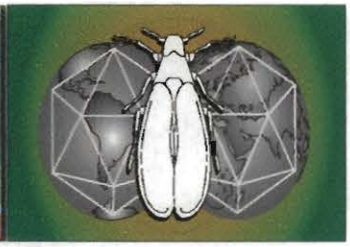
INTEGRATED PEST AND DISEASE MANAGEMENT IN MAJOR AGROECOSYSTEMS

CIAT

PROJECT-PE1
Annual Report 2000

System-wide Programme on
Integrated Pest Management



PROJECT PE-1

INTEGRATED PEST AND DISEASE MANAGEMENT IN MAJOR AGROECOSYSTEMS

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PROJECT PE-1: INTEGRATED PEST AND DISEASE MANAGEMENT IN MAJOR AGROECOSISTEMS

PROJECT DESCRIPTION

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

Outputs:

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

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

Milestones:

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

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

2003 Research on soil-borne arthropods and pathogens advanced and coordinated with systemwide programs. Research on invasive pests defined and underway. Utilization of cassava frogskin tolerant varieties in breeding and IPM programs. Biological control through entomopathogens developed for soil borne pests. Whitefly natural enemies 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 phytophothora root rot resistance using marker aided selection. Epidemiological validation of specified whitefly-transmitted geminiviruses.

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

Collaborators: IARCs (IITA, ICIPE, CIP, USDA, Crop and Food Research Institute), Advanced research institutes (e.g., CATIE, NRI, Univeristy of Florida, Wisconsin, Cornell, Iowa and São Paulo, John Innes Center, ETH/IRD/CIRAD, Boyce Thompson Institute), NARS (e.g., EMBRAPA, CORPOICA, ICA, INIAP, INIVIT, NARO), NGOs, private industries (CENIPALMA, BIOCARIBE, S.A.).

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

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

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

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Critical Assumptions beyond control of Project Team
Program Goal: To increase crop yields and reduce environmental contamination through the effective management of major pests and diseases.	-Increased cassava yields -Reduction in environmental degradation due to adoption of improved technology -Reduced losses due to several major diseases	-Production statistics -Adoption and impact studies -Project reports	-National policies favorable to adoption of IPM strategies (i.e. increased support to extension, reduction of subsidies to pesticides). -National programs are active and strong in key countries
Project Purpose: To develop and transfer knowledge systems and pest and disease management components for sustainable productivity and healthier environment.	-Adoption of germplasm with resistance to biological constraints -Establishment of release natural enemies -Use of environmentally friendly control strategies -Improved understanding major biotic constraints	-End of project reports -Refereed publications, book chapters -Adoption and impact studies	-Financial resources are mobilized -Active collaboration with NARs -Active collaborations with other IARCs and Developed countries research organizations -Active collaboration with advanced research institutions
Outputs:		All areas: -Project reports and refereed publications, book chapters, etc.	
1. Pest and disease complexes described and analyzed.	-Pests, diseases, natural enemies and vectors characterized -Host/pest/natural enemy/vector interactions analyzed -Better diagnostic tools available -Biological control agents established -Better understanding of the influence of drought in hot/pest interactions -Identification of cassava with tolerance to diseases -Pest and disease distribution (maps) determined	-Reports with maps, economic damage, biological information. -Analysis of experiments -Transfer of tools to seed health facilities -Analysis of experiments -Guidelines for IPM -Reports on field effectiveness and probability of adoption of components -Field oriented brochures	-NARs have the needed resources -Adequate interaction with other disciplinary scientists -Successful experiments -Continued development of new varieties that are commercially acceptable -Farmers have adequate access to extension agents, credits and other factors that impact on adoption -Collaborative with NARS possible -Evaluation, screening, exploration sites accessible
2. Pest and disease management components and IPM strategies and tactics developed.	-Testing of components for effectiveness. -Control strategies recommendations clearly identified and crop management practices determined -Farmer testing of components -Guides on IPM strategies published -Disease detection methods available -Web site published	-Reports on training courses -Concept notes and projects prepared with partners -Electronically published web pages and databases	
3. NARS capacity to design and execute IPM research and implementation strengthened.	-Training especially in FPR -Development of projects with NARs -Training materials developed		
4. Global IPM networks and knowledge systems developed.	-Network of researchers established -Preparation of web pages and databases with relevant IPM information		
Inputs: **Core** **Non-core** Senior staff: 193.020 Support staff: 100.700 Operations: 20.170 Total: 313.890 852.890	Senior Staff: 3.8 Support Staff: 6.5 Secretaries: 0.5 Field Workers: 4.5	-Accounting of budgets -Project reports -Donor reports	-Administration commitment to stable core support -Ability to attract continued donor support -Project office support

PROJECT SUMMARY

World wide, crop yield losses due to pests (arthropods, diseases, and weeds) are estimated to be as high as 50%, and valued at hundreds of billions of dollars per year. Chemical pesticides continue to be an important tactic for reducing pest induced crop losses. The pesticide market is estimated at more than US$30 billion per year and about 80% of the pesticides in use are applied in developing countries. As we move into the future with a growing population, especially in the developing world, we face the daunting challenge of trying to increase crop yields and reduce chemical pesticide use.

Pesticide use in CIAT commodities varies with the crop species; moderate to high levels are applied to beans, especially snapbeans, moderate levels are applied to rice, low levels to cassava and very low to none on tropical pastures. However as cropping systems change, for example the increase in cassava plantation size in Colombia, Venezuela, Brazil and other countries, the tendency to apply more pesticides will also increase.

