviposition of the populations and ANOVA and a Riant-Welch test were carried out.

Results and discussion

Results from crosses between the three populations Cajibío (cj), Guajira (g) and Copacabana (cp), show oviposition for all crosses. Average oviposition for 10 days was very similar for the two crosses (Cajibío x Copacabana = 32.3 and Cajibío x Guajira = 32.9); the average preoviposition time was greater for the cj x cp cross (0.75) than the cj x g (0.49). In the cj x cp cross, the greatest number of eggs were obtained with populations cp x cj (35.3) and the backcross control cj x cj (35.2). These show a significant difference from the cp x cj by the male of cj (29.3) (**Tables 3.2 and 3.3**).

In the cj x g cross, preoviposition time was similar for all crosses and backcrosses, with significant differences for the cj x g backcross, when it was crossed with the cj male (1.13 days) and the backcross g x cj when copulating with males of Guajira (1.0 days). Significant ovipositional differences occurred between the controls, with highest eggs counts for Cajibío (36.4) and Guajira (35.0), and with the backcrosses when the female was offspring of the cj x g cross and was crossed with males of the two populations; with males of Guajira, 29.7 eggs were obtained in 10 days (**Table 3.3**).

The proportion of females in the majority of the populations were similar, showing an approximately normal relationship (70:30). In heterogamic crosses of populations of Cajibío and of Guajira, and when crosses with Copacabana the proportions were 50:50 (**Tables 3.2 and 3.3**).

The lowest percentage of mortality and egghatch was in the cj x g cross (**Table 3.3**) and highest mortality was in the cj x cp crosses and control crosses with 17.6% for cp x cp (control) and lower percentages for the backcrosses. Considerable contrast was observed between the controls in the Cajibío and Copacabana populations with 12.2% and 14.3% respectively and the controls of the backcrosses with 0.0 and 2.9%. This demonstrates that variations can be present in the populations in percentage of egg hatch (**Table 3.2**).

The development time for crosses of cj x cp did not show differences, with an average of 6.0 days **(Figure 3.1)**. In the crosses of populations from Cajibío and Guajira, a lower development time was obtained for the g x cj cross and its female progeny when crossed with males from the two populations (5.5 days).

Similarly, female progeny from the g x cj cross when crossed with males from cj, and backcrosses resulted in a similar time **(Figure 3.2)**.

In these crosses with the three populations, no type of reproductive isolation was observed, and the individual behavior was very close to normal as presented by the controls.

The behavior of Cajibío population was normal in these crosses, which is contrary to previous studies when crossed with populations from Brazil (Cruz das Almas) and no oviposition resulted for the majority of the individuals (Annual Report, Project PE-1, 1998). This may demonstrate that there exists some type of reproductive isolation between the two populations, but when crossed with other populations, this isolation does not persist. It appears that the results obtained

by Hoy, that shows 3 groups as such: one from Cruz das Almas, one from Guajira and a third from Cajibío and Copacabana, has not been determined that crosses cannot be made between these groups. Studies done with AFLPs with several populations of *T. manihoti* show differences in their polymorphie bands in comparison to other species such as *Neoseiulus idaeus*, which are very homogenic in their monomorphyc bands.

These differences have also occurred in studies using isozymes where, when population groups were crossed, they did not demonstrate reproductive incompatibility (Cuéllar, 1992).

A form of reproductive isolation is also caused by the presence of a bacteria in females called *Wolbachia*, that impedes ovipositon (Werren, 1997). Therefore, representations of the four populations of *T. manihoti* used in this study (Guajira, Cruz das Almas, Cajibío and Copacabana) were sent to Dr. Hans Breuwer, at the University of Amsterdam in Holland, to determine if *Wolbachia* exists in these predator mites. A the moment we are awaiting his reply.

Table 3.1. Crossing scheme of populations of *Typhlodromalus manihoti.*

Populations	Crosses
Cajibío (cj) x Copacabana (cp)	cj x cj cp x cp *♀ (cj) x ♂ (cp) ♀ (cp) x ♂ (cj) ♀ offspring (cjxcp) x ♂ cj ♀ offspring (cjxcp) x ♂ cp ♀ offspring x ♂ offspring (cjxcp) ♀ offspring (cpxcj) x ♂ cj ♀ offspring (cpxcj) x ♂ cp ♀ offspring x ♂ offspring (cpxcj) Co cp x cp Co cj x cj
Cajibío (cj) x Guajira (gu)	cj x cj g x g *♀ (cj) x ♂ (g) ♀ (g) x ♂ (cj) ♀ offspring (cjxg) x ♂ cj ♀ offspring (cjxg) x ♂ g ♀ offspring x ♂ offspring (cjxg) ♀ offspring (gxcj) x ♂ g ♀ offspring (gxcj) x ♂ cj ♀ offspring x ♂ offspring (gxcj) Co g x g Co cj x cj

*♀=female, ♂=male, Co=control offspring

Table 3.2. **Effect of population crosses of *T. manihoti* (Cajibío, Colombia, (cj) and Copacabana, Colombia (cp) on fecundity and progeny development.**

Cross		Non Eclosion Egg Hatch		Mortality	Preoviposition Time (Days)		Total Oviposition (10 Days)		•Proportion of Females
Female	Male	N	%	%					
Control Cajibío		98	12.2	14.3	0.84	*B	31.6	*ABC	0.6
Control Copacabana		91	14.3	17.6	0.86	B	31.1	ABC	0.7
Cajibío x Copacabana		105	9.5	14.3	0.68	BC	34.5	AB	0.5
Copacabana x Cajibío		109	9.2	13.8	0.62	BC	35.3	A	0.5
Offspring/Female x Male (cjxcp)		106	0.9	2.8	0.30	C	32.4	ABC	0.7
Female offspring (cjxcp) x Male Cajibío		102	2.9	3.9	0.71	BC	30.5	BC	0.7
Female offspring (cjxcp) x Male Copacabana		99	0.0	0.0	0.85	B	30.5	BC	0.7
Offspring/Female x Male (cpxcj)		100	7.0	7.0	1.43	A	32.8	ABC	0.7
Female offspring (cpxcj) x Male cj		100	3.0	3.0	0.96	B	29.3	C	0.7
Female offspring (cpxcj) x Male cp		107	3.7	3.7	0.85	B	32.3	ABC	0.6
Control offspring Cajibío		106	0.0	0.0	0.61	BC	35.2	A	0.6
Control offspring Copacabana		104	2.9	2.9	0.33	C	32.1	ABC	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 3.3. **Effect of population crosses *Typhlodromalus manihoti* (Cajibío, Colombia (cj) x Guajira, Colombia (g) on fecundity and progeny development.**

Cross		Non Eclosion Egg Hatch		Mortality	Preoviposition Time (Days)		Total Oviposition (10 Days)		•Proportion of Females
Female	Male	n	%	%					
Control Cajibío		107	0.0	0.0	0.44	*CD	36.4	*A	0.6
Control Guajira		110	0.0	0.0	0.39	CD	35.0	A	0.6
Cajibío x Guajira		107	0.0	0.0	0.29	CD	33.0	AB	0.5
Guajira x Cajibío		112	4.5	4.5	0.46	CD	31.9	AB	0.5
Offspring/Female x Male (cjxg)		90	0.0	0.0	0.44	CD	32.9	AB	0.6
Female offspring (cjxg) x Male Cajibío		95	0.0	1.1	1.13	A	30.0	B	0.6
Female offspring (cjxg) x Male Guajira		94	0.0	0.0	0.52	CD	29.7	B	0.6
Offspring/Female x Male (gxcj)		90	0.0	0.0	0.37	CD	34.0	AB	0.6
Female offspring (gxcj) x Male cj		93	0.0	0.0	0.69	ABC	33.0	AB	0.7
Female offspring (gxcj) x Male g		101	0.0	1.0	1.00	A	30.0	B	0.6
Control offspring Cajibío		95	2.1	2.1	0.09	D	32.7	AB	0.6
Control offspring Guajira		92	1.1	1.1	0.03	D	35.6	A	0.7

* 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).

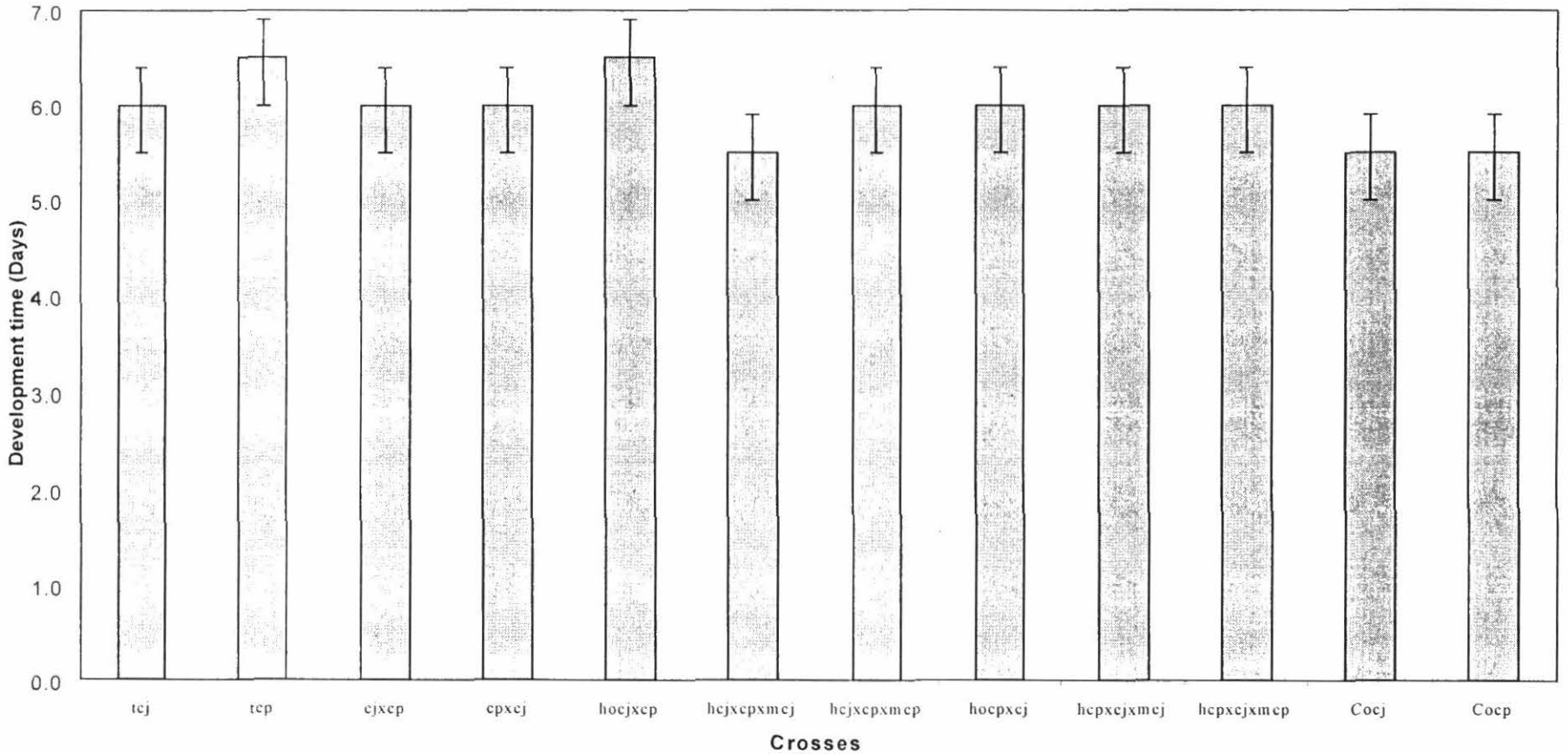

Figure 3.1. Development time for populations crosses of *Typhlodromalus manihoti* Cajibío (cj) x Copacabana (cp)) - t=control, h=female, m=male, ho=offspring, Co=offspring control.

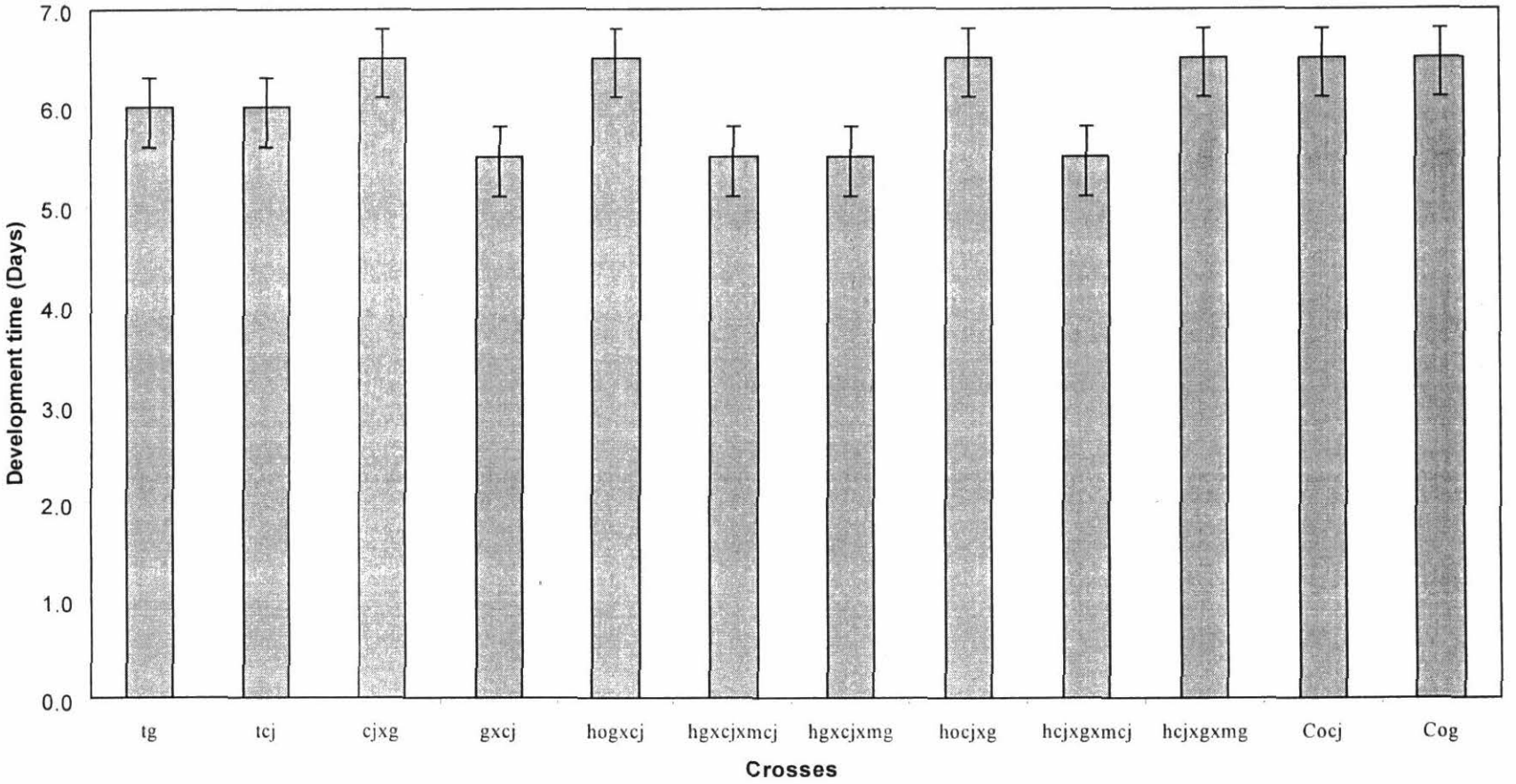

Figure 3.2. **Development time for populations crosses of *Typhlodormalus manihoti* Cajibío (cj) x Guajira (g)) - t=control, h=female, m=male, ho=offspring, Co=offspring control.**

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Activity 4. The Potential use of phytoseiid mite predators for biological control of *Thrips palmi*

Thrips palmi Karny (*Thysanoptera: Thripidae*) native to the Malasia-Indonesia region, has now diseminated throughout Asia, Africa and the Americas. This pest is reported attacking more than 50 crop plants, including solanaceas, cucurbitaceas and leguminasas (Hall, 1992). Once introduced into Colombia, *T. palmi* disseminated rapidly and has become a major pest problem on several crops. Plant damage is caused by both larvae and adults; initially silvery streaks or spots are observed on the leaf under surface, and as damage increases leaves become russet or bronzed, leaf veins necrose and turn brown. Plant growth is strunted or retarded and may dry up (Cermili et. al. 1993).

The introduction of natural enemies into newly invaded areas could help control this pest. The mite predator *Neoseiulus* (Amblyseius) *cucumeris* has been widely used to control other thrips species such as *Frankliniella occidentalis* and *Thrips tabaci* (Gillespie, 1989; Steiner, 1990; Hoy and Glenister, 1991, Shipp and Whitfield, 1991; Wittmann and Leather, 1997). Some studies with *T. palmi* have also been done in Florida, USA (Castineiras et. al. 1997). In addition, *Typhlodromulus aripo,* another pytoseiid mite predator, often found on cassava and occasionally on beans, has demonstrated to have a good capacity to consume *Scirtothrips manihoti* in cassava (CIAT, 1998).

The CIAT Acarology Laboratory has had considerable success in biological control studies utilizing phytoseiid mite predators (Bellotti et. al. 1999); Smith and Bellotti, 1996) and has advanced basic research on the biological control of thrips.

This research has the following objectives:

1. Determine the fecundity and development of *N. cucumeris*. and *T. aripo* when feeding on *T. palmi* and compared to phytophagous mite prey.
2. Quantify the consumption of first instar larvae of *T. palmi* by *N. cucumeris* during its development stages.
3. Quantify the consumption of *N. cucumeris* and *T. aripo* during their reproductive phase when feeding on *T. palmi* larvae.
4. Determine the feeding capacity of *N. cucumeris* in first instar *T. palmi* offered at different prey densities.

Material and methods

Prey and predator colonies

A colony of *T. palmi* was established in the laboratory (25± 2°C; photoperiod 12 hrs. light, 12 hrs dark) on beans (*Phaseolis vulgaris*; var. ICA-Pijao). For experimental purposes *T. palmi* prey individuals were obtained from the laboratory colony or directly from the field (Municipio de Pradera, Valle). The phytophagous mite species offered in comparative studies were

Tetranychus urticae and *Mononychellus caribbeanae*, both obtained from a CIAT screenhouse colony on cassava.

The colony of *N. cucumeris* was obtained from New York (USA), and established at CIAT using the McMurtry and Scriven rearing method, where predator mites feed on *T. urticae* eggs, pollen and bees honey. *T. aripo* comes from CNPMF/EMBRAPA, Cruz das Almas, Brazil and is reared at CIAT using the Mesa Bellotti method, where they are maintained on cassava leaves containing *M. caribbeanae* as prey. Both phytoseiid colonies were maintained under the same environmental conditions as the *T. palmi* colony.

Predator development and feeding studies

Development studies were carried out by introducing five *T. palmi* first instar larvae into an experimental arena (a 2 cm diameter plastic vial covered with seranwrap) containing a cassava leaf disc. (Although cassava appears to be an accidental host of *T. palmi*, in laboratory studies it was observed that *T. palmi* will feed and complete its developmental cycle to adult on cassava leaves). Into each arena, predator eggs from the same cohort (n>80) for each species were introduced and prey numbers were reestablished daily once feeding was initiated. At the same time *N. cucumeris* and *T. aripo* eggs were placed in mite infested cassava leaves in jars. Predator developmental changes was recorded daily.

The experimental arena described above was also used for fecundity studies. Five larvae of both the first and second instars was introduced into the arena. Predator females, one day after copulation (n>20), were individually released in each arena and oviposition and feeding were recorded daily. Thrips numbers were replenished daily until female predator death. Predator fecundity feeding on the phytophagons mite prey was recorded in jars with cassava leaves infested with *T. urticae* (for *N. cucumeris*) and *M. carribeanae* (for *T. aripo*). The use of the phytophagous mite hosts allows comparison of development and fecundity with that obtained on *T. palmi* as a prey host to the predator species.

The prey densities offered to the predator species were 1, 3, 5, 10, 20 and 40 first instar *T. palmi* larvae. The phytoseiid mite predators employed were in the third day of their reproduction cycle. Prey consumption was recorded for a 24 hrs period and there were 15 repetitions at each prey density.

Result and discussion

1. Predator development and fecundity

The effect of *T. palmi* consumption on the development and fecundity of *N. cucumeris* and *T. aripo* compared favorably to mite prey hosts.

The development time of *N. cucumeris* feeding on *T. palmi* and *T. usticae* is 8.9± 1.5 and 8.6± 0.8 days respectively presenting no significant difference in development cycle duration, regardless of prey species (**Table 4.1**).

Table 4.1. The effect of two prey species on the development of *Neoseiulus cucumeris*.

Prey species	N	Development time		Survival %	Females %
		Average	DS		
Thrips palmi	85	8.9	1.5	81.2	65
Tetranychus urticae	89	8.6	0.8	92.1	49

The percent survival of *N. cucumeris* when feeding on *T. palmi* and *T. urticae* was 81.2 and 92.1 respectively (**Table 4.1**). In both cases survival to the adult stage is considered high. Predator mortality, when it did occur, was usually during the protonymph stage, and in some cases, was due to the predator not feeding on the prey offered. During the first two days (larvae stage) the predators did not feed; they were probably able to survive for two days due to the reserve energy source that they bring from the eggs stage. When larvae moult to the protonymph stage they may be emaciated and if they do not initiate feeding rapidly, the can die. Five *T. palmi* were offered to the phytoseiid predators each day and at no time were the protonymphs able to consume more than one thrips larvae per day, indicating that the amount of prey quantity available was not significant in total consumption. This suggests that the protonymph did not have sufficient energy to search and/or capture the prey available and eventually died.

A higher percentage of females of *N. cucumeris* resulted when they feed on *T. palmi* compared to *T. urticae* **(Table 4.1)** which favors population increases of *N. cucumeris*.

The development time of *T. aripo* feeding on *T. palmi* and *M. caribbeanae* is 7.71± 0.79 and 6.8± 0.6 days respectively, also presenting no great difference in life cycle duration when feeding on the two prey species **(Table 4.2)**. Survival was also very high when *T. aripo* fed on *T. palmi*.

Table 4.2. The effect of two prey species on the development of *Typhlodromalus aripo*.

Prey species	N	Development time		Survival %	Females %
		X	DS		
Thrips palmi	88	7.71	0.8	89.8	80
Mononychellus caribbeanae	87	6.8	0.6	97.8	75

The average total number of eggs oviposited by *N. cucumeris* when feeding on *T. palmi* and *T. urticae* was 24.9± 9.7 and 21.3± 7.6, respectively. The average total oviposition for *T. aripo* when feeding on *T. palmi* and *M. caribbeanae* is 14.6± 4.3 and 13.0± 8.5 respectively **(Table 4.3)**. In both cases, predator oviposition was not greatly influenced by the prey species, but was slightly higher when feeding on *T. palmi*.

Table 4.3. **Total eggs oviposition of two Phytoseiidae species feeding on different prey.**

	N. cucumeris		*T. aripo*	
Prey species	**X**	**DS**	**X**	DS
T. palmi	24.9	9.7	14.6	4.3
T. urticae	21.3	7.6	--	--
M. caribbeanae	--	--	13.0	8.5

The ovipositional period for both predator species is not greatly altered by the host prey species offered. The ovipositional period ranged from 27 to 34 days but oviposition is greatest during the initial 15 to 17 days and than declines significantly **(Figures 4.1 and 4.2)**. Daily oviposition by female *N. cucumeris* feeding on *T. palmi* and *T. urticae* was highest during days 2 to 16; feeding on *T. palmi* oviposition continued until day 32 and until day 27 when feeding on *T. urticae* **(Figure 4.1)**. Highest daily oviposition occurred when *N. cucumeris* fed on *T. urticae*, 2.5 eggs (on day 6) while highest on *T. palmi* was 1.5 (on day 3).

The ovipositional period for *T. aripo* when feeding on *T. palmi* and *M caribbeanae* is similar to that described for *N. cucumeris* **(Figure 4.2)**. The maximum period for oviposition is between days 2 and 16 and the highest daily oviposition was 1.5 eggs for both prey species. For *T. aripo* the ovipositional curve is almost identical for the two prey species whereas with *N. cucumeris* the curves were distinct for the two prey species **(Figures 4.1 and 4.2)**.

Since both mite prey species selected for this experiment are very acceptable host to the two predator species, the results obtained indicate that *T. palmi* is a very acceptable host to the phytoseiid predators in tems of development and fecundity.

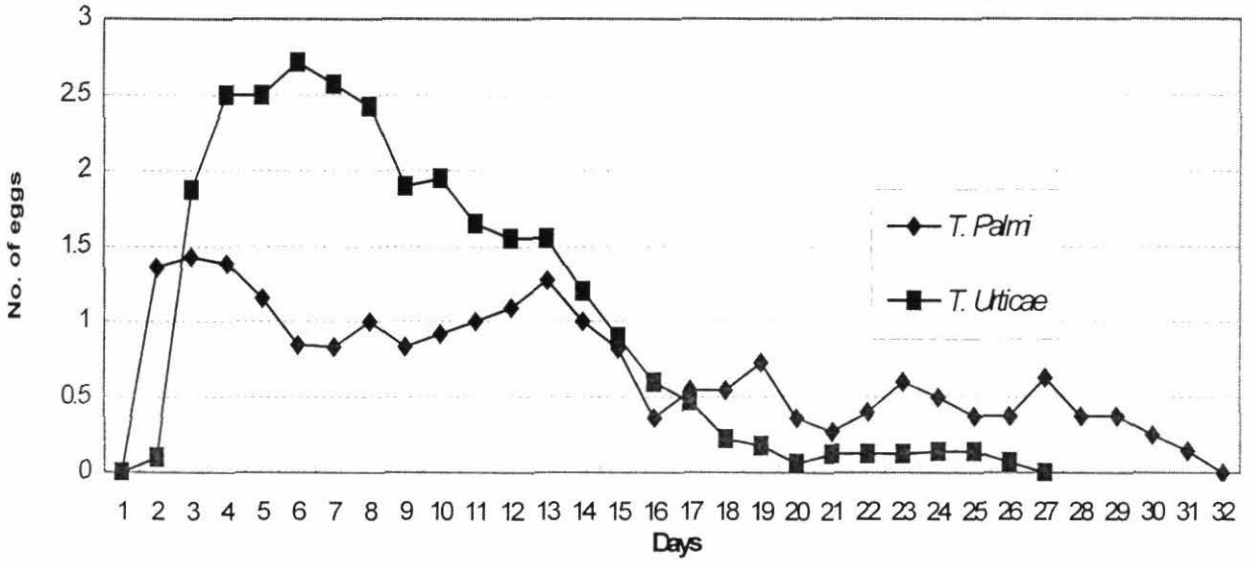

Figure 4.1. **Daily ovipositional of *Neoseiulus cucumeris* feeding on two prey species.**

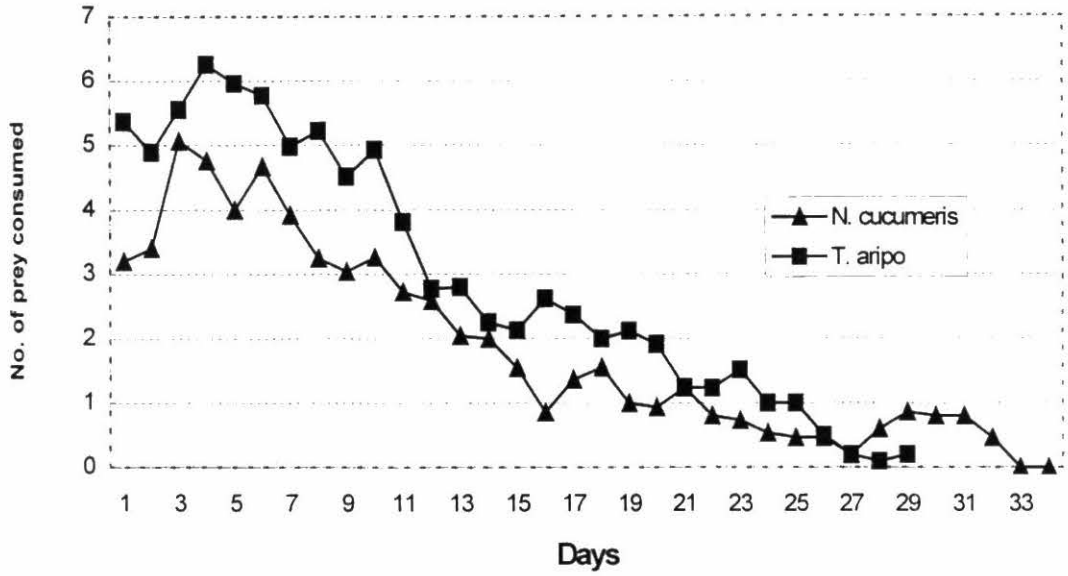

Figure 4.2. Daily consumption of females of two Phytoseiidae species (*Neoseiulus cucumeris* and *Typhlodromalus aripo*) feeding on *Thrips palmi* during their reproductive cycle.

2. Consumption of *T. palmi* by *N. cucumeris*.

It was difficult to exactly determine the prey consumption by each immature stage of *N. cucumeris* as it was not possible to record changes in the immatures from one stage to the next. However it can be confirmed that the larvae of *N. cucumeris* did not feed, supporting literature reports that larvae of certain phytoseiid species do not feed (Shipp and Whitfield, 1991).

N. cucumeris from protonymph to adult, will consume 10± 2.6 first instar larvae of *T. palmi*. Both males and females predate equally; 10.1± 2.8 for males and 9.8 ± 2.6 for females. The predator immatures (protonymph and duetonymph) were easily able to capture, manipulate and consume *T. palmi* larvae during feeding.

3. *N. cucumeris* and *T. aripo* consumption of *T. palmi* during their reproductive cycle.

