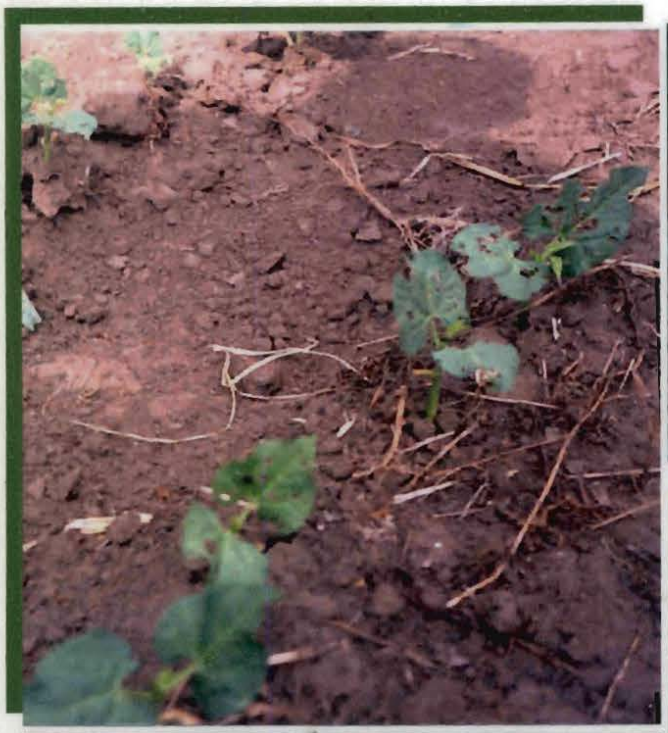
INTEGRATED PEST AND DISEASE MANAGEMENT IN MAJOR AGROECOSYSTEMS



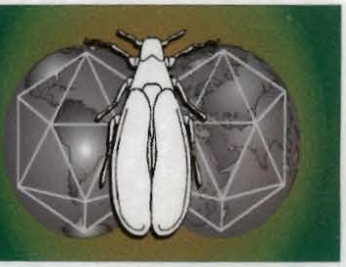
CIAT

PROJECT-PE1

Annual Report 2001

System-wide Programme on

Integrated Pest Management



SUMMARY ANNUAL REPORT

2001

PROJECT PE-1

INTEGRATED PEST AND DISEASE MANAGEMENT IN MAJOR AGROECOSYSTEMS

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

SUMMARY ANNUAL REPORT 2001

PE-1 PROJECT

Title: Integrated Pest And Disease Management In Major Agroecosystems

Inputs:

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Budget:

LINE ITEM	TOTAL US$	Proportion
Unrestricted	331,351	32.0%
Unrestricted carryover	76,100	7.3%
Sub-Total	407,451	39.4%
Unrestricted Sustitution		
Carryover		
Sub-total	0	
Restricted	627,965	60.6%
Total Project	**1,035,416**	
All sources:		
Personnel	526,944	50.9%
Operations	508,472	49.1%
Total	**1,035,416**	
Number of IRS	2.70	
Number of NRS	14.43	
Total personnel	**17.13**	

Research Highlights in 2001

Research highlights are presented in two sections, the first part consists of short narrative that relates a brief description of important achievements that have taken place in the project activities.

The second section presents additional highlights in the shortened "bullet" form that points to specific results in research and management of biotic stresses.

Biological control of cassava green mite

The cassava green mite (CGM) is an important pest of cassava in the Americas and Africa. Biological control of CGM with phytoseiid mite predators shows considerable promise and is being evaluated in Colombia and Africa. It was found that densities of CGM (*Mononychellus tanajoa*) prey have a significant influence on the behavior and reproductive development of phytoseiid predator mites. Prey consumption by *Euseius ho*, *Typhlodromalus aripo*, and *T. tenuiscutus* continued increasing up to the highest prey densities evaluated. Prey densities also had a significant influence on the number of eggs oviposited by all phytoseiid species. *Galendromus annectens, Neoseiulus californicus* and *N. idaeus* were the most efficient predators at converting their prey into progeny. In conclusion, it appears that among the predatory species studied, when *M. tanajoa* population increases markedly or during an outbreak, the use of *E. ho, T. aripo* or *T. tenuiscutus* species should be recommended. In contrast, when the mite population is

low on cassava, the use of *N. californicus, N. idaeus* or *G. annectens* should be better because they may be able to multiply more rapidly.

Development of an artificial diet for the cassava burrower bug

The use of artificial diets can provide a better knowledge of the nutritional requirements of various insect species. They can also be used to bioassay the activity of allelochemicals against insect pests to determine factors involved in resistance mechanisms in host plants. Field and laboratory studies indicate that the cassava burrower bug, *Cyrtomenus bergi*, reacts to different cassava root contractions of HCN, as well as possible other root allelochemicals. An artificial diet has been developed to rear *C. bergi* through the adult stage. This liquid diet can now be used to test potentially active molecules, such as HCN on *C. bergi.*

Using Molecular Markers to Identify Cassava Mealybugs

The mealybugs are important cassava pests in Latin America and Africa. *Pseudococcidae herreni* and *P. manihoti* are morphologically similar, and it is difficult distinguish between these species. Populations of *P. herreni* and *P. manihoti* were tested using randomly amplified polymorphic DNA (RAPD) analyses, and molecular markers that rapidly identify each species were developed. This is additional evidence that *P. herreni* and *P. manihoti* are distinct species, and that the African and Latin American populations of *P. manihoti* are closely related. This new detection method is an important diagnostic tool. For example, if *P. herreni* would be introduced into Africa, this method would be able to confirm its presence thereby allowing corrective measures including the introduction of natural enemies to begin on a prompt and scientific basis.

IPM in forage grasses: spittlebug bioecology for advancing management in Neotropical pastures

Despite a high pest status and long history in the Neotropics, an effective and coordinated program for the integrated management of spittlebugs in forage grasses does not yet exist. Among the challenges are a poor basic understanding of biology and ecology at the species and family level, a high diversity of insect/host/habitat associations, and IPM tools that are rudimentary or absent. To overcome these limitations we have completed a 5-year diagnostic phase where we sought to (1) acquire new bioecological information on this pest complex and the family Cercopidae, (2) develop five contrasting ecoregions in Colombia as model sites for advancing the diagnosis and management of spittlebugs, and (3) develop and evaluate research methodologies and technologies to promote higher quality research from NARS. The pest complex in each ecoregion (Cauca River Valley, Caribbean Coast, Orinoquian Piedmont, Amazonian Piedmont, South Pacific Coast) was characterized through comparative biological and population ecology studies to establish the patterns of variation in biology, behavior and ecology, fundamental for advancing management by tailoring control tactics to the diverse habitats, regions and production systems where spittlebugs are economically important. The biology and habits of nine previously unstudied species was described, representing the genera *Aeneolamia, Mahanarva, Prosapia* y *Zulia*. The population dynamics of the pest complex in 4/5 ecoregions was studied over 2-4 years to describe variation in certain components of population ecology at the level of farm, region and year. The degree of variation in

certain aspects such as oviposition sites (soil, litter, plant stem), duration of the life cycle (45-75 days), and number of generations per year (3-6) is relevant to pest management. In general, compared to regions with high and continuous humidity, regions with low and seasonal rainfall can be characterized by lower diversity of spittlebugs, lower incidence of natural enemies, and more pronounced population fluctuation and synchrony. In seasonal systems management strategies should therefore focus on spatial and temporal detection of initial outbreaks to target control tactics designed to suppress focal populations. In less seasonal systems, management strategies should focus on cultural tactics to reduce habitat quality, and there are more perspectives for the use of biological control.

Among the diverse IPM components advanced in parallel to these basic studies was the evaluation of fungal entomopathogens as biological control agents. A diverse collection of 77 strains isolated from spittlebugs was established. Initial screenings identified highly promising isolates that are being characterized for virulence across different life stages and species of spittlebugs and for deployment in recently initiated field trials to establish application and evaluation techniques in two ecoregions. Given our new and detailed understanding of spittlebug bioecology, we expect to overcome the challenges and limitations of previous studies and serious assess the biological control potential of fungal entomopathogens for spittlebug management in pastures.

Indigenous Amazonian communities increase cassava yields and quality through improved crop management practices

Nine indigenous communities in Mitú (Vaupés, Colombia) located in the area around the road Mitú – Monfort are composed by 250 families that cultivate more than 450 ha of cassava. The increase of indigenous population and its dependence of the road and town have caused a pressure on forest, with the reduction of time for adequate forest regeneration. As consequence, soil fertility has decreased, while ants and cassava root rots have increased. Other crop problems include the reduction of cassava cutting quality. In order to improve the sustainability of cassava and associated crops established in the *chagra*, trials were conducted applying participatory research tools with evaluation by women, where soil amendments were applied, consisting in ash, organic matter from dead leaves and a mixture 1:1 of both materials. In addition, stem cutting selection was done, choosing healthy material from the middle stem.

Cassava yield was increased by soil amendment with ash mixed with organic matter by 44.6%, while adding ash alone increased yields 27.2 %. With cutting selection, the yield increased 36.6%. Compared to traditional management, cassava root rots were reduced on 100 %, 94.2% and 89.7%, by application of a mixture of ash and organic matter, ash alone and cutting selection, respectively.

Cassava varieties from CIAT, resistant to root rots, chosen by women applying participatory research methodologies in 1998, are being adopted by indigenous persons from different communities not only in communities close to the road but also in settlements close the river Vaupés. With these varieties indigenous persons have improved quality and quantity of starch and better foods prepared from roots.

Developing cassava resistance to cassava frogskin disease

Although the causal agent of cassava frogskin disease (CFSD) remains elusive, the search for resistance is surpassing our expectations. Six years ago, the decision was made to test the entire CIAT cassava core collection for resistance to cassava frogskin disease (CFSD), and tolerance was found to be widespread in cassava. We have identified more than 100 CFSD tolerant lines. Many of these are resistant to other pests and diseases. Some of them have high yields and produce good stem cuttings for the propagation of the crop. Besides being sources of resistance in breeding programs, several of these may be suitable to release as cassava varieties for those areas most affected by CFSD.

Developing Sequence Characterized Amplified Regions (SCARs) to identify whiteflies

In previous studies RAPD markers were developed and used to distinguish between *B. tabaci* biotype A, *B. argentifolii* (biotype B) and *T. vaporariorum*. Since they amplified multiple products, the RAPD markers are often difficult to interpret. We have developed sequence characterized amplified regions (SCARs) for each of these whiteflies. The simplicity of this test permits laboratories with PCR capability to use these primers to distinguish between these whiteflies. Because there is no easy way to distinguish between the indigenous whitefly *B. tabaci* biotype A and the introduced *B. argentifolii* (biotype B), the achievement of this milestone is an import advance that now can be used by our partners throughout the region.

Management of* Pythium *root rot of beans in Africa

Several species of *Pythium* occur in soil as pathogens, saprophytes or potential bio-control agents. In east and central Africa, Pythium root rot is an important soil-borne disease of beans and management efforts developed are aimed at reducing the soil pathogen population, one of the key factors that influences occurrence and severity. To assess effectiveness of management options, there is need to first develop tools and methods to facilitate rapid, accurate and reliable detection assays and quantification of the pathogen in soil. Reverse Dot Blot Hybridization (RDBH) is a diagnostic technique (based on species-specific oligonucleotides designed and blotted onto a membrane array) that has been developed to detect most known species (in the temperate) of *Pythium*. Its use requires to first characterize representative *Pythium* species and inclusion (if any) of novel strains on the membrane array. Given the wide genetic variation within the *Pythium* genus, some strains (pathogenic or beneficial) particularly in the tropics could be novel

Over 100 isolates (from Kenya, Uganda and Rwanda) were characterized by amplifying DNA templates with primers which target the ITS regions of the ribosomal genes and specific to *Pythium* spp. The PCR fragments were sequenced and compared to sequences of known *Pythium* species. Sequence analysis of the isolates characterized identified 12 *Pythium* species. Out of the four species known to infect beans, two (*Pythium ultimum* and *P. irregulare*) were identified, with *Pythium ultimum* var *ultimum* being the most prevalent. New putative species having sequences significantly different from their closest match among the neo (type) strains were observed, implying that they could be novel. Two isolates were characterized as *Pythium oligandrum* a known potential biological control agent against a number of soil borne pathogens including *Pythium*

species. More sampling and characterization is underway to increase both coverage and representation.

No one has monopoly over good ideas: experiences in participatory IPM development with farmers in northern Tanzania

Technologically sound and effective integrated pest management (IPM) strategies are often not adopted because farmers' production circumstances are frequently not well understood, or sometimes neglected in the generation and packaging of technologies. To ensure that IPM technology is appropriate to farmers' production circumstances we have initiated a participatory technology generation process with farmers and other stakeholders in agricultural production.

Bean foliage beetle (*Ootheca* spp.) is a pest that defoliates bean seedlings in Africa; the larvae also feed on roots and poach nodules. It is a pest that is gaining in importance in many of the bean production environments in the region and farmers are beginning to abandon beans in favour of other crops as they fail to control the pest. Farmers from Masama in Hai District, northern Tanzania requested for assistance to diagnose and develop solutions to this pest that was constraining bean production in the area. It was observed that farmers often did not understand the pest adequately to take appropriate preventive or management action. We engaged them in participatory surveys, problem diagnosis and monitoring of pest biology and ecology. The understanding of the pest biology and ecology enabled them to identify appropriate opportunities for its management. These opportunities focussed on cultural strategies, including the use of botanical pesticides, timely planting, crop rotation and post-harvest tillage. These were further evaluated with the farmers, who were then able to select strategies that they felt were compatible with their production circumstances. Detailed researcher-designed comparisons confirmed the efficacy of many of these products to be as good as a commercial neem oil formulation.

The result of this process has been a boost in farmers' confidence such that they are revisiting their traditional resources for more technologies to address their production problems. It has also enabled them to contribute ideas in research and technology dissemination. The process is spreading as farmers take it upon themselves to discuss and brainstorm for problem solving strategies on their own. Already farmers in Hai District have started a program on a local radio program for the dissemination of IPM.

Systemwide Whitefly IPM Project

This Systemwide Project, coordinated by CIAT and carried out under the umbrella of the Systemwide Program on Integrated Pest Management, was presented to the plenary session at International Centers Week in Washington, D.C. October 2000. In addition to presenting the advances achieved by the Project (reported in the previous PE-1 Annual Reports), the presentation communicated to the CGIAR plenary that funding was not yet available for advancing the SP-IPM Whitefly Project to Phase 2.

As a consequence of the ICW2000 plenary talk, DFID's Rural Livelihoods Department communicated to CIAT in April 2001 it's intent to provide core funding for Phase 2 of

the Project in Africa and Latin America. Discussions have concluded, project proposal and budget have been presented. Phase 2 of the SP-IPM will become operational as soon as the Project Documentation is approved in the UK, which should be before the end of the 2001 calendar year.

First reported outbreak of the whitefly, Bemisia afer in the Neotropics

The first outbreak of *Bemisia afer* sens. Lat. In an agricultural situation in the Americas is reported on sweetpotato (*Ipomoea batatas*) in the Cañete Valley in the Central Coast of Peru. *B. afer* is also an important pest of cassava in East Africa and therefore could threaten the cassava crop in the neotropics. It is a wide spread species feeding on a wide variety of hosts in the Mediterranean Region, the Middle East, Africa, South East Asia and Australia.

Additional Highlights

Output I

Cassava Arthropod Pests

- Surveys in Venezuela have confirmed three whitefly species feeding on cassava, *Aleurotrachelus socialis*, *Bemisia* sp. and *Trialeurodes* sp. These surveys have also identified seven parasitoid species associated with the three whitefly species. Two species *Euderonphate* sp., and *Metaphycus* sp. are new recordings for Venezuela.
- Surveys for cassava whiteflies and associated parasitoids have been completed in Colombia, Venezuela and Ecuador. The greatest species richness for both whiteflies and their parasitoids was found in Colombia. Five whiteflies and 11 parasitoid species were identified feeding on cassava in Colombia. Several appear to be new or unrecorded species that are being named by taxonomists.
- The cassava hornworm, a serious pest of cassava, can be efficiently controlled by the use of a naturally occurring baculovirus. This baculovirus, previously unavailable to, or not easily accessible to cassava producers, is now being formulized for the commercial market. Through a joint agreement between CIAT, Biocaribe, a commercial biopesticide company and MADR (Colombian Ministry of Agriculture and Rural Development), an economic, environmentally safe, commercial product will be marketed during early 2002. Product evaluations done at CIAT with a wettable powder and oil based formulation, resulted in a 90% mortality of hornworm larvae.
- Evaluations of isolates of fungal entomopathogens has identified several isolates for potential control of cassava whiteflies. Mortality in adult pathogenicity studies reached an average of 88% for four fungal isolates.
- Isolates of the fungal entomopathogens *Metarhizium anisopliae, Beauveria basssiana* and *Paecilomyces liliacenus* have demonstrated pathogenic abilities on the cassava burrower bug *C. bergi*. Fifth instar nymphal mortality with certain isolates reached 100%, 17 to 20 days after treatment in laboratory studies.

Tropical Pasture Arthropod Pests

- Comparative biological studies completed for two spittlebug species (*Mahanarva andigena*, *Prosapia simulans*).
- Studies initiated to describe the population dynamics and phenology of *Prosapia simulans*, a newly detected *Brachiaria* pest in the Cauca Valley.
- A two-year study on the population dynamics and phenology of *Zulia carbonaria* in pastures of the Cauca Valley completed.
- Second year of data gathered on the early season population dynamics of spittlebug nymphs and adults in three ecoregions to measure the correlation between phenology and rainfall and to gauge potential to predict the timing of outbreaks.
- Host plant water stress and age were found to have no detectable effect on the incidence and duration of diapause in *Aeneolamia varia* eggs.
- Documented an increase in the incidence of egg diapause towards the end of the dry season in field populations of spittlebugs from two seasonal sites with unimodal precipitation; incidence of diapause was minimal throughout the year in a site with bimodal precipitation.
- An artificial diet validated as effective for maintaining adult *Aeneolamia varia*.
- Strengthened the collection of fungal entomopathogens of major insect pests which now includes 73 isolates from cassava pests (burrower bugs, stem borers, whiteflies) and 77 from forage grass pests (spittlebugs)
- Eighteen fungal entomopathogen isolates were screened for virulence to burrower bug nymphs and adults showing high levels of virulence in certain strains.
- Determined that virulence of fungal entomopathogen isolates varies among spittlebug species.
- Protocols established for determining LC_{50} and LC_{90} of fungal entomopathogen isolates to spittlebug nymphs.
- Field studies initiated in two contrasting regions to test the number and timing of applications of a formulated fungal entomopathogen product to suppress spittlebug populations.
- New information on the bioecology and management of spittlebugs shared in a 5-day workshop for sugar cane entomologists in Guatemala.
- Reference collection of 675 papers on the spittlebugs and the superfamily Cercopoidea strengthened for conversion to an on-line bibliographic database.

Cassava Diseases

- RAMS and AFLP were applied to *Sphaceloma manihoticola*, the causal agent of cassava super elongation disease and optimum conditions for PCR amplification were resolved.
- Genetic variation in the Brazilian population of *Sphaceloma manihoticola* was very limited compared with that of Colombia. There was a high correlation between geographical origin (country and municipality) and genetic variation.

Bean Diseases

- Sixty-six Pythium isolates from Uganda were characterized using RFLPs, and 38 by sequencing the ITS1 region of ribosomal DNA. The latter gave better results and grouped 38 isolates into 9 species
- Forty-six isolates of *Fusarium solani* f. sp. *phaseoli* from Uganda were characterized in two broad groups using cultural, pathogenicity, and AFLPs. Two primers that can distinguish the two groups were designed, synthesized and evaluated.

- Extensive collection of Pythium samples was initiated in Kenya, Uganda and Rwanda. Ten Pythium species were identified from 50 isolates from the three countries by sequencing the ITS and ITS2 regions of the ribosomal DNA. New putative Pythium species were also identified.
- Extensive collection of *Phaeoisariopsis griseola* samples from beans was initiated in Kenya, Uganda and Rwanda. Nine isolates were identified as Mesoamerican, 6 Andean and 1 Afro-Andean using host differential interactions.
- Genetic variation of *Phaeoisariopsis griseola* in bean varietal mixtures was assessed using Random Amplified microsatellites

Output II

Cassava Arthropod Pests

- A survey of cassava procedures in the Valle del Cauca and Cauca Departments indicate that they do not employ a uniform criteria in cassava crop management. At least 20 different varieties are grown, 13 different fertilizers and five herbicides are applied. A complex of nine cassava pests were identified including hornworms, mites, leafcutter ants, shooflies, thrips, whiteflies, white grubs and burrower bugs. Pest frequency was higher on smaller plantations (< 5ha.).
- Preliminary results with biological and chemical pesticides indicate that cassava whitefly control will be difficult and may be costly. Confidor (Imidacloprid) was the only product that gave effective control of whitefly nymphs in field experiments with cassava farmers.

Plant Diseases

- Bud Rot disease progress reduced by disease management in oil palm in Colombian Eastern Plains.
- New alternatives to manage powdery mildew of roses were generated by using a plant extract, resistance inducers and watery compost extracts.
- Under high inoculum pressure of powdery mildew of roses, a tabog (*Swinglea glutinosa*) based plant extract reduced incidence and severity in six field trials at three municipalities of the Sabana de Bogotá.
- Treatments with monobasic and potassium bibasic phosphate for control were more efficient than the commonly used fungicides Stroby and Rubigan.
- The mixture of the tabog extract and potassium monobasic phosphate is an efficient alternative, for powdery mildew of roses control taking into account that the percent of leaflets per plant affected by the disease was reduced, even more than the fungicide Rubigan.

- A liquid plantain compost extract at a concentration of 0.5% of three months affected sporulation of *Sphaerotheca pannosa* var. *rosae* and reduced disease incidence.
- A liquid fruits and vegetables compost at 20% and 30%, reduced CBB severity in cassava greenhouse experiments.

Bean Diseases

- Intensive surveys were conducted to determine farmer perception and indigenous knowledge and management of bean root rots in southwest Uganda.

Bean Insect Pests

- *Ootheca,* the bean foliage beetle, biology better understood and explained to farmers who now disregard local myths about the pest: Beetle populations develop in the soil within their own fields.
- More information gathered to explain the factors that influence BSM (Bean Stem Maggot) population dynamics: Populations are lowest during April (high rainfall) and highest during July and August (low rainfall).
- The relationship between bean pod borer damage and bruchid infestation elucidated to bean farmers in target communities are now aware that bruchid (*Acanthoscelides obtectus*) infestation starts in the field and that delayed harvest increases infestation levels.

Output III

Bean Insect Pests

- Three Farmer Research Groups continued to do trials on several issues such as evaluation of climbing beans, use of indigenous methods for pest control, bean and maize varieties, wheat and safflower cultivation.
- Farmers are interested to re-evaluate their Indigenous Technical Knowledge on crop management and are willing to contribute their own treatments.
- Climbing beans are appreciated by farmers in Northern Tanzania.
- Increasing African farmers knowledge about the biology and ecology of their common pests of beans and other crops has enabled them to develop appropriate management practices.
- Farming communities empowered to make IPM decisions with focus indigenous knowledge systems and to rediscover value in their traditional IPM strategies in bean based agroecosystems in Eastern Africa.
- Farmers' best bet traditional pest management practices prove to be effective against bean foliage beetles but duration of effectiveness is short (around 3 to 5 days).
- Farmers understand some of the causes of pest outbreaks in their production system
- Farmer preferred dissemination channels better understood and feedback from stakeholders enable better orientation of project processes.

Problems encountered and their solutions: Frogskin

Plans for next year:

1. One of the major problems encountered during the year was the presence of Cassava Frogskin Disease in CIAT cassava fields. This led to the suspension of cassava planting at CIAT and resulted in seeking off station fields to carry out cassava activities.

2. Reduction of input by IRD (France) into IPM activities, leading to the loss of an important French Scientist.

Project PE-1: Integrated Pest and Disease Management

Project Description

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

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 farms. Increased knowledge of the biology and ecology of pests and diseases and of the damage they cause. Molecular characterization of major pathogens and diagnostic kits made available. Whitefly biodiversity characterized. FPR methods for IPM developed and implemented. Biological control agents established in new regions.

Milestones:

2002 A global network and website for information on tropical AES developed. Evaluation and dissemination of biological control agents of major pests of restricted crops. IPM projects developed for AES. Components of the IPM package for global whitefly project ready for diffusion. First crop viruses identified and diagnostic tools developed. Whitefly resistance mechanisms in cassava identified.

2003 IPM for cassava viruses and root-rot diseases implemented. Cassava germplasm with resistance to CBB identified by use of molecular markers. Research on soil-borne arthropods and pathogens advanced and coordinated with systemwide programs. Research on invasive pests defined and under way. Use of cassava varieties tolerant of frogskin disease in breeding and IPM programs.

2004 Biological control through entomopathogens developed for soil-borne pests. Natural enemies of whitefly available for IPM programs. Leader in information and technologies for implementing phytosanitary certification programs for cassava and other crops. Molecular markers tagging resistance to CBB available. Germplasm screened for resistance to *Phytophthora* root rot, using marker-assisted selection. Epidemiological validation of specified whitefly-transmitted geminiviruses.

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

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

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

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

Log Frame Work Plan for PE-1, 2002-2004

Area: Genetic Resources
Manager: Anthony Bellotti

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To increase crop yields and reduce environmental contamination through the effective management of major pests and diseases.	• Increased cassava yields. • Reductions in environmental degradation through adoption of improved technology. • Reduction of losses 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.
Purpose To develop and transfer knowledge systems and pest and disease management components for sustainable productivity and healthier environments.	• Adoption of germplasm with resistance to biological constraints. • Establishment of released natural enemies. • Use of environmentally friendly control strategies. • Improved understanding of major biotic constraints.	End-of-project reports. Refereed publications, book chapters. Adoption and impact studies.	Financial resources are mobilized. Active collaboration with NARS. Active collaboration with other IARCs and DC research organizations. Active collaboration with AROs.
Output 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 host-pest interactions. • Identification of cassava with tolerance of diseases. • Pest and disease distribution (maps) determined.	All areas: Project reports, refereed publications, book chapters. Reports with maps, economic damage, biological information. Analysis of experiments. Transfer of tools to seed health facilities.	NARS have the needed resources. Adequate interaction with other disciplinary scientists Successful experiments. Continued development of new varieties that are commercially acceptable. Farmers have adequate access to extension agents, credit lines, and other factors that influence adoption. Collaboration with NARS possible Evaluation, screening, and exploration sites accessible
Output 2 Pest and disease management components and IPM strategies and tactics developed.	• Testing of components for effectiveness. • Control strategy recommendations clearly identified and crop management practices determined. • Farmer testing components. • Guides on IPM strategies published. • Disease detection methods available. • Web site published.	Analysis of experiments. Guidelines for IPM. Reports on field effectiveness and probability of adoption of components Field-oriented brochures.	
Output 3 NARS' capacity to design and execute IPM research and implementation strengthened.	• Training, especially in FPR. • Development of projects with NARS. • Training materials developed.	Reports on training courses. Concept notes and projects prepared with partners.	
Output 4 Global IPM networks and knowledge systems developed.	• Network of researchers established. • Preparation of web pages and databases with relevant IPM information.	Electronically published web pages and databases.	

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OUTPUT I. PEST AND DISEASE COMPLEXES DESCRIBED AND ANALYZED

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

Introduction

As direct feeding pests and virus vectors, whiteflies cause major damage in cassava-based agroecosystems in the Americas, Africa and to a lesser extent in Asia. There is a large complex associated with and feeding on cassava, 11 species in the neotropics. *Aleurotrachelus socialis* is the predominant species in Northern South America (Colombia, Venezuela and Ecuador) where it causes considerable crop damage. *Aleurothrixus aepim* is the major species causing crop damage in Brazil. Both species cause direct damage to cassava by feeding on the phloem of leaves, causing chlorosis and leaf fall, which results in crop loss. Neither species is known to transmit virus diseases. *Bemisia tuberculata* and *Trialeurodes variabilis* feed on cassava in many regions of the neotropics, including Northern South America, Brazil and Paraguay. Both species are usually found in lower populations and probably do not cause crop loss. *B. tuberculata* is the suspected vector of cassava Frog Skin Disease (CFSD) (see this report).

Bemisia afer occurs on cassava in many countries of Africa, especially East Africa (Kenya, Uganda, Malawi, etc.) where, until recently it was reported as a minor pest. However, more recently, it has been described as occurring in higher populations and causing crop damage. It is also suspected as being the vector of cassava brown streak virus. This species has now been reported in the Americas, attacking sweet potato in Perú (See Output 4 of this report). Although it has not yet been reported attacking cassava, it is considered as a potential threat to cassava, based on its history in East Africa.

B. tabaci has a pantropical distribution, feeding on cassava and several countries in Asia, and is the vector of Africa Cassava Mosaic Disease (ACMD). Prior to 1990, the *B. tabaci* biotypes found in the Americas did not feed on cassava and it was therefore speculated that the absence of ACMD in the Americas was partially due to the inability of its vector, *B. tabaci* to colonize cassava. Since the early 1990s a new biotype (B) of *B. tabaci*, considered by some, a separate species (*B. argentifolii*), has been found feeding on cassava in the Neotropics. It is considered that ACMD now posses a more serious threat to cassava production given that most traditional cultivars in the Neotropics are highly susceptible to the disease. In addition the *B. tabaci* biotype complex is the vector of several viruses of crops often gown in association with cassava or near it. The possibility of virus diseases moving among these crops or the appearance of new viruses represents a potential threat.

Research efforts in the Neotropics have concentrated on *A. socialis* and *A. aepim*. Populations of both species are highest during the rainy season but may be present throughout the crop cycle. CIAT research has emphasized both biological control and host plant resistance (HPR) to attain effective management of *A. socialis*. Research at CNPMF (Centro Nacional de Pesquisa em

Mandioca y Fruticultura)/EMBRAPA, in Bahia, Brazil has concentrated its efforts on *A. aepim*, also emphasizing biocontrol and HPR. Collaborative research between CIAT and CNPMF has been in effect for several years and may intensify in the future. Research on HPR of *A. socialis* is reported in a separate project report (IP-3).

Surveys

Surveys in recent years in the Neotropics – especially in Colombia, Venezuela, Ecuador and Brazil – have identified a considerable number of natural enemies associated with the cassava whitefly complex. During 2001 explorations were carried out in Venezuela, complementing those done in previous years in Colombia and Ecuador (See Annual Reports PE-1, 1998, 1999, 2000). Surveys in Venezuela was primarily carried out in the Northeastern region of the country, including the states of Monagas, Bolívar, Anzoátegui, Aragua, and Lara, and sampling was done in 21 localities. The primary objective of these surveys is to determine the whitefly species present and identify the natural enemy complex associates with each species.

Each zone is characterized by taking data on m.a.s.l., rainfall, temperature range, vegetation type, associated crops, latitude, etc. From each collection site 40 leaves are randomly collected; a one square inch leaf area was examined to determine the whitefly species preset and the number of nymphs and pupae is recorded.

The rate of parasitism is determined by collecting 40 leaves randomly from each field and removing a one-inch square leaf sample. Only one whitefly species is allowed to remain on each leaf square and the emergence of parasitoids is recorded for each whitefly species. Each leaf square is taped to a cardboard strip, avoiding a rapid leaf deterioration and loss of specimens. These were placed in emergence vials (transparent 1 x 2 inches). Each vial is coded for rapid identification as to date, locality, etc. During explorations, collected materials were transported in a styro foam container, placing ice in it each day to maintain lower temperatures to avoid sample deterioration and transported to the laboratory. Parasitoids collected from these samples were sent to taxonomists or in some cases, identified at CIAT.

Parasitoids are prepared for shipment by placing individual specimens (dried) in small gel capsules with a piece of facial tissue; each gel is placed in a glass vial containing cotton, and coded with site, date, collector, host, etc. Specimens were sent to collaborating taxonomists; Gregory A. Evans, Gainesville Systematic Entomology Laboratory, University of Florida, a specialist in Aphelinidae, Eulophidae and Platygasteridae families; and Mike Rose, of Texas A&M University, College Station, Texas, specialist in Aphelinidae, especially the genus, *Eretmocerus*.

Results

Numerous species of parasitoids of whiteflies were recovered from surveys in Venezuela (**Table 1.1**). Most (5) were from the genus *Encarsia* and included *E. hispida, E. bellottii, E. cubensis* and *E. tabacivora*, collected from cassava, and *E. sofia* that was collected from eggplant and cole. Two new species for Venezuela were also collected; *Metaphycus* sp. (Fam: Encyrtidae) and *Euderomphale* sp. (Fam: Eulophidae); individuals from the genus *Eretmocerus* (Fam:

Aphelinidae) were also found. These are being identified to species by Mike Rose of Texas A&M University. In addition the hyperparasite, *Signiphora aleyrodis* was also recovered. All of the aforementioned species have also been recovered from surveys in Colombia and Ecuador (See PE-1 Annual Report, 2000).

Table 1.1. Taxonomic classification of parasitoide species collected from whiteflies on cassava and associated crops in Venezuela 2001.

Order	Super family	Family	Genus	Species
Hymenoptera	Chalcidoidea	Aphelinidae	*Encarsia*	*hispida* De Santis
"	"	"	"	*bellottii* Evans & Castillo
"	"	"	"	*tabacivora* Viggiani
"	"	"	"	*cubensis* Gahan
"	"	"	"	*sofia* (Girault & Dodd)
"	"	Encyrtidae	*Metaphycus*	sp . * ne
"	"	Eulophidae	*Euderomphale*	sp. * ne
"	"	"	*Signiphora*	*aleyrodis* Ashmead **

* New species
** Hyperparasite

Three whitefly species on cassava were collected during exploration, *A. socialis*, *Bemisia* sp. Probably *B. tuberculata*) and *Trialeurodes* sp. (probably *T. variabilis)* **(Table 1.2)**. All parasitoids were collected from *A. socialis* and *Bemisia* sp.; no parasitoids were collected from *Trialeurodes* sp. During the time of survey, March 2001, *Trialeurodes* populations were very low **(Table 1.3)**, and this probably accounts for the absence of parasitoids. *E. hispida* and *Euderomphale* sp. were collected from both *A. socialis* and *Bemisia* sp. while *E. bellottii* and *E. cubensis* were collected only from *A. socialis* and *E. tabacivora* and *Metaphycus* sp. were collected only from *Bemisia* sp. **(Table 1.2)**. *E. sophia* was collected form *Bemisia* sp. on eggplant and cole.

Table 1.2. The association between parasitoid species and cassava whitefly species collected from numerous sites in Venezuela during 2001.

	Whiteflies		
Parasitoids	*A. socialis*	*Bemisia* sp.	*Trialeurodes* sp.
Encarsia hispida De Santis	x	x	-
Encarsia tabacivora Viggiani	-	x	-
Encarsia bellottii Evans y Castillo	x	-	-
Encarsia cubensis Gahan	x	-	-
Encarsia sophia (Girault and Dold)	-	x	-
Euderonphate sp.[1]	x	x	-
Metaphycus sp.[1]	-	x	-
Signiphora aleyrodis Ashmead[2]	x	-	-

[1] New species
[2] Hyperparasite

Nearly 99% of the whitefly specimens collected from cassava were *A. socialis* **(Table 1.3)**. *Bemisia* sp. Represented about 1.0% collected and *Trialeurodes* sp. only 0.2%. *A. socialis* is also the predominant species recovered in Colombia and Ecuador and, as noted earlier, is the predominant species in Northern South America. In addition *A. socialis* was recovered from nearly all of the localities surveyed; it was not observed in only two sites, one in Monagas, the other in Lara. *Bemisia* sp. was collected in about 50% (12) of the 20 localities surveyed, but in

much lower populations. *Trialeurodes* sp. was collected from only 5 localities and a total of only 17 specimens were recovered **(Table 1.3)**.

The highest populations of *A. socialis* were observed in Caripitos-Los Mangos (Monagas St.), Caripitos-Los Barrancos (Monagas) and Maturín-El Respiro (Monagas) with 2577, 1726, and 1465 pupae collected, respectively **(Table 1.3)**. In general, localities in Monagas State had much higher populations than the other states surveyed. In the agroindustrial locality, "Mandioca" in Libertador (Monagas), *Bemisia* sp. was the only species collected.

Table 1.3. Cassava whitefly species distribution collected from several localities in Venezuela during 2001.

Locality	State	No. *A. socialis*	No. *Bemisia* sp.	No. *Trialeurodes* sp.
Barranca - La Malcanera	Monagas	1260	-	-
Barranca km. 1	"	369	-	-
Piedras de Uracoa	"	34	10	10
Caripitos Los Barrancos	"	1726	-	-
Caripitos - Los Mangos	"	2577	-	-
Puncere	"	121	8	4
Maturín – Los Mulatos	"	356	1	-
Maturín – Jusepin	"	135	7	-
Guayana - San Felix	Bolivar	133	-	-
Upata - Los Posos	Bolivar	33	-	-
Upata – Crucero	Bolivar	25	3	-
Maturín San Agustín	Monagas	272	4	-
Maturín – Carrizal	Monagas	135	3	-
Maturín - El Respiro	Monagas	1465	-	-
Maturín – Corocito	Monagas	50	-	-
Libertador - Mandioca	Monagas	-	40	-
Maracay - Linares Alcantara	Aragua	134	6	1
Zamora - Santa María	Aragua	1	2	1
Maracay - Inia	Aragua	17	9	1
Federman - Cantarrana	Lara*	-	120	-
Total of Individuals		8843	93**	17
% of species		98.8 %	1.0 %	0.2 %

* Sample collected from cole crop.

** Number of *Bemisia* sp. collected from cassava.

Additional Cassava Pests

Other cassava pests observed and collected during surveys in Venezuela were stemborers (*Chilomima clarkei* and *Chilozela* sp.) lacebugs (*Vatiga* spp.), shootflies (*Silba pendula*), gallmidges (*Jatrophobia brasiliensis*), scales (*Aonydomitilu albus* and *Saissetia miranda*), the hornworm (*Erinnyis ello*), mites (*Mononychellus tanajoa, M. caribbenae, Tetranychus* spp. and *Oligonychus peruvianus*, thrips, the fruitfly (*Anastrepha* sp.). Leaf cutter ants were found causing damage in several localities.

Activity 2. Parasitoid identification of cassava whiteflies in the Caribbean region of Colombia

Whitefly and parasitoid collections were made from cassava on the Colombian Atlantic Coast, the Caribbean Region, during 1999 and 2000. Specimens collected were sent to different taxonomists for identification. The following is an up-to-date report on the progress with the identification of these species.

Parasitoids collected from *A. socialis* include *E. bellottii, E. hispida, Encarsia* sp., and *Eretmocerus* sp. **(Table 2.1)**. In addition new registers were reported for *Amitus macgowni* and *Encarsia americana* in Colombia.

Table 2.1. Taxonomic classification of parasitoid species collected from cassava whiteflies in the Colombian North Coast.

Order	Superfamily	Family	Genus	Species
Hymenoptera	Platygasteroidea or Proctotropoidea	Platygasteridae	*Amitus*	*macgowni* Evans & Castillo
"	Chalcidoidea	Aphelinidae	*Encarsia*	sp.
"	"	"	"	*hispida* De Santis
"	"	"	"	*bellottii* Evans & Castillo
"	"	"	"	*sofia* (Girault & Dodd)
"	"	"	"	*luteola* Group
"	"	"	"	*Strenua* Group
"	"	"	"	*americana* (De Bach & Rose)
"	"	"	*Eretmocerus*	sp.
"	"	Encyrtidae	*Metaphycus*	sp.
"	"	Eulophidae	*Euderomphale*	sp.

Parasitoids collected from *Trialeurodes variabilis* include *E. tabacivora* and *Eretmocerus* sp. In addition *E. bellottii* and *E. hispida* are new reports on *T. variabilis*. *Encarsia americana* is a new report for *Tetraleurodes* sp. **(Table 2.2)**. The presence of the genus *Eretmocerus* was confirmed on *B. tuberculata* and the parasitoid *Euderomphale* sp. is a new register for *B. tuberculata*.

Encarsia americana is a new species for Colombia and there are several species that still need to be identified and are probably new species. The taxonomist, Greg Evans, has requested additional samples. A record of all identification and collections are held in the CIAT arthropod collection and data base.

The data from the Caribbean region of Colombia shows considerable parasitoid species richness associated with cassava whiteflies **(Table 2.2)**, greater than that observed in Ecuador and Venezuela. The greatest parasitoid diversity is in the genus *Encarsia* and especially on the whitefly species *A. socialis* and *Trialeurodes* sp. This contrasts somewhat with Venezuela, where no parasitoids were collected from *Trialeurodes* and Ecuador where 3 parasitoid species were collected (See PE-1 Annual Report, 2000).

In Colombia, Venezuela and Ecuador the greatest parasitoid species richness is from *A. socialis*. The parasitoids *Amitus macgowni, E. americana, E. luteola* group and *E. strenua* group, found

on the Colombia Caribbean coast were not collected from Venezuela. The whitefly species *Tetraleurodes* sp., found in Colombia, was also not collected in Venezuela.

Table 2.2. The association between cassava whitefly species and parasitoids collected on the Colombian Caribbean Coast.

Parasitoids	Whitefly Species				
	A. socialis	*Aleurodicus* sp.	*B. tuberculata*	*Tetraleurodes* sp.	*Trialeurodes* sp.
Amitus macgowni	X				
Eretmocerus sp.	X		X	X	X
Encarsia sp.	X	X	X	X	X
E. americana	X			X	
E. hispida	X				X
E. tabacivora					X
E. bellottii	X				X
E. luteola Gr.	X				X
E. sofia	X		X		X
E. strenua Gr.					X
Euderomphale sp.			X		
Metaphycus sp.			X		

Activity 3. Cassava mites and biological control

Mites are a universal pest of cassava, causing serious yield losses in the Americas, Africa, and to a lesser degree in Asia. Of the >40 species reported feeding on cassava, the most frequent are *Mononychellus tanajoa* (syn=*M. progresivus*) (The Cassava Green Mite or CGM), *M. caribbeanae*, *Tetranychus cinnabarinus* and *T. urticae* (also reported as *T. bimaculatus* and *T. telarius*). Cassava is the major host for the *Mononychellus* species, while the *Tetranychus* species have a wide host range. Other mite species (e.g. *Oligonychus peruvianus*, *O. beharensis*, *Eutetranychus banksi* or *M. mcgregori*) are usually not economically important, feeding on cassava only sporadically.

CIAT maintains a collection of phytophagous mites and, mostly collected from cassava, as well as a collection of mite predators. These collections were initiated nearly 25 years ago and are now represented by more than 12,709 specimens, consisting of 96 species. During the 1980 and 1990 systematic surveys were made throughout the neotropics and specimens were brought in from limited trips to Asia and Africa. This systematic surveying and collecting have allowed us to determine the geographic distribution of cassava mites and their natural enemies.

During the past two years (2000-2001), while surveys were carried out primarily for whitefly species and natural enemies on cassava, considerable mite damage and corresponding populations were also noted. During this period and additional 28 samples, 23 from cassava, were added to the collection and corresponding data base **(Table 3.1)**. Collections were made in Colombia and Venezuela and *M. tanajoa* and *M. caribbeanae* were the two predominant species collected from cassava. *T. urticae* was collected from two sites in Venezuela and one in Colombia.

Table 3.1. Phytophagous mite species collected from cassava and other hosts during 2001-2002 and added to CIAT collection.

Sample	Country	Dept.	Site	Host	Species
2538	Colombia	Valle	Caicedonia	Cassava	*Allonychus braziliensis*
2539	Colombia	Valle	CIAT	Jatropha	*Tetranychus urticae*
2540	Puerto Rico		Isabella	Papaya	*Eotetranychus lewisi*
2541	Colombia	Cordoba	Ciénaga de Oro	Cassava	*Eriophydae: Calacarus sp.*
2542	Colombia	Valle	Candelaria	Cassava	*Tetranychus urticae**
2543	Colombia	Valle	Cali	Flowers	*Eotetranychus neolewisi*
2544	Colombia	Valle	CIAT	Rice	*Schizotetranychus oryzae*
2545	Venezuela	Monagas	Malcanera	Cassava	*Mononychellus caribbeanae**
2546	Venezuela	Monagas	Barrancas	Cassava	*M. tanajoa* *M. caribbeanae* *T. urticae*
2547	Venezuela	Monagas	Caripito	Cassava	*M. caribbeanae*
2548	Venezuela	Monagas	Maturín	Cassava	*M. caribbeanae*
2549	Venezuela	Monagas	Jusepin	Cassava	*M. caribbeanae* *M. tanajoa*
2550	Venezuela	Guayana	San Felix	Cassava	*M. tanajoa*
2551	Venezuela	Monagas	San Agustín	Cassava	*M. tanajoa*
2552	Venezuela	Monagas	Carrizal	Cassava	*M. tanajoa*
2553	Venezuela	Monagas	Carosito	Cassava	*M. tanajoa* *M. caribbeanae** *T. urticae**
2554	Venezuela	Monagas	Temblador	Cassava	*M. caribbeanae** *M. tanajoa**
2555	Venezuela	Aragua	Linares	Cassava	*M. tanajoa*
2556	Venezuela	Aragua	Maracay	Cassava	*M. tanajoa*
2557	Venezuela	Anzoátegui	La Bombita	Cassava	*M. caribbeanae* *M. tanajoa*
2558	Venezuela	Anzoátegui	Cantaura	Cassava	*M. tanajoa* *M. caribbeanae*
2559	Venezuela	Anzoátegui	El Tigre	Cassava	*M. caribbeanae* *M. tanajoa*
2560	Venezuela	Lara	Barquisimeto	Cassava	*M. tanajoa*
2561	Colombia	Valle	CIAT	Cassava	*M. caribbeanae* *M. tanajoa*
2562	Colombia	Valle	Rozo	Cassava	*M. tanajoa** *M. caribbeanae*
2563	Colombia	Valle	Cali	Cassava	*M. caribbeanae* *M. tanajoa*
2564	Colombia	Risaralda	Pereira	*Cyrtomenus bergi*	*Acaridae*
2565	Colombia	Norte de Santander	Los Patios	Cassava	*M. tanajoa* *M. caribbeanae*

* Neozygites pathogen infesting tetraniquid mites.

Collections were also made from Jatropha, Papaya (sent from associates in Puerto Rico for identification), roses, and rice **(Table 3.1)**. The species *Schizotetranychus oryzae*, was observed in high populations on rice at CIAT.

The fungal pathogen *Neozygites*, pathogenic to tetraniquid mites, was detected at several sites in Colombia and Venezuela (marked by an asterisk *, in Table 3.1) infecting at least three different mite species. The presence of epizootics of this fungus reflects its potential importance as a

natural biocontrol agent under favorable environmental conditions for the fungus (See PE-1 Annual Report, 2000 for additional information).

Activity 4. Consumption and oviposition rates of six phytoseiid species feeding on eggs of the cassava green mite

The cassava green mite, *Mononychellus tanajoa* Bondar (Acari: Tetranychidae) is an important pest of cassava, *Manihot esculenta* Crantz (Euphorbiaceae) in dry regions of South America (Farias et al., 1982, Byrne et al., 1983, Veiga, 1985). In the early 1970s, this mite species was accidentally introduced into Africa, spreading rapidly across the Subsaharan zone in the absence of its natural enemies (Yaninek & Herren, 1988) and causing severe yield losses (Yaninek et al., 1990, Bonato et al., 1994). Classical biological control (i.e., through the use of introduced natural enemies) was developed to control *M. tanajoa* in Africa (Mégevand et al., 1987, Yaninek & Herren, 1988). Among ten phytoseiid species released in Africa from 1984 to 1993 three of them are now well established but only one is spreading well and affecting the green mite population (Bellotti et al., 1999). It is therefore necessary to release more phytoseiid species or strains from South America. Meanwhile, CIAT began exploration and evaluation of phytoseiids from coastal Colombia and Ecuador, which has a dry climate similar to target areas in Africa.

Two factors that affect the success of phytoseiid mites in controlling their mite prey are their functional and numerical responses (Sabelis, 1985). These factors must be considered when the importance of the phytoseiid species is to be evaluated. First described by Solomon (1949), the functional and numerical responses were defined as follows. The functional response refers to the change in the number of prey consumed per unit time in relation to the change in prey density. The numerical response refers to the increase in numbers of predators in response to increases in prey density and is thus positively correlated with the ovipositional rate. A good candidate for controlling mite populations should have both increased prey consumption and oviposition rates in proportion to the available prey density.

The aim of this study was to evaluate, under optimal laboratory conditions, prey consumption and oviposition rates of six phytoseiid predatory mite species in relation to prey density. The objective was to estimate the maximum number of prey consumed and the maximum number of eggs laid as well as their maximum efficiency at converting food energy into egg production of six phytoseiid species.

Materials and Methods

Six phytoseiid mite populations (Acari: Phytoseiidae) were collected from coastal areas of Colombia and Ecuador **(Table 4.1)**. All predatory mite species were maintained in the laboratory on cassava leaves infested by *M. tanajoa* at 25±1°C, 75±5% RH and 12-h photoperiod. Immediately after emergence, individual females were placed with a male in the predation arena, described below, with an uncontrolled egg prey density (generally > 100) for 3 days. Gravid female predators from the predation arenas were then used for the experiments.

Prey for the phytoseiid species, *M. tanajoa*, were reared on 2-month-old cassava plants, var. CMC-40, in a greenhouse under natural conditions of temperature and relative humidity and 12-h photoperiod in Palmira, Colombia.

Table 4.1. Origin of six phytoseiid mite species used in the experiments and collected from different areas of South America.

Species	Country	Region	Location	Altitude (m)	Collection Date
Neoseiulus idaeus Denmark & Muma	Colombia	Guajira	Fonseca	180	2-97
Typhlodromalus aripo De León	Colombia	Magdalena	Pivijay	3	6-97
Galendromus annectens De León	Ecuador	Manabí	Crucita	–	12-95
Neoseiulus californicus McGregor	Ecuador	Manabí	Portoviejo	50	11-94
Typhlodromalus tenuiscutus McMurtry & Moraes	Ecuador	Manabí	Puerto Cayo	40	12-95
Euseius ho De León	Ecuador	Manabí	Rocafuerte	16	12-95

All experiments were conducted under laboratory conditions at 25±1°C, 75±5% RH and 12-h photoperiod [optimal conditions to rear all phytoseiid species studied in laboratory (M. E. Cuellar, unpublished data)]. The experiments were performed on 3.14 cm^2 greenhouse-collected cassava leaf discs of var. CMC-40, containing controlled egg densities of *M. tanajoa*. The leaf disc floated abaxially on water-saturated filter paper in plastic dishes (diam., 2 cm, height, 1.5 cm). Individual predatory mite females were placed on the leaf discs, and the predation arena was sealed with transparent plastic wrap.

The number of egg prey consumed per predatory mite female was counted at 24 hours. The same predatory mite female was then transferred to a new predation arena with the same egg density of prey as on previous day, and the number of eggs laid by the predatory female was counted after 24 hours. For each predator species, 14 to 18 predatory mite females were used at each egg prey density.

The following egg densities were tested: 1, 3, 7, 15, 30, 105 or 200 eggs per leaf disc.

Statistical tests were performed with Statview software (Abacus Concept, USA). For two-way analyses of variance (2-way ANOVA), the factors "prey density" and "phytoseiid species" were considered as fixed factors. Homogeneity of variance and data normality were examined by the F-test and Kolmogorov-Smirnov method, respectively, before running the ANOVA. Only the number of eggs consumed was normalized by log (X+1) transformation. The Fisher's PLSD (Protected Least Significant Difference) test following the ANOVA was used to compare means post-hoc.

Results

Prey density had a significant influence on the number of eggs consumed regardless of the phytoseiid species [result of 2-way ANOVA: F=1177.7, df=(6, 616), P<0.05 for the factor "prey density"]. There was a general increase in egg consumption with increasing prey density **(Table 4.2)**. This indicated that all predator species responded functionally to *M. tanajoa* egg density and thus curves of functional responses can be plotted **(Figure 4.1)**. Prey consumption by *E. ho*, *T. aripo* and *T. tenuiscutus* continued increasing up to the highest density of prey evaluated (200 prey eggs per leaf disc). In fact, linear regression coefficients (r^2) between consumption and prey density were high for these species, about 0.99, 0.94 and 0.89, respectively. Furthermore, they presented the highest consumption rates, consuming a maximum of 93, 101 and 59 prey in 24 h, respectively. In contrast, lowest correlations were found for *N. californicus*, *N. idaeus* and *G. annectens* (linear regression coefficients: r^2= 0.79, 0.32 and 0.26 respectively). Nevertheless, at lowest prey densities (≤30 prey eggs per leaf disc), high correlations were also obtained (linear regression coefficient: r^2 = 0.94 to 0.99). This indicated that prey consumption by these species increased linearly up to 30 prey eggs offered and then leveled off at a plateau **(Figure 4.1)**. Consequently, they exhibited lower consumptions, consuming a maximum of 40, 35 and 18 eggs in 24 h, respectively.

Prey density also had a significant influence on the number of eggs laid by all phytoseiid species [result of 2-way ANOVA: F=601.6, df=(6, 614), P<0.05 for the factor "prey density"]. There was a general increase in eggs oviposited by female predator with increasing prey density, regardless of the phytoseiid species **(Table 4.2)**. Nevertheless, little increase was generally noted at highest densities evaluated, so daily fecundity appeared to reach a plateau for all species. Highest maximum oviposition rates were registered for *T. tenuiscutus*, *N. californicus*, *N. idaeus* and *G. annectens*, ovipositing a maximum of 3.9, 3.6, 2.9 and 2.8 eggs in 24 h; whereas *E. ho* and especially *T. aripo* oviposited no more than 2.2 and 1.4 eggs in 24 h, respectively.

The number of eggs laid per prey consumed was calculated (i.e. mean number of eggs oviposited/mean number of prey eggs consumed), and presented in Table 4.2. As mentioned above, this ratio reflects in a straightforward way the efficiency of a predator at converting their prey into progeny. In general highest ratios were obtained for *G. annectens, N. californicus* and *N. idaeus* showing a maximum of 35.6, 14.5 and 12.0, respectively, suggesting that these species presented highest efficiency at converting prey into progeny. In contrast, lowest ratios were generally registered for *T. aripo*, *T. tenuiscutus* and *E. ho* showing a maximum of 6.7, 9.9 and 11.6, respectively, indicating that these species were the least efficient.

Discussion

All predator species studied responded functionally to *M. tanajoa* egg density **(Figure 4.1)**. Holling (1959) proposed three types of functional response curves: Type 1, a linear rise to a plateau; Type 2, a curvilinear rise to an asymptote; and Type 3, a sigmoid curve rising to an asymptote. These curves, which have been extensively used in predator-prey interactions, are used to evaluate the effectiveness of a predator [see Sabelis (1985) for review]. At lowest prey densities (≤30 prey eggs per leaf disc), curves fitted well to a typical Holling Type-1 functional

response for all phytoseiid species. Nevertheless, at higher densities, a flat response was clearly observed and can be regarded as a "plateau" for *N. californicus*, *N. idaeus* and *G. annectens*.

Table 4.2. Influence of seven levels of egg prey availability on the number of eggs consumed and number of eggs laid per predator (means[1] ± se) in 24 h by females of six phytoseiid species and on eggs laid/prey consumed ratio.

Species	Egg Prey Densities	Eggs Consumed	Eggs Laid	Ratio (x 100)
Euseius ho	1	0.93 ± 0.07 a	0	0
	3	3.00	0.23 ± 0.12 a	7.8
	7	6.30 ± 0.34 b	0.73 ± 0.12 ab	11.6
	15	10.90 ± 0.70 c	1.25 ± 0.21 bc	11.5
	30	23.93 ± 1.30 d	1.60 ± 0.23 c	6.7
	105	52.80 ± 5.73 e	2.23 ± 0.25 d	4.2
	200	93.40 ± 9.84 f	2.07 ± 0.30 cd	2.2
Typhlodromalus aripo	1	0.93 ± 0.07 a	0.30 ± 0.12 a	32.2
	3	2.90 ± 0.08 b	0.20 ± 0.10 a	6.7
	7	6.83 ± 0.12 c	0.40 ± 0.12 a	5.8
	15	14.94 ± 0.06 d	0.62 ± 0.12 a	4.1
	30	28.60 ± 0.40 e	1.30 ± 0.13 b	4.5
	105	81.30 ± 3.40 f	1.22 ± 0.13 b	1.5
	200	101.31 ± 7.60 g	1.40 ± 0.20 b	1.4
Typhlodromalus tenuiscutus	1	0.91 ± 0.09 a	0.09 ± 0.09 a	9.9
	3	3.00	0	0
	7	6.85 ± 0.11 b	0.30 ± 0.10 a	4.4
	15	14.53 ± 0.24 c	1.13 ± 0.21 b	7.8
	30	26.12 ± 1.80 d	2.40 ± 0.40 c	9.2
	105	48.22 ± 4.60 e	2.41 ± 0.30 c	5.0
	200	59.20 ± 5.90 f	3.93 ± 0.21 d	6.6
Neoseiulus californicus	1	1.00	0	0
	3	3.00	0.07 ± 0.07 a	2.3
	7	6.90 ± 1.12 a	1.00 ± 0.09 b	14.5
	15	14.50 ± 0.35 b	1.90 ± 0.30 c	13.1
	30	24.80 ± 1.10 c	3.13 ± 0.32 d	12.6
	105	25.00 ± 1.60 c	3.10 ± 0.30 d	12.4
	200	39.72 ± 5.30 d	3.60 ± 0.20 d	9.1
Neoseiulus idaeus	1	0.92 ± 0.08 a	0	0
	3	3.00	0.25 ± 0.14 a	8.3
	7	6.73 ± 0.15 b	0.60 ± 0.13 a	8.9
	15	12.93 ± 0.93 c	1.40 ± 0.30 b	10.8
	30	24.53 ± 1.20 d	2.94 ± 0.22 d	12.0
	105	34.73 ± 3.61 e	2.90 ± 0.35 d	8.3
	200	18.60 ± 1.61 f	2.12 ± 0.15 c	11.4
Galendromus annectens	1	1.00	0	0
	3	2.53 ± 0.30 a	0.90 ± 0.20 a	35.6
	7	6.20 ± 0.50 b	1.80 ± 0.20 b	29.0
	15	11.31 ± 0.90 c	2.31 ± 0.22 cd	20.4
	30	15.53 ± 1.40 d	2.80 ± 0.14 d	18.0
	105	18.10 ± 3.21 d	2.70 ± 0.20 cd	14.9
	200	11.50 ± 1.95 c	2.23 ± 0.20 bc	19.4

[1] Means followed by different letters are significantly different at 5% level using Fisher's PLDS test following the ANOVA (when means or SE = 0, no statistical test can be performed then no letter was given).

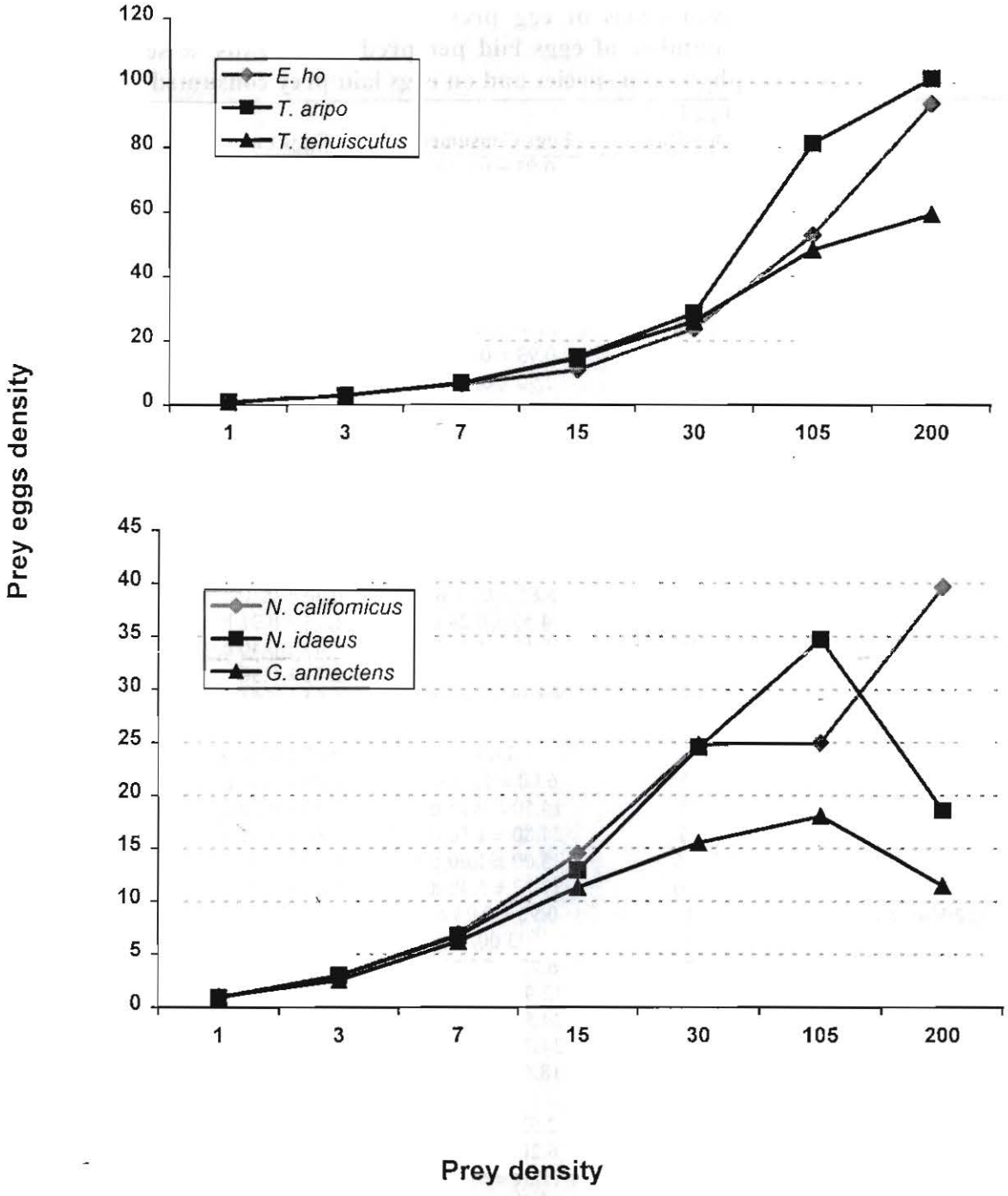

Figure 4.1. **Functional response of six phytoseiid species (A) *E. ho, T. aripo, T. tenuiscutus* and (B) *N. californicus, N. idaeus, G. annectens,* to increases in density of *M. tanajoa* eggs in 24 hours.**

Various factors influence the plateau level of the functional response curve [see Sabelis (1985) for review]. For example, it is well known that the plateau level depends to a major extent on the prey stage supplied and the age of the predator. In this study, the prey stage and the age of female predator were held constant. The single factor varying in the experiments was the phytoseiid species. Therefore, the differences in the plateau level of curve are mainly a consequence of differences in the phytoseiid species. The fact that the curves do not rise clearly to a plateau for *E. ho*, *T. aripo* and *T. tenuiscutus* **(Figure 4.1)** indicates that these species exhibit higher consumption in the range of high prey densities consuming a maximum of 93, 101 and 59 preys in 24 h, respectively. In contrast, *N. californicus*, *N. idaeus* and *G. annectens*, whose curves rose more clearly to a plateau at the density 30. have a low consumption capacity among the high egg densities tested, consuming no more than 40, 35 and 18 eggs in 24 h, respectively. These results suggested that when the prey population is high, *E. ho*, *T. aripo* and *T. tenuiscutus* will be more efficient to control *M. tanajoa*.

Daily fecundity rates at the prey density 30 (the density where functional response curves reached a plateau) were higher for *N. californicus*, *N. idaeus* and *G. annectens*. In fact, it was at 3.1, 2.9 and 2.8 eggs in 24 h for *N. californicus*, *N. idaeus* and *G. annectens*, respectively; whereas it was only at 1.6, 1.3 and 2.4 eggs in 24 h for *E. ho*, *T. aripo* and *T. tenuiscutus*, respectively. This suggests that *N. californicus*, *N. idaeus* and *G. annectens* may be able to multiply well at low prey densities. Furthermore, by their higher oviposition/consumption ratios at this prey density, these phytoseiid species converted prey to predator progeny efficiently at the lower levels of prey eggs availability. As emphasized by Friese & Gilstrap (1982) for three other phytoseiid species, predator species which require fewer preys should be better able to survive as an effective searching population at low prey density and therefore better able to maintain the population at low prey density.

In conclusion, it appeared that among the predatory species studied, when *M. tanajoa* population increases markedly or during at outbreak, the use of *E. ho*, *T. aripo* or *T. tenuiscutus* phytoseiid species should be recommended. In contrast, when the mite population is low on cassava, the use of *N. californicus*, *N. idaeus* or *G. annectens* should be better because they may be able to multiply well. The fact that all phytoseiid strains or populations used in this study came from semi-arid areas of South America suggests that they may establish well in semi-arid areas of Africa to help control cassava green mite populations.

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The aforementioned information has been excerpted from a manuscript of the same title submitted to the Florida Entomologist for publication with the following authors:

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Activity 5. Development of a commercial biopesticide for control of the cassava hornworm

The cassava hornworm *Erinnyis ello,* (Lepidoptera: Sphingidae) is one of the most serious pests of cassava in the Neotropics. It has a broad geographic range, extending from southern Brazil, Argentina and Paraguay to the Caribbean basin and the southern United States. The migratory flight capacity of *E. ello,* its broad climatic adaptation and wide host range probably account for its wide distribution and sporadic attacks. Severe attacks cause complete plant defoliation, bulk root loss and poor root quality.

E. ello's migratory flight capacity and high oviposition often make effective control difficult to achieve. Farmers often respond to hornworm eruptions with excessive, ill-timed applications of pesticides, leading to severer and repeated attacks. Pesticides can give adequate control if hornworm populations are detected early, and treated during the first three instars. Larval populations in the forth and fifth instars are not only difficult to control but uneconomical because considerable defoliation has already occurred. Pesticide use also disrupts natural enemy populations, leading to more frequent attacks.

Although there is an extensive complex of natural enemies associated with *E. ello* (more than 40 species have been identified), their effectiveness is greatly reduced, most likely due to the migratory behavior of the hornworm adults. The key to the effective use of biological control agents is the ability to synchronize the large number of predators or parasites during the early stages, preferably the egg or the first to third larval instars. Predator and parasite effectiveness is limited by poor functional response during hornworm outbreaks, which are of short duration (about 15 days). Successful control therefore requires monitoring of field populations to detect immigrant adults or larvae in the early stages and having access to a cheap, storable biological pesticide.

A granulosis virus (Baculoviridae) was found attacking *E. ello* in cassava fields at CIAT in the early 1970's. Subsequent research, including pathogenicity studies in the laboratory and field resulted in nearly 100% mortality of hornworm larvae. Techniques were developed to macerate diseased larvae and the subsequent virus infected liquid, mixed with water and applied to hornworm infested cassava fields. The hornworm baculovirus has been used most successfully on larger cassava plantations where the virus can be stored under refrigeration for long periods and applied when hornworm eruptions occur. However there is a need for a finished, commercial, quality product that is available to most cassava producers when the need arises.

CIAT does not have the capacity, nor is it its role to develop or produce, marketable biopesticidas. This requires a link to, and collaboration with the biopesticide industry. CIAT has entered into a collaborative agreement with local biopesticide companies to research, develop, evaluate and eventually market biopesticidas for control of arthropod pests of cassava (and other crops). Production models and protocol have been, and are being developed (see PE-1 Annual Report, 2000). The cassava hornworm baculovirus is the first product destined for commercial release under this agreement. This agreement involves CIAT, BIOCARIBE S.A. a Colombian, commercial biopesticide company, and the Universidad de Antioquia in Medellín, Antioquia.

Production models for biopesticidas have been established (see PE-1 Annual Report 2000) for the cassava hornworm baculovirus and for Entomopathogens of other cassava pests, especially whiteflies and the burrower bug (*C. bergi*).

The *E. ello* baculovirus (PBv) was formulated by BIOCARIBE S.A. and its efficacy evaluated by CIAT. The process for virus purification consists of collecting virus infected hornworms (from colony) macerating the larvae in a blender, separating, larval parts through decanting and filtering, centrifuging (4500 r.p.m.) for 30 minutes, protein digestion and lypholization (**Figure 5.1**).

The baculovirus was produced in two formulas, one in wettable powder form, the other, an oil based liquid formula. Both the powder and liquid formulas was evaluated at three concentrations, 0.003, 0.0045 and 0.006% BvL. These concentrations were sprayed over first and third instar larvae feeding on cassava leaves in the laboratory. Evaluations of *E. ello* larval mortality were made every 24 hours.

Larval mortality reached 90% or higher for both the wettable powder and oil based baculovirus formulations (**Figure 5.2**). The wettable powder formulation mortality reached 90% in about 72 hours after application. With the oil based liquid formulation, mortality was delayed, reaching the 90% level after 120 hours. In the latter case mortality levels were highest with the higher concentration (6%). In both cases, mortality was observed during the first 24 hours after application.

This initial success of the product will lead to a more accurate doses recommended for application. The product is now being evaluated in the field in different localities, with cassava producers, in Colombia (The Llanos Orientales, Villavicencio; the Coffee zone, Armenia-Tebaida; the Atlantic Coast, Sucre, as well as Valle del Cauca). Numerous training courses, involving producers and technicians have been carried out to introduce cassava farmers to this method of hornworm control.

It is expected that the final commercial product available to cassava producers will be on the market by the end of 2001.