It is therefore appropriate that the major goal of the CIAT Integrated Pest and Disease Management Project (PE-1) is to increase crop yields while decreasing or avoiding pesticide use, thereby reducing environmental contamination. This can be accomplished through the effective management of major pests and diseases on CIAT's mandate crops as well as other important food crops such as fruits and vegetables in other major agroecosystems.

The development of low cost technologies and components for effective pest and disease management and sustainable productivity is a primary purpose for the research activities carried out in the PE-1 project. During 1999, project activity expanded from being primarily cassava based, to also include East African bean system entomology and pathology research. During 2000 the project again expanded to also include grass and legume systems, particularly basic research on the spittlebug complex that causes serious losses in tropical rangelands.

Project PE-1 now consists of eight senior scientists, including four entomologists, three plant pathologists and one virologist. In addition, numerous other scientists are involved in, or collaborate with the project since the System-wide Whitefly IPM project is housed within PE-1. Research activities in PE-1 are still primarily based on CIAT's commodity crop mandates: cassava, beans and tropical forage. About 75-80% of the research output involves pests and diseases of these three crops. The project has expanded into other crops, however, such as fruits (citrus, melons), vegetables (mostly through the system-wide whitefly IPM project), oil palms, potatoes and cut flowers.

CIAT is the convening center, of the CGIAR System-wide Whitefly IPM Project. The first phase is now complete in Latin America (LA) and Africa, and is nearing completion in Asia where activities were initiated more than one year after LA and Africa. Phase I concentrated on the formation of a pan-tropical research network for whiteflies (WFs) and whitefly - transmitted viruses (WTVs) as well as the extensive diagnosis and characterization of the WF/WTV problem in LA and Africa. Some of the successes of Phase I include the formation of the whitefly IPM Network; the identification of "hot spots" in several countries where Phase II activities can be targeted; the identification of numerous serious viruses being transmitted by *Bemisia tabaci* in

several crops; the development of a mathematical model to capture the dynamics of a pathosystem; the identification of numerous potential biological control agents; and successful control of Cassava Mosaic Diseases in Africa. The Whitefly IPM Network includes 5 IARC's, 10 basic research organizations, 54 NARS institutions and a link to numerous researchers, institutions, universities, NGO's, and extension services, around the world.

As will be noted in this Annual Report, there is an increasing emphasis in the identification and evaluation of entomopathogens for biopesticide development. Priority is being given to pests of cassava (hornworm, burrower bugs, mites) tropical forage grasses (spittlebugs) and vegetable crops (whiteflies). A bottleneck in biopesticide use has been the unavailability of commercial products for producer use. A collaborative agreement has been signed with a private biopesticide company in Colombia; they will formulate and commercially produce biopesticide products, and make these available to farmers, but at the same time reinvest a percentage of the profits in entomopathogen research. In addition, the entomopathogen collections from the cassava and tropical forages entomology research projects were merged into one "Ceparium" or working collection with over 100 isolates from several entomopathogen species. Numerous new isolates from spittlebugs and burrower bugs were added to the collection.

The characterization of cassava frogskin disease (CFSD). The presence of CFSD in a very high proportion of cassava germplasm on the CIAT farm has limited field and laboratory research during the past year. Several steps are being taken to clean up the germplasm including the use of meristem tissue culture, limiting planting at CIAT to certain periods of the year, and finding off-campus sites to plant clean germplasm and avoid CFSD infection. These measures and others are being widely adopted and it is expected that by mid-2001, levels of CFSD on the CIAT campus will be greatly reduced. In addition, a continued research effort over several years, has identified resistance to CFSD in cassava germplasm, thereby offering a long-term solution to this problem. Additionally, within the IPM project, progress was made on characterization of strains on citrus tristeza virus. This objective is to develop systems for the certification of budwood including using mild strain cross protection as a control strategy.

Molecular techniques are allowing pathologists to move forward rapidly in characterizing genetic diversity of fungal pathogens, identifying genetic markers, and describing genetic variation. Cassava superelongation disease caused by *Sphaceloma manihoticola* is a serious production problem in several regions. Using AFLP's and RAM's, high genetic variation was found among *S. manihoticola* isolates. The RAM techniques showed polymorphism between isolates of the same municipality, between municipalities and between countries, confirming that genetic diversity of the pathogen exists. These techniques were also, applied to detect and characterize genetic diversity of *Ceratocystics paradoxa* (bud rot) in oil palm. Both RAM and RAPD were used for the molecular characterization of *Phytophthora* and *Pythium* isolates obtained from cassava at different locations, and several of the important morphological characteristics of these root rot fungi and their pathogenicity were described.

Pest and disease management components for IPM strategies have been, or are being, developed for several crops. A hot water treatment (49°C for 49 min.) of cassava stakes, greatly reduces CBB incidences without affecting germination. In addition, farmer participatory research with Mitu

indigenous communities demonstrated, that wood ash applied to the soil around cassava plants reduces the severity of *Phytophthora* root rot.

In Africa, evaluation of bean germplasm lines from the CIAT breeding program showed that several materials had superior performance against the bean stem maggot. These lines, distributed to NARS in Kenya and Congo DR, combined good resistance to the pest and with high yield. These lines also show good performance in Malawi, Tanzania and other sites. The superior lines are being multiplied and will be disseminated more widely.

IPM strategies for bean pests, many of these developed by CIAT and the Bean Network, are available, but scaling these up to be employed in a wider area has been slow. In order to improve adoption, village level strategies, are being developed by African scientists with farmers and extensionists in Tanzania. Dissemination pathways selected by villages include: on farm demonstrations, demonstrations at schools, training through farmer research groups, distribution of extension information, and "awareness" seminars and field tours. Farmer research groups, and extension agents implement techniques and together with monitoring of farmers perceptions.