As mentioned previously, 5 first and 5 second instar larvae of *T. palmi* were offered each predator species. Both *T. aripo* and *N. cucumeris* will predate on both the first and second larvae instar of *T. palmi*; initially attacking the first instar and later the second. Female adult predators have no difficulty in searching or capture of the prey, although they may not consume the total body fluid of the thrips, they will kill them.

The total thrips consumption during the reproductive period was 48± 13.3 larvae of *T. palmi* by *N. cucumeris* and 70.6± 16.3 by *T. aripo*. Although the total consumption of the two predator

species is quite different, with the high standard deviations, the differences are not significant. If only first instar *T. palmi* were offered, predator consumption would be even higher.

Highest thrips consumption occurred during the initial stages of the reproductive cycle, or about 13 days **(Figure 4.3)**. After that period oviposition is reduced and logically, prey requirements are also reduced. Peak consumption occurs between days 3 and 6, when *T. aripo* consumes six thrips per day and *N. cucumeris* five. As can be observed **(Figure 4.3)** both predator species have similar requirements of the prey during the reproductive period. The predation by females during their reproductive period is superior, in comparison, to the consumption of the immature predators, or for females that have terminated oviposition. This is due to the increase in body size of reproductive females and the energy demands made by egg development, making this period the most efficient stage for predation.

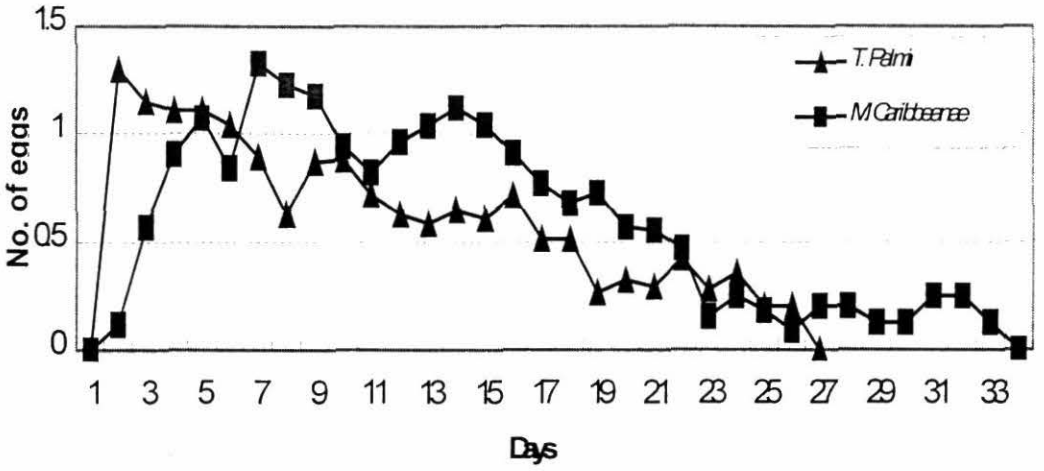

Figure 4.3. Daily oviposition of *Typhlodromalus aripo* feeding on two prey species.

4. Consumption capacity of female *N. cucumeris* feeding on different densities of *T. palmi* larvae.

Prey (*T. palmi*) consumption by N. *cucumeris* increases as prey density also increases **(Figure 4.4)**. When up to 10 prey were offered, *N. cucumeris* has the capacity to consume all 10. With a density of 20 prey, 13.1± 3.1 were consumed and at a density of 40, 16.7± 2.9 were consumed in a 24 hr period. Although there was no significant difference between densities of 20 and 40, the results indicate that at higher densities, the probability of prey capture increases, which results in a higher consumption.

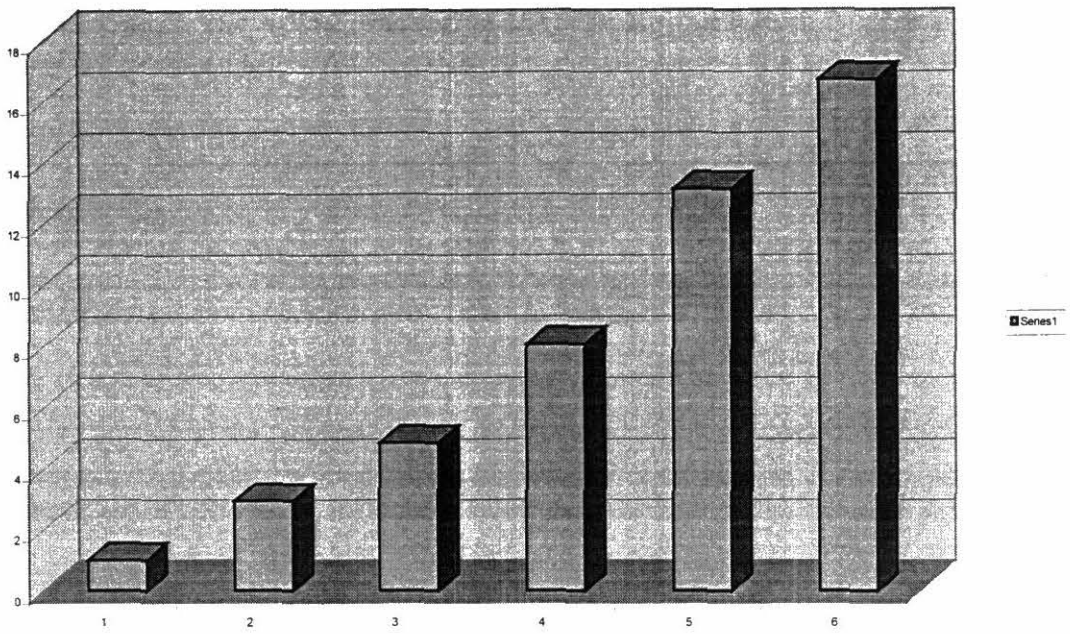

Figure 4.4. Consumption capacity of *Neosiulus cucumeris* females feeding on different larval densities of first instar larvae of *Thrips palmi*.

Shipp and Whittfield (1991) found that pregnant females of *N. cucumeris* can consume up to 10 first instar larvae of *F. occidentalis* on pepper leaf discs, and up to 7 on cucumber leaf discs at a density of 40 in 24 hours. These results suggest that yuca leaves could influence consumption rates by the predator species, indicating that the leaf structure, for example, the presence of tricomes or other factors or substances on the foliar surface could influence predation rates. To test this, studies could be conducted with other plant species.

Considering that *N. cucumeris* is being used successfully for the control of *F. occidentalis* in greenhouses, and the promising results obtained with *T. palmi*, the use of *N. cucumeris* as part of biological control/IPM programs for *T. palmi* is a future possibility. However, the high (excessive ?) use, at present, of agrochemicals for control of *T. palmi* is a disconcerting factor and makes the release of predator natural enemies less favorable. However past successes in biological control in Colombia (Guarin and Parra, 1999) and other countries (Hirose, et al 1993) indicate that the biological control of *T. palmi* is a worthy and accessible goal.

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Activity 5. Research on the development and use of entomopathogens for biological control of arthropod pests

There has been a growing interest in recent years in the use of entomopathogens as "biopesticides" for the control of arthropod pests (Lacey and Brooks, 1996). We have carried out extensive surveys of pest problems in varied agroecosystems, in numerous countries, for more than 20 years, especially on cassava. These explorations have often led to the discovery that there are several entomopathogens that could play a positive role in IPM program. In past annual reports, through publications and thesis, we have reported and described research on the use of entomopathogens (including entomopathogenic nematodes) for control of mites, hornworms, whiteflies, burrower bugs and other pests.

It is estimated that continued exploration and research in this area could lead to the discovery and development of additional species and strains of entomopathogens. Research on virulence and pathenogenicity of entomopathognes, is being carried out and planned on a complex of arthropod pests. In order to support this research, emphasis is being given to the development of methods for conserving and rearing entomopathogens. The identification of entomopathogens on whiteflies will be given priority.

Collection and maintenance of fungal entomopathogens

Insects that show diseased symptoms are collected in the field.
Diseased insects are desinfested with a 5% hyporchloride/distilled water solution.
Insects are placed on growth media, usually a Saboaraud-dextrose Agar with a yeast supplement (SDAY).
Infectous pathogens present on insects are cultured and slide mounted for identification.
For storage of fungal pathogens, pieces of filter paper are placed on SDAY media and inoculated with the pathogen, when conidia appear, the pieces of filter paper are wrapped in aluminium foil, placed in paper envelops and maintained at 4°C.

Maintenance of Entomopathogenic Nematodes

For nematode maintenance there are both *in vitro* and *in vivo* methods available that we have used in laboratory cultures (Caicedo and Bellotti, 1996, Barberena and Bellotti, 1998). At present we are utilizing the *in vivo* method; this method uses larvae of *Galleria mellonella* (Lepidoptera). *G. mellonella* larvae are placed in soil samples collected from the field and after 10 days they are dissected to observe if they have been parasitized by nematodes.

A colony of *G. mellonella*, to facilitate these studies, is maintained in the laboratory. Adult *G. mellonella* are placed in large glass jars with honey for feeding, and fan-folded paper for oviposition. The paper, containing eggs, is removed daily and stored in other jars until larval eclosion (approximately 5 days). Larvae are placed on an artificial diet (310 gms wheat bran, 90 gms brewers yeast, 225 gms bees honey, 45 gms bees wax, 150 mil glicerine=1000 larvae). A permanent colony of *G. mellonella* has now been established, continually producing larvae.

The above *in vivo* methodology is adequate for nematode production for laboratory and small field studies, but not adequate for mass production needed to treat large acreage. For mass production an *in vitro* method is more practical and efficient.

Results

Fungal pathogens were field collected from whiteflies, *G. mellonella*, mites and other host arthropods. These were identified and stored as described above. In addition, fungal pathogens were obtained from other research institutes (i.e. CENICAFE) as part of collaborative studies using entomopathogens.

Neozigytes spp isolates were obtained from Africa and Brazil and collected from cassava fields in Colombia **(Table 5.1)**. Fungal pathogens were collected from numerous localities in Colombia **(Table 5.2).**

Future research activities will include:

Field collection of diseased arthropods and identification of pathogens
Multiplication and storage of entomopathogens
Pathogenicity tests and bioassays to evaluate the effectiveness of pathogens
Soil sampling in different ecoregions to collect entomopathogenic nematodes
Reactivate pathogen isolates in the laboratory
Survey for, and collect, whitefly entomopathogens from different regions and countries
Multiplication of *Neozygites* fungus for laboratory and fields studies.

Table 5.1. Isolates of the *Neozygites* fungal pathogen stored in CIAT laboratory.

Strain	Host	Origin
Mtaf	*M. tanajoa*	Benin, Africa
Mtcd	*M. tanajoa*	Cruz das Almas, Bahía, Brazil
Mtml	*M. tanajoa*	Media Luna, Magdalena, Colombia
Mtml	*M. tanajoa*	Piritiba, Brazil
Mtml	*M. tanajoa*	Caruaru, Brazil
Tuct	*T. urticae*	CIAT, Valle, Colombia
Tuaf	*T. urticae*	Benin, Africa

Table 5.2. Fungal pathogens collected from several hosts and regions of Colombia and stored in CIAT laboratory.

Pathogen Code		Original Host	Site
CENICAFE CIAT			
Metarhyzium anisopliae			
9001	01	Unknown	CIMIC
9004	02	White grub (Coleop: Scarabaeidae)	Antioquia
9108	03	Cargdia arana (Homop: Geometridae)	Caldas
9206	04	*Aenolamia reducta* (Homop: Cercopidae)	Carimagua
9207	05	*Mocis* sp. (Lepidop: Noctuidae)	Carimagua
9211	06	Zulia colombiana (Homop: Cercopidae)	S. de Quilichao
9236	07	Unknown	Colombia
9237	08	*Plectris* sp. (Colep: Scarabaeidae)	Colombia
9301	09	*Ancognatha scarabaeidae* (Colep: Scarabaeidae)	Antioquia
		Hypothenemus hampei (Coleop: Scolytidae)	
9303	10		Antioquia
9304	11	*Cosmopolities sordidos* (Coleop: curculionidae)	Colombia
9401	12	Unknown	Colombia
9501	-	*Cyrtomenus bergi* (Hemip: cydnidae)	CIAT-Palmira
-	-	Galleria mellonella	Cajibío
Beauveria bassiana			
9002		*Hypothenemus hempei* (Coleop: Scolytidae)	Caldas
Strains not identified			
-	-	*Trialeurodes vaporariorun*	Pradera
-	-	Tarántula	Valle del Cauca
-	-	*Cyrtomenus bergi* (Hemip: Cydnidae)	Popayán

Activity 6. Identification of pests associated with the asparagus crop in Cajibío, Cauca, Colombia

Traditionally asparagus (*Asparagus officinalis*) has been cultivated in the temperate regions of the world. In more recent decades, production has moved into the tropical and subtropical regions, including several countries in South (Perú, Chile, Argentina and Colombia) and Central (Guatemala, Costa Rica) America. At present Colombia cultivates only 700 to 1000 ha, 80% of this in the Department of Cauca. This is a high value crop, mostly for export, and it is estimated that production area will steadily increase.

It is only in the last 7 or 8 years that the crop has become established in Colombia where climatic conditions are optimal to produce 2 to 3 crops (harvests) per year. The United States is the principal importer of Colombia asparagus; quality requirements are high and the product is subject to a rigorous evaluation for arthropod pests or pesticide residues, upon entrance to the USA. As a somewhat "novel" crop in Colombia, there is no information in the literature on the pest complex associated with the crop nor crop damage. CIAT was approached by three asparagus companies in the Department of Cauca, Compañía Agrícola de Espárragos, Espárragos Chayaní S.A. and Agrícola Palacé E.U. and subsequently entered into an agreement with them to provide technical assistance. A three phase project was developed and funded by the 3 asparagus companies.

Phase 1. Diagnostic phase; diagnostic evaluation of the principal arthropod pests and natural enemies associated with the asparagus crop.

Phase 2. Research on the biology, ecology and behavior, and damage of the major pest species, based on analysis of Phase 1 diagnostic data.

Phase 3. Development of integrated pest management strategies and technologies for major pests.

Phase 1 was designed as a six month duration and was carried out during 1999. It was also envisioned that these three phases would be overlapping; for example pest and natural enemy identification would continue throughout the duration of the project, and the development of IPM strategies and technologies would begin during phase 2.

Results Phase 1

A literature review of asparagus was made; this included crop history, areas of production, crop establishment, varieties, fertilization, crop management, phytosanitary problems, harvest, post harvest and quality aspects. Although not included in this report, this information is available in a separate document.

Since asparagus is a perennial crop, pest problems can build up overtime and it is difficult to break pest cycles. The literature indicates that there are 6 major diseases of asparagus, but it appears that these can be controlled or prevented through the use of resistant varieties and

phytosanitary practices. The situation with arthropod pest problems is less advanced; a complex of pests is reported in the literature, mostly belonging to the Orders, Homoptera, Lepidoptera, Coleoptera, Diptera, Hemiptera and Thysanoptera. One of the objectives of the present study is to compare the arthropod complex we find in Colombia with that of other asparagus growing regions.

Aphids are considered a major problem in asparagus production as they can damage the crop directly by injecting a toxin into the plant during feeding that can cause plant stunting and distortion and aphid honeydew excretion will attract high ant populations. In addition, aphids can vector at least two known virus diseases that reduce plant vigor and/or cause plant death. The two aphid species of major importance in the Americas are *Brachycorynella asparagi* and *Bracycolus asparagi*.

Coleopteran (*Crioceris asparagi, C. duodecimpunctata*) and Lepidopteran (*Spodoptera exigua*) species will feed on the buds, leaves and stems and if this occurs during harvest, it results in considerable commercial damage. In addition the presence of coleopteran and lepidopteran egg masses on exported stems can result in product rejection or fumigation during export. Lepidopteran cutworms (*Euxoa scandems* and *E. messoria*) are subterranean pests that damage roots and young stems, reducing yield and quality. Thrips (*Thrips tabaci*), by their rasping feeding habits, can cause stunting, a loss of vigor or death of growing points.

Diagnostic methodology

Surveys were carried out on the three aforementioned asparagus plantations, located in the Department of Cauca between 1700 and 1840 m.a.s.l. Daytime temperature ranged from about 24 to 31°C and nighttime from 12 to 14.6°C and rainfall was highest (196 to 358 mm) during the months of Oct. Nov. Dec. and Jan.

Field sampling was initiated in September, 1998 and continued through early 1999. Prior to collecting, an evaluation of the cropping system (planting, pruning and harvesting dates, etc.) of each plantation was done, in order to select appropriate fields for sampling. An attempt was made to sample each plantation twice each month but this schedule was not always feasible due to guerilla activities and security problems in the region. In a six month period, each plantation was sampled about 8 times. "Plots" or fields were sampled at numerous locations, primarily using a sweepnet, or by direct observation of adult and immature stages. Each sample was collected from a 10 linear meter area with 10 double sweepnet passes.

Specimens collected were placed in plastic bags, coded, and placed in a styrofoam container (cooled) and transported to the CIAT entomology laboratory. Field collected insects were immobilized at 3°C and placed in vials with 70% alcohol. Immature stages were placed in plastic boxes with asparagus leaves and shoots or lettuce, and reared to adult stage for identification. Specimens were morphologically classified (first to Order), numbers collected recorded, pin mounted or placed in alcohol. A preliminary screening of the specimens collected was made, based on the following criteria:

1. That the same specimen (species) was collected from each plantation.
2. More than five individuals of each species was represented.
3. In agreement with the literature review specimens of the families of the pest arthropod or beneficial were registered on asparagus.

An initial identification of the species was done through comparison to specimens already present in the CIAT arthropod collection. Unidentified specimens were sent to appropriate taxonomist, including those of the British Museum in London or USDA at Beltsville, Maryland.

Preliminary results

During the six months of sampling at the three plantations approximately 1440 specimens were collected. These are represented by the Orders of Coleoptera, Hemiptera, Neuroptera and Thysanoptera. The most common families observed were Chrysomelidae, Noctuidae, Artiidae, Coccinelidae, Cleridae, Rhipiphoridae, Pentatomidae, Tripiidae, Aphiidae, Membracidae, Tachinidae, Syrphiidae, Ichneumonidae, Braconidae and Chrysopidae. Using the above mentioned criteria the total sample of 1440 specimens was reduced to 72 species including beneficials, of major importance for identification purposes **(Table 6.1).** Of this group, we were able to identify 17 to species, using our own reference collection, leaving 55 species that will require identification by the appropriate taxonomists. A description and field observations pertaining to this group of species is outlined in (**Table 6.2**). These are divided into pest species and beneficials. Several of these appear to be new or unrecorded species.

The importance of collecting these aphid species cannot be ignored. As mentioned earlier, aphids are vectors of virus diseases in asparagus. Recommendations on the importance of quarantine and the care required in introducing new germplasm, has been emphasized with these asparagus growers. One of the virus diseases is also seed transmitted and could easily be introduced on uncertified seed.

The diversity and strength of the natural enemy complex associated with the crop is notable. This indicates that pesticide use has been moderate. This observation was confirmed by the three growers. Pesticide applications were made on all three plantations for thrips control. Trap cropping, hand collecting, baited traps and applications of *Bacillus popillae*, a biopesticide were additional methods of pest control.

A study was also made of weed species associated with the crop. These are possible alternate host to the asparagus pests or they may harbour natural enemies. This information is available in a separate report.

Recommended activities for the next phase of the project include

1. The diagnostic evaluation should also be carried out during the drier season.

2. Biology and behavior studies for major pests and associated natural enemies should be initiated.

3. Observations and evaluation of the virus/aphid interactions.

4. Damage description for major white grub species associated with asparagus crop and their relation to soil pathogens.

5. Determine pest damage and population fluctuations for principal insect pests, especially thrips, aphids and white grubs.

6. Initiate studies on pest and natural enemy interactions, including the introduction of biological control agents such as mite predators for thrips control and the use of entomopathogenic fungi, bacteria or nematodes for white grub control.

7. Initiate studies on the use of parasites for control of aphid and lepidopteran pests.

Table 6.1. Arthropods, by order, considered of major importance, from diagnostic surveys of the asparagus plantation in Cauca, Colombia, and selected for identification to species.

Order	Selected	Identification	To Identify
Coleoptera	20	9	11
Hemiptera	18	6	12
Lepidoptera	6	0	6
Diptera	10	2	8
Hymenoptera	8	0	8
Neuroptera	2	0	2
Thysanoptera	3	0	3
Homoptera	5	0	5
Total	72	17	55

Table 6.2. Observations on 72 selected arthropod specimens collected from three asparagus plantations in Cauca, Colombia.

Order	No. species	Observations
Pest Species:		
Lepidoptera	6	Of the six species collected, four are of the Family Noctuidae, belonging to the genus *Spodoptera*. Two are *possibly S. fugiperda* and *S. ornithogali*. Of this family, only *S. exigua* is reported as a pest on asparagus. Two species are of the Family: Artiidae, one is *Estigmene acrea*, the other is a new species for asparagus. These lepidopterans are found in all biological stages and all stages of crop development. Eggs and first instar larvae are found on recently emerged asparagus spears at harvest and later instar larvae, pupae and adults are found in mature asparagus stands.
Thysanoptera	3	Taxonomists need to confirm presence of 2 or 3 species, possibly *Thrips palmi*, *T. tabaci* and *Frankliniella occidentalis*. Thrips are found on all development stages of the crop and persist on the processed product. Yellowing, due to thrips feeding is observed on recently germinated spears and at harvest causes bracket apertures.
Homoptera	5	Three aphid species were collected and appear to be distinct from, *B. asparagi*, probably the most common aphid species found on asparagus. Aphids were collected from branches and spears at harvest. In addition two Membracidae species were collected.
Hemiptera	18	This, the most numerous order of pests collected, was observed throughout all crop growing cycles. In addition egg masses, in different forms, were found on harvested spears. Adults and instars were observed feeding on crop foliage. Only one species was identified, *Thyanta perditor*.
Coleoptera	17	Six species of Chrysomelidae, foliage feeders, were selected, and none corresponded to the two registered species in the literature (*Crioceris asparagi* and *C. duodecempuntacta*). These are probably newly registered for asparagus and may contain unrecorded species. Several other families, including Curculionidae and Scarabaeidae were also selected. White grubs (Scarabaeidae) are probably feeding on asparagus roots and may be causing considerable damage.
Beneficial Species:		
Coleoptera	3	Two species of coccinelids, *Hipodamia convergens* and *Cicloneda sanguinea* were identified. These are generalist egg and larvae predators. One species needs to be identified.
Hymenoptera	8	Of the numerous hymenopterans collected, only those species of two families Braconidae and Ichneumonidae, both containing registered parasites of asparagus pests were selected. Two species were collected parasitizing Spodoptera. In addition numerous species of ants and bees (*Aphis mellifera*, especially during floration) were collected.
Diptera	10	In this group, parasitoids of the family Tachinidae, were most numerous. Two species were collected parasitizing *E. acrea* and other unidentified species of the Artiidae family. Four species of the family Syrphidae were selected; these are aphid predators. Three species of the family Agromizydae, economically important were selected, two appear to be unrecorded species.

Contributors – Sub-output 1 - Entomology

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Sub-output 2. The Influence of Cassava Plants under Water Deficiency on the Development of the Cassava Mealybug *Phenacoccus herreni* Determined.

Introduction

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). Previous works showed that cassavas under water deficit conditions induced a reduction in the duration on female development and an increase in fecundity, intrinsic natural growth rate (rm) and in weight (CIAT, 1998; Polanía *et al.*, 1999). This phenomenon might be a response to biochemical changes in cassava leaves induced by water deficiency, which enhances insect development at nutritional aspect. 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 in order to protect cellular structures. These changes in the trophic quality of cassava induced by water stress should favor insect growth. In order to verify this phenomenon, it was first necessary to estimate carbon and nitrogen modifications in cassava leaves during water deficiency.

Activity 7. Estimation of carbon and nitrogen modifications in cassava leaves during water deficiency

Two genotypes of cassava (*Manihot esculenta* Crantz) CM 1585-13 and CM 507-37, were used in this study. Drought stress was imposed by decreasing the irrigation volume from 1200 ml (three times a week per plant, control) to 80 ml (two times a week per plant, stress). Three-month-old plants (10 per treatment) were used for the experiment. Both young leaves (2 weeks old) and old leaves (8-10 weeks old) were collected for metabolite and enzyme activity analysis. The experiment was duplicated.

After 45 days of water deficiency, shoot development was affected for both varieties. The stems stopped growing from the onset of the period of water limitation. The area of the mature leaves was reduced and the stomatal conductance was very low under water limitation (all data not shown). These modifications of plant growth and stomatal conductance indicate that our experimental conditions mimic water stress and thus validate the approach used here to study the adaptive reaction of cassava to water limitation.

Carbon concentration in dry mater was not affected by the water availability **(Table 7.1)**. For both genotypes, the $\delta^{13}C$ values were higher in young leaves as compared to old leaves. All these values were increased under water limitation. It is well known that stomatal conductance influences $\delta^{13}C$ since stomata aperture reduces discrimination against ^{13}C during CO_2 fixation. Thus, the positive effects of drought on $\delta^{13}C$ in leaves could result from the lowest conductance of these organs in water limited plants. In addition, the high $\delta^{13}C$ values in young leaves of drought-adapted cassava plants was associated with a high phospho*enol*pyruvate carboxylase (PEP Case) activity **(Table 7.3)**. The later enzyme expresses a low isotope fractionation. Since

young leaves exhibited higher stomatal conductance than old leaves in water limited plants, the high $\delta^{13}C$ values in young leaves could also result from intensive carbon fixation through PEP Case activity. However, in control plants, $\delta^{13}C$ was higher in young leaves as compared with old leaves. PEP Case activity was not different suggesting some stomatal effect in relation to the rate of CO_2 fixation by the photoreductive cycle.

For both genotypes and leaf ages, the $\delta^{15}N$ values were lower but the nitrogen concentration in dry mater was slightly higher under low water availability **(Table 7.1)**. This was associated with higher free amino acid level in stressed plants **(Table 7.2)**. Serine, glutamic acid, glutamine and arginine reached very high levels. As already observed in previous works (CIAT, 1998; Polanía *et al.*, 1999), the amino acid composition was also affected showing a decrease in aspartic acid, glutamic acid and alanine proportion and an increase in that of asparagine and arginine (data not shown).

Glucose, fructose and sucrose showed modifications of their accumulation levels but the total amount of the three carbohydrates increased following water limitation (data not shown). This was associated with higher sucrose phosphate synthase activity, both maximal and limited activities **(Table 7.3)**.

Phosphoglycolate amount in leaves was depressed under water limitation (data not shown). This was associated with higher phosphoglycolate phosphatase activity suggesting higher photorespiration **(Table 7.3)**. An increase in malate, succinate and citrate levels was also observed in leaves of water depressed plants (data not shown). This was associated with higher PEP case activity **(Table 7.3)**. Based on the increase in serine and glycine levels and in phosphoglycolate phosphatase activity in leaves of drought-adapted cassava plants, is hypothesized that the high phospho*enol*pyruvate carboxylase activity found under water limitation is due to an increase in photorespiration leading to an acceleration of respiratory ammonium assimilation requiring a high anapleuretic phospho*enol*pyruvate carboxylase functioning.

In conclusion, after limited water supply for 45 days in glasshouse conditions, cassava leaves exhibited an increase in the level of some metabolites which could favor mealybug growth. In fact, under water limitation cassava leaves exhibited an increase in phospho*enol*pyruvate carboxylase activity and thus in the level of some organic compounds such as malate, succinate and citrate. The free amino acids level was higher in water starved plants. Furthermore, water limitation induced an increase in the sucrose phosphate synthase activity and thus in the level of sucrose.

These same changes in enzyme activities and metabolites levels were observed in infested cassava leaves by *P. herreni* under low water availability (data not shown, CIAT, 1998; Polanía *et al.*, 1999) and suggest us to test in artificial conditions the effect of the increase in the level of these metabolites on mealybug growth.

Table 7.1. **Natural isotope compositions (δ13C and δ15N) and proportions of carbon and nitrogen of young and old leaves of two cassava varieties (CM 1585-13 and CM 507-37) grown under sufficient (NS) or limited (S) water availability (mean from five leaves).**

Variety	Treatment	Age of leaves	δ13C	δ15N	%C	%N
CM 1585-13	NS	Young	-28.2	2.9	51.0	5.4
		Old	-31.0	4.0	51.0	3.0
	S	Young	-24.2	1.8	51.4	5.8
		Old	-26.5	2.2	50.9	4.2
CM 507-37	NS	Young	-29.7	4.2	51.0	5.8
		Old	-30.1	4.0	48.1	3.5
	S	Young	-24.6	2.6	49.6	6.1
		Old	-28.1	2.5	51.6	4.6

Table 7.2. **Free amino acid contents (expressed in nmol/mg Chl) of young and old leaves of two cassava varieties (CM 1585-13 and CM 507-37) grown under sufficient (NS) or limited (S) water availability (mean from five leaves).**

	CM 1585-13				CM 507-37			
	NS		S		NS		S	
	Young	Old	Young	Old	Young	Old	Young	Old
Asp	102.8	63.5	143.6	80.2	118.2	39.3	124.2	75.1
Thr	39.8	21.4	63.7	51.2	40.2	21.7	78.5	26.9
Ser	98.4	39.9	203.4	94.5	95.8	33.8	263.3	60.6
Asn	15.4	Nd*	114.0	46.2	67.8	20.7	351.5	152.5
Glu	182.6	148.8	282.7	315.7	189.8	135.8	293.0	254.4
Gln	62.9	14.3	226.0	83.9	97.1	15.6	205.9	72.9
Pro	5.3	2.5	10.4	13.4	4.0	2.5	10.8	11.3
Gly	17.2	18.9	31.4	76.9	9.4	5.9	33.6	37.1
Ala	79.0	41.6	109.6	74.6	91.2	55.8	116.5	44.3
Val	10.8	6.3	48.4	25.6	8.8	6.2	48.2	17.1
Cys	18.9	12.8	34.0	32.6	23.0	8.8	35.9	30.9
Met	0.8	Nd*	nd*	2.4	0.6	0.1	3.0	1.8
Ile	1.4	2.4	8.3	8.5	1.3	1.5	13.9	8.6
Leu	1.4	1.0	8.1	8.9	1.4	0.9	13.0	9.8
Tyr	1.9	1.0	7.4	22.4	2.1	0.9	12.2	8.8
Phe	5.5	3.2	10.7	12.3	5.6	2.9	10.8	13.9
Lys	2.3	3.0	12.3	7.9	2.6	3.1	16.5	6.9
His	50.6	0.8	21.4	29.5	29.4	1.9	34.3	23.9
Arg	183.6	1.2	474.2	56.4	5.2	0.9	734.4	220.8
Total	880.6	382.6	1809.6	1043.1	793.5	358.3	2399.2	1077.6

*nd: not determined.