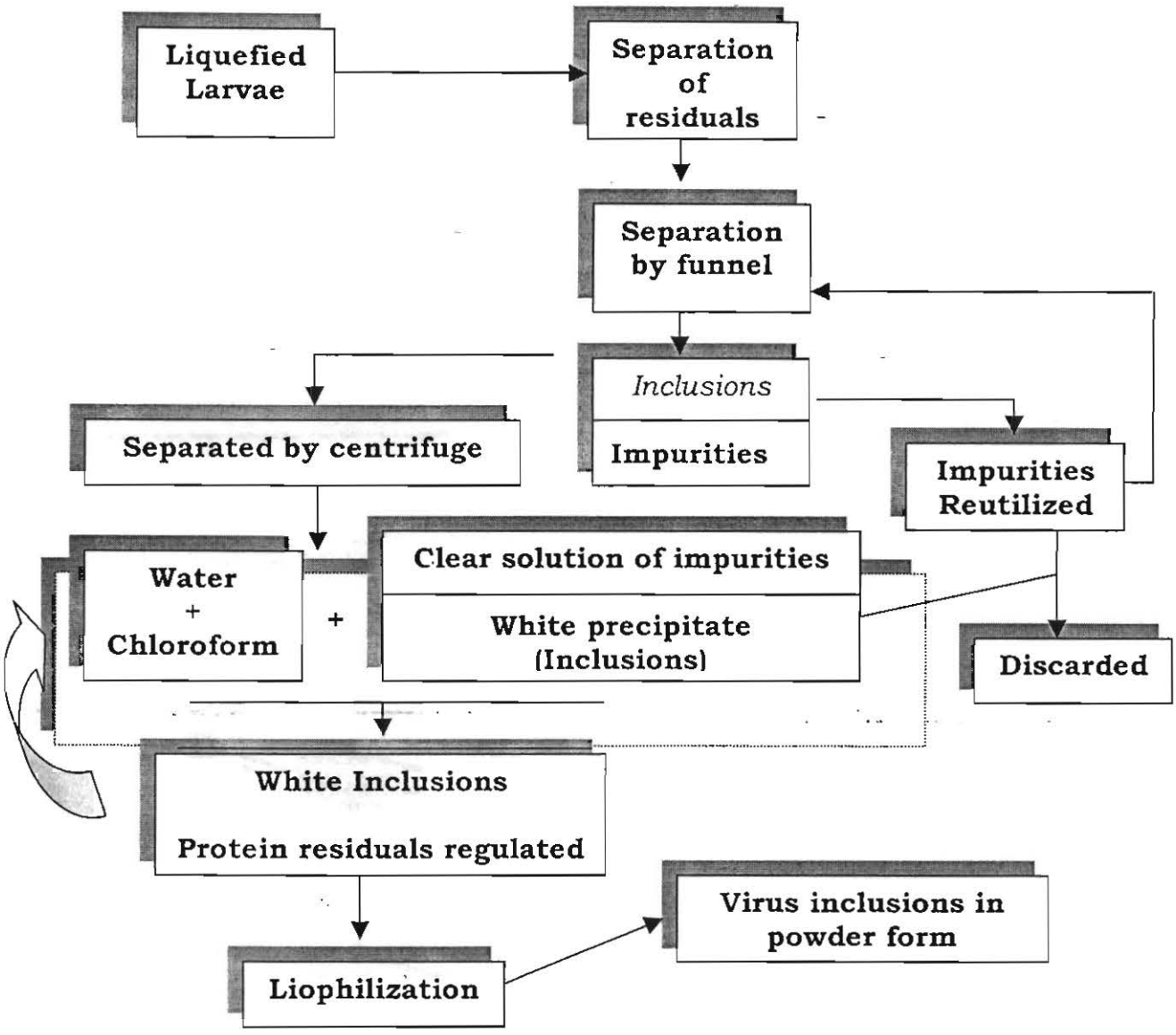

Figure 5.1. Flow diagram for *Erinnyis ello* baculovirus purification (Upali Jayasinghe method).

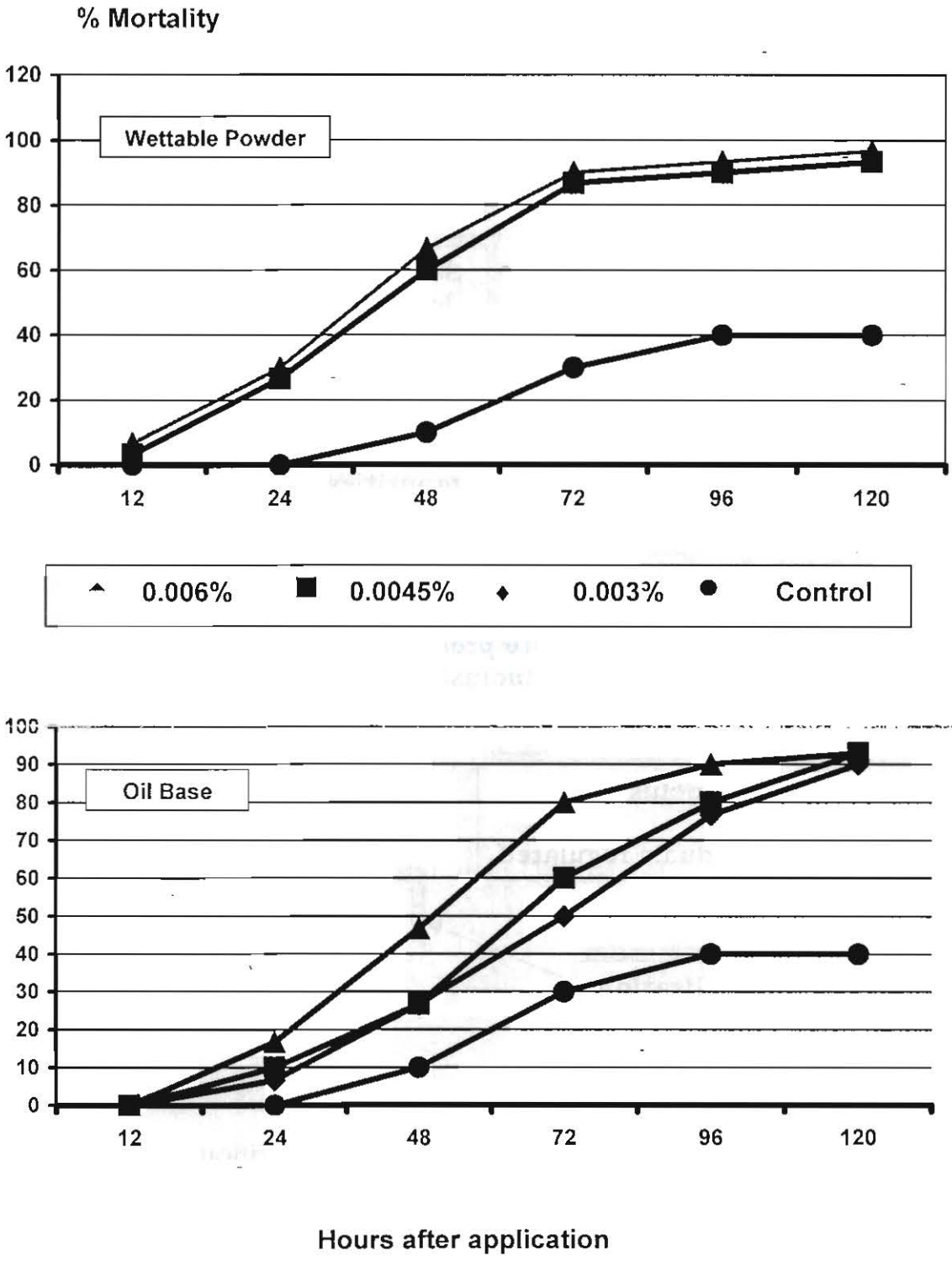

Figure 5.2. *Erinnyis ello* mortality due to applications of wettable powder and oil based formulations of three concentrations (0.006%, 0.0045% and 0.003%) of the cassava hornworm baculovirus.

Activity 6. Development of entomopathogens for biopesticidas research and cassava pest control

Isolates of Entomopathogens have been, and are being field collected from different areas of Colombia. In addition isolates have also been obtained from several different entities (The Coffee Federation, CORPOICA, Universities, and commercial biopesticide companies) for evaluation on controlling cassava pests, primarily whiteflies and burrower bugs. The CIAT collection now contains approximately 73 isolates from numerous fungal pathogens. The major objective of this work is to identify Entomopathogens for control of too major cassava pests, whiteflies (especially *Aleurotrachelus socialis*) and burrower bugs (*C. bergi*). It is also planned that this research will be expanded into control of white grubs (Scarabidae), probably during 2002.

Activity 7. Evaluation of entomopathogens for control of whiteflies

Whiteflies cause major damage in cassava based agroecosystems in the Americas, Africa and to a lesser extend in Asia. In Africa, *Bemisia tabaci* is the vector of Africa Cassava Mosaic Diseases (ACMD); this disease reduces cassava yields considerably. In the Americas, whiteflies reduce cassava yields through their direct feeding and are not as important as virus vectors. Given this scenario, biological control can play an important role in reducing yield losses by reducing pest populations.

Aleurotrachelus socialis is the most damaging species in Colombia and northern South America, while *Aleurothrixus aepim* is the most damaging species in Brazil. EMBRAPA/CNPMF (The Brazilian Cassava Research Center) in Bahia, Brazil, has also mounted a major effort to evaluate cassava whitefly biological control and CIAT and CNPMF are closely collaborating our efforts.

Female *A. socialis* adults oviposit bananas shaped egg individually on the apical leaves of cassava plants. There are three Nymphal instars followed by the pupal (4^{th} instar) and adult stages. The first Nymphal instars, the crawler stage, are highly mobile, moving about the plant to find a fixed position for feeding. During the third instar, nymphs change from cream colored to black and encircled with an abundant, white, waxy cerosine that may cover the whole nymph; the pupal stage is black, making this species easy to distinguish in the field. This waxy secretion may be acting as a barrier toward natural control agents such as predators and parasites. It may also serve as a protect purpose against pesticide applications.

In biological control employing Entomopathogens, fungal pathogens are probably the most common ally. More than 20 entomo-fungal species are reported infecting whiteflies; these include *Aschersonia* sp., *Verticillium lecanii* (Zimmerman), *Beauveria bassiana* (Bálsamo), *Paecilomyces farinosus, Paecilomyces fumosoroseus* (Landa, et. al. 1994). The development of fungal entomopathogen as a biological pesticide requires a careful selection of the most appropriate species and isolate (Heale, 1988). With this in mind we have entered into a strategic program to identify and evaluate Entomopathogens for whitefly (as well as other pests) pest management. Emphasis is being given to native isolates of fungal Entomopathogens, those

collected primarily in Colombia but also those from neighboring countries such as Venezuela, Ecuador and Brazil.

1.1. Fungal isolates. Fourteen fungal isolates collected between 1996 and 2001, from whiteflies are presently stored in the CIAT "ceparío" and will be evaluated in the laboratory **(Table 7.1)**. Seven of these have now been reactivated on *A. socialis*, and others are in the process of being "cleaned" and multiplied (cultured). The methodology being used for culturing the fungi includes a Sabouraud & PDA enriched media, and maintained in incubators at 27°C and 12:12 hour photoperiod with 40±10% relative humidity **(Figure 7.1)**.

As can be observed in **Table 7.1**, fungal pathogens have been collected from at least three different whitefly species, *T. vaporariorum, T. variabilis* and *A. socialis*. Seven fungal pathogen species have been identified with others pending identification. Collections are from three countries, Colombia, Ecuador and Brazil.

Table 7.1. Entomopathogenic fungal isolates recovered from whitefly species and stored in CIAT's "ceparío."

Isolate	Origin	Date of Collection	Host	Identification*
CIAT 210	Pradera-Valle	26-Jul.-99	*T. vaporariorum*	*Paecilomyces fumosoroseus*
CIAT 211	Pradera-Valle	14-May-99	*T. vaporariorum*	*Paecilomyces fumosoroseus*
CIAT 212	Pradera-Valle	10-Jun.-99	*T. vaporariorum*	*Paecilomyces fumosoroseus*
CIAT 215	CIAT	04-Dec.-97	*A. socialis*	*Verticilliun lecani*
CIAT 216	CIAT	27-Apr.-97	*A. socialis*	*Paecilomyces fumosoroseus*
CIAT 217	CIAT	16-Nov.-96	*A. socialis*	*Beauveria bassiana*
CIAT 244	Ecuador-Imbabura	May-00	Whitefly	*Metarhizium* sp.
--	Colombia-Ibagué	11-Jun.-01	*T. variabilis*	*Cladosporium* sp. *Fusarium*
--	Colombia-Biocaribe	Apr.-01	Whitefly	*Verticillium lecanii*
--	Colombia-ICA-Palmira	Jun.-01	Whitefly	*Cladosporium*
--	CIAT	Aug.-01	Whitefly	
--	Brazil-Guajerú	12-Jul.-01	Whitefly	

* Identifications are made through collaboration with Cornell University, Ithaca, NY, USA.

1.2. Reactivation of fungal pathogens: Several methods were evaluated to determine the best procedure for reactivating fungal pathogens on whiteflies. With the first method evaluated, pathogens were reactivated on the whitefly pupal stages (Landa, 1994) by placing pupae (4 instar) on a glass slide (10 x 3mm) with a microdrop (3µl) of the fungal solution, and placed in a petrel dish with moistured (0.2ml of ADE) filter paper. All material must be sterile and maintained under constant light and temperature of 25°C. It is evaluated after 7 days and cultured on Sabouraud media and maintained in an incubator at 27°C, 12:12 hr. photoperiod at 40±10% RH. This method often led to considerable contamination and was therefore altered.

The second method consisted of spraying the fungal pathogen on cassava leaves infested with first instar whiteflies. However this method resulted in considerable leaf deterioration and no fungal effect on the whitefly was observed, and therefore also discarded.

Figure 7.1. Incubator for culturing and multiplying isolates of fungal entomopathogens.

The third method evaluated was with adult whiteflies. One month cassava plants were placed in acetate cages. All leaves except one were removed and adult whiteflies were released into the cages. Applications of the different fungal pathogens were made by an air brush with a 10-psi compressor. Cages were maintained in room at 25°C and 100% RH. This method resulted in reactivation of the pathogen, but also presented considerable contamination.

The forth method consisted of medium sized (9 x 2mm) petri dishes with moist filter paper; 30 whitefly adults, removed from the colony, were placed on the filter paper and a fungal solution was applied. Sporulation of the pathogen could be observed after 7 days and this was cultured on Sabourand and PDA media. All materials were autoclaved prior to use. Using this procedure, different pathogen were obtained; there were recultured until a pure culture could be obtained, subsequently multiplied and used in pathogenicity studies.

1.3. Pathogenicity studies. Pathogenicity of fungal isolates was evaluated on two whitefly stages, nymphs and adults. These were carried out in the greenhouse (26°C and 100% RH) under a 12:12 hr. photoperiod.

For nymphs, one month potted cassava plants in the greenhouse. 30 female and 10 male, two day old whitefly adults were introduced into small leaf cages and allowed to copulate and oviposit on cassava leaves for 48 hours **(Figure 7.2)**. Cages and adults were then removed and eggs allowed to hatch (about 12 days). A solution of the fungal entomopathogen (conc. 1 x 10^8 conidian/ml) was applied to the first instar nymphs. Plants were placed in a growth room with controlled humidity and adult emergence was evaluated. The control consisted of a 0.1% tween application on infested leaves; in a second control no application was made.

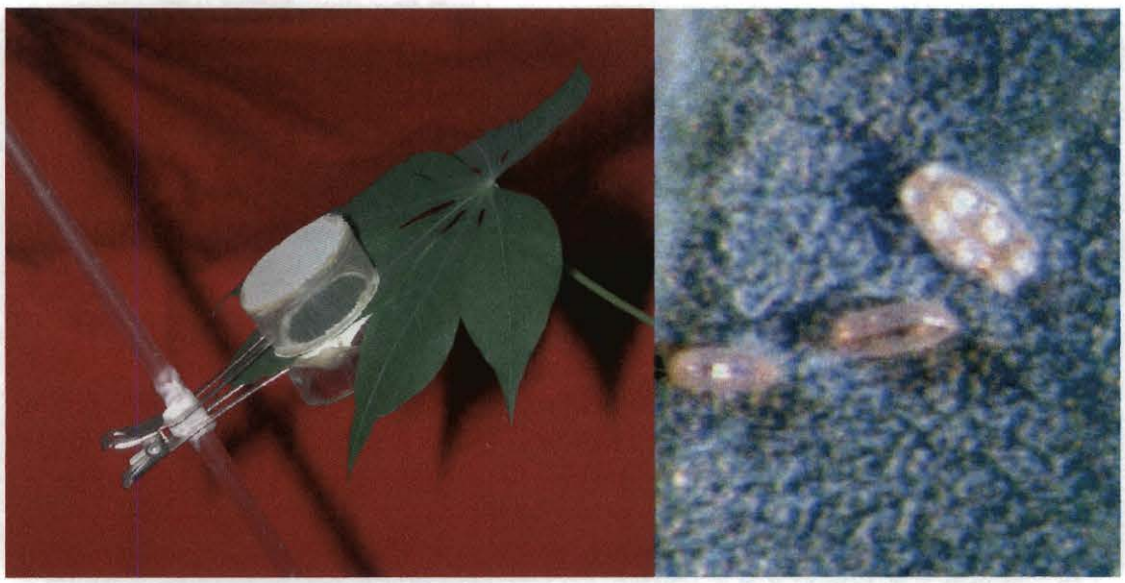

Figure 7.2. A. Leaf snap cages on cassava leaves to facilitate whitefly oviposition. B. First instar nymph or "crawler" of *A. socialis*.

Adult pathogenicity was evaluated by using acetate cages (17w x 40hcm) with a fine cloth covered opening on the superior part of the cages. A second opening (also cloth covered) on the lower part of the cage allowed for manipulation of whiteflies and fungal applications. Two month plants were introduced into the cages; only one leaf at the apical part of the plant was left. A black cloth was placed on soil in the pot (7 inches), allowing for the easy collection of infected adults. This pot was placed in a 7 in. pot and hermetically sealed **(Figure 7.3)**. These units were also placed in growth rooms and 30 female whiteflies were introduced into each unit. Evaluations were made 5 days after fungal application. Dead adults were placed on Sabourand media to determine fungal identification. A control similar to that described for nymphs was employed.

Fungal solutions of the different isolates were prepared using the culture from the petri dishes on Sabourand media. Tween (0.1%) was added to the solution and sifted with a sterile gas and homogenized in a vortex, the concentration of conidia was determined with a hermacitometro (Camara de Neubauer). Solutions were adjusted to a concentration of 1 x 10^7 and 1 x 10^8 con/ml.

A completely random design with 10 repetitions and four treatments (two isolates and two controls) was used **(Table 7.2)**. The Analyses were made using SAS software, versions R 6.12, to determine difference between treatments pertaining to percent emergence of adults and percent mortality. Fisher LSD was used to measure treatment efficiency; Henderson and Tilton, Efficiency Test was used too.

Table 7.2. Evaluation of fungal entomopathogen isolates for pathogenicity on *Alerotrachelus socialis* adults and nymphs in the greenhouse.

Spore	Spore Concentration	Origin	Collection Date	Host	Identification
CIAT 211	$7.25x10^8$ con/ml	Pradera-Valle	14-May-99	*T. vaporariorum*	*Paecilomyces fumosoroseus*
CIAT 216	$8.25 x10^7$ con/ml	CIAT	27-Apr.-97	*A. socialis*	*Paecilomyces fumosoroseus*
Control 1*					
Control 2+					

* Tween Solution, 0.1%; + Absolute control.

Figure 7.3. Acetate cages with potted cassava plants for release of *A. socialis* adults and application of fungal entomopathogens.

1.4. Results and discussion. Significant differences were observed in adult emergence in experiments where the fungal pathogens were applied to first instar nymphs **(Table 7.3)**. Isolate CIAT 211 had the lowest adult emergence (89.9%). There was no significant difference between the two controls. Percentage emergence was highest when the isolate CIAT 216 was applied (95.7%). Using the Henderson and Tilton Efficiency Test, nymphal control for isolate CIAT 211

was 5.8% and for CIAT 216, 3.2%. There are both considered very low, leaving two choices, either the method of application is not adequate or the isolates have a very low pathogenicity on whiteflies and additional isolates need to be evaluated.

Table 7.3. Percent *A. socialis* adult emergence from nymphs treated with two fungal entomopathogen isolates in the greenhouse.

Treatment/Control	Percent Adult Emergence[1]
CIAT 211	89.9 B
CIAT 216	95.7 A
Control 1*	92.9
Control 2+	94.2

* Tween Solution at 0.1%.
\+ Absolute control.
[1] Averages followed by different letters are significantly different.

Adult pathogenicity experiments gave similar results **(Table 7.4)**. Table 6.4 shows that adult mortality was not influenced by either isolate. In fact mortality was higher in the two controls, than with the fungal isolates. Mortality however, was higher with adults than nymphs **(Table 7.5)**.

Table 7.4. *Aleurotrachelus socialis* nymph and adult mortality after treatment with two entomofungal pathogens in greenhouse screening.

	Treatment			
	CIAT 211	CIAT 216	Control 1*	Control 2+
Nymphs	16.7	14.3	11.7	6.7
Adults	92.0	92.0	87	83

* Tween Solution at 0.1%; + Absolute control.

Table 7.5. *Aleurotrachelus socialis* nymph and adult total average mortality of four entomopathogenic fungal treatments.

Stage	N (Treatments)	Average*
Adults	40	88.5 A
Nymphs	40	12.3 B

* Averages followed by different letters are significantly different.

In preliminary trials, using pupae, it was observed that the waxy cerosine surrounding the pupal stage may offer protection against the action or penetration of fungal pathogens. For this reason it was decided to use first instar or "crawless" in pathogenicity experiments.

Experimental methodology will also need to be re-evaluated and changed. It was observed that numerous whitefly adults become "struck" to cage walls, probably due to excess humidity, often resulting in high mortality.

Activity 8. Biological control of the Burrower Bug, *Cyrtomenus bergi* with entomopathogenic fungi

Introduction

The burrower bug, *C. bergi*, is one of the few arthropod pests that feed directly on the commercial, swollen root of cassava. It was first recorded as a cassava pest in Colombia in 1980; more recently it has been reported causing commercial losses in Panama, Costa Rica and Venezuela. *C. bergi* is probably present in many other areas of the Neotropics, but feeding on other hosts, including onions, groundnuts, maize, potatoes, *Arachis pintoi* (forage groundnut), sorghum, sugarcane, coffee, coriander, asparagus, beans, peas, pastures and numerous weeds. This polyphagous species has probably not coevolved with cassava and some hosts are strongly preferred over others. *C. bergi* develops faster on maize and groundnuts than on cassava and prefers maize to cassava. Optimal fecundity, survival and intrinsic rate of population increase occurred on groundnut and maize, not on cassava.

C. bergi nymphs and adults feed on cassava roots by penetrating the peel and parenchyma with a thin strong stylet. This feeding introduces several soil-borne pathogens (e.g. *Aspergillas, Diplodia, Fusarium, Phytophora,* etc.) into the root parenchyma. Brown-to-black lesions begin to develop on the roots within 24 hours after feeding is initiated, resulting in starch reduction and a serious loss in commercial value. As damage is not detected until roots are harvested and peeled, producers can lose the value to the crop as well as labour, time and land use. *C. bergi* populations are present in the soil throughout the crop cycle, and root damage starts in the first month of plant growth. Feeding can continue throughout the crop cycle and can result in 70 to 80% total root damage with >50% reduction in total starch content. The economic injury threshold-the point where cassava root purchasers will reject a shipment-is when 20 to 30% of the roots are damaged given that the "cosmetic" damage of rot spots is not acceptable for the fresh food market.

C. bergi is difficult to control due to the polyphagous nature of the pest and its adaptation to the soil environment. Pesticide applications can reduce pest populations and damage, however frequent applications may be required, which are costly, environmentally hazardous, and often fail to reduce damage below economic injury levels.

The potential of biological control of *C. bergi* needs to be investigated and alternatives to chemical pesticides need to be developed. The nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* successfully parasitized *C. bergi* in the laboratory. Isolates of entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria basssiana* and *Paecilomyces lilacinus* have demonstrated pathogenic abilities on *C. bergi*.

The objectives of the present study are to evaluate the pathogenicity and virulence of different (several) isolates of entomopathogens such as *Metarhizium* sp., *Beauveria* sp., and *Paecilomyces* sp., on nymphs and adults of *C. bergi*.

Methodology

A laboratory colony of *C. bergi* was established by collecting nymphs and adults from onion fields in the Risaralda Department of Colombia. The colony is maintained in sterilized soil in plastic boxes in the laboratory (25-30°C). *C. bergi* nymphs and adults feed on groundnut that has been sterilized with a 3% sodium hypocloride solution for 3 hours, then hydrated with distilled water for 5 hours. Groundnut seeds, prior to planting, are pregerminated in plastic boxes with moist paper towels for two days.

Fungal isolates of *M. anisopliae, B. bassiana* and *P. lilacinus* were collected from several sources. These isolates came from CENICAFE in Caldas, Colombia; 19 isolates were collected from the Department of Cauca (Municipality of Popayán), from *C. bergi* infected asparagus fields during the 1990s; and six of the isolates were collected in the Department of Risaralda (Municipality of Pereira) during 2000 **(Table 8.1)**. The selected isolates were cultured and multiplied on Saburand Dextrose Agar, enriched with Peptone, sucrose and yeast extract (SADY), and with Potato Dextrose Agar, enriched with yeast extract, peptone and agar at 0.1% (PADY). Isolates are maintained in petri dishes stored in incubators (25±2°C) to allow for growth and sporalation. Conidia are harvested from each isolate and placed in sterilized water with Tween (0.05%).

Table 8.1. Entomopathogenic fungal isolates collected from *Cyrtomenus bergi* and stored in CIAT's collection.

Catalogue	Host		Origin of Sample			Date of Selection	State of Collection
	Genus	Species	County	Dept.	Collector		
CIAT 214	*Cyrtomenus*	*bergi*	Colombia	Caldas	CENICAFE	30-Oct.-95	Paper
CIAT 224	*Cyrtomenus*	*bergi*	Colombia	Caldas	CENICAFE	30-Oct.-95	Paper
CIAT 225	*Cyrtomenus*	*bergi*	Colombia	Caldas	CENICAFE	30-Oct.-95	Paper
CIAT 226	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 227	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 228	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 229	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 230	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 231	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 232	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 233	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 234	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 235	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 236	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 237	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 238	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 239	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 240	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 241	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 242	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 243	*Cyrtomenus*	*bergi*	Colombia	Cauca	Caicedo *et al*	July-94	Paper
CIAT 250	*Cyrtomenus*	*bergi*	Colombia	Risaralda	Rendón *et al*	12-July-00	Medium
CIAT 251	*Cyrtomenus*	*bergi*	Colombia	Risaralda	Rendón *et al*	12-July00	Medium
CIAT 258	*Cyrtomenus*	*bergi*	Colombia	Risaralda	Rendón *et al*	12-July-00	Medium
CIAT 259	*Cyrtomenus*	*bergi*	Colombia	Risaralda	Rendón *et al*	12-July-00	Medium
CIAT 260	*Cyrtomenus*	*bergi*	Colombia	Risaralda	Rendón *et al*	12-July-00	Medium
CIAT 261	*Cyrtomenus*	*bergi*	Colombia	Risaralda	Rendón *et al*	12-July-00	Medium

Three methods for pathogen application were evaluated:

1. 250 gr. of sterile soil containing 2 *A. pintoi* seeds was placed in rounded box (6 x 7 cm) and infested with 10 5th instar or adult *C. bergi* (10 replications per treatment). Fungal application (aspersion) was made 24 hours later by spraying the soil surface. Evaluations were made 20 days after treatment.

2. 500 gr. of sterile soil was placed in plastic boxes (30x20x10cm) with cassava roots (var. CMC-40) and infested with 20 5th instar and adult *C. bergi*. Fungal application by liquid spraying the insects five times. Evaluations were made every two days for 20 days.

3. 500 gr. of sterile soil with groundnut seed or cassava was placed in plastic boxes (as above) and infested with 20 5th instar and adult *C. bergi*. Fungal application by liquid spraying was applied to both insects and the soil. Evaluations as above (2).

All fungal isolates were applied in liquid formula containing conidia (Tween 0.05%; ADET) at a concentration of 1×10^7 and 1×10^8 conidia/ml., 10ml of conidia solution were applied to each experimental unit, in concentrations of $5.72 \times 10^7 \pm 9.02 \times 10^6$ conidia/ml. and $1.45 \times 10^8 \pm 9.18 \times 10^7$ conidia/ml. A completely random experimental design was used with 5 repetitions for each treatment. ANOVA analysis was used for percent mortality, and the Tukey test to determine the effect of treatment on mortality.

Results

Best results were obtained using the method that included the use of plastic boxes and spraying the fungal pathogen on insects and the soil. Mortality reached 100% for fifth instar nymphs and 60% for adults with certain isolates. These experimental units provided for easy manipulation of *C. bergi* combined with minimal contamination. This methodology was therefore utilized to evaluate the different fungal isolates. Based on preliminary results, 20 fifth instar nymphs and 20 adults were included in each treatment (each experimental unit).

Pathogenicity and virulence evaluations

Eighteen isolates divided into two groups were evaluated at two dates, nine isolates at each date, March 6 and May 1, 2001. In the control treatment, insects and soil was sprayed with a 0.05% Tween (ADET) solution. However fungal contamination occurred in the control treatments leading to some mortality in these treatments (**Tables 8.2 and 8.3**). Nymphal mortality in the control treatments reached 22 and 19% for Group 1 (March 6 evaluation) and Group 3 (May 1 evaluation). For adults, mortality in Groups 1 and 2 were 28.3 and 17.0% respectively.

Nymphal Mortality. Of the 18 fungal isolates evaluated, nymphal mortality ranged from 30 (CIAT-241) to 100% (CIAT 224 and CIAT 245) (**Table 8.2**). The highest mortality was obtained with isolates CIAT 224, CIAT 245, CIAT 230 (89%), CIAT 261 (74%) and CIAT 240 (74%). Nymphal mortality was observed occurring soon after treatments were initiated. For example isolates CIAT 230, CIAT 261, CIAT 224, CIAT 245, CIAT 239 and CIAT 228, recorded mortalities above 15% just two days after treatment (DAT). Mortality with isolate

CIAT 245 reached 94%, 8 DAT and 100% 12 DAT **(Table 8.2)**. Mortality with isolate CIAT 224 reached 94% 10 DAT and 100% 17 DAT. Mortality in the control treatments remained low during the early days of the experiment and rose to over 20% DDAT. These results indicate a possible problem with contamination as the experiment progressed.

Table 8.2. Fifth instar *C. bergi* nymphal mortality after exposure to 18 entomopathogenic fungal isolates.

		Nymphal Mortality (%) Groups I & II								
		Days After Treatment								
Isolate	Conidias/ml.	2	4	6	8	10	12	14	17	20
CIAT 227	5.45X107	0	0	0	0	1	2	2	28	66
CIAT 231	6.65X107	1	1	2	3	3	3	3	20	53
CIAT 233	4.65X107	1	4	5	5	5	6	6	41	67
CIAT 234	6.50X107	0	0	0	0	0	0	3	40	58
CIAT 241	5.45X107	0	0	2	4	7	8	9	19	30
CIAT 242	4.50X107	4	11	11	11	14	16	16	45	55
CIAT 250	6.75X107	2	5	6	9	11	11	11	35	52
CIAT 258	6.75X107	2	4	5	5	5	6	8	40	58
CIAT 259	4.75X107	1	2	5	6	6	7	7	31	51
Control	ADET	0	0	6	6	8	8	8	12	22
CIAT 230	4.75X107	15	42	50	56	66	74	79	81	89
CIAT 237	6.70X107	8	8	9	9	9	30	45	49	58
CIAT 261	1.45X107	19	23	23	24	24	37	47	67	74
CIAT 224	2.68X108	20	40	60	84	94	96	99	100	100
CIAT 245	1.80X108	25	50	63	94	99	100	100	100	100
CIAT 239	2.2X108	20	21	24	30	35	36	38	40	56
CIAT 228	2.65X108	18	21	26	28	28	31	40	45	55
CIAT 238	6.75X107	5	7	12	14	15	21	30	36	50
CIAT 240	1.75X108	5	9	9	9	12	21	31	37	74
Control	ADET	1	2	6	6	8	8	12	19	29

Twenty DDAT, the control treatment in group 2, had 29% mortality. The isolates that had a significantly higher mortality were CIAT 245 (100%), CIAT 224 (100%), CIAT 230 (89%), CIAT 261 (74%) and CIAT 240 (74%) **(Figures 8.1 and 8.3)**. The coefficient of variation was 24.1% **(Table 8.4)**.

Table 8.3. *Cyrtomenus bergi* adult mortality after exposure to 18 entomopathogenic fungal isolates.

		Adult Mortality (%) Groups I & II								
		Days After Treatment								
Isolate	Conidias/ml.	2	4	6	8	10	12	14	17	20
CIAT 227	5.45X107	0	5	6.7	15	16.7	20	21.7	30	56
CIAT 231	6.65X107	0	1.7	3.3	5	5	8.3	15	20	48.3
CIAT 233	4.65X107	0	1.7	3.3	3.3	5	11.7	11.7	35	53.3
CIAT 234	6.50X107	0	1.7	5	8.3	10	11.7	13.3	15	31.7
CIAT 241	5.45X107	0	3.3	3.3	8.3	15	23.3	28.3	30	58.3
CIAT 242	4.50X107	0	11.7	11.7	13.3	16.6	21.6	30	35	50
CIAT 250	6.75X107	0	6.7	15	16.7	18.3	20	21.7	30	56.7
CIAT 258	6.75X107	0	5	5	8.3	13.3	20	21.7	30	55
CIAT 259	4.75X107	0	5	5	11.7	15	18.3	18.3	45	65
Control	ADET	0	0	0	0	3.3	5	8.3	10	28.3
CIAT 230	4.75X107	7	10	10	13	18	25	30	35	53
CIAT 237	6.70X107	14	14	16	19	21	29	34	35	50
CIAT 261	1.45X107	9	13	17	20	22	26	32	33	49
CIAT 224	2.68X108	14	17	17	18	19	19	22	24	47
CIAT 245	1.80X108	14	16	19	20	21	23	26	26	47
CIAT 239	2.2X108	7	8	11	13	13	13	19	20	33
CIAT 228	2.65X108	3	4	6	8	8	10	14	15	23
CIAT 238	6.75X107	1	2	3	5	5	8	12	13	20
CIAT 240	1.75X108	2	3	4	5	5	8	11	12	21
Control	ADET	0	0	0	2	2	5	6	6	17

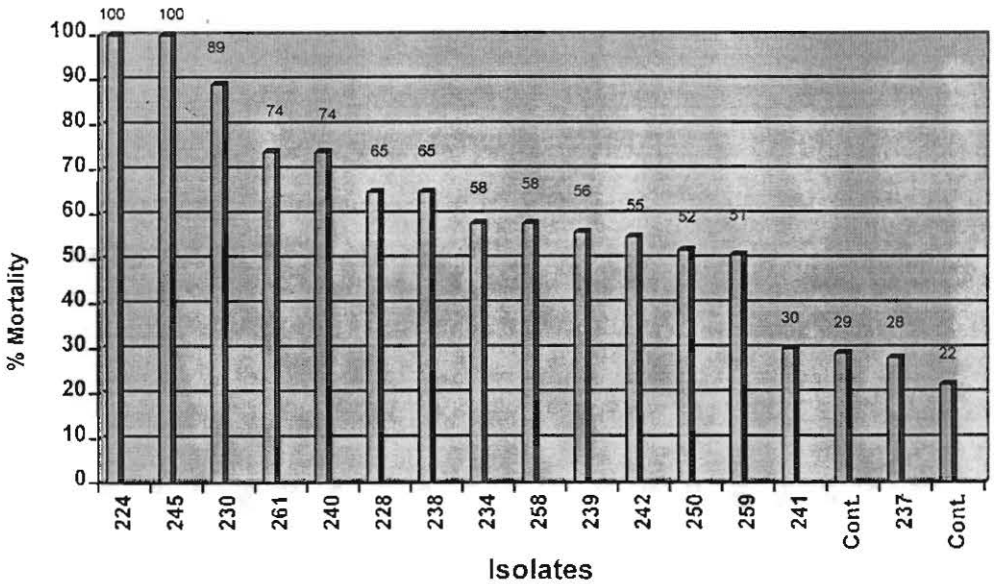

Figure 8.1. Fifth instar *Cyrtomenus bergi* nymphal mortality 20 days after exposure to 18 entomopathogenic fungal isolates.

Table 8.4. Statistical design and ANOVA for the means of the five best entomopathogenic fungal isolates causing *C. bergi* nymphal mortality.

Dependent Mortality Variable of 5th Instar Nymphs 20 Days After Treatment					
Source	DF	ANOVA SS	Mean SQ	F Value	Pr > F
Model	16	52861.1	3303.1		
Error	64	14727.1	230.1	14.36	0.0001
Corrected total	80	67588.1			
CV: 24.09				Nymphs Mean: 62.96	
Source	DF	ANOVA SS	Mean SQ	F Value	Pr > F
Isolate	12	52484.3	4373.1	19.01	0.0001
REP	4	377.2	94.3	0.41	0.8009
ALPHA: 0.05		Df: 18		Mse: 100.3	
	Range: 4.86			Difference: 31.826	

Tukey Grouping			Mean	N	Isolate	
	A		100	5	CIAT	245
	A		100	5	CIAT	224
B	A		89	5	CIAT	230
B	A	C	74.17	5	CIAT	261
B	A	C	74	5	CIAT	240
B		C	65	5	CIAT	228
B		C	65	5	CIAT	238
		C	56	5	CIAT	239
	D		29.25	5	Control	
	D		28	5	CIAT	237

Adult *C. bergi* when exposed to the two groups of isolates were in general, lower than the mortality observed for the nymphs (**Table 8.3**). The isolates presently significantly higher mortality in group one than the control (28.3%) at 20 DAT were, CIAT 259 (65%) and CIAT 241 (58%) (**Figure 8.2**). The coefficient of variation was 19.9% (**Table 8.5**). In the second group of isolate evaluated, only one, CIAT 230, with 53% mortality was significantly different from the control treatment (17% mortality). In this experiment the coefficient of variation was much higher (39.3%) than in the other experiments (**Table 8.5**).

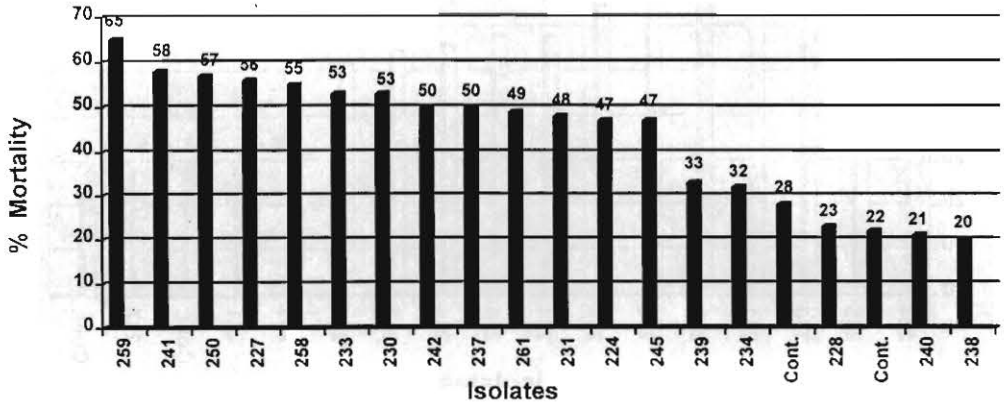

Figure 8.2. Adult *Cyrtomenus bergi* mortality (%) 20 days after exposure to 18 entomopathogenic fungal isolates.

Comparing mortality across isolates for control of nymphs the most effective isolates were CIAT 245, CIAT 224, CIAT 230, CIAT 261 and CIAT 240 **(Figure 8.3)**. CIAT 245 and CIAT 224 were the most outstanding, causing 100% mortality **(Table 8.4)**. These isolates were significantly superior to the control.

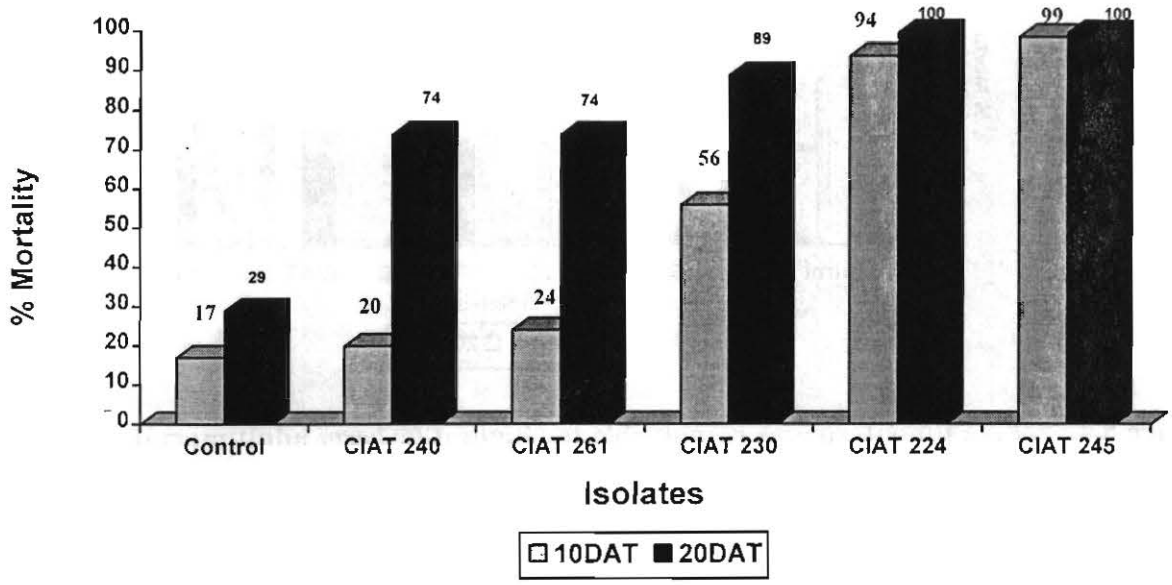

Figure 8.3. Five entomopathogenic fungal isolates responsible for highest *C. Bergi* mortality (%) 10 and 20 days after treatment (DAT).

These isolates have been identified by Cornell taxonomists as follows:

CIAT 259 = *Fusarium* sp.
CIAT 241 = *Asprgillas neosartorga*
CIAT 250 = *Fusarium* sp.
CIAT 227 = *Aspergillas flavus*
CIAT 258 = *Metarhizium anisopliae*

For adult control efficiency the isolates causing highest mortality were CIAT 259, CIAT 241, CIAT 250, CIAT 227 and CIAT 258 **(Figure 8.4)**. CIAT 259 (65% mortality) and CIAT 241 (58.3%) were significantly different from the control **(Table 8.5)**.

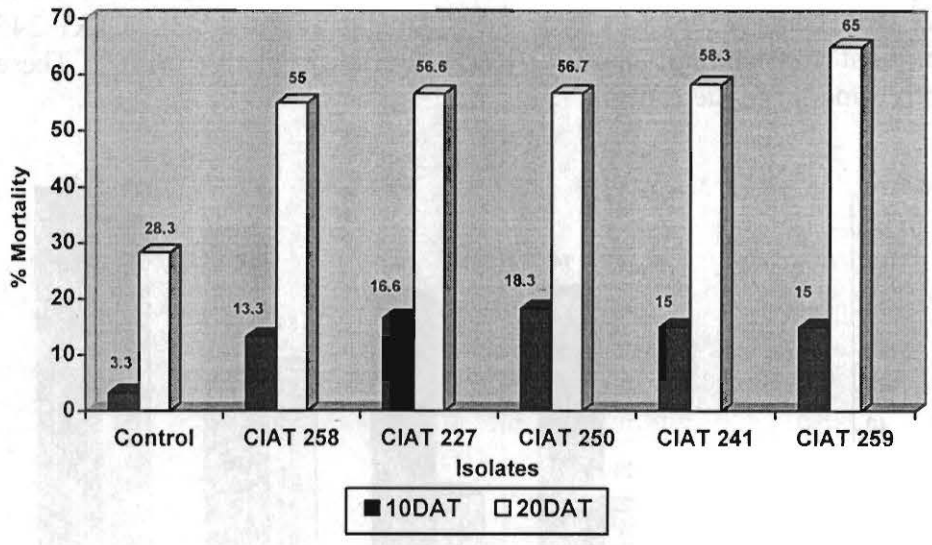

Figure 8.4. Five fungal isolates responsible for highest *C. bergi* adult mortality 10 and 20 days after treatment (DAT).

Table 8.5. Statistical design and ANOVA for the means of the five best fungal isolates causing *C. bergi* adult mortality.

Dependent Mortality Variable of 5th Instar Nymphs 20 Days After Treatment					
Source	**DF**	**ANOVA SS**	**Mean SQ**	**F Value**	**Pr > F**
Model	11	3891.7	353.8		
Error	18	1805	100.3	3.53	0.0088
Corrected total	29	5696.7			
CV: 19.9				Adults Mean: 50.3	
Source	**DF**	**ANOVA SS**	**Mean SQ**	**F Value**	**Pr > F**
Isolate	9	3680	3680	4.08	0.0054
REP	4	211.7	211.7	1.06	0.3686
ALPHA: 0.05		Df: 18		Mse: 100.3	
Range: 5.1				Difference: 29.3	
Tukey grouping			**Mean**	**N**	**Isolate**
	A		65	5	CIAT 259
B	A		58.3	5	CIAT 241
B	A	C	56.7	5	CIAT 250
B	A	C	56.6	5	CIAT 227
B	A	C	55	5	CIAT 258
B	A	C	53.3	5	CIAT 233
B	A	C	50	5	CIAT 242
B	A	C	48.3	5	CIAT 231
B	A	C	31.7	5	CIAT 234
B	A	C	28.3	5	Control

All 18 isolates were evaluated both with 5th instar nymphs and adult *C. bergi*. The five isolates selected, as the best for causing nymphal mortality were different than the five best selected for causing adult mortality. If one tries to combine the mortalities of each isolate for both nymphal and adult mortality, the three best isolates are CIAT 245, CIAT 224 and CIAT 230; the second best group could be CIAT 261, CIAT 227 and CIAT 259. It is notable the best isolates for

nymphal mortality did not match the best for adult mortality, and that nymphal are some sensitive or susceptible to infection and morality than adults.

It is recommendable that the best isolates be multiplied at commercial level and field trials for control of *C. bergi* with these isolates be initiated.

Activity 9. Rearing the burrowing bug, *Cyrtomenus bergi* , on a defined diet

The burrowing bug, *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae), is considered to be one of the most important pests living in the soil, especially in Panama and Colombia. It causes severe damages to cassava roots, as well as roots of other cultivated crops such as onions, coffee, sugarcane, potatoes, peanuts, and maize. The nymphs and adults of this insect cause damage to the fleshy roots of cassava by inserting their strong stylet to feed. The wounds left in the roots by the stylet are good opportunities for soil-borne pathogens to invade which therefore makes the roots commercially unacceptable. Controlling *C. bergi* is difficult because of its polyphagous nature and its adaptation to the soil environment. Pesticide applications are costly, environmentally hazardous, and not always effective. Intercropping cassava with *Crotalaria* sp. (sunne hemp) was found significantly more effective than pesticide treatments, reducing root damage to 4%, compared with 61% in cassava monoculture. Recent studies indicate that entomopathogenic nematodes and fungi may offer a more acceptable solution for controlling *C. bergi* (Bellotti et al., 1999).

A recent screening of cassava germplasm indicates that HPR (Host Plant Resistance) may be also available. In fact, field trials have shown that low-HCN clones suffer more damage than high-HCN ones, indicating that cyanogenesis in cassava may acts as a deterrent. Nevertheless, further research is required as results are not conclusive, because attempting to test HCN as deterrent factor to *C. bergi* in artificial conditions have failed, due to the fact that an artificial diet was not available for this insect species.

Since the 1950's, the use of artificial diets has provided a better knowledge of the nutritional requirements of various insect species. Artificial diets have been very widely used to bioassay the activity of allelochemicals against various insect pests and have played a particularly important role in the bioassay of individual factors involved as resistance mechanisms. Therefore, when studying plant resistance, artificial diets of known chemical composition (holidic diet) have been shown to be indispensable tool. Therefore the purpose of this activity was to develop a holidic diet and a system unit for rearing *C. bergi* under artificial conditions.

The standard medium used, was diet derived from A0 of Febvay et al. (1988), except for sucrose and cholesteryl benzoate which were adjusted to 6 g/100mL and 5 mg/100mL respectively **(Table 9.1)**. pH was adjusted to 7.0 with potassium hydroxide, and the medium was then filter-sterilized (0.22 µm Millipore units). Pieces of foam rubber (1cm x 1cm) were used to supply the liquid diet to the insects **(Figure 9.1)**.

Table 9.1. Composition of the diets used to rear the burrower bug, *Cyrtomenus bergi* (derived from A0, Febvay et al. (1998).

L-Amino Acids (mg/100mL of Diet)		Vitamins (mg/100mL of Diet)	
Ala	178.71	p-aminobenzoic acid	10.00
ß-Ala	6.22	L-ascorbic acid	100.00
Arg	244.90	Biotin	0.10
Asn, H_2O	298.55	D-calcium pentothenate	5.00
Asp	88.25	Choline chloride	50.00
Cys	29.59	Folic acid	1.00
Glu	149.36	i-Inositol	42.00
Gln	445.61	Nicotinamide	10.00
Gly	166.56	Pyridoxin HCl	2.50
His, HCl, H_2O	136.02	Riboflavin	0.50
Ile (allofree)	164.75	Thiamine di-HCl	2.50
Leu	231.56		
Lys mono HCl	351.09	Others (mg/100mL of diet)	
Met	72.35	$CuSO_4$, 5 H_2O	0.47
Orn mono HCl	9.41	$FeCl_3$, 6 H_2O	4.45
Phe	294.53	$MnCl_2$, 4 H_2O	0.65
Pro	129.33	NaCl	2.54
Ser	124.28	$ZnCl_2$	0.83
Thr (allofree)	127.16	Calcium citrate	10.00
Try	42.75	Cholesteryl benzoate	5.00
Tyr	38.63	$MgSO_4$, 7 H_2O	242.00
Val	190.85	KH_2PO_4	250.00
Sucrose (g/100mL of diet)	6.00		

Note: pH = 7.0 for all diets.

With the diet and rearing technique, we reared *C. bergi* from newly hatched larvae to Vth instars (**Table 9.2**) and also to adults (data in process). For each developmental stage, the developmental time was longer on artificial diet than recorded on a natural diet i.e. maize. The weights of insect reared on the liquid medium were close to those obtained on maize. Similar observations were done on larval lengths (data not shown).

In conclusion, although the duration of larval period on the artificial diet was longer than recorded on maize, the fact that the weight and length were similar; indicated that the use of our technique and liquid diet revealed to be useful to test potentially active molecules such as HCN on the burrowing bug *C. bergi*.

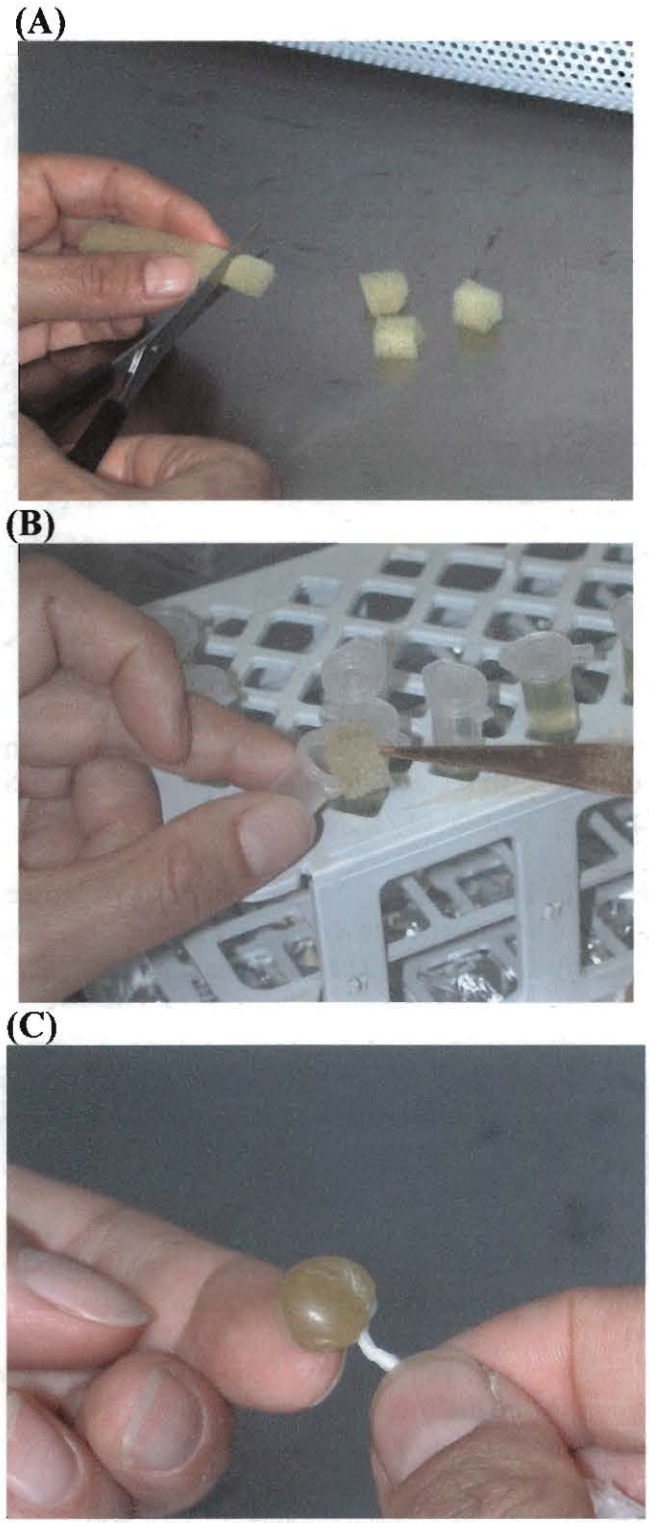

Figure 9.1. **Pieces of foam rubber were cut (A) and soaked to the liquid diet (B). Then, they were enclosed in sterile parafilm sachets (C) and put on about 10 cm^3 of soil.**

Table 9.2. Biological performance of *C. bergi* on different diets.

Diet	Developmental Stage	Developmental Time (Days, Mean ± SE)	Weight (mg. Mean ± SE)
Maize	II	12.5 ± 0.5 a	4.0 ± 0.3 a
Diet	II	15.1 ± 0.9 b	4.6 ± 0.2 a
Maize	III	22.8 ± 0.5 a	9.9 ± 0.9 a
Diet	III	26.8 ± 0.8 b	10.4 ± 0.6 a
Maize	IV	34.1 ± 0.6 a	24.5 ± 5.3 a
Diet	IV	45.6 ± 1.2 b	21.3 ± 1.1 a
Maize	V	48.5 ± 0.8 a	67.7 ± 14.6 a
Diet	V	69.8 ± 3.2 b	41.6 ± 2.9 a

Within a column, means followed by different letters are significantly different ($P<0.05$; Student's t-test, diet comparisons).

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Awards

Distinción Especial, 2001. La Sociedad Colombiana de Entomología (SOCOLEN) for a continued and fructiferous contribution to Colombia Entomology.

Premio "Hernán Alcaraz Viecco". First Prize, 2001. Awarded by Sociedad Colombiana de Entomología, SOCOLEN for outstanding scientific research. (Shared with co-workers).

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Sub-output 2. Determine Alternative Methods for Control of the Cassava Mealybug. (P.-A. Calatayud)

Activity 1. Identification of toxic protein to *Phenacoccus herreni*

Toxic proteins in plants are considered to have the potential to function as chemical defensive factors 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*.

Leaves of *Jatropha gossypiifolia* (Euphorbiaceae) showed a strong toxicity to *P. herreni* (CIAT, Annual Report, 1999). In fact, after 24 hours of infestation this plant showed 95% mortality and after 48 hours, 100% mortality. Therefore the purpose of this activity was to identify toxic protein in leaves of *J. gossypiifolia*.

The standard medium used, was diet derived from A0 of Febvay et al. (1988), except for sucrose which was adjusted to 200 g/L (see the composition below in Table 1.2). For identifying protein toxicity in *J. gossypiifolia* leaves different diets **(Table 1.1)** were based on A0 and differed only by their extract compositions. All diets were enclosed in sterile parafilm sachets and stretched on the top of a standard film box (black; height, 5 cm; diameter, 3.2 cm), which constituted the rearing unit. Groups of 50-60 neonate larvae were placed directly in experimental rearing units. After 48 hours, higher mortality was recorded with extract of young leaves of *J. gossypiifolia* **(Table 1.1)**. KCN was used as positive control for toxicity.

In conclusion, toxicity was evidenced only in young leaves of *J. gossypiifolia*. Nevertheless, when this extract was boiled the toxicity disappeared suggesting that protein(s) are involved. Further studies are in process to confirm the presence of toxic proteins in the extract and to purify the protein for their identification.

Table 1.1. Mortality of neonate (in percentage) reared on different diets after 48 hours.

Diet	% Mortality (Mean ± SE)
Control	6.9 ± 3.1 a
Extract of young leaves of *J. gossypiifolia* (1)	82.8 ± 4.9 b
Extract (1) boiled	13.0 ± 2.3 a
KCN	100 c
Extract of CMC 40 leaves	9.9 ± 3.1 a
Extract of mature leaves of *J. gossypiifolia*	11.6 ± 2.6 a

Means with different letters are significantly different at 5% level (PLSD Fisher's test following ANOVA).

Activity 2. Screening of digestive enzymes in the gut of *Phenacoccus herreni*

In recent years a number of different classes of proteins have been reported to promote toxic effects when they are ingested by plant-sucking Homoptera. To date, extensive work has been done with aphids and whiteflies, reporting that lectins generally promote a more toxic effect than the δ-endotoxin proteins of *Bacillus thuringiensis* or the common enzyme inhibitors such as protease and α-amylase inhibitors. In contrast, no work in pseudococcids has been reported to our knowledge.

The natural food source of Homoptera feeding strictly on phloem sap is mainly constituted by free amino acids and sugars (mainly sucrose)[see Srivastava (1987) for review]. Therefore the protease or α-amylase inhibitors, which show considerable specificity toward these target enzymes, should not be toxic to phloem-feeder insects because they do not require such enzymes in their gut. Although some information on the digestive enzymes has been reported for aphids, no work has been done for whiteflies or mealybugs.

Therefore, in order to determine the kind of enzyme inhibitor that could be toxic to the cassava mealybug, it was first necessary to complete the analysis of the enzymes present in the *P. herreni* gut. This study reports on the types of enzymes present in the insect's digestive tract. Determination of the intestinal pH, crucial information for evaluating enzyme activities, was done first.

By using pH indicators, we consistently observed values between pH 6.8 and 7.6 for *P. herreni* intestine. As a control the pH of the distilled water where the intestine was extracted was determined using the same procedure. We observed values between pH 4.6 and 5.2, distinct from the range obtained for the intestinal fluids. Using a pH meter, the distilled water had a pH of about 4.7, within the range estimated using pH indicators, thereby validating the methodology used.

It is well known that aphids respond to the pH of the diet and that they prefer and generally perform better on diets with a slightly alkaline pH of 7.3-7.6 (Auclair, 1969). This can also be valid for pseudococcids because it is easy to rear them on an artificial diet at pH 7.5. Furthermore, the mealybug digestive system has a filter chamber (Pesson, 1943). This structure plays an important role in regulating the osmotic pressure and in accumulation of nutrients. This is very important for insects that ingest a large volume of phloem sap and that show a significant proportion of sap nutrients excreted as compared to the amount of nutrients actually ingested. Therefore it should be more likely for them to present an intestinal pH in a range similar to that the range of the ingested diet. For many plants species it has been reported that the pH of the phloem sap is from slightly-to-moderately alkaline, 7.2-8.5 (Ziegler, 1975). All these results reported in the literature are consistent with the estimate of the pH intestine range of *P. herreni* as being slightly alkaline, 6.8-7.6. Moreover, this estimate is also in agreement with the enzymes in the digestive tract of *P. herreni* having an optimum pH from 6.5-8.5 as revealed by the API system.

A fast semi-quantitative analysis of enzymatic activities was performed using the API system (see CIAT Annual Report, 2000). By comparing the enzymatic activities in the gut with those

identified in the body after having extracted the digestive tract, we found that the major activities exhibited by whole *P. herreni* digestive tracts were for alkaline phosphatase, esterase (C4) and leucine aminopeptidase.

The presence of these three major intestinal enzymes revealed by the API-ZYM system was confirmed by the development of protein zymograms **(Figure 2.1)**. The presence of alkaline phosphatase, esterase and leucine aminopeptidase activity in the digestive tracts of the insect was clearly evidenced (see lane 2). Two bands of activity were evidenced for esterase and were more clearly visible for leucine arylamidase; whereas a single major band was clearly observed for alkaline phosphatase.

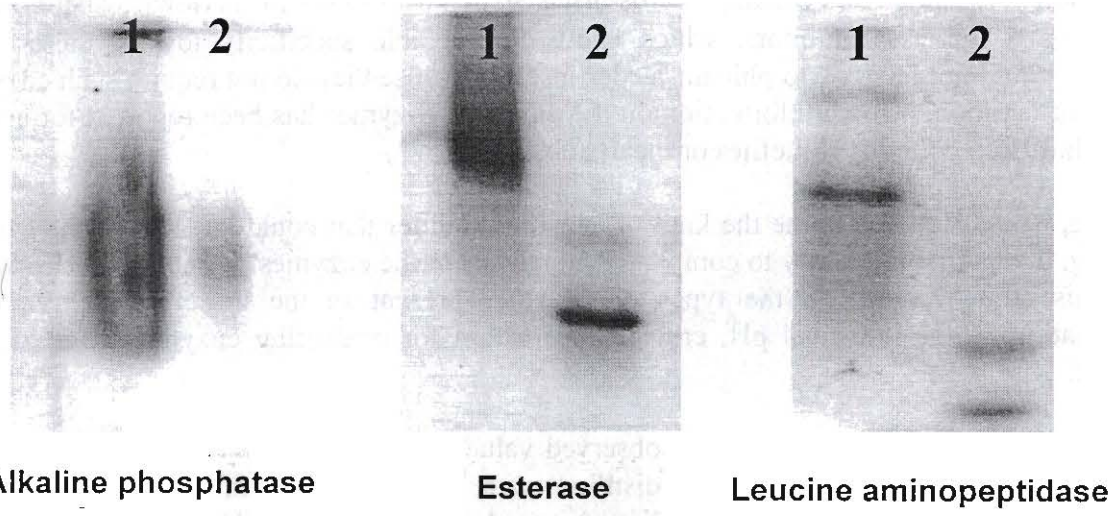

Figure 2.1. Zymograms of digestives enzymes in the gut of *Phenacoccus herreni*.

As expressed by Srivastava (1987), enzyme production in Homoptera is related to the type of food ingested. As mealybugs are mainly phloem feeders (i.e. with simple food constituents such as free amino acids), only a few digestive enzymes should be detected. In fact, among the 19 common enzymes analysed by API-ZYM system (CIAT, Annual Report, 2000), only 3 (alkaline phosphatase, esterase (C4) and leucine aminopeptidase) were clearly evidenced in the digestive tracts of *P. herreni*. Alkaline phosphatase and leucine aminopeptidase were also detected in the digestive tracts of an aphid species *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), using the same API-ZYM system. In contrast to *A. pisum*, the α-glucosidase (or invertase) was not clearly evidenced in *P. herreni*. This is not surprising if we assume that a significant proportion of sucrose is excreted, a large amount of sucrose is generally found in the honeydew of Homoptera sap feeders, and that similar to aphids (Srivastava, 1987), this carbohydrate plays more of a phagostimulant than nutritive role. Furthermore, similar to *A. pisum* and to aphids in general (Srivastava, 1987), no significant levels of endoprotease activity (trypsin- or chymotrypsin-like activity) were detected in the digestive tracts of *P. herreni*. Thus, not surprisingly, most protease inhibitors, especially trypsin inhibitors, will be inactive in such

insects. In contrast, an endopeptidase (leucine aminopeptidase) was evidenced in the digestive tracts of *P. herreni*. This enzyme has also been detected in the digestive tracts of two whitefly species: *Aleurotrachelus socialis* (Bondar) and both biotypes (A and B) of *Bemisia tabaci* (Gennadius) (data not shown).

Other endopeptidases are also reported in several aphid species (Srivastava, 1987). The principal nitrogenous sources in the phloem sap are free amino acids (Ziegler, 1975), which often show an exceptionally unbalanced composition. In cassava the pattern of amino acids in the phloem sap is so unbalanced that glutamine and asparagine together account for up to 55% of the total free amino acids (Calatayud P.A., unpublished data). Due to the unbalanced amino acid composition of their diet, amino acid metabolism is crucial. It is hypothesized that, similar to aphids, all essential amino acids are provided by their bacterial endosymbionts. Based on the results obtained in this study and the fact that the phloem sap contains some oligopeptides (Ziegler, 1975), essential amino acids can also be provided by digestive aminopeptidases, indicating the importance of such enzymes in Homoptera. Additional research is needed to confirm this hypothesis.

Activity 3. Using Molecular Markers to Identify Cassava Mealybugs

Abstract

Several species of mealybugs are important cassava pest in Latin America and Africa. *Pseudococcidae herreni* and *P. manihoti* are morphologically similar but *P. herreni* reproduces sexually while *P. manihoti* reproduces parthenogenesis. The similar morphology has made the determination of these species difficult. Populations of *P. maderensis*, *P. herreni* and *P. manihoti* were tested using the Operon primers H9 and H16 in RAPDs (randomly amplified polymorphic DNA) analyses. It was easy to identify molecular markers to distinquish these species of mealybugs implying that they are not closely related. To further study the phylogenetic relationships of these mealybugs, a region of the 16S ribosomal DNA was cloned and sequenced. Parsimony and distance analyses were performed and the phylogenetic relatedness of these species were determined. The molecular data confirms the placement of *P. herreni* and *P. manihoti* in distinct species and confirms that the African and Latin American populations of *P. manihoti* are closely related.

Introduction

The mealybugs *Phenacoccus herreni* Cox & Williams (Homoptera: Pseudococcidae) and *P manihoti* are both important pests of cassava; *Manihot esculenta* Crantz (Euphorbiaceae). These mealybug feeds on the phloem and prefers the growing tip of the plant. They can cause severe defoliation of existing leaves, prevent the expansion of new leaves, malformation of the growing tip, and in severe cases the stem dries out and turns necrotic. This causes loss of the vegetative stem cuttings used for propagation, and yield loss in the roots as high as 80%. High populations are often associated with drought.

There are few morphological differences between *P. herreni* and *P. manihoti*. They are considered different species on the basis of their biological characteristic, but there relationship has not been investigated on the basis of molecular characteristic. The ITS region of the nuclear 16S rDNA gene has been used in many studies of phylogenetic relationships. Here, we tested the utility of RAPD PCR to identify these mealybugs and have begun the characterization of the ITS of the nuclear 16S rDNA gene from five populations representing three species of mealybugs in Africa and South America.

Materials and Methods

Collection of the mealybug samples. The samples from Africa, Brazil and Paraguay were collected in the respective countries, preserved in 70% EtOH, and imported to Colombia with the permission of ICA. A colony in CIAT was the source of *P. herreni* from Colombia and the *P. maderensis* were collected from the field at CIAT headquarters.

RAPD PCR anaylsis. Total DNA was isolated from individual mealybugs using a method developed for plants (Gilbertson et al. 1991) with volumes of reagents appropriate for the small weight of the whiteflies. The DNA was amplified using the polymerase chain reaction (PCR). The primers used were Operon H9 (5'TGTAGCTGGG3')(Operon, Alameda, CA), and H16 (5'TCTCAGCTGG3'). The reactions were carried out using Taq polymerase and programmable thermal controllers (PTC-100, MJ Research, Waltham, MA). The reaction conditions for the first cycle were five min at 94°C, two min at 40°C and three min at 72°C. This was followed with 39 cycles of one min at 94°C, 1.5 min at 40°C, and two min at 72°C. The PCR products were run in agarose gels, stained with ethidium bromide and visualized using UV light.

PCR, cloning and sequence anaylsis of a region of the 16S mitochondrial DNA. The mitochondrial DNA was amplified using the polymerase chain reaction (PCR). The primer 4119 (5' CGCCTGTTTAACAAAAACAT) was the forward primer and primer 4118 (5' CCGGTCTGAACTCAGATCACGT 3') was the reverse primer (Xiong and Kocher 1991). The PCR reaction conditions were 30 cycles of 1 min at 95°C, 50 sec at 50°C, and 50 sec at 72°C. In the last cycle, the 72°C reaction was for 10 min. The products were purified using the Wizard™ PCR purification columns (Promega, Madison, WI) and were visualized by agarose gel electrophoresis with ethidium bromide. The PCR products were cloned into the plasmid PCR script amp SK(+)™ (Stratagene, La Jolla, CA). Plasmid DNA was purified using Wizard™ plasmid purification columns (Promega). Nucleotide sequences were determined using an ABI Prism 377 sequencer (Perkin-Elmer,) by the dideoxynucleotide chain termination procedure (Sanger et al. 1977) using the ABI dye terminator reaction ready kit. The sequence data were analyzed using DNAMAN Version 4.13 (Lynnon Biosoft, Vaudreuil, Quebec).

Results and Discussion

PCR RAPD analysis of mealybugs. Populations of *P. maderensis*, *P. herreni* and *P. manihoti* were tested using the Operon primers H9 and H16 in RAPD analyses. For *P. herreni*, a populations from Colombia and Brazil were tested, and the amplified products from both sets of primers had very similar banding patterns **(Figure 3.1 and 3.2)**. For *P. manihoti*, a population from Paraquay was compare to a population from the Republic of Congo. Both set of primers

amplified nearly identical set of products from both populations. Only one population of *P. maderensis* was tested and the patterns of amplified products for both set of primers were very distinct from the other mealybugs tested.