The advent of CLAYUCA in Colombia, with considerable involvement of the private as well as the public sector, has had a very positive influence on the availability of funding for cassava research and technology development and transfer. This demand driven research has already resulted in producer demands for low cost and environmentally sound pest and disease management technologies. For example, there is a considerable desire for biopesticides, pest and disease resistant germplasm, and cultural practices that will reduce pest incidence. CLAYUCA now has 10 participating countries, and it is anticipated that this will increase funding opportunities for research and facilitate the transfer and adoption of pest and disease management technologies and IPM implementation.

HIGHLIGHTS

- Agroecozones defined for two whitefly species, *Bemisia tabaci* and *Trialeurodes vaporariorum* feeding on numerous crop hosts, in Colombia and Ecuador.
- Three genera of whitefly parasitoids, *Amitus, Encarsia* and *Eretmocerus* collected from *B. tabaci* and *T. vaporariorum* on several host crops, in Ecuador and Colombia.
- Surveys in Colombia and Ecuador confirm five whitefly species feeding on cassava, *Aleurotrachelus socialis, Bemisia tuberculata, Trialeurodes* sp. (possibly *T. variabilis) Tetraleurodes* and *Aleurodicus* sp.
- Surveys in Colombia have resulted in the identification of at least 10 parasitoid species associated with whitefly species collected on cassava. In Ecuador four parasitoid species were collected.
- The biology of the whitefly, *Aleuroglandulus malangae*, reported for the first time on cassava in Colombia, was determined on *Colocasia*, one of its hosts.
- As part as System-wide Whitefly IPM project, a whitefly species and natural enemy reference collection has been organized, with accompanying database and made available to collaborators.
- Production models for commercially produced biopesticides for control of cassava hornworm and other pests have been established with private biopesticide industry.
- CIAT database established on mites and natural enemies; it contains more than 6000 records from more than 2400 sites surveyed, available to national and international collaborators.
- Fifty three species of Phytoseiidae mite predators have been identified associated with cassava phytophagous mites, and maintained in reference collection.
- Collaboration with apple producers (NGO's) in Chile for biological control of mites has been established, and a specialists trained.
- Mass rearing techniques for selected phytoseiid (predator mite) species have been developed.
- Potential natural enemies of the Colorado potato beetle have been identified from a related species *Leptinotarsa decemlineata* found on a wild relative of potato, *Solanum torvum*, as part of collaboration project with Iowa State University.
- A contact kairomone synthesized and identified mediating host recognition and inducing oviposition by a generalist parasitoid *Aenasius vexans*
- The leucine arylamidase, a major aminopeptidase enzyme detected in the digestive tracts of the cassava mealybug *Phenacoccus herreni* and the whitefly *Aleurotrachelus socialis*, useful information for identifying proteinase inhibitors
- Further evidence that *Phenacoccus manihoti* and *Phenacoccus herreni*, the cassava mealybugs causing severe damages on cassava in Africa and in South America respectively, are distinct species by a molecular-based approach study
- Comparative biological studies on three spittlebug species (*Zulia carbonaria, Zulia pubescens, Zulia* sp. nov.) were completed and studies initiated on a fourth (*Prosapia simulans*).
- Detected and preliminary assessment was made of the presence of the Central American forage grass and sugar cane pest, *Prosapia simulans*, in *Brachiaria decumbens* of the Cauca Valley, which is a first report of the species and genus in Colombia and South America.

- Substrate communication in spittlebug adults was further characterized by describing the male courtship calls of three species and confirming significant differences in call structure among taxa.
- Data was gathered on early season population dynamics of spittlebug nymphs and adults in three contrasting sites to measure the correlation between phenology and rainfall and to gauge potential to predict the timing of outbreaks.
- New studies established on egg diapause including an experiment to test the effect of preoviposition conditions on diapause incidence and a study to document seasonal changes in diapause incidence among field populations in three contrasting sites.
- Patterns of variation in the biology, behavior, and ecology in the spittlebug species complex, fundamental for advancing management tactics further established.
- Confirmed 18 species and 7 genera of spittlebugs associated with graminoids of Colombia and Ecuador (15 species in Colombia, 9 in Ecuador, 6 in both countries), including 7 species for Colombia and 4 for Ecuador not yet reported in the literature, data on 27 host plants, and distribution data for 21 of 32 Colombian departments.
- Demonstrated the effectiveness of an artificial diet for maintaining spittlebug adults and thereby its potential as a tool to screen factors of interest to genetic transformation in *Brachiaria*.
- Strengthened the collection of fungal entomopathogens of spittlebugs, which now includes 71 strains, from 10 genera and 12 species of fungus, isolated from 4 genera and 7 species of Colombian spittlebugs collected in 6 departments.
- Screened 28 new fungal entomopathogen isolates to adults of *Aeneolamia varia*, obtaining high virulence measures of up to 95.1% adult mortality for *Metarhizium*, 62.8% for *Paecilomyces* and 53.5% for *Fusarium*.
- Developed and evaluated new methodology to screen fungal entomopathogens for virulence to spittlebug nymphs, obtaining up to 87.1% mortality compared to 24.6% in the control.
- IPM components relevant to spittlebug management in forage grasses and other graminoids better understood
- The amplified fragment length polymorphism (AFLP) technique was implemented to characterize the genetic diversity of *Sphaceloma manihoticola* in cassava and *Ceratocystis paradoxa* in oil palm.
- AFLPs and random amplified microsatellites (RAMS) proved their usefulness in identifying genetic markers for virulent strains of *C. paradoxa* and *S. manihoticola*.
- *Phytophthora melonis*, isolated from cassava at Sergipe, Brazil, was identified through the internal transcribed spacer (ITS) region sequence analysis of ribosomal DNA. This is the first time that *P. melonis* was reported as causing root rot in cassava.
- High genetic variation was detected among isolates of *Sphaerotheca pannosa* var. *rosae*, causal agent of powdery mildew of rose, by using the RAMS technique.
- No significant differences were found between rose plants affected by powdery mildew treated with foliar applications of a swinglea-based biofungicide, KH_2PO_4, and a commercial fungicide.
- Wood ash applied to the soil around cassava plants was effective as an alternative control for root rot at Mitú (Vaupés, Colombia).