Table 7.3. **Phosphoenolpyruvate carboxylase (PEP Case expressed in µmol.(mg Chl)-1.min-1), sucrose phosphate synthase (SPS expressed in nmol.(mg Chl)-1.min-1) and phosphoglycolate phosphatase (PGP expressed in nmol.(mg Chl)-1.min-1) activities of young and old leaves of two cassava varieties (CM 1585-13 and CM 507-37) grown under sufficient (NS) or limited (S) water availability (mean from five leaves).**

	CM 1585-13				CM 507-37			
	NS		S		NS		S	
	Young	Old	Young	Old	Young	Old	Young	Old
PEP Case	0.4	0.7	4.4	0.8	0.8	0.3	5.6	2.4
SPS								
Vmax	41.0	14.3	26.7	26.5	23.3	10.9	82.4	30.3
Vlim	9.3	6.5	15.7	5.9	8.7	4.3	9.4	6.3
PGP	tr*	tr*	168.1	3.7	tr*	tr*	358.2	68.0

*tr: traces of activity.

Activity 8. Influence of carbon and nitrogen modifications in cassava leaves during water deficiency on mealybug growth

It is well known that for rearing phloemophagous insects with an holidic diet, copying the phloem sap concentration of sucrose and free amino acid do not allow a complete development of these insects probably due to the fact that some of them feed on the phloem sap and also on mesophyll cell contents. To elaborate an holidic diet it is necessary to have higher levels of sucrose (phagostimulant compound) and free amino acids than levels found in host plants. With *P. herreni*, we observed in artificial conditions that the increase in the level of sucrose and free amino acids favor mealybug growth (data not shown).

Furthermore, it is well known in the literature that the ratio "sucrose/free amino acids" influence the development of phloemophagous insects. According to the results obtained in activity 1.1, we tested in artificial conditions the effect of the change in the molar ratio "sucrose/free amino acids" induced by water limitation (which changed from 5 to 2.5 when cassava are under low water availability) on mealybug weight. It appeared that the molar ratio at 2.5 found in cassava leaves under low water availability favor the mealybug growth **(Table 8.1)**. In fact, the weight of adult females was significantly higher. This result confirm that cassava plants under water limitation favor mealybug growth because the leaves are better balanced (ratio "sucrose/free amino acids").

Table 8.1. Biological performance of females of P. herreni on different diets. Weight of females reared on different diets during 35 days were determined. Data are means* ± SE.

Diet**	Weight (mg)	Number of replicates
Ratio 5	0.244 ± 0.009 a	110
Ratio 2.5	0.384 ± 0.014 b	76

* Means followed by the same letter are not different at 5 % level (U-Mann Whitney test). a,b : column comparison (diet factor)

** Molar ratio "sucrose/free amino acids" (5 found on well-irrigated cassavas and 2.5 found on water-stressed cassavas).

In conclusion, the change in the trophic quality of cassava induced by water limitation favor mealybug growth because plant nutrients are either more concentrated in sucrose and free amino acids and better balanced (ratio "sucrose/free amino acids"). The effect of the increase in the level of some organic compounds such as malate, succinate and citrate on mealybug growth is presently in course in artificial conditions.

Since 1997 at CIAT, our main objective was to study 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 parasitoid developments.
In laboratory conditions, we showed that cassava leaves in water deficiency exhibited an increase in the concentration of some compounds such as free amino acids, carbohydrates (sucrose) and organic acids (malic acid, succinic acid) contributing to decrease the osmotic potential of the tissues in order to protect cellular structures. These changes in the trophic quality of cassava favor the cassava mealybug growth and reproduction because plant nutrients are either more concentrated in sucrose and free amino acids and better balanced (ratio "sucrose/free amino acids"). Furthermore, cassava in water deficit enhances the cassava mealybug immune's system against parasitoid egg or larva. In contrast, cassava in drought conditions has a negative effect on parasitoids affecting the parasitism success and, depending on parasitoid species, disfavoring the parasitoid development. All these results explain in part the increase in the population of *P. herreni* on cassavas during long dry season.

We assume that some cassava genotypes are more drought tolerants because they admit a higher soil water limitation and thus they react less quickly to the changes in soil water availability. There is no evidence that cassava genotypes more drought tolerant are more favorables to mealybug development and more disfavorables to the biological control. Furthermore, mealybug infestations induce a water stress on cassava plants (personal observations, Calatayud *et al.*, 1994). Then, drought tolerant cassavas should be more tolerant to mealybug infestation.

Activity 9. Determine some aspects of the photosynthesis in cassava wild species

Cassava endures several months of water limitation during its seasonal cycle (Cock *et al.*, 1985; Yao *et al.*, 1988; El-Sharkawy *et al.*, 1992; El-Sharkawy, 1993). It resists drought by reducing its evaporative surface. In addition, water use efficiency is enhanced through stomata closure (Cock

et al., 1985; Chaves & Pereira, 1992; El-Sharkawy *et al.*, 1992; El-Sharkawy, 1993). These mechanisms of stress avoidance are not unusual, and are extremely effective in this species. In cassava, the photosynthetic CO_2 assimilation pathway has been a matter of debate (Angelov *et al.*, 1993). In early work, cassava was classified as a C3-C4 intermediate (El-Sharkawy & Cock, 1987). Later, it was demonstrated that the plant displayed C3 photosynthesis (Edwards *et al.*, 1990). Nevertheless, leaves of *Manihot* species have distinct green bundle sheath cells which are unusual in C3 leaves and they show particular photosynthesis characteristics (Angelov *et al.*, 1993).

It is suggested in the literature that C4 plants are evoluted from C3 plants (Edwards & Ku, 1987). Then, this evolution occurred through a number of steps, since the modifications in biochemistry and anatomy associated with C4 photosynthesis are relatively complex. It now appears more likely that we are seeing evolution in progress; that is, at least some intermediates originate from C3 species and are at various stages in the process of becoming C4 plants (Hatch & Boardman, 1987). It could be possible that we are seeing this evolution with *Manihot* species. Our purpose in this study was to determine the photosynthesis (C3, C4 or C3-C4 intermediate) in cassava wild species availables at CIAT.

Twenty six different species of *Manihot* were used and obtained by *in vitro* culture (except for *M. crassisepala* and *M. grahami* obtained from cutting materials). After 4 months of culture at the same growth conditions and without water limitation, the plants were used. Young leaves (2 weeks old) were collected for ^{13}C and enzyme activity analysis.

It is well known that both C3 and C4 plants discriminate against $^{13}CO_2$ during photosynthesis, but C3 plants discriminate against $^{13}CO_2$ to a larger extent, and thus have a more negative $\delta^{13}C$ value relative to C4 plants. Typically, the $\delta^{13}C$ values in C3 plants range from -25 to -30‰ and in C4 plants from -11 to -16‰ **(Table 9.1)** (Edwards & Ku, 1987). The values of $\delta^{13}C$ obtained on various cassava wild species, ranging from -23.7 to -33.6‰, indicate that they displayed more a C3 photosynthesis.

An analysis of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)(enzyme implied in the fixation of CO_2 into 3 carbons), phospho*enol*pyruvate carboxylase (PEP Case)(enzyme implied in the fixation of CO_2 into 4 carbons) and phosphoglycolate phosphatase (enzyme initiating the photorespiration) activities in young leaves confirmed the fact that all cassava genotypes studied displayed more a C3 photosynthesis (data not shown). Nevertheless, for *M. crassisepala* and *M. aesculifolia*, the lowest values of $\delta^{13}C$ and the no detection of phosphoglycolate phosphatase activity found in *M. aesculifolia* leaves suggest that they could display an intermediate C3-C4 photosynthesis. This hypothesis is presently in course by anatomic observations.

Table 9.1. Natural isotope compositions (δ13C) of young leaves of 26 cassava wild species (mean from three leaves) and leaves of Arachis pintoi (C3 plant) and Brachiaria dictyoneura (C4 plant).

Plant species (genotype number)	$\delta^{13}C$ (‰)
M. aesculifolia (1)	-24.8
M. alutacea (11)	-31.3
M. anomala (3)	-30.9
M. brachyloba (1)	-26.9
M. caerulescens (13)	-31.6
M. carthaginensis (297)	-30.7
M. cecropiaefolia (3)	-30.8
M. chlorosticta (10)	-27.2
M. crassisepala (4)	-23.7
M. epruinosa (1)	-29.8
M. flabellifolia (36)	-29.3
M. fructiculosa (6)	-30.0
M. grahami (3)	-25.2
M. guaranitica (6)	-31.2
M. hastatiloba (2)	-33.6
M. irwinii (6)	-30.7
M. jacobinensis (9)	-31.2
M. longepetiolata (5)	-32.4
M. orbicularis (18)	-30.8
M. pseudoglaziovii (1)	-32.2
M. purpureo-costata (4)	-29.1
M. rubricaulis (19)	-31.9
M. sparsifolia (4)	-30.1
M. triphylla (21)	-32.2
M. tristis (12)	-29.6
M. violacea (3)	-31.5
Arachis pintoi (C3)	-28.3
Brachiaria dictyoneura (C4)	-12.1

Activity 10. Determine alternative mealybug control methods other than biological control

Introduction

At present, proteinase inhibitors, lectins and alpha-amylase inhibitors are the three classes of proteins considered to have the potential to function as chemical defensive factors in plant against attacking insect. These proteins should be considered as important factors in plant-insect interactions when developing host plant resistance programs. In fact, such proteins representing direct gene products could most directly lend themselves to genetic engineering manipulations for crop improvement.

Our objective in this sub-project is to identify some "toxic" proteins to *P. herreni*.

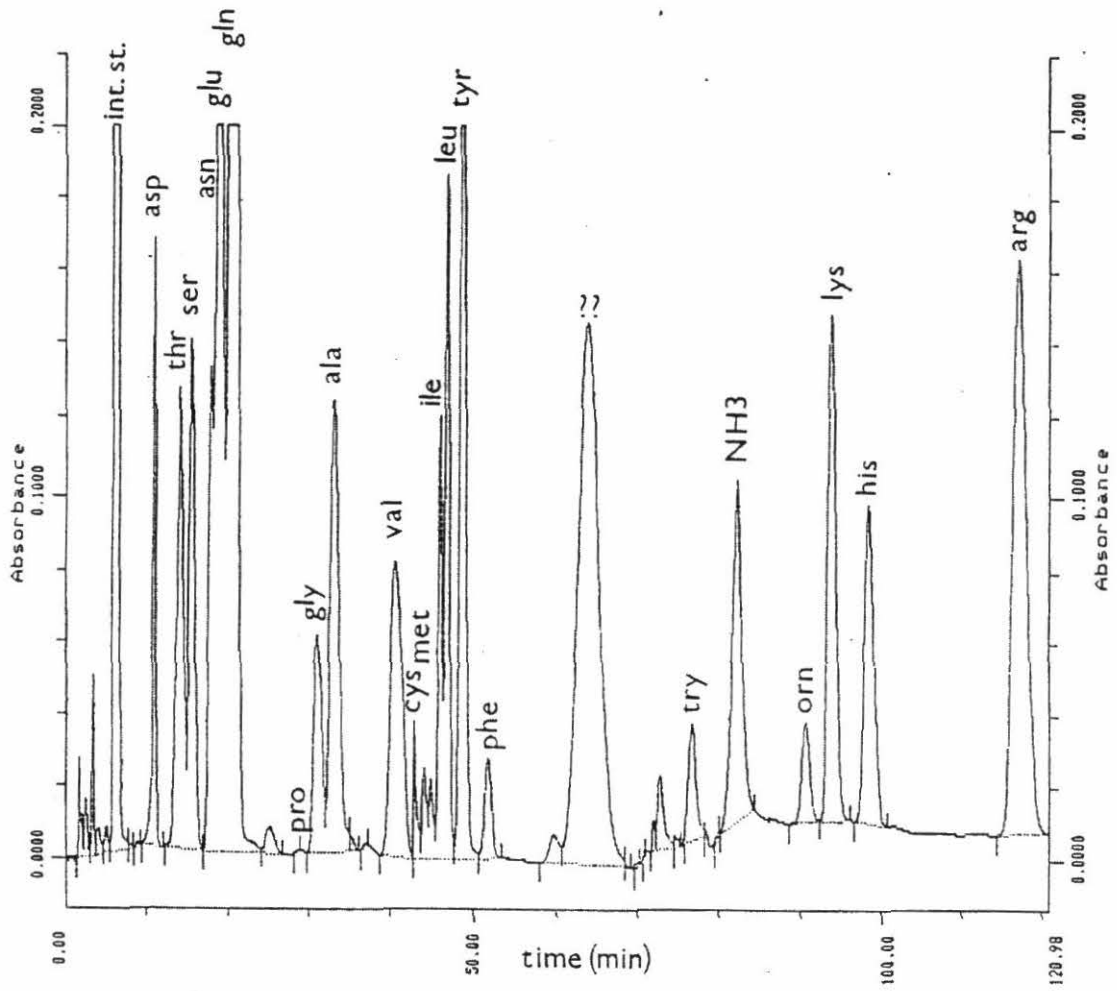
Activity 11. Compound detection implied in mealybug physiology: A new phenolic compound detected in *Phenacoccus herreni*

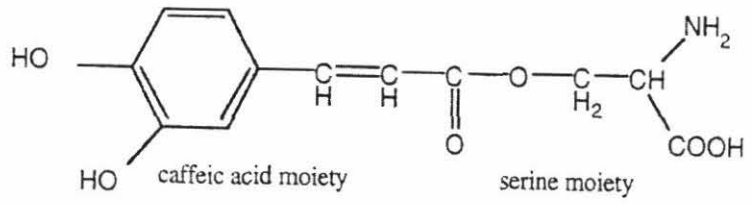
Free amino acid analysis of each developmental stage of *P. herreni* reared on cassava, revealed the presence of an unknown compound with a retention time of 64 min appearing after tyrosine and phenylalanine in the chromatogram **(Figure 11.1)**. In our chromatographic conditions, its elution time was approximately that of a standard ß amino-butyric acid. Setting up its colorability as a standard α-amino acid, its concentration in nmol/mg of fresh weight was estimated through the development of the insect : it varied significantly, from 26.4 in the eggs, to 14.8 in neonates, 4.4 in second instars (including both males and females), 4.9 in females at third instars, 9.6 in female adults and 14.1 in male adults ($p<0.0001$ of ANOVA). It was also detected in adult female mealybugs reared on an holidic diet during 35 days, at 9.5 nmol/mg of fresh weight. It was detected in other mealybugs species like *P. manihoti*, *P. madeirensis*, *Planococcus citri* and *Icerya* sp., but was not detected in *Rastrococcus invadens*, *Pseudococcus jacbeardsleyi* and *Pseudococcus longispinus*. Furthermore, this compound was not detected in free amino acid extracts of host plant leaves (*M. esculenta*).

These results suggest that this compound is not provided directly by the host plant, but rather that it is synthetized by the insect itself, or with the help of its symbiotic bacteria. The identification of its precursor(s) is presently under study. Furthermore, this unknown compound could not provide "good" taxonomic character at the family level for the Pseudococcidae.

This compound disappeared in samples purified from cassava-reared *P. herreni* after acid hydrolysis, and the presence of serine was revealed (from its chromatographic behaviour in column or TLC systems, and from its colourability ratio at the two wavelength channels used in autoanalysis). A purification of the compound and its partial identification by NMR showed that it consisted also of a caffeic acid moiety (**Figure 11.1**), ^{1}HNMR (D_2O)(200 MHz): δ7.72 (d, 1H, *J*=15.9 Hz, CHCOOH), 7.24-7.46 (3H, m, aromatic), 6.42 (d, 1H, *J*=15.9 Hz, olefinic H)). The ninhydrine positivity of this compound indicates that the NH_2 group of serine is free, and thus that this amino acid could be linked either by its carboxylic function to one of the two OH groups of the aromatic nucleus of caffeic acid, or more probably by its own hydroxyl group to constitute the O-caffeoylserine. The confirmation of this link is being investigated.

Although numerous caffeic acid esters occur in plants, O-caffeoylserine has not been reported in the literature. Nevertheless, a caffeic acid ester was already found in an other Homoptera. O-caffeoyltyrosine, the ester of caffeic acid and tyrosine, was identified in the California red scale *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae)(Millar & Hare, 1993; Hare *et al.*, 1993). The concentration of this ester varies according the developmental stages of *A. aurantii*.





putative O-caffeoylserine (??)

Figure 11.1. Typical chromatogram obtained after autoanalysis of free amino acids of cassava mealybug. Int. st.: internal standard (glucosaminic acid); ??: putative O-caffeoylserine.

The physiological role of our compound in the cassava mealybug is being studied. As suggested by Hare *et al.* (1993) for O-caffeoyltyrosine, the caffeic acid moiety could be a precursor of the quinone compound involved in cuticle sclerotization and tanning. Non tyrosine precursors of sclerotizing agents was hypothesized for explaining the incorporation of plant phenolics in the cuticle of a locust (Bernays *et al.*, 1983), but no firm evidence has since been given to our knowledge. The serine moiety could be precursor of bristle (serine polymer) constitutive of the ovisacs.

If we confirm these physiological roles of our putative ester, it could be possible that the phenylalanine present in the phloem sap is the precursor of the caffeic acid moiety and thus *P. herreni* possess an enzyme for the synthesis of this compound such as phenylalanine ammonia-lyase (PAL), commonly involved in plants in the synthesis of phenolic compounds. Consequently, a PAL inhibitor and/or a L-serine arylamidase inhibitor (according to the putative role of the serine moiety) should perturb the physiology of *P. herreni* and its development.

Activity 12. Identification of host plant resistant to *Phenacoccus herreni*

The objective of this study was to identify a high level of plant resistance to *P. herreni* focused on antibiosis (*ie* affecting the insect nutrition or toxic to the insect). It is well known that no cassava varieties was found to be resistant to *P. herreni*. Then, some cassava wild species studied in the Table 9.1 were used in this study. Five-months-old plants were used and two young leaves were infested by 100-200 neonates of *P. herreni* newly hatched. After 48 hours of infestation, for each leaf the number of surviving insects were counted. At day 15 after the infestation, the number of surviving insects were recorded and the proportion of mortality was calculated.

The percentage of mortality for all cassava species range from 3.3 to 14.3% indicating that no wild cassava species used in this study showed high level of resistance to *P. herreni* **(Table 12.1)**. Some species like *M. longepetiolata* and *M. hastatiloba* showed higher mortality but due to the fact that some infested leaves dried up as a result of a reaction to mealybug infestation which induce a water stress phenomenon (data not shown for *M. hastatiloba)*. Nevertheless, these species are not considered resistant because on the live leaves the highest insect mortality was only 13.9%.

In the same experiment, we used another plant species which the common name of PIAO ROXO. It is a *Jatropha* sp. (species not yet determined) (Euphorbiaceae). This species showed a strong toxicity to *P. herreni*. In fact, after 24 hours of infestation this plant showed 95% mortality and after 48 hours, 100% mortality. We established with additional experiments and observations that this mortality is mainly due to a toxic effect probably located in mesophyll cells (data not shown). It is well known in the literature that *Jatropha* species possess toxic protein curcin (Morton, 1981).

Table 12.1. Proportion of mortality (mean ± standard error, mean from three plants) obtained on 26 cassava wild species and on *Jatropha* sp.

Plant species (genotype number)	% Mortality
M. aesculifolia (1)	10.9 ± 1.3
M. alutacea (11)	12.8 ± 1.1
M. anomala (3)	9.0 ± 0.6
M. brachyloba (1)	5.5 ± 1.3
M. caerulescens (13)	7.7 ± 1.1
M. carthaginensis (297)	10.3 ± 2.3
M. cecropiaefolia (3)	3.3 ± 0.9
M. chlorosticta (10)	5.5 ± 1.6
M. crassisepala (4)	12.3 ± 1.9
M. epruinosa (1)	12.3 ± 0.7
M. flabellifolia (36)	11.6 ± 2.7
M. fructiculosa (6)	12.3 ± 2.2
M. grahami (3)	10.2 ± 1.3
M. guaranitica (6)	14.0 ± 1.2
M. irwinii (6)	14.3 ± 1.2
M. jacobinensis (9)	11.4 ± 2.4
***M. longepetiolata* (5)**	**13.9 ± 1.9**
M. orbicularis (18)	9.1 ± 0.3
M. pseudoglaziovii (1)	12.5± 1.0
M. purpureo-costata (4)	9.7 ± 0.9
M. rubricaulis (19)	5.1 ± 1.9
M. sparsifolia (4)	3.8 ± 0.8
M. triphylla (21)	8.8 ± 0.5
M. tristis (12)	10.9 ± 0.9
M. violacea (3)	3.8 ± 0.8
Jatropha sp.	100

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Sub-output 3. Biological Control and Plant Interactions of Cassava Mealybugs Reducing Populations.

Activity 13. Ecological cultivation of cassava: To render natural enemies of sucking pests more effective and reliable

Reducing populations of the cassava mealybug *Phenacoccous herreni* in Latin America relies on the biological control with natural enemies as cassava germplasm with adequate resistance to this sucking pest is not available. Two encyrtid parasitoids *Aenasius vexans* and *Acerophagus coccois* have been mass-reared and released. Often mass-reared natural enemies lose behavioural traits related to successful host searching and host location. However, efficient host searching capabilities are important elements in the assessment of natural enemies as biological control agents.

While searching for hosts many natural enemies respond to volatile chemicals released by the plant upon which the host is feeding, the host itself or chemicals released as a result of interaction between the host and its food plant. Experience with host or host related products can render natural enemies more responsive to certain host/plant derived odours and result in improved host handling skills of a host selecting female. Furthermore they are able to learn these cues. Consequently experienced natural enemies find their hosts better and faster thus increasing paratism success. Enhancing this behavioural trait of insect learning by exposing parasitoids in the pre-release phase to host related cues may be one possibility to render biological control organism more effective and reliable.

The parasitoids degree of dietary specialisation is hypothesised to influence the behavioural variability. Parasitoids appear to use different host searching strategies according to their degree of dietary specialisation. The behaviour of generalists is supposed to be more flexible and affected by learning than that of specialists. It is hypothesised that learning in generalist parasitoids is more important than in specialist parasitoids as they deal with greater host and environmental variation. Generalists are expected to learn stimuli of host and environmental characteristics where they successfully encountered hosts 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.

A.vexans is a specialist on *P. herreni* whereas *A. coccois* is a generalist on different mealybug species. In a comparative cage experiment the learning abilities of two encyrtid parasitoids of cassava mealybug, *A. vexans* and *A. coccois* are being assessed. Naïve and experienced females are released in the cage containing a mealybug infested cassava plant. In order to obtain experienced females, the wasps were allowed to walk over a mealybug infested cassava leaf. Mummy formation was assessed.

Preliminary results indicate that naïve and experience wasps differed in their parasitisation success. Percentage parasitism of naïve *A. vexans* females and 2.5 times lower than of experienced females. Naïve *A. coccois* females parasitizes 3.5 times less mealybugs than

experienced ones. Parasitism of *A. coccois* was lower than of *A. vexans* regardless of parasitoid treatment **(Table 13.1)**.

Table 13.1. Percentage parasitisation of naïve and experienced *Aenasius vexans* and *Acerophagus coccois* in a cage bioassay.

Parasitoid	Mealybugs alive	Mummies	Total
A. vexans naïve	165	32 (16.2%)	197
A. vexans experienced	89	57 (39%)	146
A. coccois naïve	183	8 (4.2%)	191
A. coccois experienced	137	28 (17%)	165

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Sub-output 4. Diseases Complexes Described, Characterized and Analyzed.

Activity 14. Genetic characterization and induction of resistance to *Sphaerotheca pannosa*, the causal agent of powdery mildew in rose in Colombia

Introduction

Rosa sp. is an extremely popular flowering ornamental, and is an important export product for Colombia, Ecuador, Costa Rica, Kenya, and other developing countries. This plant is attacked by several genera of fungi that cause powdery mildew, an economically major disease because its control is costly. The most common agent is *Sphaerotheca pannosa* var. *rosae* Wall. Fr. The infection reduces the flowers' vigor and lowers their esthetic value. This obligate parasite is difficult to study, with the result that no information exists on the presence of races. In the first phase of this project, we characterized the pathogen genetically with molecular tools, then developed rapid tests for pathogenicity. The aim was to provide valuable information toward developing more efficient and durable control practices.

Activity 15. Establishing a collection of isolates, and developing a PCR technique to detect and characterize *Sphaerotheca pannosa* var. *rosae*

Twenty-six isolates of *Sphaerotheca pannosa* var. *rosae* were collected from the Sabana de Bogotá (Cundinamarca), Palmira, and Dagua (Valle del Cauca) in Colombia **(Table 15.1)**. A quick DNA extraction procedure was adopted, following the protocol described by

Williams and Walsh. Several hundred conidia were added to 50 µL of 5% Chelex resin (BioRad Laboratory, Hercules, CA) in a 1.5-mL microcentrifuge tube and incubated at 56°C for 2 h. After mixing vigorously, the extract was incubated at 96°C for 8 min. The supernatant was transferred to another tube and used as DNA template.

The regions ITS-1 and ITS-2, and the 5.8 rRNA gene of the ribosomal DNA were amplified by PCR. Before amplification, bovine serum albumin (BSA) was added to the mixture, which reduced inhibition effects occurring during amplification. The PCR product was subjected to electrophoresis in 1.5% agarose gel in TBE 0.5X buffer (TBE = Tris borate EDTA). All isolates produced a DNA product of 290 bp. This result confirms that the PCR is an accurate technique and highly valuable for detecting *S. pannosa*.

We used RAPD markers to study the genetic diversity of 26 isolates of *S. pannosa* obtained from rose plants infected by powdery mildew in Colombia. A group of 18 oligonucleotides from Operon Technologies Inc. (Alameda, CA) were tested **(Table 15.2)**. Of each isolate, 5 ng of genomic DNA was used and the amplified products were loaded on 1.5 % agarose gels, containing 0.5 mg/mL of ethidium bromide. Eight primers yielded clear, intensive, reproducible, and polymorphic patterns. These primers suggest an intermediate level of polymorphism among the isolates **(Figure 15.1)**.

Table 15.1. Isolates of *Sphaerotheca pannosa* var. *rosae* used in the present study to detect andcharacterize this fungus with the aim of controlling it.

Isolate	Origin (site, municipality, department)	Source
Sp-1	Los Samanes, Palmira, Valle del Cauca	Leaf
Sp-2	Los Samanes	Leaf underside
Sp-3	Los Samanes	Leaf bud
Sp-4	Los Samanes	Leaf underside
Sp-5, Sp-7	La Zapata, Palmira, Valle del Cauca	Leaf underside
Sp-6, Sp-8	La Zapata	Stem
Sp-9, Sp-10	Km. 30, Dagua, Valle del Cauca	Bud
Sp-11, Sp-12	Km. 30	Stem
Sp-13, Sp-14, Sp-15, Sp-16	Km. 30	Leaf bud
Sp-19, Sp-20, Sp-21, Sp-22, Sp-23, Sp-24, Sp-25, Sp-26	Sabana de Bogotá, Cundinamarca	Leaf bud

Table 15.2. Nucleotide sequences of the 18 primers used in this study. The total number of RAPD bands and the number of polymorphic DNA fragments for each primer are presented.