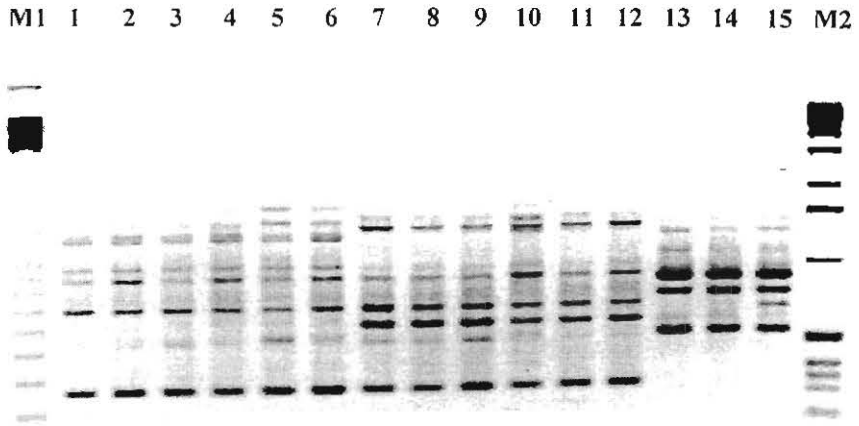

Figure 3.1. **RAPD PCR products from individual mealybugs with H9 primer. M1: 123 bp Markers, 1 - 3: *P.herreni* CIAT, 4 - 6: *P. herreni* Brasil, 7 - 9: *P. manihoti* Congo (Africa), 10-12: *P. manihoti* Paraguay, 13-15: *P. maderensis* CIAT, M2: 1 Kb Markers.**

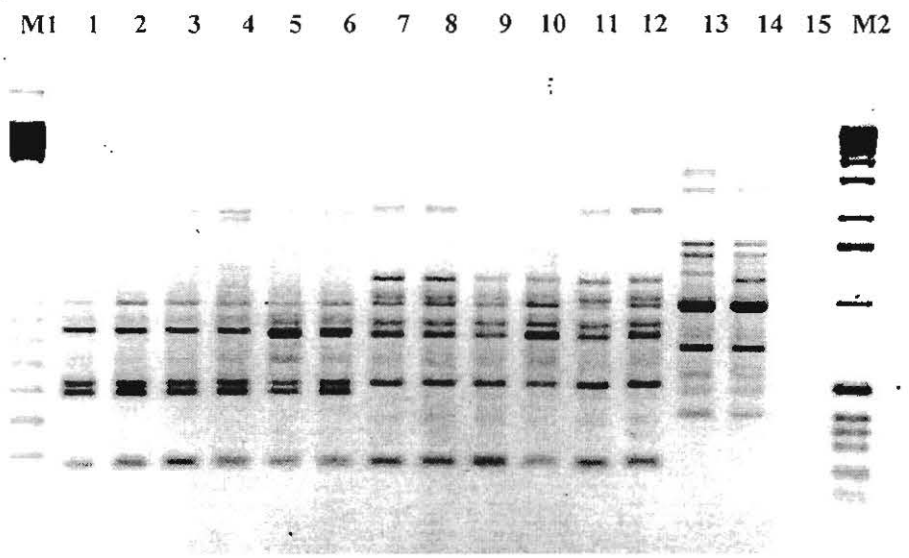

Figure 3.2. **RAPD PCR products from individual mealybugs with H16 primer. M1: 123 bp Markers, 1 - 3: *P.herreni* CIAT, 4 - 6: *P. herreni* Brasil, 7 - 9: *P. manihoti* Congo (Africa), 10-12: *P. manihoti* Paraguay, 13-15: *P. maderensis* CIAT, M2: 1 Kb Markers.**

The first two primers that were tested both proved efficient for distinguishing between the three species. This implies that there are these species are sufficiently evolutionarily different at the molecular level to produce multiple unique amplified products. Both primers proved useful in confirming that the populations in Latin America and Africa of *P. manihoti* were of the same species. The two Latin America populations of *P. herreni* also appeared nearly identical using RAPD analyses, and it was concluded that they are the same species. Since the morphology of these two species is very similar and it is not easy to distinguish between them using morphological characteristics, the RAPDs are a diagnostic method that can be used for the rapid identification of these species. Also the ease of distinguishing between these *P. herreni* and *P. manihoti* was additional evidence that these are indeed unique species.

Mitochondrial 16S gene cDNA cloning. From the amplified products, cDNA clones were produced for the *P. herreni* Colombian population, the *P. manihoti* Congo and Paraguay populations and the *P. maderensis* Colombian population. We have not yet completed in all of the cDNA clones especially for the *P. herreni* Brazilian population. Also, we need to produce some cDNA clones to have at least 2 or 3 independent clones for each population. Although several of the clones have been sequenced, the analysis of this data is pending the completion of representatives of all five populations.

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Sub-output 3. Biological Control and Plant Interactions of the Cassava Mealybug, *Phenacoccus herreni.*
(B. Dorn)

Activity 1. Biological control in complex agro-ecosystems: tritrophic effects of a mixed herbivore species infestation in cassava

Introduction

Recent epidemic outbreaks of cassava mealybug, *Phenacoccus herreni*, in Latin America caused cassava root yield losses of up to 80%, and required the release of natural antagonists. *Aenasius vexans* Kerrich is a specialist and *Acerophagus coccois* Smith a generalist enyrtid endo-parasitoid of *P. herreni* (Dorn *et al.*, 2001). Classical approaches to biological control focus on a simple tritrophic system, consisting of a single parasitoid species and a single target host species infesting a certain crop plant. However, in the field, the majority of plants are commonly attacked by a complex of herbivores that can be host and non-host species for the parasitoid. Mixed species infestations are common in the field and can have significant effects on foraging parasitoids.

In Latin America, additional common pests of cassava (*Manihot esculenta*) are the cassava green spider mite, *Mononychellus tanajoa* and the whitefly species *Aleurotrachelus socialis*. These mixed species infestations may affect the mealybugs and the efficiency of host searching parasitoids.

Material and Methods

Herbivore-herbivore interaction. Mealybug development and reproduction was assessed by following the life-cycle of mealybugs on cassava plants infested by mealybugs only and on cassava plants infested by mealybugs and spider mites or whiteflies respectively. The distribution of populations of mealybugs on plants infested by mealybugs only and on plants infested by mealybugs and spider mites or whiteflies, was determined by assessing numbers of mealybugs on the plants over their life-cycle.

Herbivore-parasitoid interaction. The searching behavior of *A. vexans* and *A. coccois* was observed on cassava plants infested by mealybugs only and on cassava plants infested by mealybugs and spider mites or whiteflies respectively.

Results and Discussion

Total female mealybug development was accelerated when an additional herbivore species was feeding on the same plant than when only mealybugs were present. In the mealybug-spider mite interaction this effect was most apparent in an accelerated development of the 3rd larval instar, and in the mealybug-whitefly interaction of the 2nd larval instar **(Figure 1.1)**.

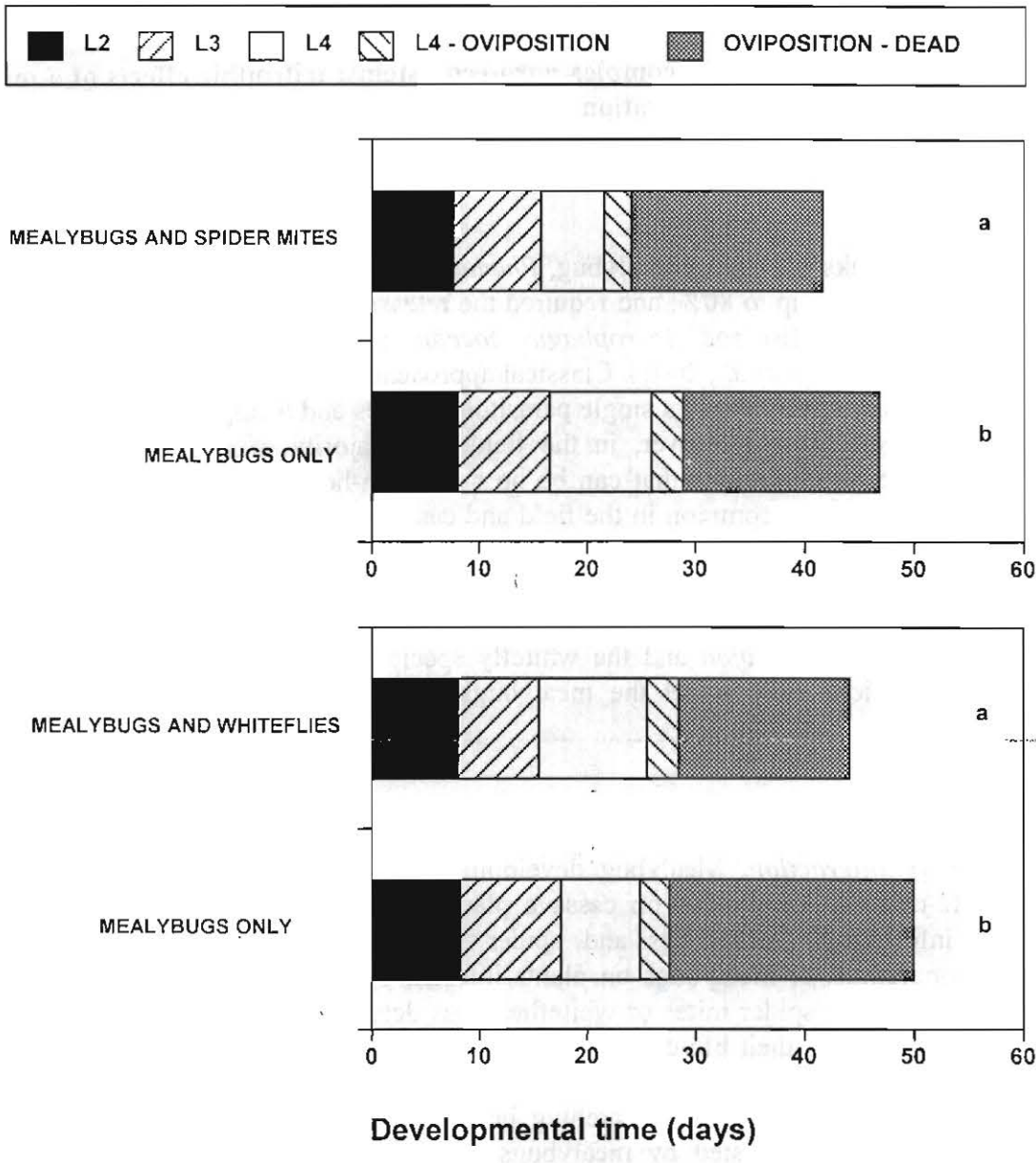

Figure 1.1. **Total developmental time and duration of each life stage of *Phenacoccus herreni* on plants infested by mealybugs only and on plants infested by spider mites, *Mononychellus tanajoa*, or whiteflies, *Aleurotrachelus socialis*, respectively. Abbreviations: L2 to L4 = 2nd to 4th instar larvae of mealybugs.**

The number of mealybugs on the plant decreased in the course of the observational period on plants with mealybugs only and on plants either infested by spider mites or whiteflies and mealybugs. Mealybugs moved in greater number from an infested leaf to other plant parts in a mixed herbivore species infestation with whiteflies than with spider mites.

The foraging behavior of the specialist parasitoid *A. vexans* was different between the simple and the complex tritrophic systems. The preference of the females for the adaxial leaf side in the single species infestation disappeared completely in either of the two herbivore species infestations. In contrast, the foraging behavior of the generalist parasitoid *A. coccois* was similar in the simple and the complex tritrophic systems. Reproduction of the two parasitoid species did not differ between the simple and the complex tritrophic systems. We therefore conclude that both parasitoid species were able to respond to the different level of complexity in their habitat and find their host on plants infested by various herbivores. We strongly suggest evaluating biological control organisms within complex agro-ecosystems, including the host and non-hosts, as they are common in the field.

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Sub-output 4. Bioecology of Spittlebug Species in Contrasting Environments. (D. Peck)

Activity 1. Biology and habits of Mahanarva andigena

Introduction

Mahanarva andigena was detected for the first time in Colombia in 1999, augmenting the known diversity of spittlebugs associated with graminoids. Up to now, this species is only known in Colombia from the south Pacific coast of Dept. Tumaco at C.I. El Mira of CORPOICA (1°33'10.001 N, 78°42'05.849 W, 50 m elev.). Hosts in that region are *Sorghum halepensis* (Johnson grass) and *Saccharum officinarum* (sugar cane). *Mahanarva andigena* is also known from sugar cane in Ecuador where this spittlebug species is of increasing concern in cane production in the coastal and interior regions of the country.

No biological, behavioral or ecological studies have yet been carried out on this economically important species. We therefore studied certain aspects of the basic biology including description and recognition of the life stages, duration of the life stages and oviposition sites to obtain information on the habits of this species and thereby guide advances in pest management.

Materials and Methods

Biological studies were carried out according to methodologies previously established at CIAT emphasizing morphological characterization of the life stages, duration of the life stages and reproductive biology. To have access to all life stages, a small colony was established in the greenhouse with eggs collected from field-caught adults during a visit to C.I. El Mira. With the aid of a stereoscope and ocular micrometer, certain aspects of the external morphology were measured for four developmental stages of the eggs, five nymphal instars, both sexes of late instar V (Vb) and both adult sexes. Adult specimens were obtained from the field, nymphs from the colony, and eggs from ovipositing adults in the colony.

To measure the duration of the life stages, field conditions were replicated in the screenhouse for controlled observations of adults and nymphs. For the adults, tenerals (<12 hours old) from the colony were confined in cohorts of four individuals under acetate sleeve cages over pots of *Brachiaria ruziziensis*; mortality was assessed daily. For the nymphs, recently emerged first instars (<12 hours old) were placed in individual pots of *B. ruziziensis* established with abundant surface roots required as feeding sites. Transformation from one instar to the next was determined by direct observation of the molted exuvia or the nymph itself. The mean longevity of each life stage was calculated from observations of 40 individuals. Duration of the egg stages was determined under controlled incubation conditions (27°C, 100% RH, total darkness). Recently laid eggs (<24 hours old) were maintained on moist filter paper in petri dishes and observed daily. The mean duration of each of the four generalized developmental stages was calculated from observations on 100 eggs.

To study oviposition sites as part of the description of reproductive biology, field conditions were replicated in the screenhouse. The soil surface was specially prepared with soil oviposition

substrate dispersed on top with 2 g leaf litter. Each pot was infested with two females and two males from the colony and 10 days later eggs were recovered from four oviposition substrates: uncovered soil, soil covered by leaf litter, leaf litter and the plant surface.

Results

Mahanarva andigena eggs conformed to the four generalized developmental stages (S1, S2, S3, S4) established for *Aeneolamia varia* and other spittlebug species. Certain externally visible characteristics accompanied these stages. In S2 a spot of red pigment was visible. In S3 the chorion opened to expose the black operculum and the red spot was no longer visible. In S4 two pairs of red spots were visible, the posterior representing the Batelli glands of the abdomen and the anterior representing the eyes of the developing nymph. Each progressive stage was accompanied by a statistically significant increase in both length and width **(Table 1.1)**. Total development time was 16.4 days; S2 was the shortest development stage and S4 was the longest **(Table 1.2)**.

Table 1.1. Width and length (mm) of development stages of *M. andigena* eggs (mean±S.E., range, n=93-100).

	Development Stage			
Parameter	S1	S2	S3	S4
Length	1.22 ± 0.03 a	1.24 ± 0.04 b	1.26 ± 0.03 c	1.30 ± 0.04 d
	(1.14-1.29)	(1.16-1.34)	(1.20-1.34)	(1.21-1.40)
Width	0.31 ± 0.01 a	0.33 ± 0.01 b	0.35 ± 0.02 c	0.39 ± 0.01 d
	(0.29 -0.34)	(0.30-0.41)	(0.31-0.39)	(0.37-0.43)

For each parameter, means followed by different letters are significantly different (P<0.05).

Table 1.2. Duration (days) of *M. andigena* eggs by development stage (mean±S.E., range, n=96-105).

	Development Stage				
	S1	S2	S3	S4	Total
Mean±S.E.	4.97 ± 0.46 c	1.57 ± 0.52 a	3.41 ± 0.61 b	6.44 ± 0.60 d	16.39 ± 0.85
Range	(4-6)	(1-3)	(2-5)	(5-8)	(15-19)

Means followed by different letters are significantly different (P<0.05).

Nymphs increased in size from one instar to the next for each parameter measured. There was no overlap in head capsule width or stylet length among the five instars confirming these to be the most useful measures for instar determination **(Table 1.3)**. Sexual dimorphism was observed in instar Vb (nymphs within a few days of molting to adults) where females were larger than males in all four parameters. Total development time was 46.5 days; instar I was the shortest and instar V was the longest representing 32.4% of the entire nymphal stage **(Table 1.4)**.

The behavior of nymphs differed from other Colombian species studied to date because nymphs of all age classes sought feeding sites in the upper portions of the plant such as leaf axils. This behavior resulted in large cohabited spittle masses and increased aggregation of individuals. The

behavior in laboratory is similar to observations on the two known hosts in the field and to reports from sugar cane studies in Ecuador.

Table 1.3. Morphological characterization (mm) of nymphal life stages of *M. andigena* (mean±S.E., range, n=15-40).

Instar	Head Capsule Width	Body Length	Anterior Wing Pad Length	Stylet Length
I	0.42 ± 0.03 a	1.80 ± 0.30 a	---	0.30 ± 0.02 a
	(0.36-0.46)	(1.21-2.19)		(0.29-0.34)
II	0.67 ± 0.02 b	2.90 ± 0.30 b	---	0.39 ± 0.02 b
	(0.61-0.71)	(2.22-3.47)		(0.36-0.43)
III	1.01 ± 0.04 c	4.25 ± 0.44 c	0.39 ± 0.03 a	0.59 ± 0.03 c
	(0.94-1.07)	(3.04-5.09)	(0.34-0.44)	(0.53-0.64)
IV	1.53 ± 0.05 d	6.93 ± 0.80 d	1.05 ± 0.07 b	0.87 ± 0.04 d
	(1.45-1.63)	(5.14-8.79)	(0.89-1.19)	(0.80-0.95)
Va	2.12 ± 0.10 e	9.57 ± 0.72 e	2.52 ± 0.14 c	1.20 ± 0.04 e
	(1.96-2.37)	(7.93-11.0)	(2.14-2.79)	(1.13-1.27)
Vb Female	2.27 ± 0.05 f	11.28 ± 1.16 g	2.79 ± 0.16 d	1.26 ± 0.04 f
	(2.19-2.37)	(8.79-13.71)	(2.36-3.07)	(1.16-1.33)
Vb Male	2.09 ± 0.08 e	10.70 ± 0.84 f	2.57 ± 0.16 c	1.19 ± 0.03 e
	(1.93-2.28)	(9.29-12.64)	(2.14-2.86)	(1.13-1.30)

For each column, means followed by different letters are significantly different (P<0.05).

Table 1.4. Duration (days) of *M. andigena* nymphs by instar (mean, n=40).

	Instar					
	I	II	III	IV	V	Total
Mean±S.E.	6.35 ± 1.03 a	8.64 ± 1.10 c	8.18 ± 1.30 b	10.14 ± 1.50 c	15.05 ± 3.80 d	46.52 ± 9.85
Range	(5-8)	(7-10)	(8-11)	(8-13)	(9-23)	(41-54)

Means followed by different letters are significantly different (P<0.05).

Adults were significantly larger than instar Vb nymphs of the same sex in terms of head capsule width and forewing length, but smaller in terms of body length without wings and stylet length (P<0.05). Sexual dimorphism was observed in the adults, expressed as the greater size of females in every parameter measured **(Table 1.5)**. Overall adult longevity was 24.0 ± 11.1 days with 25.5 ± 12.9 (8-27) days for females and 20.6 ± 9.0 (8-27) for males (difference not statistically significant). Under the conditions of this study, duration of the life cycle of *M. andigena* was 74.9 d (=16.4+46.5+12.0, egg+nymph+½ adult).

Table 1.5. Morphological characterization (mm) of *M. andigena* adults by sex (mean±S.E., range, n=40).

Sex	Head Capsule Width	Stylet Length	Body Length with Wing	Body Length without Wing	Anterior Wing Length	Body Width
Female	2.54 ± 0.08 a	1.14 ± 0.06 a	10.97 ± 050 a	10.16 ± 0.92 a	8.61 ± 0.45 a	5.19 ± 0.28 a
	(2.29 – 2.64)	(1.05 – 1.28)	(9.71 –11.93)	(8.43 – 12.00)	(7.71 – 10.07)	(4.14 – 5.71)
Male	2.28 ± 0.09 b	1.03 ± 0.06 b	9.96 ± 0.46 b	9.05 ± 0.78 b	7.95 ± 0.32 b	4.72 ± 0.24 b
	(2.07 – 2.50)	(0.91 – 1.14)	(9.07 – 10.71)	(7.14 – 10.36)	(7.21 – 8.50)	(4.21 – 5.21)

For each column, means followed by different letters are significantly different (P<0.05).

Mahanarva andigena exhibited some flexibility in oviposition substrates. Most eggs (67.6%) were recovered from the soil with 51.2% from uncovered soil and 16.4% from soil under litter. Nearly a third of eggs (32.4%), however, was recovered from the plant surface. None were recovered from leaf litter.

Discussion

Like other graminoid spittlebugs studied to date, *M. andigena* eggs pass through four egg development stages that increase in size and are distinguished by externally visible characteristics; nymphs pass through five morphologically distinguishable instars best differentiated by width of the head capsule and stylet length; and adults exhibit sexual dimorphism expressed as greater size of females.

The life cycle of 74.9 d is longer than other spittlebugs studied to date with the same methodology, including *Aeneolamia* (45.3-52.6 d) and *Zulia* (61.5-69.6 d), and is 9.3 days longer than its congener *Mahanarva* sp. nov. *Prosapia simulans* is the only other described Colombian species with a life cycle >70 days.

A preference for laying eggs in the soil is common to most other species studied (*A. lepidior, A. reducta, A. varia, Mahanarva* sp. nov., *Z. carbonaria, Zulia* sp. nov.). The tendency of *M. andigena* to lay eggs (32.4%) on the surface of the plant stem is greater in *Z. pubescens* (59.2%) and *P. simulans* (82.6%).

Biology and habits of *Prosapia simulans*

Justification

The first detection of the Central American spittlebug, *Prosapia simulans*, in Colombia has serious economic ramifications for ranchers and cane producers of the Cauca River Valley. The insect has already reached economically damaging levels in *Brachiaria* pastures in the Dept. Valle del Cauca. In sugar cane, *P. simulans* represents a potential threat since it is the second most important spittlebug cane pest in Central America and since changing cultural practices in Cauca Valley cane production (prohibition of burning) may enable this species to get a foothold in cane fields. Up to now spittlebugs have not been present in sugar cane of this region.

We have previously documented that *P. simulans* occurs over a large elevation range, extending from the Cauca Valley floor (1100 m elev.) to just over the western cordillera of the Andes (1621 m elev.). Multiple visits to the same farms have shown *P. simulans* populations to be persistent especially in improved pastures of *Brachiaria decumbens*. Despite its importance in pastures and cane of Central America, little is known about this insect's biology and ecology. To support advances in management, we carried out initial biological studies of *P. simulans* in the Cauca Valley focusing on differentiation of the life stages, duration of the life stages and reproductive biology.

Materials and Methods

The biology of *P. simulans* was characterized using previously established methods **(see Biology and habits of *Mahanarva andigena* - Pag. 52)**. To differentiate among the life stages, these were characterized morphologically using different measures of body size. To quantify duration of the life stages, the development of individual eggs, nymphs and adults was observed under controlled conditions. To begin to describe the reproductive biology, oviposition site preferences were determined.

Results

The eggs conformed to the four generalized developmental stages (S1, S2, S3, S4) described in other spittlebug species. Both size and width of eggs increased from one stage to the next **(Table 1.6)**. Total development time was 18.0 days; S2 was the shortest development stage and S1 the longest **(Table 1.7)**. Diapause was not detected among individuals of the study population, however diapause during stage S2 was observed in eggs collected from a lower elevation site during the course of other studies (1100 m elev., Santa Helena, Dept. Valle del Cauca); maximum time to eclosion of these diapause eggs was 128 days.

Table 1.6. Width and length (mm) of development stages of *P. simulans* eggs (mean±S.E., range, n=75-100).

Parameter	Development Stage			
	S1	S2	S3	S4
Length	1.16 ± 0.03 a	1.18 ± 0.03 b	1.21 ± 0.03 c	1.25 ± 0.03 d
	(1.09-1.24)	(1.10-1.26)	(1.14-1.30)	(1.19-1.34)
Width	0.32 ± 0.02 a	0.34 ± 0.01 b	0.39 ± 0.03 c	0.42 ± 0.01 d
	(0.29-0.36)	(0.31-0.37)	(0.30-0.47)	(0.39-0.46)

For each parameter, means followed by different letters are significantly different (P<0.05).

Table 1.7. Duration (days) of *P. simulans* eggs by development stage (mean±S.E., range, n=66-100).

	Development Stage				
	S1	S2	S3	S4	Total
Mean±S.E.	6.90 ± 1.09 d	2.13 ± 1.69 a	3.98 ± 0.77 b	5.18 ± 0.58 c	17.99 ± 1.27
Range	(6-13)	(1-9)	(2-5)	(4-7)	(16-23)

Means followed by different letters are significantly different (P<0.05).

For the nymphs, each of the morphological parameters measured (head capsule width, body length, anterior wing pad length, stylet length) increased in size from one instar to the next **(Table 1.8)**. There was no overlap in head capsule width among the five instars confirming this to be the most diagnostic character for instar determination. Total development time was 45.6 days; instar I was the shortest and instar V was the longest, representing 28.8% of the entire nymphal stage **(Table 1.9)**.

Table 1.8. Morphological characterization (mm) of nymphal life stages of *P. simulans* (mean±S.E., range, n=40).

Instar	Head Capsule Width	Body Length	Anterior Wing Pad Length	Stylet Length
I	0.45 ± 0.02 a (0.36-0.50)	1.66 ± 0.17 a (1.24-1.96)	-	0.36 ± 0.04 a (0.28-0.44)
II	0.65 ± 0.03 b (0.50-0.69)	2.33 ± 0.31 b (1.55-2.88)	-	0.47 ± 0.03 b (0.37-0.52)
III	0.96 ± 0.03 c (0.89-1.01)	3.16 ± 0.21 c (2.64-3.70)	0.32 ± 0.02 a (0.27-0.37)	0.62 ± 0.05 c (0.53-0.73)
IV	1.42 ± 0.06 d (1.28-151)	5.99 ± 0.57 d (4.91-7.52)	0.90 ± 0.08 b (0.71-1.10)	0.97 ± 0.04 d (0.89-1.04)
Va	1.92 ± 0.08 e (1.78-2.13)	7.79 ± 0.43 e (6.64-8.50)	2.23 ± 0.13 c (1.78-2.43)	1.16 ± 0.05 e (1.04-1.27)
Vb Female	2.01 ± 0.08 f (1.84-2.13)	8.12 ± 0.95 e (6.07-10.86)	2.27 ± 0.13 c (1.78-2.55)	1.30 ± 0.05 f (1.19-1.39)
Vb Male	1.92 ± 0.09 e (1.78-2.19)	7.99 ±0.65 e (6.43-9.43)	2.26 ± 0.09 c (1.99-2.43)	1.28 ± 0.06 f (1.04-1.42)

For each column, means followed by different letters are significantly different (P<0.05).

Adults were larger than instar V in all parameters with the exception of male body length and male and female stylet length (shorter in adults). Sexual dimorphism was observed in the adults, expressed as the greater size of females in every parameter measured with the exception of forewing length **(Table 1.9)**. Overall adult longevity was 17.8 ± 8.2 days with 19.9 ± 8.6 (6-32) days for females and 14.5 ± 5.1 (5-21) days for males (difference not statistically significant). Under the conditions of this study, duration of the life cycle of *P. simulans* was 72.5 days (=18.0+45.6+8.9, egg+nymph+½ adult).

Table 1.9. Duration (days) of *P. simulans* nymphs by instar (mean, n=40).

	Instar					
	I	II	III	IV	V	Total
Mean±S.E.	6.75 ± 1.16 a	7.54 ± 2.16 a	9.30 ± 2.79 b	10.04 ± 2.26 b	13.14± 2.70 c	45.59 ±5.45
Range	(5-11)	(4-13)	(5-17)	(5-14)	(10-20)	(35-57)

Means followed by different letters are significantly different (P<0.05).

Prosapia simulans exhibited a marked preference for laying eggs on the surface of the plant stem; 82.6% of eggs were recovered from this substrate. Only 17.4% was recovered from the soil with 3.6% from uncovered soil and 13.8% from soil under litter. No eggs were recovered from leaf litter.

Discussion

Prosapia simulans conforms to the developmental and morphological patterns established in graminoid spittlebugs **(see Biology and habits of *Mahanarva andigena* - Pag. 52)** including diapause expressed as an extended S2 egg stage. The life cycle of 72.5 days in the Cauca Valley is longer than what is reported from Central America (58.4 and 58.0 days). It is also longer than all other species studied to date in Colombia (*Aeneolamia lepidior, A. reducta, A. varia, Mahanarva* sp. nov., *Z. carbonaria, Zulia pubescens, Zulia* sp. nov.), comparable only to *M.*

andigena from the south Pacific coast (74.9 days; **(see Biology and habits of *Mahanarva andigena* – Pag. 52)**. The marked preference for oviposition sites on the plant stem is different from other Colombian species studied to date which all prefer to oviposit in the soil. Only *Z. pubescens* has also been shown to lay a majority of eggs (59.2%) on the plant stem.

Table 1.10. Morphological characterization (mm) of *P. simulans* adults by sex (mean±S.E., range, n=40).

Sex	Head Capsule Width	Stylet Length	Body Length with Wing	Body Length without Wing	Anterior Wing Length	Body Width
Female	2.31 ± 0.06 a	0.98 ± 0.33 a	8.71 ± 0.33 a	8.18 ± 0.61 a	6.80 ± 0.22 a	4.63 ± 0.15 a
	(2.21-2.43)	(0.89-1.16)	(7.29-9.29)	(7.29-9.29)	(6.36-7.21)	(4.36-5.07)
Male	2.04 ± 0.06 b	0.89 ± 0.03 b	8.52 ± 0.31 b	7.23 ± 0.32 b	6.84 ± 0.28 a	4.16 ± 0.14 b
	(1.93-2.14)	(0.82-0.94)	(7.36-9.29)	(6.57-8.14)	(5.93-7.43)	(3.79-4.43)

For each column, means followed by different letters are significantly different (P<0.05).

Population dynamics and phenology of *Prosapia simulans*

Introduction

The graminoid spittlebug, *Prosapia simulans*, is a new arrival to the Cauca Valley of Colombia **(see section Biology and habits of *Prosapia simulans* – Pag. 55)** and has been detected in four municipalities to date: Santander de Quilichao (Dept. Cauca), Cerrito, Calima Darién and Yotoco (Dept. Valle del Cauca). In many of the sites where it has been detected, *P. simulans* shares pastures with *Zulia carbonaria* and *Zulia pubescens* particularly where *Brachiaria decumbens* is the dominant forage grass host.

In general, the management of graminoid spittlebugs has been compromised by a lack of bioecological information specific to the species and habitats of concern, and by a tendency to over generalize among the diverse insect/host/habitat associations in which these pests have economic impact. Advances in spittlebug management requires a detailed understanding of aspects such as differentiation and duration of the life stages, correspondence between population fluctuations and precipitation, habitat and host plant preferences, and the incidence of natural enemies.

Biological studies on *P. simulans* in the Cauca River Valley have been initiated **(see Biology and habits of *Prosapia simulans* - Pag. 55)**. In this report we summarize recent phenological studies of *P. simulans*. This research was carried out to provide baseline data for field studies on fungal entomopathogens **(see Field evaluation of fungal entomopathogens in two contrasting regions – Pag. 87)** and assesses various components of population ecology including population fluctuation, diapause, natural enemies and precipitation.

Materials and Methods

Observation plots were established in *B. decumbens* pastures at Hacienda Piedechinche, Municipality El Cerrito, Dept. Valle del Cauca. Methods for surveying nymphs, adults, diapause

eggs and natural enemies were modified from previously established protocols used in studies with similar objectives (CIAT Annual Report 1999, 2000).

Five 0.16-ha plots were established, each in a different paddock and under the same typical management regime of the farm in terms of grazing pressure, fertilization and weed suppression. To facilitate sampling, each plot was divided into 16 subplots in which nymphs were collected from two $0.0625m^2$ quadrats to measure absolute density, and adults from two series of 10 sweeps of an insect net to measure relative density. All nymphs were determined to instar and adults to species and sex. The abundance and incidence of natural enemies were measured as part of the same surveys. Surveys were carried out once weekly and were initiated 25 January 2001. The first 7 months (through 30 August 2001) are summarized in this report. Data were analyzed to determine patterns and variation at the farm level in population fluctuation, correspondence with precipitation, population synchrony, number of generations and incidence of natural enemies.

To help interpret phenology, parallel data were collected on the incidence and duration of egg diapause, a physiological condition that enables the insect to synchronize its life cycle with the humid environmental conditions necessary for development and reproduction. Every 15 days a group of females (1-10 individuals, depending on availability) was collected from each plot and allowed to oviposit for a period of 3 days in moist filter paper lining the bottom of a large petri dish. Petri dishes and their eggs were kept under incubation (27°C, 100% RH, total darkness) and evaluated twice weekly for empty chorions (indicating nymphal emergence) and inviable eggs. The incidence and duration of diapause were quantified with eggs eclosing after 30 days considered diapausing.

Results

Over this 7-month period a total of 190 nymphs and 1465 adults were collected. Both life stages were found on every survey date until julian day 172 and 179 when nymphs and adults, respectively, were no longer detected. Abundance of these life stages coincided with the wet season, in particular March-May, historically the three wettest months of the year where 35.6% of the total annual precipitation falls. Nymphs and adults disappeared in the driest months of June-August when the insect presumably survives as diapausing eggs.

At the farm level (combined plot data), *P. simulans* exhibited one well-differentiated pair of nymph and adult population peaks **(Figure 1.1)**. The major nymph peak occurred days 81-130 and was preceded and followed by periods of abundance days 25-67 and 158-164. The major adult peak occurred days 109-158 but was also preceded by the apparent tail end of a previous peak days 25-38. These results suggest a pair of population peaks at the start of the new year just before initiation of the surveys. Although these population peaks probably represent discrete and consecutive generations a precise interpretation depends on future analysis according to nymphal life stages.

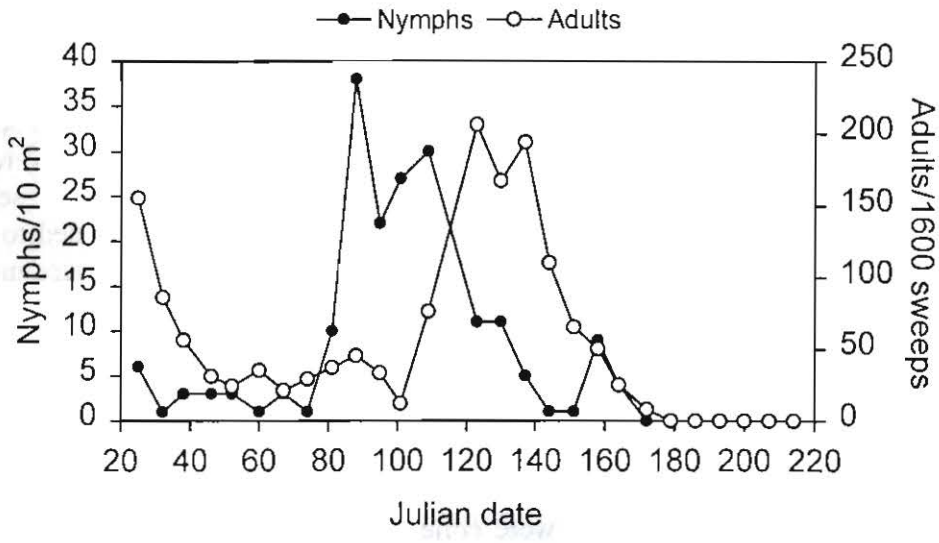

Figure 1.1. Population fluctuations of nymph and adult *P. simulans* populations in Piedechinche, Dept. Valle del Cauca in 2001.

Fluctuation curves for the five individual plots reveal the degree of on-farm variation in spittlebug phenology **(Figures 1.2, 1.3)**. In terms of nymph abundance, the plot with the highest insect load (Plot 2) had 4.7 times more than the plot with the lowest (Plot 5), or 32.1% versus 6.8% of the nymph population. For the adult life stage Plot 1 ranked first and Plot 5 again ranked last with 7.4 times more adults in Plot 1, or 37.4 and 5.1% of the adults, respectively.

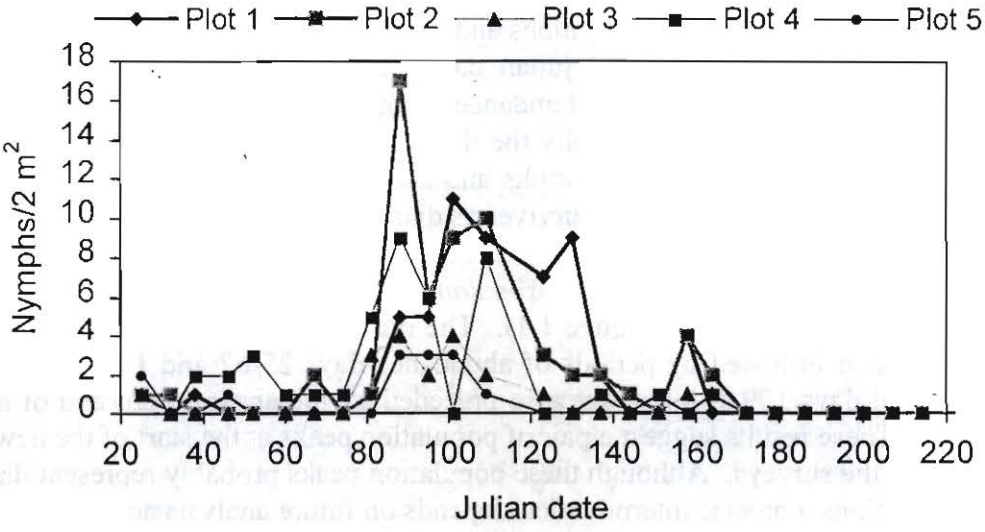

Figure 1.2. Population fluctuation of *P. simulans* nymphs in five *B. decumbens* pastures, Piedechinche, Dept. Valle del Cuaca (2001).

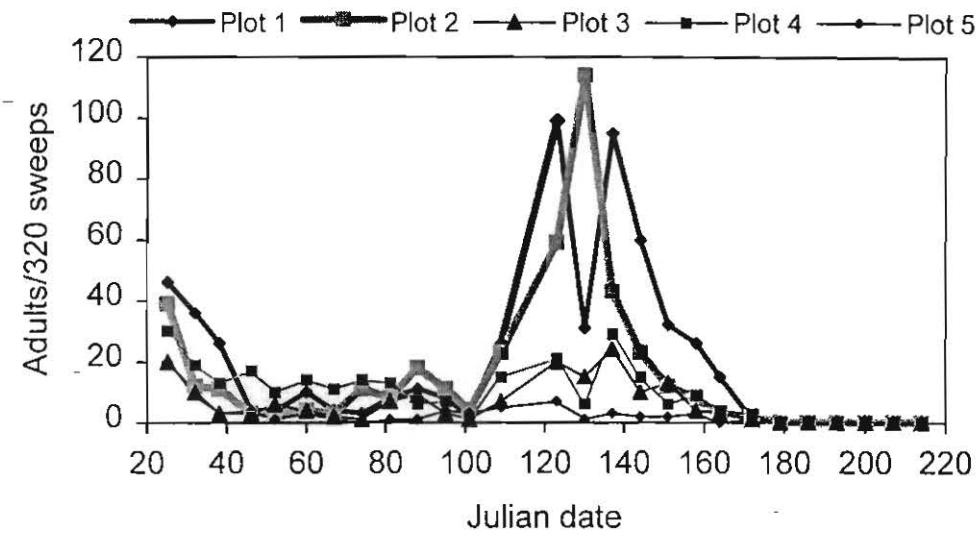

Figure 1.3. Population fluctuation of *P. simulans* adults in five *B. decumbens* pastures, Piedechinche, Dept. Valle del Cuaca (2001).

All plots had the same general periods of peak abundance as in the overall farm fluctuation curves. However, some individual plots exhibited evidence of bimodal peaks in the period March-April. This included nymphs in Plots 2 and 4 and adults in Plots 1, 2 and 4. This suggests overlapping generations that are obscured in the overall farm fluctuation curve.

Very few natural enemies were detected. Only parasitic mites (Acari: Erythraeidae) on adults were found over the survey period. Overall, 9.5% of adults had mites with a maximum of 10 mites per individual. For the entire population of adults mite load was 0.19 per adult or 0.20, 0.47, 0.21 and 0.40 per adult over the months of March, April, May and June, respectively. Mite load according to sex was 0.19 per male and 0.16 per female.

For the 1249 eggs collected over eight dates during the first five months of the study (January-May), overall mortality was 5.5% and incidence of diapause eggs was 69.8%. With the exception of two dates (22 March, 31 May), the proportion of eggs in diapause exceeded 70% in each collection date (**Table 1.11**). There was a trend toward lower diapause incidence in the second half of the period. Eggs eclosed over a period of 18-128 days (**Figure 1.4**). Mean time to eclosion was 24.0 days for non-diapausing and 79.5 for diapausing eggs. There was a trend towards longer eclosion times for eggs in the second half of the period.

Discussion

Prosapia simulans populations coincided with the wetter months of this initial survey period (January-May), and then declined and disappeared coincident with the dry season. The end of an initial generation and a complete second generation was documented based on nymph and adult peaks, but a precise determination of generations depends on future analysis of the separate life

stages of the nymphs. This further analysis will also shed light on apparent phenological differences observed among the five survey plots and help us measure the degree of on-farm variation.

Table 1.11. Seasonal changes in the incidence and time to eclosion of nondiapausing and diapausing *P. simulans* eggs in Piedechinche, Dept. Valle del Cauca (2001).

Collection Date	n	Proportion (%)		Time to Eclosion all Eggs (Days)	
		Nondiapause	Diapause	Mean ± S.E.	Range
22 Jan	204	6.4	93.6	43.0 ± 14.89	18-68
10 Feb	138	14.5	85.5	40.0 ± 11.98	20-60
23 Feb	84	3.6	96.4	53.0 ± 15.44	27-79
15 Mar	46	0.0	100.0	63.0 ± 2.16	60-66
22 Mar	69	100.0	0.0	23.0	---
5 Apr	190	29.5	70.5	74.5 ± 31.32	21-128
3 May	482	26.6	73.4	67.0 ± 28.72	18-116
31 May	36	61.1	38.9	48.5 ± 13.42	26-71
Overall	1249	30.2	69.8	18-29	30-128

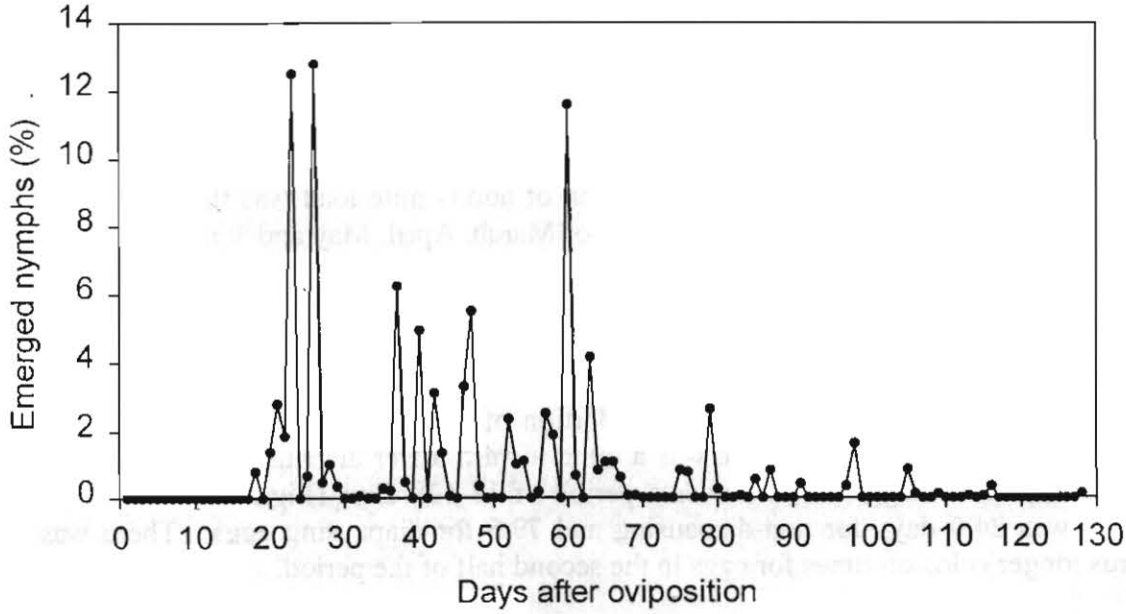

Figure 1.4. Pattern of eclosion of *P. simulans* eggs summed over eight collection dates (every two weeks) from January to May 2001 in Piedechinche, Dept. Valle del Cauca.

The majority of eggs collected over this period were diapausing despite the apparently adequate humid conditions for population development. This differs from other species studied to date that exhibit very little diapause during the wet season. The relationship between the incidence of diapause and season for *P. simulans* is unclear and requires continued studies. It is expected, for example, that we will document an even higher incidence of diapause in eggs collected from the field in June, start of the dry season. These methods should prove adequate for documenting the phenology of *P. simulans* populations in the field for the first time in Colombia. The new information should help us interpret the relationship between habitat and spittlebug presence and lead to predictions of the spatial and temporal arrival of outbreaks.

Population dynamics and phenology of *Zulia carbonaria*

Introduction

Over the past several years the impact of spittlebugs has apparently increased in forage grasses of the Interandean valleys and hillsides of Colombia, such as pastures of *Brachiaria* spp. in the Cauca River Valley. This area has a bimodal precipitation pattern and thereby represents an environment for studying spittlebug seasonality that is distinct from previously studied lowland sites of the highly seasonal Caribbean coast, intermediate seasonal Orinoquian Piedmont, and the continuously humid Amazonian Piedmont. The first information on the phenology of the spittlebug complex in the Cauca Valley was presented in 2000 (CIAT Annual Report). In this report we summarize results from detailed population surveys of the spittlebug *Zulia carbonaria* over two complete years.

Materials and Methods

This study was carried out on a representative farm of the Cauca River Valley, Hacienda Las Palmas, Municipality Santander de Quilichao, Dept. Cauca. This site featured pastures of *Brachiaria decumbens* in association with the forage legume *Centrosema* sp.

The methods were the same as in previously established protocols. Three 0.5-ha plots were established in separate pastures and divided into four subplots (0.125 ha) to facilitate sampling. Nymph surveys comprised counts in two 0.25m^2 quadrats in each subplot while adult surveys comprised 50 sweeps of an insect net in each subplot. Nymphs were counted and classified to life stage while adults were counted and classified to sex and species. Natural enemies were also recorded and identified. Surveys were carried out weekly during two years (20 January 1999 to 19 January 2001). Data were analyzed to determine patterns and variation at the farm level in population fluctuation, correspondence with precipitation, population synchrony, number of generations and incidence of natural enemies.

Results

A total of 10,546 nymphs and 2,247 adults were collected during the course of this study. With the exception of one female *Prosapia simulans*, all adults were *Zulia carbonaria*.

The abundance, or insect load, of *Z. carbonaria* varied greatly from one year to the next; there were 6.2 and 3.0 times more nymphs and adults, respectively, in 1999 compared to 2000 **(Table 1.12)**. There was also significant variation among the individual plots in total abundance. Insect load was 7.5 and 3.0 times greater for nymphs and adults, respectively, between the plot of lowest (Plot 1) and highest (Plot 2) abundance in 1999, and 3.2 and 2.4 times for 2000 (Plot 2 versus Plot 1). The plots of highest and lowest abundance were not consistent from one year to the next, in fact their ranking switched between 1999 and 2000.

Table 1.12. Variation in insect load of *Z. carbonaria* between years and among plots in Santander de Quilichao, Dept. Cauca.

		Insect Load[1]	
Year	Plot	Nymphs	Adults
1999	Plot 1	760	253
	Plot 2	5704	754
	Plot 3	2612	676
	Sum	9076	1683
2000	Plot 1	703	251
	Plot 2	218	106
	Plot 3	549	207
	Sum	1470	547
Overall		10,546	2,247

[1] Measured as total number of individuals collected in surveys.

Nymph and adult populations were most abundant during the first half of each year, coincident with the wettest months. *Z. carbonaria* essentially disappeared the second half of the year in 1999 after the two extremely dry months of June and July **(Figure 1.5, Table 1.13)**. Populations did not recover until early 2000. Populations again declined severely after the dry months of June and July in 2001.

In 1999, population fluctuation curves revealed three well-defined peaks for each plot with a correspondence between nymph peaks and the subsequent adult peaks **(Figure 1.6)**. In 2000, peaks were much less defined **(Figure 1.7)**. To more precisely interpret these data and resolve different generations, population data were analyzed according to nymphal life stage. Recruitment patterns from one life stage to the next revealed three generations of *Z. carbonaria* in 1999 and four in 2000. Cumulative insect day calculations were used to quantify the arrival of discrete generations of nymphs and adults for each plot. Peak abundance was designated as the date of 50% accumulation of the insect days or the area under the population fluctuation curve. In 1999 there was little variation in the timing of generations across plots. The three generations of nymphs peaked at a mean of julian day 52.7, 132.0 and 194.0 while adults peaked at 66.0, 144.0 and 207.0 **(Table 1.14)**. The time between subsequent nymph peaks and adult peaks is the generation time calculated as a mean of 70.6 (n=12, range 45-89) for the farm in 1999 with little variation among plots **(Table 1.15)**.

0In 2000, the phenology of spittlebug populations was best interpreted as two overlapping periods of emergence of the initial generation. The first outbreak of nymphs was day 17 leading to adults day 37. The next peak of nymphs was day 61, too early to represent progeny of the previous adult generation, and thereby probably representing the eclosion of an additional group of eggs that were late in contributing to the first generation. These dual peaks led to early and late groups of a second and third generation, and then lost any detectable separation in the fourth generation. With this interpretation, the mean generation time for 2000 was calculated as 63.9 (n=18, range 45-83) days **(Table 1.15)**.

Over both years (1999 and 2000), mean generation time was 67.3 days (n=31), corresponding very well with the time determined from greenhouse biology studies (69.6 days).

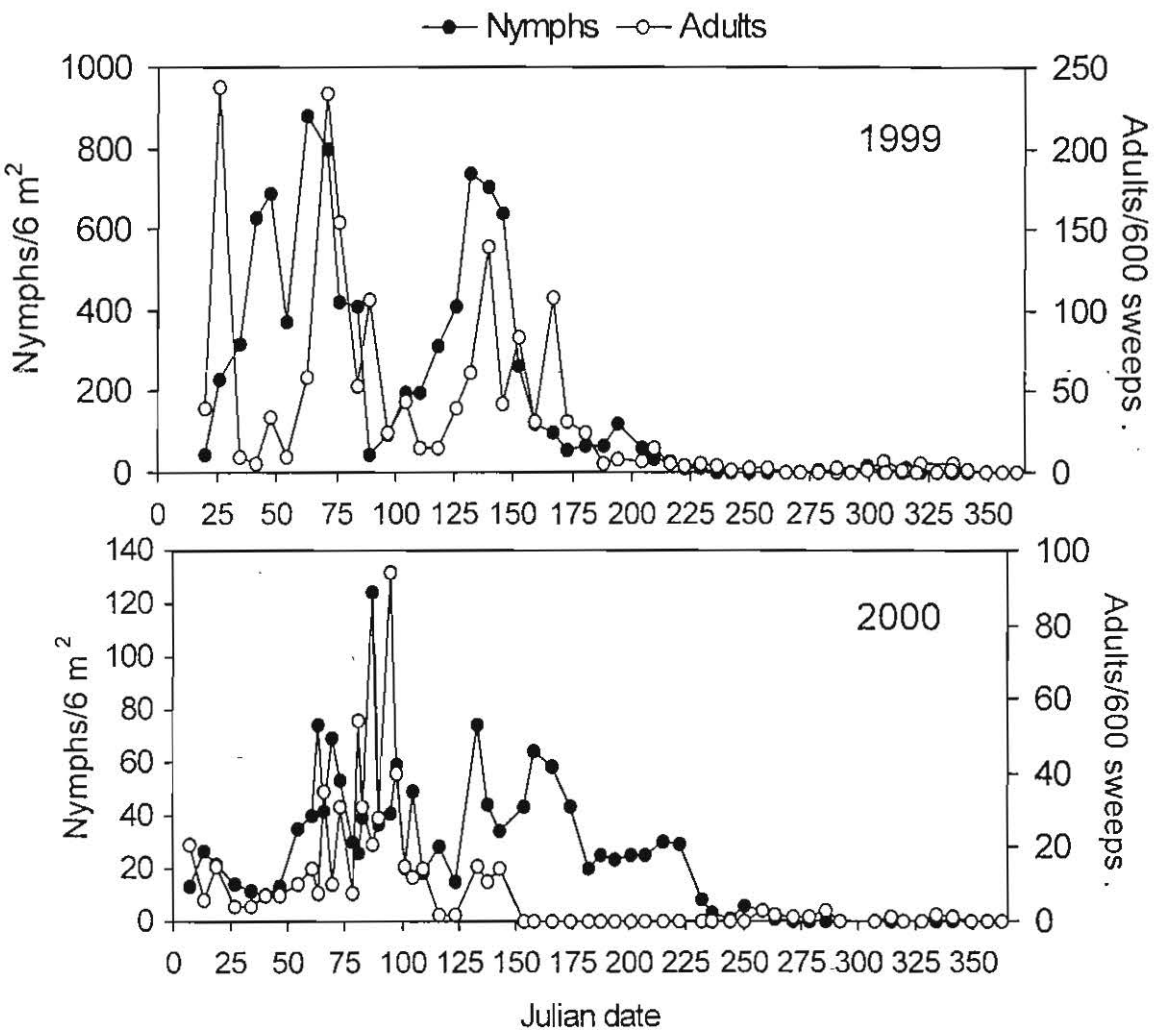

Figure 1.5. Population fluctuation of nymph and adult *Z. carbonaria* populations in Santander de Quilichao, Dept. Cauca over two years.

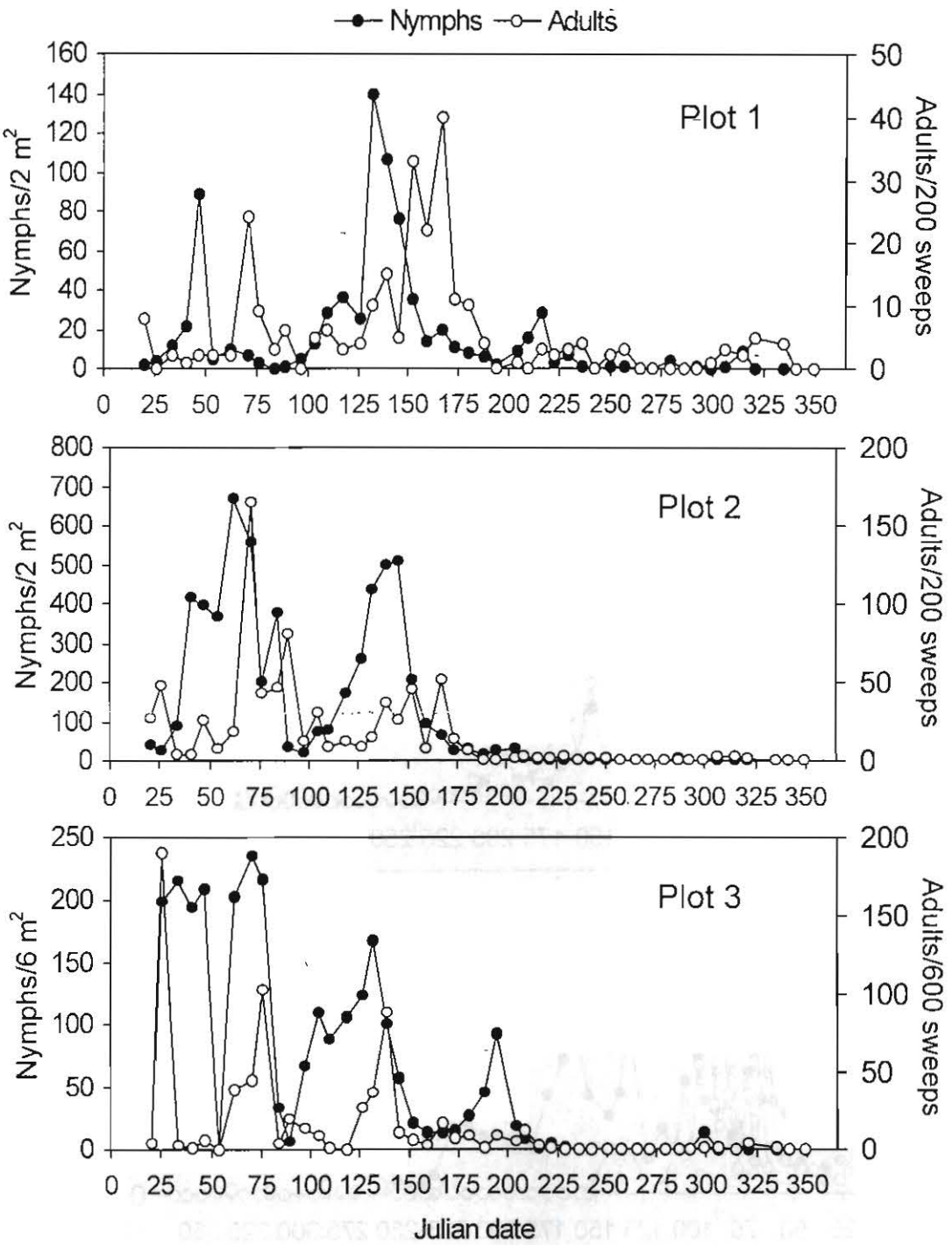

Figure 1.6. Population fluctuations of *Z. carbonaria* nymphs and adults in three survey plots in Santander de Quilichao, Dept. Cauca (1999).

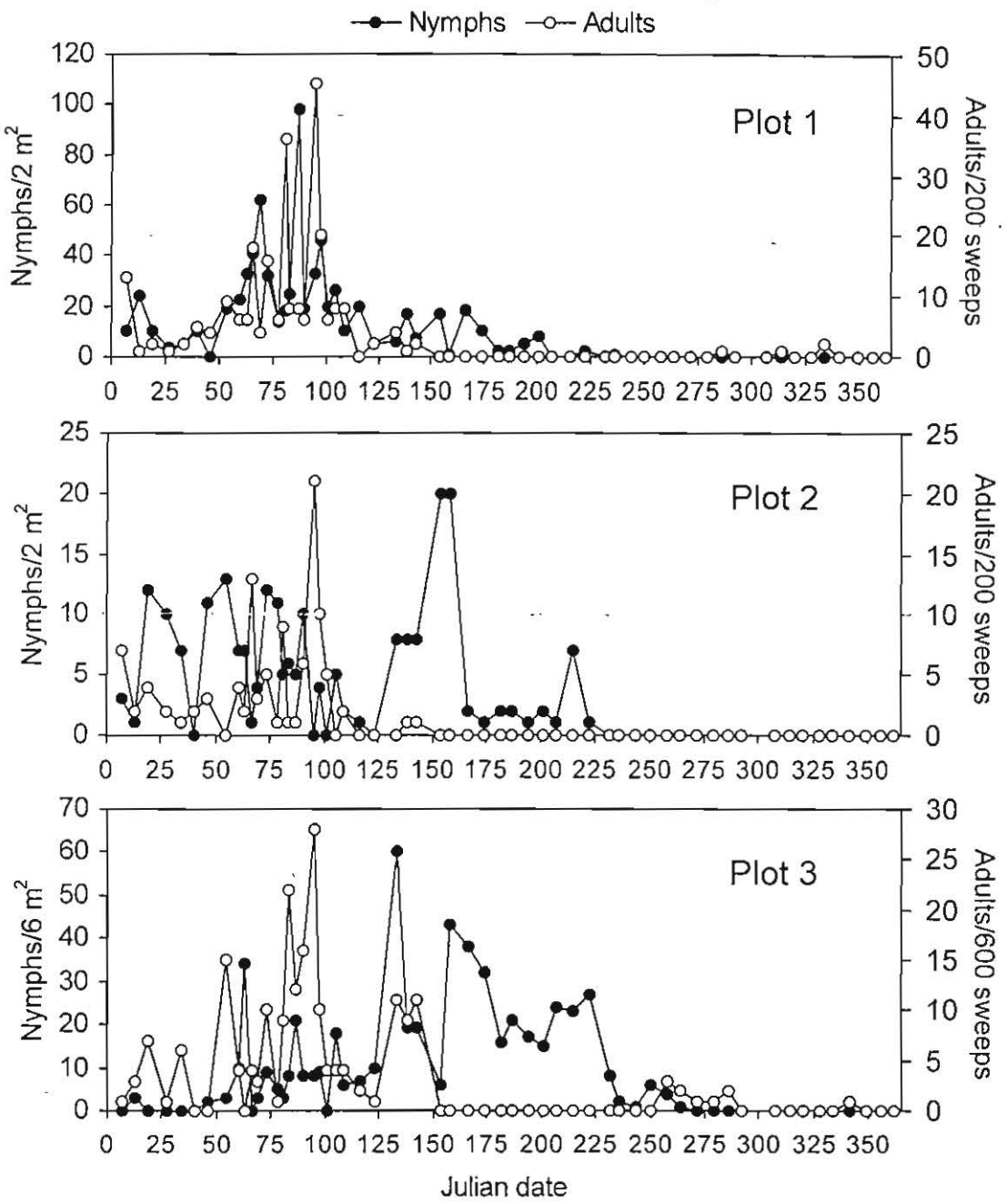

Figure 1.7. **Population fluctuations of *Z. carbonaria* nymphs and adults in three survey plots in Santander de Quilichao, Dept. Cauca (2000).**

Table 1.13. Monthly precipitation in survey sites, Santander de Quilichao, Dept. Cauca.

Month	Precipitation (mm)			
	Mean (11 years)	1998	1999	2000
Jan	155.9	20.0	305.0	241.7
Feb	141.8	126.0	345.0	179.8
Mar	216.9	125.2	265.0	327.3
Apr	251.0	304.0	236.3	319.9
May	163.7	210.0	170.7	230.6
Jun	83.6	32.0	120.2	148.0
Jul	51.4	76.0	21.5	71.5
Aug	56.1	128.0	53.2	72.2
Sep	135.9	277.0	202.6	173.1
Oct	181.6	248.0	153.0	121.0
Nov	238.6	414.0	183.7	159.0
Dec	150.0	70.0	147.8	163.0

Discussion

Zulia carbonaria achieves 3-4 generations a year in *Brachiaria* pastures of the Cauca River Valley, increasing in abundance at the onset of the wet season, and decreasing with the dry season. Small populations were still detectable during the driest months indicating that the insect is capable of finding microhabitat suitable for maintenance of nymphs and adults despite the dry pasture conditions.

The dramatic decline in population between 1999 and 2000 may have been caused by habitat alterations. Grazing bouts were more frequent and heavy in 2000 compared to 1999, degrading much of the pasture to turf-like conditions and offering poor spittlebug habitat.

Zulia carbonaria populations were shown to be highly synchronous, indicating a response to environmental variables such as mass eclosion of eggs upon return of the wet season rains. The very low frequency of diapause in eggs, however **(see Preoviposition determinants of egg diapause – Pag. 73)**, means that *Z. carbonaria* may rely on quiescence to synchronize life cycle with humid conditions. These data will be analyzed further for the correspondence between precipitation and phenology and begin to clarify how *Z. carbonaria* phenology tracks bimodal precipitation in the Cauca River Valley.

Table 1.14. Time of arrival (calculated as 50% cumulative insect days) of *Z. carbonaria* populations in three survey plots, Santander de Quilichao, Dept. Cauca.

			50% Cumulative Insect Days (Julian Date)			
Year	Generation	Life Stage	Plot 1	Plot 2	Plot 3	Mean
1999	1	Nymph	47	60	51	52.7
		Adult	70	71	57	66.0
	2	Nymph	136	136	124	132.0
		Adult	156	140	136	144.0
	3	Nymph	205	185	192	194.0
		Adult	201	215	205	207.0
2000	1 a	Nymph	17	24	10	17.0
		Adult	48	38	25	37.0
	1 b	Nymph	67	53	63	61.0
		Adult	77	66	61	68.0
	2 a	Nymph	94	77	93	88.0
		Adult	97	94	92	94.3
	2 b	Nymph	142	106	133	127.0
		Adult	140	107	138	128.3
	3 a	Nymph	168	132	170	156.7
		Adult	---	140	---	140.0
	3 b	Nymph	195	154	215	188.0
		Adult	---	---	---	---
	4	Nymph	229	205	255	217.0
		Adult	---	---	267	---

Table 1.15. Generation time of *Z. carbonaria* calculated from population dynamics studies in three survey plots, Santander de Quilichao, Dept. Cauca.

	Generation Time (Days)			
Year	Plot 1	Plot 2	Plot 3	Overall
1999	72.3	67.3	72.3	70.6
n	4	4	4	12
range	45-89	49-76	68-79	45-89
2000	65.2	50.4	76.0	63.9
n	6	7	6	19
range	49.1-77.7	40.7-55.7	66.9-82.8	44.7-82.8
Overall	68.7	58.8	74.15	67.3
n	10	11	10	31

First generation population phenology in two lowland sites

Introduction

In seasonal pasture systems, the first generation of spittlebugs is of critical importance to forage production because it leads to subsequent generations (as many as six generations per year) and is the source of infestation of previously unaffected areas of the farm. Suppressing this initial outbreak depends on our ability to predict when and where focal populations of nymphs and adults will manifest on the farm and thereby more effectively target application of available control tactics. With information on environmental factors and population fluctuation of the first generation, we hope to generate a predictive model correlating the timing of initial outbreaks with precipitation patterns at the onset of the rainy season. In this report we summarize results of the first generation population phenology at two lowland sites of Colombia over two years.

Materials and Methods

The first generation population dynamics were documented in three contrasting ecoregions of Colombia over two years (2000, 2001). Survey methods were similar to those used in previous studies to document population fluctuations in forage grasses **(see Population dynamics and phenology of *Prosapia simulans* – Pag. 58)**. The survey period was limited to two months starting at the beginning of the rainy season. Surveys were carried out twice weekly in three 0.5 ha focal plots each established in a separate pasture. These studies were carried out on the Caribbean Coast (pronounced seasonality, unimodal precipitation) at Finca Tarapacá, Corozal, Dept. Sucre with the collaboration of the Universidad de Sucre; in the Orinoquian Piedmont (intermediate seasonality, unimodal) at C.I. La Libertad, Villavicencio, Dept. Meta with the collaboration of CORPOICA; and the Cauca River Valley (pronounced seasonality, bimodal) at Hacienda Las Palmas, Santander de Quilichao, Dept. Cauca.

Results

The results from Cauca are summarized elsewhere **(see Population dynamics and phenology of *Zulia carbonaria* – Pag. 63)**.

In Meta, 64 nymphs and 566 adults were captured and assessed in 2000, 698 and 1883 in 2001 **(Table 1.16)**. Populations of nymphs were very low in Plots 2 and 3 because no nymphs were detected there in 2000 and 97% of total nymphs came from Plot 1 in 2001. Adults were also much more abundant in Plot 1 where 77 and 76% of adults were recovered in 2000 and 2001, respectively. *Aeneolamia varia* comprised 75.5 and 97.8 of adult populations in the two years while *Aeneolamia reducta* comprised 22.0 and 2.2%, and *Zulia pubescens* 2.5 and 0.0%. The date of first detection of nymphs and date of peak abundance of the first nymph generation (date of 50% accumulated insect days) was julian day 108 and 117 in 2000, and ranged from 92-99 and 104-108 in 2001 across the three plots **(Table 1.17, Figure 1.8)**. The corresponding dates for adults were 101-104 and 121-122 in 2000, and 99 and 115-117 in 2001. At the farm level over the two years, the first adult generation reached its peak 5 and 7 days, respectively, after the nymph generation.

Spittlebug populations were much higher in Sucre where 1097 nymphs and 36,396 adults were captured and assessed in 2000, 384 and 19,638 in 2001. There were large populations across the three plots over each year. *Aeneolamia reducta* was the only species detected. The date of first detection of nymphs and date of peak abundance of the first nymph generation (date of 50% accumulated insect days) was julian day 132-143 and 145-152 in 2000, and 137-140 and 147-154 in 2001 across the three plots **(Table 1.17, Figure 1.8)**. The corresponding dates for adults were 143-148 and 155-161 in 2000, and 140-148 and 158-162 in 2001. At the farm level over the two years, the first adult generation reached its peak 10 and 13 days, respectively, after the nymph generation.

Table 1.16. Comparative abundance of first generation spittlebugs surveyed in two regions over two years.

			Plot 1		Plot 2		Plot 3		
Site	Year	Life Stage	No.	%	No.	%	No.	%	Total
Meta	2000	Nymphs	64	100.0	---	---	---	---	64
		Adults[1]	436	77.0	65	11.5	65	11.5	566
	2001	Nymphs	650	93.0	32	5.0	16	2.0	698
		Adults[2]	1423	76.0	270	14.0	190	10.0	1883
Sucre	2000	Nymphs	658	60.0	64	6.0	375	34.0	1097
		Adults[3]	20065	55.0	7318	20.0	9013	25.0	36396
	2001	Nymphs	157	41.0	123	32.0	104	27.0	384
		Adults[3]	7121	36.0	8418	43.0	4099	21.0	19638

[1] *A. varia+A. reducta+Z. pubescens*
[2] *A. varia+A. reducta*
[3] *A. reducta*

Table 1.17. Time of arrival (calculated as 50% cumulative insect days) of the first spittlebug generation in two regions over two years.