- Resistance to cassava frogskin disease is widespread in cassava germplasm and needs to be systematically incorporated into cassava breeding efforts.
- Sequence characterized amplified region (SCAR) markers were developed to distinguish *B. tabaci* biotypes A and B. In a PCR assay, these marker amplify only one product and makes the interpretation of the results much simpler than using RAPD markers.
- Population studies of citrus tristeza virus isolates from different regions of Colombia were begun and will be the foundation for regional projects on citrus viruses.
- The measures to control cassava frogskin disease in CIAT germplasm have been widely adopted, and it is expected that by the middle of 2001 the levels of CFSD on the CIAT campus will be very low.
- Half of the 33 *P. griseola* isolates from southern Tanzania, and Ethiopia are of the Afro-Andean pathogen group, an increasing evidence of its importance in Africa. Two isolates from Tanzania infected Mex-54, which is resistant to almost all isolates from Africa characterized so far.
- Molecular studies based on microsatellites differentiated isolates of *P. griseola* from varietal mixtures, in groups, that varied within them. Correlation with variation in virulence and implication for pathogen diversity is yet to be determined
- Eight species have been identified in studies to characterize *Pythium* spp prevalent in areas having serious root rot problem in Uganda. However, *Fusarium oxysporum* f.sp *phaseoli* was the predominant fusarium species identified.
- The presence of bean stem maggot and nematodes increased incidence and severity of Fusarium wilt in beans, implying that development of management strategies of the disease should consider possible influence of these pests.
- Several CIAT bred bean lines tested in the Congo DR and Kenya by NARS scientists, show superior performance against bean stem maggot and some combine this attribute with high yield.
- In Tanzania IPM strategies for bean foliage beetles, selected by stakeholders include: 1) adjust of planting time (delayed planting) to avoid high BFB infestations, 2) the application of botanical pesticides, and traditional technologies such as fermented cow urine (worked best) and ashes.
- Internet access by our national partners is becoming an increasing important means to distribute information. Collaborating with Ecoport (a free site with information on plant pest and diseases) and converting documents into Adobe Acrobat PDF formats are two ways that we are making information accessible using the Internet.
- Strategic links for IPM research and development coordinated with ICIPE and IACR-Rothamsted for basic research and in depth studies on bean pests.
- For Bean IPM pests were successfully disseminated through a decentralized system with extension and bean farmers in a pilot trial in northern Tanzania.
- Whitefly IPM Network consisting of 5 IARC's, 10 basic research organization and 54 NARS institutions in 30 countries across the tropics, consolidated.
- Based on Phased I of Whitefly IPM project diagnostic surveys several "hot spots" were identified in Colombia, Ecuador, El Salvador, Guatemala, Mexico, the Dominican Republic, Sudan, Tanzania, Uganda, Kenya and Nigeria.

- During Phase I of Whitefly IPM project it was determined that *Bemisia tabaci* is transmitting serious viruses infecting tomato, beans, cassava, sweet potato, peppers, melons, eggplant, cotton and tobacco.
- During Phase I of Whitefly IPM project, in collaboration with Harvard University, a mathematical model that captures the dynamics of a pathosystem with one whitefly species and one virus in one crop, was computer verified.
- Progress was made on a Geographical Information System (GIS) for whitefly/gemenivirus problems in Latin America.
- Whitefly IPM project Phase I survey revealed that the greenhouse whitefly, *Trialeurodes vaporariorum*, is by far the most important species affecting annual crops as a direct pest in the Andean Highlands of Colombia and Ecuador.
- Several promising IPM tactics were identified for managing *T. vaporariorum* in highland beans and tomatoes and are ready to be tested.
- Phase I of the whitefly IPM project identified numerous potential biological control agents of *B. tabaci*, including 20 wasp parasitoids, 10 predators and 6 entomopathogens.
- During Phase I of the Whitefly IPM project major control successes for CMD (Cassava Mosaic Diseases) in Africa have been achieved through the deployment of CMD resistant varieties.