Primer	Nucleotide Sequence	Scored bands (no.)	Polymorphic bands (no.)	Primer	Nucleotide Sequence	Scored bands (no.)	Polymor-Phic Bands (no.)
OPA-01	CAGGCCCTTC	8	3	OPH-02	TCGGACGTGA	17	9
OPA-04	AATCGGGCTG	18	7	OPH-04	GGAAGTCGCC	14	8
OPA-08	GGGTCACGCC	8	3	OPH-05	AGTCGTCCCC	12	7
OPA-18	AGGTGACCGT	16	7	OPH-07	CTGCATCGTG	10	4
OPB-08	GTCCACACGG	11	6	OPH-19	CTGACCAGCC	14	7
OPO-09	TTCGAGCCAG	13	5	OPF-01	ACGGATCCTG	13	6
OPC-08	TGGACCGGTG	15	6	OPN-01	CTCACGTTGG	11	5
OPA-02	AGTCAGCCAC	13	8	OPN-10	ACAACTGGGG	5	0
OPA-13	CAGCACCCAC	16	7	OPN-17	TGACCCGCCT	6	0

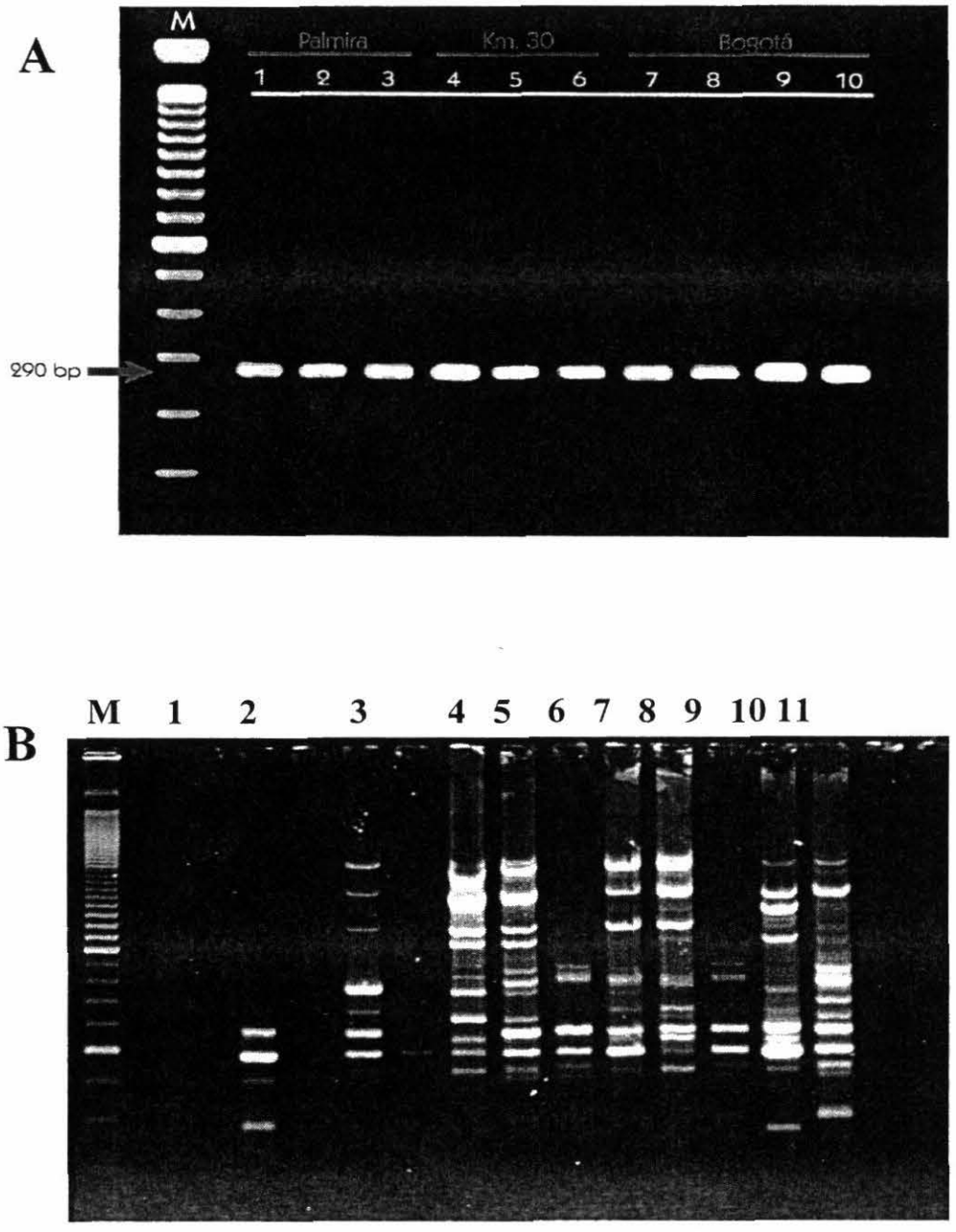

Figure 15.1. A: Direct PCR amplification of the ITS region. Lane M= 100bp ladder, lanes 1-10= *Sphaeroteca pannosa* isolates. B: Variation among *S. pannosa* isolates detected with primer OPH-02. Lane M= 100bp ladder, lane 1= H_2O control; lanes 2-11= *S. pannosa* isolates.

Activity 16. Rapid pathogenicity test developed

Tests were developed to evaluate the pathogenicity and virulence of *S. pannosa* isolates. Rose plants, placed in a growth chamber, were successfully infected by *S. pannosa*. An isolate of this pathogen, collected from diseased rose plants from the plains of Santafé de Bogotá, produced colonies within 10 days, on leaves of different rose cultivars. Inoculation was by a small fan, which blew the conidia from the inoculum source to the leaves. Several factors were important in obtaining these results: before being inoculated, plants propagated in the screenhouse at CIAT, Palmira, had to be climatized for 2 days to the low light and temperature of the growth chamber. The leaves to be inoculated had to be carefully rinsed with deionized water to remove possible contaminants. Inocula had to be fresh (no more than 4 days old). The presence and viability of the conidia of the inocula had to be monitored daily to ensure that environmental conditions were optimal. To guarantee successful infection, relative humidity during the first 48 h had to be 100%, then between 60% and 75%. Temperatures had to vary between 20°C in the day and 16°C at night.

Similar results were obtained for the *in vitro* evaluation, for which detached rose leaves were inoculated. To maintain the leaves fresh for 2 weeks, the petioles were placed in a 0.005% sucrose solution. This solution was changed twice a week. Incubation conditions were similar to those described above. This method will be used to evaluate the effectiveness of biocontrol agents on the Colombian isolates of the pathogen. Virulence of isolates will also be scored.

During the first evaluation, the cultivars Aalsmeer Gold, Libia, and a local cultivar were susceptible (white colonies grew on several leaves of each plant). 'Bryer', 'Classy', and 'Confetti' did not become infected. Young leaves were more susceptible than older ones **(Table 16.1)**.

Table 16.1. Results obtained after inoculating rose cultivars with different grades of field resistance to powdery mildew.

Cultivar	Disease incidence (% of plants infected)	Severity (No. of colonies per plant)
Bryer	0	0
Classy	0	0
Confetti	0	0
Charlotte	14	24
Tineke	20	6
Libia	25	17
Aalsmeer Gold	67	29
Unknown cultivar	100	28
Average	28	13

Activity 17. Characterizing Bud-Rot-causing pathogens in oil palm: Genetic analysis of the *Ceratocystis paradoxa* complex in oil palm

Ceratocystis paradoxa is one of the causal agents of the bud rot complex in oil palm. This disease is the major production constraint in Colombia, Brazil, and Central America, causing economic losses of US$140 million per year. A species of *Thielaviopsis* was found associated with the disease. This genus, now regarded as synonymous with *Chalara*, largely comprises asexual states of *Ceratocystis* species. In this study, isolates obtained from oil palm grown in Colombia, Ecuador, and Brazil were compared, using morphology, phylogenic analysis, asexual mating systems, and pathogenicity.

The species primarily responsible for the disease in oil palm is *C. paradoxa sensu stricto*. This species forms thick-walled, pigmented conidia (chlamydospores or aleuriospores), arranged in chains on top of conidiophores. Spore walls are smooth. Crossing among 168 isolates revealed two mating types (arbitrarily assigned MAT 1 and MAT 2), and resulted in perithecia and ascospores that met descriptions of *C. paradoxa*.

Self sterile.One isolate (C1021) differed substantially from *C. paradoxa* in its ITS sequence of the ribosomal DNA. It had a cucumber-like smell, was self-fertile, and produced rough-walled (not smooth-walled) chlamydospores. This species is thus clearly distinct from *C. paradoxa* and should be described as new. Its ITS sequence and morphology suggest that this species is intermediate between *C. paradoxa* and *C. radicicola*, and is closely related to isolate C914, which was obtained from coconut in Brazil. Progeny of a selfing of a C1021 isolate, obtained from oil palm grown in the Department of Magdalena, Colombia, were all self-fertile.

Morphological measurements were done for eight *Ceratocystis* isolates to describe the isolates C1021 and C914 as possibly belonging to a new species. These two isolates were also compared with *C. musarum* isolates from banana, and with the *C. paradoxa sensu stricto* isolates C1107 and C1502 and a cross between them. The isolates were grown on carrot agar, modified by thiamine, and incubated at room temperature (22°C) for 6 days. Perithecia of the isolates C1021, C914, and C1107 × C1502 were measured, using a microscope (20 ×), 5 days after crossing. Fifty *Chalara* conidia (40 ×), 50 chlamydospores (40 ×), 25 conidiophores and their phialides (40 ×), the base and neck of 25 perithecia (40 ×), 4 appendages of the perithecial base for each perithecium (40 ×), 4 ostyole hyphae for each perithecium (20 ×), and 100 ascospores (100 ×) were measured. The chlamydospore wall was described as either rough or smooth **(Table 17.1)**.

Table 17.1. Morphological measurements of different structures of *Ceratocystis* isolates.

	Isolates					
	Undescribed species		*C. musarum*[a]	C. paradoxa sensu stricto		
Parameters (μm)	**C1021**	**C914**		**C1107**	**C1502**	**C1107 × C1502[b]**
Chalara conidia						
Length	10.2	11.7	11.2	9.6	10.8	9.8
Hyaline length	No	7.0	8.0	7.0	8.0	8.5
Chlamydospores						
Length	14.0	14.3	14.9	18.0	15.3	16.3
Width	11.5	11.0	9.8	10.7	11.3	10.3
Wall[c]	R	R	S	R	R	R
Conidiophore Length	57.9	31.4	26.1	39.1	46.4	24.6
Conidiophores						
Septae	1.5	1.7	3.6	2.2	2.6	3.2
Base width	7.6	7.7	9.7	7.9	8.3	7.7
Length	71.5	76.9	193.5	175.1	120.7	188.0
Phialides						
Base width	6.9	7.0	7.5	6.3	6.5	6.0
Tip width	4.3	4.3	4.1	4.1	3.7	3.9
Length	47.9	62.2	111.6	107.0	78.7	99.0
Perithecia						
Base length	281.4	273.0	-[d]	-	-	292.6
Base width	282.7	276.0	-	-	-	294.2
Appendages	20.9	21.1	-	-	-	22.1
Base neck width	49.8	53.2	-	-	-	57.6
Tip neck width	23.9	29.3	-	-	-	29.7
Neck length	1278.4	1215.0	-	-	-	797.4
Ostyole hyphae Length	99.4	106.3	-	-	-	115.5
Ascospores						
Cell length	7.6	7.8	-	-	-	7.9
Cell width	2.5	2.8	-	-	-	2.6

a. Averages of isolates C915, C1510, and C1511.
b. Fertile cross.
c. R = rough; S = smooth.
d. Self sterile

Hyaline conidia were not present in the C1021 isolate. No differences in size were found among conidia of *Chalara elegans* isolates. The undescribed species (isolates C1021 and C914) had shorter conidiophores and phialides than the others. Chlamydospores were longer in the C1107 isolate. The perithecial neck of the C1021 and C914 isolates were longer than the perithecial neck of the *C. paradoxa sensu stricto* fertile cross.

Twenty-one *Ceratocystis* isolates from different crops were grown in malt extract agar medium (malt 1.5%, agar 2%), incubated for 7 days, and inoculated on banana fruit, pineapple fruit, carrot, cassava, and *Colocasia* sp. to evaluate their pathogenicity. Isolates from banana (C915, C1480, C1510, and C1511) were less pathogenic on banana fruit substrate than were isolates from oil palm (C1090), peach palm (C1492), and wheat (C1512). The isolates C1090, C1454, and C1512 were the most pathogenic on pineapple substrate. Isolates showed low pathogenicity on *Colocasia* sp. and carrot, although C1503 and C1003 were most pathogenic on *Colocasia* sp., while C870 and C1395 were most pathogenic on carrot **(Table 17.2)**. No isolate was pathogenic on fragments of cassava root.A growth rate × temperature experiment was conducted with 12 *C. paradoxa,* 4 *C. musarum*, and 1 *C. fimbriata* isolates to distinguish between the species. Three replications per isolate were grown on malt extract agar (1.5% malt extract, 2% agar) at 22°C (room temperature) and at 32°C. The radius of the growing mycelia was measured after 3 days **(Table 17.3)**. *Ceratocystis musarum* did not grow well at 32°C, but no differences were found between *C. paradoxa* and *C. fimbriata*.

Table 17.2. **Pathogenicity of 20 *Ceratocystis* isolates, inoculated on banana fruit, pineapple fruit, *Colocasia* sp., and carrot fragments.**

Species Isolate	Banana			Pineapple (infected tissue, %)	*Colocasia* sp. (lesion size in cm)[a]	Carrot (lesion size in cm)
	Skin (lesion size in cm)	Peeled fruit				
		Infected tissue (%)	Lesion size (cm)			
C. radicicola						
C869	1.95	30.9	6.6	0	-	0
C870	5.0	35.8	9.0	73.3	0.3	2.3
Unknown						
C914	3.1	37.5	7.0	66.7	0	0.4
C1021	2.7	51.7	9.6	55	0	0.3
C. fimbriata						
C1003	5.2	60.9	9.1	95	2.2	1.2
C. paradoxa						
C915	3.15	21.7	5.9	0	0	0
C1001	3.8	59.2	10.2	56.7	-	0
C1002	1.6	7.5	4.8	6.7	0	0
C1090	8.3	86.7	14.3	100	-	0
C1107	3.7	61.7	11.0	75	0.5	0
C1395	3.8	46.7	8.8	40	0.3	1.5
C1454	5.6	60.9	9.2	100	0.2	0.6
C1479	2.6	21.7	5.7	21.7	0	0
C1480	2,6	10.0	2.9	0	0.3	0
C1481	4.3	41.7	10.6	81.7	0.93	1.2
C1492	6.1	66.7	11.2	71.7	0	0
C1503	5.5	66.5	8.9	95	2.4	0.4
C1510	3.6	23.4	4.9	6.7	0	0.4
C1511	4.9	33.3	8.2	13.3	0	0
C1512	6.0	75	12.6	100	0.27	0
Control	0.2	4.2	1.1	0	0	0

a. - = Not determined.

Table 17.3. **Growth of *Ceratocystis* spp. *in vitro* at two different temperatures. Observations were made 3 days after placing the fungus on malt extract agar medium.**

Species Isolate	Host	Origin	Incubation temperature 22°C Radius (mm)	Incubation temperature 32°C Radius (mm)
C. musarum				
C915	Banana	Brazil	27.8	2.8
C1480	Banana	Honduras	17.0	1.0
C1510	Banana	Ecuador	23.4	3.3
C1511	Banana	New Zealand	17.6	1.6
C. fimbriata				
C1003	Date palm	Spain	24.6	24.3
C. paradoxa				
C1481	Date palm	Israel	21.2	8.0
C1002	Air	Brazil	14.1	13.3
C1395	Unknown	Japan	23.0	22.2
C1492	Peach palm	Costa Rica	26.7	24.1
C1001	Pineapple	Brazil	19.7	19.3
C1107	Oil palm	Colombia	14.4	17.8
C1094	Oil palm	Colombia	23.2	20.3
C1090	Oil palm	Ecuador	27.4	28.6
C1164	Oil palm	Ecuador	22.2	23.4
C1457	Oil palm	Brazil	25.7	27.3
C1458	Oil palm	Brazil	25.8	27.4
C1479	Sugarcane	USA	21.3	27.1

Activity 18. Establishing the pathogenic and genetic variation of *Phytophthora* spp., the causal agent of bud rot in oil palm in Colombia, Ecuador, and Brazil

Several fungi have been considered to be causal agents of this disease. The Cassava Pathology Section was the first to isolate *Phytophthora* spp. from infected oil palms, using techniques that had been developed for cassava research.

We obtained 28 isolates from the most important oil-palm-producing regions in Colombia, Ecuador, and Brazil by using a direct planting method and techniques in which fragments of carrots and green apples were used as baits. That all isolates belonged to the *Phytophthora* genus was confirmed by using the genus-specific immunoassay detection kit (Agri-Screen®, Lansing, MI), conducting a PCR of the ITS 1-4 region, and hybridizing with a *Phytophthora*-specific probe. We examined the potential of the PCR and RAPD techniques to generate molecular markers that would differentiate *Phytophthora* isolates from different geographic regions.

We also conducted a pathogenicity study, and characterized all the isolates according to morphology. Characterizing by morphology was difficult because not all isolates produced fungal structures. By PCR the amplified product of the ITS region was about 900 bp. According to the RAPD analysis, the Colombian *Phytophthora* populations are highly diverse and the isolates group into four main phylogenetic clusters **(Figure 18.1)**. The RFLP analysis was also conducted, using the enzymes *Msp*I, *Alu*I, *Taq*I, and *Hind*I. The results showed high genetic diversity **(Figure 18.2)**.

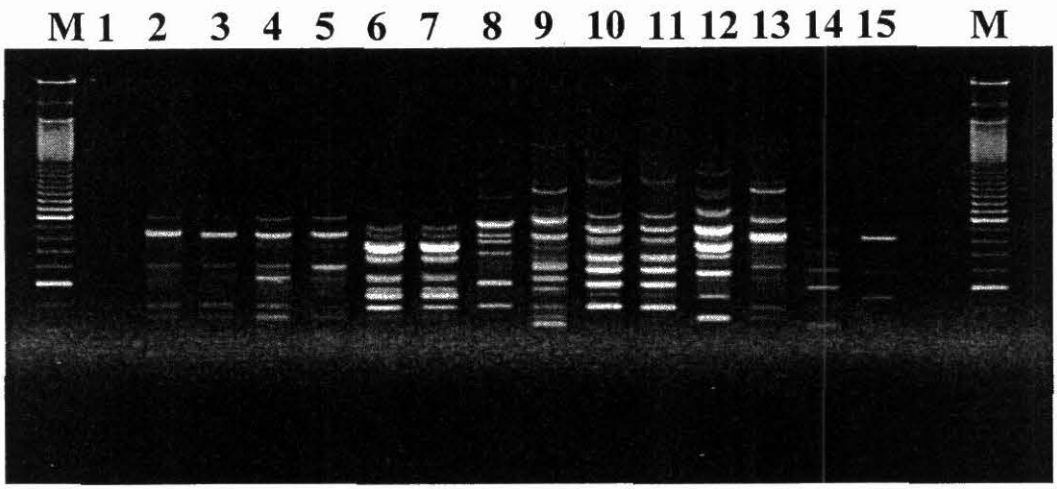

Figure 18.1. **Detected variation (using primer OPH-02) among isolates of Phytophthora spp. obtained from oil palm. Lane M = 100 bp marker; Lane 1 = water control. Lanes 1 to 15 = Phytophthora isolates.**

At CIAT, the disease was successfully reproduced by inoculating young palms with *Phytophthora*. That is, 4-month-old palms of the cultivar Camerún were inoculated with eight isolates from Villavicencio. Three different methods were used: (1) placing a fungal plug on a small wound made with a bistoury on the spear, or first leaves if the spear was still too small; (2) the same as 1, but adding sterile distilled water to the inoculum at the time of inoculation; and (3) puncturing the bud and placing a fungal plug on the wound. The age of the inocula varied. After 30 days, cultures presented oospores and oogonia (isolates CPP22, CPP28, CPP29, CPP30, and V/VP22), while cultures of 15 days possessed sporangia (isolate 14). Isolates V/VP13 and 3Fe produced only hyphae. After 6 days, plants inoculated with V/VP13, 3Fe, and CPP22 showed necrosis of the inoculated spear tissue, but only mycelium was found. The isolate 3Fe was more aggressive than the other two isolates. After 10 days, the third inoculation method had caused necrosis, with V/VP13 causing a soft rot of bud tissue, characterized by a pungent odor. The other isolates did not produce symptoms, probably because they lost pathogenicity by cultivation *in vitro*.

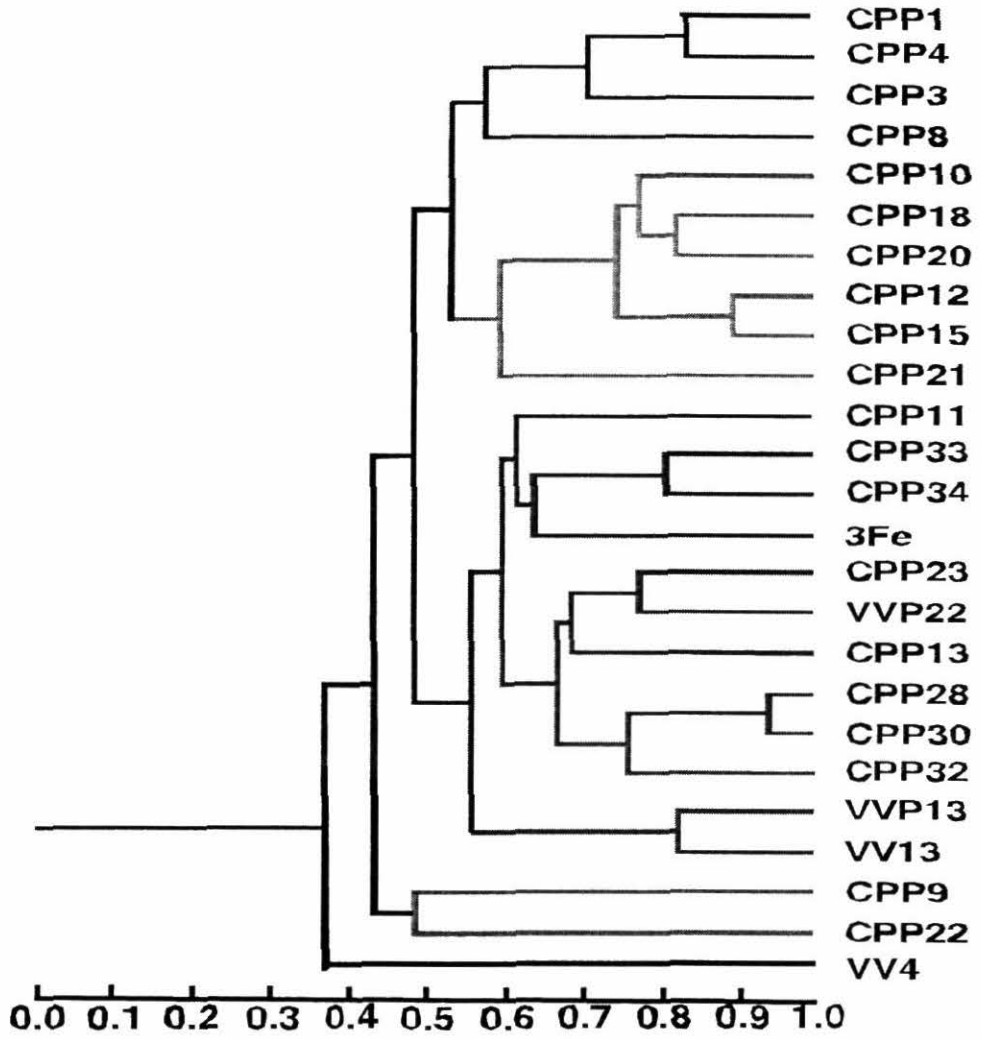

Figure 18.2. Phenogram of *Phytophthora* spp. isolated from oil palm, and corresponding to a hierarchical cluster analysis of data. Clusters were fused, using the unpaired group mean average. The similarity scale shown corresponds to the average similarity at which clusters were fused.

Zoospores are considered to be the most infectious propagule of the many diseases caused by *Phytophthora* spp. Different solutions were therefore compared for sporangia. Abundant formation of sporangia for almost all isolates was obtained by using a sterile soil extract (100 g soil from CIAT, Palmira, and distilled water, making a total volume of 1 L) and continuous light for 8 days. Isolates V/V13 and 3Fe produced only small quantities of sporangia. Experiments will be carried out to inoculate palms with zoospores.

To understand the source of genetic variability of virulence for *Phytophthora*, that is, the production of new pathotypes, all isolates were evaluated for their capacity to produce oospores. Although isolates were tested on different culture media (including V8 agar = 2% V8 and 2% agar; and oatmeal agar = 2% oatmeal and 2% agar), most isolates produced abundant oospores after 10 days on carrot agar medium with thiamine at 29°C. The isolates CPP14, V/VP13, and 3Fe did not produce oospores and were therefore regarded as heterothallic. A few isolates produced sporangia (e.g., isolate CPP14). Oospore production was not achieved in liquid media, whether based on oatmeal broth, V8 juice broth, or carrot broth. Pairing of the three heterothallic isolates or dual cultivation with *Thielaviopsis paradoxa* neither produced oogonia nor oospores *in vitro*.

The information we have so far gained will contribute significantly to the design of more effective control methods.

Activity 19. Plant disease diagnosis

Farmers have requested that major limiting diseases in several crops be identified. Diagnosis consisted in isolating the causal agent and confirming by pathogenicity tests. **Table 19.1** summarize the diseases that were studied. Disease symptoms were reproduced after inoculation with the following pathogens: *Pseudomonas andropogonis*, *P. aeruginosa, Cercospora dendrobii*, *Erwinia crysantemi*, and *Phytophthora cryptogea*.

Table 19.1. Important diseases identified on request of farmers.

Host Sample source	Symptoms	Disease name	Isolated pathogen(s)
Bermuda grass Soil and plant	Yellow to red-brown, irregular shaped patches of thin turf. Leaves may appear yellow to gray, starting at leaf tip. Stolons and leaf sheaths may be rotted.	Curvularia blight	*Curvularia* spp.
	Irregular shaped, light green or yellow patches of turf up to several feet in diameter. Leaves may be yellow or brown from the tip. Root may be thin, stunted, or knotted.	Nematodes	*Criconemoides* spp.
Rubber tree Epidermic tissue and soil			*Phytophthora* spp.
Brachiaria Leaves	Small seedlings and young plants present chlorotic striping and bleaching, and narrow chlorotic to straw-colored lesions restricted by veins. They form elongated streaks.	Bacterial leaf stripe	*Pseudomonas andropogonis*
	Oval to circular, tan-colored lesions, sometimes surrounded by chlorotic tissue lesions vary in size and may coalesce, causing leaves to be blighted.	Fungal leaf spot and leaf blight	
Dendrobium Leaves	Initially soft, light yellow, circular lesions, which later turn brown or black.		*Pseudomonas aeruginosa* And *Cercospora dendrobii*
Cattleya Leaves			*Erwinia crysantemi*
Chrysanthemum Leaves			*Erwinia crysantemi*
Potato Leaves and soil	Soft rot of the stem, brown or black.		*Erwinia carotovora*
	Grayish spots on the leaves and stems. Necrotic lesions and soft rot of the tubers.	Late blight	*Phytophthora* sp.

Activity 20. Control of *Phytophthora* root rot through potassium sulfate, soil humidity, and soil type

On-farm trials were established in Santander de Quilichao, Buenos Aires (Cauca), Caicedonia, La Tabaida (Valle del Cauca), and Montenegro (Quindío) to select agricultural practices that reduce *Phytophthora* root rot and are easy for the farmer to implement. At the same time, greenhouse trials were started to explore the potential of control of *Phytophthora* by applying organic and chemical fertilizers. The effect of soil humidity and soil type on the progress of the disease was also determined.

The experiments were conducted in a greenhouse at CIAT with soil from the northern Valle del Cauca (Caicedonia) and Cauca (Santander de Quilichao). Only the local and commonly used cassava genotypes of each region were used: Algodona (M Col 1522; Cauca) and ICA Catumare (CM 523-7; northern Valle del Cauca).

The following fertilizers were applied to soil placed in plastic pots before cassava was planted: potassium chloride (KCl; 50 and 100 kg/ha) and two types of wine lees— Sucromag (100 kg/ha, rich in magnesium) and Sucrocal (100 kg/ha, rich in calcium)— elaborated by Sucromiles (Palmira, Valle del Cauca, Colombia). Thirty day-old plants were inoculated by a stem-wounding method, using the highly pathogenic isolate P12 and B10, identified as *P. vignae*. Two controls were included: plants only inoculated with the pathogen and plants inoculated with culture medium. Damage caused by the pathogen was evaluated 7, 14, 21 and 28 days after inoculation.

Potassium chloride at 50 kg/ha significantly inhibited the disease and was not significantly different than the control without the pathogen in both experiments. Potassium sulfate did not have any effect, compared with the plants inoculated with the pathogen **(Figures 20.1 and 20.2)**. Plants treated with wine lees presented larger lesions than did the positive control. This result may have been due to the high nitrogen content of the lees, which made the plants more succulent and so more susceptible.

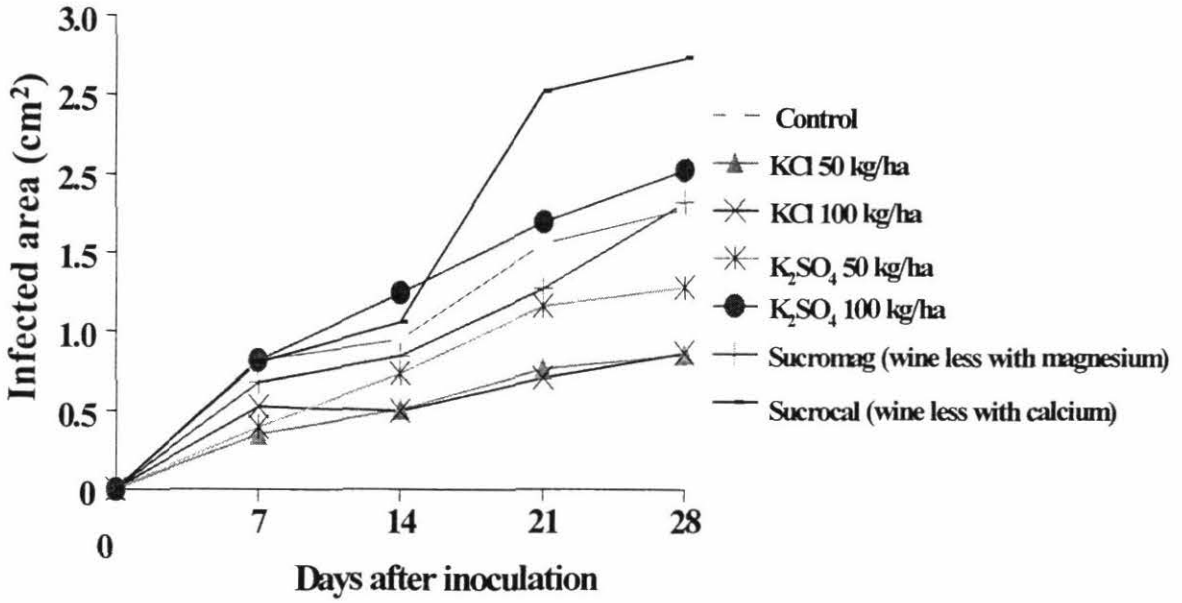

Figure 20.1. Disease progress curves over time versus fertilization treatments, in a soil from northern Valle del Cauca, Colombia.