			Date First Detected			Date of Abundance Peak			
Site	Year	Life stage	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Sum
Meta	2000	Nymphs	108	---	---	117	---	---	117
		Adults	104	101	101	121	123	122	122
	2001	Nymphs	92	99	99	108	104	104	108
		Adults	99	99	99	115	117	116	115
Sucre	2000	Nymphs	143	143	132	148	152	145	147
		Adults	143	143	143	155	161	158	157
	2001	Nymphs	137	140	140	147	154	154	147
		Adults	140	148	144	158	161	162	160

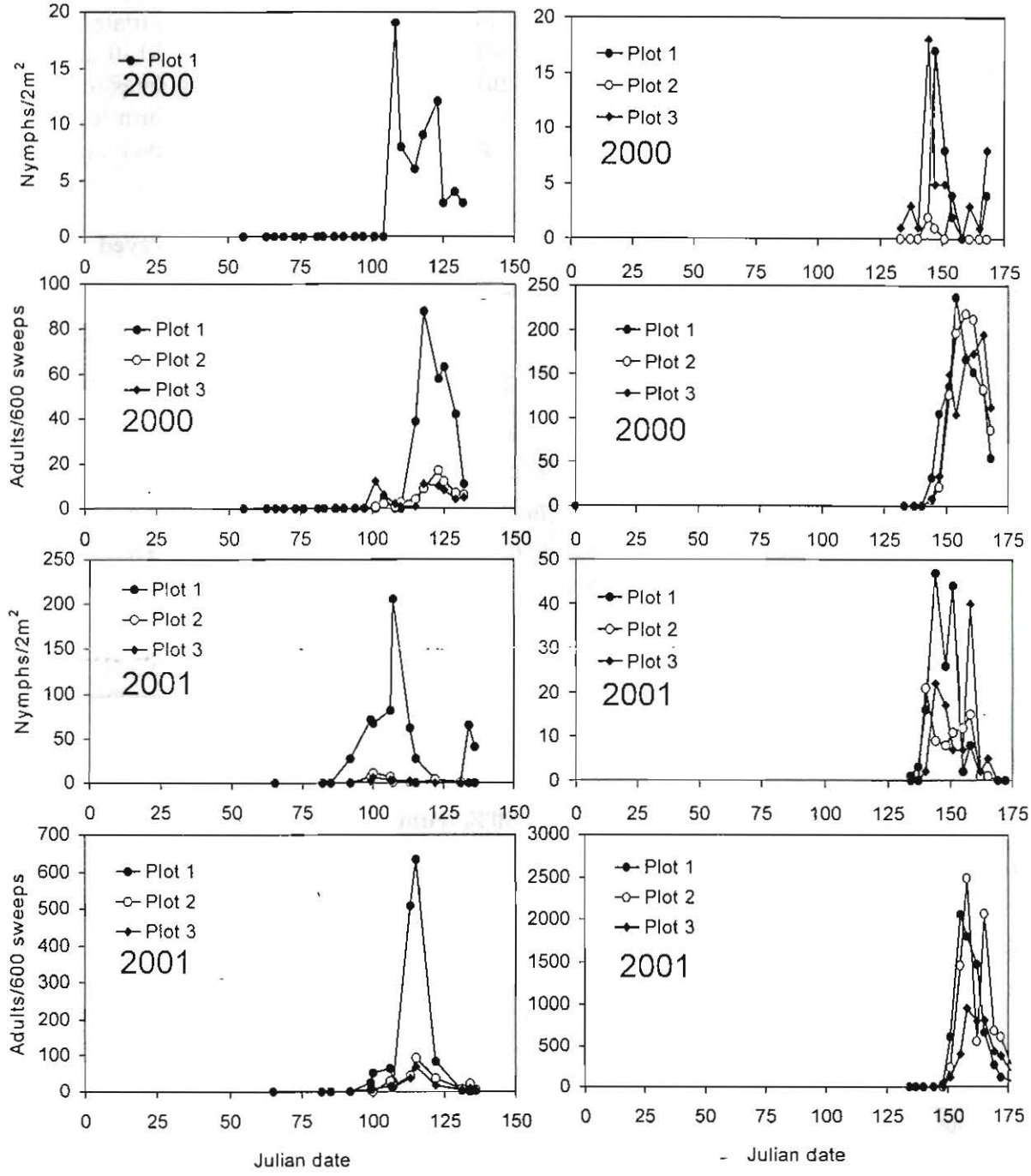

Figure 1.8. **Population fluctuations of the first generations of spittlebug nymphs and adults in Meta (left column) and Sucre (right column) over two years.**

Discussion

In both survey sites there was little variation in the timing of the first generation among plots and between years. In Meta, the difference in arrival of nymph and adult populations between years was only 9 and 6 days. In Sucre the difference was only 8 and 5 days. From these results we predict that the timing of the return of the wet season rains was similar in 2000 and 2001 since post-diapause quiescent eggs in the soil continue their development and hatch in direct response to the return of humid conditions. These population data will be combined with data from previous years (1997-1998 in Meta and 1997-1999 in Sucre) as repetitions to establish a predictive model, based on precipitation patterns, of when the first generation of spittlebugs is expected to appear in pastures of these regions. Predicting when and where the first outbreaks occur is critical information for targeting spittlebug management tactics in highly seasonal ecosystems.

Preoviposition determinants of egg diapause

Introduction

Female spittlebugs generally lay an increased proportion of diapausing eggs in response to the approaching dry season and the conditions unfavorable for spittlebug development and reproduction. The pest survives these adverse conditions of drought and high temperature as diapausing eggs that hatch upon return of the rains in the subsequent wet season. The immature stage (nymph) is responsible for predicting the extreme conditions of the future by perceiving token environmental stimuli that induce diapause in the adult stage. In temperate zones, photoperiod and temperature are dominant stimuli involved in the induction and regulation of diapause in many insects. In the tropics, however, the precise token stimuli that induce diapause in graminoid spittlebugs remain unknown. Photoperiod probably does not play a role in Colombia due to its proximity to the equator. In this report we summarize advances in assessing the role of plant age, water stress and their combination in inducing diapause in *Aeneolamia varia*.

Materials and Methods

Plants of *Brachiaria ruziziensis* were established in wooden boxes (1.4 x 0.6 x 0.1 m) with a proliferation of surface roots required as feeding sites by the nymphs. This arrangement was described previously as a component of an improved mass rearing design (CIAT Annual Report 2000). Boxes served as units of repetition for four treatments based on combinations of two factors: host plant age (4 and 8 weeks after transplanting) and water stress (field capacity and stressed). Boxes at field capacity were watered daily at the rate of 6 l/m^2 while stressed plants were watered as the rate of 3 l/m^2 every 3 days. Each box was infested with eggs of *A. varia* collected from adults in the field in the Orinoquian Piedmont (C.I. La Libertad, Villavicencio, Dept. Meta). The CIAT colony was not used as the source for these eggs because the insects were not regarded as fully receptive to token stimuli; colony management selects strongly against diapause eggs and the most recent genetic addition to the colony were individuals from

the Amazonian Piedmont (Dept. Caquetá) where conditions are continuously humid and diapause may not be important to species survival.

Each treatment had three repetitions. Boxes were infested with 1600 eggs and water treatments initiated one week later once first instars had emerged and established spittle masses. Once the adults began to appear the box was covered with an emergence cage (1.4 x 0.6 x 0.9 wooden frame covered in mesh) and individuals were collected with an aspirator and transferred to a separate small oviposition cage assigned to each repetition. After enough adults had emerged they were allowed 3 days to oviposit on fresh substrate following which eggs were extracted, disinfected and stored in petri dishes on humid filter paper under controlled conditions (27°C, 100% RH, darkness). As more females emerged or stayed alive, a second batch of eggs was collected. Eggs were evaluated twice weekly to score chorions (emerged nymphs) and inviable eggs. Viable eggs remaining after 30 days were classified as diapausing. To confirm that treatments had an affect on the quality of the host plant, plant material in each repetition was assessed for dry weight and dry matter digestibility.

Results

Of a total 9277 eggs evaluated, 12.2% were considered diapausing. Among the four treatments, the incidence of diapause varied from 8.3-13.8 % and the time to eclosion 33.2-37.0 days **(Table 1.18)**. For non-diapause eggs time to eclosion was 20.4-21.6 days and for diapause eggs 32.7-37.0 days. Analysis of variance did not detect an effect of water stress or plant age on diapause incidence.

Table 1.18. Influence of host plant age and water stress on the incidence and duration (mean±S.E., range) of diapause eggs in *A. varia*.

	Eggs	Mean Proportion (%)		Mean Time to Eclosion (Days)	
Treatment	Observed	Non-Diapause	Diapause	Non-Diapause	Diapause
Field capacity	2183	86.8 ± 0.5	13.2 ± 0.5	21.6 ± 1.4	37.0 ± 0.3
4-wk old		(86.5-87.2)	(12.8-13.5)	(20.5-226)	(36.8-37.2)
Field capacity	5017	86.5 ± 8.3	13.5 ± 8.3	20.6 ± 0.8	34.1 ± 3.5
8-wk old		(71.5-95.8)	(4.2-28.5)	(19.4-21.7)	(30.4-37.7)
Water stress	964	86.5 ± 4.7	13.5 ± 4.7	20.6 ± 1.4	33.2 ± 3.3.
4-wk old		(82.6-91.8)	(8.2-17.4)	(19.8-22.2)	(31.2-37.0)
Water stress	1113	91.7 ± 5.5	8.3 ± 5.5.	20.4± 1.9	34.9 ± 3.7
8-wk old		(83.7-96.1)	(3.9-16.3)	(18.3-22.7)	(31.4-38.2)

Mean dry matter (g), percent dry matter, and digestibility were measured to gauge differences in expression of the treatments on plant quality **(Table 1.19)**. These means have not yet been statistically tested for differences.

Table 1.19. Influence of host plant age and water stress on percent dry matter and in vitro digestibility of *B. decumbens*.

	Field Capacity		Water Stress	
Measure	4-wk old	8-wk old	4-wk old	8-wk old
Dry weight (g)	0.043	0.048	0.044	0.044
Dry weight (%)	60.8	63.8	64.4	54.0
Digestibility (%)	61.0	59.4	64.5	64.0

Discussion

Under the conditions of these experimental treatments, no effect of host plant age or water stress was detected on the incidence or duration of diapause eggs in *A. varia*. Other factors are probably responsible for the documented increase in diapause incidence at the end of the wet season **(see Seasonal changes in the incidence and duration of egg diapause – Pag. 75)**. The incidence of diapause of eggs from Meta populations was far higher than those previously examined in females from Caquetá (0.24%), a continuously humid site. This reinforces the idea that diapause is more expressed in seasonal sites and that studies should use insects originating directly from these populations.

Seasonal changes in the incidence and duration of egg diapause

Introduction

For various spittlebug species in seasonal environments it has been shown that the incidence of diapause eggs increases at the end of the wet season in anticipation of the unfavorable dry season. To complement studies on the population dynamics of spittlebug nymphs and adults in contrasting regions of Colombia, changes in the incidence and duration of diapause eggs was documented over the season in three contrasting ecoregions: the Caribbean Coast, the Orinoquian Piedmont and the Cauca River Valley.

Materials and Methods

One year of data has been analyzed for *Aeneolamia reducta* (Dept. Sucre, Caribbean Coast), *Aeneolamia varia* (Dept. Meta, Orinoquian Piedmont) and *Zulia carbonaria* (Dept. Cauca, Cauca River Valley). These data were collected from the same focal paddocks established in these sites for population dynamics studies **(see sections Population dynamics and phenology of *Prosapia simulans*, - Pag. 58, Population dynamics and phenology of *Zulia carbonaria* – Pag. 63)**. In each of the three plots per farm, two groups of females (1-5 for Cauca, 1-25 for Meta and Sucre, depending on availability) were caught with sweep nets, brought to the laboratory and confined to large petri dishes (2 cm tall, 15 cm diameter) lined on the bottom with moist filter paper that served as oviposition substrate. Females laid eggs over 3 days, the filter paper was disinfected for 2-3 min with Clorox and washed with distilled water, and petris were express mailed to CIAT for incubation under controlled conditions (27°C, 100% RH, darkness).

Collections were made every 2 wk over the season when females were available in the field. Petri dishes were evaluated twice weekly for chorions (emerged nymphs) and inviable eggs.

Results

Of 38,088 eggs collected and evaluated from January to December 2000, 12.95% were diapausing **(Table 1.20)**. The incidence of egg diapause in *Z. carbonaria* was extremely low throughout the year **(Figure 1.9)**. Diapausing eggs were only detected on one date (July) and at very low incidence. The six diapause eggs eclosed 36, 36, 46, 46, 53 and 57 days after oviposition. In Sucre and Meta the incidence of diapause increased at the end of the year at the start of the dry season achieving rates as high as 85.0 and 49.5%, respectively.

Overall, the time to eclosion for non-diapause eggs was 22.7 ± 5.5 for *A. reducta*, 18.2 ± 4.6 for *A. varia* and 18.0 ± 1.9 for *Z. carbonaria* **(Table 1.20)**. The time to eclosion for diapausing eggs was 96.9 ± 43.6, 71.5 ± 32.7 and 48.2 ± 12.5 d, respectively. In Meta the time to eclosion of diapause eggs did not vary over the season, however in Sucre there was a noticeable increase in duration toward the end of the season.

Table 1.20. Seasonal changes in diapause incidence and time to eclosion of spittlebug eggs in three ecoregions of Colombia (bars indicate periods where no females were available).

Month	Incidence of Diapause (%)			Time to Eclosion (Days)			n (Eggs Examined)		
(2000)	Sucre	Meta	Cauca	Sucre	Meta	Cauca	Sucre	Meta	Cauca
Jan	---	---	---	---	---	---	0	0	0
Feb	---	---	---	---	---	---	0	0	0
Mar	---	---	---	---	---	---	0	0	0
Apr	---	0.0	0.0	---	---	---	0	305	183
May	---	0.0	0.0	---	---	---	0	645	315
Jun	0.01	0.0	0.0	55	---	---	3781	1986	531
Jul	2.0	0.1	3.3	50	---	48	4274	2651	174
Aug	2.0	0.6	0.0	55	74	---	1916	6244	110
Sep	---	8.1	---	---	43	---	0	2991	0
Oct	57.0	11.2	---	137	89	---	1419	4953	0
Nov	72.0	2.7	0.0	127	53	---	3692	513	289
Dec	85.0	49.4	0.0	134	68	---	914	166	0

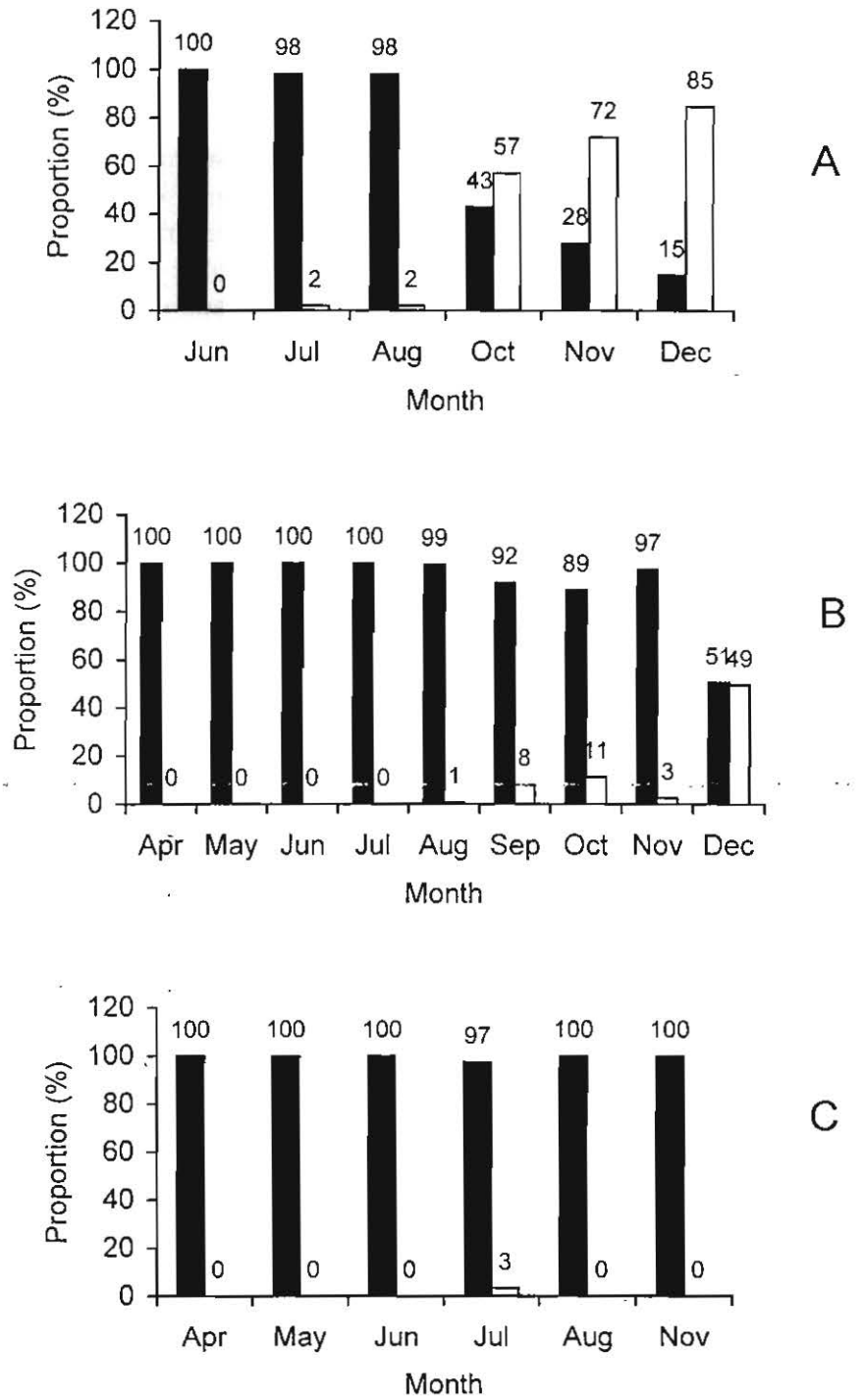

Figure 1.9. **Seasonal changes in the incidence of spittlebug egg diapause in (A) the Caribbean Coast with *A. reducta*, (B) the Orinoquian Piedmont with *A. varia*, and (C) the Cauca River Valley with *Z. carbonaria* during 2000. Black bars are non-diapause and open bars are diapause eggs.**

Discussion

In the Caribbean Coast and the Orinoquian Piedmont the incidence of diapause increased from zero at the beginning of the wet season to 85 and 50%, respectively, at the start of the dry season. In both of these sites the rainy season is highly seasonal with 2-4 months of extremely dry months annually and in accordance with our expectations, the principal spittlebug species in these regions use diapause as a means to survive the adverse conditions. In the Cauca River Valley, precipitation is bimodal and although dry periods are severe, they are comparatively brief. These seasonality conditions may allow for different mechanisms for surviving the adverse conditions because no seasonal changes in the incidence of diapause or the time to egg eclosion was detected. In this region, *Z. carbonaria* may adopt a different strategy, such as maintenance of low population levels in localized humid areas with subsequent recolonization of pastures. Alternatively, instead of diapause *Z. carbonaria* could depend on drought-tolerant quiescent eggs where development is temporarily delayed in direct response to adverse conditions, and reinitiated once adequate humid conditions return. These possibilities will be explored in future studies.

Activity 2. Developing IPM components for spittlebug management

Artificial diet for maintenance of spittlebug adults

Introduction

The development of an artificial diet for maintenance of spittlebug adults would permit the evaluation of feeding deterrents or deleterious compounds such as lectins that could be incorporated into *Brachiaria* though genetic transformation. Here we summarize results of three trials of a particular formula chosen as the best option from among a series of diets developed and assessed in 2000.

Materials and Methods

Trials consisted of three treatments: vial, plant and diet. In the plant treatment, adults were enclosed inside cylindrical acetate cages (40 cm tall, 15 cm diameter) over potted *Brachiaria ruziziensis* plants that provided a food source. In the other treatments adults were held in large petri dishes (2 cm tall, 15 cm diameter). In the vial treatment, the food source was stems of *B. ruziziensis* kept with their base in a vial of water. In the diet treatment, 500 µl of the liquid diet was sealed in parafilm sachets (3 x 3.5 cm). Thirteen repetitions were carried out over three trial periods. Each of the three trials and all their treatments were performed in an insect growth chamber with mean (± S.E.) temperatures of 23.8 ± 1.5, 22.5 ± 3.2 and 23.21 ± 1.4°C, respectively. Each treatment repetition had four adult *Aeneolamia varia* taken as tenerals (<12 hours old) from the CIAT colony.

The composition of the diet was modified from Hagley 1967. To make the diet easier and cheaper to prepare, yeast and hydrolyzed casein were substituted as the sources of amino acids and p-aminobenzoic acid substituted as the source of vitamin B_{12}. The list of ingredients in the

diet were (1) amino acids: yeast extract, hydrolyzed casein, (2) vitamins: biotin, calcium pantothenate, choline chloride, folic acid, inositol, nicotinic acid, pyridoxine, thiamine, riboflavin, ascorbic acid, ρ-aminobenzoic acid, (3) carbohydrates: sucrose, (4) salts: $MgCl_2$, KH_2PO_4, Wesson's' salts, and (5) lipids: cholesterol benzoate.

Adult mortality was assessed daily. Mean adult longevity was determined with Probit analysis and differences among treatments were tested with an ANOVA.

Results

Mean longevity among repetitions varied from 6.0-11.0 days. Analysis of variance detected no significant differences among the three diet treatments with mean longevity of 7.4, 8.7 and 9.2 days for vial, diet and plant treatments, respectively. Probit analysis, however, indicated that more trials will have to be carried out due to low X^2 values in several individual trials.

Discussion

Replacement of certain components in the original diet (Hagley 1967) did not affect the maintenance and longevity of adults *A. varia*. Use of yeast extract and hydrolyzed casein as the sources of amino acids made preparation easier and could significantly reduce costs. Whether the diet is effective for maintenance of nymphs remains to be tested; Hagley's original diet was designed and tested only for adults. Regardless, the current formulation should be adequate for future studies on substances of interest in adult feeding or toxicity.

Table 2.1. Results from Probit analysis for three trial of three diets.

Treatment	Trial	n	X^2	Prob. X^2	b	L_{50}	L_{90}
Vial	1	585	7.6377	0.6642	6.7316	7.0661	10.9537
	2	780	30.0965	0.0027	7.6551	8.6623	12.7364
	3	728	44.5463	0.0000	5.1588	6.5599	11.6229
Diet	1	784	15.5965	0.2716	8.3733	9.9825	14.2001
	2	780	6.7797	0.8718	8.1406	8.7894	12.6295
	3	888	215566	0.0882	6.0587	7.5096	12.2219
Plant	1	550	43.3962	0.0000	7.0250	6.0452	9.2011
	2	714	9.4180	0.5834	14.7956	10.4654	12.7753
	3	901	5.2907	0.9813	10.6372	10.9781	14.4879

Maintenance of a ceparium for fungal entomopathogens of major forage grass and cassava pest

Introduction

Ongoing field studies on the major insect pests in diverse regions of Colombia have allowed us to collect, isolate, propagate and store a diverse collection of fungal entomopathogens. This

ceparium was established last year (CIAT Annual Report 2000) and is designed to serve as a source of pathogenic material for studies on biological control. Maintaining and strengthening this collection is of utmost importance for advancing non-toxic alternatives to insecticides and other effective tactics as components of integrated pest management. In this report we summarize maintenance and diversity of the ceparium with a particular focus on the fungal entomopathogens of forage grass pests (spittlebugs) and cassava pests (burrower bugs, stem borers, whiteflies).

Materials and Methods

There are two main activities related to the ceparium. The first consists of the isolation, maintenance, propagation and storage of isolates based on previously established protocols (see CIAT Annual Report 2000). The second is the multiplication of isolates for reactivation and studies on virulence and pathogenicity.

Results and Discussion

The CIAT ceparium now houses a total of 150 different isolates of fungal entomopathogens. In the area of cassava pests, 34 new isolates of fungal entomopathogens were added to the 39 strains already purified and stored on filter paper **(Table 2.2)**. Of the 73 isolates, 28 were reactivated on the burrower bug *Cyrtomenus bergi* (Heteroptera: Cydnidae) and 18 of these have been evaluated for virulence to nymphs and adults in laboratory studies. Once the efficiency of these isolates is calculated in comparison to mortality in the controls, the five most virulent isolates will be selected for future studies. The levels of control are highly promising with up to 100% mortality in nymphs and 58% in adults **(Table 2.3)**.

Seven other isolates of diverse fungi including *Paecilomyces* spp. (CIAT 210, 211, 212, 216), *Verticillium lecani* (CIAT 215), *Beauveria bassiana* (CIAT 217) and *Cladosporium* sp. (CIAT 272) were reactivated on nymphs and adults of the whitefly *Aleurotrachellus socialis*. These isolates are currently in the multiplication phase to provide material for the first applications to determine pathogenicity and virulence.

In the area of spittlebug pests of forage grasses, the main activities were related to selection and characterization of isolates for field trials **(see Field evaluation of fungal entomopathogens in two contrasting regions – Pag. 87)** including (1) multiplication for virulence studies on different species of adult spittlebugs **(see Variation in the virulence of fungal entomopathogens among spittlebug species – Pag. 82)**, (2) multiplication for determination of LC_{50} and LC_{90} in nymphs **(see Characterization and formulation of select fungal entomopathogen isolates for field evaluation – Pag. 84),** (3) and quality control studies of formulated material developed in collaboration with BioCaribe, S.A. with the goal of achieving a product of higher quality.

Table 2.2. Accession, host and origin of fungal isolates entomopathogenic to different cassava insects.

	CIAT Accession	Origin	
Host Species	Numbers	Department	Municipality
Aleurotrachellus socialis	CIAT 215-217	undet.	undet.
Brassoly sp.	CIAT 246	Casanare	Villanueva
Chilomima clarkei	CIAT 249, 252-257, 263-267, 269	Tolima	Espinal
Chilomima clarkei	CIAT 274	Tolima	Ibague
Chilomima clarkei	CIAT 277	Tolima	Nataima
Coleoptera	CIAT 262	Cauca	undet.
Corinus sp.	CIAT 219	Valle	La Cumbre
Cosmopolites sordidus	CIAT 247	Valle	Jamundi
Cyrtomenus bergi	CIAT 200	Cauca	Timbio
Cyrtomenus bergi	CIAT 214, 224, 225	undet.	undet.
Cyrtomenus bergi	CIAT 226-243	Cauca	Popayan
Cyrtomenus bergi	CIAT 250, 251, 258-261, 268, 275, 276	Risaralda	Pereira
Erinnys ello	CIAT 218	undet.	undet.
Galeria melonella	CIAT 208, 213	Valle	Pradera
Galeria melonella	CIAT 270	Risaralda	Pereira
Galeria melonella	CIAT 271, 273	Tolima	Guamo
Galeria melonella	CIAT 278, 279	Cauca	Cajibio
Hymenoptera	CIAT 248	Valle	Palmira
Trialeurodes vaporariorum	CIAT 210-212	Valle	Pradera
Trialeurodes variabilis	CIAT 272	Tolima	Espinal
Whitefly	CIAT 244	undet.	Imbabura
undet.	CIAT 209	Valle	Palmira
undet.	CIAT 220-222	undet.	undet.

In terms of ceparium maintenance, viability tests of stored material are continuously carried out with the goal of reactivating on culture media the isolates that have lost vigorous growth characteristics. Three new isolates from spittlebugs were incorporated into the collection this year: CIAT 076 isolated from a nymph collected in C.I. Macagual, Dept. Caquetá; and CIAT 077 and 078 isolated from an adult of *Mahanarva andigena* collected in Tumaco, Dept. Nariño.

In addition to these activities, "Access" software was used to establish a database to manage all information related to ceparium isolates. This program allows easy consultation of the information by interested scientists.

Table 2.3. Virulence (% mortality) of 18 fungal entomopathogen isolates to nymphs and adults of the burrower bug *C. bergi*.

Accession	Nymphs	Adults	Accession	Nymphs	Adults
CIAT 227	66.0	56.0	CIAT 230	89.0	53.0
CIAT 231	53.0	48.3	CIAT 237	81.0	50.0
CIAT 233	67.0	53.3	CIAT 261	74.0	49.0
CIAT 234	58.0	31.7	CIAT 224	100.0	47.0
CIAT 241	30.0	58.3	CIAT 245	100.0	47.0
CIAT 242	55.0	50.0	CIAT 239	76.0	33.0
CIAT 250	52.0	56.7	CIAT 228	55.0	23.0
CIAT 258	58.0	55.0	CIAT 238	50.0	20.0
CIAT 259	51.0	65.0	CIAT 240	74.0	21.0

Variation in the virulence of fungal entomopathogens among spittlebug species

Introduction

A major challenge for the implementation of an integrated management plan for graminoid spittlebugs is the taxonomic diversity of species that contribute to this pest complex. In Colombia, for instance, 15 species from six genera have been identified with graminoid host plants. Management is limited by the extent that a particular control tactic can be tailored to different species, further complicated by the presence of 2-3 species in the same local pastures. We are obtaining new information on the biology and ecology of major species in contrasting ecoregions of Colombia, and this information is broadening our understanding of the variation across this group and of the different strategies for their management in different habitats. From these results we predict that particular control tactics will also need to be tailored to the particular spittlebug species and habitat in which control is required. In the specific case of fungal entomopathogens as biological control agents, effectiveness of a given isolate may also vary across species. Advancing the use of fungal entomopathogens in an IPM program for spittlebug management will therefore depend on gauging the variation in virulence across different spittlebug species.

Materials and Methods

Methods were based on protocols established and described in 2000 (CIAT Annual Report). Evaluation units were 30-day old plants (7-10 stems) of *Brachiaria ruziziensis* (CIAT 654) in pots (15 cm diameter) covered by acetate cylinders (40 cm tall, 15 cm diameter). These plants were infested with 10 adult tenerals (< 24 hours old) of *Aeneolamia reducta*, *Aeneolamia varia*, *Zulia carbonaria* and *Zulia pubescens* obtained from colonies maintained at CIAT. Two to three hours after infestation plants were sprayed with 5 ml of a concentrated conidial suspension (10^8 conidia/ml) with an airbrush and compressor (10 PSI). Four isolates were evaluated: CIAT 007C, CIAT 009, CIAT 054 and CIAT 055, identified as *Metarhizium anisopliae*, *Paecilomyces*

farinosis Metarhizium sp. and *Metarhizium* sp. respectively. These isolates were selected from among 48 strains as the most virulent to *A. varia* adults (see CIAT Annual Report 2000).

For each spittlebug species, 10 repetitions (pots) were evaluated for each isolate and a control (water with tween at 0.05%). After spraying, plants and insects were maintained in a growth chamber (27°C ± 2°C, RH 80% ± 10%). Virulence was evaluated 5 days later when all insects were scored as alive, dead, and dead with evidence of mycosis. Dead insects with no visible signs of fungus attack were stored in petri dishes with moist filter paper for 3-4 days to ascertain whether they were infected with fungus. Differences were evaluated with an ANOVA and Tukey multiple range test.

Results

Mortality in the control varied from 3.9-28.5 among the four spittlebug species. The lowest mortality was experienced by *Z. pubescens* and the highest by *A. reducta*, corresponding to the species of longest and shortest adult longevity according to greenhouse biology studies.

Virulence of isolates varied significantly among species with *A. varia* being most susceptible, followed by *A. reducta*, then *Z. carbonaria* and *Z. pubescens* **(Figure 2.1)**. As expected, control for all four isolates was significantly higher on *A. varia* since this species was used for preselecting the most virulent strains used in this study. Mortality ranged from 62.8-95.1%. Mortality in *A. reducta* ranged from 42.5-61.9%. For *Z. carbonaria* and *Z. pubescens*, mortality ranged from 20.2-33.6 and 16.1-30.4%, respectively, and in most cases this was not significantly different than the control. In the case of *Z. carbonaria*, none of the isolates achieved higher mortality than the control. The *Paecilomyces* isolate (CIAT 009) achieved relatively higher mortality (33.6%) in *Z. carbonaria* compared to the *Metarhizium* isolates.

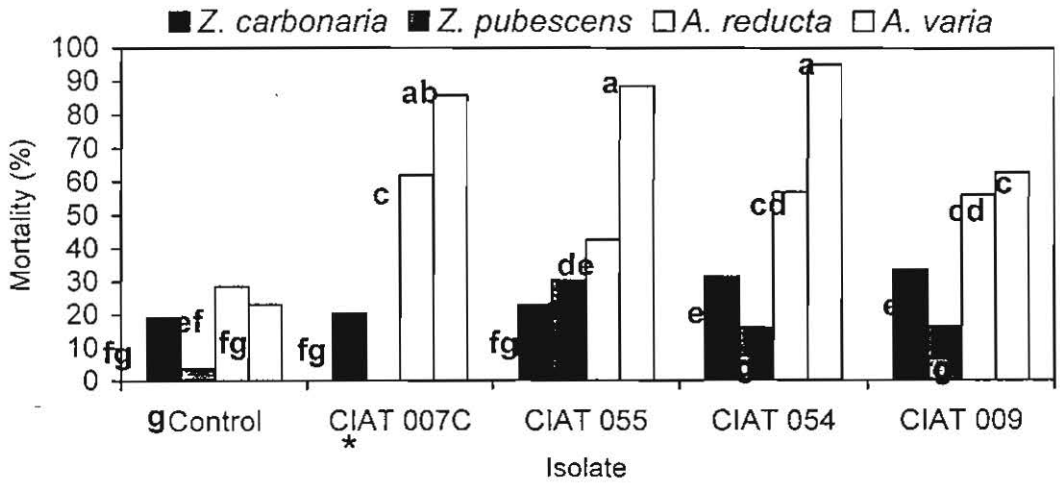

Figure 2.1. Mortality (absolute percent) of four isolates of fungal entomopathogens on four spittlebug species. Means followed by different letters are significantly different at P<0.05. * This isolate was not evaluated on *Z. pubescens*.

Control was also significantly higher in the genus *Aeneolamia* compared to *Zulia* for all four isolates evaluated. For instance, CIAT 054 achieved a mortality of 56.8 and 95.1% for *A. reducta* and *A. varia*, respectively but only 31.4 and 16.1 for *Z. carbonaria* and *Z. pubescens*. This suggests that some of the variance in virulence among spittlebug species may be expressed as differences at the genus level.

Discussion

Virulence of fungal entomopathogen strains varies among spittlebug species. Deploying these pathogens as agents of biological control therefore depends on an understanding of the species complex in the area where control is desired, selecting isolates specific to spittlebug species, and reassessing the broad effectiveness of commercial products. On the other hand, results indicate that the diverse collection of isolates in CIAT's ceparium probably has strains highly virulent to species other than *A. varia*, which up to this point has been used as the model species for developing evaluation methodologies. The most efficient screening process might therefore be evaluating a diversity of isolates to the particular spittlebug species of interest, rather than using preselection (with a model species such *A. varia*) with subsequent confirmation of high control on other species. One particular screening focus should be *Paecilomyces* isolates with *Z. carbonaria* since this fungus was relatively more virulent against this species than *Metarhizium*. At present there are three *Paecilomyces* strains in the ceparium that were originally isolated from *Z. carbonaria*.

These results confirm the need to continue documenting the patterns of variation among graminoid spittlebugs given that effectiveness of control tactics such as insect pathogens may be species specific. Significant variation in host plant resistance among spittlebug species is further corroboration of this observation.

Studies are under way to continue evaluating variation in virulence. Adults of *P. simulans* are under evaluation and variation between adults and nymphs are being explored with *A. varia*, *P. simulans*, *Z. carbonaria* and *Z. pubescens*.

Characterization and formulation of select fungal entomopathogen isolates for field evaluation

Introduction

Four isolates have been selected from CIAT's fungal entomopathogen collection for experimental field trials designed to test application techniques. These isolates are the three *Metarhizium* and one *Paecilomyces* strains screened from 49 isolates (see CIAT Annual Report 2000) as the most virulent to adults of *Aeneolamia varia* **(Table 2.4)**. Before deploying in the field, these isolates must be characterized for their biological and virulence activity on different species and life stages of spittlebugs. Variation in virulence among adults of four species was described elsewhere **(see Variation in the virulence of fungal entomopathogens among spittlebug species – Pag. 82)**. Here we summarize results of studies to determine the LC_{50} and LC_{90} on nymphs of *A. varia*.

Table 2.4. Identification and origin of fungal entomopathogen isolates selected for field trials.

	CIAT Accession Number			
	CIAT 054	**CIAT 055**	**CIAT 007C**	**CIAT 009**
Fungal isolate :				
Genus	*Metarhizium*	*Metarhizium*	*Metarhizium*	*Paecilomyces*
Species	sp. 1	sp. 2	*anisopliae*	*farinosis*
Spittlebug host:				
Genus	*Aeneolamia*	*Aeneolamia*	*Zulia*	undet.
Species	*varia*	*varia*	*pubescens*	
Sex	male	undet.	female	undet.
Life stage	adult	nymph	adult	nymph
Department	Valle del Cauca	Valle del Cauca	Caquetá	Caquetá
Municipality	Palmira	Palmira	Albania	Florencia

Materials and Methods

Evaluation methods for nymphs were based on previously established protocols (see CIAT Annual Report 2000). Evaluation units were the same small-scale PVC tubes (1.5" diameter) now standard for host plant resistance screening. At 6 weeks after planting with *Brachiaria ruziziensis* (CIAT 654), surface roots were sufficiently established for nymph development and egg infestation. Eggs of *Aeneolamia varia* about to hatch were prepared for treatments and infestation by placing 10 on each of 10 small pieces of filter paper in a petri dish that corresponded to one treatment. Nine different concentrations of conidial suspensions ($1x10^4$, $5x10^4$, $1x10^5$, $5x10^5$, $1x10^6$, $5x10^6$, $1x10^7$, $5x10^8$, $1x10^9$ conidia/ml) were prepared for three isolates (CIAT 007C, CIAT 054, CIAT 009) with a control (water and tween at 0.05%) **(Table 2.5)**. Applications were made on the substrate before infestation and on the eggs in petri dishes before infestation. An airbrush and compressor (10 PSI) were used at a volume of 1 ml for substrate and <1 ml for direct egg application. Plants were maintained in the greenhouse until evaluation of mortality 30-32 days after infestation. During this period, plants were fertilized twice (just before and 15 days after infestation) with urea at 2g/l. There were ten repetitions per treatment. Mortality data were analyzed with Probit (SAS).

Results

For CIAT 054 and CIAT 007C, *A. varia* nymph mortality increased with increasing conidial concentration as expected **(Figure 2.2)**. For CIAT 009, however, the relationship between mortality and concentration was not clear, showing irregular activity along the concentration gradient.

Probit analysis showed low X^2 values and acceptable X^2 probability values for CIAT 054 and CIAT 007C. The LC_{50} and LC_{90} were $8.0x10^6$ and $8.9x10^7$ conidias/ml for CIAT 054, and

$4.6x10^5$ and $3.6x10^8$ for CIAT 007C. Given the high X^2 value for CIAT 009, the calculated concentrations are inaccurate and the trial must be repeated.

Table 2.5. Probit analysis of mortality caused by three fungal entomopathogen isolates to nymphs of *A. varia*.

Isolate	n	LC_{50} (95% CI)	LC_{90} (95% CI)	X^2	Prob X^2	b (S.E.)
CIAT 054	900	$8.0x10^6$ ($3.7x10^6$-$1.3x10^7$)	$8.9x10^7$ ($5.2x10^7$-$2.0x10^8$)	7.3	0.290	1.2 (0.19)
CIAT 009		-	-	22.3	0.66	2.4 (0.25)
CIAT 007C	900	$4.6x10^5$ ($1.6x10^4$-$3.0x10^6$)	$3.6x10^8$ ($6.7x10^7$-$4.4x10^9$)	12.2	0.057	0.44 (0.06)

Comparative studies are currently underway with the same three isolates and nymphs of *Prosapia simulans*, the major spittlebug species in one of the two field evaluation sites. An additional phase before field evaluation is the formulation of strains. This is being carried out by BioCaribe, S.A. in a formal agreement with CIAT. To confirm the quality of this material, studies are underway to compare the effectiveness of formulated versus unformulated product.

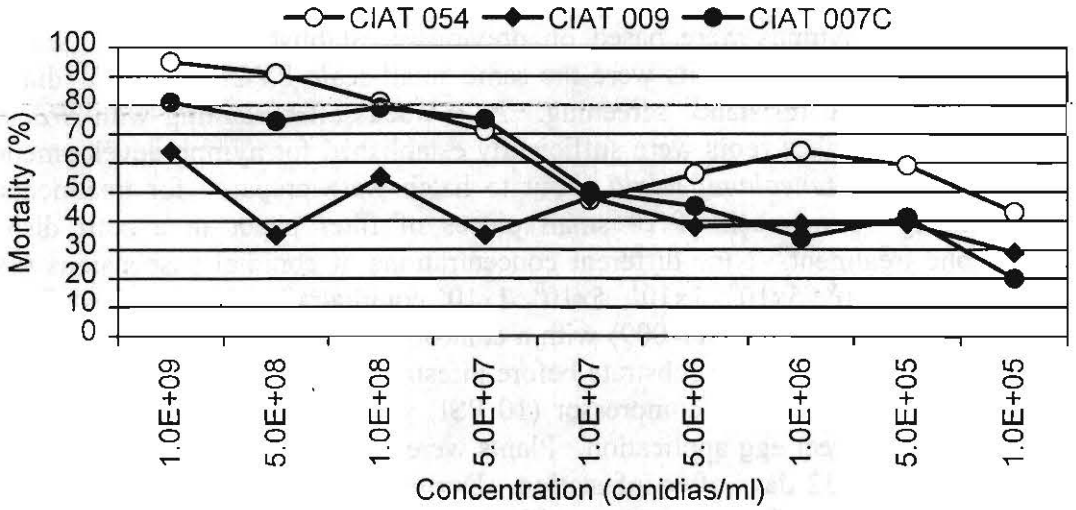

Figure 2.2. Mortality in *A. varia* nymphs caused by three fungal entomopathogen isolates at different concentrations.

Discussion

Even though identical methodologies were used for the isolates, different LC_{90} were expected given the different origin of the strains. Applications of fungal entomopathogens in upcoming field trials will be based on the LC_{90} determined here to avoid the situations where too little material is applied to have an effect, or too much is added and material is wasted. Ongoing

studies will corroborate this information and establish whether formulation has altered virulence. Plans are also underway to evaluated LC_{50} and LC_{90} on adults of *A. varia*.

Field evaluation of fungal entomopathogens in two contrasting regions

Introduction

In general, previous attempts to evaluate the efficiency of fungal entomopathogens as biological control agents of spittlebugs in pastures have been focused on laboratory assays. The few that have gone to the field have demonstrated highly variable and low levels of control due to a variety of factors including poor evaluation and applications techniques. Aspects such as the number of applications and the timing of applications in relation to phenology of the life stages have received no attention. To seriously evaluate the potential of fugal entomopathogens as an alternative for managing pasture spittlebugs, we are combining a detailed knowledge of the biology and phenology of spittlebugs with a series of studies to collect, screen, characterize, and formulate select isolates for deployment in field trials. In this report, we summarize the field trials established in two contrasting ecoregions of Colombia, the Amazonian Piedmont and the Cauca River Valley.

Materials and Methods

The Amazonian Piedmont ecoregion is continuously humid, corresponding to presence of spittlebug nymphs and adults throughout the year with little population synchrony. In this site the number of applications required to achieve an effect will be evaluated. The Cauca River Valley ecoregion is a highly seasonal site with bimodal precipitation and here spittlebug nymphs and adults are present only during the rainy months and have a high population synchrony. In this site the timing of the applications in relation to the insect's life cycle will be evaluated. The premise is that the diverging environmental conditions of these two ecoregions will require different strategies and control tactics for management of spittlebugs in pastures.

Five plots each were established in Hacienda Piedechinche, Santa Helena, Dept. Valle del Cauca (1600 m^2) and C.I. Macagual of CORPOICA, Florencia, Dept. Caquetá (1200 m^2). Each plot is located in a separate pasture under the same fertilization, grazing and weed management regime established for the rest of the farm. Each plot was subdivided into subplots (100 m^2) for application of treatments. Applications began 7 September 2001 in Macagual and will begin 15 days after the start of the next wet season in Piedechinche, predicted to be around the third week of September. Treatments are summarized in Tables 2.6 and 2.7 and are in a completely randomized block design with 5 repetitions. The isolates CIAT 054 (*Metarhizium* sp.) and CIAT 007C (*Metarhizium anisopliae*) were selected from among 49 isolates as the most virulent to adult *A. varia*.

Weekly population surveys are being carried out before the treatments to establish baseline data that verifies and gauges presence of the insect, and for six months after application to measure treatment effect. Surveys consisted of nymph counts in two 0.0625 m^2 quadrats and adult counts in two series of 10 sweeps with an insect net. In the laboratory nymphs were determined to

instar and adults to species and sex. Natural enemies were also collected as part of the spittle mass and sweep net surveys. Pretreatment surveys began 25 January 2001 in Piedechinche and 23 March 2001 in Macagual. The start of the experiment in Macagual was postponed due to a long delay in delivery of the formulated product based on technical difficulties related to production by our commercial collaborator BioCaribe, S.A. The start of the experiment in Piedechinche depends on start of the wet season.

To gauge field mortality due to entomopathogens, 5 adults will be collected from each plot repetition with a sweep net and confined to petri dishes lined with moist filter paper to assess mycosis after 5 days.

Table 2.6. Field treatments applied in Macagual, Dept. Caquetá.

Product	No. Applications per Month for Six Months
Entomopathogen (CIAT 054)	0.5
	1
	2
Entomopathogen (CIAT 007C)	0.5
	1
	2
Insecticide (Malathion)	0.5
	1
	2
Control	0

Table 2.7. Field treatments applied in Piedechinche, Dept. Valle del Cauca.

Product	Weeks after Appearance of First Generation Outbreak
Entomopathogen (CIAT 054)	1
	2
	3
	4
	5
	6
Insecticide (Malathion)	1
	2
	3
	4
	5
	6
Control	0

Treatment effects on spittlebug nymph and adult populations will be tested by measuring insect load, or the number of insects under the population fluctuation curves, using cumulative insect days analysis.

Results

As confirmed in previous studies, three species occur in Macagual: *Aeneolamia varia*, *Zulia pubescens* and *Mahanarva* sp. nov. Of 1207 adults collected to date, 97.0% were *A. varia*, 2.6% *Z. pubescens* and 0.4% *Mahanarva* sp. nov. at overall mean relative densities of 2.87, 0.07 and 0.01 adults/10 sweeps, respectively. Over this same period a total of 795 nymphs and 8 adult tenerals were collected **(Table 2.8)**.

Table 2.8. Number of nymphs per life stage and teneral adults collected in spittle mass surveys at two contrasting field evaluation sites.

Instar	Macagual	Piedechinche
I	151	10
II	190	25
III	167	52
IV	114	31
Va	113	29
Vb	60	38
Tenerals	8	5
Total	803	190

In Piedechinche, only *Prosapia simulans* (1465 adults) has been detected to date, although previous populations surveys before the start of this experiment detected the presence of *Zulia carbonaria* and *Z. pubescens* at lower abundance. Phenological analysis of these data is summarized elsewhere **(see Characterization and formulation of select fungal entomopathogen isolates for field evaluation – Pag. 84)**.

Discussion

Initial population data confirm that the selected sites have sufficient populations of nymph and adult spittlebugs for this field experiment. Application of treatments has only just begun in Macagual and will begin in Piedechinche at the start of the next rainy season.

Activity 3. Information and technology transfer for spittlebug management in graminoids

Guatemala Workshop on the Bioecology and Management of Spittlebugs in Graminoid Crops

Despite the impact of spittlebugs in forage grasses, sugar cane and other graminoid crops in the New World, there is little expertise on their biology and management outside of CIAT and EMBRAPA. Access to information is also extremely limited because there is no text that summarizes our knowledge of the family Cercopidae and existing guides to grassland spittlebugs are outdated, imprecise and ignore family level bioecology **(see Reference collection and on-line bibliography of the Cercopoidea – Pag. 90)**. To partially fill this gap, five workshops on the Bioecology and Management of Grassland Spittlebugs have been carried out from 1997 to 2001, three in CIAT, one in Ecuador and one in Guatemala.

The fifth workshop took place 13-17 August, 2001 at CENGICAÑA (Centro Guatemalteco de Investigación y Capacitación de la Caña de Azúcar), Santa Lucía Cotzumalguapa, Guatemala, sponsored by ATAGUA (Asociación de Técnicos Azucareros de Guatemala) and CAÑAMIP (Comité de Manejo Integrado de Plagas de la Caña de Azúcar). Unlike past events, the main interest of this group was information regarding spittlebugs pests in sugar cane. In Guatemala, spittlebugs are considered the most damaging pest in this crop. There are proposed IPM programs that achieve relatively good control largely through cultural techniques and biological control based in fungal entomopathogens.

The event was attended by 20 agronomists and entomologists representing the major sugar cane farms in southern Guatemala, the Ministry of Agriculture and Ranching, and CENICAÑA. The workshop was five days of intensive lectures, labs and discussions to provide a theoretical and practical foundation on spittlebugs as insects so that they can be better interpreted as pests. A 150-page manual with supporting information and notes was prepared for each participant as well as a compilation of 34 relevant articles.

Reference collection and on-line bibliography of the Cercopoidea

Introduction

A major limitation to advances in the management of spittlebugs in forage grasses and sugar cane is difficult access to information. First, there are no published reviews of the insect family Cercopidae or the superfamily Cercopoidea despite their economic significance in cultivated graminoids such as forage grasses and sugar cane. Such material exists for other groups of economically important Homoptera such as the leafhoppers, planthoppers, aphids, scales and whiteflies, but students of the spittlebugs and froghoppers must turn to articles and gray literature to acquire an understanding of this group of insects. Second, reviews of the biology and management of spittlebugs are inadequate. The few that exist are not widely disseminated, are outdated, and contain overgeneralizations and erroneous information, particularly regarding taxonomy. Third, much of the available information is in gray literature sources that are difficult to access. The quality of research from small and isolated universities or research teams is

challenged by not being able to acquire the information necessary to support studies on this pest group.

Materials and Methods

To start to overcome some of the limitations in information dissemination, we are strengthening our reference collection on the Cercopoidea. References have been gathered over the last 10 years. In 2001 we began working with CIAT Information Services to make this information source available on-line.

Results

At present, we have physical copies of 675 references related to the superfamily Cercopoidea. Of these, 468 are directly related to spittlebugs in graminoids, 320 related to forage grasses, 145 related to sugar cane and 23 related to other graminoids such as rice and turfgrass. At present, all references are housed alphabetically in filing cabinets of the Spittlebug Bioecology and IPM Research Group.

All citations are entered into an electronic database (EndNote). This bibliography has been printed and deposited in the CIAT library. Key words were assigned to each citation to facilitate searching from within the program software **(Table 3.1)**. Categorical labels were also assigned to facilitate subgrouping of references in the initial on-line database **(Table 3.2)**.

For the on-line interface, references were converted from EndNote to ProCite. The initial version is a rigid (non-searchable) database divisible into categories with relevant references listed alphabetically. This version will probably be available on the CIAT web page by the end of the year.

Discussion

The reference collection and on-line bibliography will be further improved in the following steps: (1) continual acquisition of new references with a focus on neotropical spittlebugs in graminoids, (2) continual updating of the electronic database, (3) housing physical references in the CIAT library, (4) adding information to the on-line site on how to order copies of references from the CIAT library, and (5) making the on-line database completely searchable by author, category and key words.

Table 3.1. Key words assigned to references in the Cercopoidea bibliography for key-word search in EndNote.

Region	Crop	Management	Habitat	Biology	Classification
Africa	Alfalfa	Ants	Biogeography	Aggregations	Aphrophoridae
Asia	Arachis	Biocontrol	Cover crops	Aposematism	Cercopidae
Australia	Beans	Burning	Dispersion	Bioacoustic behavior	Cercopoidea
India	Cacao	Cultural control	Distribution	Color polymorphism	Clastopteridae
Indonesia	Cassava	Cutting	Endophytes	Comparative phenology	Machaerotidae
New Zealand	Centrosema	Disease transmission	Habitat selection	Copulation	Pipunculidae
Canada	Citrus	Disturbance	Host plant selection	Defense	Procercopidae
Caribbean	Coffee	Economic impact	Host plants	Diapause	Taxonomy
Central America	Conifers	Economic threshold	Litter arthropods	Egg development	
Costa Rica	Cowpea	Entomopathogens	Original habitat	Fecundity	
Panama	Fruit trees	Fertilization	Pasture management	Feeding strategies	
Europe	Grasslands	Grazing	Plant architecture	Life table	
Mexico	Maize	Herbivore competition	Plant quality	Lights	
South America	Marijuana	IPM	Rainfall	Longevity	
Brazil	Millet	Marking	Vegetational diversity	Morphology	
Colombia	Oil palm	Mites		Movement	
Ecuador	Pecans	Natural enemies		Nymph development	
Peru	Rice	Nematodes		Oogenesis-flight syndrome	
Venezuela	Sorghum	Pasture assessment		Oviposition	
U.K.	Strawberry	Pasture pests		Preference-performance	
U.S.	Stylosanthes	Pesticides		Pheromones	
	Sugar cane	Phytotoxemia		Population dynamics	
	Turfgrass	Plant impact		Protandry	
		Plant resistance		Reflex bleeding	
		Rearing		Reproduction	
		Salpingogaster		Spittle mass	
		Sampling		Stadia	
		Spiders		Tenerals	
		Trampling			

Table 3.2. Codes assigned to references in the Cercopoidea bibliography for subdivision of references in rigid (non-searchable) on-line database.

A01 - Cercopids in graminoids
A02 - Other Cercopoidea
B00 - Bioecology
- B01 - Behavior
- B02 - Biology
- B03 - Diapause
- B04 - Ecology
- B05 - Population dynamics
- B06 - Taxonomy & Systematics

C00 - Management
- C01- Biological control & Natural enemies
- C02 - Chemical control
- C03 - Cultural control
- C04 - Host plant resistance
- C05 - Impact
- C06 - Integrated pest management
- C07 - Rearing
- C08 - Sampling & Monitoring

D00 - Host plants
- D01 - Forage grasses
- D02 - Other grasses
- D03 - Sugar cane
- D04 - Non-graminoid host

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Rodriguez, J. 2001. Biología comparada de tres especies de salivazo del género *Zulia* (Homoptera: Cercopidae) [Universidad del Tolima, Ibague, Colombia]. 99 pp.

Donors

Colombia – PRONATTA
Fungal entomopathogens as an alternative for spittlebug management (2000-2002).
Interpretation and prediction of spittlebug phenology (2000-2002).

Collaborators

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Sub-output 5. Disease Complexes Described, Characterized and Analyzed.
(E. Alvarez)

Activity 1. Molecular identification of *Phytophthora* species from different host plants

Introduction

Amplified fragment length polymorphism (AFLP) a molecular fingerprinting method proved their utility in this preliminary study of 12 *Phytophthora* species were differentiated based on the amplification of polymorphic fragments using combinations from primers sequence complementary to the *Eco*RI end of the DNA template, E + AC (5'-GACTGCGTACCAAT TCAC-3'), was used in combination with the *Mse*I primer M + C (5'-GATGAGTCCTGAGT AAC-3'). Another *Eco*RI primer, E + AC (5'-GACTGCGTACCAATTCAC-3') was used combination with the *Mse*I primer, M + A (5'-GATGAGTCCTGAGTAAA-3'). For each primer, a subset of accessions was re-run through the whole process (DNA extraction, digestion - ligation, amplification, and band scoring) **(Figure 1.1).**

AFLP fragment analysis of 12 Phytophthora species. AFLP patterns were complex and consisted of multiple unique polymorphic amplicons for each species. AFLP proved useful in discrimination Phytophthora, species and between isolates of *Phytophthora tropicalis*, based on amplicon banding patterns. DNA Sequence analysis of PCR amplification, product and confirmed the reliability of this method **(Figure 1.2).**

Comparison between Dice similarity index and multiple correspondence analysis, showed that the results were similar. In multiple correspondence analysis **(Figure 1.3)**, the three dimensions show differences between the group, grouping clearly Phytophthora isolates and differentiating possible species.

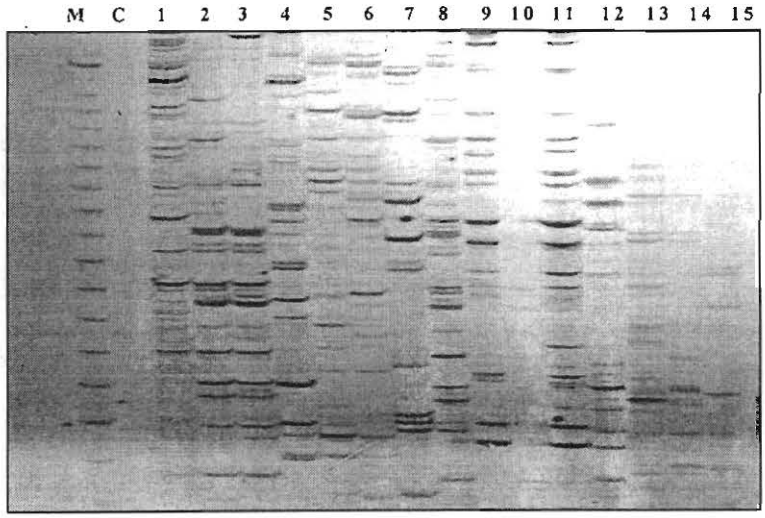

Figure 1.1. Illustration of the principle of EcoRI - MseI fragments. AFLP fingerprints are shown of *Phytophthora spp.* DNA using primer combinations with a single selective base for the EcoRI primers and two selective bases for the MseI primer, refer to the primer combinations EAC/MA.

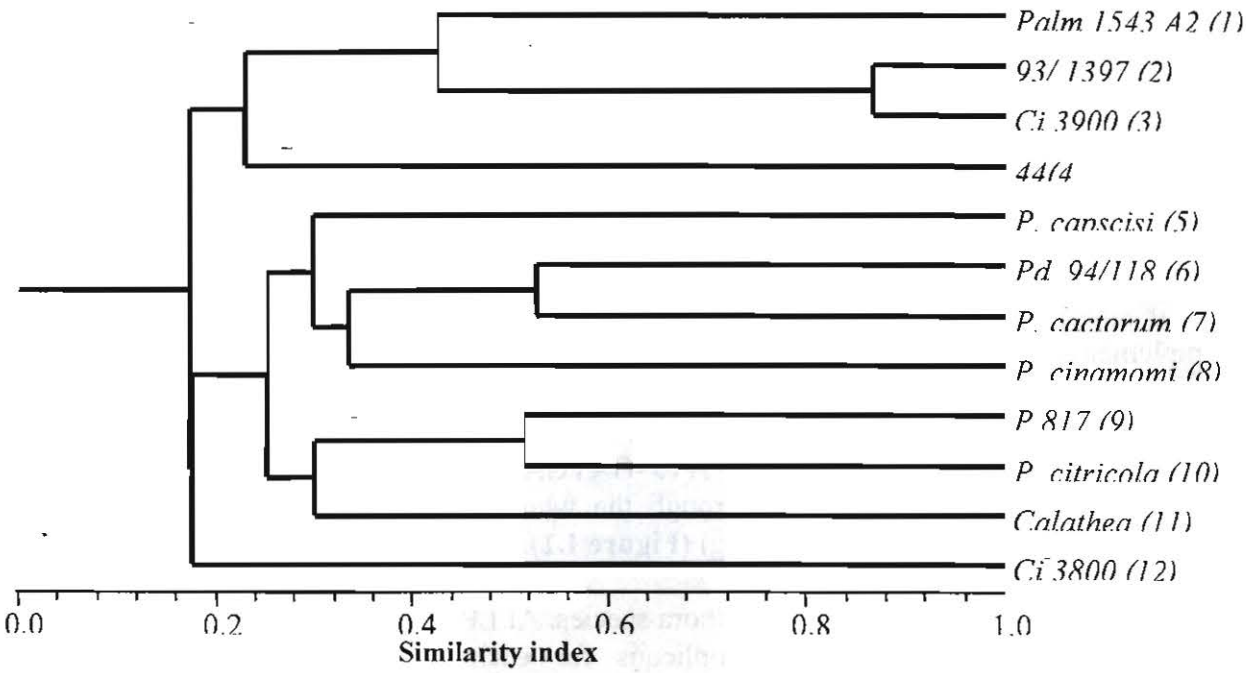

Figure 1.2. **Similarity dendrogram of 12 isolates of *Phytophthora*, based on Amplified Restriction Fragment Length Polymorphism. Phenogram was constructed using the UPGMA method and DICE index, of the NTSYS-pc package.**

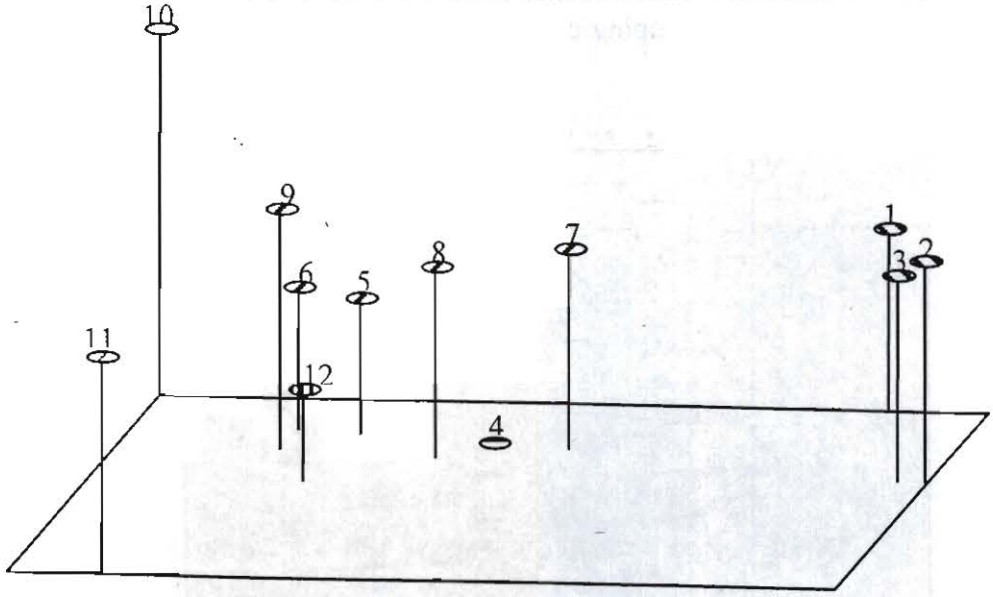

Figure 1.3. **Multiple Correspondence Analysis of 12 isolates of *Phytophthora*, based on Amplified Restriction Fragment Polymorphism. The graph was constructed using of the NTSYS-pc package.**

PCR-RFLP restriction patterns of rDNA

Amplification of the internal transcribed spacer (ITS) region of the rDNA was obtained with template DNA from the isolates, using extracted DNA. The amplified product for the ITS region of all species was about 900 bp. Restriction digestion with *Alu*I, *Msp*I, and *Taq*I of the product amplified for the ITS region showed different restriction patterns, which corresponded to the species tested. In this study, 8 *Phytophthora* species, obtained from different crops **(Table 1.1)**, were identified by molecular techniques, based on ITS rDNA sequences **(Figure 1.4)**.

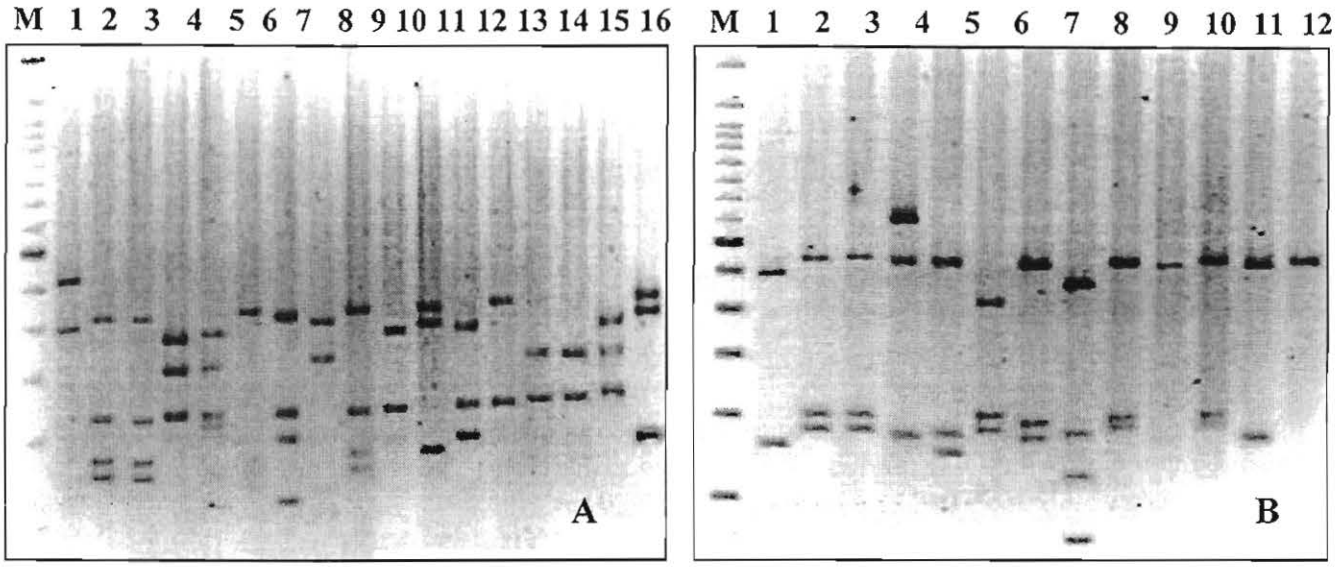

Figure 1.4. **RFLP profiles from different *Phytophthora* species isolates using the enzymes *Msp*I (A), *Alu*I (B). Lane M= 100bp ladder (Gibco BRL) marker; lanes 1-17 (A) and 1-13 (B) *Phytophthora* isolates.**

Table 1.1. **Identification of *Phytophthora* isolates obtained from different crops, based on restriction banding patterns with the enzymes *Alu*I, *Msp*I and *Taq*I.**

Isolate No.	Specie	Host
1	*Phytophthora palmivora*	*Manihot esculenta*
2	*Phytophthora cinnamomi*	Calluna
3	*Phytophthora cinnamomi*	Calluna
5	*Phytophthora capsici*	*Capsicum annuum*
6	*Phytophthora megasperma*	*Rubus ideaw*
7	*Phytophthora cactorum*	Fragaria
8	*Phytophthora lateralis*	
9	*Phytophthora cinnamomi*	Calluna
11	*Phytophthora cinnamomi*	Calluna
12	*Phytophthora citricola*	
17	*Phytophthora vignae*	
	Phytophthora melonis	
	Phytophthora sinensis	

Molecular Phylogeny Analysis of Phytophthora

Phytophthora is a major genus of plant pathogens responsible for serious disease of economically important crops. In traditional taxonomy Phytophthora were differentiated on the structure of the sporangium, the form of the antheridium and on whether the taxon is inbreeding (homothallic) or outbreeding (hetherothallic).

In this study, we present a ITS – based phylogenetic analysis of 14 described taxa, of Phytophthora, a diverse group of Pythium. The ITS information will strengthen and extend current PCR – based diagnostic, detection and identification of Phytophthora species.

Materials and Methods

The ITS regions of 14 Phytophthora isolates have been sequenced in this study (**Table 1.1**). Isolates were store on corn meal agar rDNA amplification and sequencing. Isolates were grown in classified V-8 medium.

DNA was amplified using the universal primers ITS 6 and ITS 4, amplification products were purified by QIA quick PCR purification kit protocol direct sequencing of PCR products was initiated using primers ITS4 and ITS5.

PCR always resulted in a single band of 900 pb when using the set of primers ITS 6 and ITS 4. Despite the fact that the PCR products were sequenced directly. The phylogram was based on the complete ITS1, 5.8S and ITS2 sequences.

These trimmed sequence, along with ITS sequences of related species in the database Gen Bank, were included in the analysis. From this analysis single isolates representative of each taxon were selected for further detailed phylogenetic analyses. In the case of *Pythium*, the selected taxa represented much of the diversity within the genus, as revealed by a comparison of our ITS sequences with those previously published.

Phylogenetic Analysis

Computer assisted sequence alignments revealed that the ITS sequences from Phytophthora isolates, were identical to from those of related species in the database those Gen Bank.

These trimmed sequence, along with ITS sequences of related species in the database Gen Bank, were included in the analysis. From this analysis single isolates representative of each taxon were selected for further detailed phylogenetic analyses. In the case of *Pythium*, the selected taxa represented much of the diversity within the genus, as revealed by a comparison of our ITS sequences with those previously published.

ITS sequence data is in good accord with previous analyses of *Phytophthora* subgroups using other molecular criteria. Bootstrap analysis corroborate the results obtained with other molecular markers such as RFLPs and AFLPs (**Figures 1.1, 1.4 and 1.5**).

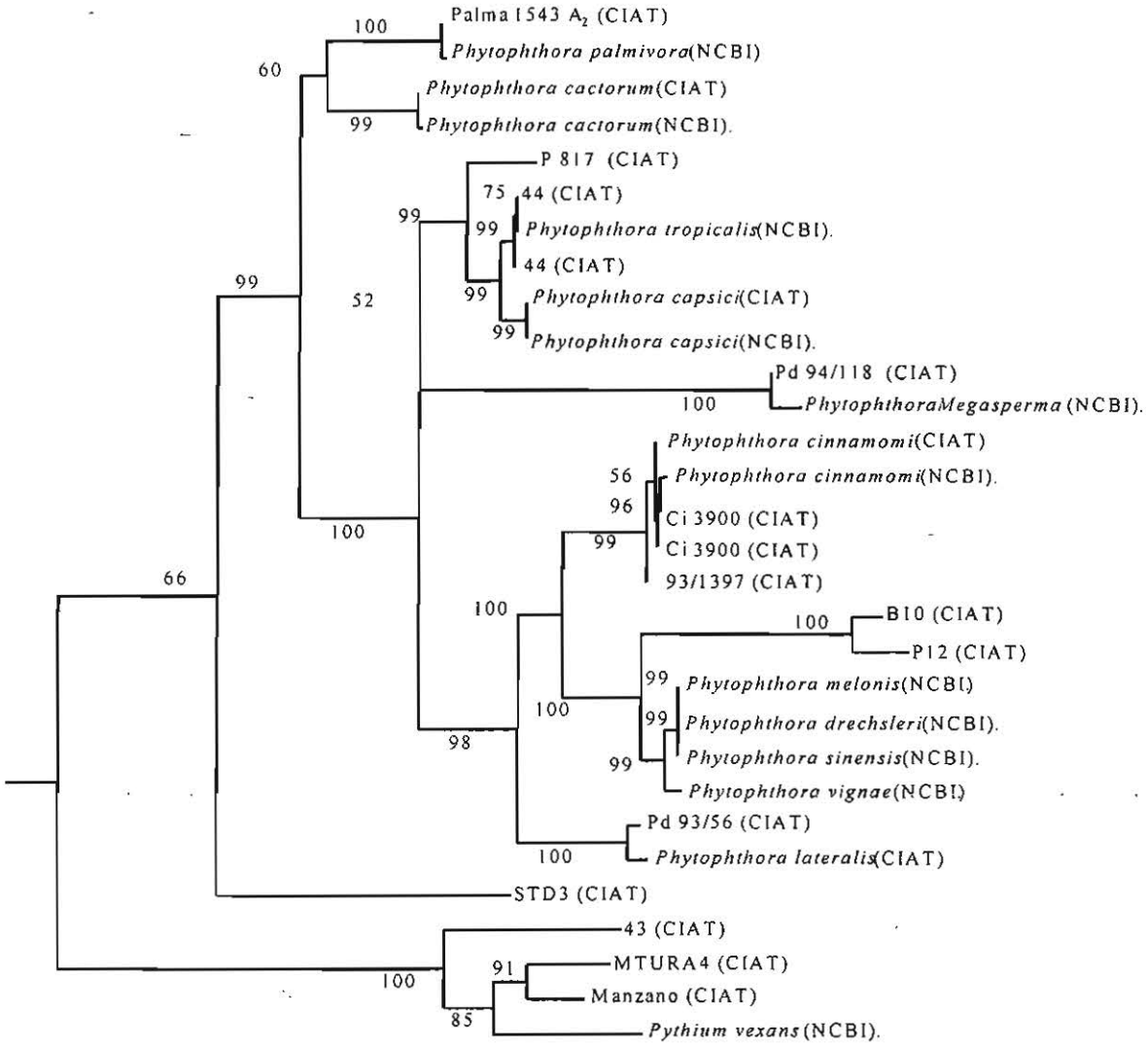

Figure 1.5. **Detailed phylogram of 14 Phytophthora taxa. The phylogram was constructed after DNA distance based analysis of the combined ITS1, 5.8S subunit, and ITS2 regions of the genomic ribosomal RNA tandem gene repeat. The numbers at the branch points indicate the percentages of bootstrap values (based on 1000 bootstraps).**

Activity 2. Assessing virulence and genetic variability of *Sphaceloma manihoticola*, causal agent of superelongation in cassava (*Manihot esculenta*), in Brazil and Colombia, using RAMS and AFLP

Introduction

Sphaceloma manihoticola is the causal agent of superelongation, one of the diseases that most limits cassava production. Studies have demonstrated pathogenic and genetic variability between isolates of Brazil and Colombia using RFLP and RAPD. The level of polymorphism can be visualized more precisely, however, with molecular markers such as amplified fragment length polymorphisms (AFLP) and random amplified microsatellites (RAMS). This study aimed to develop efficient DNA fingerprinting protocols for *S. manihoticola* and to use RAMS and AFLP markers for analyzing the genetic variability in single-spore cultures of *S. manihoticola*.