Table of Contents

OUTPUT I. PEST AND DISEASE COMPLEXES DESCRIBED AND ANALYZED

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

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

Introduction

Whiteflies, considered one of the world's major agricultural pests, are particularly damaging to crops in the tropical regions of the world. Eleven species are reported on cassava: *Aleurotrachelus socialis, Trialeurodes variabilis, Bemisia tuberculata, Aleurothrixus aepim, Bemisia tabaci, Bemisia argentifolii, Trialeurodes abutiloneus, Aleurodicus dispersus, Paraleyrodes* sp., *Aleuronudus* sp. *and Tetraleurodes* sp. The whitefly complex reported from other crops, such as vegetables, fruits, cotton and legumes, is too extensive to list. However, important species collected from the Andean region of South America include *Bemisia tabaci* and *Trialeurodes vaporariorum.*

B. tabaci has a pantropical distribution, feeding on numerous crops throughout the tropical regions of the world. It feeds on cassava throughout Africa where it is the vector of ACMD (Africa Cassava Mosaic Disease, caused by several geminiviruses), and is also reported from India and Malaysia. Since the early 1990's a new biotype (B) of *B. tabaci*, considered by some a separate species (*B. argentifolii*) has been found feeding on cassava in the neotropics. Recent reports, and personal observations, indicate that *B. tabaci* is feeding on cassava in several areas of Brazil, Northern South America, Central America and the Caribbean. Although ACMD has not been reported from the Americas it is considered that ACMD now poses a more serious threat to cassava production, as most traditional cultivars in the regions are highly susceptible to the disease. In addition, the *B. tabaci* biotype complex is the vector of several virus of crops, especially vegetables and legumes, that are often grown in association with cassava, posing a potential threat for these viruses to move to cassava.

Whiteflies cause direct damage to cassava and other crops by feeding on the phloem of leaves, inducing chlorosis and leaf fall, which can result in crop loss. Yield losses of this type are common owing to *A. socialis* and *A. aepim*, feeding on cassava in Colombia, and Brazil respectively. There is a correlation between duration of whitefly attack and root loss; losses over 70% have been reported from Colombia, and over 40% from Brazil.

A project, designed to determine the complex of indigenous South American parasitoids and other natural enemies on cassava, beans and selected horticultural crops, is being carried out in northern South America. The objective of these surveys and subsequent research is to determine the best potential natural enemies and to compare the efficiency of indigenous species to that of exotic whitefly parasitoids being recommended for introduction into the region.

This is a USAID funded collaborative project between CIAT and the University of Florida. This collaboration will provide training and improved incountry capacity for research, production, delivery and management of biological control agents. The University of Florida will provide expertise and input on parasitoid taxonomy, biology, behavior, collecting, rearing, identification and data analysis.

Survey Methodology

Two geographic areas were originally selected for exploration for whitefly species and their natural enemies in Colombia; the Caribbean Coast (the Departments of Atlántico, Córdoba, Bolívar and Magdalena), the mid-altitude highlands (Departments of Cauca, Valle del Cauca, Caldas, Quindío and Risaralda). Internal political problems have rendered several of these areas inaccessible during the last year. This resulted in a shift in emphasis from the Colombian to Ecuadorian sites. Additional surveys are planned for selected sites in Venezuela during the 2000-2001 period.

Sampling is being done on several crop hosts, including cassava, beans, tomato, eggplant cotton, cucumber and snap bean in Colombia. An expanded crop list for Ecuador also includes soybean, sesame, cabbage, lima bean, peanut, pepper, melon, watermelon, "Archucha" and "Escoba." The Ecuadorian sites represent two distinct zones; one is lowland, 40 to 60 m.a.s.l., and the second is highland, > 1500 m.a.s.l. Each zone in Colombia and Ecuador is characterized by taking data on m.a.s.l., rainfall, temperature range, vegetation type, latitude, geographic area, etc. From each collection site, 100 leaves are randomly collected; a one square inch leaf area was examined to determine the whitefly species present and the number of nymphs and pupae is recorded.

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

Results: Diverse Crops

Whitefly and parasitoid surveys and collections, during 1999-2000, were continued in different Colombian zones, and expanded into Ecuador. The Colombian departments surveyed were Atlántico, Caldas, Córdoba, Quindío, Risaralda and Valle del Cauca; Ecuadorian departments included De los Ríos, Guayas, Imbabura and Manabí. The Colombian departments range from 20 to 1500 m.a.s.l. with average temperatures of 19°C (Caldas and Quindío) to 33°C (Atlántico). The Ecuadorian departments ranged from 47 to 1550 m.a.s.l.; Guayas, Manabí and De los Ríos are similar ranging from 47 to 60 m.a.s.l., while Imbabura is distinct from these at 1550 m.a.s.l. The crops surveyed in Colombia were tomato, egg plant, cucumber, snap bean, beans and cotton. In Ecuador, tomato, pepper, cabbage, melon, watermelon, beans, lima beans, soybean, peanut, sesame, "escoba" and "archucha" were sampled.

The whitefly species complex associated with the diverse cropping systems surveyed in Colombia and Ecuador is distinct from that described on cassava. Only two whitefly species were collected in both countries, *Bemisia tabaci* and *T. vaporariorum*. In Colombia, *T. vaporariorum* is the

predominant species; *B. tabaci* was found only in the departments of Córdoba and Atlántico (see PE1 Annual Report, 1999, for additional data and information). Meanwhile in Ecuador *B. tabaci* was the predominant species and *T. vaporariorum* was collected only from the province of Imbabura (**Table 1.1**).

Whitefly population in Ecuador and Colombia were similar in number of individuals collected (**Table 1.1**), but, as noted, species domination was reversed. Both whitefly species were collected from beans and tomatoes in both countries. Differences in the occurrence of the two whitefly species are more related to the agroecozones where collected than to that of the host crops (also see PE1 Annual Report 1999).

Table 1.1. Whitefly populations collected from several crop hosts in different agroecozones of Colombia and Ecuador during 1999-2000.