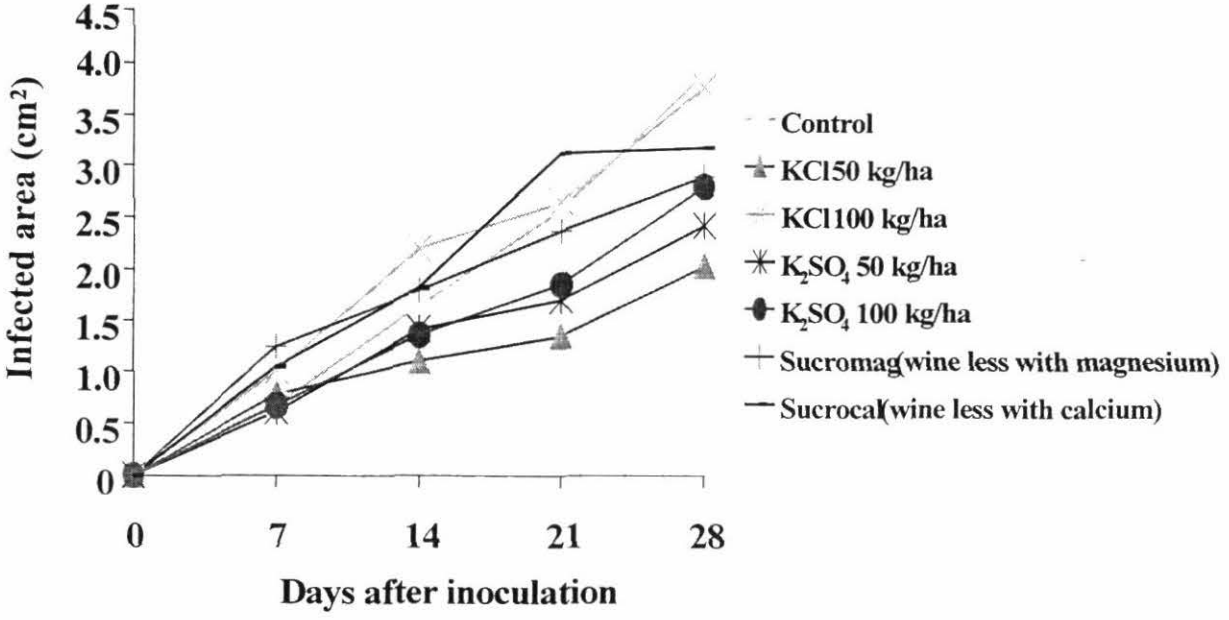

Figure 20.2. Disease progress curves over time versus fertilization treatments in a Cauca soil, Colombia.

Activity 21. Efficient *Trichoderma* isolates for controlling *Phytophthora* in common cassava genotypes

Greenhouse bioassays were performed to evaluate the efficiency of *Trichoderma* isolates. Before planting, cassava stems were immersed for 10 min in a suspension of conidia of *Trichoderma*, grown in liquid Richard medium. After planting the treated stakes, the remaining *Trichoderma* suspension was poured around the planted cutting. Thirty days after planting, the sprouts of the young plantlets were inoculated with the *Phytophthora* isolates 71 and P12. After inoculation, the progress of the disease was observed at weekly intervals. Fourteen cassava genotypes were inoculated with 34 *Trichoderma* isolates. Results are presented in **Table 21.1**. The interactions host × *Trichoderma* and control × *Trichoderma* were significant.

Table 21.1. Disease caused by *Phytophthora* isolates 44 and P12 was significantly controlled by six *Trichoderma* isolates.

Treatment[a]	Area below the disease progress curve[b]
19TSM-3A	2.43937 a
Control	2.05719 b
41TSM-4	1.19419 c
TV2CENI	1.17831 c
T8 CENI	1.09063 c
4 TSM-1B	1.04731 c
1644 CENI	1.04638 c
14 PDA-4	0.59975 d

a. Cassava genotypes used: M Cub 74, CG 1-37, M Col 1684, M Col 1522, M Col 1468, CM 3306-4, CM 523-7, M Col 2215, CG 1141-1, and M Col 2066.

The numbers followed by different letters are highly significant different ($P < 0.01$) according Duncan's Multiple Range Test.

Activity 22. Root-rot-causing pathogens identified: Causal agents isolated and conserved

Phytophthora isolates were successfully obtained from infected tissues of cassava plants collected from different agroecosystems, using the methodology described in the reports of previous years. To improve the rate of isolation of *Phytophthora* spp., a baiting technique was modified. Fragments of infected plant tissue were macerated and placed in sterile distilled water in Falcon tubes. Segments of in vitro propagated cassava plantlets, leaves and stems of young lupine (*Lupinus* spp.), or carrot roots were used as baits. After 3 days of incubation at room temperature, the fragments were disinfected, rinsed with sterile distilled water, dried, placed on selective media for *Phytophthora*, and incubated at 17°C. The rate of isolation of *Phytophthora* was more efficient from cassava plantlets or carrot roots than from lupine.

Phytophthora spp. were readily isolated from infected soil, following the method used by Goodwin *et al.* This method involved passing soil through a 2-mm sieve, diluting it with sterile distilled water, leaving it for 30 h, adding sterile soil, placing the mixture into a plastic bag with holes for draining, and incubating the mixture for 96 h at room temperature. The mixture was then passed to a plastic bag without holes, and the soil saturated with sterile distilled water. Small pieces of carrot root were added as bait. After incubating for 48 h at 22°C, the carrot fragments were removed, washed, dried, and placed on selective culture media.

All the *Phytophthora* isolates were conserved in sterile distilled water at 14°C.

Activity 23. Molecular techniques developed to identify and assess genetic diversity among isolates of *Phytophthora* spp.

Nuclear ribosomal DNA (rDNA) was obtained from 15 isolates of *Phytophthora* spp. obtained from cassava, oil palm, rubber, and apple **(Table 23.1)**. Internal transcribed spacer (ITS) regions were then sequenced, using ITS-4 and ITS-5 primers and the automated dideoxy sequencing (Sanger) at the Iowa State University's DNA Sequencing and Synthesis Facility. The following reference isolates were included: L41381 (*P. megasperma*), L41383 (*P. nicotianae*), L41376 (*P. cryptogea*) and L41388 (*P. vignae*).

Reactions were set up, using the Applied Biosystems (Foster City, CA) Prism Big Dye terminator-cycle-sequencing kit and Ampli*Taq* DNA polymerase. Electrophoresis was carried out on an Applied Biosystems Prism 377 DNA sequencer.

Table 23.1. Description of 15 *Phytophthora* isolates (13 from Colombia), for which ITS regions were sequenced in a study to assess the genetic diversity of these fungi.

Host crop Isolate	Location	*Host crop* Isolate	Location
Cassava		*Cassava*	
P12	Brazil	Mitú TR A4	Vaupés
27	Quindío	Mitú TR A6	Vaupés
44	Quindío	Mitú TR A7	Vaupés
66	Valle del Cauca	B10	Sergipe, Brazil
923 Cauca	Cauca	*Rubber tree*	
Santander de Quilichao 3	Cauca	Caucho 2	Caldas
5Circasia	Quindío	*Apple tree*	
Meta	Meta	Manzano	Caldas
		Oil palm	
		VV13	Meta

a. Database reported by National Center for Biotechnology Information.

Each sequence was aligned, using the Sequence Navigator Program, and comparisons made against a *Phytophthora* spp. database managed by the National Center for Biotechnology Information (NCBI; Internet address = http://www.ncbi.nlm.nih.gov). To get a better sequence,

ITS-2 and ITS-3 primers were also used for nine isolates. The isolates' phylogenetic tree was made with PAUP (Phylogenetic Analysis Using Parsimony) **(Figure 23.1)**.

The isolates Meta, Mitú TR A4, Mitú TR A6, Mitú TR A7, 27, Caucho 2, Manzano, and 5Circasia have similar sequences. The isolates 66, VV13, and 923 Cauca form another group. P12 and B10 have the same sequence, and both are close related to *Phytophthora vignae*. The isolate 44 is highly similar to *P. citricola* (97% of the bases are identical). Santander de Quilichao 3 shares 95% of its bases with *P. nicotianae*.

An AFLP fingerprinting protocol was standardized and shown to be a powerful tool for characterizing *Phytophthora* spp. isolates from cassava and oil palm. To achieve high levels of polymorphisms, different primer combinations of the AFLP primer kit for microorganisms (Gibco BRL) were evaluated. The protocol used was described by Vos et al., with modifications by Tohme et al. The manufacturer's specifications were also followed. Results, using the isolates P12 (cassava, Brazil), 129 (cassava, Quindío, Colombia), 32 (cassava, Quindío), Cp010 (oil palm, Meta, Colombia), Mono 115 (cassava, Valle del Cauca, Colombia) are presented in **Figure 23.2**.

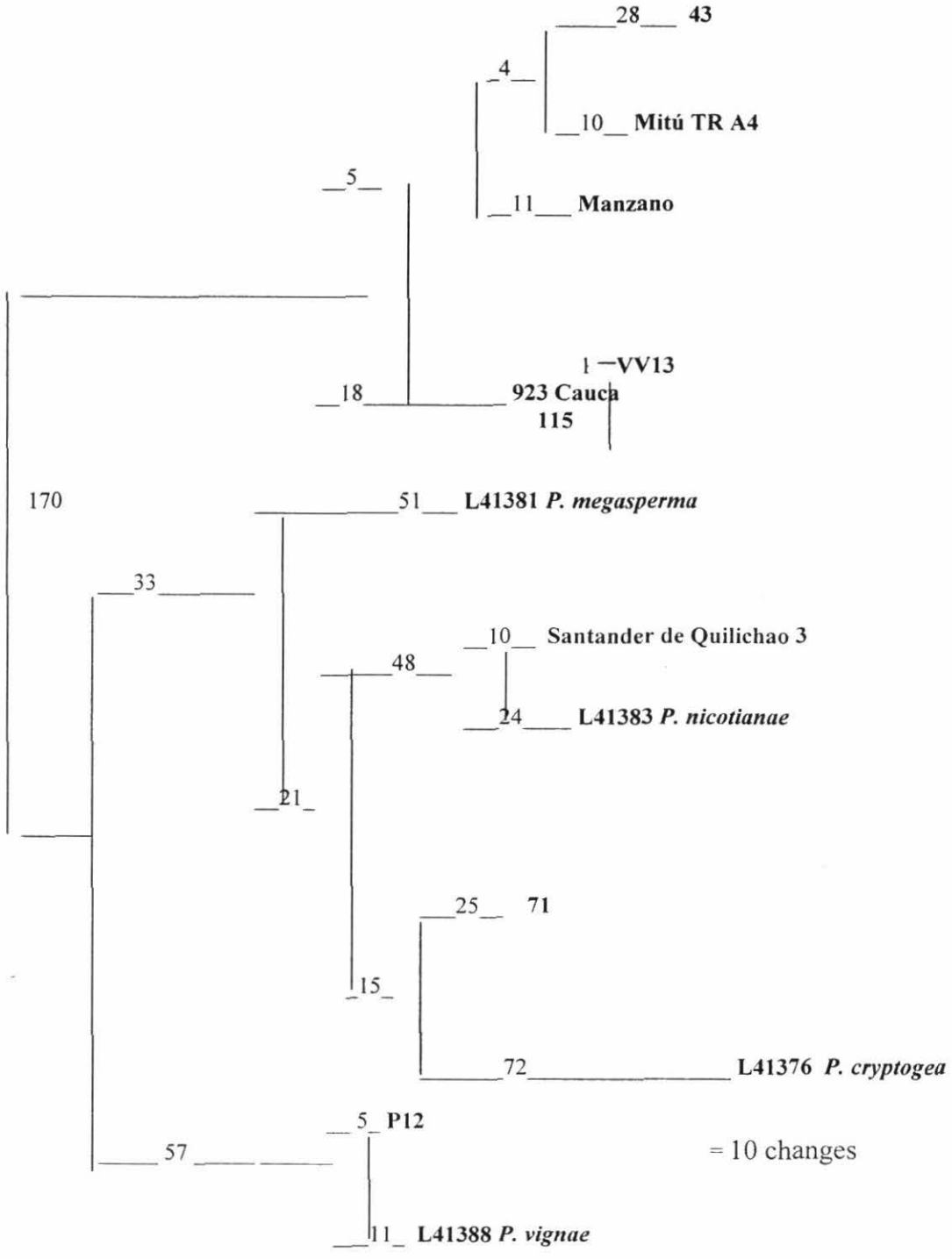

Figure 23.1. Species identification based on the comparison of amino acid sequences of the ITS-2 and ITS-3 regions of *Phytophthora* species isolated from cassava and oil palm. The phylogeny is constructed with the p-distance model and NJ tree-building method. Numbers along the branches are bootstrap values.

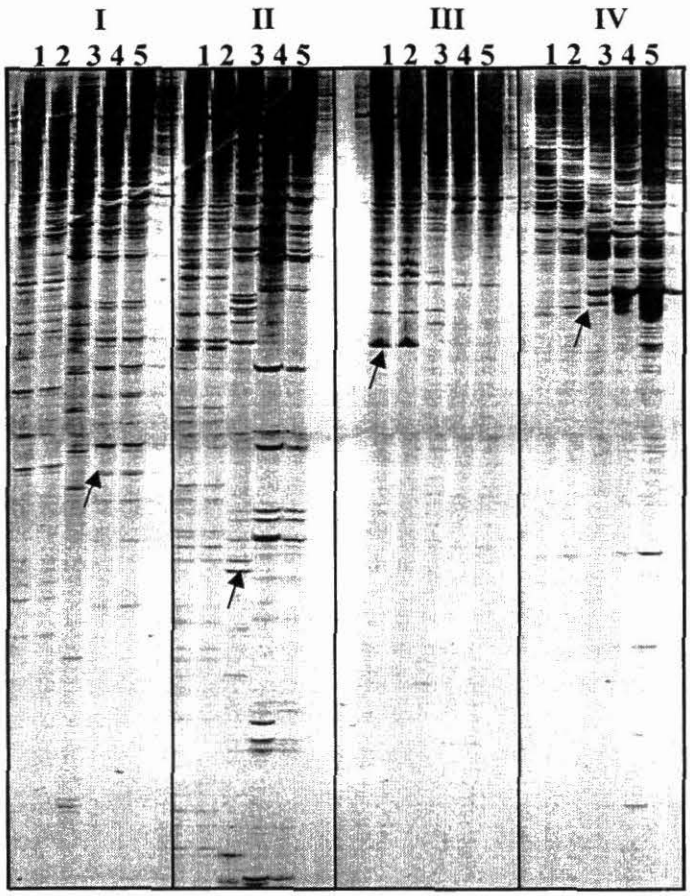

Figure 23.2. AFLP fingerprint of fungal DNA using for different primer combinations. Arrows indicate polymorphisms among five *Phytophthora* isolates tested.

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Sub-output 5. Identification and Characterization of Major Virus Diseases.

Activity 24. Molecular characterization of CFSV

Last year, the clone FSD-pol was reported to contain a section that had a high degree of homology with an RNA dependent RNA polymerase. This is a typical polymerase gene of an RNA virus. To test if this clone contained a part of the genome of cassava frogskin virus, total RNA was purified and amplified using specific primers in a reverse transcriptase-PRC assay. Several sets of primers were developed and in many cases, a specific 450 base PCR product was amplified in the healthy control. The current set of experiments used primers that only amplified a 250 base fragment that had the highest degree of homology with the RNA dependent RNA polymerase. This set of oligonucleotide primers did not amplify a product from the seven healthy controls of cassava nor from *N. benthamiana* (healthy or CsXV infected plants). The other negative control was passionfruit infected with a potyvirus. All these controls were negative (**Table 24.1**).

Fifteen cassava lines known to be infected with CFSD were tested using these primers. The PCR products were amplified under stringent conditions of 62 C for the temperature of annealing. In six of these a PCR fragment of the expected size (ca. 250 bases) was amplified. In nine of the isolates, there was no PCR product. Three of the isolates were tested in five separate experiments and FSD 5 and 80 isolates were consistently positive (except for experiment 4) and FSD CM 5460-10 was consistently negative. There is growing evidence that this RNA dependent RNA polymerase clone is associated with some isolates of CFSD. When using specific oligonucleotides, a change a single base can cause the reaction to be negative. While the current set of primers is not suitable for a general diagnostic assay, they are an advance in the identification of the causal agent of CFSD.

Table 24.1. A series of experiments using a potential molecular marker for cassava frogskin disease.

Isolate	Test 1	Test 2	Test 3	Test 4	Test 5
Healthy Per 385	-	-	-		
Healthy Tailandia 3	-	-	-		
Healthy Secundina	-	-	-	-	-
Healthy Per 324				-	
Healthy Per 325				-	
Healthy Per 197					-
Healthy Ecu 145					-
FSD 5	+	+	+	-	+
FSD 80	+	+	+	-	+
FSD 86			+	+	
FSD 29			+	+	
FSD 23			-	-	
FSD 14			+	+	
FSD CM 5460-10	-	-	-	-	-
FSD Regional Tolima			-	-	
FSD Amazonas 9				-	
FSD Amazonas 16				-	
FSD Helena 4				-	
FSD Mcol 306					-
FSD Bra 243					+
FSD Bra 110					-
FSD Mcol 1505					-
Healthy N. Benthamiana	-	-			
N. benthamiana CsXV	-	-			
Healthy Passionfruit	-	-			
Passionfruit-Potyvirus	-	-			
Whitefly DNA			-		

All of the experiments used the cDNA plasmid containing FSD-Cla I fragment as the positive control.

Activity 25. Screening the core collection to identify CFSD resistant germplasm

Cassava frogskin disease is highly destructive because it affects the roots. It can cause drastic reductions in both yield and quality. The roots affected with CFSD tend to have much less starch and are more fibrous. This is the third cycle of an experiment to select CFSD tolerant germplasm.

The starting materials were the 630 lines of the cassava core collection. Each year the cassava lines with severe root symptoms are eliminated from the experiment. This is the first year, that most of the lines had very light or no symptoms **(Table 25.1)**. Although the 52 lines that have never shown symptoms need to be analyzed for the presence of the causal agent, there are 91 lines that are known to be infected and that continue to show either light or no symptoms. Cassava frogskin symptoms are variable depending on environmental factors. In hot dry areas, the symptoms tend to be milder. This year the climate at the experiment site (Santander de Quilichao, Cauca, Colombia) had more rain and the climate was ideal for the expression of symptoms. This gives more confidence to the results and indicates that this selection process is achieving the goal of selecting CFSD tolerant germplasm.

Sixty of the 91 lines known to be infected with CFSD are from Colombia and Brazil. Twenty lines from Peru have been in the trial for two years and have not shown any symptoms. Since CFSD is thought to have originated in the Amazon region of Brazil, Peru or Colombia, it is not surprising that there is tolerance to the disease in germplasm from these countries.

Selection of CFSD resistant lines is a long-term experiment but with three years of consistent data, it is time to begin to change the strategy. These lines need to be tested in different localities and they need to be challenged with a second source of CFSD. The lines that perform best after the second phase of this experiment (at least two more years) should be recommended as potential varieties in area where CFSD in endemic and should be used as parents in breeding for CFSD resistance.

Table 25.1. Lines of the cassava core collection that were evaluated for their reaction to cassava frogskin disease.

Lines Evaluated	Total 152	100%
Lines with severe symptoms	5	3.3%
Lines with moderate symptoms	4	2.6%
Lines with light symptoms	18	11.9%
Lines showing no symptoms	125[1]	82.2%

1. In 1998, 72 of the lines had roots with either light or moderate symptoms on the roots. 53 of the plants were negative in both years.

Activity 26. To determine if the genome of CVMV is integrated into the cassava genome

Cassava vein mosaic virus (CVMV) is a disease that is endemic in the northeast region of Brazil. The virus was characterized at CIAT using non-infectious cDNA clones. There is no antiserum to this virus and four sets of PCR primers (designated SST, RBD, RT, and HS) were developed to detect CVMV from either fresh or dried leaves of cassava. The vein mosaic symptoms of CVMV

are produced in flushes. This means that there are times that infected plants do not have visible symptoms. The PCR test is highly sensitive and detects the virus in asymptomatic leaves.

As the PCR test was used to screen *in vitro* plants, there were many more PCR products than expected. In the primer set SST, there was a PCR product of the expected size even in the control "healthy" plants. In many of the *in vitro* plants tested appeared to be positive for CVMV. These were analyzed using a transmission electron microscope. All of the samples appeared to be negative for CVMV.

CVMV is in the Plant Pararetrovirus family. Another member of this family, Banana Streak Virus (BStV) is reported to be incorporated into the genome of banana. There is also some evidence that the genomic BStV DNA may be able to cause and active virus infection. Therefore, it became important to know if CVMV is incorporated into the genome of cassava.

The other three sets of primers were tested and all of them amplified bands from the *in vitro* plants, but the amplified products were not of the expected size. The expected size was determined by using the plasmid pCVMV-141 as the template. To determine if the amplified products were related to CVMV, they were transferred to filter and specific ^{32}P-labeled probes prepared from pCVMV-141 were used in hybridization assays. Using highly stringent conditions, only the PCR product amplified from the clone pCVMV was detected. The other PCR products did not hybridize and are thought to be non-specific. To further assure that these clones were non-specific, the PCR products from the SST reactions were cloned and sequenced. None of the clones were homologous with CVMV.

Another curious phenomena was that there were few or no non-specific bands when DNA was extracted from greenhouse grown plants **(Figure 26.1)**. The primer sets are oligonucleotides of 20-25 bases and should be highly specific. It is not clear why there should be more non-specific PCR products from *in vitro* plants, but that is the case for all four primers.

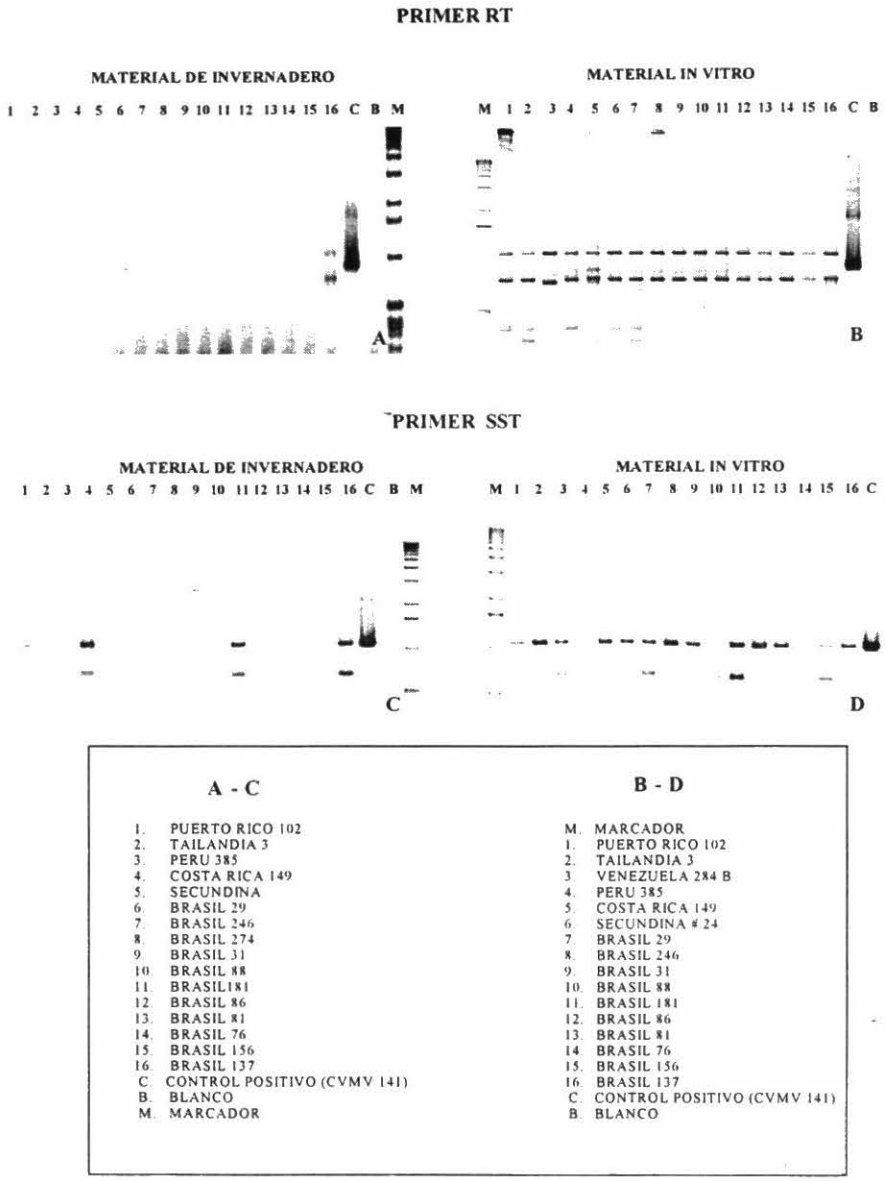

Figure 26.1. Using PCR primers in the detection of cassava vein mosaic virus.

There was one variety from Costa Rica that had PCR products of the expected size for three of the four primer combinations. This variety was rated negative using TEM and there are no symptoms. Further investigation is needed to understand why there bands of the expected size. There are also several clones from Brazil that are positive in the PCR tests and are being used as the positive controls.

CIAT is committed to the safe movement of cassava germplasm. The efforts here to assure that the test for CVMV meets the highest standards of confidence is evidence of our commitment of sending disease free germplasm. Often PCR tests are the best method available to detection of plant viruses. PCR is a highly sensitive method and one must be careful in analysis of the results. The conclusion is that CVMV is not incorporated into the genome of cassava and that the PCR test using the RT set of primers is an effective method to assure that the tested clones are not infected with CVMV.

Activities 27 & 28. Using molecular techniques to map the distribution of whiteflies with emphasis on biotypes of *B. tabaci* and development of SCARs for identification of whiteflies

Introduction

Knowing the distribution and range of whiteflies has become increasingly important with the spread of the *B. tabaci* biotype B (*B. argentinfolii*). It is not known how many biotypes of *B. tabaci* are present in the Americas. Since there are no reliable morphological characteristics, biotypes are distinguished using both biological and molecular methods. In the United States, the principal *B. tabaci* before the introduction (probably from the Middle East) of the B biotype is called the A biotype. At the beginning of this survey, most of the data on the *B. tabaci* other than the B biotype was biological and esterase isozyme data. There was significant variation in the esterase isozyme patterns, and the *B. tabaci* populations had narrow host ranges. Combined with geographical isolation, the speculation was that there were many *B. tabaci* biotypes. Whenever there is an increase in whiteflies or they begin affecting additional crops, the introduction of the B biotype is suspected. Often this is the case but whitefly populations are affected by many environmental factors, the cropping system and the varieties grown, therefore the presence of the B biotype should be confirmed through biological data and/or molecular techniques. This survey was undertaken to map the distribution and range of whiteflies in various countries in Latin America. In order to be successful, it was important to develop rapid methods to distinguish the biotypes of *B. tabaci*. In the process, more information on the populations of *B. tabaci* became known. This report describes the progress on understanding the whitefly complex and their distribution.

Mitochondrial 16S gene analysis

Total DNA was isolated from individual whiteflies of *B. tabaci* biotype A and biotype B, *B. tuberculata, A. socialis, T. vaporariorum, and T. variabilis*. The 3' region of the mt16S gene was amplified using primers (5'CGCCTGTTTAACAAAAACAT & 5'CCGGTCTGAACTCAGAT

CAGCT). The amplified products were cloned into the bacterial plasmid PCR script KS+ and sequenced. The various whitefly sequences were aligned using the ClustalW 1.7 program and parsimony and distance analysis were performed using Phylip version 3.57 (Felsenstein, 1993).

At least two independent clones from each whitefly population of the 3' region of the mitochondrial 16S gene were prepared and sequenced. These were compared with sequence data available in the Genbank (Frohlich *et* al., 1999). The mt16S sequences for the *B. tabaci* biotype A of Arizona (Genbank accession AF110722), Costa Rica (Genbank accession: AF110715) and Puerto Rico (Genbank accession: AF110719) were compared with the Colombian *B. tabaci* biotype A. In the analysis, the different populations of *B. tabaci* biotype A were grouped together, and there was at least 97% identity with the maximum mean distance of 0.02 **(Table 28.1)**. Although the data is still limited, it appears that the *B. tabaci* biotype A is widespread throughout the region of the whitefly survey. Since the expression of esterases can be induced by environmental factors such as applications of insecticides, the maternally inherited genetic marker appears to be a better indicator of diversity between populations of whiteflies. Additional studies using the carboxy oxidase subunit I mitochondrial gene are underway and these will be even more useful than the mt16S gene in understanding diversity within the *Bemisia* complex.

In the distance analysis the Israel *B. tabaci* isolate (Genbank accession: AF110717) was 98.8% identical and a mean distance of 0.01 with the *B. tabaci* biotype B isolates from Colombia. Comparisons were also made with individuals from Colombia, Arizona, Israel and Yemen and all were at least 98.2% identical (data not shown). Given the rapid spread of the B biotype, the lack of diversity between populations in Arizona USA and Colombia was expected.

Table 28.1. Mean distances for a 3' region of mitochondrial 16S ribosomal gene in fifteen individual whiteflies representing different species and populations.