Materials and Methods

Isolates. Twenty-five isolates were collected from cassava plants infected with superelongation at different sites in central-south Brazil and Colombia. Isolates were conserved at 4 °C in inclined vials containing natural PDA. All isolates were pathogenic on cassava genotypes M BRA 12 and M BRA 703.

Virulence. Variation in virulence was determined by inoculating wounded plantlets of 15 cassava genotype differentials. Inoculum consisted of six selected isolates from different regions of Brazil. Inoculated plantlets were incubated for 5 days at 95% relative humidity and 27 °C, then transferred to the greenhouse and observed for symptom development at 7, 14, 21, and 28 days after inoculation. A split-plot experiment design with five replicates was used, where the main plots were the varieties and the subplots, the isolates. Each experimental unit had one cassava plants.

DNA Extraction. Isolates were placed in a liquid medium (obtained by filtering V8 juice), and then incubated under constant agitation for 15 days at 25 °C. Colonies were harvested according to a modified Lee and Taylor protocol, in which 400 ml of a solution of phenol, chloroform, and isoamyl alcohol (25:24:1) were used to precipitate the DNA, which was then centrifuged at 10,000 rpm for 15 min. The pellet was resuspended in 100 ml of Tris Base EDTA and incubated with 10 ml of ribonuclease (10 mg ml^{-1}) at 37 °C for 30 min. The DNA concentration was determined with a fluorometer (Hoefer DyNA Quant 200).

RAMS. The reaction was performed in a final volume of 25 µl that contained 0.2 mM of each nucleotide; 3 mM $MgCl_2$; 0.5 µM of each of the primers, 1X PCR buffer; and 0.625 U the enzyme *Taq* polymerase. Everything was diluted in sterilized distilled water. The PCR reactions were carried out under the following conditions: samples were denatured by 10 min incubation at 95 °C, after which 37 cycles of amplification were carried out (30 s denaturation at 95 °C, 45 s annealing at a temperature depending on the primer, and 2 min primer extension at 72 °C). The annealing temperature for each primer was as follows: CCA primer = 64 °C, CGA primer = 61 °C, GT primer = 58 °C, and ACA primer = 45 °C (Hantula *et al*, 1997). After the cycles, the

reaction was ended with a 7-min extension at 72 °C. The electrophoresis chamber was maintained at a constant 90 V for 90 min.

AFLP. Isolates were evaluated using three combinations of primers: EAC/MA, EAC/MC and EG/MA (Gibco BRL, AFLP Analysis System for Microorganisms). The amplification products were mixed at equal volume, denatured at 95 °C, and 6 µl loaded into a 6% (w/v) polyacrylamide gel in 1X TBE electrophoresis buffer.

Data Analysis. Isolates were clustered into groups according to area under disease progress curve (AUDPC) for the 15 inoculated varieties, using the Ward minimum variance analysis (SAS Statistical Package, Version 6.0. Cary, North Carolina). To estimate genetic relationships between isolates, a phylogenetic tree was constructed, using the unweighed paired grouped mean arithmetic average method with the SAHN and TREE options of the NTSYS-pc 2.01 (F.J. Rohlf, Exeter Software, New York).

Results

Twenty-five single-spore cultures of *S. manihoticola* from central-south Brazil and NE Colombia were used to study the variation in virulence and genetics of the fungus.

Virulence. Variation in virulence was determined in the greenhouse by inoculating cassava plantlets of 15 cassava genotype differentials. A high variation in virulence was observed among the six Brazilian isolates because these were grouped into 5 pathotypes **(Figure 2.1)**.

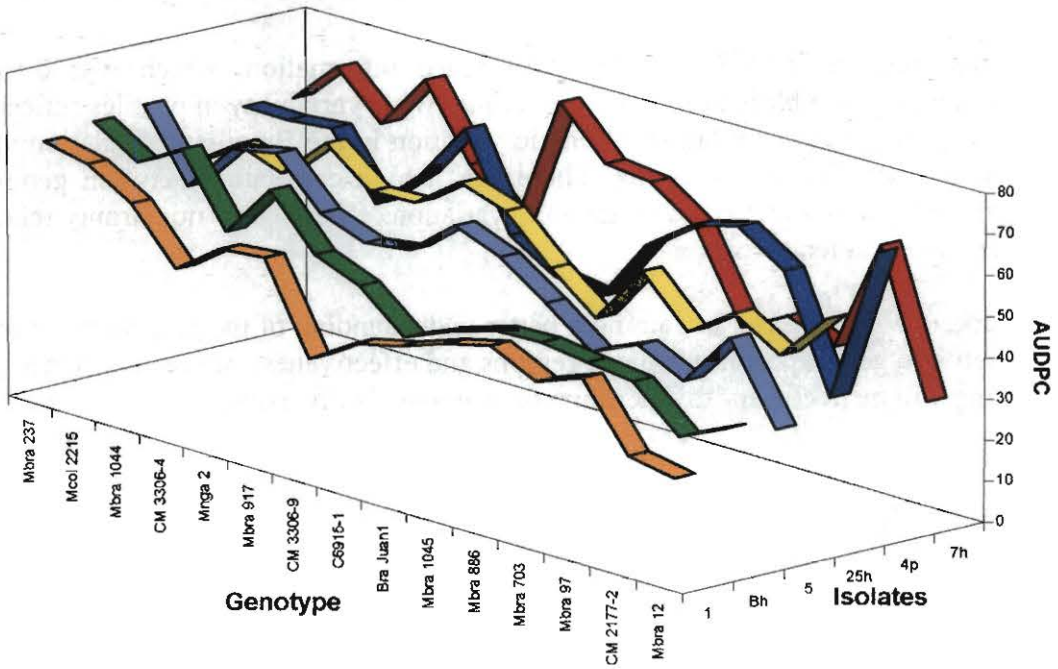

Figure 2.1. Identification and virulence phenotypes of six isolates of *Sphaceloma manihoticola*, causal agent of superelongation diseases in cassava, used to inoculate 15 cassava genotypes in the greenhouse.

Five cassava genotypes were identified as differential varieties. Isolate 4p was found to infect 12 of the 15 genotypes and was considered as the most virulent. Isolate Bh was the least virulent, infecting only five differentials. Of the cassava genotypes, M Bra 1044, M Bra 237, and M Col 2215 were ranked as highly susceptible, the most susceptible being M Bra 237. The most tolerant genotype was CM 2177-2.

Genetic Variability. AFLP (three primer combinations) and RAMS (four primers) techniques were optimized for *S. manihoticola* and reproducible bands were observed in most isolates **(Figures 2.2, 2.3, 2.5)**. The results obtained demonstrate polymorphism among isolates, indicating the pathogen's genetic variability. Polymorphism with these single primers differentiated the isolates, and five genetic groups were distinguished among the isolates. Results are shown in **Figure 2.2**, where the patterns obtained with the ACA primer are presented. Similar results were obtained with other primers **(Figure 2.3)**. Based on RAMS and AFLP, Brazilian isolates were not found in Colombia and vice versa. The results suggest all sample sites have their own genetic group of *S. manihoticola*. The correlation observed between geographical origin and polymorphism detected by RAMS and AFLP was +0.83. The dendrogram constructed by both molecular techniques evidences the separation of isolates into five groups with a similarity level of 0.7 **(Figure 2.4)**.

Conclusions

RAMS and AFLP were tested and optimum conditions for PCR amplification were resolved. Both techniques, originally described by Hantula et al. (1997) and Vos et al. (1995), can be applied to *S. manihoticola*.

The results obtained by RAMS and AFLP provided information, which was difficult or impossible to obtain on the basis of morphology alone. Wide variability in profiles reflected high polymorphism in the fungal populations. Genetic variation in the Brazilian population was very limited compared with that of Colombia. There was a high correlation between geographical origin (country and municipality) and genetic variation. There was no strong relationship between genetic and virulence markers.

Molecular markers will be useful in gaining a better understanding of the movement of pathogen populations between geographically isolated regions and effectiveness of host resistance. Further isolate sampling will be necessary to determine the pathogen's diversity.

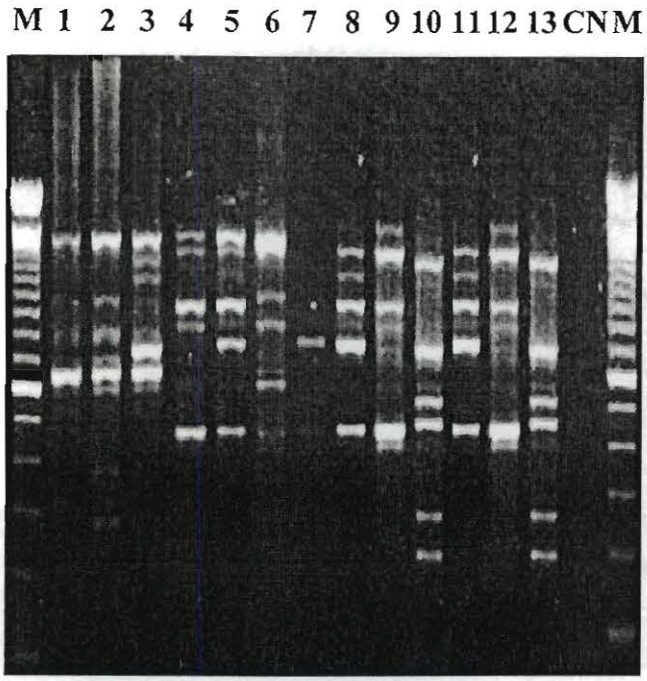

Figure 2.2.

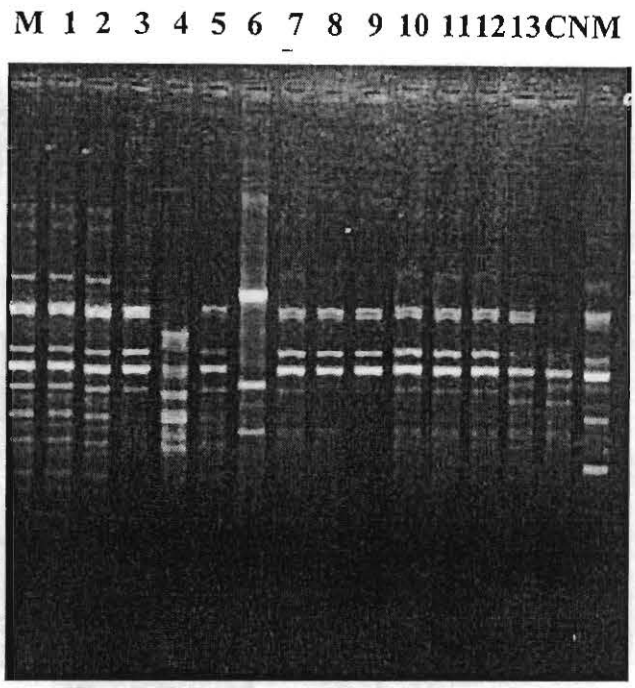

Figure 2.3.

Figure 2.2. Patterns of bands obtained with the RAMS technique using ACA primer. Isolates were collected from (1 to 7) Campos Novos, Paranavaí, Conchal, and Platina, Brazil, and (8 to 13) Puerto López, Granada, and Carimagua, Colombia. CN = Negative Control; M = marker (100 bp).

Figure 2.3. RAMs profiles observed within the isolates of *Sphaceloma manihoticola* with CCA primer Isolates were collected from (1 to 7) Campos Novos, Paranavaí, Conchal, and Platina, Brazil, and (8 to 16) Puerto López, Granada, and Carimagua, Colombia. CN = Negative Control; M = marker (100pb).

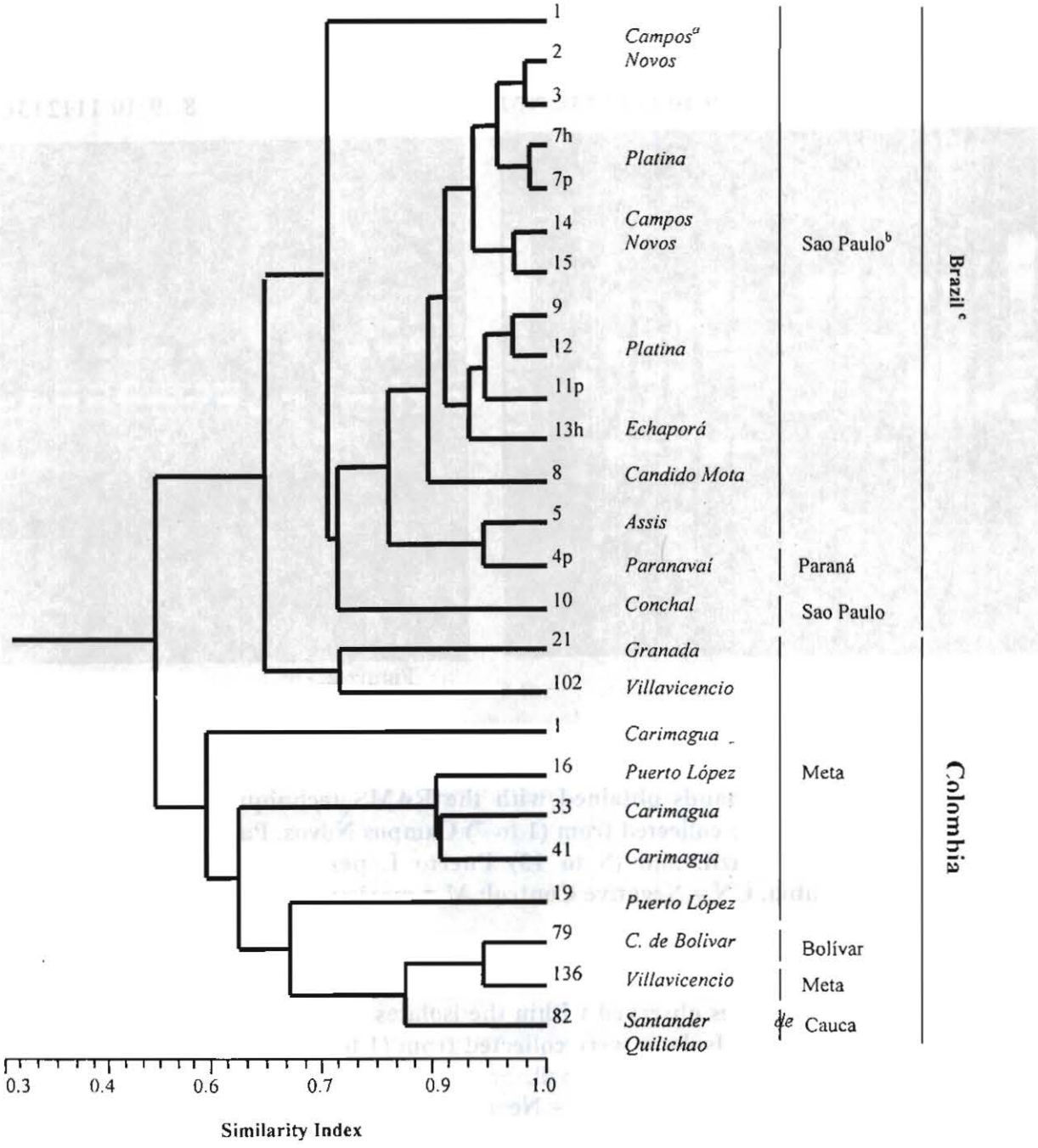

Figure 2.4. **Similarity dendrogram (AFLP-RAMS) of 25 isolates of *Sphaceloma manihoticola*, a fungus that causes superelongation disease in cassava (*Manihot esculenta* Crantz). Bra (Brazil), Col (Colombia). [a] municipality, [b] Department, [c] Country.**

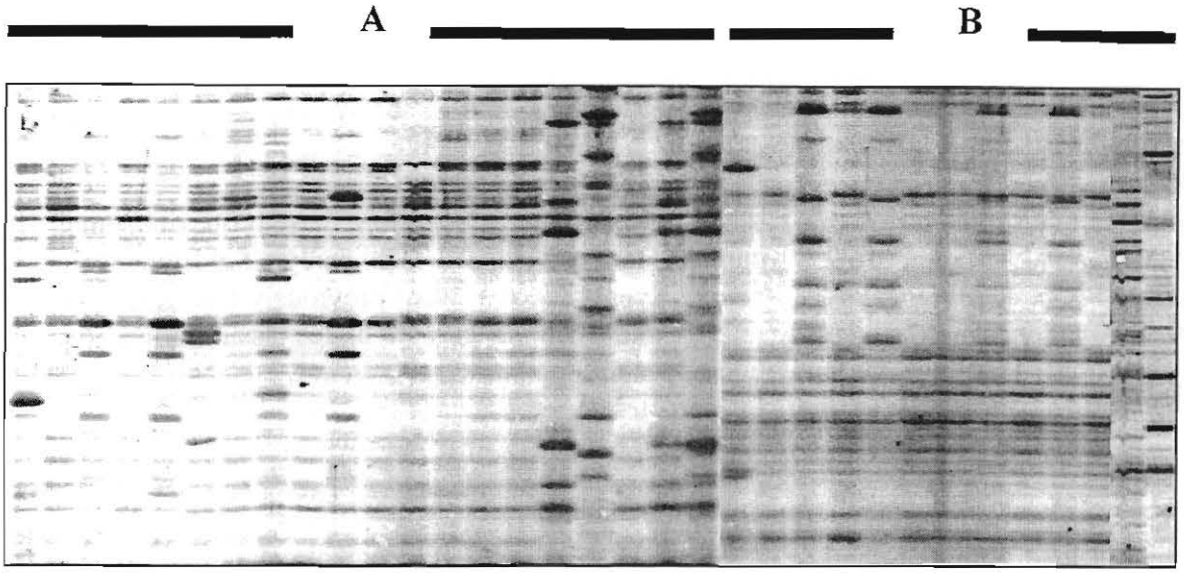

Figure 2.5. Illustration of *Eco*RI-*Mse*I fragments. AFLP fingerprints of *Sphaceloma manihoticola* DNA were made, using primer combinations with a single selective base for the *Eco*RI primers and two selective bases for the *Mse*I primer. Panels A and B refer to the primer combinations EAC/MA and EAC/MC, respectively.

References

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Lee SB; Taylor JW. 1990. Isolation of DNA from fungal mycelia and single spores. In: PCR protocols. p 282-287.

Vos P; Hogers R; Bleeker M; Reijans M; Van de Lee T; Pornes M; Frijters A; Pot J; Peleman J; Kuiper M; Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23(21):4407-4414.

Activity 3. Characterization of yeast strains using molecular markers (PCR-RFLP and RAPD)

Yeast are true fungi of the phylum *Ascomycetes,* class *Hemiascomycetes*. The classification of yeast is a specialized field using cell, ascospore, and colony characteristics for distinguishing genera, and physiological characteristics, particularly the ability to ferment individual sugars to identify species (Jones et al., 1992).

The best-known and commercially significant yeasts are the related species and strains of *Saccharomyces cerevisiae*. These organisms have long been utilized to ferment the sugars of rice, wheat, barley, and maize to produce beverages, and in the baking industry to expand or raise dough (Broach et al., 1993).

A simple and rapid method for yeast strains' characterization, based on ribosomal DNA restriction analysis and RAPD markers, was used in this study. Selected isolates of *Saccharomyces cereviciae*, as well as other yeast species, were previously classified by several physiological and biochemical characteristics such as carbohydrates assimilation, invertasic activity, copper tolerance, UV radiation exposure, temperature tolerance, etc. The purpose of this work was to use molecular markers to characterize the strains.

Materials and methods

ITS. Amplification of the ITS region (ITS 1-2) and the 5.8S ribosomal gene of 14 selected yeast strains were obtained with extracted DNA. The PCR reactions were carried out under the following conditions: samples were denatured by 3 minutes at 95°C, after which 24 cycles of amplifications were performed (30 s denaturation at 95 °C, 30 s annealing at 57° C, and 2 min primer extension at 72°C).

PCR-RFLP. Eleven restriction enzymes were tested for polymorphisms among 14 selected yeast strains. The enzymes *Alu*I, *Cfo*I, *Eco*RI, *Hinf*I, *Msp*I, *Taq*I, *Hae*III, *Xba*I, *Dra*I, *Hind*III, and *Ase*I were used at 2 units per reaction and added to the PCR reaction mix (12.5 µl) after amplification. Digestion was allowed to proceed for 12 hours at 37 °C. The digested products of the amplified DNA were analyzed on 1.5% agarose gels in a TBE buffer system and detected by staining with ethidium bromide.

RAPD analysis was performed following the procedure described by Vezinhet et al., 1990.

Data analysis. A phylogenetic tree was generated using the unweighted pair grouping method of averages (UPGMA) with the SAHN and tree options of the NTSYS-pc 2.02 I statistical package (F.J. Rohlf, Exeter Software, New York).

Results

ITS. Amplification of the ITS region was obtained with all strains (**Figure 3.1).**

Figure 3.1. Amplification of the ITS region, using extracted DNA; lane M = 100bp marker, lanes 1-50 = strains of *Saccharomyces cereviciae*, lane 51 = *Candida albicans*, lane 52 = *Candida* sp., and lane 53 = *Rodotorula* sp.

Restriction patterns of rDNA. The amplified products were digested with restriction enzymes to differentiate species. The restriction patterns were obtained with 11 restriction enzymes—*Alu*I, *Cfo*I, *Hinf*I, *Eco*RI, *Msp*I, *Taq*I, *Hae*III, XbaI, *Dra*I, *Hind*III, and *Ase*I. Restriction of the rDNA yielded two bands depending on the enzyme and the strain (**Figure 3.2**). Four different restriction pattern types were recorded using the restriction enzymes *Alu*I, *Msp*I, *Cfo*I, *Hinf* I; and the patterns corresponded to *S. cereviciae*, *Candida albicans*, *Candida* sp., and *Rodotorula* sp. **Figure 3.2.** shows the *Hinf* I restriction patterns.

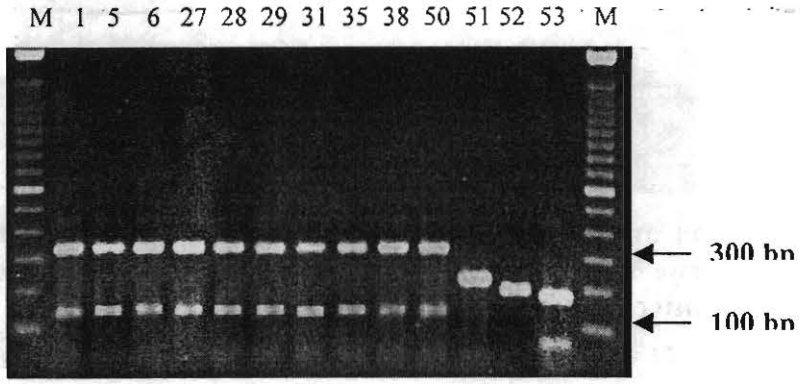

Figure 3.2. Restriction of the ITS amplified fragments in yeast species with *Hinf* I enzyme; lane M = 100 bp marker, lanes 1-50 = strains of *Saccharomyces cereviciae*, lane 51 = *Candida albicans*, lane 52 = *Candida* sp., and lane 53 = *Rodotorula* sp.

RAPD. A moderate degree of polymorphism was detected in the strains of yeast evaluated. Thirty-eight decaprimers were tested with 14 strains of yeast, of which the four most polymorphic, consistent, and reproducible banding patterns were used for statistical analysis. The primer OPC01 reflected the degree of polymorphism that was detected in the yeast species. **Figure 3.3.** shows the RAPD patterns of this primer. The intraspecific variation in *S.cereviciae* was detected with PPP1 and OPB04 primers (**Figures 3.4 –3.5**).

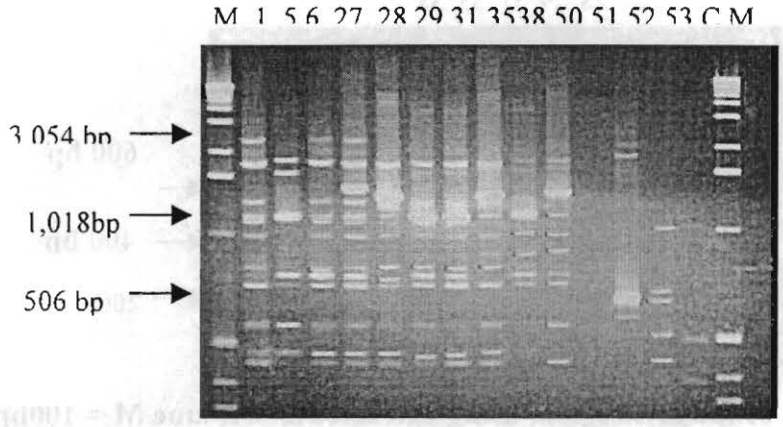

Figure 3.3. **RAPD patterns obtained when using the primer OPC 01; lane M = 1kb marker, lanes 1-50 = strains of *Saccharomyces cereviciae*, lane 51 = *Candida albicans*, lane 52 = *Candida* sp., lane 53 = *Rodotorula* sp., and lane C = negative control.**

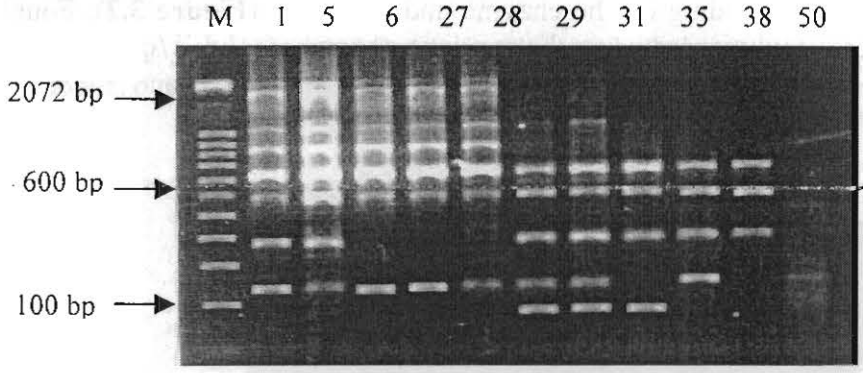

Figure 3.4. **Variation among strains of *Saccharomyces cereviciae* detected with primer PPP1; lane M = 100 bp marker, lanes 1-50 = strains of *S. cereviciae*, and lane C = negative control.**

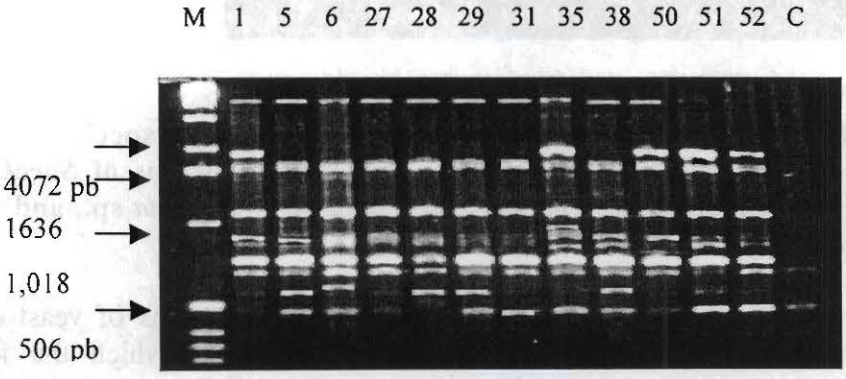

Figure 3.5. **RAPD patterns obtained when using the primer OPB04 with DNA from 12 yeast strains; lane M = 100 bp marker, lanes 1-50 = strains of *Saccharomyces cereviciae*, lane 51 = *S. boulardii*, and lane C = negative control.**

PCR amplifications followed by restriction digest provided means for differentiation of strains. RAPD analysis was also used to characterize yeast strains and some variation within species was seen. The cluster analysis at 0.55 of Dice similarity index grouped strains in four clusters designated RAPD groups G1 to G4—G1 = *C. albicans*, G2 = *Candida* sp., G3 = *Rodotorula* sp., G4a = *S. cereviciae* (strain 50), and G4b = *S. cereviciae* (strains 1, 6, 27, 28, 5, 29, 31, 38, 35) (**Figure 3.6**). Comparison of the results obtained with RAPD and PCR restriction assays showed that either method could be used to identify yeast strains. The results demonstrated that PCR could be used to identify yeast strains to the species level, and RAPD assay could be used to detect genetic variation within species.

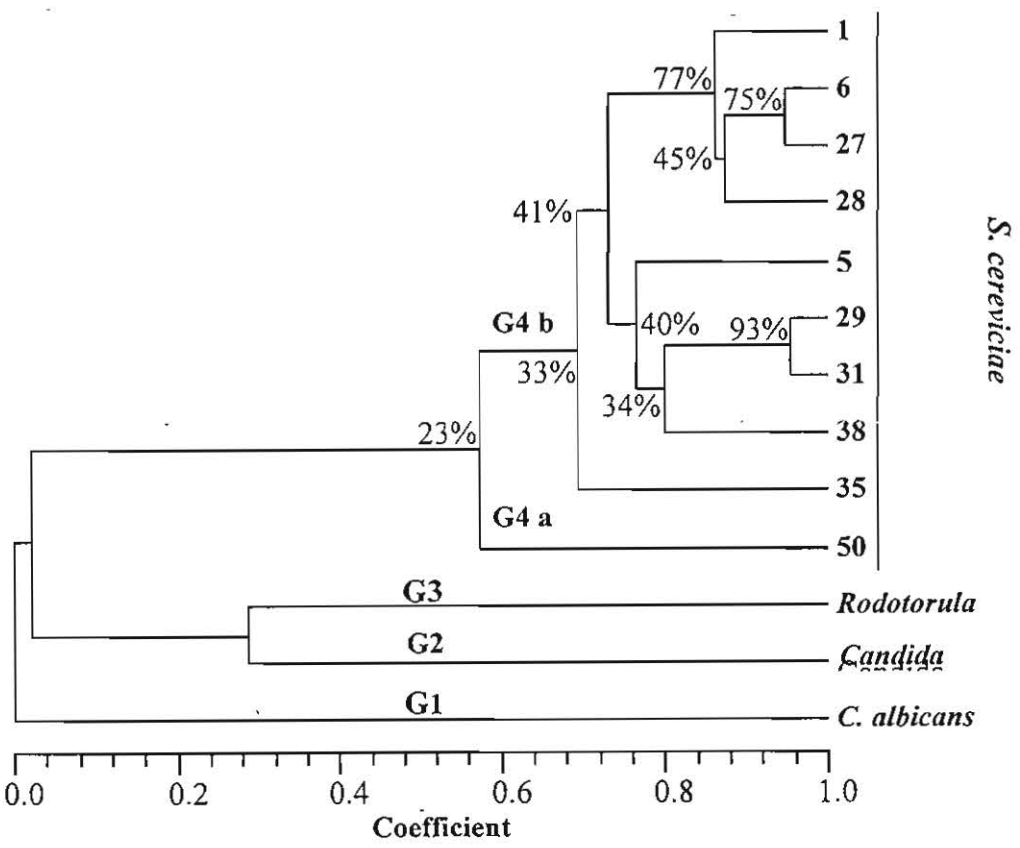

Figure 3.6. Phenogram from hierarchical cluster analysis of data and Bootstrap analysis. Clusters were fused using the unpaired group mean average (UPGMA). The similarity scale shown corresponds to the average similarity at which clusters fuse.

RAPD fragment analysis of 10 isolates of *S. cereviciae* consisted of unique polymorphic amplicons for each species. RAPD proved useful in discrimination of yeast species. Based on amplicon banding patterns, isolates 5 and 50 of *S. cereviciae* are different.

Comparison between Dice similarity index and multiple correspondence analysis showed very similar results. In multiple correspondence analysis (**Figures 3.7. and 3.8.**), the three dimensions show differences between four groups, grouping *S. cereviciae* isolates 5 and 50 as two different groups.

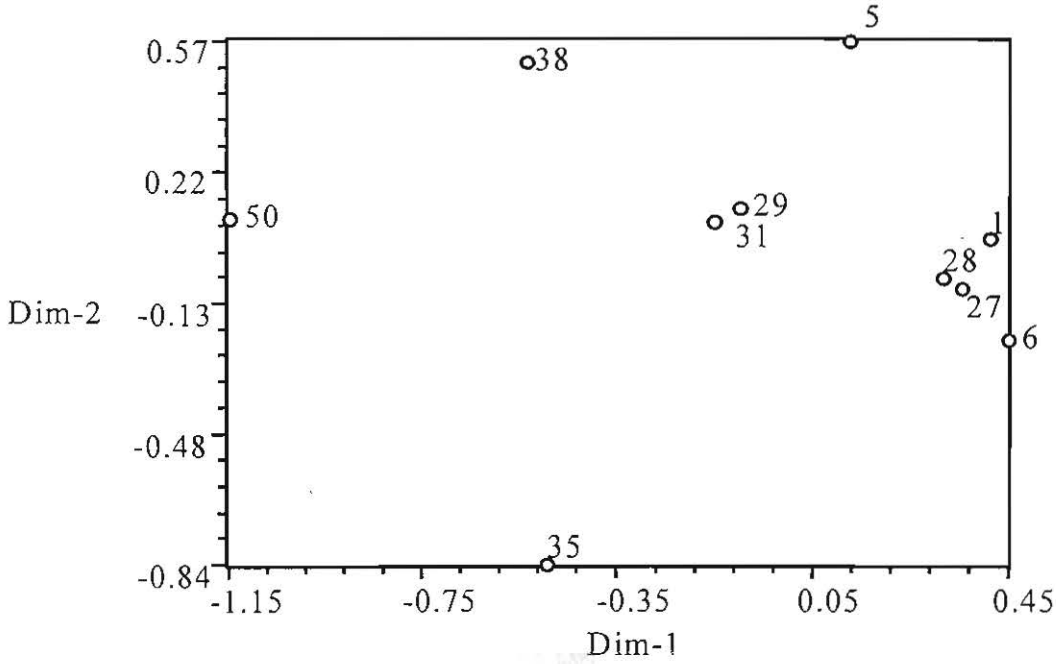

Figure 3.7. Multiple Correspondence Analysis in two dimensions of 10 isolates of *Saccharomyces. cereviciae*, based on Random Amplified Polymorphism. The graph was constructed using the NTSYS-pc package.

RFLP fragment analysis of 13 isolates of yeast separated the isolates into four groups that corresponded to the species tested. RFLP patterns consisted of multiple unique polymorphic amplicons for each species. RFLP proved useful in discrimination of yeast species. Using RAPD analysis, isolates of *S. cereviciae* were differentiated based on amplicon banding patterns. Isolates 5 and 50 were clearly separated from the other isolates evaluated, and formed different groups.

RAPD data agrees well with previous analyses of yeast subgroups using other molecular criteria (RFLPs). Bootstrap analysis corroborates the results obtained with RFLP markers. Bootstrap values were generally strong for all major branch points. Isolates 5 and 50 are clearly separated into two groups **(Figure 3.6.).**

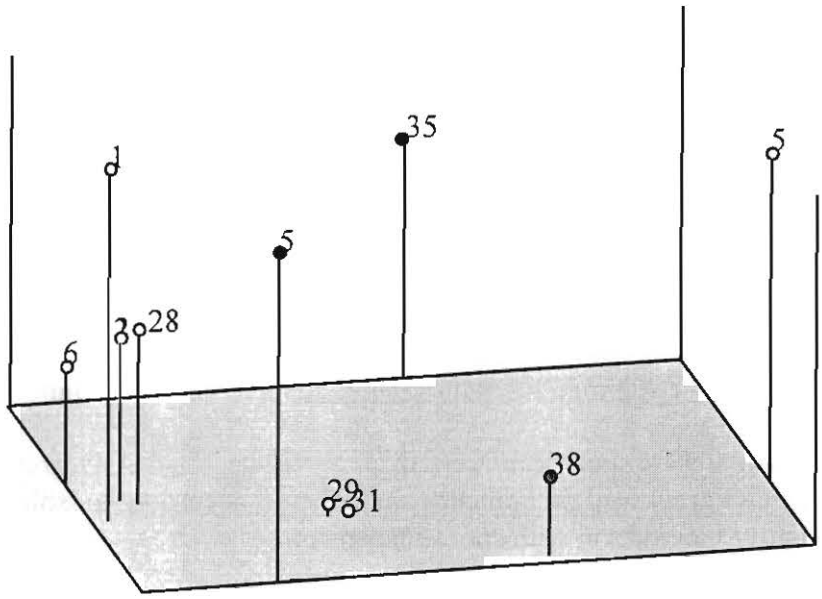

Figure 3.8. Multiple Correspondence Analysis of 10 isolates of *Saccharomyces cereviciae*, based on Random Amplified Polymorphism. The graph was constructed using the NTSYS-pc package.

References

Broach JR, Pringle JR, Jones EW. 1993. The molecular and cellular biology of the yeast *Saccharomyces cereviciae*: genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory Press, NY.

Jones EW, Pringle JR, Broach J, eds. 1992. The molecular and cellular biology of the yeast *Saccharomyces cereviciae*: gene expression. Cold Spring Harbor Laboratory Press, NY.

Vezinhet F, Blondin B, Hallet JN. 1990. Chromosomal DNA patterns and mitochondrial DNA polymorphism as tools for identification of enological strains of *Saccharomyces cereviciae*. Appl Microbiol Biotechnol 32:568-571.

Activity 4. Morphological and molecular characterization of *Colletotrichum* isolates from citrus

Anthracnose of citrus, caused by *Colletotrichum* spp., is often the major constraint to production in regions where the crop is grown, causing up to 90% production losses. Early studies have shown two or more complex fungal populations associated with the disease that can be clearly separated on the basis of morphology, growing rate and colony characteristics. Genetic variability of the pathogen increases the difficulty of breeding for resistance and effectively deploying available cultivars. A quick method for characterizing genotypes within the pathogen

population would aid citrus research by providing information on the pathogen's genetic diversity.

Two hundred and fifty five *Colletotrichum* spp. isolates, obtained from citrus grown in different edaphoclimatic Colombian zones, were compared for colony morphology and growing rate 48 h after plating on PDA modified with copper hydroxide media, incubated at 26 °C. Two well-defined groups of isolates were observed ranging from 0.8 cm to 1.8 cm for one group and from 2.7 cm to 3.8 cm for the other. These results confirmed the presence of two *Colletotrichum gloeosporioides* groups, as described by Agostini et al. (1992), for Florida, Brazil, and Basile (Costa Rica), where those groups were defined as SGO (slow growth orange) and FGG (fast growth gray). **Table 4.1** summarizes some characteristics for each group.

Each group of isolates is specific to certain plant tissue. The SGO grew well on buttons and flowers, while FGG grew well on branches and calyxes, according to isolate origin. These results were obtained after inoculation with spore suspension.

Amplification with a specific set of primers for *Colletotrichum acutatum* (CaINT2 – ITS 4) and for *C. gloeosporioides* (CgINT - ITS 4) defined two groups of isolates corresponding to two species. The fragment amplified by CaINT2 - ITS 4 had a size of 490 bp, while the fragment amplified by CgINT - ITS 4 had a size of 450 bp (**Figures 4.2. and 4.3.**).

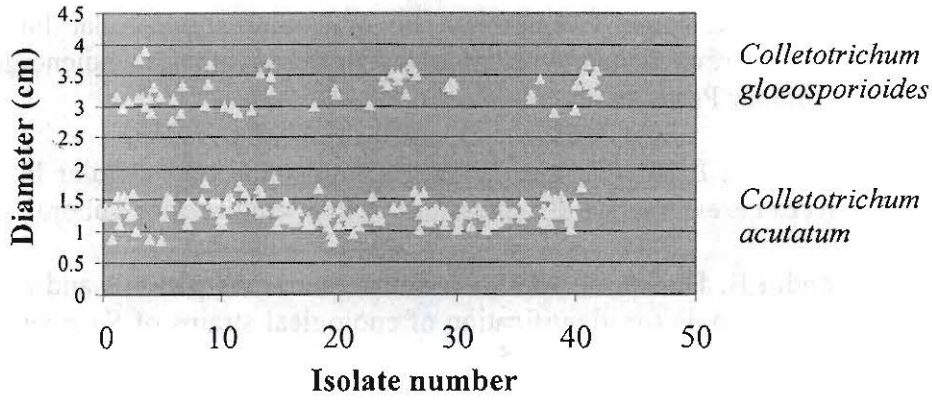

Figure 4.1. Growth rate comparison of two *Colletotrichum* species on PDA modified with copper hydroxide media.

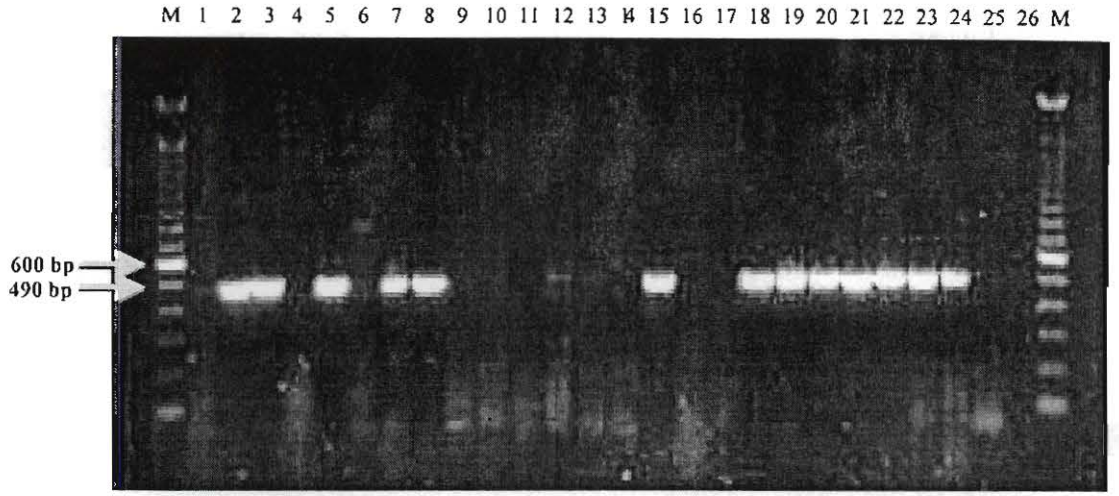

Figure 4.2. Amplification of specific fragments (490 bp) from fungal DNA, using primers ITS4 and CaInt2; M = 100 bp marker. Amplified products from lanes 1 to 25 correspond to *Colletotrichum acutatum* isolates, lane 26 to the negative control.

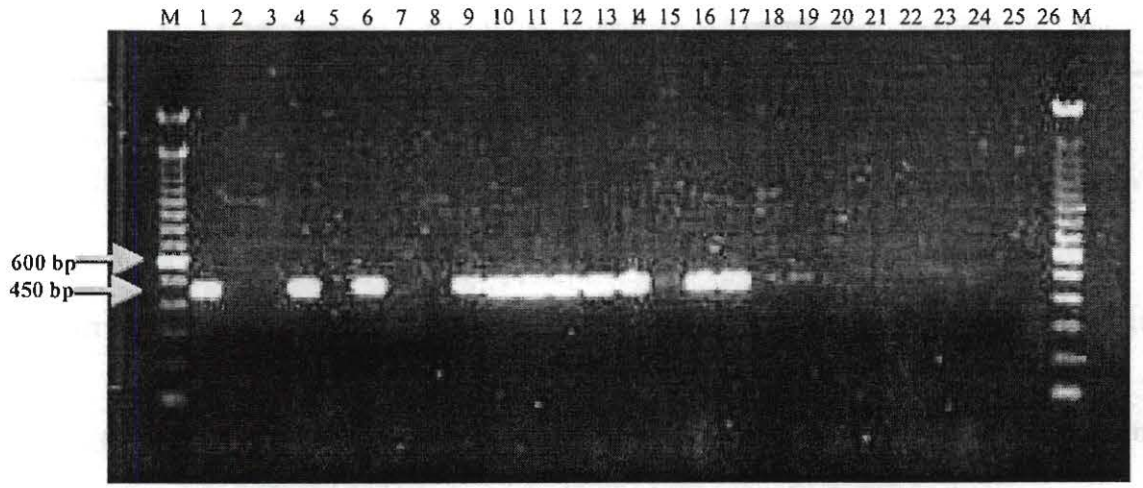

Figure 4.3. Amplification of specific fragments (450 bp) from fungal DNA using primers ITS4 and CgInt; M = 100 bp marker. Amplified products from lanes 1 to 25 correspond to *Colletotrichum gloeosporioides* isolates, lane 26 to the negative control.

Table 4.1. Host, geographic location, and source of *Colletotrichum* isolates from citrus.

Lanes	Isolate	Colony Morphology[a]	Location	Host Plant	Source	Presence of species specific band[e]	
						C. gloeosporioides CgInt	*C. acutatum* CaInt2
1	58	FGG	Caicedonia (Valle)	Tahiti lime[b]	Branches	+	-
2	94	SGO	Montenegro (Quindío)	Sweet Orange[c]	Button	-	+
3	272	SGO	Pereira (Risaralda)	Sweet Orange	Button	-	+
4	411	FGG	Pereira	Tahiti lime	Branches	+	-
5	270	SGO	Pereira	Sweet Orange	Button	-	+
6	11	FGG	Caicedonia	Tahiti lime	Button	+	-
7	377	SGO	Pereira	Tahiti lime	Button	-	+
8	302	SGO	Caicedonia	Tahiti lime	Button	-	+
9	408	FGG	Pereira	Tahiti lime	Calyx	+	-
10	293	FGG	Caicedonia	Sweet Orange	Fruit	+	-
11	414	FGG	Pereira	Sweet Orange	Branches	+	-
12	409	FGG	Pereira	Sweet Orange	Branches	+	-
13	417	FGG	Pereira	Sweet Orange	Fruit	+	-
14	256	FGG	Andalucía (Valle)	Pajarito lime[d]	Flower	+	-
15	271	SGO	Pereira	Tahiti lime	Button	-	+
16	41	FGG	Caicedonia	Sweet Orange	Fruit	+	-
17	413	FGG	Pereira	Sweet Orange	Branches	+	-
18	254	SGO	Andalucía	Sweet Orange	Flower	-	+
19	300	SGO	Caicedonia	Tahiti lime	Button	-	+
20	291	SGO	Pereira	Sweet Orange	Flower	-	+
21	285	SGO	Pereira	Tahiti lime	Flower	-	+
22	280	SGO	Pereira	Sweet Orange	Flower	-	+
23	389	SGO	Pereira	Tahiti lime	Button	-	+
24	388	SGO	Pereira	Sweet Orange	Button	-	+

[a] SGO = slow growing, orange colored, and FGG = fast growing, gray colored.
[b] Tahiti Lime (*Citrus aurantifolia*).
[c] Sweet orange - Valencia (*Citrus sinensis* (L.) Osbeck.
[d] Local name.
[e] PCR

Activity 5. Detection of DNA of plant pathogenic phytoplasma by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene

Introduction

Phytoplasmas are nonculturable microorganisms associated with diseases of many plant species. Until recently, detection of phytoplasmas was mainly based on electron microscopy and a fluorescence technique with the DNA fluorochrome 4'-6-diamidino-2-phenylindole (DAPI). Neither method allows differentiation of the organism. Moreover, their suitability depends very much on the phytoplama numbers in the phloem tissue. Thus, electron microscopy can only successfully be used for detection in hosts with a relatively high phytoplasma titer. The DAPI technique is considerably more sensitive but is limited when the phytoplasma population is very low. Among the most important plant diseases caused by phytoplasma organisms are aster yellows, apple proliferation, coconut lethal yellowing, elm phloem necrosis, and many more.

The subject of this work was the improvement of phytoplasma detection on Oil Palm samples by the use of in vitro DNA amplification through the polymerase chain reaction (PCR). This technology is used to detect nucleic acids and is highly sensitive. In addition the phytoplasma was identify by sequencing.

Materials and Methods

Sources of Phytoplasmas. The phytoplasmas included in this study were either obtained from periwinkle (*Catharanthus roseus* (L.) G. Don) maiz, soybean and coneflower as positive control host or from diseased oil palms. (**Table 5.1**).

DNA isolation. The DNA was estracted following the procedure described by Gilbertson and Dellaporta, 1988.

Primer selection. All the primers used in this study are described in Table 5.2.

PCR amplification. Total nucleic acid was extracted from healthy or phytoplasma-infected Oil Palm tissue as described by Lee, et al (1993). PCR products were analyzed by electrophoresis through 2% agarose gel followed by staining in ethidium bromide and visualization of DNA bands using a UV transilluminator. Primer pairs R16mF2/R1 and R16F2n/R2 were evaluated separately in the PCR assays for sensitivity in detection of phytoplasmas. Primer pair R16mF2/R1 was evaluated for its ability to sensitively amplify phytoplasma.

The 1,400-bp band was excised from the gel, recovered from the gel matrix and purified. DNA fragments were sequenced. The sequences were aligned by using a sequence navigator program.

Nested PCR assays

Nucleic acid samples extracted from field-collected phytoplasma-infected plants **(Table 5.1)** were used as template for PCR assays. In the nested-PCR assay, PCR products initially amplified using the universal primer pair R16mF2/R1 were diluted (1/40) with sterile deionized water and used as template for a subsequent series of 35 PCR cycles in which reaction mixtures contained the universal primer pair R16F2n/R2.

Cloning and DNA sequencing of PCR amplification products. PCR-Amplified DNA fragments were subjected to agarose gel electrophoresis. The 1,400-bp band was excised from the gel, and eluted. Purified fragments were cloned into *Escherichia coli*, and positive clones were sequenced using the ABI Prism Foster City, CA Big Dye cycle sequencing Kit and associated protocols.

Table 5.1. Description of Oil Palm samples evaluated in this study.

Samples No.	Description	Location	Source	Disease Severity
1	*Catharanthus rouseus*	Palmira	Leaf	Healthy
2	*Catharanthus rouseus*	Palmira	Leaf	Highly infected
3	Oil Palm	Palmas de Casanare	Leaf	Healthy
4	Oil Palm (10)	Palmas de Casanare	Inflorescence	Highly infected
5	Oil Palm (11)	Palmas de Casanare	Meristem	Highly infected
6	Oil Palm –1	Palmas de Casanare	Raquis	Healthy
7	Oil Palm –2	Palmas de Casanare	Spear	Healthy
8	Oil Palm –3	Palmas de Casanare	Inflorescence	Healthy
9	Oil Palm –4	Palmas de Casanare	Meristem	Healthy
10	Oil Palm –5	Palmas de Casanare	Low meristem	Healthy
11	Oil Palm –6	Palmas de Casanare	Medium stipe	Healthy
12	Oil Palm –7	Palmas de Casanare	Low stipe	Healthy
13	Oil Palm –8	Palmas de Casanare	Raquis	Highly infected
14	Oil Palm –9	Palmas de Casanare	Spear	Highly infected
15	Oil Palm –10	Palmas de Casanare	Low stipe	Highly infected
16	Oil Palm –11	Palmas de Casanare	Medium stipe	Highly infected
17	Oil Palm –12	Palmas de Casanare	Low stipe	Highly infected
18	Oil Palm #1	Palmar del Oriente	Floral primordium	Initial
19	Oil Palm #2	Palmar del Oriente	Base of the leaf	Initial
20	Oil Palm #3	Palmar del Oriente	Low meristem	Initial
21	Oil Palm #4	Palmar del Oriente	Meristem	Initial
22	Oil Palm #5	Palmar del Oriente	Root	Initial
23	Oil Palm #6	Palmar del Oriente	Inflorescence	Intermedium
24	Oil Palm #7	Palmar del Oriente	Base of the spear	Initial
25	Oil Palm #8	Palmar del Oriente	Base of the spear	Intermedium
26	Oil Palm #9	Palmar del Oriente	Meristem	Highly infected
27	Oil Palm #10	Palmar del Oriente	Inflorescence	Highly infected
28	Rose #1	Sabana de Bogotá	Leaf	Intermedium
29	Rose #2	Sabana de Bogotá	Leaf	Healthy
30	Purple coneflower	Iowa	Leaf	Highly infected

Table 5.2. Primers used for PCR amplification and sequencing of 16S rRNA genes of plant pathogenic phytoplasmas.

Primer	Sequence 5' - 3'	Source
R16F2	ACG ACT GCT GCT AAG ACT GG	Gen 16S rADN
R16R2	TGA CGG GCG GTG TGT ACA CCC G	Gen 16S rADN
R16MF2	CAT GCA AGT CGA ACG GA	Gen 16S rADN
R16MR1	CTT AAC CCC AAT CAT CGA C	Gen 16S rADN
R16F2N	GAA ACG GCG GTG TGT ACA AAC CCC G	Gen 16S r ADN
P1(General Phytoplasma)	AAG AGT TTG ATC CTG GCT CAG GAT T	
LD16-1	CGG AAA ACC TTC GGG TTT TAG	Gen 16S rARN
23S	TCT TTT CCT GCG GTT ACT TAG AT	Gen 23S rARN
P4	GAA GTC TGC AAC TCG ACT TC	Gen 16S rARN
P7	CGT CCT TCA TCG GCT CTT	Gen 23S rARN
ITS – 4	AAG GGC AGT GAA GAT GGA GTA G	Gen 5.8S rADN
ITS – 5	GGA AGT AAA AGT CGT AAC AAG G	Gen 5.8S rADN
M13 Universal	TGT AAA ACG ACG GCC AGT	PGEM –T
M13 Reverse	CAG GAA ACA GCT ATG ACC	Easy vector

Results

From the Oil Palm samples phytoplasmas were detected using PCR and Nested PCR in 10 of 15 palms. The Oil Palms were located in Villavicencio (Casanare).

Primer pair for amplification of phytoplasma-specific 16S rDNA sequences. Of the primer pairs designed for PCR, two primer pairs, R16MF2-R16MR1and amplified a 16S rDNA sequences from DNA samples extracted from infected plant tissue. The primer pair R16MF2-R16MR1 amplified a fragment of about 1.4 kb.

The amplified DNA fragments were confirmed to be specific for phytoplama-infected plants and homologous to the 16S rRNA genes of phytoplasma by Aster Yellows

Determination and comparison of amplified phytoplasma 16S rRNA sequence.

The aproximately 1,377-nucleotide sequence of each PCR fragment was determined by direct sequencing **(Figure 5.1)** of both strands by using the oligonucleotide primers presented on Table 5.2. On the basis of the results of an extensive analysis (maximum matching and homology search) in which the Sequence navigator program was used. The phytoplasma analyzed in this study, had levels of sequence homology with group I (Aster Yellows) of 99%.

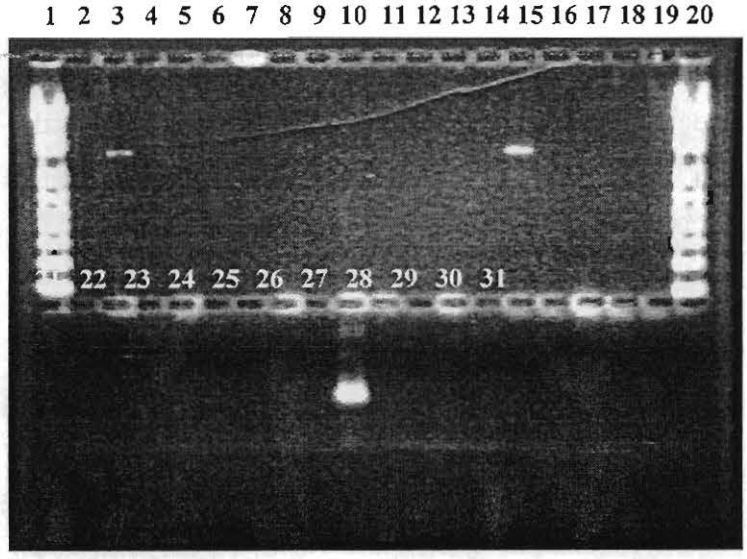

Figure 5.1. **Polymerase chain reaction (PCR) amplification of a 16S rDNA sequence using primer pair R16MF2-R16MR1. Lane 1 = 100 bp marker, lane 2 = healthy Oil Palm (Casanare), lanes 3 and 15 = disease Oil Palm (Casanare), lanes 4 to 14 = Oil Palm samples (Casanare), lanes 16 to 26 = Oil Palm samples, (Palmar del Oriente), lanes 27 and 28 = Rose samples, lane 29 = Oil Palm sample (Casanare), lane 30 = positive control (Purple coneflower), and lane 31 = negative control (water).**

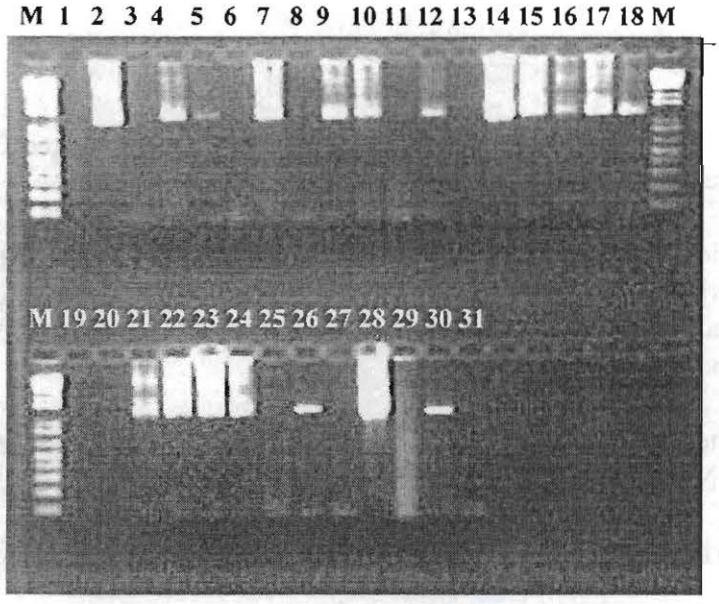

Figure 5.2. **PCR products from symtomatic Oil Palms amplified using Nested PCR (P1-P7) external primers and (R16F2N-R16FR2) internal primers. M = 100 bp. marker. Lane 1 = healthy Oil Palm (Casanare), lane 2 = disease Oil Palm (Casanare), lanes 3 to 14 = Oil Palm (Casanare), lanes 15 to 24 = Oil Palm Palmar del Oriente), lane 25 = healthy rose, lane 26 = disease rose, lane 27 = Oil Palm (Casanare), lane 28 = positive control (coneflower), lanes 29 and 30 = disease Oil Palm (Casanare), lane 31 = negative control (water).**

Figure 5.3 shows the Nested PCR amplified products obtained with primers R16MF2-R16MR1/ R16F2N-R16FR2. Selected fragments were purified and sequenced directly.

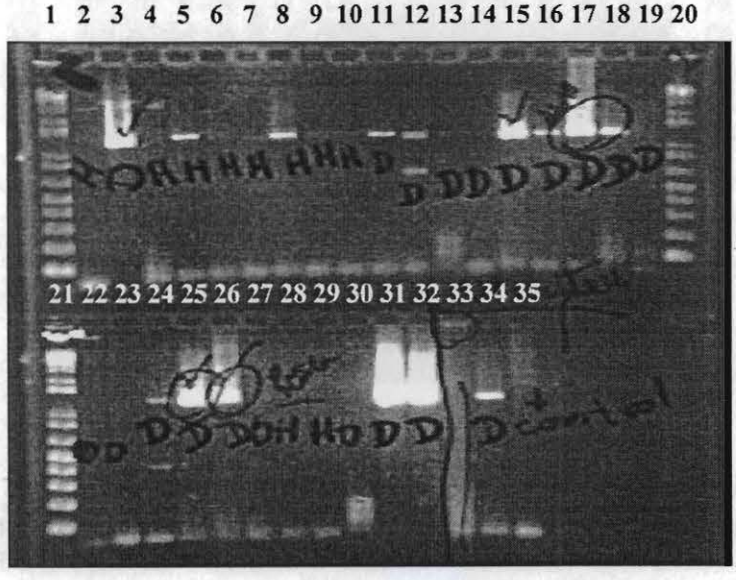

Figure 5.3. **Nested PCR R16MF2-R16MR1 external primers and R16F2N–R16FR2 internal primers. Lanes 1, 20 and 21 = 100 bp. marker. Lanes 2 to 18 and 22 to 34 = DNA amplified products from Oil Palm samples.**

Figure 5.4 shows in lane 3 and 4 the right fragments from Pc2(10)-2 and 14R-2 clones, obtained after restriction with Eco R1 enzyme. These two plasmid purified samples were sequenced with vector primers (Universal and Reverse).

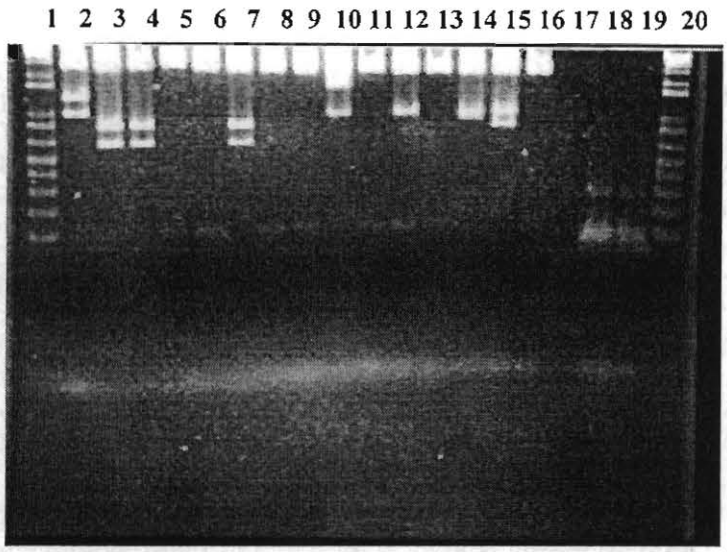

Figure 5.4. Restriction with the enzyme EcoR1of purified plasmids. Lanes 3 and 4 correspond to PC2 (10)-2 y 14 R-2 clones from Palmas de Casanare.

Figure 5.5 shows the right restriction fragments from Oil Palm samples selected to be cloned for further sequencing.

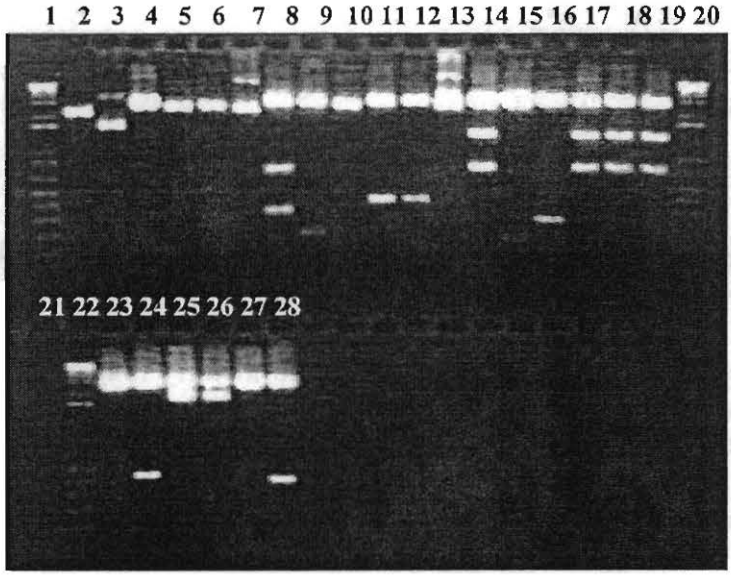

Figure 5.5. Restriccion of the purified plasmids with *Eco*RI enzyme. Lane 14 = Palmas de Casanare, and lanes 17 to 19 = Palmar del Oriente.

Computer assisted sequence alignment revealed that the nucleotide sequence of 16S ribosomal RNA and tRNA from Oil Palm phytoplasma was identical to that of 16S rRNA and tRNA gen bank accession U89378 from a phytoplasma associated with group I Aster Yellows disease in Oil rape seed.

1 57 PC 2 – 10
GAATTGNATACGACTCACTATAGGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCGGC
CGCGGGAATTCGATTCATGCAAGTCGAACGGAAGTTTAAGCAATTAAACTTTAGTGGCGAACGGGTGA
GTAACGCGTAAGCAATCTGCCCCTAAGACGAGGATAACAGTTGGAAACNACTGCTAAGACTGGATAG
GAGACAAGAAGGCATCTTCTTGTTTTTAAAAGACCTAGCAATAGGTATGCTTAGGGAGGAGCTTGCGT
CACATTAGTTAGTTGGTGGGGTAAAGGCCTACCAAGACTATGATGTGTAGCCGGGCTGAGAGGTTGAA
CGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATTTTCGGCAA
TGGAGGAAACTCTGACCGAGCAACGCCGCGTGAACGATGAAGTATTTCGGTACGTAAAGTTCTTTTAT
TAGGGAAGAATAAATGATGGAAAAATCATTCTGACNGTACCTAATGAATAAGCCCCGGCTAACTATGT
GCCAGCAGCCCGCGGTAATACATAGGGGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGTGCGT
AGGCGGGTAAATAAGTTTATGGNCTAAGTGCCAATGCTCAACANTGNGATGCTNTNAAAACTGTTTAC
TAGAGTAAGATAGAAGNCAGTGGAATTCNTTGTNTAGNGGNAAAATGCGTAAATTTTGGAGGAACNC
CATNGCCGAAGGNGGGTTGCTGGGCTTTCTGNCCTNAGGNCCAAACGTGGGACNAACNGATAANNCC
CTGTATTCCCCCCNAAACATAATCTAACGTGGNAAACCCTNGAATTACCATATCTCCC

This complete Oil Palm phytoplasma sequence belongs to group 16SrI (Aster Yellows). This is the first study in which a phytoplasma is associated with Oil Palm, phylogenetically analyzed by sequencing their 16S rRNA and tRNA genes.

An approach for producing phytoplasma-specific diagnostic reagents will be the molecular cloning of phytoplasma chromosomal fragments extracted from phytoplasma-infected plants. The cloned DNA fragments obtained in this study will be used as hybridization probes to detect pathogenic phytoplasmas in oil palm plants.

Activity 6. AFLP analysis of isolates of *Ceratocystis paradoxa*, causal agent of dry basal rot disease in oil palm

Introduction

Oil palm (*Elaeis guineensis* jacq.) is a widely cultivated crop in Colombia. Dry basal rot is an important disease that affects oil palm on every major commercial cultivar.

Molecular techniques are being increasingly applied to identify fungal species and characterize genetic variability of many fungi. The identification of variability in fungal pathogens is traditionally based on morphology and pathogenicity studies, as well as through molecular markers such as PCR-RAPD and RFLP (Alvarez et al., 1997). Low genetic variability among *Ceratocystis paradoxa* strains was detected in a study conducted previously at CIAT using RAPD markers. Isolates of *Ceratocystis paradoxa* were characterized using Amplified Restriction Fragment Length Polymorphism (AFLP) to be able to detect a high level of polymorphism.

Samples' preparation and DNA synthesis

Isolates obtained from diseased oil palms were grown in potato dextrose agar (PDA) medium with lactic acid, and incubated at 24 °C (room temperature) for 7 days. Isolates were grown on liquid medium of malt yeast extract (malt 2%, yeast 0.2%) and then incubated at 28 °C for 7 days without agitation until enough mycelia were formed. Colonies were harvested according to the Lee and Taylor protocol (1990), modified as follows: 400 mL solution of phenol, chloroform, and isoamyl alcohol (25:24:1) was used to precipitate DNA, which was then centrifuged at 10,000 rpm for 15 min. The pellet was resuspended in 80 µl of TE and incubated with 25 µl of ribonuclease (10 mg/ml) at 37 °C for 30 min. The DNA concentration was determined with a fluorometer (Hoefer DyNA Quant 200) and checked by agarose gel 0.8%.

DNA-AFLP analysis

The restriction of samples for AFLP analysis was prepared according to Bachem et al. (1996) using *Eco*R I and *Mse* I, ligation of adapters. Following heat inactivation of the restriction endonucleases, the genomic DNA fragments are ligated to *Eco*R I and *Mse* I adapters to generate template DNA for amplifications. PCR is performed in two consecutive reactions. Preamplification was carried out with one *Eco*R I and one *Mse* I adaptor (with no selective bases) or with primer containing one selective base (AFLP microorganism primer Kit, Gibco BRL). The subsequent amplifications were made with a pair of primers with one or two selective bases (12 and 20 combinations, respectively). Selective amplification products were separated on a 6% denaturing poliacrylamide (sequencing) gel run at 100 W, 50 °C, for 2 h 30 min. They were processed with the silver staining technique (Promega).

Interpretation of results

The resultant banding pattern ("fingerprinting") was analyzed for polymorphisms manually, using criteria such as presence or absence and individual band intensity. Size distribution of amplified product and overall pattern should be similar for AFLP analysis with the same primer pairs and the same DNA template. We take into account differences in banding pattern caused by DNA polymorphism (Figure 6.1.).