			Species	
Country	**Locality**	**Host**	*B. tabaci*	*T. vaporariorum*
Colombia	Atlántico	Tomato	155	
	Caldas	Snap bean		138
		Cucumber		56
		Tomato		150
	Córdoba	Cotton	44	
	Quindío	Tomato		48
	Valle	Beans		100
		Snap bean		237
		Cucumber		63
		Tomato		158
Ecuador	Guayas	Cabbage	100	
		Escoba	42	
		Pepper	41	
		Watermelon	36	
		Soy bean	80	
		Tomato	54	
	Imbabura	Beans		100
	Manabí	Beans	75	
		Lima bean	100	
		Peanut	83	
		Melon	82	
		Tomato	173	

B. tabaci, in both Colombia and Ecuador is collected from topical lowland zones, while *T. vaporariorum* is collected from agroecozones above 900 m.a.s.l. (**Figure 1.1**). In no case were both species found at the same site surveyed. Similar results were reported last year when *B. tabaci* was collected in Colombia only from sites below 400 m.a.s.l., and *T. vaporariorum* from sites above 1000 m.a.s.l. At altitudes between 400-800 m.a.s.l. both biotypes of *B. tabaci* and *T. vaporariorum* can be found. During the past years survey we did not collect from the mid altitude range (400-800 m.a.s.l.).

The parasites collected during this phase belong to three genera, *Encarsia*, and *Eretmocerus* (Aphelinidae), and *Amitus* (Platygasteridae). Several of the parasitoids collected have now been identified to species; these include *Amitus fuscipennis, Encarsia sofia, Encarsia hispida, Encarsia*

nigricephala, and *Encarsia tabacivora*. Several species of *Eretmocerus* collected in both countries are in the hands of taxonomists and awaiting identification.

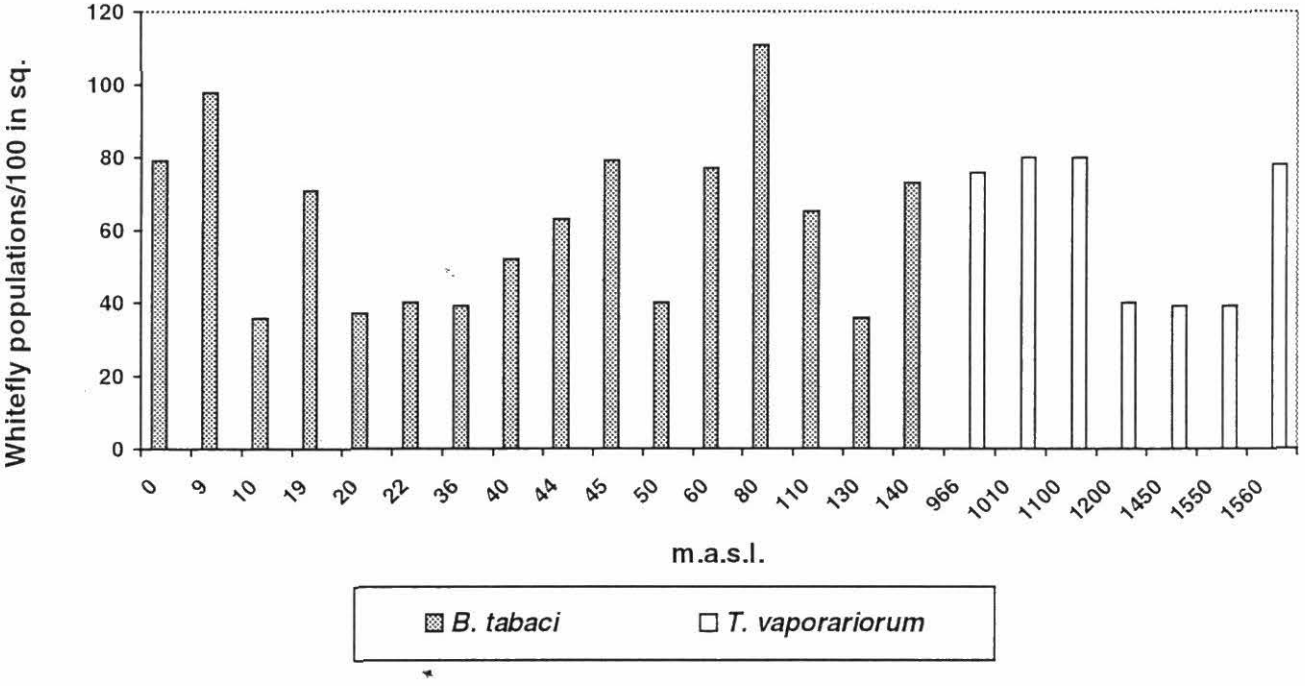


Figure 1.1. Whitefly populations collected in Colombia and Ecuador, related to meters above sea level (m.a.s.l.) (1999-2000).

The species identified from Colombia are *E. sofia, E. nigricephala, E. tabacivora, E. hispida* and *A. fuscipennis*. *A. fuscipennis* is the species most frequently collected in Colombia, and *E. hispida* the species least collected. *E. nigricephala* and *Encarsia* sp. were also frequently observed in Colombia. *E. nigricephala* was the most frequently collected species in Ecuador, followed by *Encarsia* sp., while *A. fuscipennis* was the least observed (**Figure 1.2**).

B. tabaci was parasitized by *E. hispida* and *E. sofia*, and *T. vaporariorum* by *E. tabacivora*. The remaining species parasitized both whitefly species (**Figure 1.3**). *B. tabaci* was most frequently parasitized by *E. nigricephala*, followed by *Encarsia* sp., while *T. vaporariorum* was most frequently parasitized by *A. fuscipennis* followed by *E. nigricephala* and *Encarsia* sp. These results indicate that *E. nigricephala* will actively parasitize both whitefly species.