	Whitefly	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	B. tabaci B Sucre	0.00	0.01	0.01	0.01	0.10	0.10	0.09	0.10	0.10	0.22	0.35	0.35	0.37	0.37	0.36
2	B. tabaci B CT cass		0.00	0.00	0.00	0.08	0.09	0.08	0.09	0.09	0.21	0.34	0.34	0.36	0.36	0.35
3	B. tabaci B CT bn			0.00	0.00	0.09	0.10	0.09	0.09	0.09	0.21	0.34	0.35	0.37	0.36	0.35
4	B. tabaci Israel				0.00	0.08	0.09	0.08	0.09	0.09	0.21	0.34	0.34	0.36	0.36	0.35
5	B. tabaci A CT 1					0.00	0.01	0.01	0.02	0.00	0.22	0.34	0.35	0.36	0.35	0.38
6	B. tabaci A CR						0.00	0.01	0.02	0.01	0.23	0.35	0.36	0.37	0.36	0.39
7	B. tabaci A AZ							0.00	0.02	0.01	0.22	0.34	0.36	0.37	0.36	0.38
8	B. tabaci A PR								0.00	0.02	0.22	0.35	0.36	0.36	0.36	0.38
9	B. tabaci CT 2									0.00	0.22	0.34	0.35	0.36	0.36	0.38
10	B. tuberculata CT										0.00	0.28	0.28	0.37	0.36	0.38
11	A. socialis Mon											0.00	0.07	0.40	0.39	0.43
12	A. socialis CT												0.00	0.41	0.40	0.41
13	T. vaporariorum CT													0.00	0.00	0.39
14	T. vaporariorum AZ														0.00	0.38
15	T. variabilis CT															0.00

A representative from each species or biotype was used in the analysis to find the most parsimonious tree **(Figure 28.1)**. *B. tabaci* biotype A and B were always grouped together. *B. tuberculata* was grouped with the *B. tabaci* 92% of the time. *T. vaporariorum* and *T. variabilis* were grouped together 86% of the time. Using parsimony analysis, the relationship between the three genera of *Bemisia*, *Trialeurodes*, and *Aleurotrachelus* was not clear. The results of the distance analysis (Table 3) show that *Aleurotrachelus* was closer to the genus *Bemisia* than to *Trialeurodes*. An unexpected result was that relatively large mean distance between *T. vaporariorum* and *T. variabilis*.

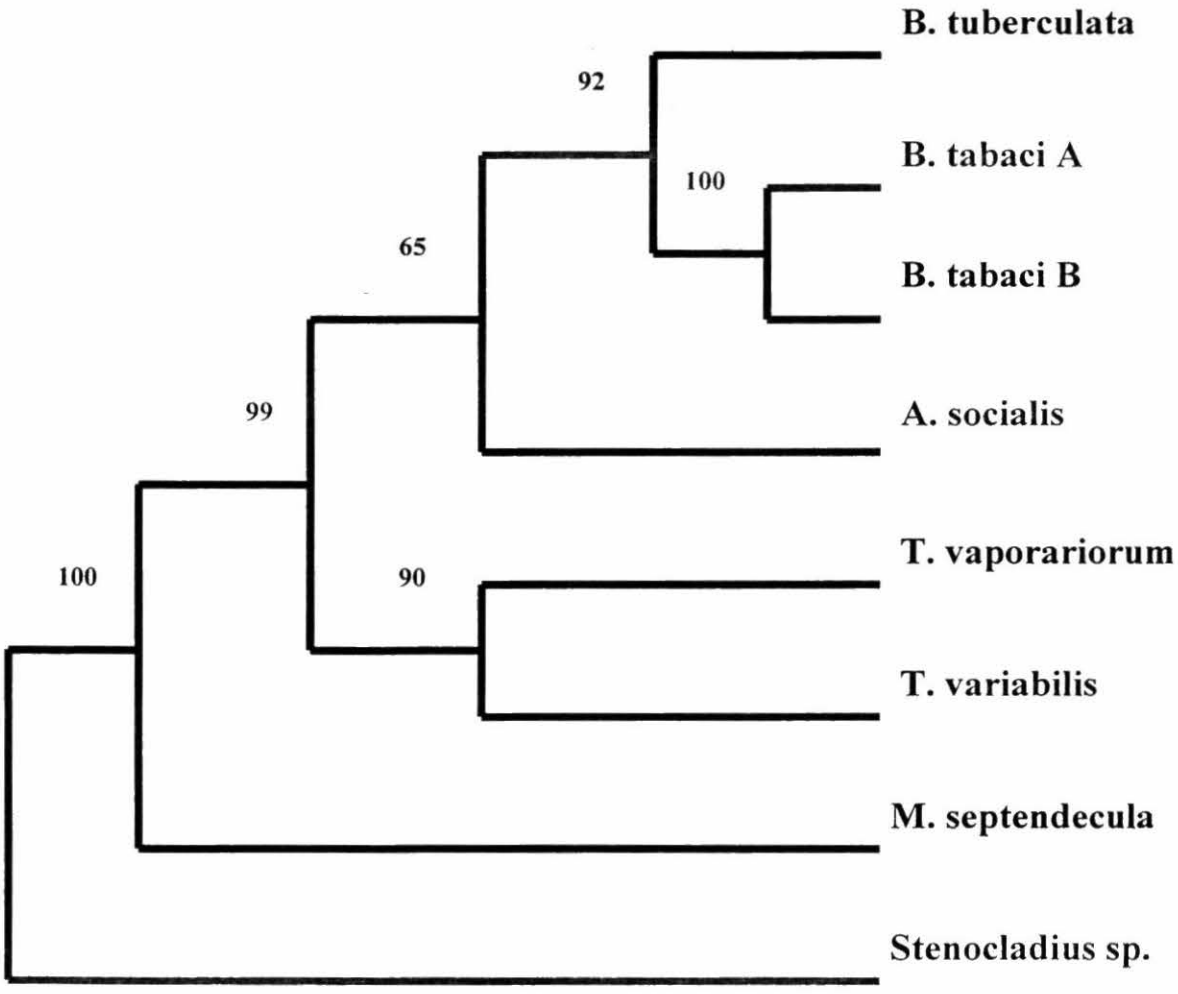

Figure 28.1. Cladogram showing the relationship between the whiteflies in this study. The cladogram is based on the most parsimonious tree inferred from the analysis of 485 base sites of a region of the mitochondrial 16S gene. Numbers above the branches indicate the level of statistical support for the corresponding node from 10,000 bootstrap replicates (Phylip 3.57)

RAPD analysis

Randomly amplified polymorphism analysis (RAPD) was used (De Barro and Driver,1997) to distinguish indigenous Australian populations of *B. tabaci* from the introduced B biotype. They screened many and reported on four oligonucleotide primers that they considered the most useful to distinguish the *Bemisia* complex in Australia. These were tested using different populations of whiteflies from various countries include controls of a the CIAT colonies of *B. tabaci* biotype A and biotype B, *B. tuberculata, A. socialis, T. vaporariorum, and T. variabilis.*

Four PCR primers were tested for their utility in distinguishing whitefly species. In the analysis of PCR products using the primer H9, there were differences for the range of whitefly species tested **(Figure 28.2)**. For *B. tabaci* B biotype and *T. vaporariorum*, there are prominent PCR products ca. 600 and 800 bp that can sometimes make distinguishing the two species difficult. The is a unique PCR product in *B. tabaci* B biotype ca. 950 bp, and *T. vaporariorum* has a unique PCR product at ca. 500 bp that are important for distinguishing between these two species. Similarly there were PCR products of ca. 350, 550, and 600 bp that were similar in *B. tabaci* biotype A and *B. tuberculata*. These species could be identified by a unique 250 bp PCR product in *B. tuberculata*, and a ca. 900 bp product in *B. tabaci* A biotype. Although there can be some confusion in the interpretation between some species, the H9 primer was most useful in distinguishing between *B. tabaci* biotypes A and B. At 600 bp, there are PCR products of similar in size in both biotypes, but the biotype A has several unique PCR products including doublet bands at 300-350 bp. The B biotype has unique PCR products at ca. 600, 700 and 900 bp compared with one product of ca. 850 bp in the A biotype.

The primer H16 was most useful in distinguishing between the whitefly species. While there can be some common bands in the 500 to 1000 bp range for both *B. tabaci* biotypes, there were three products in *B. tabaci* biotype B of ca. 350, 450 and 550 bp that were consistently useful for identification of the B biotype.

When primers F2 and F12 were used, there were larger numbers of PCR products. These can be used for distinguishing the whitefly species, but due to the large numbers of bands were generally less useful than H9 and H16. When using RAPDs for the identification of the whiteflies in this study, it is recommended to analyze the individual whiteflies with both the H9 and H16 primers.

The distribution of whitefly species and biotypes

The whitefly specimens were analyzed by light microscopy and RAPD analysis. At least one and often two primers were used in the RAPD analysis and the results were compared to the morphological identification. The RAPD data was most useful to distinguish biotypes in the *Bemisia* complex. Most of the samples of that were determined to be in the *B. tabaci* complex by morphological were classified as either biotype A or B. This survey was extensive but not exhaustive. These results should be interpreted as a representation of the predominate whiteflies populations in various regions of Cuba, Dominican Republic, Guatemala, Honduras, Costa Rica, Panama, Colombia and Venezuela. Since the pupae were collected, the data also represents hosts of the whiteflies.

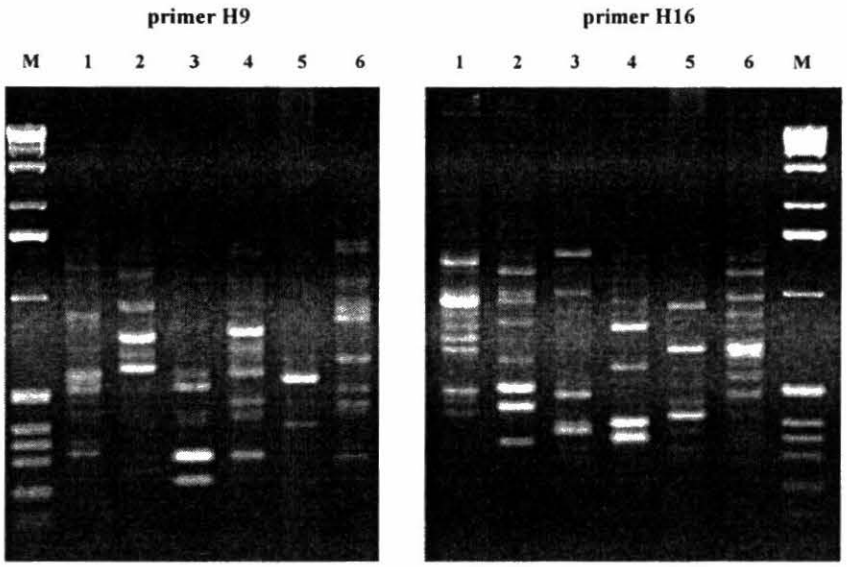

Figure 28.2. RAPD PCR products from individual whiteflies are shown in lane 1: *B. tabaci* biotype A, 2: *B. tabaci* biotype B, 3: *B. tuberculata*, 4: *T. vaporariorim*, 5: *T. variabilis*, 6: *A. socialis*, and M: 1Kb Markers.

Dominican Republic

In the departments of Azua, Barahona, Peravia, Santiago, Montecristo and San Juan, the predominant whitefly was *B. tabaci* biotype B. Plants tested include *Solonum melongena*, tomatoes, okra, eggplant, melons, cucumbers, and hibiscus. Only one sample of tomatoes in Azua were classified a biotype A. In the lowland tropics of the Dominican Republic, the biotype B was introduced nearly a decade ago and has nearly exclude the indigenous biotype A. In the department of Vega, beans, tomatoes and potatoes were tested and *T. vaporariorum* was the only whitefly found.

Guatemala

In the department of Zacapa, the predominant whitefly was *B. tabaci*. In Zapcapa, the B biotype was present on all the host tested which included okra, melon, watermelon, and cucumber. In Japala both *B. tabaci* biotype B and *T. vaporariorum* were found. The B biotype was found on okra, melon, watermelon, cucumber, tomatoes, eggplants, and tobacco and weed species. *T.*

vaporariorum was the predominate whitefly in several host plants including papaya, tomate-manzana, tomate-cerezo, beans as well as some samples of tomatoes.

Honduras

In Honduras, the predominant whitefly was *B. tabaci* biotype A. Most of the samples were beans, tomatoes, and cucumber from the Department Comayagua. The papaya in Comayagua was host to *T. vaporariorum.* In the Department Fco Morazan on chili peppers, the A biotype was also the only whitefly identified.

Costa Rica

In the lowland tropics of Costa Rica, *B. tabaci* biotype A is still the predominate whitefly. In the Departments of Guanacaste, San Jose, Heredia, and Alajuela most of the samples were biotype A. The A biotype was found on beans, melons, watermelons, tomatoes, chili peppers, cucumbers and others. In the Departments of Arajuel and Puntarenas, the B biotype was present. It was only whitefly found in the two samples from Arajel, but in Puntarenas both biotype A and B were present. *T. vaporariorum* was present in the Department of Alajuela and was very common in Cartago where it was found on chili peppers and tomatoes.

El Salvador

Only a limited number of samples (15) have been tested and all were *B. tabaci* biotype B. Additional testing is being done to make a more complete analysis.

Panama

The principal whitefly in the Department of Chiriqui was *T. vaporariorum.* In the other regions of the country *B. tabaci* biotype B was the predominant whitefly. There are still populations of *B. tabaci* biotype A and in some areas the populations are mixed. This suggests a relatively recent introduction of *B. tabaci* biotype B in Panama.

Cuba

B. tabaci biotype B was the only whitefly found in the samples from Cuba. The B biotype has been in Cuba for nearly ten years and has clearly become the predominant whitefly.

Colombia

Most of the samples tested were from the north coastal region of Colombia. In the region, the principal whitefly is *B. tabaci* biotype B. The B biotype is affecting tomatoes, col, eggplant, melons, and cassava. The A biotype is still present but the introduction of the B biotype is relatively recent. This is the first report of the B biotype on cassava in Colombia. *B. tuberculata* and *A. socialis* were also found on cassava in this region. This contrasts with the Departments of Cauca and Valle in the Andean region where *A. socialis* is the principal whitefly on cassava and

B. tuberculata is much less common. The B biotype has not been found on cassava in the Andean region.

Venezuela

Samples were collected from the Departments of Zamora and Jimenez, the principal whitefly was the B biotype. In the Department of Urdaneta, there was a mixture of A and B biotypes. Only a few samples were analyzed and more are needed before conclusions should be made.

Conclusions

B. tabaci biotype A, *B. tabaci* biotype B and *T. vaporariorum* were the principal whiteflies found in the region **(Table 28.3)**. Even at the level of Departments, a predominate whitefly could usually be identified. Occasionally within the same region mixed populations were present, but these were normally separated being found on different hosts. *T. vaporariorum* was present in many regions, but tends to be at higher elevations. Only in the mid altitudes do the *Bemisia* and *T. vaporariorum* populations overlap. In countries including Cuba and the Dominican Republic, the exclusion of the *B. tabaci* biotype A is almost complete at least on the principal crop species tested in this survey. The pattern in Central America is more complex. In Cuba, Guatemala, Panama and the north cost of Colombia, the process of domination of the B biotype appears to be well advanced. In Honduras and Costa Rica, the A biotype is still predominate. It is probably just a matter of time until the B biotype becomes the principal whitefly in those areas. Nevertheless, these are the zones that need to be monitored closely and strategies to decrease the probability of introduction of the B biotype should be developed.

Table 28.3. A summary of the RAPD whitefly data by country.

Country	Number of samples	*B. tabaci* Biotype A (%)	*B. tabaci* Biotype B (%)	B. tuberculata (%)	T. vaporariorum (%)	NI[1]	NB[2]
Guatemala	185	8.1	60.5	0	26	5.4	0
Cuba	44	0	100	0	0	0	0
Dominican Rep.	106	13.2	81.1	0	0	0	5.7
Colombia	179	0.56	61.45	3.35	13.96	5.58	15.1
Venezuela	262	4.6	18.3	0	0	0	77.1
Costa Rica	160	61.25	6.9	0	12.5	3.1	16.25
Panama	198	4.6	33.3	0	40.9	91.8	12.1
El Salvador	15	0	100	0	0	0	0
Honduras	138	88.4	2.9	0	0	7.3	1.4

[1] Samples with amplified PCR products but were unable to identify.
[2] No products were amplified

Activity 29. Develop diagnostic capabilities for citrus viruses

Citrus is a high value tree crop with the potential to prevent erosion in the hillsides and provide a source of income for the small farmer. There are both large domestic and international markets. Citrus viruses are a major limitation on maintaining highly productive trees. This year in collaboration with CORPOICA, the CIAT virology laboratory began increasing our diagnostic capabilities for the major viral pathogens affecting citrus. Biological, serological and PCR methods are being used as diagnostic tools. Citrus tristeza virus, citrus psorsis virus, blight and the viroid exocortis are some of the main pathogens. The University of Florida at the Citrus Research and Education Center is a key contact and links have been established to collaborate and jointly develop projects. The virology laboratory is developing the capacity to act as a region diagnostic laboratory and the expertise to aid in the establishment of budwood certification programs.

Bibliography

Morphological and mitochondrial DNA marker analysis of adult whiteflies (Homoptera: Aleyrodidae) colonizing cassava and beans in Colombia.

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OUTPUT II. PEST AND DISEASE MANAGEMENT COMPONENTS AND IPM STRATEGIES AND FACTORS DEVELOPED

Sub-output 1. An Integrated Control Method for Cassava Root Rots in Colombia.

Activity 1. Participatory disease diagnosis, description of farming systems, and loss assessment, together with cassava growers and technicians

Partners identified.

Pilot regions identified where root rot is endemic:

Vaupés, Cauca, Valle del Cauca, Bolívar, Sucre, Córdoba, Atlántico, Meta, Casanare, Cundinamarca, and Quindío.

Introduction

A diagnostic survey was conducted with the collaboration of cassava farmers, the Ministry of Agriculture, UMATAs (Unidades Municipales de Asistencia Técnica Agropecuaria) of Montenegro, Caicedonia, Santander de Quilichao, and Mitú, and farmer associations. Crop diseases and farming systems were described as a means toward developing efficient control practices that are easy for farmers to adopt.

Between October 1998 and April 1999, 38 interviews were conducted in the North Coast region of Colombia, in the Departments of Bolívar, Sucre, and Córdoba, and another 21 in Quindío and 32 in Valle del Cauca. Cassava crops were also visited, and diseased plants and soil samples were taken to isolate root-rot-causing agents.

North Coast

We interviewed various cassava farmers in the North Coast, as follows:

Department of Córdoba (17 farmers) in the municipalities of:
Chinú (5), Montería (3), Ciénaga de Oro (3), San Pelayo (1), Catorra (1), San Andrés de Sotavento (1), Cereté (1), Canalete (1), Sahagún (1)

Department of Bolívar (10 farmers) in the municipalities of:
San Jacinto (2)m María La Baja (2), Carmen de Bolívar (2), San Juan de Nepomuceno (1), Córdoba (1), Zambrano (1), Arjona (1)

Department of Sucre (11 farmers) in the municipalities of:
Palmito (2), San Onofre (2), Betulia (1), Morroa (1), Ovejas (1), Sampués (1), San Pedro (1), Sincé (1), Tolú Viejo (1)

The average size of the farmer plot in the different departments is, in Bolívar, 0.5-1 ha and in Córdoba, 4 ha, ranging from 1 to 9 ha. In Sucre, however, plots ranged from 1 to 45 ha.

In all the departments surveyed, cassava is usually planted in the rainy season (April and May). In Bolívar and Sucre, cassava is commonly associated with maize and yam, and in Bolívar, it is also associated with hot (or chili) pepper. In Sucre, farmers with large planting areas monocrop cassava.

The commonest cassava genotypes planted are:

Department of Córdoba: Venezolana, Brasilera, and Chiroza, with the less frequently found ICA Negrita, ICA Costeña, and Cogollo Colorado.

Departments of Bolívar and Sucre: Venezolana.

Department of Sucre: P-12 on some farms.

According to farmers, the most limiting constraints to cassava production are:

In Córdoba: bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis*, the Lepidoptera *Chilomina* spp. (stemborers), root rots, and whitefly (*Aleurotrachelus socialis*). Less common constraints are superelongation disease caused by *Sphaceloma manihoticola*, termite (*Coptotermes* spp.), hornworm (*Erynnis ello*), mites, and thrips (*Thrips* spp.).

In Bolívar: stemborers (*Chilomina* spp.), bacterial blight, and root rots.

In Sucre: bacterial blight, stemborers, root rots, followed by termites, hornworm, and thrips.

Córdoba farmers reported that 'Venezolana' is the most susceptible genotype to root rots. In Bolívar, 'Venezolana' was also considered to be susceptible to bacterial blight.

All farmers were interested in planting new early maturing genotypes (harvested at 6 to 10 months) and, in Córdoba and Bolívar, highly productive genotypes.

Farmers in Córdoba need new cassava genotypes, especially for starch production. In almost all Bolívar and Sucre municipalities, cassava is produced for fresh consumption.

Farmers in Córdoba and Sucre prefer cassava plants of intermediate height and little branching to facilitate labor. Resistance to bacterial blight and to root and stem rot diseases was considered as highly important by farmers in all departments.

Department of Quindío

We interviewed a group of 21 farmers from the municipalities of Quimbaya (4 farmers), Circacia (3), and Montenegro (15).

The average area planted to cassava for this department is 2 ha per farm, varying from 0.3 to 19 ha. Of the farmers, 65% grow cassava in fields where coffee trees had recently been pruned. The commonest genotypes grown are Chiroza, ICA, and HMC 1, all produced for the fresh market of

Santafé de Bogotá. According to the farmers, the principal cassava problems are fruit fly (*Anastrepha* spp.), root rots, and whitefly, and less important are hornworm and thrips. 'Chiroza' is the most susceptible genotype to root rots.

Most of the farmers were not interested in introducing cassava genotypes other than 'Chiroza'. New genotypes useful for agroindustry and the fresh market will be important in the nearby future.

Department of Valle del Cauca

We visited 26 cassava farmers in northern Valle del Cauca, in the municipalities of Caicedonia (17) and Sevilla (9). The average area planted to cassava per farmer is 1.2 ha, ranging from 0.3 to 6.4 ha.

In the municipality of Sevilla, all farmers plant cassava between pruned coffee trees. Production in this region is characterized by the use of high quantities of fertilizers, which are applied to soil and foliage. Both small and large-scale farmers frequently use Sistemin (dimetoate).

The commonest cassava genotypes are Chiroza, ICA, and HMC 1, which are all used for fresh consumption.

The commonest and most severe problems are "viruela" (cassava root rot spot), another root rot caused by a complex of *Phytophthora* spp. with *Cyrtomenus bergi* (burrowing bug), the fruit fly *Anastrepha* spp., other root rots, and whitefly. Less common problems are hornworm and thrips.

In Caicedonia, small areas of lowlands are often planted to cassava and, in hillside areas, cassava is associated with coffee.

We also visited 6 farmers in the municipality of Buenaventura, situated on the Pacific coast, to the southeast of the department. The farmers clear tropical forest, then plant cassava, usually in association with *chontaduro* (or peach palm, *Bactris gasipaes*), maize, and dwarf banana (*Musa textiles*). The cassava crops that we visited were free of diseases or pests and were vigorous.

Loss assessment

Root rots, wilt, and blight are reported as the most important production constraints in most of the visited departments. *Phytophthora* can cause rots although abrupt changes in temperature, excess soil humidity, and several pests can also induce rots **(Table 1.1)**. Stem rot, wilt, and/or blight of leaves can also be caused in Colombia by several diseases and pests, like *Phytophthora*, *Xanthomonas axonopodis* pv. *manihotis*, *Erwinia carotovora* pv. *carotovora*, stemborers, fruit fly, or drought. Cassava bacterial blight is a very common pathological problem in the Colombian North Coast. Fruit fly (*Anastrepha* spp.), whitefly, and the burrowing bug are frequently mentioned as the most important production constraint. Cauca, North Coast, and Vaupés have the most troublesome phytosanitary problems.

Table 1.1. The most important production constraints according to cassava farmers in the major production regions of Colombia[a].

Constraint	Cauca	North Coast[b]	Vaupés	Valle del Cauca	Quindío	Eastern Plains[c]	Average
Root rots and wilt	39	8	65	3	10	27	31
Cassava bacterial Blight	0	61	0	0	0	0	9
Fruit fly (*Anastrepha* spp.)	13	0	0	7	11	0	8
Whitefly	8	3	0	7	8	0	6
Burrowing bug	0	3	0	37	26	0	6
Hornworm	0	15	0	13	3	0	4
Mites	0	3	0	3	3	0	1
Stemborers (*Chilomina* spp.)	0	3	3	0	0	7	1
Superelongation Disease	0	3	0	0	0	0	0
Other, or farmer did not reply	40	1	32	30	39	66	34
Number of farmers Interviewed	120	38	31	26	21	15	251

a. Of the farmers interviewed, percentages are given of those who mentioned the problem as a major constraint to cassava production.
b. Departments of Bolívar, Sucre, Córdoba, and Atlántico.
c. Departments of Meta, Casanare, and Cundinamarca.

Of the farmers, 30% ranked rots and blight as the most limiting production factors. During the visits to Cauca, in the first semester of 1998, extremely high losses to root rots and blight were reported. After this period, damage diminished considerably, but the reasons for this reduction are unknown. Except for Cauca, the average loss in production caused by rots and blight in the most important cassava-producing regions is 22%. Cauca and Vaupés can be considered as the most affected departments. Losses in Quindío are also relatively high, compared with other departments. As reference, the results of successful isolation of *Phytophthora* are also included in **(Table 1.2)**.

Table 1.3 shows that two practices-crop rotation and adopting a different crop are negatively correlated with losses caused by rots, wilt, or blight, taking into account that the production systems and agroecological conditions across a department or region are similar. According to the survey, maize, pasture, coffee, bean, tobacco, cotton, soybean, passion fruit, plantain, sesame, yam, and several vegetable crops (e.g., *Cucurbitacea* spp.) are commonly rotated with cassava. Although farmers recognize the potential usefulness of changing cassava genotype, they often do not practice it, thus reducing the possible effectiveness of proposing this practice.

Collecting infected plants, planting different cassava genotypes in the same field, and fallowing for 3 to 4 years are useless for effective disease control.

In all, farmers use 14 cassava genotypes **(Table 1.4)**, with the highest genetic diversity being found in the North Coast and Valle del Cauca. Farmers in Cauca tend to exclusively plant 'Algodona', which is highly susceptible to *Phytophthora* root rot. The genotypes Chiroza and ICA Catumare are grown in several agroecological zones.

Table 1.2. Survey of losses in cassava crops in Colombia, 1998 and 1999.

Department	Farmers interviewed (no.)	Percentage of cassava farmers who recognized root rot[a]	Percentage of losses caused by root and/or stem rot[b]	*Phytophthora* isolates obtained (no.)
Cauca	120	39	70	5
North Coast[c]	38	8	14	1
Vaupés	31	65	28	3
Valle del Cauca	26	3	7	28
Quindío	21	10	38	26
Eastern Plains[d]	15	17	30	1
Total	251	30	45	64

a. Average percentage of farmers who recognized symptoms of the disease and/or who considered root and/or stem rot as the most important production constraint.
b. The average percentage of production lost to root and/or stem rot, based on affected production and/or area.
c. Departments of Bolívar, Sucre, Córdoba, and Atlántico.
d. Departments of Meta, Casanare, and Cundinamarca.

Table 1.3. Diagnosis of farmer management of root rots infecting cassava in Cauca, North Coast, Vaupés, the Coffee Zone (northern Valle del Cauca and Quindío), and Eastern Plains, Colombia[a].

Recommended practice for reducing root rots	Cauca	North Coast[b]	Vaupés	Valle	Quindío	Eastern Plains[c]	Average
Crop rotation	21	50	0	21	40	0	23
Change genotype	18	27	0	30	60	7	21
Adopt different crop	17	27	0	17	0	27	16
Select healthy planting material	27	71	0	7	36	87	33
Destroy infected plants (e.g., by burning)	12	13	0	10	0	13	10
Destroy crop residues	7	0	0	0	20	20	6
Collect infected crop residues	35	0	0	15	30	53	24
Before planting, treat stakes with pesticides	25	0	0	0	52	7	17
Cultivate on ridges or hills	66	20	0	16	0	20	37
Collect infected plants	43	0	0	0	0	20	22
Plant different cassava genotypes in the same field	30	15	100	0	0	67	33
Leave fallow for 3 to 4 years	26	10	94	0	15	27	28
Number of farmers interviewed	120	38	31	26	21	15	251

a. Percentage of farmers who apply the control practice.
b. Departments of Bolívar, Sucre, and Córdoba.
c. Departments of Meta, Casanare, and Cundinamarca.

Table 1.4. Genetic diversity of cassava grown by 251 farmers in Colombia[a].

Genotype	CIAT	North Coast[b]	Valle del Cauca	Eastern Plains[c]	Quindío	Cauca
Algodona	M Col 1522	0	0	0	0	63
Chiroza	M Col 2066	0	47	40	50	0
Brasilera	-[d]	0	0	47	0	0
ICA Catumare	CM 523-7	0	24	0	43	24
Llanera	-[d]	0	0	20	0	16
Venezolana	M Col 2215	47	0	0	0	0
ICA Negrita	CM 3306-4	22	0	0	0	0
P12 (Verdecita)	M Col 1505	18	0	0	0	0
H1	HMC 1	0	10	0	5	0
Mona Blanca	M Col 40	9	0	0	0	0
Valluna	M Col 113	0	5	0	0	0
Sardina	M Col 2962	4	0	0	0	0
ICA Costeña	CG 1141-1	2	0	0	0	0
Palmirana	M Col 139	0	2	0	0	0
Diversity (number of Different genotypes Per department)		6	5	3	3	3
Number of farmers Interviewed		38	26	15	21	120

a. Percentage of farmers who planted the genotype.
b. CIAT code unknown.
c. Departments of Bolívar, Sucre, Córdoba, and Atlántico.
d. Departments of Meta, Casanare, and Cundinamarca.

Activity 2. Participatory plant breeding in Brazil

A participatory research project with farmers in 50 communities in the State of Sergipe (northeast Brazil) has recently started. Two new cassava genotypes—hybrids resistant to several pathotypes of *Phytophthora*—were selected from a series of candidate test genotypes. Farmers across the State are now adopting the new materials.

The project consists of several phases: identifying the problem, studying the pathogen, making a preliminary selection of resistant cassava genotypes in the greenhouse, and, finally, on-farm selection of the best genotypes.

In a diagnostic survey, farmers, including women, were interviewed, their fields were visited, and samples of diseased plants collected. This study revealed some underlying problems: farmers were not rotating their crops; they were unaware of the advantages of using good-quality, uninfected stakes; and they were exchanging stakes between farms, making disease control more difficult.