Data analysis

The data matrix was obtained by presence or absence of bands on a 1 or 0 basis, and analyzed by two methods. First, genetic distance was calculated according to the Ney-Li definition of similarity. The matrix of distances was analyzed by the UPGMA method of the software NTSYS. Dendrograms were originated with the TREE program of NTSYS (**Figure 6.2.**).

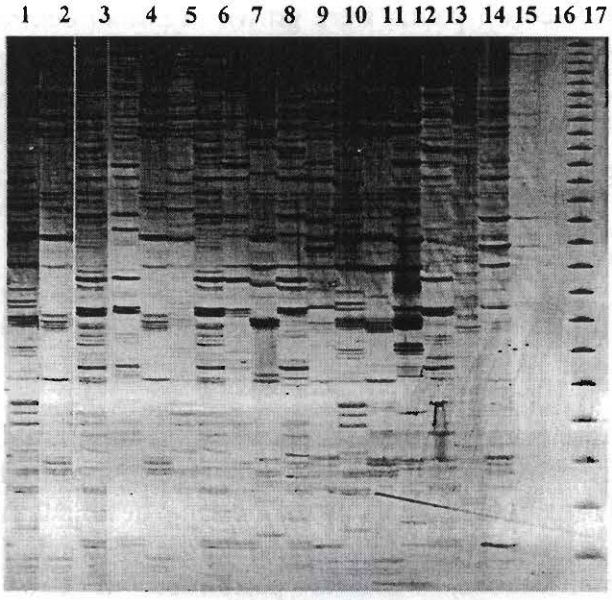

Figure 6.1. **Polymorphisms detected with the AFLP primer combination EAC/MA in 17 isolates of *Ceratocystis paradoxa* from Colombia; M = 30-330 bp marker, tracks 1 to 17: T 1021, T 14, T 1546, Th i24, Thi 049, Th 012, Th i040, Thi 014a, Thi 062, Mec tumaco, Thi 045, Thi014b, Thi 092, Thi 094, Thi 060, Thi Cpt 113b, and Manavire 112.**

In addition, Multiple Correspondence Analysis was applied, using the CORRESP procedure of SAS version 6.09 to visualize the dispersion of individuals in relation to the first three principal axes of variation. For this purpose, the active variables were those corresponding to the presence or absence of bands (**Figure 6.3.**).

Results and Discussion

Seventeen selected isolates from three *Ceratocystis* species were characterized by AFLP methodology, and 92 polymorphic bands were scored. The isolates were screened with 32 primer combinations and one (EAC/MA) was found that generated polymorphism and consistently produced the same banding patterns in replicated trials. Both analyses, matrix of distances method and dendrograms with NTSYS as well as Multiple Correspondence Analysis, reflected the high level of polymorphism detected among 17 isolates evaluated. The phenogram of *Ceratocystis paradoxa* separated the isolates into two molecular groups (Cluster 1 and Cluster 2) (**Figure 6.2.**) at 45 % of similarity, and showed high genetic variation within each one.

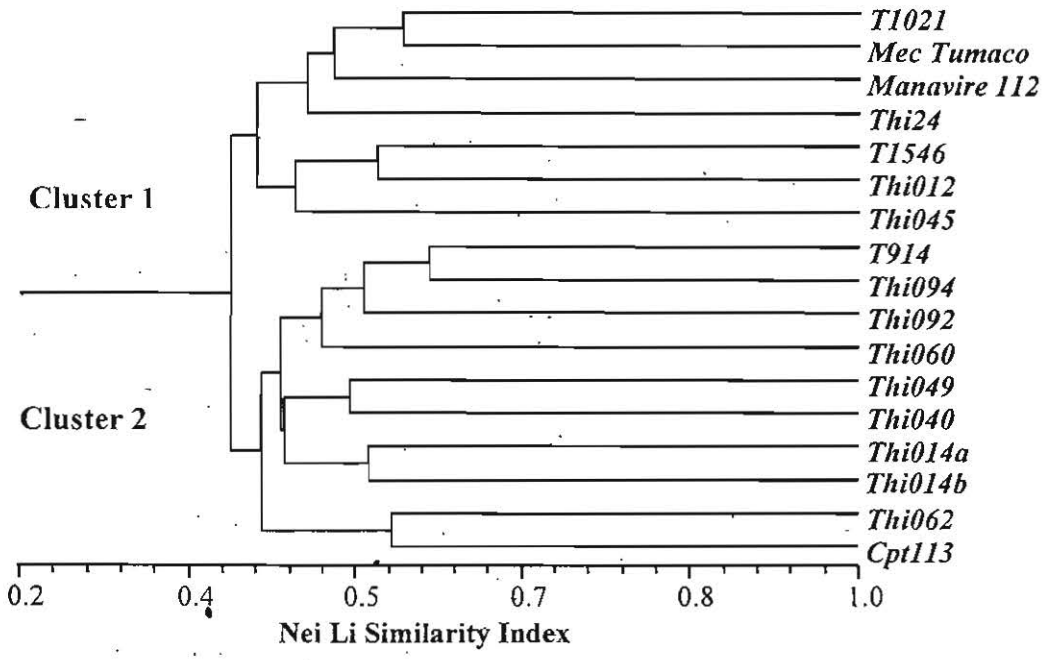

Figure 6.2. **Dendrogram developed from the Nei – Li similarity distances. Figures at right refer to clusters discussed above. Cluster 1 refers to *T1021, Mec Tumaco, Manavire 112, Thi 24, Thi 1546, Thi 012*, and *Thi 045* isolates. Cluster 2 refers to *T914, Thi 094, Thi 092, Thi 060, Thi 049, Thi 040, Thi 014a, Thi 014b, Thi 062,* and *Cpt 113* isolates.**

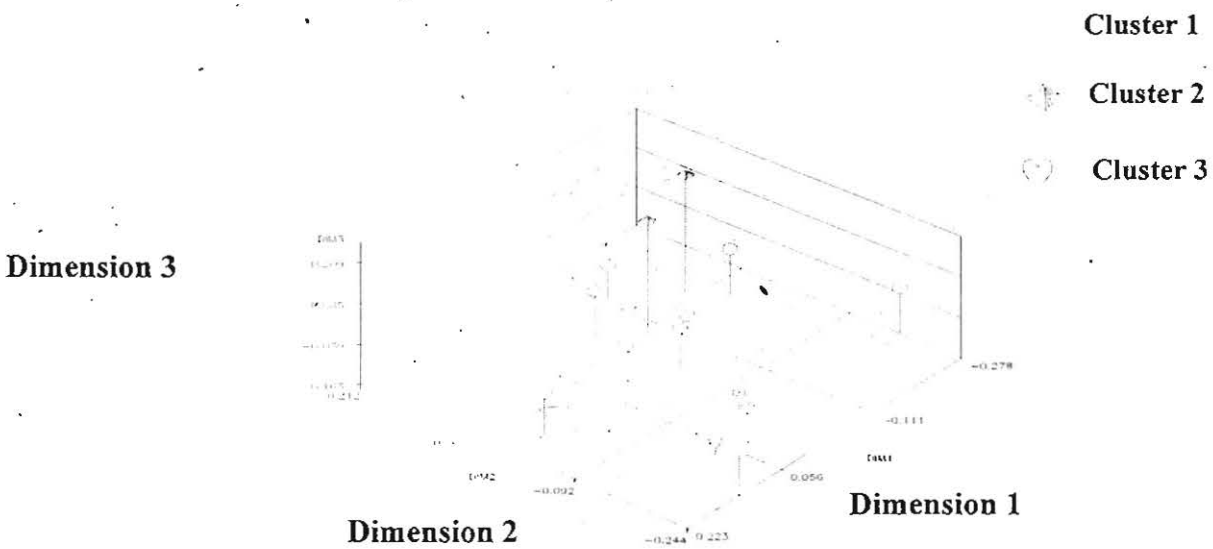

Figure 6.3. **Multiple correspondence analysis showing the separation of isolates in three dimensions from three principal axes.**

According to these results, high genetic variability in these fungus isolates are in contrast to results obtained in previous work, with other molecular markers evaluated (PCR and RAPD) in the same species, in which a lower genetic diversity was detected. Compared to other reported methods of species classification and genetic diversity; AFLP analysis allowed much more detailed analysis of variability in *C. paradoxa*, generating new elements to understand the genetic variability present in this genus.

References

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Activity 7. Plant disease diagnosis

This year, we received samples for bacteriological and fungal diagnoses; MLO and nematodes were also detected. **Table 7.1.** presents relevant data from bacteria that have been isolated and identified.

Table 7.1. Disease-causing pathogens identified on different crops by the Diagnostic Service of the CIAT Cassava Pathology Section[a].

Host	Symptoms	Detection methodology	Result
Cassava (*Manihot esculenta*)	Root rots with black necrotic spots	Visual	*Rossellinia* sp.
	Root rots	Isolation	*Diplodia manihotis*
	Large black or brown necrotic lesions on the stems	Isolation	*Thielaviopsis* sp., *Fusarium* sp.
	Leaves affected by necrotic lesions	Isolation and pathogenicity test	Bacteria were isolated from stems of affected plants
	Necrotic stem and die back	V8 agar	*Colletotrichum* sp.
	Root rots	V8 agar	Oomycete
Grape (*Vitis labrusca*)	Necrotic inflorescence lesions	Direct observation by microscope	*Phyllosticta* sp.
Sugar cane (*Saccharum officinarum*)	Necrotic lesions, damping off	Direct observation by microscope, isolation	*Curvularia* sp., *Pythium* sp., *Fusarium* sp. *Bipolaris* sp.
Orchid (*Dendrobium* and *Oncidium*)	Necrotic lesions and chlorotic leaves	Isolation	*Fusarium* sp.
	Black spots on both sides of the leaves	Direct observation by microscope	*Cladosporium* sp.
	Chlorosis of the flowers	Isolation	Unidentified bacteria
Rose (*Rosa* sp.)	Leaves and stems affected by black necrotic spots and chlorosis	Selective culture Medium for bacteria and fungus	Bacteria and fungus (e.g., *Fusarium* sp., *Cladosporium* sp., *Botrytis* sp., *Phoma* sp.)
Chrysanthemum (*Chrysanthemum* sp.)	Yellowing of the leaves, thin roots which easily fall off	Isolation	*Fusarium* sp. and bacteria
	V-shaped necrotic lesions	Direct observation by microscope	*Pratylenchus* spp. (nematode)
Heliconia (*Heliconia* sp.)	Brown wilting	Selective culture medium for bacteria	*Ralstonia solanacearum*
Oil palm (*Elaeis guineensis*)	Bud rot	Isolation using selective culture medium	*Phytophthora* sp., Pythiaceae, *Fusarium* sp.
	Leaves yellowing, ascendant wilting	PCR and DNA sequencing	Aster Yellow Phytoplasma
	Leaves yellowing, ascendant wilting	PCR and DNA sequencing	Phytoplasma related Phytoplasma related with a *cucurbitaceae*

[a] Isolation of *Phytophthora* spp. and *Xanthomonas axonopodis* pv. *manihotis* from cassava field trials of the ongoing research projects are not included.

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Scottish Crop Research Institute, UK (Dr. J. Duncan)
The Royal Veterinary and Agricultural University, Copenhagen, Denmark (Dr. D. Collinge)

Sub-output 6. Cassava Virus Disease and their Vectors Described and Analyzed. (L.A. Calvert)

Activity 1. Developing new diagnostic methods for cassava frogskin disease

Introduction

The identification of cassava frogskin disease (CFSD) has remained elusive. There is evidence that the disease is caused by a virus-like agent. The disease is readily graft transmitted and there is evidence for transmission by an aerial vector. The symptoms of hyperplasia are similar to those caused by reoviruses. Despite the evidence of reo-like virus particles, it has not been possible to associate any of these particles with nucleic acids and structural proteins. Because of the difficulties with the purification of the causal agent, clone double stranded RNA (dsRNA) extracted from affected cassava plants has been tried. Most of the cloned fragments have been plant products. Other cloned products include an RNA dependant RNA polymerase gene of an unknown mycovirus and a region that has amino acid similarity with the RNA 5 of rice ragged stunt reovirus. It appears this latter cDNA product is part of the genome of cassava.

The cloning of ds-RNA products has not yet identified the causal agent or led to a rapid diagnostic procedure. Since the milestone of developing rapid diagnostic methods has not been met, it was decided to try a novel method to achieve the goal. This report describes the preliminary use of reverse transcriptase-AFLP (Vos *et al.* 1995) of ds-RNAs extracted from healthy and infected plants to identify amplified products that are associated with the cassava frogskin diseases.

Materials and Methods

Source and extraction of dsRNA. The source of CFSD affected is from the cassava line CM 5460-10 with mosaic symptoms on the leaves. The "healthy control" are from tissue culture derived Secundina that were maintained in greenhouses and had no CFSD symptoms. The disease causes both mosaic symptoms in the leaves and roots in both these cassava lines. Stem cuttings were collected and treated to produce flushes of roots. Three grams of root tissues were collected at 27 days. The dsRNA was extacted (Morris and Dodds, 1979). The ds-RNAs were treated with DNase (10µg/ml) for 40 minutes at 40°C. The samples were then subjected to an ethanol precipitation and analyzed on TAE agarose gels.

The synthesis of cDNA from the dsRNAs. For each sample, 5µg dsRNA, 500 ng of random primers and 500ng of 18mer-oligo(dt) (Gibco BRL) for a total volume 13µl where denatured by the addition of 13µl of 40Mm methylmercuric hydroxide (Jelkmann et al. 1989). The mixture was incubated for 10 minutes at room temperature and frozen using liquid nitrogen. The samples were the allowed to thaw out and were processed immediately.

The first strand synthesis was done in a final volume of 40µl containing 50Mm Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM each dATP, dCTP, dGTP, dTTP, 40 U of RNasin (Promega) y 400 U of SuperSript II RT (Gibco, BRL). The mixture was incubated for 60 min a 37° C. Then an additional 200 U of SuperScript II RT was added to the mixture and the

reaction was allowed to continue for another 30 minutes. The reactions were then subjected to 70° C for 1 minute and placed in ice water for 2 minutes.

To the 40µl of the first strand reaction 25 mM Tris-HCl (pH 7.5), 100 mM KCl, 5 mM $MgCl_2$, 10 mM$(NH_4)_2SO_4$, 0.15 mM β-NAD^+, 0.25 mM each dATP, dCTP, dGTP, dTTP, 1.2 mM DTT, 25 U *E. coli* Ligase, 40 U *E. coli* Polymerase, 4 U *E. coli* Rnase H were added and the final volume was 150µl. After the mixture was incubated for 3 h at 16° C, 30 U of T4 DNA Polymerase was added and the reaction was continued at 16° C for 10 minutes. The reaction was stopped by the addition of 10 µl of 0.5 M EDTA, pH 8.0 and treated with phenol:chloroform:isoamyl alcohol (25:24:1). The cDNAs were precipitated with 1/10 volume of 7 M ammonium acetate and 2.5 volumes of 95% ethanol.

AFLPs analysis. The AFLP method (Vos et al. 1995) was implement by digesting the cDNA using restriction enzymes *Eco*RI-*Mse*I and ligating it with corresponding adaptors. The PCR preamplification PCR was done with selective *Mse*I-adaptors. The profile was 30 cycles consisting of 94°C for 30 sec, 55°C for 30 sec, and 1minute at 72°C. In the cascade PCR reaction, seven combinations of primers (with 2 and 3 selective nucleotide for the *Eco*RI y *Mse*I sites respectively) were used. The amplified products were analyzed using a 6% polyacrylamide gel and visualized by silver staining.

Elution and amplification of selected cDNAs. The polymorphic cDNA amplified products were eluted from pieces of the polyacrylamide gels. The selected pieces of the gels were soaked in water for 10 minutes at room temperature then an additional 15 minutes at 65°C. The aqueous portion containing the cDNA was subject to an ethanol precipitation and the pellet was resuspended in sterile water.

Five µl of each sample was amplified using 10mM dNTPS, 1X PCR buffer, 2mM $MgCl_2$, 0.2µl de 10U/µl Taq polymerase final volume of 20 µl. The PCR profiles were the same used in the original amplification and were visualized used agarose gel electrophoresis.

Results and Discussion

In the seven restriction site-primer combinations used to amplify rt-AFLP products from the dsRNA from healthy and affected roots, a total of 37 polymorphic products were identified. Of these 37 products, the majority (need a number) were present in the CFSD affected plants. These products range in size from ca. 190 to 1400 base pairs. The re-amplifications of 23 products were successful, but in most cases multiple products were amplified. This prevents the direct sequencing of these products. These preliminary experiments show that this method has the power to distinguish differences between affected and apparently healthy plants.
Five different cassava lines have been selected with both healthy and CFSD affected plants. The same technique will be used to determine which polymorphisms are consistently associated with the diseased plants. The selected polymorphic bands will be cloned, sequenced, and analyzed. The cloned regions that appear to be specific to the pathogen will be tested to see if they can be developed into rapid diagnostic probes. These should also prove useful in identifying the causal agent.

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Activity 2. Developing cassava resistance to cassava frogskin disease

Abstract

Six years ago, it was decided to test the CIAT cassava core collection to cassava frogskin disease (CFSD). The results have shown that tolerance to CFSD is widespread in cassava germplasm. More than 100 tolerant lines have been identified and are potential sources of resistant to CFSD. During the last cycle, agronomic characteristics and resistance to other pests were used to reduce the number of lines to 66. Of these 61 continued to display a high level of resistance to CFSD. All these lines have been infested with CFSD for at least four growing cycles. Some commercial varieties and landraces are tolerance to CFSD. Since these can act a reservoirs of the disease, we will need to do more investigation to understand the influence of tolerance on the endemic problem of CFSD.

Introduction

Cassava frogskin disease (CFSD) was first reported in 1971 from southern Colombia (Lozano & Nolt, 1989). CFSD is endemic throughout Colombia and in the Amazon regions of Peru, Brazil. Often there are varieties that appear healthy that are planted next to fields that have high levels of CFSD. Cassava farmers often think that CFSD is a physiological disorder, and some landraces have been named for the root symptoms. In the Amazonian region, one common name for landraces is "Jacare" which translates to caymen. Cassava is a vegetatively propagated crop, and CFSD is transmitted efficiently through stem cuttings. The disorder can be transmitted by grafting, and there is evidence that *B. tuberculata* is a vector of the disease (Angel, 1990). The predominate whitefly in the Cauca region of Colombia is *Alureotrachlus socialis*, and normally the populations of *B. tuberculata* are only 1-2% of those of *A. socialis*. Nevertheless, where CFSD is endemic, the disease spreads persistently and can affect new plantings of cassava.

In most varieties, symptoms only develop in the roots, and even roots with moderate symptoms accumulate less starch than normal. These roots contain more fibers and are of lower cooking quality than roots that are not affected. In areas where the disorder is endemic the yield losses can be 100%. In a limited number of cassava landraces, the leaves of affected plants develop mosaic systems and the plants are often stunted. Secundina is a variety that develops mosaic symptoms in the leaves and is used to index cassava for CFSD.

In 1995, we started the testing of the 640 lines in the CIAT core collection. This report describes the recent advances to select for cassava lines with resistance to CFSD.

Materials and Methods

Testing for resistance to CFSD. The plants tested were from the core collection of 640 cassava lines that are representative of the CIAT cassava collection that consists of over 6000 lines. All plants in this trial were inoculated using stem cuttings form the hybrid CT5460-10 that were positive for CFSD as determined from mosaic symptoms in the leaves. Five plants from each line were inoculated by grafting with the CFSD affected stem cuttings of line 5460-10. In the subsequent years, 10-20 plants per line were grown for 12 months and evaluated visually for root symptoms. Selected lines were assayed for the presence of CFSD by grafting stem cuttings (rootstock) to Secundina (scion), grown at temperatures below 30°C, and the new leaves were examined for mosaic symptoms. Those plants with leaf symptoms were rated as positive for CFSD.

Results and Discussion

Evaluation of cassava for resistance to CFSD. The graft transmission of CFSD was highly efficient and allows the screening of cassava germplasm to proceed in a systematic manner. Over 100 of the lines in the core experiment were eliminated from the trial because they were known to be highly susceptible to CFSD. Each year cassava lines with either moderate or severe symptoms were eliminated from the experiment. At the end of the 1999-2000 season, nearly 111 of the clones in the core collection consistently were scored as having no root symptoms or very

mild symptoms (**Table 2.1**). The widespread tolerance to CFSD was not expected, but it helps to explain observations of varieties in adjacent fields with very different incidence of CFSD. It is an important consideration when developing control strategies for the disorder. Since there were ample number of lines with significant tolerance to CFSD, during the last cycle, agronomic characteristics were used to select the best lines under the growing conditions at Santander de Quilichao, Cauca, Colombia. At the planting for the year 2000, some lines that were resistant were eliminated from the experiment because they did not grow well in the location of the trial. All of the lines chosen for the 2000-01 cycle were tested for the presence of CFSD by grafting them to Secundina. Sixty-six lines were selected and evaluated during the last growing cycle and five of these had either moderate or severe symptoms of CFSD (**Table 2.1**). More than 92% of the cassava lines had either very mild or no root symptoms. This was expected since all the cassava lines have been in this long term experiment for at least three years. More than 40% of these lines are still without any visible symptoms. All of the cassava lines were tested for the presence of CFSD by grafting to the indicator clone Secundina. Only one clone of these was negative for CFSD and it will be reinfested to assure that this line indeed resistant or immune to the disease. Since the causal agent is apparently still present, the resistance is probably a type of tolerance. Until a test is developed to quantify the levels of an infectious agent, it will not be known what affect the resistance is having on the levels of a pathogenic agent. The origin of the disease is believed to be the Amazon region, and cassava has been cultivated for at least 5,000 years in the region. One hypothesis for the widespread tolerance is that there has been a long period of selection of varieties that had high yield and no obvious root symptoms even when the disease is present. The high percentage of lines that have a significant level of resistance after several years of being affected with the disease indicates that the resistance is useful to control disease losses. Since tolerance to CFSD is presence in some commercial varieties and many

landraces of cassava, mixed croping may increase disease incidence in susceptible varieties. Since these tolerant lines have very mild or no symptoms, these varieties may end up with a high percentage of infected plants in the field the tolerant cassava acts as a reservoir for CFSD.

Table 2.1. Summary of five years of testing the cassava core collection to cassava frogskin disease.

	1995-1996		1996-1997		1997-1998		1998-1999		1999-2000	
No. of cassava clones evaluated	267		180		214		159		141	
Negative for root symptoms	148	55.4%	56	31.11%	100	46.73%	125	78.6%	68	47.51%
Very mild symptoms	45	16.86%	58	32.22%	77	36.01%	18	11.30%	53	38.29%
Moderate symptoms	17	6.4%	48	26.66%	31	14.5%	4	2.50%	8	5.7%
Severe symptoms	3	1.12%	16	8.9%	0	0%	5	3.2%	8	5.7%
Clones that died	54	20.22%	2	1.11%	6	2.80%	7	4.40%	4	2.80%

The tolerance is widespread throughout the cassava germplasm. While many of the tolerant lines are from Brazil and Peru, others are from China, Malaysia, Argentina, Paraguay, Mexico, and Colombia. Many of these produced both high yields and superior number of commercial stem cuttings as compared to typical varieties grown in Colombia **(Table 2.3)**. The high yields can be explained by the fact that all the selected lines have been grown in the same site for at least three years, and agronomic performance was used to select the cassava lines that remain in the experiment.

The long term experiment is continuing and 53 of the lines were planted for the cycle 2001-02. We are in the process of multiplying CFSD free plants of these 53 lines for multi-locational testing to select clones that grow well in a wide range of agronomic systems. When to developing cassava varieties these lines need to be evaluated for quality and consumer acceptance. Those that have the agronomic characteristics and are acceptable to the consumers should be promoted as varieties. Several of these lines should also be selected for use in breeding programs.

Table 2.2. The reactions of cassava clones affected with cassava frogskin disease in field trials harvested in 2001.

No, of cassava clones evaluated	66	Percentage
Negative for root symptoms	28	42.4
Mild root symptoms	33	50.0
Moderate root symptoms	4	6.1
Severe root symptoms	1	1.5

Table 2.3. Agronomic characteristics of cassava lines that were resistant to cassava frogskin disease.

Cassava Line	Root Symptoms	Root Weight (Kg/Plant)	No. Commercial Stem Cuttings/Plant
HMC-1 ICA	Negative	1.72	5.75
M Arg 2	Very mild	4.24	7.23
M Bra 897	Very mild	3.25	7.3
M Chn 2	Negative	3.31	11.2
M Col 1468 CMC40	Very mild	2.20	6.06
M Mal 50	Negative	3.13	9.38
M Mex 95	Negative	2.79	15.9
M Par 183	Negative	3.94	17.8
M Per 184	Very mild	3.85	18.6
M Per 213	Negative	2.68	22.5
M Per 368	Very mild	6.44	10.1

Almost 20% of the core collection is tolerant to CFSD. If the core collect is truly representative of the CIAT cassava collection, there should be nearly 1000 sources of resistance and potentially CFSD tolerant varieties in it. Since tolerance is more common in certain countries such as Peru, the selection of additional tolerant lines should be a easier. Still it will take several years of field trials to select additional tolerant cassava lines. In order to reduce the total number of lines tested, the strategy will be to select lines that are known to have high yields, good cooking quality and other desirable traits and test these for their reaction to CFSD. The widespread use of CFSD varieties should be an effective control strategy to reduce loss caused by CFSD.

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Activity 3. Developing Sequence Characterized Amplified Regions (SCARs) to Identify *Bemisia tabaci* Biotype A, *B. argentifolii* (Biotype B), and *Trialeurodes vaporariorum* (Homoptera:Aleyrodidae)

Abstract

In previous studies RAPD markers were used to distinguish between *B. tabaci* biotype A, *B. argentifolii* (biotype B) and *T. vaporariorum*. The RAPD markers were often difficult to

interpret since they amplified multiple products. Sequence characterized amplified regions (SCARs) were developed for each of these whiteflies. Amplified products of 697, 567, and 303 nucleotides were produced for *B. tabaci* biotype A, *B. argentifolii* (biotype B), and *T. vaporariorum*, respectively. Although additional testing is needed to assure the robustness of these SCARs, the preliminary results using samples of *T. vaporariorum* from Guatemala, Ecuador and Colombia demonstrated that these SCARs were specific. The simplicity of this test permits laboratories with PCR capability to use these primers to distinguish between these whiteflies.

Introduction

Whiteflies are agricultural pests in most tropical and subtropical countries. *Bemisia tabaci* (Gennadius), *Bemisia argentifolii*, (Bellows and Perring), and *Trialeurodes vaporariorum* (Westwood) are three of the most important whitefly pests in tropical America. These whiteflies overlap in their range and the agricultural crops that they damage. *Bemisia tabaci* B biotype is reported to be a distinct species called *B. argentifolii* (Perring et al. 1992, 1993, Bellows et al. 1994). Studies (Brown et al. 1995, Calvert et al. 2001, Frohlich et al. 1999) suggests that *B. tabaci* should be considered mixture of cryptic species complex, and that *B. argentifolii*, a member of the complex, is a recent introduction from the Old World to the Americas. In a study based on the ribosomal internal transcribed spacer (ITS 1), the authors reached the same conclusion (DeBarro et al. 2000). *Bemisia argentifolii* is widespread in many countries of Latin America, and this pest causes hundreds of millions of dollars annually in direct damage and as a vector of whitefly-transmitted viruses (Polston and Anderson 1997). While host range, silverleaf symptoms, and increased populations of whiteflies are indicators that the population is *B. argentifolii*, species within the *Bemisia tabaci* complex cannot be distinguished by morphology (Calvert et al. 2001, Rosell et al. 1997).

Molecular detection methods using randomly amplified polymorphism DNA (RAPDs) products were developed to distinguish native Australian populations of *B. tabaci* from *B. argentifolii* (De Barro and Driver 1997). A RAPD test was developed to distinguish between *B. tabaci* biotype A and *B. argentifolii* (biotype B) (Calvert et al. 2001). While this method is convenient since the samples can be preserved in alcohol and relatively large number of samples can be processed rapidly, the banding patterns are complex and it takes experience to interpret them correctly. Sequence characterized amplified regions (SCARs) can be developed to amplify only one product and make the results easy to interpret (Ohmori et al. 1996).

Materials and Methods

Origin of whiteflies. The whiteflies used to develop the SCARs were from colonies that have been previously described (Calvert et al. 2001). The whiteflies were collected and preserved in 70% ethanol.

RAPD PCR analyses. Total DNA was isolated from individual whiteflies using a method developed for plants (Gawel and Bartlett, 1993). The DNA was amplified in a polymerase chain reaction (PCR) using Operon primer H9 (5'TGTAGCTGGG3') and H16 (5'TCTCAGCTGG3') (Calvert et al. 2001). The reaction conditions for the first cycle were five min at 94°C, two min at

40°C and three min at 72°C. This was followed with 35 cycles of one min at 94°C, 1.5 min at 40°C, and two min at 72°C. The PCR products were run in agarose gels, stained with ethidium bromide, visualized using UV light, and recorder using the Eagle Eye system (Promega, WI, USA).

Development of SCARs. PCR products were purified using the Wizard™ PCR purification columns (Promega, WI, USA), and cloned into the plasmid TA cloning™ (Invitrogen, USA). Using the ABI dye terminator kit, the sequences were determined in an automated sequencer using the dideoxynucleotide chain termination procedure (Sanger et al., 1977). The sequences were analyzed and candidate oligonucleotides were designed. These were first tested using the whiteflies from the colonies, and those primers that amplified only one PCR product in the target whitefly were tested using whiteflies collected from the field. The identities of the unknowns were checked both morphology and RAPD analysis.

Results and Discussion

The primer H9 amplified several PCR products that appeared unique to either *B. tabaci* biotype A or to *B. argentifolii* **(Figure 3.1a)**. Two of these products amplified from *B. tabaci* biotype A were cloned, sequenced and primers were designed to produce SCARs. The better set of primers was H9BA1. When tested against five species of whiteflies, they amplified a product of the expected size for *B. tabaci* biotype A and a slightly larger product in *B. argentifolii* (biotype B) **(Figure 3.2a)**. Smaller products were amplified in *B. argentifolii*, *B. tuberculata*, and *T. vaporariorum*. While this set of primers is useful and easier to interpret than the RAPDs, the PCR products of expected size from both *B. tabaci* biotype A and *B. argentifolii* (biotype B) were cloned and sequenced. The sequences were compared and a four primers were designed. One set of these primers amplified a single product of 697 nucleotides in *B. tabaci* biotype A, and did not amplify and products in the other four species of whitefly that were tested **(Figure 3.2b)**.

Three amplified products were from *B. argentifolii* were cloned and sequenced **(Figure 3.1b)**. From each sequence, a set of primers was designed and tested. One set of these primers amplified products of the same size in both *B. argentifolii* and *B. tabaci* biotype B. The second set amplified multiple products in all five whiteflies tested. The third set amplified one product of 597 nucleotides **(Figure 3.3)**. There is a product of approximately 300 nucleotides amplified from *B. tuberculata*. There is also a very poorly amplified product in *B. tabaci* biotype A of approximately 610 nucleotides.

To develop the SCAR for *T. vaporariorum* the RAPD oligonucleotide Operon H16 was chosen **(Figure 3.4a)**. One amplified product of approximately 1300 bases was cloned and sequenced. From this sequence three forward and two reverse primers were derived. Most combinations amplified multiple products in various whitefly species that were tested. The best combination of primers amplified two products that were approximately 300 nucleotides. One of these was sequenced and was 303 nucleotides. No other products were amplified in the five whitefly species tested. This set of primers was tested using *T. vaporariorum* collected from Colombia, Ecuador and Guatemala.

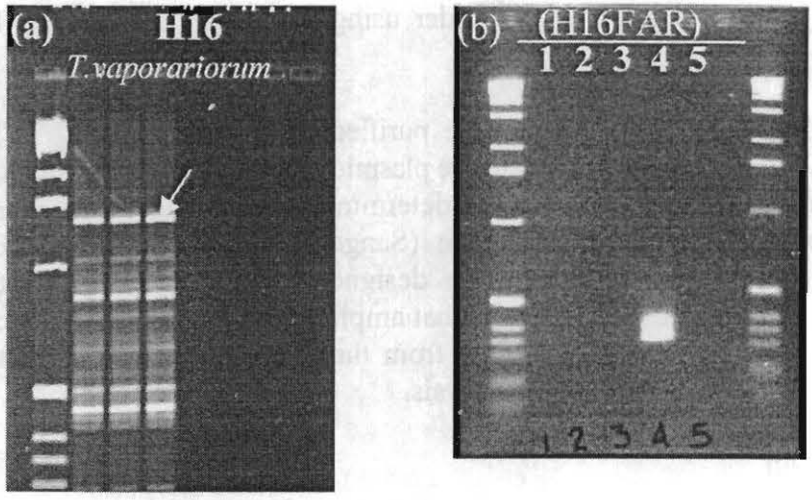

Figure 3.1. **RAPD profiles using operon primer H9. A: *B. tabacil* biotype A. B: *B. argentifolia* or biotype B. The markers are a 1 kb ladder. The arrow shows the amplified products that were cloned and sequenced.**

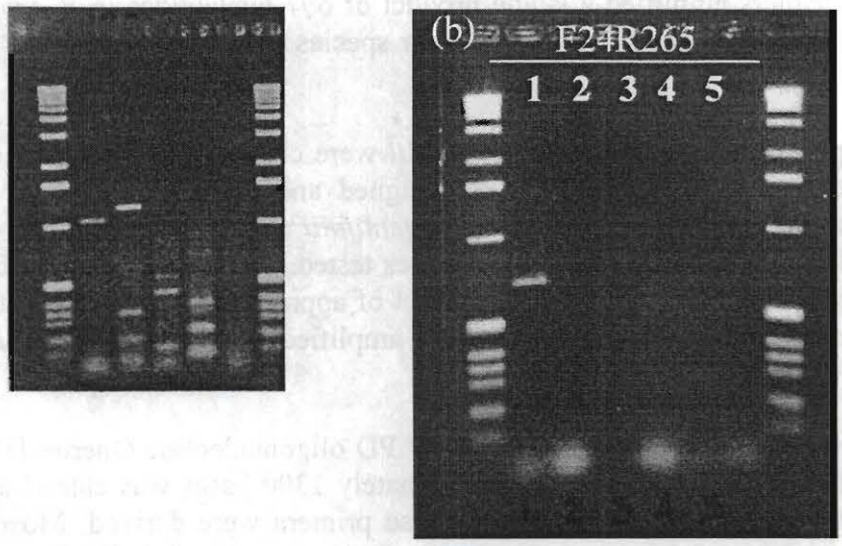

Figure 3.2. **1: *B. tabaci* biotype A, 2. *B. argentifolia* or biotype B, 3. *B. tuberculata,* 4. *T. vaporariorum*, 5. *A. scialis.* (a) first generation SCAR primer set H9BA1, (b) second generation SCAR primer set F24R265.**

Figure 3.3. 1: *B. tabaci* biotype A, 2. *B. argentifolia* or biotype B, 3. *B. tuberculata,* 4. *T. vaporariorum*, 5. *A. scialis.* first generation SCAR primer set H9BB1.

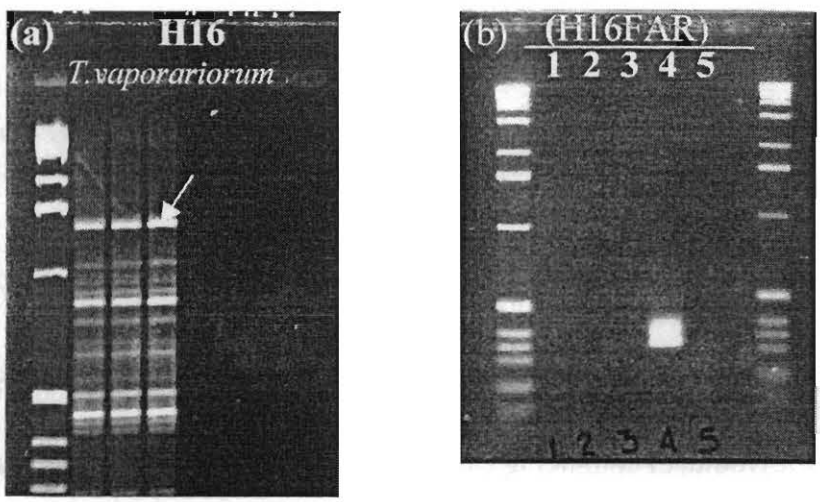

Figure 3.4. (a) RAPD profiles of *T. vaporariorum* using operon primer H16. The markers are a 1 kb ladder. The arrow shows the amplified product that was cloned and sequenced. (b) 1: *B. tabaci* biotype A, 2. *B. argentifolia* or biotype B, 3. *B. tuberculata,* 4. *T. vaporariorum*, 5. *A. scialis.* first generation SCAR primer set H16FAR.

The development of SCARs was an empirical process. In all cases, several combinations of oligonucleotides needed to be tested in order to select the most appropriate combination of primers. The experience developing primers to *B. tabaci* biotype A demonstrated the utility of using a two step process of SCAR development. In this case, the amplified product from both *B. tabaci* biotype A and *B. argentifolii* (biotype B) were sequenced and primers that only amplified a product from *B. tabaci* biotype A were successfully developed. The other strategy of designing multiply primers to the same sequence and then testing which combination was somewhat quicker, but our experience was that there were more anomalies with these primers. In the case of the primers developed for *B. argentifolii*, there was a product amplified to *B. tuberculata* and a very faint product to *B. tabaci* biotype A.

The only set of primers that was tested using whiteflies from other countries was the set developed to identify *T. vaporariorum*. In order to assure that these primers are robust, samples from many different countries need to be tested. This process is underway and will be complete soon.

The development of SCARs is important if the use of a PCR based test is to be devolved to scientist in tropical America. The simplicity of the amplified products allows the results to be clear and no interpretation of multiply and often confusing products is needed. At soon as the testing to assure that these sets of primer work over the range and diversity within tropical America, these primers will be made available to scientist in many countries and should aid in the identification of whiteflies of economic importance.

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L.A. Calvert, M. Cuervo, and B. Pineda. 2001. Cuero de Sapo: Una enfermedad que ataca la yucca. (More than 300 copies have been distributed).

Workshop

Taller Regional: Reconocimiento y Manejo de Enfermedades Transmitidas por Semilla de Yuca, Armenia, 31 de Mayo de 2001: Auditorio SENA Regional Quindío.

Collaborators

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Donor

CONTROL DE LA ENFERMEDAD DEL CUERO DE SAPO EN LA YUCA República de Colombia: Ministerio de Agricultura y Desarrollo Rural - MADR

Sub-output 7. Bean Diseases Complexes in Africa Described and Analyzed. (R. Buruchara)

Activity 1. Epidemiology of bean root rots: characterization of Pythium and Fusarium spp associated with bean roots in Uganda

Introduction

In East and Central Africa, where bean root rots are a serious problem, soil inoculum of the pathogens causing the diseases is one of the key factors thought to influence incidence and severity. Some of the management technologies under consideration are meant, in the long run, to reduce soil inoculum to below economic threshold levels. Evaluation of such technologies is usually based on disease severity, which alone is not always a good indicator of soil pathogen population because it can be influenced by environmental or host characteristics. There is therefore need to develop tools and procedures for quantifying pathogen populations particularly *Pythium* and *Fusarium* spp, to enable assessment of root rot management options (cultural, varietal and biological) on pathogen populations. However, characterization of the main root rots (Pythium and Fusarium spp) pathogenic to beans is an important prerequisite for these epidemiological studies.

Molecular characterization *Pythium* root rots from southwestern Uganda

Materials and Methods

Sixty-six Pythium isolates from Uganda characterized last year by morphological methods were characterized using restriction analysis and sequencing.

***Restriction Fragment Length Polymorphism Analysis*:** Genetic variability of among the *Pythium* isolates was determined by digesting extracted DNA using *Cfo* I, *Hinf* I and *Mbo* I endonucleases and separating fragments on 2% agarose.

***Sequencing of Amplified rDNA*:** DNA was extracted from 38 isolates representing different RFLP groups and amplification of the ITS 1 region of the ribosomal gene was done using the ITS1primer (White *et al.*, 1990). Nucleotides sequences were obtained using automated sequencer and were edited using SeqWeb Version 1.2. Multiple alignments of the reverse complement of the sequences were compared to *Pythium* species database sequences publicly available.

Results

ITS1/4 products of 66 isolates (plus 14 isolates from culture collection) analyzed grouped 63 isolates into seven RFLP groups; 17 isolates were not grouped. There was some relationship between morphological groupings and RFLP. In some instances RFLP groups contained isolates from more than one morphological group.

Although there was some consistency between RFLP and sequence analysis, results from the latter were better and the 38 isolates were grouped into nine species namely *P. vexans, P.*

tolorosum, P. spinosum, P. salpingophorum, P. ultimum, P. nodosum, P. echinulatum P. aphanidemetum and P. pachycaule. Pathogenicity studies are in progress to assess variability in virulence between and within species.

Activity 2. Characterization of *Fusarium solani* f. sp. *phaseoli* in Uganda

Materiasl and Methods

Forty *Fusarium solani* f. sp. *phaseoli* isolates from Uganda were characterized using pathogenicity and molecular methods. Five isolates from international culture collection centers and other *formae speciales* of *F. solani* were included for comparison.

***Amplified fragment length polymorphism (AFLP) analysis of F. s. f.sp. phaseoli isolates*:** Isolates were subjected to AFLP analysis using 15 primers and the products separated by agarose gel electrophoresis, visualized under UV light and captured by photography. Presence or absence of bands was scored and analyzed and a dendogram showing relative similarity was generated using NTSYS-pc (Ver. 2.01i)

***Virulence characterization of F. s. f.sp. phaseoli isolates*:** Pathogenicity of isolates was determined by planting seed of bean cultivar, K20 on artificially infested soil (3000 – 4000 conidia per gram of soil) in plastic pots in the screenhouse. Four weeks later, plants were assessed for root rot severity using a CIAT scale of 1-9.

***Development of molecular detection techniques for F. s.* f. sp. *phaseoli*:** The aim of this study was to develop specific primers that would be used to detect pathogenic isolates of *F. s.* f. sp. *phaseoli*. Eleven isolates were used; five pathogenic and four non-pathogenic but isolated from beans with fusarium root rot symptoms. *F. s.* f. sp. *fabae* (IMI 172300) and *F. s.* f. sp. *pisi* (MUCL 906), which cause fusarium root rots of broad bean (*Vicia faba*) and peas (*Pisum sativum*), respectively were included. AFLPs were used to determine unique bands within the genome of the pathogenic isolates. Six bands were recovered, purified, quantified, and sequenced. The 3 longest sequences were visually inspected for suitable sequences for primer design. Four pairs of primers were designed, synthesized (by Sigma-Genosys Ltd. (UK)) and tested for specificity on a limited number of pathogenic and non-pathogenic *F. s.* f. sp. *phaseoli*. The most promising pairs were then tested on more *F. solani* isolates and other Fusarium species.

Results

***Molecular and cultural variation*:** On the basis of AFLP analysis, the isolates could be divided into 2 broad groups with little intra-group variation (Fig 1). One group consisted of isolates with light-yellowish mycelia, with relatively fast growth rate (at least 6mm per day) on PDA at 22^0C and produced numerous micro- and macro-conidia. The second group was made of isolates that grew relatively very slowly (not exceeding 1.8mm per day), produced less mycelia that was initially whitish but later turned bluish with production of conidia and produced macro-conidia only.

***Variation in virulence*:** All slow growing isolates caused lots of root symptoms (severity above 6.5) and hypocotyls 4 weeks after inoculation while little (severity of less than 2.5) or no apparent symptoms were observed with the fast-growing isolates.

***Molecular detection technique*:** The four primers designed were tested for specificity in a PCR on pathogenic and non-pathogenic *F. solani,* other *Fusaria* and *non-Fusaria* fungi. Two primer pairs (FSP1F-BEAT/FSP1R-BEAT and FSP4F-DETT/FSP4R-DETT) were specific and amplified expected fragment sizes of 180 and 287 bp, respectively from pathogenic isolates only, while the other two pairs, (FSP2F-CETT/FSP2R-CETT and FSP3F-DETT/FSP3R-DETT) were non-specific and amplified fragments from pathogenic and non-pathogenic *F. solani*, other *Fusarium* spp. and also from other fungal species used in the study.

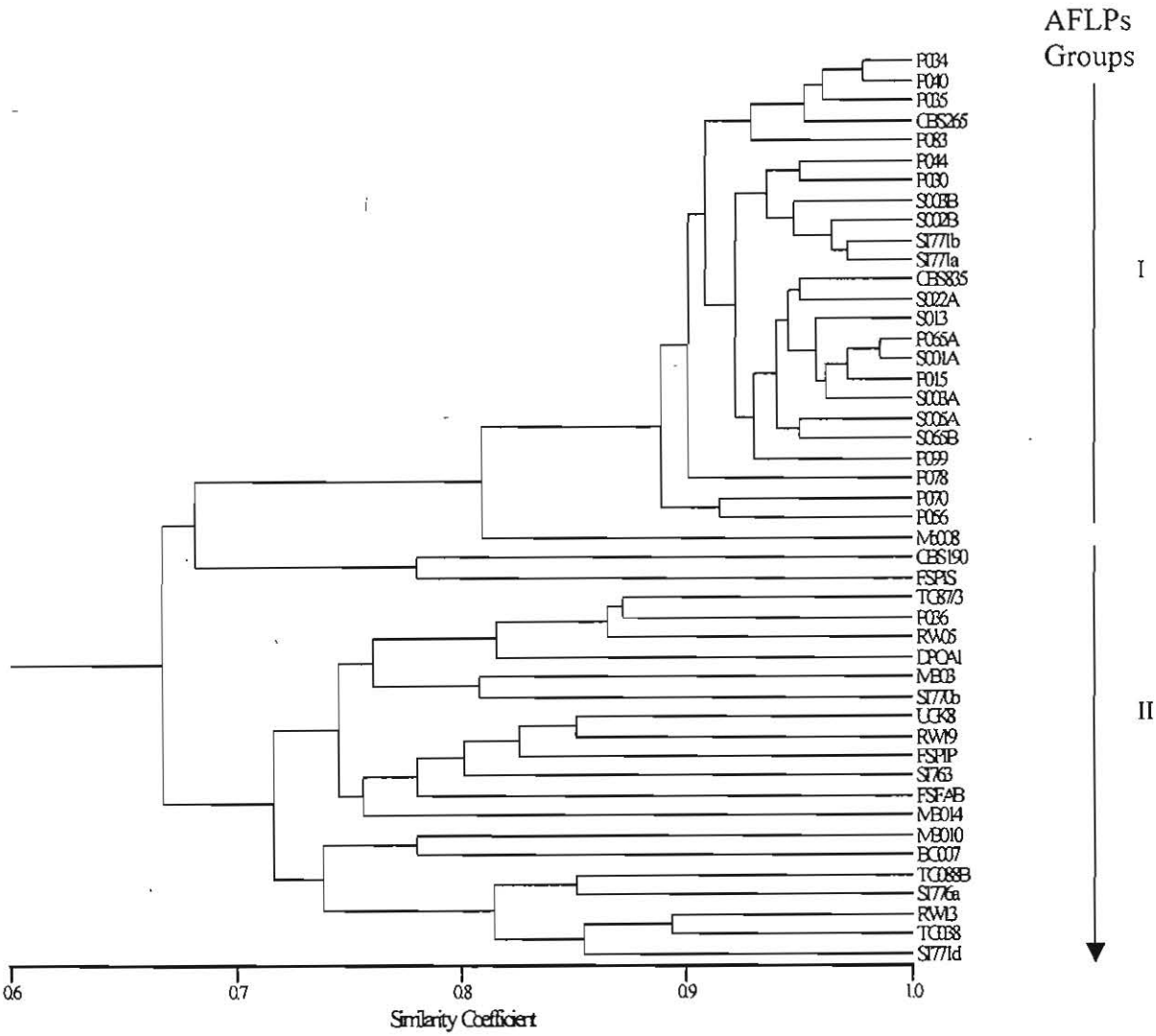

Figure 2.1. Dendogram of *F. solani* isolates based on UPGMA methods using SAHN and tree program in NTSYS showing relative similarity based on AFLPs data. Isolates in group I and II also gave pathogenic and non-pathogenic reaction respectively on Phaseolus bean cultivar K20.

Our studies resulted in the recovery of two groups (pathogenic and non-pathogenic) of *F. solani* isolated from bean plants and distinguished by pathogenicity and molecular methods. On the basis of fungal isolates tested so far, two primers developed, show promise in developing molecular diagnostic techniques for *F. solani* f. sp. *phaseoli*. Their specificity and capacity to detect pathogenic forms in the soil should complement cultural methods in facilitating subsequent epidemiological studies.

Activity 3. Adaptation of specific PCR based markers to characterize and differente *Pythium* spp

Introduction

Pythium root rot is the most destructive soilborne disease of beans in East and Central Africa and development of effective management strategies against the disease, requires accurate, reliable, and rapid detection assays. A diagnostic test has been developed for the detection of most known species (particularly in the temperate) of Pythium using Reverse Dot Blot Hybridization (RDBH) (Levesque, 1998). It is based on species-specific oligonucleotides that have been designed and blotted on to a membrane array. There are approximately 100 species in the genus *Pythium*. Some *Pythium* species are highly pathogenic, some are almost exclusively saprophytes and some species are biological control agents. Given the wide genetic variation within the *Pythium* genus, it is almost certain that some strains (pathogenic or beneficial) particularly in the tropics could be novel. This study was initiated to adopt the reverse dot blot hybridization technique to rapidly identify the different species commonly associated with bean root rots in East and Central Africa. Because the sequences used to develop the probes were obtained exclusively from *Pythium* spp found in temperate zones, the first step was to test the suitable of this assay for tropical zones. The hypothesis was that some *Pythium* populations are only found in tropical zones.

Materials and Methods

This work was done in Canada. DNA from 100 *Pythium* isolates collected from Uganda, Kenya and Rwanda was carried to Canada for analysis. All isolates were amplified with primers targeting the ITS regions of the ribosomal genes and specific to *Pythium* spp. This first step allowed the differentiation of *Pythium* and non-*Pythium* species. Direct sequencing of the PCR fragment was done using primers that annealed inside of the first fragment and the sequencing products were run on and ABI prism automated sequencer. After editing the sequences were compared to sequences of known *Pythium* species from the *Pythium* database managed by Dr A. Levesque.

Results and Discussion

Amplification with *Pythium* specific primers identified 17 isolates as *Mortierella* spp. **(Figure 3.1)**. The rest of the isolates were identified, as *Pythium* spp. *Mortierella* is a common saprophyte that can be isolated on *Pythium* specific media. Morphologically, it cannot be differentiated from *Pythium*. This molecular method (using Pythium-specific primers) is a very

useful tool that can be used to eliminate *Mortierella spp* from the collection of *Pythium*. Sequence analysis of the isolates identified 12 different species. Of the four species reported to infect beans, only *Pythium ultimum* and *P. irregulare* were identified and *P. ultimum* var *ultimum* had the highest incidence (25), showing that this species is the most prevalent in the areas under study. Other species identified included *P. acanthicum, P. dissotocum, P. indigoferae, P. oligandrum, P. salpingophorum, P. spinosum, P. tolorusum,* and *P. vexans*. Of interest were new putative species found in association with beans, and whose sequences were significantly different from their closest match (*Pythium torulosum)* among the neo (type) strains implying that they could be novel. Additional characterization studies are underway to look at the morphology and pathogenicity of these of these isolates, as well as other parts of the genome to establish if indeed these isolates represent new species within the genus *Pythium*. Also of interest was the occurrence *Pythium oligandrum,* a known potent biocontrol agent, effective against a number of soil borne pathogens including *Pythium* species. We are currently verifying its effectiveness to manage soil-borne pathogens. Other species identified, *P. vexans* and *P. indigoferae,* have been implicated as possible biocontrol agents.

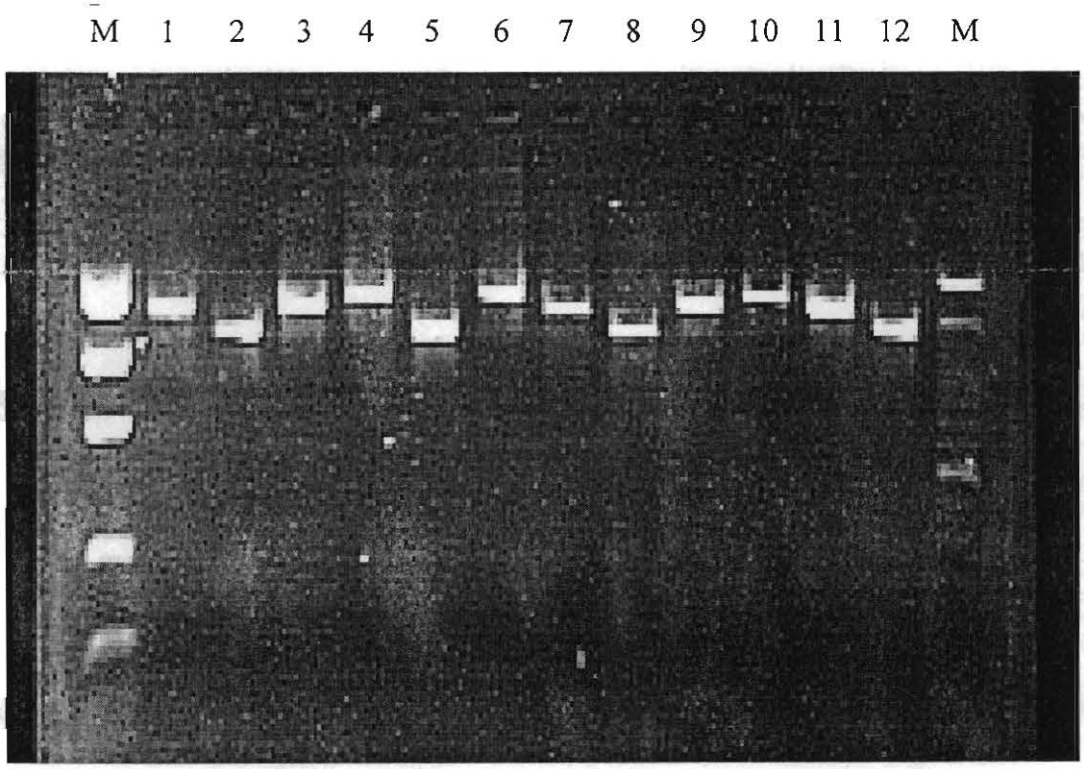

Figure 3.1. Banding patterns from amplifying ribosomal DNA spacer region using Oomycete specific primers and used to distinguish between *Mortierella* spp and *Pythium* spp. Lanes 1, 3,4, 6,7and 9 –11 represents DNA from *Pythium* spp, while DNA in lanes 2, 5, 8 and 12 contains DNA from *Mortierella* spp. Lane M a DNA molecular ladder.

Conclusion

This study demonstrated the complex nature of the genus *Pythium*. A total of 12 *Pyhtium* species were identified from bean fields in Uganda, Rwanda and Western Kenya. Of these species, *Pythium ultimum* var *ultimum* was the most prevalent. This species has been reported as the most important incitant of bean root rots. Two isolates that are potential biological control agents were identified. Studies have been initiated to test the effectiveness of these isolates as biocontrol agents against *Pythium ultimum* var *ultimum* and other pathogenic soil borne species. More samples are being collected from these areas to have an extensive coverage of the bean growing areas experiencing root rot problems. In addition, DNA microarrays are being adopted for fast detection and identification of *Pyhtium* species in order to accelerate the diagnosis process.

Activity 4. Characterization of pathogen diversity of *Phaeoisariopsis griseola* in Africa

Introduction

Pathogen variability limits deployment, effectiveness and durability of resistance. Understanding diversity structure and distribution for a variable and economically important pathogen as *Phaeoisariopsis griseola* is important in designing strategies for deploying durable resistance. Characterization done so far in Africa, show occurrence of Mesoamerican and Andean pathogen groups. In addition, an Andean subgroup, the Afro-Andean, has been identified. Knowledge of this diversity and distribution in many African countries, where ALS is important, is inadequate. Continuous monitoring for emerging new races is also essential.

Materials and Methods

An extensive collection was initiated in an effort to characterize pathogen diversity in Kenya, Uganda and Rwanda, giving consideration to spatial, ecological, cropping systems and varietal variation. A total of 16 isolates from different districts of Kenya were characterized on the basis of a set of 12 host differentials (6 Andean and 6 Mesoamerican).

Results and Discussion

Twelve races comprising Mesoamerican, Andean and Afro-Andean pathogen groups were identified in Kenya from 16 isolates collected showing a high degree of variability. Further characterization is underway and information will go towards developing a race distribution map for *P. griseola* in Africa and as a basis for monitoring of new races.

Table 4.1. Virulence diversity of *P. griseola* in Kenya.

Isolate Identification	Race	Andean						Mesoamerican					
		A[x]	B	C	D	E	F	G	H	I	J	K	L
Eb-5	10-0		b		D								
Eb-8	14-0		b	c	D								
Tt-10	14-0		b	c	D								
Mk-1	31-32	A	b	c	D	e							l
Eb-11	34-0		b				f						
Eb-15	46-0		b	c	D		f						
Eb-3	58-18		b		D		f		h			k	
Eb-2	6-0		b	c					h	i			
Tt-4	62-32		b	c	D	e	f						l
Kb-10	62-39		b	c	D	e	f	g	h	i			l
Eb-17	63-39	A	b	c	D	e	f	g	h	i			l
Eb-1	63-55	A	b	c	D	e	f	g	h	i		k	l
Eb-10	63-55	A	b	c	D	e	f	g	h	i		k	l
Mk-5	63-55	A	b	c	D	e	f	g	h	i		k	l
Eb-24	63-7	A	b	c	D	e	f	g	h	i			

[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.

Activity 5. Pathogen population structure of *Phaeoisariopsis griseola* in varietal mixtures

Introduction

Mesoamerican and Andean pathogen groups of *Phaeoisariopsis griseola* occur both in Africa and Latin America. But the occurrence, only in Africa, of the Andean sub-group (Afro-Andean) is thought to have been influenced by farming practices that include growing together or as varietal mixtures, germplasm belonging to the two major Phaseolus genepools (Mesoamerican and Andean). Studies initiated last year continued, to determine the population structure and diversity of *P. griseola* in varietal mixtures, its significance and implication in developing management strategies for ALS.

Materials and Methods

Twenty-five isolates were recovered from naturally infected bean plants grown at Kawanda research station, consisting of two variety mixtures (originally from Rwanda, and southwest Uganda) and from determinate cultivars K131 (small seeded), CAL 96 (large seeded) and a local variety Kanyebwa (medium). Kisoro mixtures consisted mainly of indeterminate medium seed sized components while the SOH mixtures consisted of determinate and semi-climber large, medium and small seeded types.

Molecular variation of the 25 isolates was assessed using two Random Amplified Microsatellites (RAMs) primers [(CA)n and (GT)n].

Results and Discussion

The isolates could be separated into two broad groups. The first group with the majority of isolates (20) was largely derived from medium and large seeded components of the two varietal mixtures while the second group (5 isolates) was recovered from mainly small seed components. Most isolates (in group 1) belonged to Andean pathogen group and a possibility of including some Afro-Andean given the infection on small seeded Mesoamerican varieties. The results so far indicate that type of varieties in mixtures influence pathogen groups recovered from them, and therefore the pattern of variation of the pathogen.

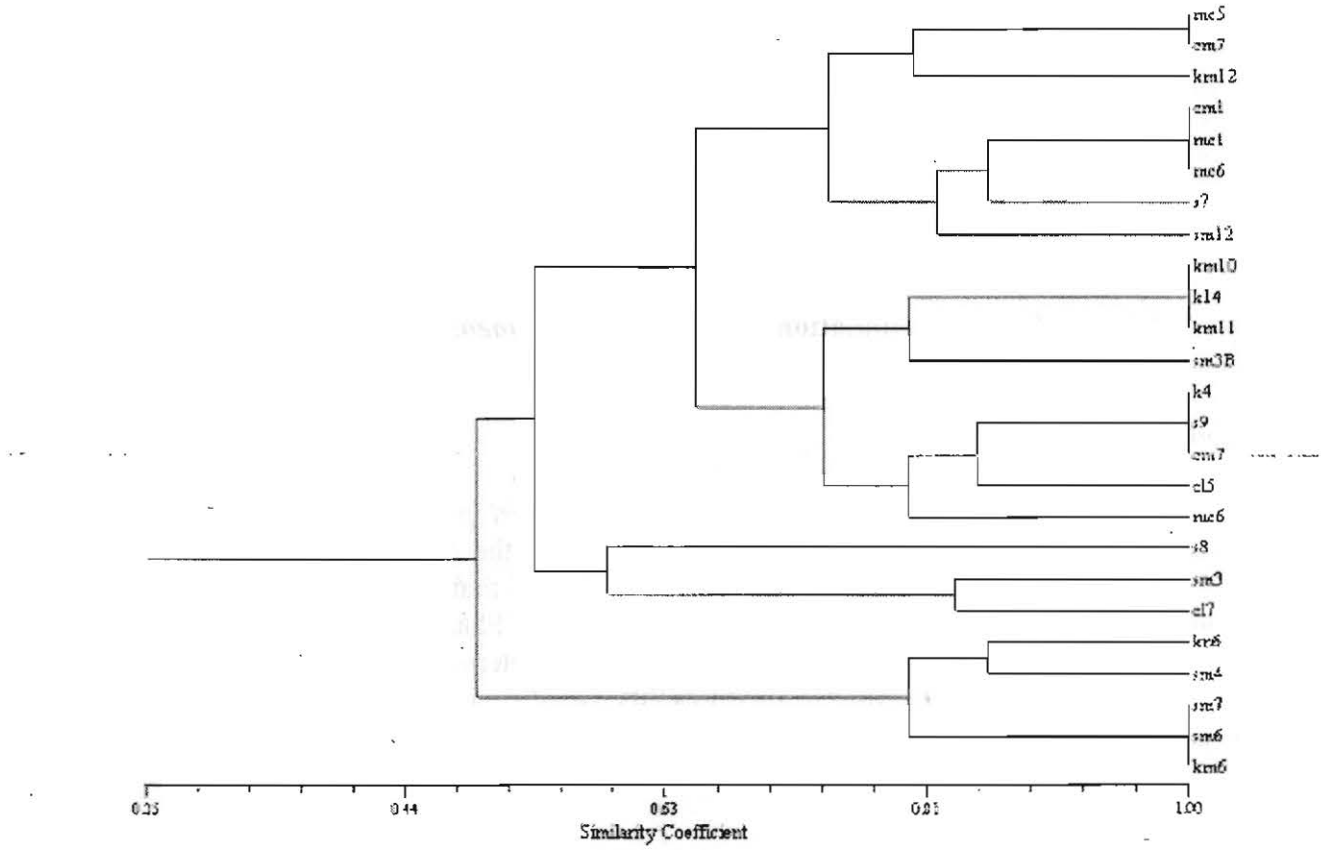

Figure 5.1. A dendogram of 25 isolates of *Phaeoisariopsis griseola* based on UPGMA analysis of random amplified microsatellites [(CA)n and (TG)n] primers data using NTSYS-pc ver 2.02i

Contributors

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

Sub-output 1. An Integrated Control Method for Cassava Root Rots in Colombia (E. Alvarez)

Activity 1. Evaluation of hot-water treatment of cassava cuttings, and the effect on CBB in the field at Santander and Quindío

Cuttings from three cassava varieties growing in Quindío Department, affected by cassava bacterial blight (CBB), were treated to evaluate the severity of CBB on shoots and saprophytes on stakes. Cuttings were immersed in water at 49 °C for different times (thermotherapy), given 5 minutes immersion in a chemical suspension (Captan, 1.5 g/Lt and Benomyl, 1.5 g/Lt) or Ecolife® 1 cc/Lt (suspension from citrus seed).

Previous treatment by thermotherapy for 10 min, 24 h before thermotherapy during 1 h, and thermotherapy for 49 min without pre-treatment, did not affect the number of shoots/cuttings nor shoot length. In CM 523-7 and HMC-1, thermotherapy increased the number of shoots per stake. Using thermotherapy for a long time increased the presence of saprophytes on stakes. Ecolife and chemical treatment were not effective in controlling saprophytes grown when cuttings were treated by thermotherapy. Although CBB severity was low, in HMC-1 (the susceptible variety) CBB had 1.8 on the severity scale, while thermotherapy over a long time after pre-treatment decreased the disease more than thermotherapy at 1 h after pre-treatment or 49 min without pre-treatment.

Table 1.1. Effect of cassava-cuttings treatment by thermotherapy to control saprophytes and *Xanthomonas axonopodis* pv. *manihotis* affecting stakes.

Variety	Treatment	Shoot length (cm)	Shoots/Cutting	Saprophytes on Stakes[a]	CBB[a]
CM 523-7	10 min + 1 h	25.6	5.0	1.5	1.3
CM 523-7	10 min + 2 h	14.3	4.2	1.8	1.2
CM 523-7	10 min + 3 h	10.3	4.4	1.8	1.0
CM 523-7	10 min + 4 h	6.0	2.4	1.8	1.2
CM 523-7	10 min + 5 h	1.1	0.6	2.5	1.0
CM 523-7	10 min + 5 h + Ecolife	0.0	0.0	3.0	-[b]
CM 523-7	10 min + 5 h + chemical	0.0	0.0	4.0	-
CM 523-7	5 h	4.9	1.3	2.0	1.5
CM 523-7	5 h + chemical	0.3	0.4	2.2	1.0
CM 523-7	49 min	24.7	3.7	1.7	1.7
CM 523-7	49 min + Ecolife	18.7	4.2	1.3	1.6
CM 523-7	49 min + chemical	17.9	4.8	1.3	1.6
CM 523-7	Ecolife	18.7	4.3	1.7	1.7
CM 523-7	Chemical	27.1	3.4	1.2	1.4
CM 523-7	Control	23.2	3.0	2.3	1.4
HMC -1	10 min + 1 h	15.4	5.0	2.2	1.4
HMC -1	10 min + 2 h	18.0	4.4	2.0	1.2
HMC -1	10 min + 3 h	5.9	4.8	2.3	1.3
HMC -1	10 min + 4 h	1.0	1.4	2.3	1.0
HMC -1	10 min + 5 h	0.1	0.3	3.0	1.0
HMC -1	10 min + 5 h + Ecolife	0.0	0.0	4.0	-

Variety	Treatment	Shoot length (cm)	Shoots/Cutting	Saprophytes on Stakes[a]	CBB[a]
HMC -1	10 min + 5 h + chemical	0.0	0.0	4.0	-
HMC -1	5 h	0.0	0.0	1.0	-
HMC -1	5 h + chemical	0.0	0.0	2.0	-
HMC -1	49 min	21.0	7.0	2.0	1.0
HMC -1	49 min + Ecolife	14.7	4.4	1.7	1.2
HMC -1	49 min + chemical	14.3	4.0	1.2	1.2
HMC -1	Ecolife	12.0	2.5	2.0	1.0
HMC -1	Chemical	16.5	4.4	1.8	1.4
HMC -1	Control	19.1	4.0	2.0	1.8
M Per 183	10 min + 1 h	18.8	4.6	1.5	1.2
M Per 183	10 min + 2 h	13.9	5.6	1.5	1.0
M Per 183	10 min + 3 h	14.3	6.6	2.0	1.0
M Per 183	10 min + 4 h	8.8	3.2	2.2	1.0
M Per 183	10 min + 5 h	2.0	1.6	2.0	1.0
M Per 183	10 min + 5 h + Ecolife	0.4	0.8	1.3	1.0
M Per 183	10 min + 5 h + chemical	0.8	0.5	2.0	1.0
M Per 183	5 h	0.2	0.3	2.0	1.0
M Per 183	5 h + chemical	0.0	0.0	2.3	-
M Per 183	49 min	11.0	5.0	2.0	1.0
M Per 183	49 min + Ecolife	12.1	5.4	1.8	1.0
M Per 183	49 min + chemical	13.9	6.0	2.0	1.3
M Per 183	Ecolife	14.3	4.5	5.0	1.0
M Per 183	Chemical	14.8	3.8	1.7	1.0
M Per 183	Control	11.3	4.4	2.0	1.4

[a] Scale: 1= no incidence, 5= high incidence and severity.
[b] Stakes not germinated.

Activity 2. Evaluating practices for managing root rots in cassava

To evaluate those crop management practices that, in previous studies, have shown promise in reducing root rots, we conducted trials with the active participation of farmers and UMATA technicians in the Departments of Cauca and Quindío.

2.1. Department of Cauca

In the Department of Cauca, two trials were established in the village districts of San Jerónimo and Mondomito, Municipality of Santander of Quilichao, to evaluate the control of some practices over *Phytophthora* spp., fungi which induce root rot.

The following treatments were evaluated for their effect on the incidence and severity of root rots:

Treatment

1. 2.5 t/ha chicken manure + 300 kg/ha of the chemical fertilizer Agropremix® (15% N, 10% P_2O_5, 12% Zn, 2% B, 0.75% Cu, 3% S, and 0.01% of Mo)
2. 2.5 t/ha chicken manure + potassium sulfate (180 kg/ha K_2O)
3. 2.5 t/ha chicken manure + potassium chloride (180 kg/ha K_2O)
4. 2.5 t/ha chicken manure + thermotherapy (stakes immersed in water heated over a wood fire to 49°C for 49 min)
5. *Trichoderma* strain 14PDA-4 (1×10^4 conidia/mL)
6. *Trichoderma* strain 19TSM-3A (1×10^4 conidia/mL)
7. Cassava variety La Reina (CM 6740-7)
8. Stake selection
9. 2.5 t/ha chicken manure (traditional farmer's practice)

For all treatments, chicken manure was incorporated at 2.5 t/ha. The cassava regional variety Verdecita (M Col 1505) was planted with vegetative seed obtained from a farm located in San Jerónimo, where the disease was present. The two best strains of the *Trichoderma* fungus were selected to control *Phytophthora* spp. in *in vitro* tests and in the greenhouse. Cassava stakes were inoculated with *Trichoderma* by immersion for 10 min in a suspension with a concentration of 1×10^4 conidia/mL. We then applied 100 mL of the suspension at the base of each plant, and again every 45 to 60 days throughout the crop's cycle. Stakes were selected for their health and from the middle parts of stems.

The experimental design used for these plantings was a randomized complete block design with three replicates and 20 plants per treatment. Treatment 6 was applied only in San Jerónimo.

Following farmers' customs, for the San Jerónimo trial, dolomitic lime was applied at 500 kg/ha and fertilizers were applied 35 days after planting. In contrast, in Mondomito, fertilizers were applied at planting and no lime was applied. The performance of the elite genotype CM 6740-7 ('La Reina') was evaluated.