Whitefly and parasitoid collection were also made from cassava in Ecuador. *Aleurotrachelus socialis* and *Tetraleurodes* sp. were the two species collected both in low populations. *A. socialis* was parasitized by *Amitus* sp. and *Eretmocerus* sp. and *Tetraleurodes* sp. by *Encarsia* sp. and *Eretmocerus* sp. (**Figure 1.3**).

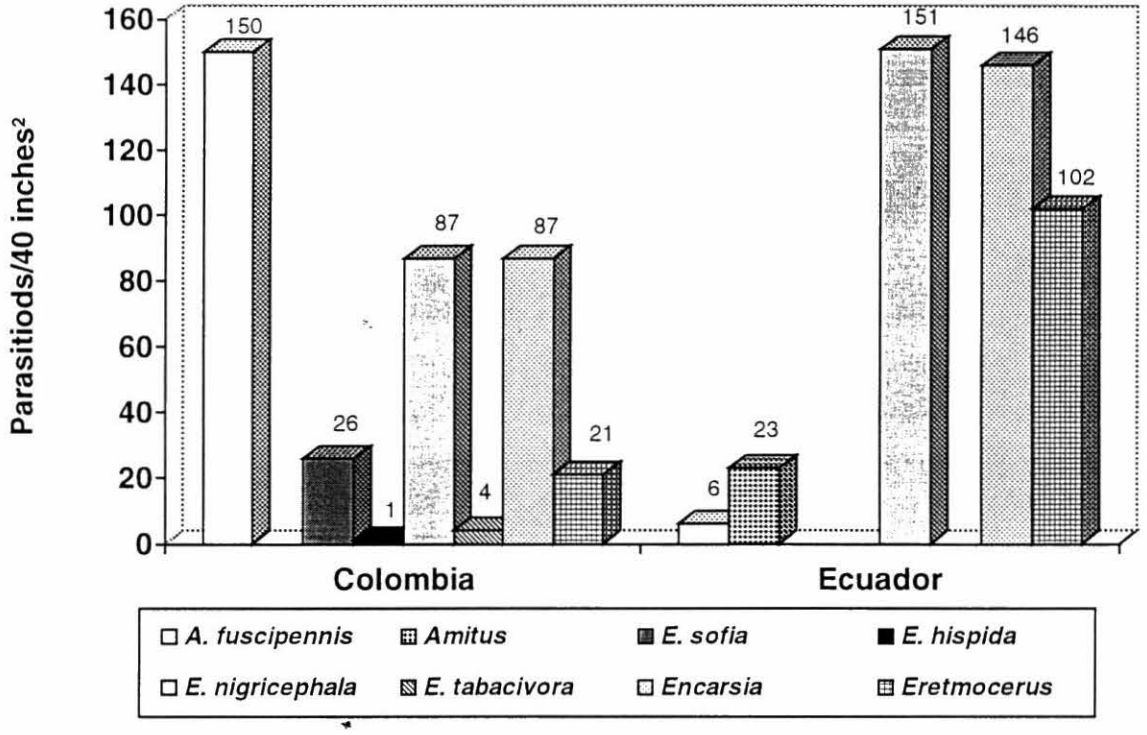


Figure 1.2. Frequency of parasitoid species collected from whiteflies on several hosts in Colombia and Ecuador (1999-2000).

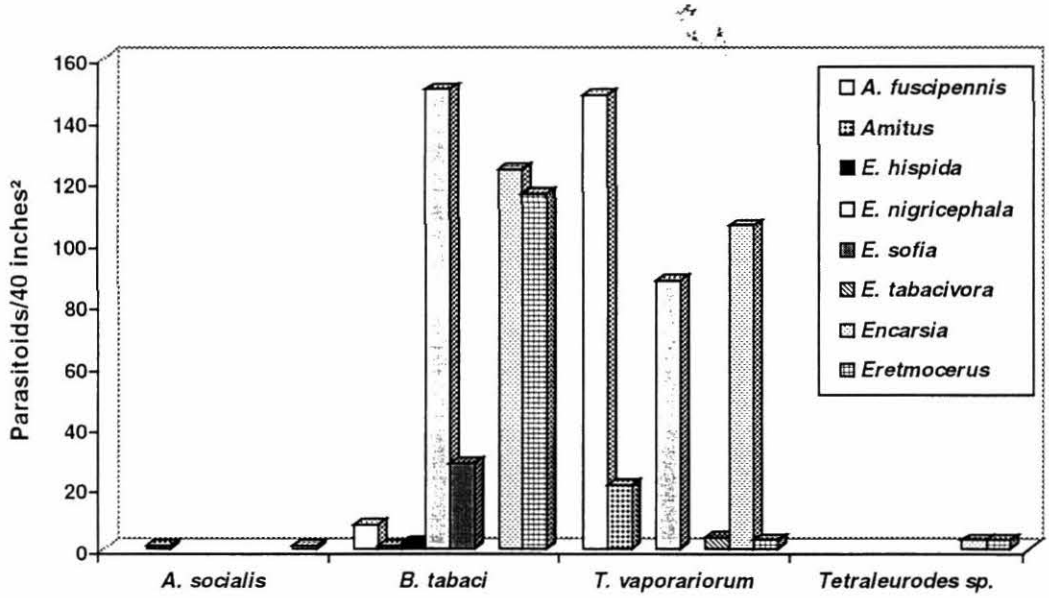


Figure 1.3. Parasitoid species associated with four whitefly species collected from several host plants in Colombia and Ecuador.