After observing the problems in the field, farmers and scientists collaborated in a plan involving different activities. The causal agent of the disease was isolated and identified in the laboratory, and its genetic diversity and virulence spectrum determined. *Phytophthora*'s high variability, observed by farmers as well as scientists, makes it possible to target clusters of genetic material to certain local pathotypes of the pathogen.

In the greenhouse, many young plantlets of local cassava genotypes and hybrids were inoculated with several pathotypes of *Phytophthora*.

Thirty genotypes with high levels of tolerance of most pathotypes were identified (to ensure broad resistance), then multiplied, and taken to farmers for testing under field conditions. These genotypes were distributed according to their adaptation to different agroecological zones. Farmers were also advised on the importance of planting uninfected stakes.

To ensure variability of cassava genotypes among the different municipalities, farmers in each ecozone planted six candidate genotypes that had been selected to suit the farmers' local needs. These were planted in infected fields so that the farmers could compare them with their own local genotypes. The scientists and farmers agreed on a set of criteria that farmers would use to score the plants by visual evaluation during the different stages of development.

Laboratory, greenhouse, and fieldwork continue to be carried out in a complementary way to solve this important problem.

Activity 3. Participatory plant breeding in the Colombian Amazon

A participatory diagnostic survey, based on pictographic sheets and field visits, was conducted in Mitú, Vaupés, Colombia. *Phytophthora* root rot was identified as the major constraint by cassava farmers, mostly indigenous women.

Local pathotypes of the pathogen were isolated from affected *chagras* (farm plots), then identified, and used in greenhouse inoculation trials to select cassava genotypes that were tolerant of the disease. From 430 genotypes from Brazil, Ecuador, Venezuela, and Colombia, eight resistant genotypes and hybrids were selected as tolerant, not only of local pathotypes but also of pathotypes from different cassava-growing regions in Colombia. These resistant cultivars were evaluated and harvested by farmers from four different communities for local selection at Mitú. The participatory research methodology was used. The genotypes eventually selected were adapted to the Amazonian conditions, and were high-yielding with good starch quality under the traditional, indigenous, conditions.

In the first year, an open evaluation was conducted. The criteria for selection by the women were identified and a field book designed for further evaluations to be conducted at Mitú. To evaluate the vegetative stage of the crop, the women most used the criteria vigor, healthiness, plant height, number of stakes per plant, and early maturity. They evaluated the harvested crop according to yield, starch content and quality, cutting (i.e., planting material) production, healthiness, and early maturity.

The women selected both sweet and bitter cassava genotypes developed at CIAT: CM 2772-3 (low cyanide and yellow pulp) and CG 165-7 (high cyanide and white pulp). The local genotypes 'Yuca de Abeja' (high cyanide and white pulp) and 'Mirití' (high cyanide and yellow pulp) were also chosen. (**Table 3.1**)

Table 3.1. Agronomic characteristics of genotypes evaluated in indigenous "chagras" in Mitú, under participatory research methodologies.

Genotypes	Origin	Pulp color	Food roots	Root rot %	Yield (Ton/ ha)	HCN[a]	# Branches	Cuttings per plant	% Dry matter	% Starch	Preference[b] Harvest	Preference[b] Starch
CM 2772-3	CIAT	Yellow	8.9	0.0	5.4	7	2.0	3.9	29.9	28.1	9.5	8.5
Mirití	Mitú	Yellow	14.3	0.0	7.7	8	2.3	4.0	31.8	29.7	8.0	8.0
M Bra97	CIAT	White	9.2	0.0	3.5	6	1.3	2.9	33.9	31.8	7.5	7.5
CM 523-7	CIAT	White	22.7	1.5	7.9	7	3.6	8.7	35.0	32.8	6.0	8.3
Abeja	Mitú	White	14.0	0.0	3.8	8	1.3	6.0	29.9	27.8	7.0	7.0
M Bra 532	CIAT	White	33.0	0.0	10.7	7	3.8	7.8	34.5	32.4	7.8	6.0
M Bra 71	CIAT	White	19.7	0.2	7.6	8	3.0	5.9	30.7	28.6	6.0	7.7
M Ven 25	CIAT	White	19.8	0.6	6.0	8	2.1	5.2	34.5	32.3	6.9	6.8
CG 165-7	CIAT	White	11.9	0.0	4.6	7	3.1	3.8	32.3	30.2	5.1	8.0
Blanca	Mitú	White	14.3	0.0	11.1	8	2.7	6.7	34.1	32.0	7.0	6.0
CG 402-11	CIAT	White	12.8	4.3	3.9	6	2.4	4.7	28.0	26.0	5.3	7.6
Abiyú	Mitú	Yellow	41.0	0.0	8.9	8	2.5	3.0	32.1	30.0	7.2	5.0
Lapa blanca	Mitú	White	27.5	3.6	17.3	9	0.5	10.0	31.7	29.6	6.4	5.0
M Bra1044	CIAT	Yellow	15.6	0.0	6.8	7	2.8	7.0	36.2	34.1	5.8	4.5
M Arg6	CIAT	Cream	6.0	0.0	3.0	7	1.8	1.3	35.0	32.8	2.5	6.0
Wasaí	Mitú	Yellow	4.0	0.0	3.7	8	0.7	3.7	32.1	30.0	5.0	3.0
Brava Blanca	Mitú	White	66.7	0.0	19.9	7	3.0	16.7	33.9	31.7	-[c]	4.0
Brava Amarilla	Mitú	Yellow	43.3	0.0	12.2	8	4.0	10.0	32.4	30.4	-	2.0
Low cyanide variety	Mitú	White	6.4	1.7	8.1	6	3.1	7.8	30.6	28.5	7.0	4.5
White native varieties[d]	Mitú	White	30.6	1.1	8.1	8	1.9	9.8	32.4	30.3	6.6	7.0
Yellows native varieties[d]	Mitú	Yellow	25.7	0.0	5.1	8	2.4	5.2	32.1	30.0	7.0	4.0

[a] Cyanide content: 1 = low; 9 = very high.
[b] Preference scale: 1= low; 10 = high.
[c] - = Not determined.
[d] White or Yellow native varieties average

At Cucura, an Indian settlement, a field day brought together 85 women farmers from eight communities close to Mitú. The farmers from the four communities who participated in the case study shared their experiences and trial achievements with the other farmers. That the field day was successful was demonstrated by the interest shown by the nonparticipant communities in the participatory evaluations and in possibly adopting the cassava genotypes being demonstrated during the field day.

Activity 4. Participatory plant breeding in the Colombian departaments of Cauca, Valle and Quindío

A participatory research approach was successfully used to develop effective disease management for controlling cassava root rots in the Colombian departments of Cauca, Valle del Cauca, and Quindío.

Eight field experiments were established in the departments Cauca, Quindío and Valle, on different agroecological zones were root rots are endemic. The experiments were conducted with farmers and extension officers using a participatory approach, **(Table 4.1)** showed treatments currently evaluated. The potential control practices were evaluated initially in the greenhouse. Previous results demostrated that potasium chloride application reduced significantly disease severity on young plantlets inoculated with the most aggressive *Phytophthora* isolate. In addition, stakes germination was not affected by thermotherapy.

Table 4.1. During 1998 and 1999 12 field experiments were established to evaluate the control of Phytophthora Root Rot disease in Colombia.

			Validation of control practices to reduce Phytophthora Root Rot[1]									
Department	**Municipality**	**Experiment**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Cauca	Santander de Quilichao	1	1 7	-	+	1	-	-	-	-	-	-
		2	1	2	+	1	-	+	+	+	+	+
Cauca	Buenos Aires	1	2	-	+	1	-	+	+	+	+	+
Valle	Caicedonia	1 and 2	4	2	+	1	+	+	-	+	+	+
Quindío	La Tebaida		4	1	+	1	+	+	+	+	+	+
	Montenegro		3	1	+	1	+	+	+	+	-	-

[1]A = Resistance (number of genotypes),
B = Hot water treatment, immersion of cassava stakes at 49°C for 49 min
C = Chemical treatment, immersion of stakes in 3 g/L Ridomil (Metalaxyl), for 5 min.
D = Biological treatment , immersion of stakes in 1 X 10^6 conidias/ml *Trichoderma* spp. (isolate 14PDA-4) for 30 min and application of 50 ml of this suspension/plant to the soil.
E = Biological treatment, immersion of stakes in MICOBIOL HE suspension for 30 min and application of 50 ml of suspension/plant to the soil. MICOBIOL HE is a mixture of micoorganisms (*Trichoderma* spp., *Bacillus thuringiensis, Beauveria bassiana, Metarhizium anisople, Paecilomyces lilacinus, Paecilomyces fumosoroseus, Nomureae rileyi, Entomophthora muscae, Hirsutella thompsonii* and *Verticillium lecanii*).
F = Potassium chloride (50 or 100 kg/ha)
G = Potassium sulphate (50 or 100 kg/ha)
H = Agropremix , chemical mixture containing (per Kg): 150 gr nitrogen, 100gr phosphate, 120gr zinc, 20gr boron, 7.5gr copper, 30gr sulphate, 1gr molybdenum and 2gr silicon.
I = Sucromag: wine less with magnesium
J = Sucrocal: wine less with calcium

At Santander de Quilichao 16 cassava genotypes were evaluated for resistance to Phytophthora Root Rot. These genotypes were selected previously in the greenhouse for resistance to several *Phytophthora* isolates. M Col 72 was the only genotype that did not perform well, because is not adapted to this agroecological zone. M Arg 6 and M Arg 9 were highly susceptible to root rots. These two varieties were also scored as susceptible in greenhouse evaluations. M Bra 1045 and

M Bra 383 did not show any symptoms of this devastating disease. In the same infected field, stakes of the local variety *Algodona* (M Col 1522) were treated with the biocontrol agent *Trichoderma* spp. and did not present any diseased plants. No differences in yield were observed comparing chemical and biological treatments.

Contributors – Sub-output 1 – Phytopathology

Elizabeth Alvarez

Sub-output 2. Sustainable Bean Production Systems: Developing IPM Components.

Activity 5. IPM development with small scale farmers

CIAT and NARS scientists are engaged in developing IPM strategies with small holder farmers to generate strategies that are more compatible with their production circumstances. The initial focus in this is often on genetic resistance where available, and supported with other cultural practices.

Rationale

IPM approaches are essential for bean stem maggot and bean foliage beetle management as tolerance alone is insufficient to combat the pest and its damage. However, improved adoption of IPM strategies would require a better understanding of the problem by farmers and complex decision on their resource allocation. Therefore participatory research approaches are needed to evaluate options.

IPM strategies for BSM management in small holder farmers in Western Kenya

Method

Cultural strategies for BSM management were tested with farming communities in Kisii, western Kenya. These included: 1. seed dressing with conventional chemicals; 2. earthing up, 3. mulch, 4. farm yard manure and di-ammonium phosphate at half the recommended rates. These are strategies that are reported to reduce BSM infestations or enhance the tolerance potential of susceptible plants against the pest. Farmers were encouraged to suggest other technologies. Such technologies were discussed and selections made among them. Farmer technologies selected for testing were; rotation with sweet potatoes (a common practice in the area), and farmers' practice (i.e. without any of the above treatments). Two bean varieties "Red Haricot" and "GLP-2" were used to determine varietal interaction with the treatments. The trial was farmer managed.

Results and discussion

Apart from the seed treatment with "Murtano" (lindane and thiram), none of the other treatments reduced BSM infestation significantly below that of the control (farmers' practice) **(Table 5.1).** For reasons that are not currently well understood, rotation with sweet potatoes reduced BSM infestation significantly below several of the other treatments. This will be investigated in future trials. Red Haricot suffered less mortality compared with GLP-2. However, they all enhanced plant tolerance to attack; the effect the combined half rates of farmyard manure and DAP enhanced plant vigour and tolerance to BSM attack and increased yield significantly above the control. Mulch and earthing up created extra labour but did not increase yields and were less preferred by the farmers.

Table 5.1. Effect of various cultural practices on BSM infestation and plant performance.

Treatment	BSM infestation/plt	% Plant mortality due to BSM	Yield/plot
Chemical seed dressing	2.6 ab	7.9 ab	573.9 ab
1/2 DAP + 1/2 FYM	2.2 ab	4.6 bc	840.0 a
Earthing up	2.0 ab	12.7 a	341.6 b
Mulch	2.3 ab	7.8 ab	323.6 b
Rotation with sweet potato	1.8 b	5.8 bc	500.1 b
Control	4.0 a	11.8 a	490 5 b
$LSD_{(0.05)}$	2.1	5.6	272.8

Results and discussion

Aphids, bean stem maggots, bruchids and foliage beetles were identified by the farmers as the key pests that constrain bean productivity in their fields. They were able to identify their importance in relation to season as well as their sequence within season. They observed that early planted beans are more attacked by bean foliage beetles than late planted crops. Bean stem maggots were more important in the second season than in the first and aphids attacked in both seasons but usually appeared late in the season. Once the problem and its development was understood farmers came up with possible solutions. Experiences from elsewhere were used to boost farmer knowledge and expand options for management. During the early parts of the season, an army worm outbreak enabled farmers to test some of their concoctions on this pest. The combination of neem leaves, ash and pepper protected maize against army worm attack. Both the neem leaf infusion and leaves of *Vernonia* sp. protected beans from foliage beetle damage but cow urine was ineffective against this pest. Improved bean varieties were distributed to the farmer research groups.

Activity 6. Dissemination of IPM information to NARS

Rationale

The lack of adequate sharing of information leads to costly duplication of research efforts across network countries.

Method

Posters on IPM strategies for BSM, BFB and storage pests developed by CIAT, farmers and the extension service in northern Tanzania were multiplied on CDs and distributed to NARS in the Bean Networks. The materials were designed in Microsoft Word and were set up for easy translation into local languages without disruption to the illustrative materials. Dissemination strategies through the individual national extension systems were discussed at Network Steering Committee meetings and a Bean Entomologists Working Group meeting held early this year.

Results and discussion

The posters were translated into Kiswahili by the extension Service in Tanzania and they are looking for funds for greater dissemination. The Bean Program in Malawi is also in the process of translating the IPM information into the local language for greater dissemination.

Contributors – Sub-output 2 – Entomology

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Sub-output 3. Disease Management Component and IPM Strategies and Tactics for Beans in Africa.

Activity 7. Characterization of pathogen diversity of *Phaeoisariopsis griseola* in Africa

Rationale

Angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* is the most widespread and important bean disease in Africa causing an estimated annual yield loss of 374,800 tones. The use of host resistance in managing the disease is the most effective and practical strategy for the majority of the poor resource farmers. However, deployment and usefulness of resistance can be adversely affected by occurrence of pathogen variability in *P. griseola*. Recent studies based on virulence and molecular characterization of isolates from Africa show that in addition to the occurrence of Mesoamerican and Andean pathogen groups, there also occurs an Andean sub-group known as the Afro-Andean. These findings are significant because the sub-group infects Mesoamerican differential cultivars, which are usually resistant to the typical Andean group. Characterization of pathogen diversity of *P. griseola* and its distribution therefore continued with the objective of extending coverage to new areas and countries.

Methods

Isolates from Madagascar were characterized for the first time using virulence methods. More isolates from Uganda, the Democratic Republic of Congo (DRC), and Malawi were also characterized. A total of 24 isolates were characterized. Virulence phenotypes of isolates were characterized based on a set of 12 bean differentials where 6 belong to the Andean and 6 to the Mesoamerican genepool.

In collaboration with IP-1 project, some aspects of molecular characterization of *P. griseola* were initiated at Kawanda. More emphasis was given to developing and establishing DNA extraction capacity and procedures that adapted to conditions and facilities in standard laboratories common in a number of national research institutions in the region.

Results and Discussion

On the basis of virulence, the six isolates from Madagascar could be grouped into three races all belonging to the Mesoamerican pathogen genepool **(Table 7.1)**. They infected at least 4 Mesoamerican differentials in addition to the Andean ones. Some of those from the DRC and Malawi belonged to Andean (infecting Andean differentials only) and others to Mesoamerican pathogen genepool. Some isolates from DRC (Z4, Z11 and Z15) and Uganda (KAB.1N, KAB.2N and KIS.1R) gave reactions associated with Afro-Andean group. Characterization of these isolates continues using molecular methods to establish occurrence and importance of the Afro-Andean group. For the moment detection of Afro-Andean races require comparison of molecular and virulence characterization. The differential MEX 54 was resistant to all isolates characterized. This has been the case with most African isolates and shows the potential of the

variety as a source of resistance to most races of *P. griseola* identified so far. Work has been initiated to determine the nature of resistance in MEX 54.

DNA extraction from *P. griseola* was successfully done on 60 isolates using modified protocols in a standard laboratory. Molecular characterization of these isolates is reported elsewhere.

Table 7.1. Virulence diversity of *P. griseola* in Madagascar, Malawi and Zaire.

Isolate Identification	Origin[y]	Host seed type	Race	Phenotypic reaction on differential cultivars[x]											
				Andean						Mesoamerican					
				A	B	C	D	E	F	G	H	I	J	K	L
M3	MWI		30-0		b	c	d	e							
Z1	DRC		61-0	a		c	d	e	f						
Z6	DRC		63-1	a	b	c	d	e	f	g					
Z15	DRC		5-4	a		c						i			
Z11	DRC		31-4	a	b	c	d	e				i			
KAB-1N	UG		62-4		b	c	d	e	f			i			
KAB-2N	UG		63-5	a	b	c	d	e	f	g		i			
KIS.1R	UG		63-6	a	b	c	d	e	f	g	h				
Z4	DRC		55-35	a	b	c		e	f	g	h				l
M1	MWI		51-38	a	b			e	f		h	i			l
Z12	DRC		63-38	a	b	c	d	e	f		h	i			l
Z5	DRC		0-39							g	h	i			l
M4	MWI		25-39	a			d	e		g	h	i			l
MDR1	MD		31-39	a	b	c	d	e		g	h	i			l
MDR2	MD		63-39	a	b	c	d	e	f	g	h	i			l
Z2	DRC		63-39	a	b	c	d	e	f	g	h	i			l
M2	MWI		53-55	a		c		e	f	g	h	i		k	l
2M	MWI		63-55	a	b	c	d	e	f	g	h	i		k	l
KAB-2	UG		63-55	a	b	c	d	e	f	g	h	i		k	l
KIS-1	UG		63-55	a	b	c	d	e	f	g	h	i		k	l
MDR4	MD		63-55	a	b	c	d	e	f	g	h	i		k	l
MDR5	MD		63-55	a	b	c	d	e	f	g	h	i		k	l
MDR6	MD		63-55	a	b	c	d	e	f	g	h	i		k	l
MDR7	MD		63-55	a	b	c	d	e	f	g	h	i		k	l

[x] = CIAT *P. griseola* differentials A = Don Timoteo; B=G 11796; C = Bolon Bayo; D = Montcalm; F = Amedoin; E = G 5686; G = PAN 72; H = G 2858; I = Flora de Mayo; J = MEX 54; K = BAT 332; L = Cornell 49-242.

[y] = Origin of *P. griseola* isolates: MD - Madagascar DRC = Democratic Republic of Congo; MWI = Malawi; UG = Uganda.

Activity 8. Course on simple molecular methods to characterize pathogen diversity of key bean pathogens

Rationale

Characterization of disease pathogens is important in order to develop effective management strategies. However, some pathogens are difficult or slow to characterize using morphological or pathogenic characteristics and require time-consuming laboratory and/or greenhouse tests. For

example, characterizing *P. griseola* by virulence method is often slow and limits the number of isolates that can be handled. Whereas, development of molecular methods has increased the number of options and tools that are faster and specific in studying pathogens, their use in Africa is limited. The latter is not only blamed on lack of expertise and facilities, but also on the common belief that this can only be done in better equipped or sophisticated laboratories. Given the generally good correspondence between molecular and virulence methods for some bean pathogens, there are opportunities and interest to develop expertise and adapt some of the molecular methods to facilities and conditions found in some of the national programme laboratories. The objective of this course was therefore to

1. Introduce simple molecular tools for characterizing bean pathogens
2. Facilitate characterization of on-going studies on bean pathogen diversity in Africa
3. Initiate a process of adapting and sharing research responsibilities between ECABREN and SABRN countries and advanced laboratories in the use and application of molecular tools.

Methods and Materials

A four-day course supported by ECABREN, SABRN and PABRA was held at Kawanda Agricultural Research Institute (KARI) in Uganda. There were eight participants (4 men and 4 women) consisting of pathologists from Burundi, Democratic Republic of Congo, Ethiopia, Kenya, South Africa, Tanzania and Uganda. Designed to be a hands-on type exercise, the primary emphasis was on DNA extraction from *P. griseola*, *Colletotrichum lindemuthianum* and *Xanthomonas campestris* pv. *phaseoli*a. PCR amplification procedures were also demonstrated and tried out. DNA extraction was based on modified procedures to adapt to available facilities.

Results and Discussion

Successful extraction of DNA using relatively unsophisticated facilities helped to build confidence among participants and change the widely held notion that biotechnology is only possible in advanced labs. The course also generated lots of interest among participants for molecular tools. They were also confident to put in practice procedures learnt with little or no extra facilities in their institutions. Some laboratories may not be self-sufficient but there was optimism of sharing facilities among departments and programmes in the different institutions represented. However modest support for expendable supplies is necessary to initiate and facilitate application of these tools in pathogen characterization. Besides a follow-up training to extend this biotechnology adaptation to PCR analysis and marker assisted selection in breeding was considered necessary. This should find wide application in the multiple constraint resistance projects supported by the bean networks. A training manual was developed and used for the course.

Activity 9. Studies to determine characteristics associated with root rot resistance against Pythium root rot

Rationale

Pythium root rot is the most import soilborne disease of beans in some of the main bean growing areas of east and central Africa. A number of the popular and commercial varieties are susceptible. Though very few in number, some varieties have been identified to have good levels of resistance and therefore offer potential for genetic improvement of susceptible varieties. Studies started last year to determine characteristics associated with resistance against Pythium root rot continued.

Methods

Twenty entries selected from previous evaluations were assessed under screenhouse conditions for root and shoot parameters associated with root rot resistance. These included root biomass, root length, shoot weight and disease severity on the hypocotly and roots. These parameters were compared in non-infected and artificially infected soils. In addition, seedling emergence, plant stand one month after germination and any other character observed were noted. Entries were further reduced to 8 and evaluated in detail. CAL 96 and RWR 719 were used as susceptible and resistant checks respectively.

Results and Discussion

There was no difference among the entries and/or between the two soil environments for plant emergence. There was significant difference in root rot severity between the susceptible cultivar, CAL 96 (8.9) and the resistant entries **(Figure 9.1a)** in infected soil. Differences among some of the resistant cultivars were also significant and varied between 3 and 6 using the CIAT scale of 1 to 9. All entries showed virtually no symptoms in non-infected soil. Severity of root infection was a good measure to distinguish between susceptible and resistant cultivars and also between infected and non-infected soils.

Root length was significantly reduced on CAL 96 and on two other cultivars in infected soil while there was no difference in 4 varieties, and was significantly longer on AND 1064 and RWR 719 (**Figure 9.1b**). The little or no effect observed on most resistant cultivars indicate that root rots least affect this parameter. On the other hand root weight on the susceptible variety (CAL 96) and most resistant varieties was significantly reduced in infected soil comparing to non-infected soil (**Figure 9.1c**). Of interest were entries MLB-17 and RWR 719, where there was no reduction in root weights in infected soil implying minimal damage of roots.

With the exception of MLB-17 and RWR 719, there was significant reduction in shoot weight with all entries in infected soil (**Figure 9.1d**). The percentage reduction varied with different entries but reduction in root weight seemed to be associated with reduction in shoot weight. These results show that disease severity is a good measure of resistance. However, the percentage reduction in root weight appeared to be associated with severity. Entries with little or no

reduction in root weight (MLB-17, RWR 719) had also better levels of resistance. There are however some exceptions.

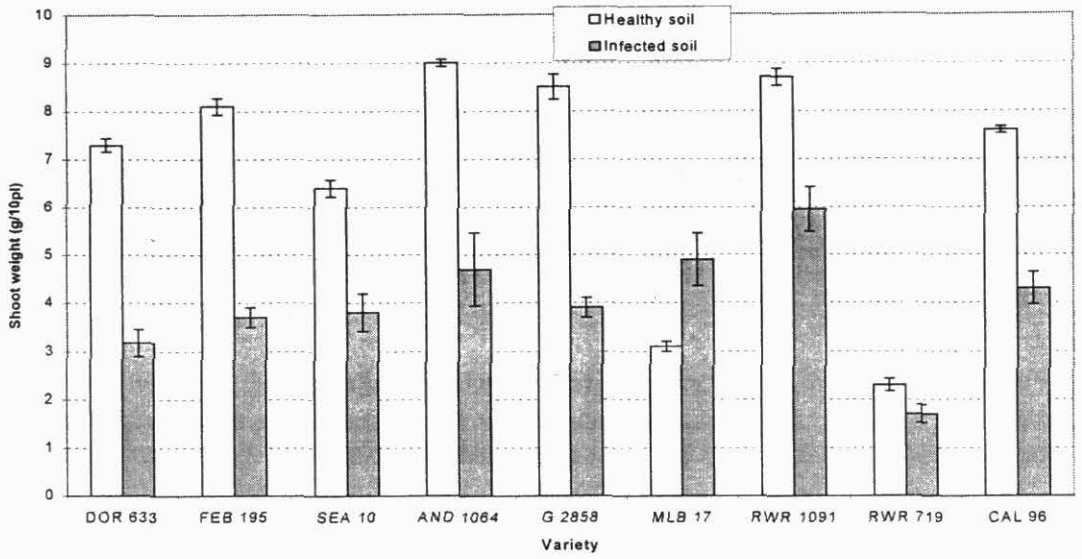

Figure 9.1a. Effect of Pythium root rot on disease severity on susceptible (CAL 96) and tolerant cultivars. Bars indicate standard errors of means. Greenhouse, Uganda, 1999.

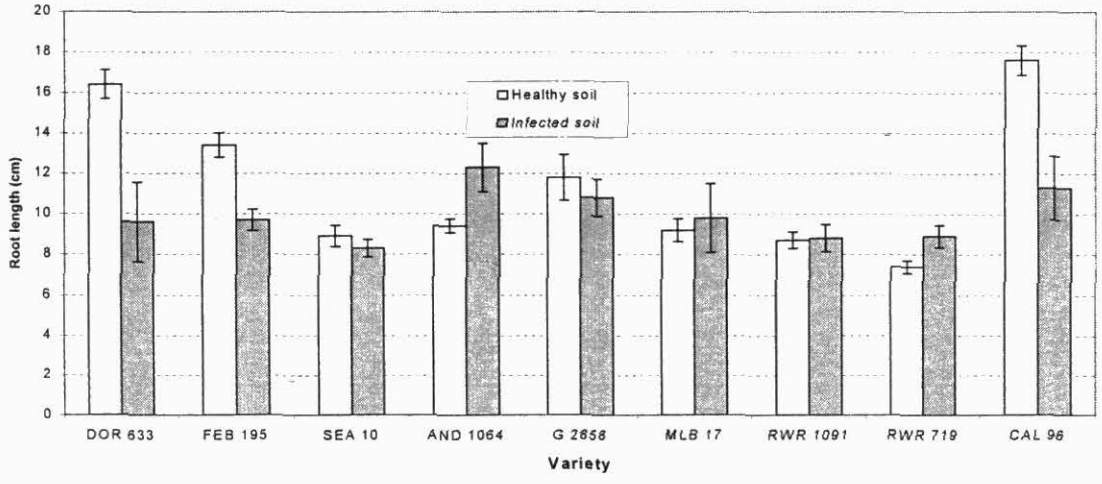

Figure 9.1b. Effect of Pythium root rot on root length of susceptible (CAL 96) and tolerant cultivars. Bars indicate standard errors of means. Greenhouse, Uganda, 1999.

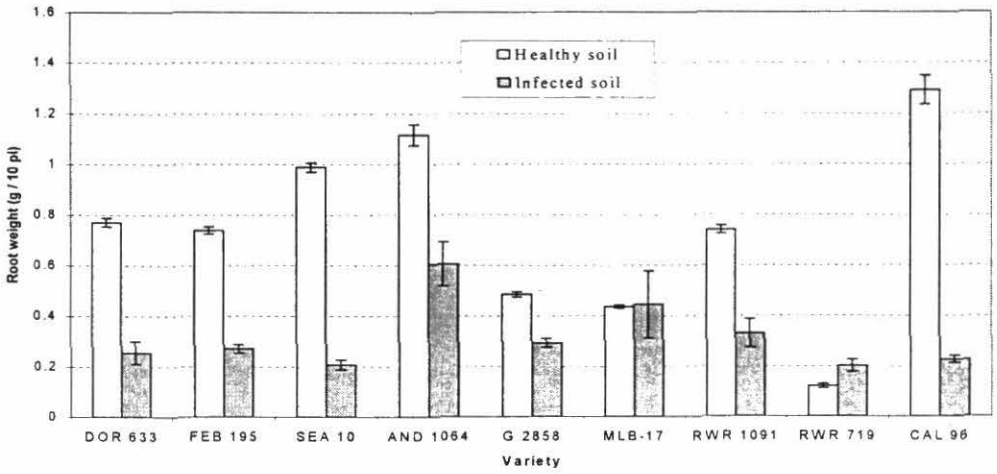

Figure 9.1c. Effect of Pythium root rot on root weight of susceptible (CAL96) and resistant cultivars. Bars indicate standard errors of means. Greenhouse, Uganda, 1999.