In the Santander de Quilichao trial, heat treatment did not affect germination (data not shown). The 'Verdecita' planting material was of lesser quality, whereas 'La Reina' had a high germination rate.

Plant height and stake production per plant were greatest when the trial was fertilized with Agropremix. **Table 2.1** shows the effect of the treatments on yield and incidence of rotten roots. All treatments surpassed the control in stake production per plant. Yield under all treatments in San Jerónimo was very low because of low-fertility soil and the plot's history of six cassava crops previous to the trial. Chemical fertilization did not increase yield, whereas treatments with *Trichoderma* 14 PDA-4 and selection of stakes improved yields by 33.6% and 25.8%, respectively, although root-rot incidence was higher than for the control. In contrast, *Trichoderma* 19 TSM-3A helped reduce root rots. Potassium sources also helped reduce rots. The variety La Reina showed no root rots.

The Mondomito trial could not be harvested because of public order problems.

Table 2.1. Effect of root-rot management on yield and incidence of rotten roots, Farm "Villa Fernanda", San Jerónimo Village District, Santander de Quilichao, Cauca.

Treatment	Plant Height (m)	Stake Production per Plant	Yield (T/ha.)	Root Rot Disease		
				Incidence (% Affected Plants)	Severity (Kg. Affected Roots/ha.)	Percentage of Affected Roots
Agropremix	2.1	10.2	3.63	14	183	4.8
K_2SO_4	1.9	8.4	3.2	5	50	1.5
KCl	2	8.5	3.6	5	67	1.8
Stake selection	2	9.4	4.38	4	150	3.3
Thermotherapy	2	8.2	3.95	23	150	3.7
Control, traditional farmer's practice	1.9	7.9	3.48	16	100	2.8
Trichoderma strain 14PDA-4	2	8.6	4.65	17	175	3.6
Trichoderma strain 19TSM-3A	2	9.1	3.15	5	33	1.0
Cassava variety Reina (CM 6740-7)	2.8	8.4	5.15	19	0	0.0

2.2. Department of Quindío

The different control practices for *Phytophthora* spp. were evaluated for disease incidence and severity, and for yield in four field trials in the Municipalities of Montenegro and La Tebaida.

Two experiments were established on the Farms "El Jardin" (La Tebaida) and "Guayaquil" (Montenegro) to evaluate the effect of some management practices for controlling *Phytophthora* spp. Variety HMC-1 was used, and the treatments were as follows:

Treatment

1 Fertilization with KCl (180 kg/ha K_2O).
2 Fertilization with K_2SO_4 (180 kg/ha K_2O).
3 Farmer fertilization: Farm "El Jardin" applied 350 kg/ha of a mixture of ammonium sulfate and borax at a rate of 50:1.5; Farm "Guayaquil" applied 500 kg/ha of a mixture of Nitrax-DAP-KCl at a rate of 1:2:2. Fertilizers were applied 45 days after planting.
4 Stakes given thermoterapy (49°C for 49 min).
5 Stakes immersed for 5 min in Orthocide® (captan, 4 g/L of the commercial product) and Ridomil® (metalaxyl, 3 g/L of the commercial product).
6 Stakes immersed in Lonlife® (ascorbic acid) at 4%.
7 Biological control: stakes immersed for 10 min in a suspension of *Trichoderma* (1×10^4 conidia/mL), strains 19TSM-3A and 41 PDA-3A. The area around the stake was treated with 100 mL/plant of the fungal suspension.
8 Varietal resistance, using genotypes 'HMC-1', 'ICA Catumare', 'M Per 183' ('Peruana'), and the local variety 'Chiroza' (M Col 2066).

The experimental design was a randomized complete block design, with three replicates and 20 plants per treatment. The treatments with thermoterapy and *Trichoderma* were as described for the trials in Cauca (Treatments 4, 5, and 6).

The highest yields were obtained with the crop management practices suggested by CIAT: stake immersion and periodic applications of a suspension of the biological agent *Trichoderma* strain 14 PDA-4. Compared with local practices, applications of potassium sulfate and potassium chloride improved yield. The incidence of drying was only 13% (scale of 2 or 3), a low level for evaluating the effects of treatments. In general, germination and plant development were good. The application of Micobiol® increased plant height considerably **(Table 2.2)**. The percentage of germination in the Quindío trials was reduced by heat treatment (40%-68.3%), compared with the same treatment in Cauca (98.3%), probably because the temperature at the bottom of the oil drum was too hot, being higher than 49°C. These results suggest that more trials should be carried out to adjust the technique. Old and/or very thin stakes are probably more negatively affected by temperatures.

Table 2.2. Effect of stake treatments, including hot water, biocontrol, chemical control, fertilizers, and varietal resistance, on cassava development, root rot disease, and cassava bacterial blight in a trial established in the Department of Quindío, Colombia.[a]

				Bacterial Blight		Root Rot Disease		
Control Practices	Plant Height (m)[b]	Root Yield (T/ha.)	Number of Stakes per Plant	Incidence (% Affected Plants)	Severity (%)	Incidence (% Affected Plants)	Severity (T Affected Roots/ha.)	% Affected Roots
Variety HMC-1								
Thermotherapy[c]	1.73	62 a	36 a	21 a	89	2 a	3.7 a	5.6
Biocontrol with Trichoderma spp.[d]	1.89	63 a	36 a	16 a	89	2 a	1.8 a	2.8
Micobiol®[e]	2.31	60 a	37 a	12 a	56	1.3 a	0.2 a	0.3
Ridomil (metalaxyl)	1.91	70 a	39 a	16 a	89	1.7 a	1.0 a	1.4
Potassium chloride (KCl)	1.90	70 a	37 a	18 a	100	2 a	0.3 a	0.4
Potassium sulfate (K_2SO_4)	1.90	80 a	38 a	24 a	100	2 a	1.1 a	1.4
Local varieties								
Manzana	1.93	41 a	36 a	21 a	100	2 a	7.1 a	14.8
HMC-1	1.86	51 a	37 a	22 a	100	1.8 a	6.1 a	10.7

a. Duncan's multiple range test, alpha <= 0.05.
b. At 7 months after planting.
c. Oil drum on wood fire, with the water's temperature at 49°C for 49 min.
d. Strain 14 PDA-4.
e. Contains *Trichoderma* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Hirsutella thompsonii*, and *Bacillus thuringiensis*.

Germination of plants treated with thermotherapy was very low (26.7%-68.3%; **Table 2.3**), compared with the same treatment in Cauca (98.3%). The temperature was possibly inappropriate because a different container was used to that in the Cauca experiments. At Farm "El Jardin", the highest cassava yield was obtained with 'ICA Catumare', which surpassed by more than 20 t/ha the varieties HMC-1, Chiroza, and M Per 183, whose yields ranged between

32.0 and 38.7 t/ha. At Farm "Guayaquil", 'ICA Catumare' and 'HMC-1' surpassed 'Chiroza' (**Table 2.4**).

Table 2.3. Germination rate of cassava on three farms, Quindío, Colombia[a].

Treatment	Farm			Average
	El Jardín	Las Mercedes	Guayaquil	
Fertilization				
KCl (30 g/plant)	88.3	66.6	96.7	83.9
K_2SO_4 (36 g/plant)	91.7	95	95	93.9
Control of the farmer[b]	91.7	85	88.3	88.3
Control without fertilization	93.3	93.3	86.7	91.1
Stake Treatment				
Thermotherapy (49°C during 49 min)	40	26.7	68.3	45.0
Orthocide® (4 g/L) + Ridomil® (3 g/L)[c]	100	96.7	73.3	90.0
Longlife® 4%	-	-	96.7	96.7
Biological Control				
Trichoderma (strain 19TSM-3A)	88.3	98.3	95	93.9
Trichoderma (strain 41PDA-3A)	96.7	71.7	90	86.1
Varietal Resistance				
HMC-1	100	100	91.7	97.2
ICA Catumare	88.3	86.7	89.8	88.3
M Per 183	93.3	95	-	94.2
Chiroza	93.3	83.3	70	82.2

a. All treatments, except Treatment 7, used variety HMC-1.
b. Farm "El Jardin": ammonium sulfate + borax (50:1.5) at 300 kg/ha. Farm "Guayaquil": Nitrax-DAP-KCl (1:2:2) at 500 kg/ha.
c. At Farm "Guayaquil", Orthocide® was replaced by copper oxychloride.
d. Oil drum on wood fire, with the water's temperature at 49°C for 49 min.

Farmers' fertilization management, which involved high doses, led to the highest yields, but also to the highest incidence of root rots. Although Farm "Guayaquil" obtained the higher yield (28.9 t/ha) with the *Trichoderma* strain 41 PDA-3A, it was not consistent with what happened on Farm "El Jardin", where yield (28.3 t/ha) was much lower than the control without fertilizer (47.9 t/ha; **Table 2.5**).

When potassium sulfate was used, root rots were not present. Stake treatment with Longlife® led to the greatest reductions of root rots. The varieties most affected by root rots were Chiroza and M Per 183, whereas variety HMC-1 had the least root rots. The *Trichoderma* strain 19 TSM-3A helped perceptibly to reduce root rots, although the resulting yields were not good (**Table 2.5**).

At Farm "El Jardin", 65-day-old plants were affected by the bacterium *Xanthomonas axonopodis* pv. *manihotis* in some treatments. The bacterium was not present in treatments with K_2SO_4, thermotherapy, nor in the genotypes 'ICA Catumare' and 'Chiroza', which have shown

acceptable resistance to the disease, whereas 'HMC-1' and 'M Per 183' are susceptible. As the crop aged, incidence of the bacterium became insignificant.

Table 2.4. Effect of management practices for root rots on plant growth in cassava, Farm "El Jardin", La Tebaida, Quindío, and Farm "Guayaquil", Montenegro, Quindío.

Treatment	El Jardín		Guayaquil		Average	
	Plant Height (m)	No. of Stakes/ Plant	Plant Height (m)	No. of Stakes/ Plant	Plant Height (m)	No. of Stakes/ Plant
Fertilization						
KCl (180 Kg/ha K_2O)	1.81	8.4	2.14	9.9	1.98	9.2
K_2SO_4 (180 Kg/ha K_2O)	1.92	10.3	1.92	7.1	1.92	8.7
Control farmer[a]	1.88	11.8	1.86	8.9	1.87	10.4
Control without fertilization	1.89	10.1	1.89	9.2	1.89	9.7
Stake Treatment						
Thermotherapy (49°C during 49 min)	1.92	9.7	1.86	6.5	1.89	8.1
Orthocide® (4 g/L) + Ridomil® (3 g/L)[b]	1.74	9.0	1.85	8.7	1.80	8.9
Lonlife® 4%	-	-	2.14	8.5	2.14	8.5
Biological Control						
Trichoderma strain 41PDA-3A	1.75	8.2	2.07	7.7	1.91	8.0
Trichoderma strain 19TSM3A	1.88	10.7	1.92	6.9	1.90	8.8
Varietal Resistance						
Chiroza	2.59	18.3	2.34	17.2	2.47	17.8
HMC-1	1.81	10.7	2.30	10.5	2.06	10.6
Ica Catumare	2.03	11.0	2.80	13.9	2.42	12.5
M Per 183	1.82	10.2	-	-	1.82	10.2

At Farms "Las Mercedes" and "El Jardin", where incidence of cassava bacterial blight is high, some 35-day-old plants were evaluated as being affected by *Xanthomonas axonopodis* pv. *manihotis* in treatments with KCl, farmers' control, *Trichoderma* spp., chemical control, and in genotypes 'M Per 183' and 'HMC-1'.

2.3. Comparing Departments

Table 2.6 compares selected trials carried out during the project. Thermotherapy of cassava stakes before planting and the use of *Trichoderma* are practices that have a good effect on yield. The use of KCl is recommended for Quindío. The variety La Reina (CM 6740-7) is a very good option for farmers in Cauca. The Chiroza, the variety traditionally planted in the Eje Cafetero, produced much less than did 'ICA Catumare' or 'HMC-1'.

Table 2.5. **Effect of root-rot management practices on yield and on incidence of rotten roots at the Farms "El Jardin" (La Tebaida, Quindío) and "Guayaquil" (Montenegro, Quindío).**

	El Jardín			Guayaquil			Average		
	Root yield	Roots affected by Root Rot		Root yield	Roots affected by Root Rot		Root yield	Roots affected by Root Rot	
Treatment	(T/ha)	(Kg/ha)	(%)	(T/ha)	(Kg/ha)	(%)	(T/ha)	(Kg/ha)	(%)
Fertilization									
KCl (180 Kg/ha K_2O)	42.6	0	0.0	23.4	439	1.8	33	220	0.7
K_2SO_4 (180 Kg/ha K_2O)	29.9	0	0.0	22.3	0	0.0	26.1	0	0.0
Control of the farmer[a]	50.5	0	0.0	23	1869	7.5	36.8	935	2.5
Control without fertilization	47.9	0	0.0	19.2	575	2.9	33.6	288	0.8
Stake Treatment									
Termotherapy (49°C/ 49 min)	35.1	123	0.3	20.8	1768	7.8	28	946	3.3
Orthocide® (4 g/L) + Ridomil® (3 g/L)[b]	37.3	0	0.0	27.9	514	1.8	32.6	257	0.8
Lonlife® 4% (ascorbic acid)	-	-	-	23.4	114	0.5	23.4	114	0.5
Biocontrol with Trichoderma									
Strain 41PDA-3A	28.3	0	0.0	28.9	247	0.8	28.6	124	0.4
Strain 19TSM 3A	32.4	0	0.0	14.7	41	0.3	23.6	21	0.1
Varietal Resistance									
Chiroza	38.6	0	0.0	15.5	3086	16.6	27.1	1543	5.4
HMC-1	38.7	0	0.0	25.2	24	0.1	32	12	0.0
ICA Catumare	59.5	597	1.0	28.9	1028	3.4	44.2	813	1.8
M Per 183	32	3009	8.6	-	-	-	32	3009	8.6

[a] Farm "El Jardin": ammonium sulfate + borax (50:1.5) at 300 kg/ha. Farm "Guayaquil": Nitrax-DAP-KCl (1:2:2) at 500 kg/ha.

[b] At Farm "Guayaquil", Orthocide® was replaced by copper oxychloride.

Table 2.6. Cassava yield under management for root rots. Averages across five trials established in the Departments of Quindío and Cauca, Colombia.

	Root yield (T/ha)						
	Quindío				Cauca		
Treatment	Montenegro (Cantores)	Montenegro (Guayaquil)	La Tebaida (El Jardín)	Average	Santander de Quilichao (San Jerónimo)	(El Turco)	Average
Thermotherapy	62	21	35	39.3	4	15	11.5
Trichoderma	63	22	30	33.5	3.9	-	3.9
KCl	70	23	43	45.3	4	-	4
K_2SO_4	-	22	30	26	-	9	9
Manzana	41	-	-	41	-	-	-
Chiroza	-	15	39	27	-	-	-
La Reina (CM 6740-7)	-	-	-	-	5	-	5
Ica Catumare	-	29	59	44	-	-	-
HMC-1	-	25	39	32	-	-	-
M Per 183 Farmer [a]	51	23	51	41.7	4	15	9.5

[a] Montenegro and La Tebaida: HMC-1; Santander de Quilichao: Verdecita

Activity 3. Effect of soil physico-chemical properties on cassava root rot

Three samples of cassava roots were taken during the harvesting of the trial carried out in the rural community of San Jerónimo near Santander de Quilichao (Cauca, Colombia). Each sample consisted of a combined sample of roots from a replicate of the eight test treatments. Replicate 1 consisted of cassava plants planted in the highest part of the test lot, which was located on a steep slope; replicate 2 was located in central part of the test plot; and replicate 3 in the lowest part of the test plot.

Based on the chemical analysis of cassava roots (**Table 3.1**), Fe, Mn, and Zn contents increased in a downward direction on the slope, where nutrient content is expected to be higher. Although fertility is expected to improve downwards on the slope, cassava yields and root rot decreased perhaps because Fe and Mn increased to levels that affected yields by interfering with the absorption of other nutrients. The coefficient of correlation between yield and Fe, Mn, and Zn contents was, respectively, -0.61, -0.99, and -0.91 (**Table 3.2**). The coefficient of correlation between percentage plants suffering root rot and Fe, Mn, and Zn contents was, respectively, -0.57, -0.99, and -0.89. The correlation between percentage rotten roots/plant was also high and negative for these same elements. Plans are to establish trials to analyze whether these elements increase the resistance to root rot.

Table 3.1. Chemical analysis of cassava roots and soils in a field trial carried out in Santander de Quilichao (Colombia), with three replicates.

Chemical Analysis of Roots				Chemical Analysis of Soils[a]		
	No. of Replicate					Qualitative
Element	1	2	3	Element[b]		Assessment
N (%)	0.220	0.250	0.260	-	-	-
P (%)	0.062	0.067	0.064	Assimilable phosphorus (ppm)	25	-
K (%)	0.595	0.610	0.670	Exchangeable potassium (meq/100 g)	0.29	-
Ca (%)	0.106	0.092	0.112	Exchangeable calcium (meq/100 g)	3.1	-
Mg (%)	0.068	0.072	0.099	Exchangeable magnesium (meq/100 g)	1.2	-
Fe (ppm)	93.3	141.7	201.3	Fe (ppm)	85.1	High
Mn (ppm)	5.49	4.71	7.01	Mn (ppm)	5.8	Intermediate
Cu (ppm)	2.27	2.40	2.20	Cu (ppm)	2.5	Intermediate
Zn (ppm)	4.90	4.83	6.91	Zn (ppm)	2.6	Intermediate
B (ppm)	2.67	2.23	2.17	B (ppm)	0	Low
S (%)	0.018	0.045	0.036	S (%)	-	-
Yield (t/ha)	4.09	4.06	3.64			
Rotten roots (%)	2.80	3.70	1.03			

[a] Trial average.
[b] '–' means not determined.

Table 3. 2. Coefficients of correlation between productivity, root rot, and chemical analysis of roots.

	Chemical analysis of roots[a]			
Evaluation parameter	Fe (ppm)	Mn (ppm)	Zn (ppm)	B (ppm)
Growth and productivity				
Established plants (%)	0.55	0.98	0.88	-0.11
Plant height (m)	0.87	0.96	1.00	-0.55
No. of stakes/plant	0.46	0.96	0.83	-0.01
No. of commercial roots/plant	-0.81	-0.98	-0.99	0.46
Weight of commercial roots/plant (kg)	-0.81	-0.98	-0.99	0.46
Yield (t/ha)	-0.61	-0.99	-0.91	0.19
Root rots				
Plants with rotten roots (%)	-0.57	-0.99	-0.89	0.13
Rotten roots/plant (%)	-0.80	-0.99	-0.99	0.43
Plants with dry buds (%)	0.05	0.75	0.51	0.41

[a] Correlation.

Activity 4. Biological control of root rots in cassava

Evaluating *Trichoderma* in soils of the Departments of Cauca and Quindío, Colombia.

To better understand the dynamics of *Trichoderma* populations in the soil, samples of soil were taken on four farms during the harvesting of trials.

Samples of soil (3 g each) were taken from each cassava plot established in the field that had been inoculated with *Trichoderma* strains and also from the non inoculated check (numeral 2), and then diluted in 27 ml distilled water (base solution) and serial dilutions prepared (from 10^{-1} to 10^{-4}). Each dilution was agitated by vortex and placed on 0.1 ml V8A and PDA culture media; 100 □g/ml penicillin, 25 □g/ml chlortetracycline, 100 □g/ml PCNB, and 25 □g/ml Bengal Rose were added. Petri dishes were incubated at room temperature with light. *Trichoderma* colony counts were performed up to two weeks after placement in culture media.

Table 4.1 shows that 19TSM-3A is the *Trichoderma* isolate that survives longest in the soil. Previous greenhouse trials indicate that a population of 10^4 CFUs/g soil is sufficient to respond to mild attacks of soil pathogens such as *Phytophthora* or *Fusarium*. However, *Trichoderma* concentrations in the soil should be built up periodically. Inoculating stakes at planting also proves very beneficial. The effect of soil on *Trichoderma* spp. populations was not significant. Plots where *Trichoderma* had not been applied presented a relatively high natural population, although lower than inoculated plots. In Cauca, the natural population of *Trichoderma* is low probably because of the soil's low organic matter content. Although the use of molecular markers could improve the detection of *Trichoderma*, it is still impossible to quantify these populations with these tools.

A high correlation was observed between yield and concentration of *Trichoderma* in the soil. The correlation with root rot incidence, although negative, was not very high, indicating that the evaluated strains do not affect pathogen control significantly. On the contrary, strain 41PDA-3A showed a correlation of 1.0 with root rot, indicating that plots with higher concentrations presented greater root rot (**Table 4.1**).

Tabla 4.1. Evaluating *Trichoderma* in soils of the Departments of Cauca and Quindío, Colombia, inoculated with this fungus.

		Treatment			
		Isolate			Non Inoculated Check of *Trichoderma*
Municipality	Farm	19TSM-3A	41PDA-3A	14PDA-4	
		Trichoderma (cfu/g Soil)			
La Tebaida	El Jardín	4.1×10^4	2.3×10^4	-	5.9×10^3
Montenegro	Guayaquil	5.7×10^4	3.9×10^4	-	2.7×10^3
Santander de Quilichao	San Jerónimo	3.9×10^3	-	3.1×10^4	9.3×10^1
Santander de Quilichao	Mondomito	-	-	1.9×10^3	1.0×10^3
Average		3.4×10^4	3.1×10^4	1.7×10^4	2.4×10^3
Yield				**Yield (t/ha)**	
La Tebaida		32.4	28.3	-	50.5
Montenegro	El Jardín	14.7	28.9	-	23.0
Santander de Quilichao	Guayaquil San Jerónimo	3.15	-	4.65	3.48
Average		16.8	28.6	4.65	25.7
Correlation[a]		0.59	1.0	-	0.99
Root Rots				**Rotten Roots (%)**	
La Tebaida	El Jardín	0	0	-	0
Montenegro	Guayaquil	0.28	0.85	-	8.13
Santander de Quilichao	San Jerónimo	1.05	-	3.76	2.87
Average		1.33	0.43	3.76	2.55
Correlation[b]		-0.36	1.00	-	-0.40

[a] Coefficient of correlation between yield and concentration of *Trichoderma* in soil.
[b] Coefficient of correlation between rotten roots and concentration of *Trichoderma* in soil.

Activity 5. Controlling *Sphaerotheca pannosa*, causal agent of powdery mildew of rose in Colombia

Introduction

Sphaerotheca pannosa var. *rosae* (Wallr.) Lév., the causal agent of powdery mildew, is a major pathogen of roses. Disease symptoms develop quickly, affecting flower quality and causing significant economic losses. Control measures include foliar applications of fungicides to reduce pathogenic inoculum. Increasing public and scientific awareness of the need for a healthy environment has encouraged the development and evaluation of new disease control measures to replace fungicides in integrated management strategies. The use of biofungicides directly affects environmental protection and, in the future, could replace the more expensive chemical fungicides. Interest in using alternative disease control methods in flowers has grown, not only because of society's continuing demand for reduced pesticide levels in greenhouse drainage water, but also because of the limited availability of resistant, commercially acceptable plants. Control methods to replace agrochemical applications should be economical, sustainable, durable, and able to control several diseases. Foliar applications of biocompatible fungicides may reduce the development of powdery mildew, for example IT was demonstrated that the foliar application of phosphate salts reduced the incidence of powdery mildew in *Rosa indica* by 79%.

In spite of being an insecticide, neem derivatives affect diseases, including rose mildew and black spot. Other biopesticides based on plants are extracts of garlic, beggar's ticks (*Bidens pilosa*) and castorbean (*Ricinus communis*).

Pastor-Corrales reported the inhibitory effect of leaf extract from tabog (*Swinglea glutinosa*) on the causal agent of anthracnose (*Colletrotrichum lindemuthianum*) and Ascochyta blight in beans. This study aims to evaluate a plant extract and several fertilizers for their effectiveness in reducing populations of *S. pannosa* var. *rosae* and in controlling disease development in roses under greenhouse conditions.

Materials and Methods

Validating the use of swinglea extract, foliar fertilizers, and fungicides for controlling *S. pannosa* in rose crops

In the first semester of 2001, six experiments were established at the farms "El Ciprés" (Gachancipá), "Megaflor" (Madrid), and "La Valvanera" (Chía), in the Department of Cundinamarca, Colombia. At "El Ciprés", four experiments were carried out on cultivars Aalsmeer Gold and Charlotte to validate the efficiency of each of 6 methods of controlling powdery mildew. At the other two farms, different packages or combinations of these have being evaluated for disease control on cultivar Aalsmeer Gold.

At the beginning of each experiment, the rose beds were heavily inoculated with the pathogen. The high inoculum pressure meant that, for these experiments, there was no need to artificially inoculate the plants. Some selected beds received no disease control throughout the experiment.

At "El Ciprés", spray applications of the various control products were carried out on all stems in selected plots, using a motor pump (Maruyam) with two nozzles and automatized pressure between 25 and 30 pounds. Foliage was wetted completely with a given product every 7 days.

Applications in Madrid and Chía were done with a 1-L pump, which was pressurized manually. Every 4 days, only selected and marked stems for evaluation were sprayed. Between 1 and 2 L were needed per plot for each application.

Products evaluated in the Sabana de Bogotá

To prepare swinglea extract, healthy leaves of shrubs established at CIAT (Palmira, Valle del Cauca) were cut. We used 100 g of swinglea leaves per liter of drinking water; then liquefied the leaves and strained them through six layers of gauze. Before application, we added 1 mL/L of Inex-A® solution as a dispersing agent and adherent. For the experiments in Madrid and Chía, 50% ethanol was used to obtain the active ingredient in greater quantities. Because of problems of plant poisoning in some rose plants, the quantity of swinglea leaves was decreased from 100 to 30 g/L. Between applications, the extract was stored in cold rooms at 2°C on the farms.

The following foliar fertilizers were evaluated: monobasic potassium phosphate (KH_2PO_4, 13.6 g/L) and dibasic potassium phosphate (K_2HPO_4, 17.4 g/L). After observing plant poisoning,

dosage was dropped to 8 and 7 g/L for monobasic potassium phosphate and dibasic potassium phosphate, respectively (experiments 3 and 4 at "El Ciprés"). In Chía and Madrid, two doses of monobasic potassium phosphate were evaluated: 8 and 1.5 g/L **(Table 5.1)**. Sodium phosphate (16.1 g/L) was not assessed because this high dosage can cause plant poisoning in roses.

In these two municipalities, we are also evaluating phosphoric acid (1 mL/L) and Nutriphite® (2 mL/L; assimilable phosphorus 434.0 g/L, soluble potassium 403.0 g/L; Biagro Western Sales, Visalia, CA, USA).

Treatments were carried out with Elosal® (80% elemental sulfur at 1 mL/L), Rubigan® (0.6 mL/L), Stroby® (kresoxim-methyl, 0.25 mL/L), and Meltafun® (Meltatox or dodemorph, 2.5 mL/L). We also applied a mixture of Meltafun® with Rubigan® (fenarimol, equal dose). We added 1 mL/L Inex-A® to all treatments, except the two with Rubigan®.

The efficiency of plant washings was also evaluated, using large quantities of water.

Activity 6. Evaluating fungicides, swinglea extract, and foliar fertilizers under greenhouse conditions, CIAT, Palmira

To date, we have established five experiments, each with treatments using healthy and infected plants. At CIAT, the effectiveness of the fungicides mentioned previously is being evaluated. Under favorable conditions, pressure of pathogen inoculum is high and artificial inoculations have been carried out only occasionally. The treatments are applied weekly (experiments 1-3) or every 3 days (experiments 4 and 5). The effectiveness of an extract, frozen and conserved at -20°C for 3 months, was compared with fresh extracts. Extracts obtained with 50% ethanol and 50%, 25%, and 10% thinner were also included.

Products evaluated at CIAT, Palmira

The following products were evaluated for their efficiency in controlling powdery mildew:

Swinglea extracts diluted in:

- Water (100 g/L)
- 50% ethanol
- 50% acetone
- 25% thinner
- 10% thinner

Swinglea extracts:

- Pulverized (after drying at 55°C overnight) and diluted in water
- Pulverized (after drying at 55°C overnight) and diluted in 50% ethanol
- Frozen and conserved at -20°C for 3 months, then prepared in water
- Mixed in a solution of water and monobasic potassium phosphate (13.6 g/L)

- Mixed in a solution of water and dibasic potassium phosphate (17.4 g/L)

Resistance inducers (fertilizers):

- Dibasic potassium phosphate (17.4 g/L)
- Monobasic potassium phosphate (13.6 g/L)

Fungicides:

- Rubigan® (0.6 mL/L)
- Stroby® (0.25 mL/L)
- Elosal® (1.0 mL/L)

Biofungicides (based on plant extracts):

- Laurel (*Laurus nobilis*)
- Hairy beggar-ticks (*Bidens pilosa*)
- French marigold (*Tagetes patula*)

The last two plant extracts were fermented for 6 h in 50% ethanol.

Biological products:

- Lixiviated worm compost (200 mL/L)
- Citric emulsion (2.5 mL/L)
- Ecolife® (1.5 mL/L)
- Lixiviated banana bunches fermented with *Acetobacter* spp. under anaerobic conditions
- Lixiviated compost of fruits and vegetables (to 50%) such as red pepper, tomato, grape, Andean blackberry, pineapple, eggplant, melon, yellow passionfruit (Grajales S.A., La Unión, Valle del Cauca) for about 3 months

Negative control was drinking water.

Evaluations

The Sabana de Bogotá

In each plot, we evaluated 20 stems ("El Ciprés") or 9 stems ("Megaflor" and "La Valvanera"), previously identified. The stems selected were those with the largest number of infected leaves. Each stem was numbered, using tape. These plants were not harvested until the experiment was completed. The stems located at the extremes of each plot and close to streets were not included in the evaluations. Each stem was evaluated for severity of powdery mildew according to the following scale:

1 = Healthy stem
2 = Some leaves on the infected stem show mild leaf symptoms,

beginning infection (lesions between 4 and 6 mm in diameter), only 1 to 2 leaves infected

3 = Stem moderately infected, lesions being larger than 7 mm on more than 2 leaves and appearing white

4 = Most leaves on the stem are infected, the area infected per leaf is relatively large, the disease attacks both leaves (more than 3) and stem, as much as half the leaf surface infected

5 = Many infected leaves, leaves totally invaded, with small colonies on more than 5 leaves per stem.

Leaves that had very small colonies of the fungus were recorded as infected. Data were stored on Excel, a computerized program for calculation.

At the end of each experiment, we evaluated incidence, number of infected leaves per stem, and sporulation (scale of 1 to 3, where 1 = no sporulation and 3 = abundant sporulation).

CIAT, Palmira

For the experiments at CIAT, we evaluated incidence, number of infected leaves per stem, and sporulation (scale of 1 to 3, where 1 = no sporulation and 3 = abundant sporulation).

Experimental Design

For all experiments in the Sabana de Bogotá, the experimental design of randomized complete blocks was used. Each trial had three blocks or replicates. A treatment was assigned at random to each plot. At CIAT, the experiments were carried out with two or three blocks.

Statistical Analysis

We used the statistical package SAS to carry out an analysis of variance for each experiment, and determined significant differences according to Duncan's $\alpha = 5\%$.

Results

Validating the use of swinglea extract, foliar fertilizers, and fungicides for controlling *S. pannosa* in rose crops

At "El Ciprés", six methods of control were evaluated under conditions of very high pressure from powdery mildew inoculum on two rose cultivars. Each experiment was repeated twice. The swinglea extract reduced incidence and severity in all experiments in "El Ciprés" **(Table 6.2)**.

The swinglea extract prepared with drinking water lowered disease incidence and the number of infected leaves per plant, especially in cultivar Charlotte. However, no significant difference in susceptibility was detected between 'Aalsmeer Gold' and 'Charlotte'.

The treatments with the monobasic and dibasic potassium phosphates fared better than those with Stroby® and Rubigan®. Monobasic potassium phosphate was slightly more effective than dibasic potassium phosphate, and was better than Stroby®, because it reduced the rate of increase in incidence and severity. Reducing the dosage of monobasic phosphate from 13.6 to 8 g/L did not diminish its effectiveness in controlling powdery mildew.

Chemical treatment with Stroby® was more effective than with Rubigan®. Elosal® had the least effect on the disease. Differences between treatments were greater for 'Charlotte' than for 'Aalsmeer Gold'. The biofungicides best controlled disease in cultivar Charlotte. For cultivar Aalsmeer Gold, differences among treatments were not detected, although the biofungicides were slightly more effective than chemical treatment with Stroby®.

In two experiments at Madrid and Chía, doses of the products used were reduced to prevent plant poisoning. Washings with water were also included. These experiments also included combinations of different control methods **(Table 6.1)** to reduce the problem of plant poisoning. The swinglea extract is now prepared with alcohol (50% ethanol), because it does not induce plant poisoning and extracts a larger volume of the active ingredient. The final concentration of alcohol is 30 mL of 50% ethanol per liter of swinglea extract. At "Megaflor" (Madrid), disease incidence and severity in cultivar Aalsmeer Gold were reduced by an application of swinglea **(Table 6.3)**. A mixture of Meltafun® and Stroby® was the most effective treatment at the two farms. Monobasic phosphate did not control the disease. No plant poisoning was observed on the farms when the modified extract of swinglea and monobasic potassium phosphate were used together. Plants under chemical control, cultural control, and the expensive integrated control in the three replicates presented green lesions that were either circular or long and narrow extending from leaf base to tip.

Note, however, that the fungicides Stroby® and Rubigan® did not control the disease effectively. That no treatment, including Stroby® and Rubigan®, lowered disease incidence or severity is of concern, even though they did reduce the rate of increase in the disease. The reason is that most of the experiments were carried out with beds carrying many infected stems, most of which were very unhealthy. The experiments indicate that initiating control while disease incidence is still low is more effective. With the more frequent applications used at "Megaflor" and "La Valvanera", for example, applications every 3 days instead of 7, efficiency of control improved. That is, the short time between infection by and sporulation of *S. pannosa* is taken into account. In the experiments at Madrid and Chía, Meltafun® was also evaluated. This fungicide was not available in Colombia when the experiments at Gachancipá were established.

Table 6.1. Treatments realized at experiments in the farms Megaflor (Madrid) and La Valvanera (Chía), Cundinamarca Department.

Treatment	Day of Evaluation and Application					
	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
Chemical control	Meltafun and Stroby	Rubigan	Meltafun	Rubigan	Meltafun and Stroby	Rubigan
Biocontrol[a]	Swinglea extract	Swinglea extract	Swinglea extract	Swinglea extract	Swinglea extract	Swinglea extract
Cultural control	Monobasic potassium phospate (8 g/L)	Phosphoric acid	Nutriphite	Washing	Monobasic potassium phospate (8 g/L)	Phosphoric acid
Integraded control, expensive option (chemical, biological and cultural control)	Meltafun and Stroby	Swinglea extract	Monobasic potassium phospate (8 g/L)	Meltafun	Swinglea extract	Monobasic potassium phospate (8 g/L)
Integraded control, economic option (chemical, biological and cultural control)	Elosal	Swinglea extract	Monobasic potassium phospate (1.5 g/L)	Washing	Elosal	Swinglea extract

[a] At CIAT currently other plant extracts are being evaluated for their efficiency to control the disease which offers different options for biocontrol.

Table 6.2. Evaluation of time in use of swinglea extract, foliar fertilizers, and fungicides to control powdery mildew in rose cultivar Aalsmeer Gold (experiments 1 and 2), "El Ciprés", Gachancipá, Cundinamarca, Colombia.

Treatments	Area below the Disease Progress Curve[a,b]			
	Aalsmeer Gold		Charlotte	
	Incidence[c]	Severity[d]	Incidence[e]	Severity[f]
Swinglea extract	594 a	36 a	129 a	27 a
Monobasic potassium phosphate	407 a	30 a	160 a	28 a
Bibasic potassium phosphate	608 a	40 a	90 a	27 a
Stroby	759 a	41 a	135 a	36 ab
Rubigan	741 a	38 a	294 b	48 b
Elosal	773 a	41 a	409 c	34 ab
Average	647	38	203	33

[a]Duncan multiple range test α=5%;
[b]Plants were evaluated 0, 9 and 16 days. After each evaluation the different products were applied;
[c]Variation coefficient = 28% and R^2 = 0.71;
[d]Variation coefficient = 17% and R^2 = 0.51;
[e]Variation coefficient = 30% and R^2 = 0.87;
[f]Variation coefficient = 29% and R^2 = 0.53.

After one semester of the project's second phase, we conclude that swinglea extract and monobasic potassium phosphate are effective (comparing the results obtained in six field experiments) low-cost alternatives, with very low toxicity to humans. In contrast, the chemical treatments Stroby® and Rubigan®, which are widely used in the Sabana de Bogotá, belong to the high toxicological categories I and II, respectively.

As explained in "Materials and Methods", more evaluations are being carried out to determine the efficiency of each control method and their efficiency when combined in different packages for disease management. In the second semester, experiments will also be established on different farms to analyze the effect of different genetic groups found on the farms on the efficiency of control methods.

Table 6.3. Evaluation of the use of different technological packages to control Powdery Mildew of the cultivar Aalsmeer Gold at the Farms Megaflor (Madrid) and La Valvanera (Chía) (Experiments no. 5 and 6).

Treatments		Disease Progress[a]					
	Control Practice	Farm Megaflor		Farm La Valvanera		Average	
Package[b]	Applied Day 4	Incidence[c]	Severity[d]	Incidence[c]	Severity[d]	Incidence[c]	Severity[d]
Economic integrated control	Elosal	30	-0.27	-103	-0.03	-37	-0.15
Biocontrol	Swinglia extract	11	-0.29	11	0.07	11	-0.11
Expensive integrated control	Meltafun and Stroby	26	-0.24	15	-0.31	20	-0.28
Chemical control	Meltafun and Stroby	30	-0.49	19	-0.21	24	-0.35
Cultural control	Monobasic potassium phosphate	26	1.93	22	0.02	24	0.97
Average		25	0.13	-7	-0.09	8	0.02

[a] Difference between d=4 and d=0;
[b] See Table 1 for more information;
[c] Percentage of the stems affected by Powdery Mildew;
[d] Range between 0 and 3, average of the stems affected, 0=healthy, without sporulation; 3=high sporulation.

Evaluating fungicides, swinglea extract, and foliar fertilizers under greenhouse conditions, CIAT, Palmira

To generate new alternatives for managing powdery mildew, CIAT is continually evaluating, in the greenhouses, the efficiency of plant extracts, resistance inducers, lixiviation, and others. Those practices that are currently the most efficient are being evaluated on farms.

We found we could improve the swinglea extract **(Table 6.4)**. The mixture of swinglea diluted in a solution of water and monobasic potassium phosphate is a highly efficient alternative, taking into account the considerably diminished percentage of infected leaflets per plant, and was more effective than chemical treatment with Rubigan®. This suggests that the combination of two mechanisms that act differently may increase effectiveness in disease control.

Table 6.4. Evaluating fungicides, swinglea extract, and foliar fertilizers for controlling powdery mildew in rose cultivar Konfetti under greenhouse conditions at CIAT (experiment 1), Palmira. The experiments were initiated with two infected plants and two healthy plants per treatment.

	% of Foliolos Affected per Plant					
Treatment	Day 0	Day 14	Difference Between Days 14 and 0	Day 21	Difference Between Days 21 and 0	Average Differences
Mixture of swinglea prepared in water and monobasic potassium phosphate	41	0	-41.4	25	-16.4	-28.9
Rubigan (0.6 ml/L)	86	63	-22.6	76	-10.0	-16.3
Swinglea extract prepared with 50% ethanol	35	8	-26.5	31	-4.0	-15.2
Drink water	73	63	-10.4	63	-10.2	-10.3
Monobasic potassium phosphate (17.4 g/L)	27	14	-12.8	21	-6.0	-9.4
Lixiviated earthworm compost (200 ml/L)	0	0	0.0	9	9.4	4.7
Monobasic potassium phosphate (13.6 g/L)	32	15	-16.8	66	34.2	8.7
Citroemulsion (2.5 ml/L)	3	0	-2.8	26	23.7	10.5
Swinglea extract prepared with water (100 g/L)	18	4	-13.6	62	44.7	15.5
Elosal (1 ml/L)	6	2	-3.7	44	38.2	17.3
Pulverized swinglea extract prepared with water (dried at 55° C during one night)	9	8	-0.5	48	39.5	19.5
Average	53	30	-22.7	43	-9.3	-16.0

The results obtained at CIAT show that extracting swinglea with 50% alcohol is a better alternative. This extract is now being evaluated on farms around Madrid and Chía.

These experiments need to be continued at CIAT. Several products cause plant poisoning, which means the problem must be minimized, together with improving product preparation and application methods, and reducing dosage.

Lixiviation of compost with 50% fruits and vegetables for about 3 months affects sporulation in the fungus and disease incidence. A lixiviated fruit compost was obtained from Grajales S.A. (La Unión, Valle del Cauca). We plan to evaluate this potential biofungicide in experiments at Madrid and Chía.

Laurel, hairy beggar-ticks, and French marigold did not show control over powdery mildew, compared with Rubigan® **(Table 6.5)**. Possibly, fermenting these plants in water for several days will improve their efficiency.

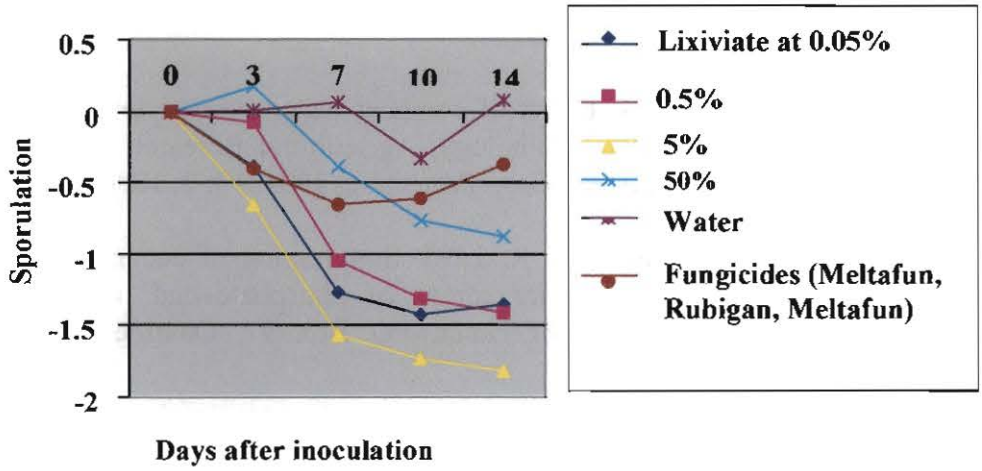

Figure 6.1. **Evaluating the treatment with lixiviated plantain compost for controlling powdery mildew in cultivar Livia under greenhouse conditions at CIAT, Palmira. Every 4 days plants were treated with four different concentrations. A chemical treatment was included as positive control. Nine plants per treatment were used. Sporulation was determined each four days using a range between 0 to 3 where 0 is no sporulation and 3 sporulated.**

Table 6.5. **First evaluation of plant extracts of swinglea, hairy beggar-ticks (*Bidens pilosa*), and French marigold (*Tagetes patula*) in controlling powdery mildew in rose cultivar Livia under greenhouse conditions at CIAT (experiment 5), Palmira. For the experiment, an average of two infected plants and two healthy plants were used, together with a chemical treatment as positive control.**

Treatment[a]	Development of Affected Leaves (%)	Development of Sporulation (%)
Hairy beggar-ticks	203	196
French marigold	108	108
Swinglea	91	163
Water	133	243
Rubigan	90	98

[a] The vegetal extracts were fermented during six hours en 50% ethanol.

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Activity 7. Evaluation of fungicides to control bud rot disease of oil palm in Meta and Cundinamarca

A trial to control bud rot in oil palm was conducted in a field of Unipalma, Paratebueno (Cundinamarca). Two and a half year old palms, genotype CIRAD C1001 F, were treated with two experimental fungicides used to control the fungi *Phytophthora* spp. in coconut palm, cocoa, and avocado in Australia. The product, coded as H, was used at 25% and 40%, and the product coded as NF was used at 100% in two ways—by injection into the trunk just under the bud, and by root absorption. Of each solution, 20 cm^3 were injected 15 cm deep into the trunk with a plastic injector. Root absorption was done by cutting two roots 1.4 m from the palm and immersing them in a plastic bag with 10 cm^3 of solution for each root. Healthy palms were also treated and a control with water was used.

The treatments reduced disease progress, although it continued increasing slowly. Solution H at 40% and NF were the best treatments for reducing disease progress, 5.5 months after application. The trunk, under the bud, was the best tissue into which to apply the solutions, although there were no significant differences with root absorption. The NF product reduced disease 2.5 and 3.5 months after treatment, although disease increased again, probably because of long application frequence period and rain favoring disease (**Figure 7.1.**).

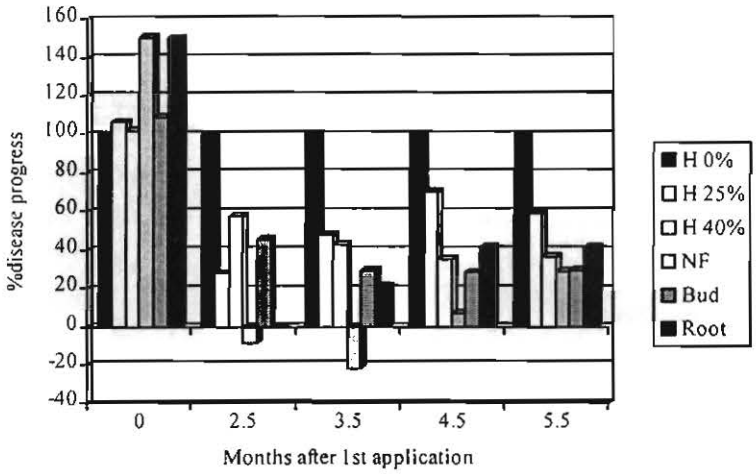

Figure 7.1. Bud rot control in oil palm by injection under bud and root absorption, at a field in the eastern plains of Colombia.

Activity 8. Participatory disease and crop management in the Colombian northeast Amazon

Ash (200 g per plant), organic matter (200 g per plant) from dead leaves taken from forest soil, and a mixture of both at a 1:1 ratio were evaluated by farmer participatory research methodologies for their effects on the yield of four native cassava varieties. Cassava was grown in *chagras*, which are small plots of slash-and-burn agriculture in rain forest. Farmers were women from four Tukano indigenous communities at Mitú (Vaupés). When ash was incorporated into the soil, the vigor scale was good (2.02 for yellow and 2.15 for white varieties), compared with traditional management (3.08 for yellow and 3.38 for white varieties). Incorporating organic matter gave yellow varieties 2.65 in the vigor scale and white varieties 2.38. Cutting selection without amendments had superior behavior to traditional management (**Table 8.1.**).

In Cucura community, the assay showed better yield with ash alone, then mixed with organic matter, and then using cutting selection. Root rot percentage was lower when ash is incorporated in the soil. Traditional management, without ammendments or cutting selection, had the lowest root yield and higher root rot. Other *chagras* will be harvested in November (**Table 8.2.**).

Table 8.1. Effect of organic matter (from dead leaves collected from forest soil), ash, and cutting selection on cassava vigor in four indigenous communities.

Treatment	Variety	Community				Average
		Seima Cachivera	Seima Central	Cucura	Puerto Palomas	
Ash	Yellow	1.0	2.5	3.3	1.3	2.02
Organic matter	Yellow	3.0	2.3	4.0	1.3	2.65
Ash + organic matter	Yellow	1.5	2.2	3.5	1.0	2.05
Cutting selection	Yellow	4.0	2.7	3.8	1.7	3.05
Traditional management	Yellow	4.0	2.5	4.5	1.3	3.08
Ash	White	2.0	2.5	2.8	1.3	2.15
Organic matter	White	1.5	3.2	2.8	2.0	2.38
Ash + organic matter	White	2.5	2.3	3.3	1.7	2.45
Cutting selection	White	4.0	3.5	3.3	2.0	3.20
Traditional management	White	4.0	4.0	3.8	1.7	3.38

[a] On a vigor scale where 1 = good and 5 = poor.

[b] Varieties: Seima Central: Yellow, Wasaí; White, Patabá. Pto. Palomas : Yellow, Yuca de Piña; White, Yuca de Rana. Seima Cachivera: Yellow, Yuca de Mico; White, Ibacabá. Cucura: Santa Catalina (low cyanide).

Table 8.2. Effect of organic matter (from dead leaves collected from forest soil), ash, and cutting selection on cassava yield and root rot percentage in Cucura, indigenous community, Mitú, Vaupés, Colombia.

Treatment	Cuttings/Plant	Yield (Kg/ha.)	Root Rot (Kg/ha.)	% Root Rot
Ash	5.4	5117	29.8	1.4
Organic matter	4.3	2943	37.2	8.3
Ash + organic matter	5.6	7497	0.0	0.0
Cutting selection	8.4	6405	206.6	2.5
Traditional management	0.9	1373	398.9	24.2

Four applications of 250 g per plant of the same treatments and chemical fertilizers (Di-ammonium phosphate at 4 g per plant and potassium chloride at 4 g per plant) were made, one per month, to evaluate the control of *Phytophthora capsici* under greenhouse conditions at CIAT, inoculating young stems of M Bra 12 by the wounding method. Plants were planted in 28-kg pots containing soil from Mitú.

Disease decreased when organic matter and chemical fertilizer were incorporated into the soil, while higher dry matter production was obtained by incorporation of a 1:1 mix of ash and organic matter (**Table 8.3.**).

Table 8.3. Effect of organic matter from dead leaves, collected from forest soil and ash, on the dry matter yield of the cassava variety M Bra 12 growing in a soil from Mitú (Vaupés, Colombia), and on the severity of *Phytophthora* rot in young stems in the greenhouse.

Treatment	AUDPC[1] (Inoculation 45 Days after Planting)	AUDPC[1] (Inoculation 90 Days after Planting)	Dry Matter (gr) 3 Months after Planting	Dry Matter (gr) 9 Months after Planting
Ash	8450	18182	26.46	97.5
Organic matter	3188	14361	26.43	90
Ash + organic matter	6267	21813	28.01	120
Chemical fertilizer	2158	12870	21.95	100
Control	4421	17397	23.37	105
No Inoculated	0	0	-	-
Duncan 5%	6795	7011	7.1	39.3

[1] AUDPC: Area under disease progress control

Activity 9. Paper submitted to Acta Agronómica Journal from the Universidad Nacional de Colombia, Palmira

Adaptation assessment of cassava varieties with resistance to Phytophthora spp., by participatory research at indigenous communities from Mitú (Vaupés, Colombia)

Elizabeth Alvarez, Germán Alberto Llano, John Loke, Raúl Madriñán, Jaime Andrés Restrepo, Jairo René Mora.

Cassava is a staple of the Amazon indigenous diet, and the marketing of its derived products provides a source of income. Because of land pressure around Mitú (Department of Vaupés, southeastern Colombia), shifting cultivation is no longer practiced with rotations, which had permitted forest regeneration, nor are plots adequately selected. One consequence is an increased incidence of cassava pests and diseases. Root rots, caused by several *Phytophthora* species, comprise a major production constraint in the region. Through surveys and meetings with communities, a crop management diagnostic was made and its relationships with root-rot incidence determined. Two thirds of the indigenous women farmers agreed that rots constituted the main cassava-production problem. The farmers were willing to try new varieties, describing the characteristics that they preferred. Based on the diagnostic, the researchers offered to indigenous to evaluate varieties with rot tolerance. Activities planning and evaluations were made with active communities participation and women diffused results. The indigenous helped the researchers to choose four *chagras* (farming plots), each from a different community. The farmers evaluated ten CIAT materials and nine local landraces according to their own selection criteria for the vegetative stage: vigor, plant health, plant height, stems per plant, and days to maturity. At harvest, the farmers also had specific criteria for adequate yield, starch content, and planting materials (stakes). The identified criteria led to a field book for evaluating cassava varieties in the zone. The farmers preferred the CIAT material CM 2772-3 (sweet, with yellow pulp) to the local landraces. Results were diffused, with farmers' participation, through meetings with the Department's Institutional Group, nongovernmental organizations, and communities.

Two field days were also conducted, and two pictographic handbooks published.

Activity 10. Evaluation of liquid compost from different sources as fertilizer in cassava

Foliar aspersion (20%) and soil application (50%) of liquid compost, twice weekly, were evaluated on cassava growth as fertilizer effect. Compost sources were plantain, fruit and vegetable, and organic matter processed by earthworms. Water was applied as control.

Plant height was measured every 2 weeks and foliar chemical analyses were made 1.5 months after the first treatment. Plants with foliar treatment were taller than with fertilized soil. The tallest plants were those with fruit and vegetable application. Plantain liquid compost had better performance in soil applications.

Mineral content in young leaves from plants treated with fruit and vegetable compost was higher than with other composts, showing high content of K, enough P, Ca, and Mg, and low N and S, according to CIAT (**Figure 10.1.**). However, mineral contents obtained by foliar aspersion of fruit and vegetable compost were higher than in the control. With this product applied to the soil, P, K, Ca, and Mg were higher in leaves, than in the control.

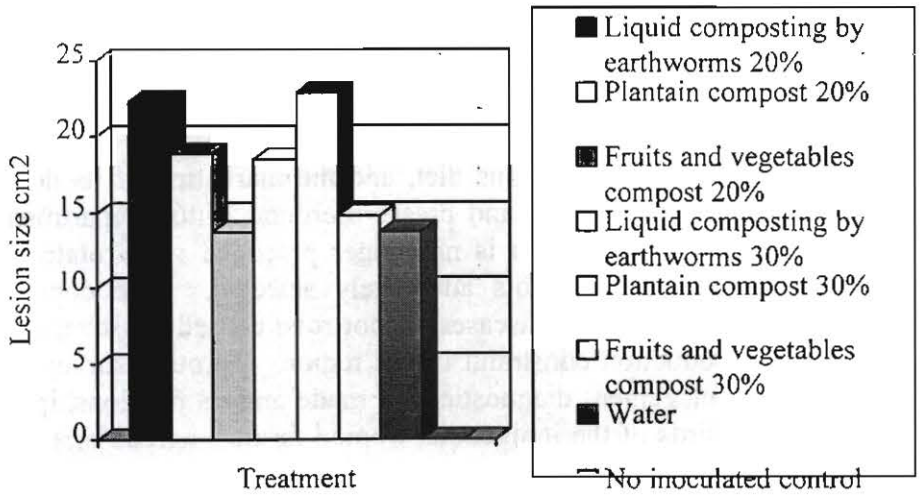

Figure 10.1. Mineral analyses of leaves (% dry matter) in cassava plants treated with different liquid compost sources.

The same treatments described above were applied to the genotype M Col 1505, susceptible to CBB, in order to evaluate the effect of liquid compost on *Xanthomonas axonopodis* pv. *manihotis*. The bacteria affected all inoculated plants, although those treated with fruit and vegetable compost were less affected by disease (**Figure 10.2.**), and its progress was lower than with other liquid compost sources.

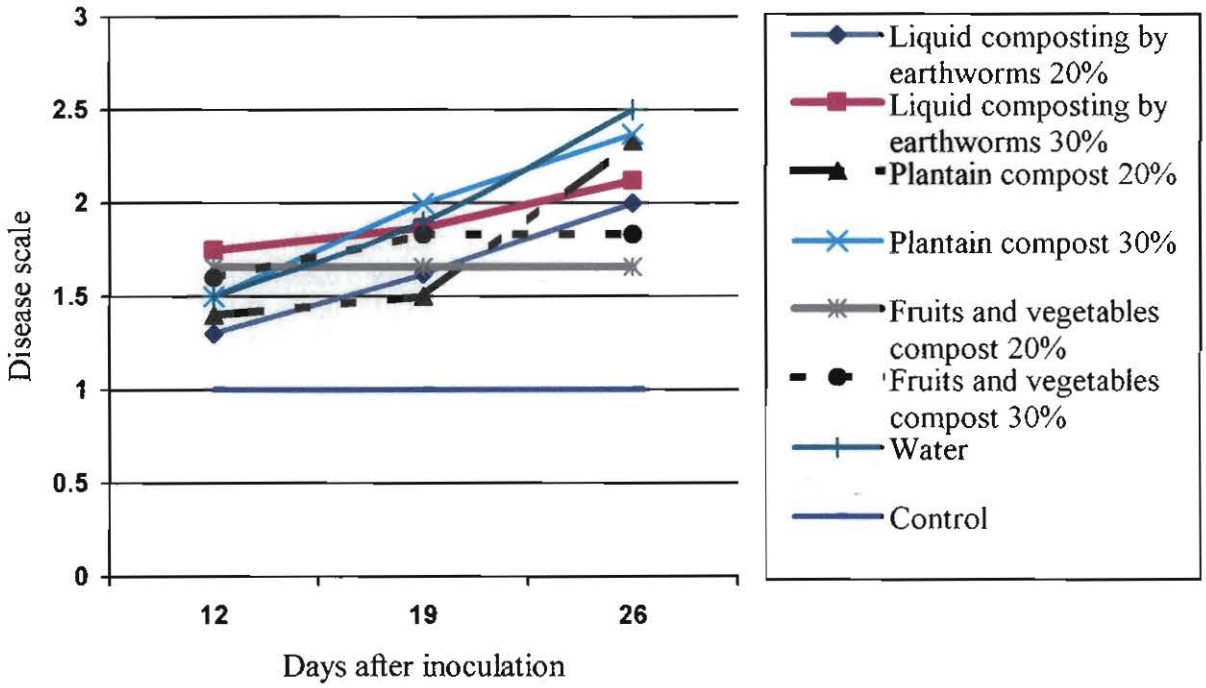

Figure10.2. Effect of different liquid compost sources on *Xanthomonas axonopodis* pv. *manihotis* control in cassava, variety M Col 1505.

Activity 11. Evaluation of liquid compost from different sources as fertilizer in cassava

Foliar aspersion (20%) and soil application (50%) of liquid compost, two times a week, were evaluated on cassava growth, as fertilizer effect. Compost sources were plantain, fruits + vegetables and organic matter processed by earthworms. Water was applied as control.

Plant height was measured each 2 weeks and foliar chemical analyses were done 1.5 months after first treatment. Plants with foliar treatment were higher than soil fertilized. The highest plants were those with fruits + vegetables application. Plantain liquid compost had better performance in soil applications.

Mineral content in young leaves from plants treated with fruits + vegetables compost were higher than other composts, showing high content of K, enough of P, Ca and Mg and low of N and S, according to CIAT (**Table 11.1**). Although, mineral contents obtained by foliar aspersion of fruit and vegetables compost were higher than control. With this product applied to soil, P, K, Ca and Mg were higher in leaves, than control.

Table 11.1. **Leaves mineral analyses (% dry matter) in cassava plants treated with different liquid compost sources.**

Sample	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Foliar aspersion						
Control. Water to foliage	3,69	0,30	2,09	0,68	0,39	0,23
Plantain liquid compost 20%	3,41	0,24	2,13	0,68	0,37	0,25
Liquid compost from organic matter processed by earthworms, 20%	3,29	0,28	1,82	0,67	0,40	0,21
Fruits and vegetables liquid compost 20%	3,83	0,34	2,17	0,73	0,42	0,26
Soil application						
Control. Water to soil	3,35	0,23	2,12	0,65	0,37	0,22
Plantain liquid compost 50%	3,39	0,23	2,12	0,57	0,37	0,21
Liquid compost from organic matter processed by earthworms, 50%	3,01	0,24	2,11	0,59	0,35	0,21
Fruits and vegetables liquid compost 50%	3,07	0,26	2,22	0,69	70,41	0,20

Activity 12. Treatment of cassava cuttings with liquid compost

Cassava cuttings from varieties M COL 1505, M PER 183, and CM 523-7, immersed for 10 min in 35%, 50%, and 70% liquid compost from plantain and organic matter processed by earthworms, were planted in a field in Ginebra (Valle). They were compared with chemical treatment with Captan (1.5 gr L^{-1}), Benomyl (1.5 gr L^{-1}), and Dimetoato (1.2 cc L^{-1}). **Figure12.1.** shows the higher effects of chemical treatment, followed by organic matter from earthworms at 70% and 50%.

Applications were made every 15 days during the first 4 months, and 21 days later, to evaluate the effect on cassava plants' growth and yield. The trial is in progress.

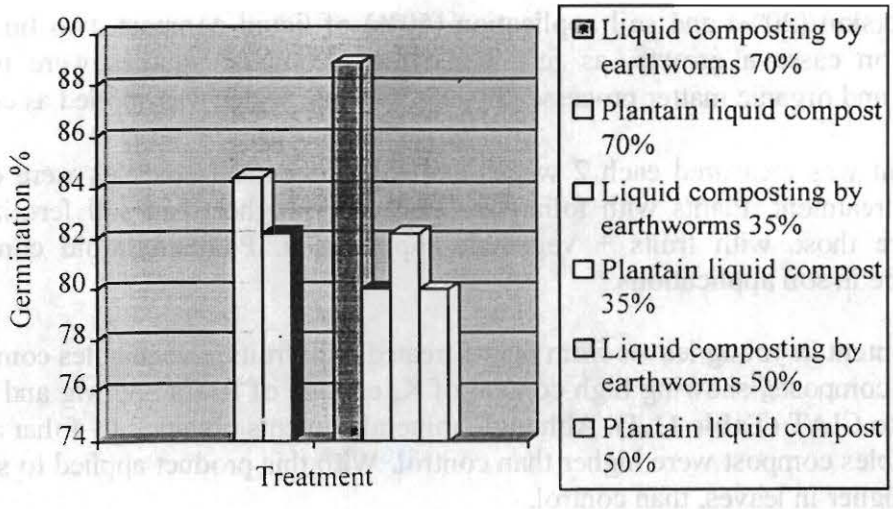

Figure 12.1. **Effect on plant germination of treating cassava cuttings by immersion in different sources of liquid compost.**

Publications

Handbook "Investigación Participativa para el control de pudriciones de yuca con comunidades indígenas de Mitú". 500 units.

"Evaluación de la adaptación de variedades de yuca con resistencia a *Phytophthora* spp., mediante investigación participativa en comunidades indígenas de Mitú (Vaupés, Colombia)". Acta Agronómica, journal from Universidad Nacional de Colombia, Sede Palmira. **In Press**.

Alvarez, E., J. L. Claroz, S. P. Cuero, and J. B. Loke. 2001. Controlling powdery mildew of roses using a plant extract and foliar fertilizers. Salt Lake 2001. APS, SON and MSA Joint Meeting August 25-29. Phytopathology 91:S101. Publication no. P-2001-0005-MSA.

La yuca en el tercer milenio. Integrated Disease Management. Chapter in Handbook for Cassava Crop. 2001. CIAT.

Pocket Handbook for Disease Diagnostic. 2001. CIAT.

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Sub-output 2. Developing IPM Strategies for Cassava Arthropod Pests.
(A. C. Bellotti)

Activity 1. The establishment of an IPM program for important cassava arthropod pests in Valle del Cauca and Cauca departments in Colombia

Introduction

The area under cassava production in Colombia is expanding due to the demand for cassava, not only as a human food, but more recently, its potential use as an animal feed. This increase in area, and in many cases, the increase in the size of cassava plantings, has led to an increase in arthropod pest problems. Populations and damage of the cassava hornworm, the cassava burrower bugs (*Cyrtomenus bergi*), white grubs and whiteflies are increasing, and in certain cases, difficult to control.

Whitefly (*Aleurotrachelus socialis*) populations have increased dramatically in recent years and this is a very difficult pest to control. It has a short life cycle (about 1 months), a high rate of reproduction, considerable flight capacity, disseminating rapidly and its waxy, cerosine, covering on the nymphal and pupal stage can act as a protective devise against natural enemies and pesticide applications. In addition, changing planting patterns, especially in larger plantations, where cassava is planted more frequently, or "escalonada" presents optimal conditions for rapid population buildup. This is also aided considerably if cassava is irrigated (more young leaves produced, favorable to whitefly oviposition and nymphal development) or rains occur more frequently, shortening the dry season. Yield losses during severe attacks can reach 80%. These conditions prompt farmer reaction, usually the indiscriminate and ill-timed use of toxic pesticides.

This scenario has prompted the Colombian Ministry of Agriculture (MADR) to release funding co cope with this situation. A project has been designed to evaluate the options, or tools available to control whiteflies, there include the use of biopesticide, cultural practices, resistant varieties, biological control, trapping, and the rational or discriminate use of chemical pesticides.

The over-all goal is to develop an IPDM program for both small and large cassava farmers based on sound economical principles and is ecologically sustainable.

The specific objectives of the program are:

1. Determine optimal cassava pest management practices with cassava producers in Valle del Cauca and Cauca.
2. Identify selective chemical pesticides and biopesticides, determine doses and time of application for effective control with minimal effect on natural enemies.
3. Carry out field trials to recommend to cassava producers the use o effective natural enemies, including biopesticidas and parasitoids.
4. Train farmers in cassava IPM practices, emphasizing the reduction of pesticide applications and the use of natural enemies.

Materials and Methods

Visits were carried out with 46 cassava farmers in Valle del Cauca and Cauca. Surveys were done to identify current pest problems, present farmer pest control and crop management practices and farmer perceptions, needs and priorities. In addition, systematic sampling was done in farmers' fields to identify pest problems and design a general pest damage scale.

Chemical Pest Control

The cassava pest complex is associated with a larger number of natural enemies that play an important role in maintaining some pest populations below economic injury levels. For this reason, chemical pest control is considered a "last option," as indiscriminate pesticide use could reduce the effectiveness of natural or applied biological control. However the very high populations of whiteflies observed in farmers fields, is an indication that natural biological control is not effective or the most effective biocontrol agents are not present. This has forced farmers to apply chemical pesticides as an alternative to whitefly control. This is considered a short term alternative until effective biocontrol agents, especially entomopathogens, can be identified and resistant cassava varieties can be multiplied and made available to producers. Four experiments have been designed and are being carried out to determine effective pesticide practices and recommendations for whitefly control. Results that will be presented are preliminary as there are on-going experiments.

Experiment

1. Evaluations of chemical and biological pesticide for whitefly (*A. socialis*) control.

Preliminary experiments were carried out to design field methodologies for evaluation of the effectiveness of chemical pesticides. These were done at the farm "El Bohio" located in Santander de Quilichao (Cauca), 1000 m.a.s.l. with the cassava variety MBra 12. One month after planting foliar applications of 8 commercial products were made. These included Biomel (refined vegetable oils), Bioneem (neem extract), Bioterpeno (natural terpenoids), confidor (Imidacloprid), oportune (Buprofezin), tamaron (Metamidofos), sistemin (Dimethoate), and cipermetrina (generic). They were applied at the commercially recommended doses. The experimental design was completed randomized blocks, with 8 treatments, a control and four replications. Evaluations were initiated at the first sign of whiteflies, using a population scale, and after the first evaluation, evaluations were made every 15 days.

2. Evaluation of product efficiency in Santander de Quilichao and Jamundí.

In this experiment (Finca El Bohio) products with new or novel active ingredients are being evaluated for whitefly control, using the cassava variety "Parrita" (MCol 2758). The experimental design is completely randomized blocks with seven treatments, a control and four replications per treatment. The products tested include, Confidor (Imidacloprid), Oportune, Eltra (Carbosulfan), Actara (Tiametoxan), Polo (Diafentiuron), Epingle (Piriproxifen) and Citronela extract, all at commercially recommended doses. Product application and evaluations were made as cited in Experiment 1.

3. Evaluation of imidacloprid (Confidor) efficiency for cassava whitefly (*A. socialis*) control.

This experiment was carried out in "Agrovelez," an industrial size cassava plantation, in Jamundí, Valle del Cauca, with the cassava variety "Reina." Eight, doses are being tested as well as several different forms of application **(Table 1.1)**. The experimental design is completely randomized blocks with 7 treatments, one control, one commercial control and four repetitions per treatment. In preliminary trials, Confidor or Imidacloprid gave the best results for *A. socialis* control.

Table 1.1. Type and doses of imidacloprid application on cassava in field trials for *A. socialis* control.

Product	Doses/ha.	Type of application
Confidor SC 350 (Concentrated suspension)	0.6 lt.	Drench* at planting
Confidor SC 350 (Concentrated suspension)	0.8 lt.	Drench at planting
Confidor SC 350 (Concentrated suspension)	0.2 lt.	Drench at emerge
Gaucho FS 600 (Stake treatment)	0.4 lt.	Stake dip
Gaucho FS 600 (Stake treatment)	0.5 lt.	Stake dip
Confidor WG 70 (Granular treatment)	0.3 kg.	Drench at planting
Confidor WG 70 (Granular treatment)	0.4 kg.	Drench at planting
Imidor (Commercial control)	0.2 lt.	Drench at emerge
Absolute control		

* Application to the base of the plant.

4. Evaluation of Imidacloprid, Tiametoxan and Carbosulfan for *A. socialis* control in the greenhouse.

In this experiment, carried out under greenhouse conditions several new products, including Actara (Tiametoxan) and Eltra (Carbosulfan) were compared to Imidacloprid for *A. socialis* control, using a completely aleatory design, with three treatments and an absolute control. Cassava leaves were immersed in the pesticide solution (commercial doses), and whitefly adults were released into leaf snap-cages. Mortality was evaluated after 48 hours. Experiments with higher than 10% mortality in the controls were discarded.

Results

Cassava Producers; Survey Results

This survey shows that cassava producers in the area surveyed do not employ a uniform criteria in cassava crop management **(Table 1.2)**. As can be seen numerous varieties and fertilizers are used within the same zone. Some varieties, such as chiroza, a very consumer popular eating variety with good fresh market value is grown throughout all the municipalities. Several types of fertilizers are applied and there is no consensus as to rate, nor time of application. Several herbicides are used for weed control, including round-up, gramoxone, karmex and estelar, as well as hand weeding or cultural practices, and there is considerable variation in doses and time of application.

Sixty Eight percent of the farmers surveyed indicated that they make their own decisions on crop management, with little or no outside technical advise. Those farmers with better economic resources, often do rely on technical assistance provided by consultants (agricultural engineers or technicians) (17%). In addition they also receive advise from agrochemical company representatives or commercial agrochemical outlets. NGO such as Fidar, Recampo and Cetec also provided technical assistance in 17% of the farms visited. CIAT has provided cassava-planting material. However 83% of the farmers surveyed have received no assistance from any organization.

Table 1.2. Cassava crop management characteristics from surveys of cassava producers in Cauca and North Valle del Cauca.

Department	Municipality	Variety	Fertilization	Weed Control	Advisors	Organization
Valle del	Alcalá	Chiroza	Lime	Round-up	Agriculturist	None
Cauca	Sevilla	Manzana	10-20-20	Gramoxone	Technician	Recampo
		Chiroza	15-15-15	Karmex	Agronomist	Cetec
	Caicedonia	HCM1	Urea	Estelar		Fidar
		Chiroza	KNO3	Cultural		CIAT
		Gallinaza	Microelements			
Cauca	S. Quilichao	523-7	DAP			
		Parrita	Cosmocel			
		Rancho Azul	Pdn. cassava			
		Verdecita	Kelatex -Zn			
		P12	KCl2			
		Algodona	Chicken manure			
	Buenos Aires	Verde	10-30-10			
		Barejona				
		Negrita				
		Roja				
		Venenosa				
		Blanquita				
		Verdecita				
	Caldono	Chiroza				
		J Jobio				
		Algodona				
		Roja				
		Varita				
		Chiroza				
		Batata				
		Raya Siete				
		P 12				

[1] 43 farmers surveyed.

Surveys of pest problems resulted in identifying nine arthropod pest species **(Figure 1.1)**. These include fruitflies (*Anastrepha* sp.), burrower bugs, hornworm, white grubs, leaf-cutter ants, thrips, shootflies (*Silba* sp.), mites and whiteflies. This is not surprising as the cassava crop is often accompanied by a diverse complex of arthropod pets. Their incidence and populations can vary from one zone to another. As can be observed **(Figures 1.1 and 1.2)** most pests are in low populations, indicting little or no economic importance. However populations of whiteflies in the North Cauca region were very high **(Figure 1.1)**, and probably causing yield losses.

A comparison between farm size and pest complex and populations indicates that cassava plantations of less than 10 ha. have a greater pest complex than larger plantations **(Figure 1.2)**. Whiteflies predominate on these smaller farms. This greater diversity if probably due to the fact that the smaller farms tend to be traditional cassava growers, planting cassava for numerous years, so pest species and populations have, over time, had the opportunity to establish. Larger farms are more recent plantings and pest problems have not yet established nor accumulated to higher populations. However, as can be observed **(Figure 1.2)**, whitefly populations are already high on these larger plantations, owing to the flight capacity or mobility, aided by wind currents, of whiteflies. These results confirm that some pests may become more problematic as plantation size increases.

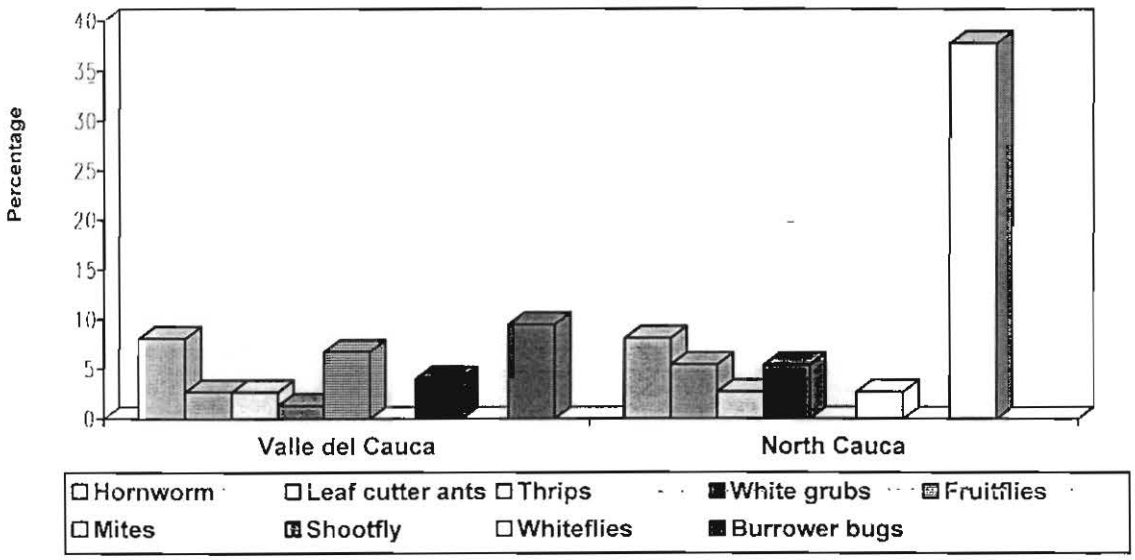

Figure 1.1. Cassava pest frequency from farm surveys in North Cauca and Valle del Cauca.

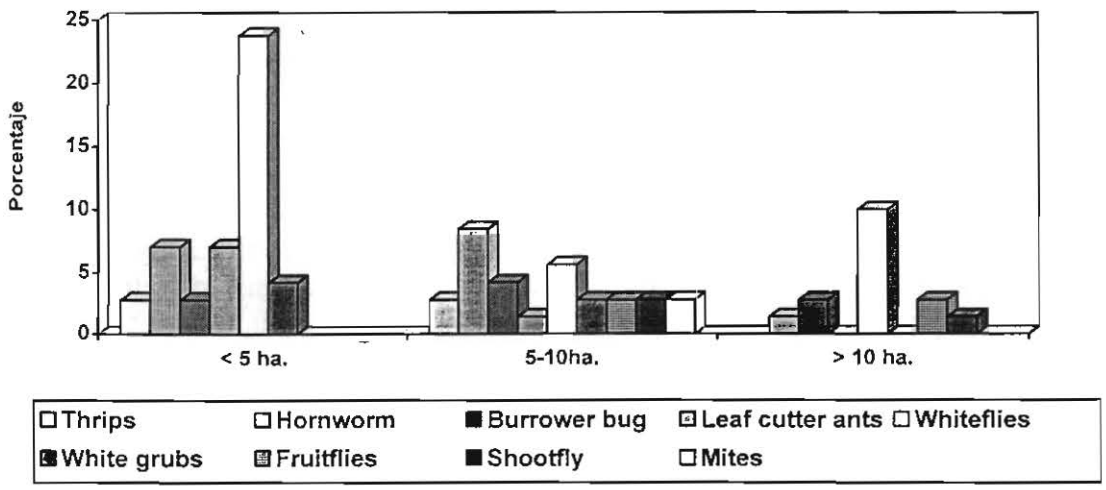

Figure 1.2. Cassava pest frequencies influenced by farm size in North Cauca and Valle del Cauca.

Evaluation of chemical and biological pesticides.

Results from the different biological and chemical pesticide evaluated for *A. socialis* control were similar **(Table 1.3)**. The biological products, biomel, bioneem and bioterpeno required additional applications (6 rather than 5 for other products). Only 3 applications of Imidacloprid (Confidor) were required to maintain low whitefly populations and this pesticide was the only treatment giving significantly different results **(Table 1.3)**. The other products did not present results significantly different from the control.

Table 1.3. The effect of different chemical and biological products on *Aleurotrachelus socialis* nymphs in the field.

Treatment	No. Applications	*A. socialis* Adult Population[3]			
		15 d.a.p.s*	45 d.a.p.s	75 d.a.p.s	105 d.a.p.s
Vegetable oils	6	1.91 ab[1]	3.42 ab	4.17 a	2.71 a
Neem	6	2.75 ab	3.62 ab	3.96 ab	2.62 a
Natural terpenoids	6	2.87 ab	3.54 ab	3.96 ab	2.48 ab
Imidacloprid	3	1.42 c***[2]	2.58 c***	3.42 b***	2.04 b ***
Buprofezin	5	2.25 b	2.96 ac	4.29a	2.48 ab
Metamidofos	5	3.21 a	4.00 a	4.37a	2.65 a
Dimetoato	5	1.92ab	3.79 ab	4.04 a	2.71 a
Cipermetrina	5	2.33 ab	3.62 ab	4.12 a	2.50 ab
Control		3.12 ab	3.58 ab	4.37 a	2.92 a

[1] Duncan test, numbers followed by the same letter are not significantly different at the 5% level.
[2] Dunnett Comparison Test of treatments vs. control.
[3] Based on population scale; 1=no whiteflies present; 2=1-200 individual per cassava leaf; 3=201-500 per leaf; 4=501-2000 per leaf; 5=2001-4000 per leaf; 6=>4000 per leaf.
* d.a.p.s.: Days after application

The effects of the different products on adult populations were not significantly different from the control (no application) **(Table 1.4)**. Buprofezin and Imidacloprid showed a significant difference in egg population from the control, but not between treatments.

These results show that it is difficult to control *A. socialis* with conventional biological and chemical pesticides. As indicated earlier this may be due to the cerosine covering of immature *A. socialis* stages, that may provide protection, rendering pesticide applications ineffective. Metamidofos (Tamaron) and Dimethoate (Systemin) were at one time effectively used to control *A. socialis*, but due to continued applications, *A. socials* may now be resistant to these pesticides. Many of the products have been continually used for a considerable number of years to control whiteflies and may now be losing their effectiveness.

The methodology employed in this experiment permitted better knowledge of whitefly behavior over time, and was used in subsequent experiments.

Table 1.4. The effect of applications of several pesticides on *A. socialis* egg and nymph survival in field trials.

Treatment	No. Applications	Eggs	Adults[3]
Vegetable oils	6	3.60 ab[1]	3.28 ab
Neem	6	3.47 ab	3.17 ab
Natural Terpenoides	6	3.55 ab	3.32 ab
Imidacloprid	3	3.38 b	3.23 ab
Buprofezin	5	3.44 b***[2]	3.10 ab
Metamidofos	5	3.63 ab	3.46 a
Dimetoato	5	3.55 ab	3.23 ab
Cipermetrina	5	3.69 ab	3.28 ab
Control		3.83 a	3.48 a

1 Duncan test, numbers followed by the same letter are not statistically different at the 5% levels using DMS tests.

2 Dunnett test; comparison of treatments with control.

3 Based on population scale; 1=no whiteflies present; 2=1-200 individual per cassava leaf; 3=201-500 per leaf; 4=501-2000 per leaf; 5=2001-4000 per leaf; 6=>4000 per leaf.

Evaluations of chemical and biological products for whitefly control in Santander de Quilichao.

Results from this experiment show that tiametoxan gave the most effective control of *A. socialis* adults. All other products gave results not significantly different from the control **(Table 1.5)**. The best treatment for reducing egg populations was imidacloprid, when compared to the other products but did not differ significantly from the control **(Table 1.5)**.

The effects of these products on Nymphal populations indicate that several, including piriproxifen, tiametoxan and imidacloprid, gave results significantly different from the control **(Table 1.5)**. Citronela, the only biological pesticide evaluated in this trial, did not provide significant differences from the control **(Table 1.5)**.

Table 1.5. The effect of foliar applications of biological and chemical pesticides on *A. socialis* eggs, nymphs and adults in Santander de Quilichao, Cauca.

Treatment	Adults	Eggs	Nymphs[3]
Imidacloprid	1.81 abc[1]	1.96 c**	1.51 d**
Buprofezin	1.89 a	2.08 ab	1.88 ab
Carbosulfan	1.87 ab	2.06 abc	1.78 bc
Tiametoxan	1.78 c **[2]	1.97 c	1.56 d**
Diafentiuron	1.82 abc	2.07 ab	1.87 ab
Piriproxifen	1.80 bc	2.04 abc	1.70 c*
Citronela	1.87 ab	2.12 a	1.91 a
Control	1.86 ab	2.01 ac	1.99 a

1 Duncan test, numbers followed by the same letter are not statistically different at the 5% levels using DMS tests.

2 Differences highly significant.

3 Based on population scale; 1=no whiteflies present; 2=1-200 individual per cassava leaf; 3=201-500 per leaf; 4=501-2000 per leaf; 5=2001-4000 per leaf; 6=>4000 per leaf.

Evaluation of chemical pesticide for *A. socialis* control in Jamundí (Valle del Cauca).

Results from this experiment show that only tiametoxan and carbosulfan gave results significantly different from the control for adult *A. socialis* control **(Table 1.6)**. For eggs, imidacloprid was the only treatment that presented significant differences. For nymphs, all treatments were significantly different from the control, with tiametoxan giving the best results, followed by imidacloprid.

Tiametoxan gave the best results over the two experimental sites, Santander de Quilichao and Jamundí, especially for adult and nymph control. Imidacloprid gave the next best results.

Table 1.6. The effect of foliar applications of chemical and biological products on *A. socialis* eggs, nymphs and adults in Jamundí, Valle del Cauca.

Treatment	Adults	Eggs	Nymphs[3]
Imidacloprid	1.55 ab	1.61 abc	1.45 c*
Buprofezin	1.51 bc	1.65 a	1.53 b
Carbosulfan	1.49 c**	1.61 abc	1.54 b
Tiametoxan	1.48 c**	1.59 abc	1.30 e**
Diafentiuron	1.51 bc	1.54 bc	1.41 dc
Piriproxifen	1.58 a	1.65 a	1.54 b
Imidacloprid (2)	1.50 bc	1.52 c**	1.35 de
Control	1.55 ab	1.62 ab	1.61 a

[1] Duncan test, numbers followed by the same letter are not statistically different at the 5% levels using DMS tests.

[2] Differences highly significant.

[3] Based on population scale; 1=no whiteflies present; 2=1-200 individual per cassava leaf; 3=201-500 per leaf; 4=501-2000 per leaf; 5=2001-4000 per leaf; 6=>4000 per leaf.

Evaluation of Imidacloprid for *A. socialis* control in cassava in Jamundí.

Confidor (Imidacloprid) was applied in three forms, a suspension, stake treatment, and as a granular.

All treatments gave results significantly different from the control for adult control of *A. socialis* **(Table 1.7)**. The drench treatment with a concentrated suspension or stake treatment gave the best results but not significantly different from the other treatments.

The best treatment for reducing egg populations was applying imidacloprid as a drench, concentrated suspension at the time of planting. (0.6 lt./ha).

Nymphal results were similar as those for adults in that all treatments differed significantly from the control. Imidacloprid applied at the time of plating in concentrated suspension in drench from gave the best results (0.6 a d0.8 lt./ha).

Results show that a drench application at the time of planting, using a concentrated suspension at a doses of 0.6 or 0.8 lt./ha. Gives the best control of *A. socialis*.

Table 1.7. The effect different treatments and doses of the pesticide imidacloprid (Confidor) on *A. socialis* eggs, nymphs, and adults in Jamundí (Valle del Cauca).

Treatment	Adults	Eggs	Nymphs[3]
Confidor SC 350 (Concentrated	1.52 b**	1.47 e**	1.44 c**
suspension)	1.55 b	1.55 d**	1.32 d**
Confidor SC 350 (Concentrated	1.58 b	1.59 dc	1.45 bc
suspension)	1.52 b	1.65 bc	1.55 b
Confidor SC 350 (Concentrated	1.59 b	1.68 abc	1.51 bc
suspension)	1.58 b	1.68 abc	1.48 bc
Gaucho FS 600 (Stake treatment)	1.55 b	1.65 bc	1.52 bc
Gaucho FS 600 (Stake treatment)	1.57 b	1.72 ab	1.52 bc
Confidor WG 70 (Granular application)	1.67 a	1.75 a	1.78 a
Confidor WG 70 (Granular application)			
Imidor (Commercial control)			
Absolute control			

[1] Duncan test, numbers followed by the same letter are not statistically different at the 5% levels using DMS tests.

[2] Differences highly significant.

[3] Based on population scale; 1=no whiteflies present; 2=1-200 individual per cassava leaf; 3=201-500 per leaf; 4=501-2000 per leaf; 5=2001-4000 per leaf; 6=>4000 per leaf.

Evaluation of imidacloprid, tiametoxan and carbosulfan for whitefly control in the greenhouse.

These results indicate that greenhouse testing gives optimal results of *A. socialis* control. All treatments gave significantly different results from the control and all products resulted in whitefly mortalities over 73% **(Table 1.8)**. These results support finding from field trials. The three products, imidacloprid, tiametoxan and carbosulfan also gave the best control in field trials. These results also indicate that those methodologies employed for greenhouse testing of products are reliable for field extrapolation.

Table 1.8. Percent mortality of *A. socialis* adults after applications of three chemical pesticides in the greenhouse.

Treatment	Experiment 1	Experiment 2	Experiment 3	Combined Experiment
Imidacloprid	84***[1]	73.75***	87.5***	83.23***
Tiametoxan	84***	73.75***	85***	82.52***
Carbosulfan	84***	73.75***	84.5***	82.23***
Control				

[1] Dunnett comparison test of treatments with control.

Conclusions

The survey of cassava producers has provided considerable information and is an aid in executing this project as it allowed us to know the needs and priorities of cassava farmers. It also provided important information on farmer crop management practices.

In addition the survey provided important information on the cassava pest complex, identifying whiteflies, *A. socialis*, as the most important pest in the complex. The differences indicated between small (<10ha) and large (>10ha) plantations, although important at present, need to be observed over time, and needs to be compared to cultural practices, especially planting dates and patterns, varietal mixtures and pesticides used, particularly on the larger plantations.

Preliminary results with biological and chemical pesticides indicate that control of whiteflies will be difficult and may be costly. Only a few products were effective and the results of the biopesticide were/are disappointing. Confidor (Imidacloprid) was the only product that gave effective control of nymphs; nymphal control is essential for reducing whitefly populations. Tiametoxan also gave adequate control for nymphs.

Drench applications with a concentrated suspension at planting time at 0.6 to 0.8 lt./ha. Effectively reduced populations of eggs, nymphs and adult whiteflies (*A. socialis*).

Collaborators

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Sub-output 3. Disease Management Components and IPM Strategies and Tactic developed for Bean Root Rots in Africa.
(R. Buruchara)

Activity 1. Indigenous knowledge, perceptions and traditional management of bean root rots in southwest Uganda

Introduction

Bean root rots are associated with intensification in agriculture, declining soil fertility and in Eastern Africa association with bean stem maggot, making diagnosis difficulty. Research efforts have been made in characterizing the diseases, their causal agents, damage caused and in developing integrated management technologies that include varietal and cultural options. However, to develop dissemination strategies and increase the rate of technology uptake by small-holder farmers, there is need to first understand local knowledge, farmer perceptions of the diseases and their relative importance to other constraints, and traditional management strategies.

Materials and Methods

In-depth informal and formal surveys were carried out in two study sites in southwestern Uganda districts of Kabale and Kisoro where bean root rots (BRR) are a serious problem but where dissemination of management technologies had never been carried out. Informal surveys were designed to target "knowledgeable" farmers (35 in both study communities) and data collection procedures used included participant observation, key informant and group interviews, informal discussions and field observations conducted over the growing period of the crop (three months). The formal survey based on a structured questionnaire was carried on 100 farmers.

Results and Discussion

The local names for root rots in the study areas (Kabale and Kisoro) are *Kiniga* and *Churisuka* respectively. Literally *Kiniga* means, "is angry and commits suicide" while *Churisuka* means "coming home with only a hoe and no harvest". The names depict the effect of the disease on beans and its importance, assessed on the basis of the effects on the crop. Generally, damage to beans (due to diseases) is categorized into "soil and rain diseases". BRR is considered a soil disease because of rotting of roots while foliar diseases are regarded as "rain diseases" because of their association with rain.

***Disease Recognition*:** Most farmers (94 %) recognize and clearly describe above-the-ground symptoms of BRR (yellowing), while a lesser but significant number (64%) associate rotting of roots with BRR. A few think above-the-ground (yellowing) and below-the-ground (rots) symptoms are two distinct and unrelated problems. Development and appearance of symptoms is a well-understood process and 85% observe symptoms at the 2nd and 3rd leaf stage. However, symptoms and effects of root rots and bean stem maggot (BSM) are largely undistinguished.

***Cause of Root Rots*:** Conditions associated or considered to cause BRR include poor soils, continuous cropping, use of poor seed, water stagnating in fields, too much rain or drought conditions. The latter further testifies the lack of distinction between the effects of BRR, BSM or even soil fertility. Too much rain implies heavy down pours resulting in high moisture content in the soil.

***Traditional Management Practices*:** The destructive effects of root rots were early on associated with bad omen by some communities, which used certain rituals to "chase it away" without success. Other traditional management practices that have been used are varietal changes and adjustment. Sixty percent of farmers have made changes to their traditional varieties. About 50% and 40% of farmers stopped growing large and medium sized seed respectively due to their susceptibility. Forty-eight percent introduced small sized seed because of their tolerance to root rots and high yields. Sixty percent adjusted components of varietal mixtures by either removing large seeded (36%), planting only medium and small seeded (17%), increasing small seed proportions within the mixture (16%) or simply reducing the proportions of big seed (14%).

A number of cultural practices are carried out routinely for reasons other than managing root rots. These include manuring (63%) to improve soil fertility, "seasonal rotation " (100%) to meet needs for other crops, fallowing (54%) in very poor plots to improve soil fertility, planting on raised beds or ridges (90% in Kisoro) to avoid flooding, growing beans as a sole crop particularly climbers (91%), intercropping (94%) and terracing (71%). Whereas some of these practices are useful IPM components (manuring or planting on raised beds) against root rots farmers do not appreciate them as such.

Conclusion and Implications: The above clearly show that:

- Farmers have a good knowledge of the above-the-ground symptoms and overall crop damage caused by BRR based on their observations
- Farmers associate the disease with certain soil and environmental factors. However, due to reliance on ("easily visible") symptoms (yellowing, wilting) effects of ("invisible") BSM and soil fertility are easily confused as being due to root rots. This is a major diagnostic weakness that can result in the use of wrong or rejection of appropriate management practices.
- Traditional management practices having useful effects have been limited to varietal manipulation leading to reduction or elimination of large and introduction of small seeded components in varietal mixtures.
- Certain practices (manuring, planting on mound or ridges) are routinely used for other reasons and are not appreciated as components in the IPM of root rots.

Results obtained clearly show the need for using a learning approach in introducing and dissemination of IPM technologies against root rots (and BSM) among the communities studied. However, the understanding of the farmer traditional knowledge of BRR gained should enable development of appropriate materials information to fill gaps in farmers' knowledge.

Contributors

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Sub-output 4. Make more Options Available for Managing Soil Productivity and Bean Pests.
(K. Ampofo)

Activity 1. Studies on *Ootheca* biology and development

Introduction

Farmers believed "*Ootheca* came with the rains and went with the rains", however, having realized that *Ootheca* developed in the soil within their own fields, they were eager to learn more about the biology and development in relation to their production circumstances. We encouraged farmers to sample soil for the appearance of the different stages of the pest in the learning plots. In addition we undertook a lab and screenhouse study to understand the details of *Ootheca* biology.

Materials and Methods

Mating pairs of *Ootheca* were collected from the field and caged with a petri dish of loose soil. They were monitored for oviposition and all eggs laid were collected, counted and incubated under ambient temperature conditions. Neonates were removed and placed in soil with a potted bean plant. There were 168 such pots. Each week 6 pots were removed randomly and sampled for larvae. Head capsule widths (HCW) of all larvae collected were measured. Frequency distribution of the HCW was plotted and the number of instars determined.

Results

The developmental parameters of *Ootheca* eggs and larvae are described in **Table 1.1**. Newly emerged females had a pre-oviposition period of 2 – 3 days and laid up to 564 eggs over about 3 weeks. The eggs took 2 – 3 weeks to hatch at ambient temperatures between 17 and 27 °C. Egg viability was 97.6%. There were three larval instars **(Figure 1.1)** with the larval stage lasting over 24 weeks from April until September. Mean head capsule widths of the different instars are presented in **Table 1.2**. Pupation started in September and early adults hatched in early October but remained in teneral diapause. These laboratory and screen house studies confirm field observations on *Ootheca* development but the lab studies shed more light on the biological parameters of the larvae

Table 1.1. Some developmental parameters of *Ootheca* in northern Tanzania.

Parameter	Mean ± SEM	Range
Pre-oviposition period	2.8 ± 0.34	2-3
Eggs laid /female	148 ± 107	20-564
Oviposition period	22 ± 13	3-52
Egg batch size	59 ± 5	53-67
Number of batches	4.5 ± 2.5	1-8
Incubation period (days)	16.9 ± 1.89	13-20
Incubation temperature (°C)	20.8 ± 2.67	17-27

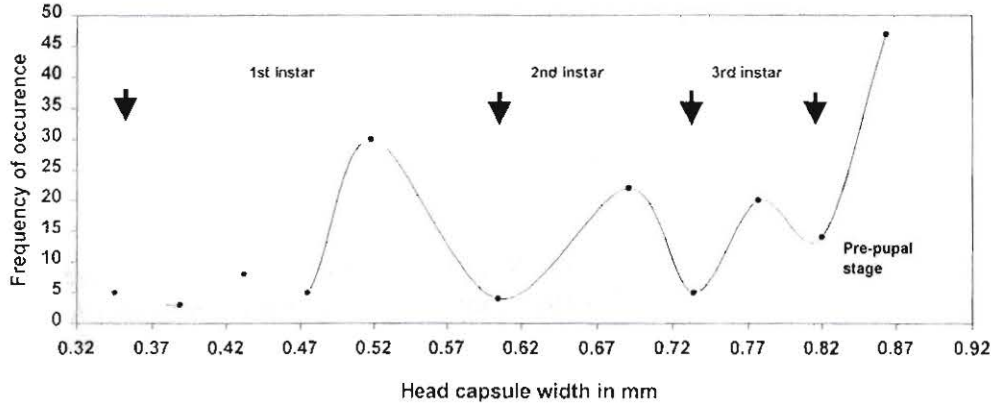

Figure 1.1. Frequency distribution of larval head capsule widths of *Ootheca.*

Table 1.2. Mean head capsule widths (mm) and duration of different larval instars of *Ootheca.*

Instar	Head Capsule Width		
	Mean ± SEM	Range	Duration in Weeks
First	0.485 ± 0.0664	0.345- 0.604	5
Second	0.695 ± 0.0244	0.604 – 0.734	8
Third	0.794 ± 0 0237	0.734 – 0.820	11

Activity 2. Understanding factors that influence bean stem maggot population dynamics

Introduction

BSM ecology is not well understood and this leads to difficulties in predicting population changes. This year we monitored BSM species population dynamics in relation to various climatic variables and the incidence of parasitism. The data could be used to develop models for advisory forecasting the pest's populations.

Materials and Methods

Beans were planted on weekly basis at Selian, Arusha (ca 3^0 S and 1380 m.a.s.l.). Plants were removed at 3 weeks after emergence and all insects extracted and sorted into species using puparial characters. Other plants from the same group on each sampling occasion were placed in a paper bag and sealed to determine adult or parasite emergence. Parasites are stored for later species determination.

Results

O. spencerella was dominant throughout the monitoring period **(Figure 2.1)**. There was always a decline in BSM populations to the lowest level during April each year, but populations were high in May to August. The population increases and decreases of the parasite population appear to follow the BSM population trends very closely. Rainfall (a) does not appear to be a major factor, even though low populations in April coincided with periods of high rainfall. These periods also coincide with the planting of beans in the Arusha area. Delayed planting often results in high BSM infestation.

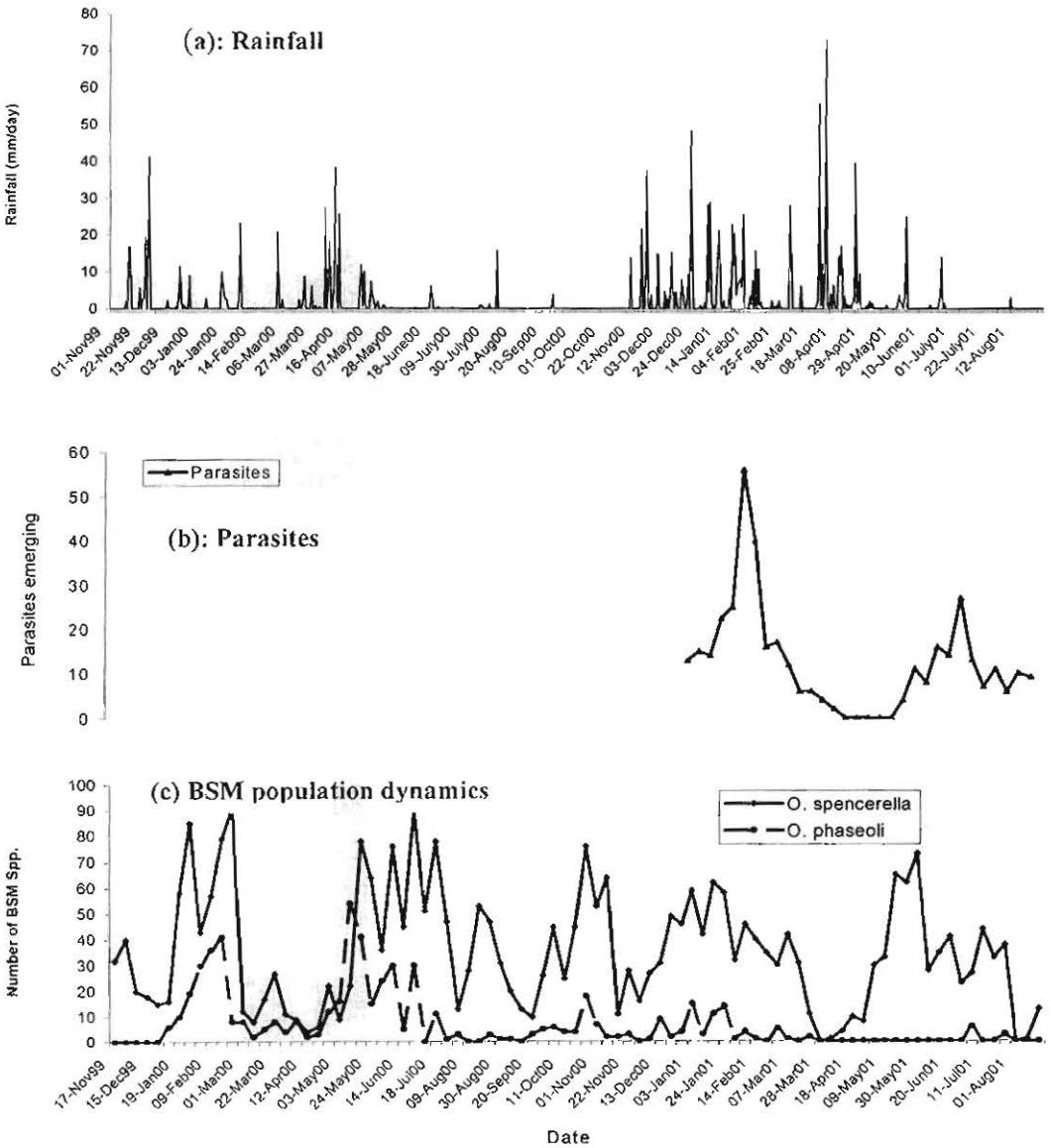

Figure 2.1. Relationship between rainfall patterns, the incidence of parasitism and BSM population dynamics in Arusha.

Activity 3. Relationship between pod damage characteristics, time of harvest and bruchid infestation

Introduction

Farmers experience high bruchid infestation in their harvested produce after a few weeks of storage. This forces them to sell their produce soon after harvest for low prices. They were unaware of bruchid infestation in the field.

Materials and Methods

A sequential harvest program was initiated with the community in Sanya Juu (N. Tanzania) to monitor the relationship between time of harvest and bruchid infestation. Beans were harvested at physiological maturity, and at two weekly intervals thereafter for 4 weeks. The harvested beans were grouped into split and intact clean pods and according to the number of holes (pod borer damage). The grains were extracted from the pods and stored in sealed paper bags for 8 weeks. The grains were then examined and all emerging insects were collected and recorded, grain damage was also recorded. The data were analyzed and discussed with farmers.

Results: Grains from pods that provided access for bruchid entry; pods with pod borer damage and split pods (as a result of delayed harvesting) were more attacked than intact pods **(Figure 3.1)**. Also pods harvested at physiological maturity had significantly less damage than later harvested pods. Grain damage increased progressively with weeks after physiological maturity **(Figure 3.2)**. Each week of delayed harvest resulted in 7 % grain infestation by Acanthoscelides obtectus.

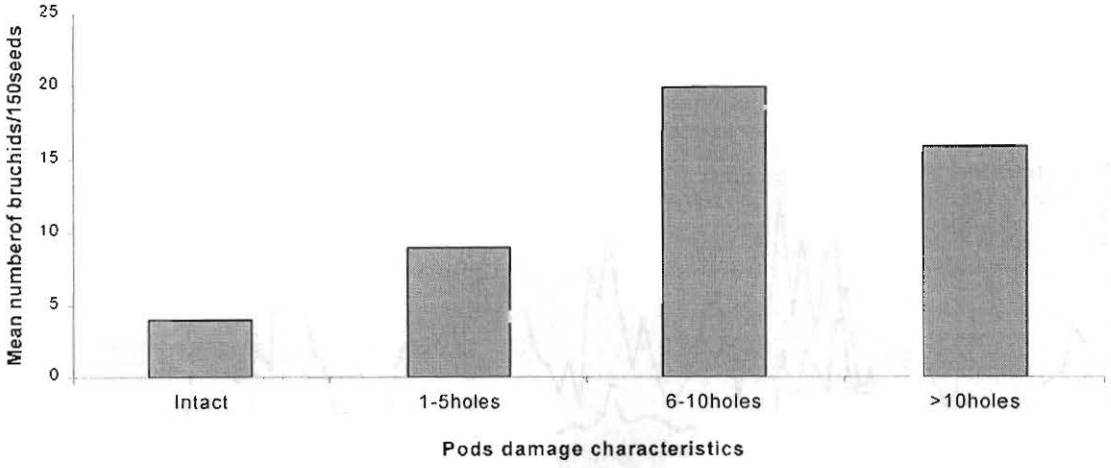

Figure 3.1. Pod damage characteristics at harvest and bruchid infestation.

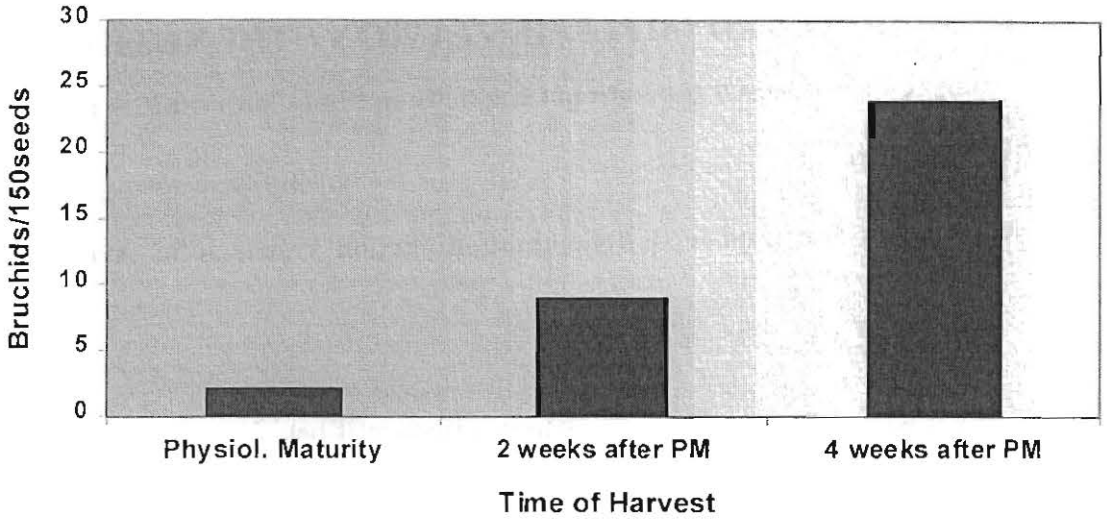

Figure 3.2. Relationship between time of harvest after physiological maturity and bruchid infestation.

Among the Wasambaa in Lushoto District of Northern Tanzania, farmers harvest soon after physiological maturity (early) as a tradition and sell their produce soon after harvest without much time in storage, either because they are aware of the high bruchid infestation associated with delayed harvest or that they sell wet seed to take advantage of the seed weight. The farmers' claim they gain more from the early harvest than if they harvest late. Grain harvested this way and air-dried was kept for 4 months without bruchid emergence. For longer term storage, farmers add dust or sun-dry or smoke the harvest and this protects the grain for much longer.

Collaborators

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

Sub-output 1. Catalyze Improved Organizational Capacity in Pilot Communities in East Africa.
(K. Ampofo)

Activity 1. Approaches for improved dissemination: action research in Arumeru district

Introduction

Disseminating agricultural technologies is more likely to be effective if based on farmers' ways of learning about new ideas and how they pass on innovations to other farmers. This action research project seeks to put theory into practice by working in close collaboration with farmers on some of their problems. It also monitors the dissemination of several technologies that are developed or adapted together. Its output is ideas and approaches for enhancing dissemination, necessary for achieving impact.

Materials and Methods

During this third phase, some trials continued in three villages in three agro-ecological zones of Arumeru District, Northern Tanzania. Our main focus was on how to phase out and to empower the farmers to continue on their own. Each village decided on the research area and the research plan was discussed together. The resulting small trials focused on evaluation of bush and climbing beans, maize, wheat and safflower varietal evaluation, production practices and pest management. The trials were farmer planned, implemented and managed. During meetings with the participating farmers, we discussed the outcome of this year's trials but also evaluated the past two years of collaboration. The researchers encouraged the farmers to continue with their research, although CIAT was leaving the area, as the project was coming to an end. We assured them, that they should continue to come to the research station for help when needed.

Results and Discussion

Two villages received varieties of climbing beans. They were planted on the grounds of the local primary school and on farmers' fields. The crop failed in one village as a result of heavy bean stem maggot infestation, in spite of the seed dressing (Murtano = lindane +thiram) used by farmers. The farmers group selected preferred varieties and the harvested seed distributed to farmers, school children and teachers for further multiplication and testing.

In collaboration with the wheat section of SARI we planted 3 wheat varieties in Kisimiri on three plots. In one plot, the plants died before flowering because of suspected manganese deficiency. The other two plots performed well and the wheat was harvested. In the same village, a few farmers continued to plant safflower (seed from last year's harvest) and had good yields. Farmers also planted 10 highland maize varieties in search for a replacement of their traditional variety that does not yield well, matures late and has many disease problems (e.g. smut). The farmers

identified preferred varieties, but the harvest data could not yet be collected. 10 farmers in two villages planted 7 drought tolerant maize varieties supplied by the maize section. Due to lack of funds, the research partners could not evaluate the trials, but the farmers identified a few preferred varieties. A collaborator from the animal husbandry section conducted seminars and taught the farmers on how to care for their animals, how to recognize diseases and how to treat them, how to build improved sheds and how to plant fodder shrubs, grasses and legumes. We gave the farmers seeds of fodder plants and they established demonstration plots.

Activity 2. Support farmers' experimentation and application of technical skills

Introduction

Farmers in Olmotonyi experience pest problems in beans, vegetables and maize. They do not want to use too much chemical pesticide and are looking for an alternative.

Materials and Methods

One farmer offered a field with diamond back moth infested cabbage field as trial site. As a group they decided that they want to test "Ormabinu" (a local medicinal plant), and fermented cow urine. The two treatments were sprayed and the farmer recorded insect abundance and damage. After two days he noticed that the treatments might not work because of the heavy rains during those days. We then decided together, to use stronger treatments (for him to be sure not to lose his crop): neem seed powder and a chemical pesticide (Selecron). He sprayed again and monitored the trials. After one week he repeated the neem treatment and after two weeks both treatments.

Results and Discussion

The farmer noticed that Selecron kills fast, but that neem needs almost 5 days to show first effects, but then it protects well. The farmer learned the need of frequent spraying with the neem extract for good results. Damage was similar in both treatments. In spite of the time lad for neem to take effect", he prefers neem to Selecron, saying that neem is a medicine but Selecron is a poison".

Activity 3. Scaling up participatory IPM development and promotion with small holder farmers through strategic alliances with specialist NGOs

Introduction

Many technologies have been developed for the management of pest problems in smallholder production systems but a lot have remained out of reach to them. Community participatory approaches to increase awareness of the availability and adoption of IPM technology were initiated with farming communities and the research and extension services in several countries in eastern and southern Africa with funding support from the DFID Crop Protection Programme.

The approaches enabled farmers to combine traditional knowledge and recent discoveries from research to make the best decision for themselves with regard to their prevailing production circumstances.

Materials and Methods

A proposal was submitted to the DFID Crop Protection Program for grant support to scale up "Participatory IPM Development And Promotion In Eastern And Southern Africa". The proposal was accepted and funded for operations in Kenya, Tanzania and Malawi. The Bean Networks made a decision to link all IPM subprojects to it and allocated funds for extension of the project to Madagascar, Mozambique and Sudan. A stakeholders' meeting (including all collaborators) was held in February to ensure a common understanding and enable collaborators to share their work plans with all. The project was initiated in April 2001. Collaborative links for dissemination have been developed with GTZ-IPM and Farm-Africa in Tanzania and with Concern Universal in Malawi.

Participating extension officers and farmer extensionists were trained in IPM methods, including pest identification, as well as the fundamentals of participatory research. Local knowledge and the available scientific knowledge were integrated and discussed for their suitability in the management of the selected priority problems. Common strategies were the use of community participatory approaches, inclusion of traditional pest management strategies for evaluation and training of extension staff and farmers on IPM concepts and approaches. The farmers groups in collaboration with their partners (research and extension staff) established learning plots at several sites within each target area to verify and learn the new management practices. At all sites there was a strong collaboration between researchers, farmers and extension, including NGOs involved in agricultural development. There were regular meetings by the farmers, extension and research staff to monitor and discuss their activities and observations from the LPs (learning plots). The project in N. Tanzania is now fully led by the extension service and their farmer communities with technical backstopping from us (CIAT and the Tanzania National Program). We help in methodology development for community research and extension activities and also undertake on-station and laboratory research to answer basic questions that could not be done in the farmers' fields.

Results

At each site farmers contributed their traditional knowledge for managing the various pests they encounter in bean production. The traditional IPM practices from the different sites were different and appeared to be related to local traditions or cultures. Among the Merus and the Chaggas of northern Tanzania, the traditional IPM practices were related to animal products (cow urine and cowshed slurry) whereas among Wasambaas (also of north-eastern Tanzania), the Nyakusi of southern Tanzania and the Sukwa, Phoka and Tumbuka of northern Malawi, the IPM practices were related to botanical products and other concoctions such as ashes of the offending pest (N. Malawi). There were differences also in farmers' knowledge about the pests they encountered: in Malawi, many farmers could describe their pests and their management strategies well but in northern Tanzania many farmers did not know their hidden pest. This difference is probably due to the level of contact with extension service.

Investigations Into The Efficacy Of Traditional IPM Methods: After confirming that their traditional technologies were effective as compared to the new scientific discoveries, such as neem **(Figure 3.1)**, the farmers were very eager to apply their traditional knowledge through the participatory IPM process to various other crops and livestock. In Lushoto District the process has been integrated with farmer training in Integrated Nutrient Management and there is demand in other sites also for integration. This process of community experimentation has been applied to the improvement of farmer understanding of the various production and pest problems they encounter.

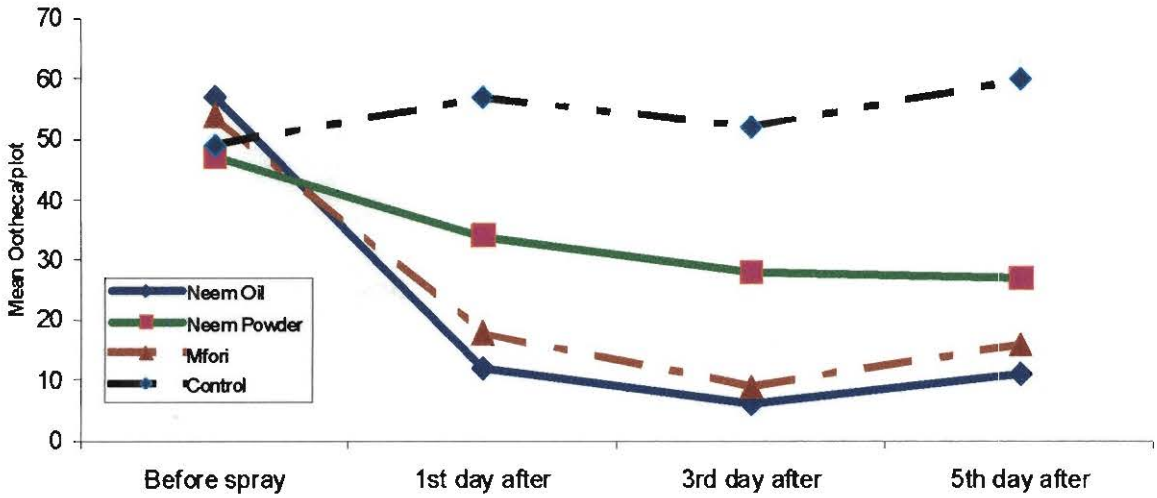

Figure 3.1. Effect of cowshed slurry and neem on *Ootheca* infestations (farmer experimentation).

Activity 4. Effectiveness of selected traditional pest management practices against bean foliage beetles (*Ootheca* spp)

Introduction

Traditional IPM strategies appear to be related to local traditions and vary from one group to another. The Wa-Sambaa use plant based concoctions (e.g. Mhasha, a *Vernonia sp.*) while the Wa-Arusha, Wa-Meru and Wa-Chagga practices are uses preferentially animal derived concoctions (e.g. fermented cow urine). These practices need to be assessed for effectiveness before they can be disseminated to other areas. We compared some of these traditional IPM practices to verify their efficacy

Materials and Methods

We evaluated the performance of some of the traditional IPM recommendations from these groups in an on-station controlled trial. The treatments were Cow urine fermented for (1) 1, (2) 5 and (3) 9 days. These were diluted at the rate of 1:3. Treatment 4 was 0.5% Neem seed oil (at 2.5 ml:1 litre of water), *Vernonia* spp. leaf extract (1:1, vol/vol). All treatments were applied at the

same day as foliar sprays. BFB were counted and damage scored before and after the treatment and then daily. When the insect counts went up and no difference between treatments could be seen, we applied a second time again all treatments.

Results and Discussion

All the treatments repelled *Ootheca* from the plots and reduced damage **(Figure 4.1)**. However, for most of them the protection only lasted about three days after which there was no effect and the pest returned. Another application of the treatments seven days after the initial application repelled the pest again. Neem seed oil and *Vernonia* leaf extract maintained infestations below the control, but the cow urine applications wore off after about five days from the initial application and needed repeated application. This confirms the efficacy of traditional IPM methods and boosts farmer confidence in their use. Farmer awareness of this has empowered them to revisit and try more traditional IPM options.

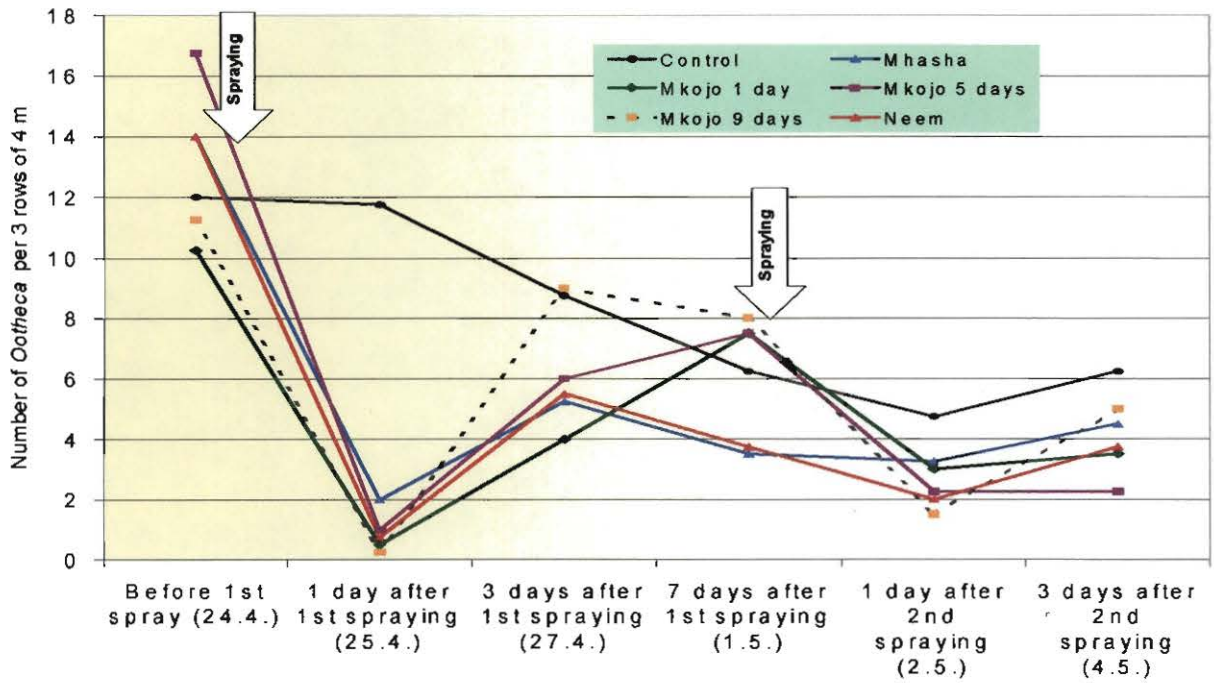

Figure 4.1. The effect of various traditional IPM recommendations on *Ootheca* infestation and damage (Figures in parenthesis e.g. 24.4, 25.4 etc. correspond to activity dates).

Activity 5. Effect of crops rotation on *Ootheca* emergence pattern

Introduction

Crop rotation emerged as an option for the management of *Ootheca* and farmers wanted to evaluate it at their learning site. They realized that part of the increasing *Ootheca* population rise

was due to the continued cropping of beans on the same plot of land and were eager to learn more about this interaction. Farmers are now aware of that *Ootheca* develops in the soil.

Materials and Methods

Learning plots were established to compare different crops: maize, cowpea, soybean, sorghum and fallow on *Ootheca* emergence from teneral diapause. Small replicated plot were planted with these crops and *Ootheca* emergence trap cages were placed over then. The traps cages were monitored at regular intervals to collect and count *Ootheca* present in them. The data was summarized and discussed with all stakeholders.

Results

Beans and cowpeas were the only crops that hatched out any significant numbers of *Ootheca* from the soils. The other crops hatched out very few adults. *Ootheca* emergence started soon after bean crop emergence and peaked at crop growth stage V3, thereafter there was a decline in the emergence pattern but emergence persisted until growth stage R5. Emergence from cowpea plots followed a similar pattern but the emerging population was low. The fact that the only beans and cowpea hatched significant numbers of *Ootheca* out of diapause suggest the presence of an emergence stimulus probably associated with root exudates. Experiments are underway to study these exudates further.

Activity 6. Monitoring and evaluation of IPM dissemination process

Introduction

Participatory research requires regular monitoring and evaluation of progress by all stakeholders to ensure that the process in on course. The objective of the project was to identify appropriate pest management strategies and dissemination pathways in smallholder production systems through participatory approaches and use lessons learned to scale up IPM technologies to the large community.

Materials and Methods

Farmers and their partners held a field day to share what they had been doing with others within the community. Farmers and other stakeholders were invited to the Sanya Juu community-learning site where they viewed different IPM practiced. The participating farmers explained the activities; other farmers shared their experiences and the whole group discussed the process and the technologies. We took advantage of the large gathering to administer a questionnaire to evaluate the dissemination process.

Results

Farmers that participated were very appreciative of the process. What they liked best was the new knowledge they had acquired and the fact that the activity was focused on issues that concerned them directly. They also liked the fact that research was working with them and that

new findings were shared directly. The farmers were very confident in sharing their knowledge and experiences with others especially as those experiences were direct. Participants in the field day were predominantly male (65.9%). A summary of stakeholders' responses (disaggregated according to gender) to issues in the questionnaire is presented in Table 6.1. Most farmers felt working in groups enhanced community cohesion, which enabled them to "educate one another", and made it easier to for them to communicate with "experts" (outsiders e.g. researchers, who are supposed to be more knowledgeable). Many group participants were however appalled by lateness and absenteeism by other; this they saw as a drag. The preferred pathway for dissemination of IPM technology was: field visits by extension officers, group demonstrations, seminars, and radio programs. The ranked the use of drama, newspapers and television quite low. One farmer group had initiated a program "Ukilima Wakisasa" with funding support from the local administration on Radio Sauti ya Njili, a local Christian station that gave them the clearest reception. Our survey suggested that the station was not the most tuned in by farmers. They are now considering a switch to a more popular station across the district. The extension service is planning to use the data to sensitize the district administration for more support and also to review the current extension pathways to focus on increased community participation.

Table 6.1. Farmer evaluation of participatory IPM development and promotion activities in Hai district, northern Tanzania.

Question/Category	Male (N=191)	Female (N=89)
	% Responding	
Desire to learn through group activities	2.6	77.5
Participation in Bean IPM and other group activities	33.0	61.0
Gets help from extension whenever needed	24.6	27.0
Frequently visited by village extension officer	17.3	9.0
Awareness of IPM promotion activities in district	41.4	50.6
Knowledge of traditional IPM methods	29.9	51.8
Recommendation for IPM dissemination channels:		
Participation in group learning activities	42.9	40.4
Visits to community learning plots	46.1	36.0
Radio programs	40.3	40.3
Visits from extension officer	33.5	27.0
Seminars	45.0	49.4
Drama	4.2	4.5
Newspapers	9.9	3.4
Radio station frequently tuned into:		
Radio Free Africa	1.0	4.5
Radio One	52.9	43.8
Radio Sauti Ya Njili	13.6	25.8
Radio Tanzania	22.5	16.9
Expectations of members of farmer groups:		
Acquisition of knowledge	12.6	25.8
Other support	1.6	9.0
How expectation was met:		
Increased yield	3.1	3.4
More knowledge	54.5	49.4

Publications

Ogecha J., J.K.O. Ampofo and J. Owuor, (Under review) Development of an integrated pest management strategy for bean stem maggot control in southwestern Kenya, Insect Science and Its Application.

Ampofo, J.K.O., U. Hollenweger, S.M. Massomo, and E. Ulicky. 2001. Participatory IPM development and extension: the case of bean foliage beetles in Hai, northern Tanzania. *A case study presented during the Workshop on Advancing Participatory Technology Development, September 17-21, 2001, IIRR, Silang Cavite, Philippines.*

Ampofo, J.K.O., S.M.S. Massomo and U. Hollenweger, 2001. Using participatory approaches for IPM development and extension: Two case studies from northern Tanzania. *Presented at the PABRA Millennium Synthesis:A workshop on Bean Research and Development in Africa over the last Decade. Arusha, May 28-June 1, 2001.*

Seminar

Ampofo, J.K.O. IPM Research and Development in the CIAT Africa Program. A seminar at the Department of Applied Entomology, Swiss Federal Research Institute (ETH) Zurich, June 2001.

Collaborators

K. Ampofo, D. Mohamed; staff of SARI (Dr. Ndondi, Mrs. Ngulu, and Dr. Mduruma, Dr. Massawe, Mr. Muhidini); Development office of the Anglican church (Mr. H. Horsch), Mr. Solomon Silongoi, Hendry Mziray, Mrs. Amanda Koola, (Hai District Extension Service), Ursula Hollenweger, Daima Mkalimoto.

Staff at the Arusha base

Dr Pyindji Mukishi, ECABREN Coordinator
Ms. Ursula Hollenweger, SDC Research Associate (Left in October 2001)

Support Staff

Mrs. Betty Travas, Finance and Administrative Assistant
Ms. Eva Ngalo, Secretary
Mr Hendry Mziray, Research Assistant (Joined in October 2000)
Mr Miraji Ndolwa, Driver/Mechanic
Mrs Julita Shirima, Office Cleaner/ Messenger
Mr Abdalla Gamba Security Guard
Mr Meseiki Laizer, Security Guard

Sub-output 2. IPM-Cassava Training
(A.C. Bellotti)

Training Offered

Manejo integrado de plagas en yuca (IPM in Cassava).
Polos de desarrollo Cauca – 30 farmers, department of Cauca. MADR . Jun. 29, Tambo, Cauca.
CIAT-CLAYUCA. Sena-Buga -15 students. Jul. 13.
CIAT-CLAYUCA - 12 technicians and farmers, department of Cauca. Ago. 31.
CLAYUCA - 60 farmers of Magdalena Medio. Sept. 3-5. Cúcuta, Santander.
CLAYUCA - 50 technicians and farmers. Sept. 18-19. Florencia, Caquetá.
CORPOICA- 80 participants, CORPOICA. Oct. 16-18. Villavicencio, Meta.
Agroempresas Rurales - 12 farmers, department of Cauca. Oct. 30. CIAT.

Acaros plaga de la yuca y su Control. CIAT-CLAYUCA. Sena-Buga – 15 students. Ago. 17.

Curso de ácaros plaga y su control. María Virginia Bertorelli. INIA-FUNDACITE-3 weeks. Sept. 17-Oct. 5, CIAT.

Manejo de baculovirus en el control de lepidópteros, *E. ello*. Students of Entomology. Universidad del Valle. Oct. 26. CIAT.

Entrenamiento en identificación de moscas blancas. Señor Octavio Zegarra de Perú, Centro Internacional de la Papa-CIP. March, CIAT.

Manejo integrado de plagas de yuca. Farmers and technicians in Santander de Quilichao, department of Cauca - UMATAS. April.

Comportamiento de mosca blanca en campo. FIDAR, farmers and technicians, department of Cauca. May.

Training Received

Hongos y nemátodos entomopatógenos en el control de plagas en Colombia. SOCOLEN. Universidad Nacional de Colombia. Oct. 12. Bogotá.

Especialización en hongos y nemátodos entomopatógenos. Universidad del Valle. Oct. Cali.

Segundo período del Master en Biotecnología. Universidad Autónoma de Madrid, España. (April-July).

Entrenamiento en técnicas moleculares aplicadas al estudio de biotipos de mosca blanca y parasitoides, y fundamentos para estudios filogenéticos. Centro de Investigación y Desarrollo Agroalimentario. CIDA, Murcia, España. June.

Seminario Nemátodos Entomoparásitos, una alternativa en MIP. Univ. Nacional de Bogotá. Ago.

Thesis in Progress

Aleán, I. Evaluación de la eficacia de diferentes hongos entomopatógenos (Hyphomycetes) en el control de la mosca blanca de la yuca *Aleurotrachelus socialis* Bondar. Tesis de Pregrado. Pontificia Universidad Javeriana, Bogotá.

Ramírez, C. Aportes al estudio de la biología, comportamiento y distribución del barrenador del tallo de la yuca; *Chilomima clarkei* A. (Lepidoptera: Pyralidae) en el departamento del Tolima. Tesis de Pregrado, Ingeniero Agrónomo. Universidad del Tolima.

Rendón, M. Control biológico de chinche subterráneo de la yuca *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) con hongos entomopatógenos Hyphomycetes. Tesis de Pregrado. Universidad de Santa Rosa de Cabal – UNISARC, Santa Rosa de Cabal, Risaralda.

Carabalí, A. Evaluación del potencial de resistencia/tolerancia de diferentes genotipos de yuca *Manihot esculenta* Crantz al biotipo "B" de mosca blanca *Bemisia tabaci* (Homoptera: Aleyrodidae). Tesis de Maestría. Universidad del Valle, Cali.

Suboutput 3. Group training of farmers, technicians, extension workers and students. (E. Alvarez)

Seminars

Advances in the project management of Powdery Mildew in rose. Asocolflores. CIAT Palmira, February.

Asociación de Micología de Colombia. Phytophthora in palms. Diagnostic, isolation, disease management. Bogotá, February.

Field Day in participatory research, incorporation of ash and organic matter (dead leaves and sticks from forest soil), variety selection. 115 participants included technicians from SENA, CDA, JER School, Secretaría de Desarrollo del Vaupés, ONGs. Seima Central (Mitú). October 18, 2000.

Research advances in the most important cassava diseases. Carlos Yepes from Congelagro. CIAT Palmira, July 5.

Seminar on Cassava diseases and pests integrated management. Cassava varieties. 19 participants: farmers, technicians from Umatas (North of Cauca), students and professor from Universidad Nacional de Colombia (Palmira). CIAT Palmira, July 13.

Field day. Root rots integrated management. 12 participants: Farmers and technicians. Farm La Elena Municipality of Montenegro, Quindío. August 8.

Training Offered

Training to four oil palm technicians in a Bud Rot control strategy. Villanueva (Casanare) and Paratebueno (Cundinamarca) January, March, April.

Training to 10 indigenous communities in participatory research, variety selection and incorporation of ash and organic matter (dead leaves and sticks from forest soil) in soil. 77 participants included technicians from SENA, CDA, JER School, Secretaría de Desarrollo Agropecuario, ONGs. Mitú. April 20th.

Cassava diseases integrated management, presented in: Curso intensivo sobre el manejo agronómico y poscosecha del cultivo de la yuca con fines industriales. Corpoica, Villavicencio, 36 participants April 24 - 26.

Molecular techniques. Universidad Nacional de Colombia (Palmira). Professors and students. 10 Professors and students participated. April 4 - 6.

Training to Mariana Valencia, Microbiologist from Levapan S. A. RAPDs and AFLP. 6 months. February to August.

Training to César Ospina, Thesis student from Corpoica, Palmira on PCR. March – November.

Cassava diseases integrated management. 31 participants: Farmers and technicians. El Tambo (Cauca), June 29.

Case study: Participatory research to control cassava root rots, presented in the course: Methods and techniques of farmer participation in research. 24 participants: CIAT research assistants, professionals from Agriculture Ministry from Cuba and Costa Rica and INIA (Chile). CIAT Palmira, June 29.

Cassava diseases integrated management. Sena from Buga (Valle). 18 participants: Students and technicians. CIAT Palmira. August 17.

Ramón Arbona (Dominician Republic). Research and management of Commom Bacterial Blight and Superelongation Disease in cassava. August 23.

Training Received

Course: Methods and techniques of farmer participation in research. CIAT Palmira, June 29.
Course: Statistical analyses for molecular markers. CIAT Palmira, August 8-10.
Course: The nature of disease resistance, mechanisms of pathogenesis and signal transduction in plants. Dr. David Collinge, Associated Professor. Royal Veterinary and Agricultural University (Denmark). CIAT, October 16 – 20.

Thesis in Progress

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

Llano, G.A. Evaluación de la homología de sondas heterólogas en el genoma de yuca y su asociación con la resistencia a *Phytophthora* spp. Tesis para Maestría en Ciencias Agrarias con énfasis en Fitomejoramiento Universidad Nacional de Colombia, Sede Palmira.

Trujillo, O.F. Producción sostenible de yuca en un sistema agroforestal indígena de Mitú (Vaupés), con participación comunitaria. Beginning: September 1.

Celis, A. Determinación del agente causal de la enfermedad Marchitamiento Letal en palma de aceite. Beginning: September 1.

Mejía, J. F. Caracterización molecular y patogénica de aislamientos de S*phaceloma manihoticola* provenientes de la región centro-sur de Brasil.

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OUTPUT IV. GLOBAL IPM NETWORKS AND KNOWLEDGE SYSTEMS DEVELOPED

Sub-output 1. Systemwide Project On Integrated Sustainable Management Of Whiteflies As Pests And Vectors Of Plant Viruses In The Tropics. (P. Anderson)

Emerging diseases and pests

The emergence and dissemination of whitefly-transmitted geminiviruses in the Americas

The proliferation and rapid dissemination of whitefly-transmitted viruses of important food and industrial crops in Latin America, have been the consequence of dramatic changes in traditional cropping systems. Bemisia tabaci has been shown to transmit at least 20 different gemiiviruses that affect different commercial and basic food crops in Latin America. Morales and Anderson (2001) reviewed the existing knowledge on the emergence of Bemisia-transmitted geminiviruses in cotton, beans, tomatoes and other vegetables over the last 30 years, and offered several explanations for the inability to control the whitefly/geminivirus problem in Latin America, despite the availability of different whitefly control tactics.

First, all the national agricultural research institutions of Latin America have suffered significant losses of experienced personnel and operational resources. This loss in human resources and technical assistance occurs at a time when Latin American farmers are trying to grow new crops they are unfamiliar with. There are no interdisciplinary research teams currently investigating the whitefly/geminivirus problem as a complex. Most of the information generated on *B. tabaci* has been produced by entomologists, without much consideration for the role of this species as a virus vector. Most of the research on tropical geminiviruses has been conducted in temperate countries by molecular biologists. Young agricultural scientists from developing countries are being trained in advanced laboratories on the use of molecular techniques. As a result, the complex interactions among geminiviruses, whitefly biotypes and new cropping systems are often ignored.

Whitefly and geminivirus specialists usually select one or few crops to work with, either because of their mandate or because of economic circumstances (i.e. availability of research funds to study geminiviruses of economically important crops, such as tomato). Thus, the cropping systems' vision required to solve these complex pest problems, is lacking. Another negative factor has been the "projectization" of agricultural research and lack of operational funds for germplasm improvement, which has prevented the development of both long-term epidemiological studies, and development of geminivirus-resistant plant germplasm. Of all the crops currently affected by WTGs in Latin America, only the common bean, and to a much lesser extent tomato, have been genetically improved for geminivirus resistance. The development of pest and disease-resistant cultivars that eliminate or reduce the need for frequent pesticide applications, is a must for the implementation of sustainable agricultural practices. The development of transgenic plants possessing resistance to geminiviruses is a promising but costly undertaking, which should not exclude the application of conventional breeding methods to combat whitefly-transmitted geminiviruses.

The control of whitefly-transmitted geminiviruses requires the concerted participation and close collaboration of molecular biologists, virologists, entomologists, plant breeders, agronomists, epidemiologists, economists, sociologists and concerned administrators, if we expect to defeat the "pest of the century".

Morales, F.J., and P.K. Anderson. 2001. The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. Archives of Virology 146: 415-441.

***Bemisia afer sens. lat.* outbreak in the Americas**

The first outbreak of *Bemisia afer sens. lat.* in an agricultural situation in the Americas is reported. *B. afer* was discovered on sweetpotato (*Ipomoea batatas* Lam.) in the Cañete Valley in the central coast of Peru.

Bemisia tabaci (Gennadius) was reported on sweetpotato (*Ipomoea batatas* Lam.) from the central coast of Peru in the late 1980s, noting that it was not a significant pest (Redolfi 1989, cited in Nuñez 1995). However, in the 1997-1998 agricultural season, unusually large populations of *Bemisia tabaci* were reported to be significantly affecting sweetpotato yields in the coastal valleys of Peru (Valencia et al. 2000). In August of 2000, P. Anderson (CIAT) made a field visit to the Cañete Valley, approximately 100 km south of Lima, with Cristina Fonseca of the International Potato Center (CIP) and Ing. Jose M. Valencia of the Cañete Experimental Station, to explore the problem. The nymphs that were actively reproducing on sweetpotato were *Bemisia*. However, the adult whiteflies, which were abundant on sweetpotato and pepino (*Solanum muricatum* Ait.) were larger and whiter (more *Trialeurodes*-like) than typical for *Bemisia tabaci*. Thus, nymphs were collected from sweetpotato for taxonomic verification.

Whitefly nymphs were slide-mounted and tentatively identified as *Bemisia afer*, by P. Hernandez at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. The identification was verified as *Bemisia afer sens. lat.*, by J. Martin at the Natural History Museum in London, UK (BMNH). Voucher specimens were deposited in the BMNH.

This is the first outbreak we have observed of *Bemisia afer sens. lat.* in an agricultural situation in the Americas. *B. afer* has been recorded from Egypt, Greece, Sicily, the Middle East, the Ethiopian region, Comoro Islands, India, Pakistan, New Guinea, Fiji, Tonga (Martin 1987), Sudan, Sierra Leone, Cote d'Ivoire, Nigeria, Niger, Chad, Cameroon, Congo, Zaire, Uganda, Rhodesia, Malawi, South Africa (Bink-Moenen 1983), and Australia (Martin 1999). *B. afer* has hitherto been considered as a common and widespread pest species, feeding on a wide variety of plants (Martin 1987).

In Belize in 1994 and 1996, plants of a papaveraceous host, *Bocconia frutescens* L., were found to be colonized by very large populations of a species of *Bemisia* with highly characteristic puparia. This belongs to *B. afer sens. lat.*, but the puparial characteristics fall outside those normally observed in areas of the world where *B. afer* is widespread. While studying the whitefly collection of the US National Museum of Natural History (housed at USDA, Beltsville, Maryland), Martin noted a small number of *Bemisia afer*-group samples that are likely to be

conspecific with the samples from *B. frutescens* in Belize. These samples were either field-collected in, or intercepted by US quarantine authorities from Honduras, Mexico and El Salvador. Quoted host plants include *Pouteria* sp (Sapotaceae), *Hibiscus* sp (Malvaceae), *Origanum* sp (Labiateae), *Ficus* sp or spp (Moraceae), *Serjania* sp (Sapindaceae) and *Psidium guajava* (Myrtaceae). There are also two additional slides from Belize in BMNH, one from an unidentified woody vine and matching the *Bocconia* puparia, and the other (possibly a smooth-leaf form of the same species) from a wild cassava plant growing on a forest track remote from agriculture. From this material, it appears that this taxon is widespread and oligophagous in Central America.

Bink-Moenen (1983) proposed the synonymy of *Bemisia hancocki* Corbett (1936) with *B. afer* (Priesner and Hosny 1934). This synonymy was based on examination of one badly damaged syntype of *B. afer* deposited in the BMNH. Based on Martin's subsequent examination of a complete syntype puparium of *B. hancocki* deposited in USNM, this synonymy may have been premature. However, with the considerable degree of puparial morphological plasticity now becoming evident within the *B. afer* group, formally resurrecting *B. hancocki* could cause further nomenclatural confusion at this point.

B. hancocki was first described from cotton (*Gossypium hirsutum* L.) in Uganda by Corbett (1936). Mound (1965) examined *B. hancocki* specimens from cotton, peanut (*Arachis hypogaea* L.), and *Vigna (catjang) unguiculata* (L.) Walp., and noted *B. hancocki* collections from cassava (*Manihot esculenta* Cranz) in Sierra Leone, Nigeria, Cameroon, and Sudan. He further described the variation in the puparial morphology of *B. hancocki* as being almost as great as that of *B. tabaci*. Personal observations made by Martin, Estrella Hernández-Suarez (ICIA, Canary Islands, Spain) and by Raymond Gill (CDFA, Sacramento, USA) indicate that the *B. afer* group actually displays considerably greater puparial morphological variation than does *B. tabaci* and its forms/biotypes.

Although specimens from the *B. afer* group have been previously discovered in non-agricultural situations in the Americas, this is the first report of a *Bemisia afer sens. lat.* outbreak on an important crop host in the New World. The extent of *B. afer* dissemination and its host-associations in Peru need to be investigated. Furthermore, the taxonomy of *Bemisia afer* and *Bemisia hancocki* should be re-visited and, the possible role of *B. afer* in virus transmission needs to be clarified.

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