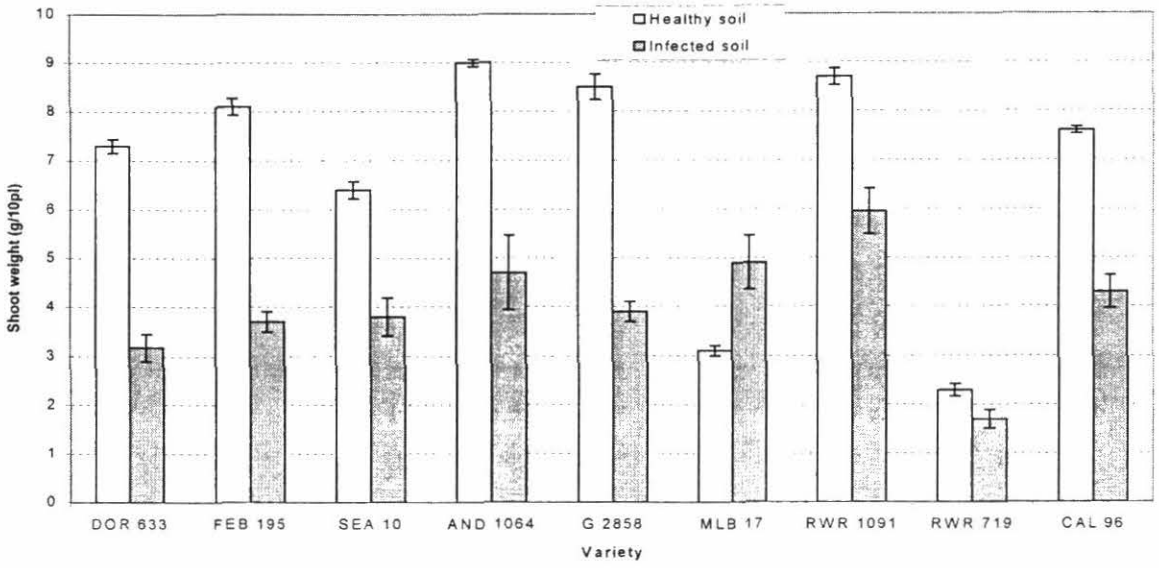

Figure 9.1d. Effect of Pythium root rot on shoot weight of susceptible (CAL 96) and tolerant cultivars. Bars indicate standard errors of means. Greenhosue, Uganda, 1999.

Activity 10. Identification of lines with resistance to *Pythium* spp

Rationale

Our past studies have shown that very few materials grown by farmers or germplasm evaluated under greenhouse conditions show good levels of resistance against Pythium root rot. On the other hand host resistance has been demonstrated to be an important component of IPM. It is because of this that evaluation of diverse types of germplasm against Pythium root rot continues to identify new sources of resistance for germplasm improvement or direct use by farmers.

Methods

A total of 622 entries were evaluated in a screenhouse under artificial inoculation. Entries evaluated included germplasm from CIATs' Core Collection (213), sources of resistance of anthracnose (70), entries from BILFA- IV (72), Rwandan germplasm (169) collection made in 1995 under the Seed of Hope (SOH) project for Rwanda, components of mixtures obtained from southwest Uganda (Kisoro) and IBN-96 (98). These materials represented both bush and climbing beans and a diverse seed characteristic.

Results and Discussion

Out of a total of 622 entries 1 was resistant, 28 (4.5%) gave intermediate reactions while 95 % were susceptible. The identity and some of the characteristics of resistant and intermediate entries are given on **Table 10.1**. Eight entries were selected for inclusion in the Regional Root Rot Nursery, which now total 68. These and previous results highlights the limitation of solely relying on varietal strategy in the management of Pythium root rots.

Table 10.1. Origin, seed types and growth habits and reaction of the best entries from the Lamb collection to Pythium root rots in the screenhouse, Kawanda, 1998.

Entry	Nursery	Seed Type	Growth Habit	Reaction
AFR 619	BILFA	M	1	4.3
CIM9313-1	BILFA	S	1	5.1
CIM9314-4	BILFA	M	1	3.9
DFA 54	BILFA	M	1	5.9
RWR 1946	BILFA	M	1	4.0
RWR 2075	BILFA	L	1	3.9
UBR(95)2	BILFA	S	1	3.7
MS1/3	SOH			6.0
199/4	SOH			5.9
G 11088	CC	M	4	5.5
G 731	CC	M	4	5.7
G 9871	CC	M	3	6.0
G 10944	CC	M	5	5.9
G 14241	CC	M	5	4.5
G 11037	CC	M	5	4.9
G 2620	CC	L	5	6.1
G 3936	CC	S	3	5.4
G 10997	CC	S	3	5.8
G 2205	CC	M	3	5.0
G 10950	CC	L	3	6.0
G 9872	CC	M	3	5.8
MX9065-3-M	IBN-96	M	BUSH	5.6
LP90-15	IBN-96	M	BUSH	3.7
FM-M-38-1	IBN-96	M	BUSH	3.6
MX9065-3-A	IBN-96	M	BUSH	4.5
ICTAJU95-19	IBN-96	S	BUSH	5.2
MX8754-22T	IBN-96	G	BUSH	5.5
LM93204453	IBN-96	M	BUSH	3.0
CB9021806	IBN-96	S	BUSH	3.6

Contributors – Sub-output 3 – Phytopathology

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Sub-output 4. IPM Tactics and Strategies for Virus Diseases Developed.

Activity 11 & 12. Safe movement and clean planting material as a control strategy for cassava frogskin disease

Cassava frogskin disease (CFSD) is an increasing problem in the cassava germplasm in the CIAT farm. The principal method of dissemination is infected stem cuttings. The whitefly *B. tuberculata* is an inefficient insect vector. To reduce CFSD, it is strategically important to develop a low cost cassava certification procedure that can be adopted by the farmers groups. The system is designed to reduce pathogens that are propagated in cassava stem cuttings. The following guidelines are for use at CIAT.

Certification Protocol and Standard field procedures

1. The roots of all cassava plants harvested at CIAT must be inspected for pathogens. Stem cuttings will only be taken from plants without any visible FSD symptoms. For plants with symptoms of FSD, the vegetative material must be destroyed.

2. It is recommended to disinfect tools with 2.5% sodium hypochloride.

1. All vegetatively propagated cassava will be analyzed for the presence of FSD and before entering into the certified disease free areas.

2. For control of Pseudomonas, Phytophthora, and Xanthomonas, stem cuttings will be subjected to a heat treatment.

3. Only cassava stem cuttings from the certified fields will be available for experiments outside of CIAT.

The certification scheme is part of a two-year plan to reduce the incidence of CFSD on the CIAT campus to a very low level. CFSD is endemic in the region and this will not eliminate the disease. Evidence from epidemiology experiments show that the rate of spread of CFSD is relatively slow and using clean planting materials is a workable strategy to control losses due to the disease.

The experience of running a center wide certification strategy to reduce CFSD will be the basis to work with NARS, NGOs and farmer groups to develop projects to produce and disseminate clean vegetative propagation materials.

Contributors – Sub-output 4 – Phytopathology

R. Buruchara

OUTPUT III. NARS CAPACITY TO DESIGN AND EXECUTE IPM RESEARCH AND IMPLEMENTATION STRENGTHENED.

Edgloris Marys received training in the virology laboratory while working on viruses of papaya on a UN University fellowship for four months.

One or more scientists from Ana Lidia Echemendia INISAV-Cuba, Nicolas Savion, INRA-Guyana, Guillermo Sibaja Ch. CATIE-Costa Rica, Orencio Fernandez & Jose Guerra IDIAP-Panama, Elsa Barrientos EAP-Honduras and Nubia Murcia Riaño CORPOICA-Colombia, Claudio Nunes UTLA-San Salvador & Leopoldo Serrano UNI.DEL SALVADOR were trained in the RAPD-PCR technique used to distinguish between the biotypes of *B. tabaci.*

Contributor – Output III - Virology

Lee Calvert

OUTPUT IV. GLOBAL IPM NETWORKS AND KNOWLEDGE SYSTEMS DEVELOPED

Sub-output 1. Project on sustainable integrated management of whiteflies as pests and vectors of plant viruses in the tropics.

Research

In the 1998 Annual Report, we outlined the Phase 1 start-up project of CGIAR Whitefly IPM Project, which has been funded by the Danish International Development Agency (Danida). Phase 1, the diagnostic phase, of the Project has been completed in Latin America and Africa.

Severe damage is being caused, throughout the pantropics, by the whitefly-transmitted viruses. One of the major results of the diagnostic phase has been the mapping and documentation of new geminiviruses and the increasing spread, or emergence, of existing geminiviruses, indicating the continuing threat that they pose to tropical and sub-tropical agriculture. An example of this is the geminivirus complex associated with tomatoes in the Neotropics. Tomatoes are grown throughout the tropical, subtropical and temperature zones of the Americas. Polston and Anderson (1997) have shown that in the early 1970s, three tomato viruses were reported from the Americas, one each from Brazil, Venezuela and Mexico (**Figure 1.1**). By the 1990s more than 17 viruses have now been described, at least two of which are reported in the USA (**Figure 1.2**). This is a dynamic situation with new viruses continually emerging (Polston and Anderson 1997).

In addition to the threat of emerging virus diseases on tomatoes, numerous other crops— including cotton, beans, cucurbits, potato, pepper, melons and soybeans— suffer from geminiviruses transmitted by whiteflies. Morales (1999, **Table 1.1**) has documented the emergence of these geminiviruses in Latin America since the beginning of the century, providing insight into the mechanisms driving disease emergence.

Table 1.1. Emergence of whitefly transmitted geminivurses in Latin America (Morales 1999).

Year	Disease	Country	Host
1935	Cotton Common Mosaic	Brazil	Cotton
1941	Bean Dwarf Mosaic	Brazil	Common Bean
1946	Infectious Chlorosis	Brazil	Malvaceae
1954	Cotton Leaf Crumple	Mexico	Cotton
1958	Infectious Chlorosis	Brazil	Tomato
1961	Bean Golden Mosaic	Brazil	Common Bean
1965	Bean Crumpling	Brazil	Common Bean
1965	Tomato Golden Mosaic	Brazil	Tomato
1966	Tomato Yellow Mosaic	Venezuela	Tomato
1971	Golden Yellow Mosaic	Puerto Rico	Lima Bean
1971	Golden Yellow Mosaic	Puerto Rico	Common Bean
1971	Chino del Tomate	Mexico	Tomato
1977	Squash Leaf Curl	Mexico	Cucurbits
1985	Tomato Yellow Mosaic	Venezuela	Potato
1988	Bean Calico Mosaic	Mexico	Common Bean
1989	Sinaloa Tomato Leaf Curl	Mexico	Tomato/Pepper
1989	Serrano Golden Mosaic	Mexico	Tomato/Pepper
1989	Pepper Mild Tigre	Mexico	Tomato
1989	Tomato Yellow Mottle	Costa Rica	Tomato
1991	Tomato Yellow Leaf Curl	Dominican R.	Tomato
1991	Tomato Yellow Vein Streak	Brazil	Tomato
1995	Tomato Mottle	Puerto Rico	Tomato
1996	Texas Pepper	Mexico	Pepper
1997	Taino Tomato Mottle	Cuba	Tomato
1998	Tomato Havana	Cuba	Tomato
1998	Tobacco Leaf Crumple	Colombia	Tobacco
1998	Melon Yellowing	Colombia	Melon
1998	Soybean Yellow Mosaic	Colombia	Soybean


Known Distribution of Tomato Geminiviruses
in the Americas, in the early 1970s
June 1997
MEXICO
Sinaloa
VENEZUELA
BRAZIL
Sao Paulo
Chino del Tomato Virus
(CdTV)
Tomato Yellow Mosaic Virus
(TYMV)
Tomato Golden Mosaic Virus
(TGMV)

Figure 1.1.


Known Distribution of Tomato Geminiviruses
in the Americas, in the mid-1990s
June 1997
USA
Texas
MEXICO
Sinaloa
Coahuila
Tamaulipas
Guanajuato
Morelos
Chiapas
Q. Roo
Florida
CUBA
DOMINICAN REPUBLIC
PUERTO RICO
JAMAICA
GUADELOUPE
MARTINIQUE
TRINIDAD
& TOBAGO
VENEZUELA
GUATEMALA
HONDURAS
NICARAGUA
COSTA RICA
BRAZIL
Minas Gerais
Sao Paulo
PHV, TPV
TPV
CdTV, PHV, TPV, SGMV, STLCV
CDTV, PHV, TPV
PHV
CdTV
TPV, TomGV1, TomGV2
TomGV1
ToYMoV
ToMoV
TYLCV, TToMoV
TYLCV
TYLCV, PYMV
ToMoV, PYMV
PYMV
PYMV, TYMV
BZ-Ig, BZ-Ub
ToYVSV

Figure 1.2.

Another virus disease that is threatening the region is cassava mosaic disease (CMD), caused by several geminiviruses transmitted by *Bemisia tabaci*. CMD is reported causing crop losses of 18-40% from all African cassava-producing countries. None of the CMD viruses have been found in the Neotropics. Until recently, the *B. tabaci* biotypes found in the Americas did not feed on cassava, and it was speculated that the absence of CMD was related to the inability of its vector (*B. tabaci*) to colonize cassava. Since the early 1990s, however, a new biotype (*B*) of *B. tabaci*, has been reported feeding on cassava in the Neotropics (Bellotti et al. 1999). Given the presence of this *B* biotype, CMD poses a serious threat to cassava production as most traditional varieties are highly susceptible to the disease. CIAT has already introduced CMD-resistant cassava germplasm from Africa and has crossed these with Neotropical varieties in anticipation of its potential introduction.

The full results and analysis from 5 of the 6 sub-projects are currently being prepared for publication. The results will be published in a traditional book format and also electronically on the World Wide Web home page that is being developed for the CGIAR Whitefly IPM Project.

Donor partners

Successful completion of a Project with the geographical, scientific, and management scope as broad as that of the Whitefly IPM Project, will require support from a team of donor partners. The Coordination Team of the Whitefly IPM Project, and the Management Team of the Coordinating Center (CIAT) have been successfully pursuing additional project funding and other linkages, with the objective of constructing a constellation of donors to support the global Whitefly IPM Project. The donor partners who have joined the Whitefly IPM Project, in addition to Danida, are outlined below. Figure 3 illustrates how these new projects and linkages are contributing to the overall Work Breakdown Structure.

The Austrialian Center for International Agricultural Research (ACIAR) has approved a project to begin a Phase 1 diagnosis and characterization for whiteflies as pests and vectors in 8 Asian countries. This project, which is just beginning field work, is parallel to and geographically complementary to the Danida Phase 1 project for Latin America and Africa - making the global Whitefly IPM Project truly pantropical. The ACIAR-funded project also brings the Asian Vegetable Research and Development Centre (AVRDC) more actively into the global Whitefly IPM Project, as a co-coordinator for this sub-project along with the CSIRO of Australia.

The United States Agency for International Development (USAID) Collaborative Research Grants Program approved a grant to CIAT (1998-2000) for studies on biological control of whiteflies pests by indigenous natural enemies. The principal objective of this project is to continue exploration of indigenous parasitoids of the major whitefly species in mixed cropping systems of South America, where cassava is a major component of the cropping system. The Whitefly IPM Task Force identified Whiteflies as Pests and Vectors in Cassava as one of the priority areas for study. However, in the Danida-funded Phase 1 project, we prioritized and proposed work on whiteflies as vectors of cassava mosaic disease (CMD) in Africa. This USAID-funded project brings the cassava pest component in South America back into the global

Project, and builds on the preliminary characterization that CIAT has already been done for whitefly pests of cassava in South America.

The New Zealand Ministry of Foreign Affairs and Trade (MFAT) has granted a project (1998-2000) to CIAT on 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 incorporation into improved African, Latin American and Asian germplasm.

The MFAT funding is complementary to a Rockefeller grant currently held by CIAT and IITA for Saturation of the Genetic Map of Cassava with PCR-based Markers and the Use of the Genetic Map in the Improvement of Cassava. The Rockefeller-funded research is mapping genes for resistance to the African cassava mosaic virus (ACMV) and developing molecular markers for their incorporation into improved African, Latin American and Asian germplasm. These last three projects mentioned (USAID, MFAT, Rockefeller) are supporting the Whitefly IPM Project activities to develop and disseminate cassava germplasm that is resistant to whitefly pests and whitefly-transmitted viruses.

The USAID Office of Foreign Disaster Assistance (OFDA) granted special funds (1998-1999) to IITA for the Emergency Programme to Combat the Cassava Mosaic Disease Pandemic in East Africa, and the funding (1999-2000) has just been renewed. 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 programme to multiply and disseminate mosaic resistant cassava. The cassava mosaic disease (CMD) epidemic in Eastern Africa has been of particular concern to the Whitefly IPM Project for humanitarian reasons. Over the past 5 years, Uganda has suffered starvation-related deaths due to the cassava mosaic disease epidemic. The situation remains serious and is spreading into neighboring countries, particularly Kenya and Tanzania.

In August 1999, CIAT signed a Scientific Cooperative Agreement with the Unites States Department of Agriculture - Agricultural Reasearch Service (USDA-ARS).

Collaboration between the Agricultural Research Service (ARS) and CIAT will be undertaken to link ARS' national research on whitefly IPM with the CGIAR Whitefly IPM Project, and to carry out research on the epidemiology of vector-borne plant viruses aimed at generating the operational knowledge necessary to develop control strategies for newly emerging crop pests and diseases, specifically the whiteflies and whitefly-transmitted geminiviruses.

Research partners

With the support of the donor partners, the research network has expanded to include: more than 50 NARS institutions in 30 countries (12 in Latin America, 10 in Africa, and 8 in Asia; 8

advanced research organizations in the United States, the United Kingdom, Germany, Australia and New Zealand; and 5 International Agricultural Research Centers (CIAT, IITA, ICIPE, AVRDC and CIP).

The Project has also made linkages with REDCAHOR (Collaborative Network for Research and Development of Vegetables in Central America, Panama and the Dominican Republic), an AVRDC-IICA initiative with support from the International Development Bank (IDB). REDCAHOR's main objective is to promote the creation of a regional network for horticultural crops, with emphasis on tomatoes, peppers, onions and, cucurbits. The Whitefly IPM Project participated in REDCAHOR's 1st IPM planning workshop. REDCAHOR representatives undertook a prioritization exercise which concluded that the most important regional IPM research project is the development of horticultural crop germplasm that is resistant to whitefly-transmitted geminiviruses. As a result of previous work by CIAT, IITA and NARS partners, resistant germplasm has been identified and is being used in breeding programs for resistance to for bean geminiviruses, cassava whiteflies, and African cassava mosaic virus. Regional programs for developing virus-resistant vegetable germplasm have been the weakest. REDCAHOR will now take on this activity, with their funding. The Whitefly IPM Project, with leadership from the AVRDC coordinators, will collaborate with information and advising.

Contributor – Sub-output 1 - Entomology

Pamela Anderson

Sub-output 2. Developing networks and partnerships in ipm for threatening pests and diseases.

The CGIAR "Whitefly IPM Project" demonstrates CIAT's capacity to link international and national (NARS) partners with universities and research institutes in a collaborative effort to solve complex regional and global problems, which cannot be effectively addressed by institutions working independently. This Project also focuses much of its attention on threatening insect pests and plant diseases.

In addition to whiteflies and whitefly-transmitted viruses, CIAT is advantageously positioned geographically, culturally and scientifically to monitor and carry out innovative research on an array of invasive pests across a wide range of crops and agroecosystems. CIAT has both a germplasm development and natural resource management base that includes a research portfolio in integrated pest and disease management. In addition CIAT has formed more than 20 research and development partnerships with nearly all countries in Central and South America and the Caribbean basin. Examples include:

- Regional collaborative Bean Program for Central America, Mexico and the Caribbean
- Cassava Biotechnology Network
- Pan American Network for Cassava Improvement
- Caribbean Rice Industry Development Network

Examples of other threatening pests include rice stripe necrosis virus, the rice mite, *Steneotarsonemus spinki*, the rice thrips, *Stenchaetotrips biformis* and *Thrips palmi*. CIAT scientists, are also involved in diagnostic surveys in regional cropping systems such as beans, vegetables, cassava, tropical pastures, rice, ornamentals, flowers and oilpalm.

Rice virus

During the early 1990s the rice stripe necrosis virus (RSNV) was detected for the first time in Colombia (Morales and Sanint 1998). The origin of the virus appears to be from the Ivory Coast of West Africa, where it was first observed in 1977. It now infests most rice growing countries in West Africa. RSNV can severely damage recently germinated rice, resulting in estimated production losses of 10-30%. Symptoms include foliage striping, severe plant malformation and seedling death. Most RSNV outbreaks are associated with periods of water stress (drought) before planting time (CIAT 1998). Molecular characterization of the virus is being carried out at CIAT.

The vector of RSNV is unique in that it is a soil-borne pathogen of the genus *Polymynxa* (possibly *P. graminis*). The virus—which can be disseminated by fungal-contaminated seeds, irrigation water or agricultural equipment—now infects the principal rice production areas of Colombia. CIAT is monitoring the movement of RSNV, which at present is reported only from Colombia although it is speculated that it may already be in other rice-growing countries. Germplasm screening methods have been developed and research to develop resistant varieties has been initiated at CIAT (CIAT 1998).

Whiteflies

Arecent introduction into the region of a whitefly species from Africa is causing concern. The spiraling whitefly, *Aleurodicus dispersus* (Russell), is now reported feeding on bananas, cassava and possibly other crops in Costa Rica and Colombia (Castillo 1996). *A. dispersus* was first reported feeding on cassava in Nigeria, the Ivory Coast and Benin (Neuenschwander 1994). This pest is presently being monitored to evaluate crop damage as well as host range. A parasitoid of the genus *Encarsia* has been observed parasitizing *A. dispersus* in Colombia; and *E.. haitiensis* (Dozier) and *E. guadeloupae* Viggiani are reported parasitizing it in Benin (D'Almeida et al. 1998).

A USAID-funded collaborative project between CIAT and the University of Florida was started in 1998 to determine the complex of indigenous neotropical parasitoids associated with the whitefly complex found on cassava, beans, cotton and selected horticultural crops. Surveys have been initiated in Northern South America (Colombia, Venezuela and Ecuador) in order to select the best potential natural enemies for continued research and to compare the efficiency of indigenous species to that of exotic whitefly parasitoids being recommended for introduction into the region.

Preliminary results show that there is a species-rich parasitoid complex associated with the numerous whitefly species collected from the aforementioned crops **(Tables 2.1 and 2.2).**

Several of the parasitoid species are unrecorded and in the process of being identified (Evans and Castillo 1998). For this reason, parasitoid species in tables 2 and 3 are identified only to genera, pending taxonomic confirmation from University of Florida taxonomists.

Rice mite

The phytophagous mite *Steneotarsonemus spinki* Smiley (Tarsonemidae) is reported as an important pest of rice in several Asian countries, including Taiwan, Philippines, Japan and China (Chow et al. 1980). The species was first collected in 1960, on *Sogata orizicola* Muir, a plant hopper pest of rice, in Baton Rouge LA, USA (Smiley 1967) lending to some question as to the actual origin of the species. After these initial report, only minimal information on this pest has appeared in the literature and it appears that it did not cause severe damage to the rice crop.

However, in 1997 it was reported causing significant damage to rice in Cuba (Ramos and Rodríguez 1998), and subsequent reports from the Dominican Republic and Haiti, indicate that the pest is causing considerable damage resulting in yield losses. It is suggested that there has been a second introduction into the area, this time on vegetables from China.

S. spinki attacks and damages new rice growth, causing necrosis and reduction in grain formation. Rice grains are partially filled, empty or hallow, and stained or blemished. In Cuba populations averaging 200 mites per cm^2 have been detected in rice fields, significantly reducing yield potential (Ramos and Rodríguez 1998). In addition, the Japanese have identified an association between *S. spinki* and virus particles similar to that of rice tarsonemid mite virus (RTMV). It is also reported that *S. spinki* can vector the fungal pathogen (*Acrocylindrium oryzae*) that causes grain rotting, and the micoplasma *Spiroplasma citri* (Chow and Liu 1985).

CIAT scientists who have been monitoring this situation are in contact with collaborators in the region. CIAT's extensive collection of natural enemies of phytophagous mites could prove useful in managing this pest. Research, including host plant resistance and biological control using phytoseiid predatory mites, are planned should *S. spinki* reach Colombia.

Thrips

Two important thrips species (Thysanoptera: Thripidae) have been introduced into Colombia and Venezuela in recent years; one is becoming a serious pest of rice, and the second is causing damage on a wide range of hosts.

The rice thrips *Stenchaetothrips biformis* (Bagnall) was first reported from Venezuela in 1995 (Cermelt et al. 1995) and has since spread into Colombia and Guyana, where it is causing considerable concern among rice producers. The origin of *S. biformis* appears to be Southeast Asia and is reported as a rice pest in India, Pakistan, Nepal, Japan and China.

Table 2.1. Survey of Whiteflies and Associated Parasitoids on Several Crops in three Colombian Departments.

Department	Host	Whitefly species	Parasitoids	Total pupae	Emerged Parasitoid
Atlántico	Tomato	*Bemisia tabaci*	*Encarsia*	11	8
			Eretmocerus		1
			Signiphora		1
		Bemisia tabaci	*Encarsia*	168	49
			Euderomphale		2
			Metaphycus		1
Caldas	Cucumber	*T. vaporariorum*	*Encarsia*	75	25
			Amitus		45
	Tomato	*T. vaporariorum*	*Amitus*	85	3
Córdoba	Cotton	*Bemisia tabaci*	*Eretmocerus*	70	44
			Encarsia		5
	Egg Plant	*Bemisia tabaci*	*Encarsia*	69	24
Valle	Beans	*T. vaporariorum*	*Encarsia*	1336	24
			Amitus		866
	String Bean	*T. vaporariorum*	*Encarsia*	208	122
			Amitus		2
	String Bean	*T. vaporariorum*	*Encarsia*	645	74
	Tomato	*T. vaporariorum*	*Eretmocerus*	9	1

Table 2.2. Whitefly species and their associated parasitoid complex collected on cassava from three geographical regions of Colombia.

Area	Whitefly species	Parasitoid species
Atlantic Coast	*Aleurotrachelus socialis*	*Encarsia sp.*
		Eretmocerus sp.
	Bemisia tuberculata	*Encarsia sp.*
		Eretmocerus sp.
		Metaphycus sp.
	Trialeurodes sp.	*Encarsia sp.*
		Eretmocerus sp.
	Tetraleurodes sp.	
Valle del Cauca	*Aleurotrachelus socialis*	*Encarsia sp.*
		Eretmocerus sp.
	Bemisia tuberculata	
Cauca	*Aleurotrachelus socialis*	*Encarsia bellottii*
		Eretmocerus sp.
		Signiphora aleyrodis
	Bemisia tuberculata	*Encarsia pergandiella*
		Eretmocerus sp.
		Euderomphale sp.
		Signiphora aleyrodis
	Trialeurodes sp.	*Encarsia hispida*
		Encarsia pergandiella
		Eretmocerus sp.

S. biformis attacks both irrigated and upland rice, infecting mostly young plants. It causes yellowish lines or silvery streaks on the leaves; and plants become stunted, wilted, necrotic and may die. *S. biformis* is reported feeding on grasses, maize and millet. Attacks are severer on irrigated rice, and older plants suffer less damage.

Thrips palmi Karny: This species has now become established in several countries in South and Central America, including Colombia and Venezuela. The pest is native of the Malaysia-Indonesia area and is now found in many areas of Asia, Africa and the Americas. *T. palmi* has a wide host range, attacking more than 50 plant species. In Colombia it has become a serious pest of cut flowers and ornamentals as well as legumes and solonaceae crops (Vergara 1999).

At CIAT we are presently evaluating numerous species of predator mites (Phytoseiidae) for biological control of thrips and mite pests. Of the numerous phytoseiid species evaluated in laboratory studies, *Neoseiulus cucumeris* has given the most positive results for control of *T. palmi*. When feeding on *T. palmi*, it has the highest fecundity of any predator species evaluated on any of the phytophagus mite hosts. *N. cucumeris* also had very high oviposition and consumption rates feeding on *T. palmi*. *N. cucumeris* is also reported predating on *T. palmi* on eggplant in South Florida (Castineiras et al. 1997).

CIAT has done extensive exploration and surveys of predator mites in the Neotropics (Bellotti et al. 1994). Collections have been made from more than 2400 sites (Bellotti et al. 1987), primarily on cassava but also on other crops. Considerable species biodiversity has been identified; nearly 80 phytoseiid species have been studied and evaluated. Colonies of 15 phytoseiid species are maintained at CIAT and are available for biological control programs. Several species have already been introduced into Africa (Yaninek et al. 1993), Brazil (Bellotti et al. 1999) and Europe. A taxonomic key is being prepared in collaboration with a Brazilian colleague.

CIAT has traditionally researched four commodity crops, rice, beans, cassava and pastures. More recently, CIAT's research has expanded into additional cropping systems, especially in the area of integrated pest and disease management. We are currently involved in research projects with fruits, vegetables, cut flowers, oil palm and ornamentals and others. Examples of this research include:

- Powdery mildew of roses, caused by *Sphaerotheca pannosa* var. rosae
- Bacterial blight of orchids, caused by *Erwinia chrysanthemi*
- Wilting root and crown rot of gerbera, caused by *Phytophthora cryptogea*
- Bud rot disease of oil palm, caused by *Ceratocystis paradoxa* and *Phytophthora* spp.
- Diagnosis of arthropod pest problems of asparagus
- Exploration for potential natural enemies of the Colorado potato beetle, *Leptinotarsa decemlineata* in Northern and Western South America
- Study of the bioecology of the spittlebug species complex in contrasting environments (mass rearing techniques, phenology, IPM components and management strategies).

It is evident that several diseases, viruses, and insect pests pose serious threats for neotropical food crops, ornamentals and oil palm, among others. There is an ever-growing need for coordinated regional and global efforts to study these pests and diseases before they become

widespread. Among the IARCs, CIAT is in an excellent position to assume a leading position of this nature. There are well-prepared staff with expertise in these areas as well as in GIS, biotechnology, laboratory facilities, already established networks that can be built upon, a project-oriented focus that facilitates collaboration of this nature, and good donor relations. CIAT, because of its unique geographic location and historic focus and activity in the Caribbean region can play a major role as the first line of defense against invasive pests.

Contributors – Sub-output 2 - Entomology

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