Results Cassava

Whitefly species collected from the sites surveyed in Colombia and Ecuador **(Figure 1.4)** were preserved in 70% alcohol; preliminary identification was made using the Caballero (1992) and Martin (1987) taxonomic keys. Definitive identification is made by Dr. Avas Hamon of the Florida State Collection of Arthropods in Gainesville, USA. Preliminary parasitoid identification was made using the Polaszek (1992) (*Amitus, Encarsia, Eretmocerus, Signiphora* and *Metaphycus* genera), La Salle (1999) (*Euderomphalini* genera), Rose and Zolnerowich (1997) (*Amitus, Signiphora, Encarsia, Eretmocerus* and *Euderomphalini* genera), and Evans and Castillo (1998) (Aphelinidae Fam.) keys. Definitive identification was made by Dr. G.A. Evans, University of Florida, and Dr. Mike Rose, Texas A&M University, USA.

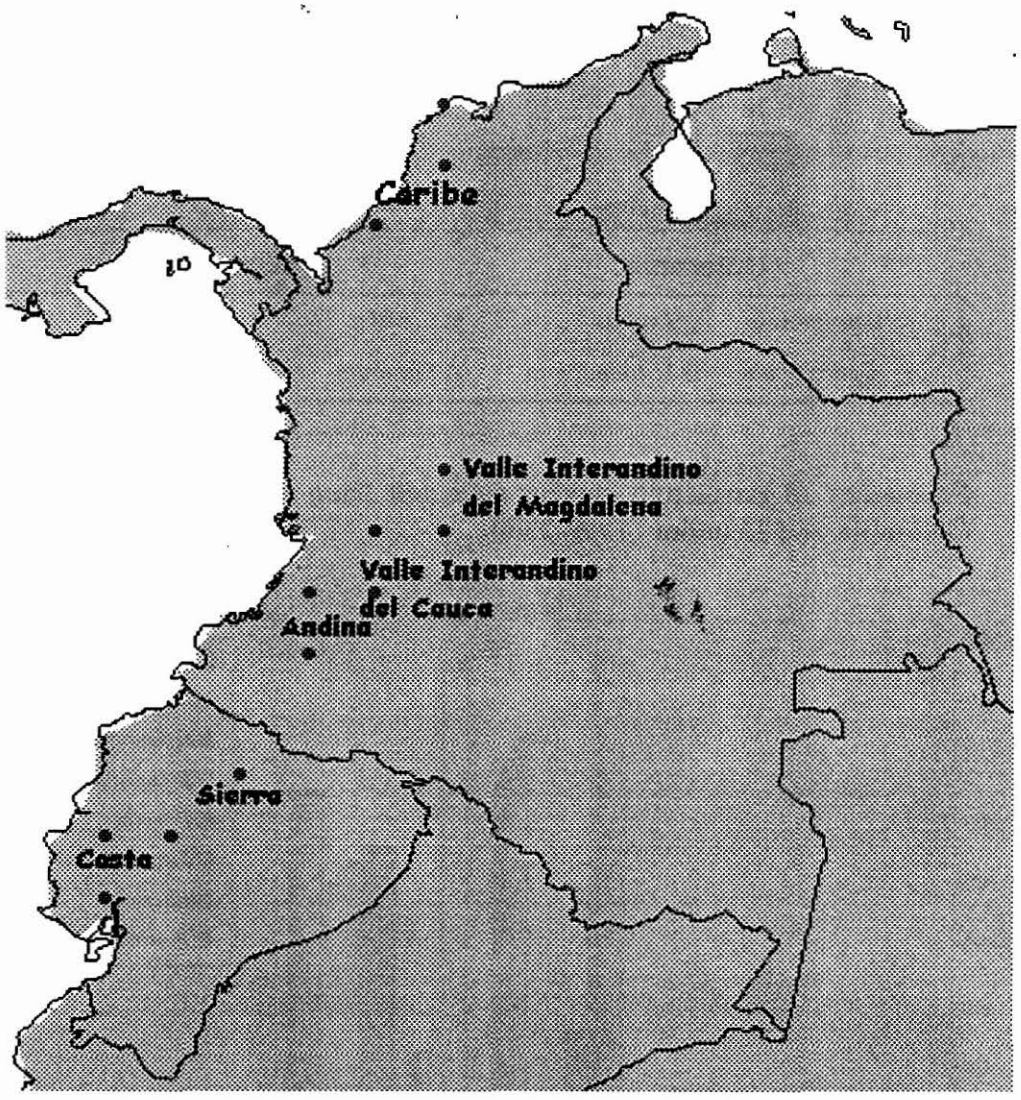


Figure 1.4. Regional sites surveyed in Colombia and Ecuador for cassava whiteflies and their corresponding parasitoids.

Surveys during the 1999-2000 season confirm that there are four whitefly species consistently found feeding on cassava (*Aleurotrachelus socialis, Bemisia tuberculata, Trialeurodes* sp.; possibly *T. variabilis,* and *Tetraleurodes* sp.), and a fifth species *Aleurodicus* sp., sporadically observed on cassava. Survey data is expressed for four regions of Colombia, the Caribbean, the Andean, the Interandean Cauca Valley and the Interandean region of Magdalena (**Figure 1.5**).

A. socialis was found in all four regions and except for Region II (Andean) was the predominant species observed. *Trialeurodes* sp. was the most frequent species in the Andean Region with 70% of the specimen collected (**Figure 1.5**). *B. tuberculata* was observed in all regions and the second most frequent species observed. *Tetraleurodes* sp. was collected only in the Caribbean (I) region, and only in low (<5%) populations.

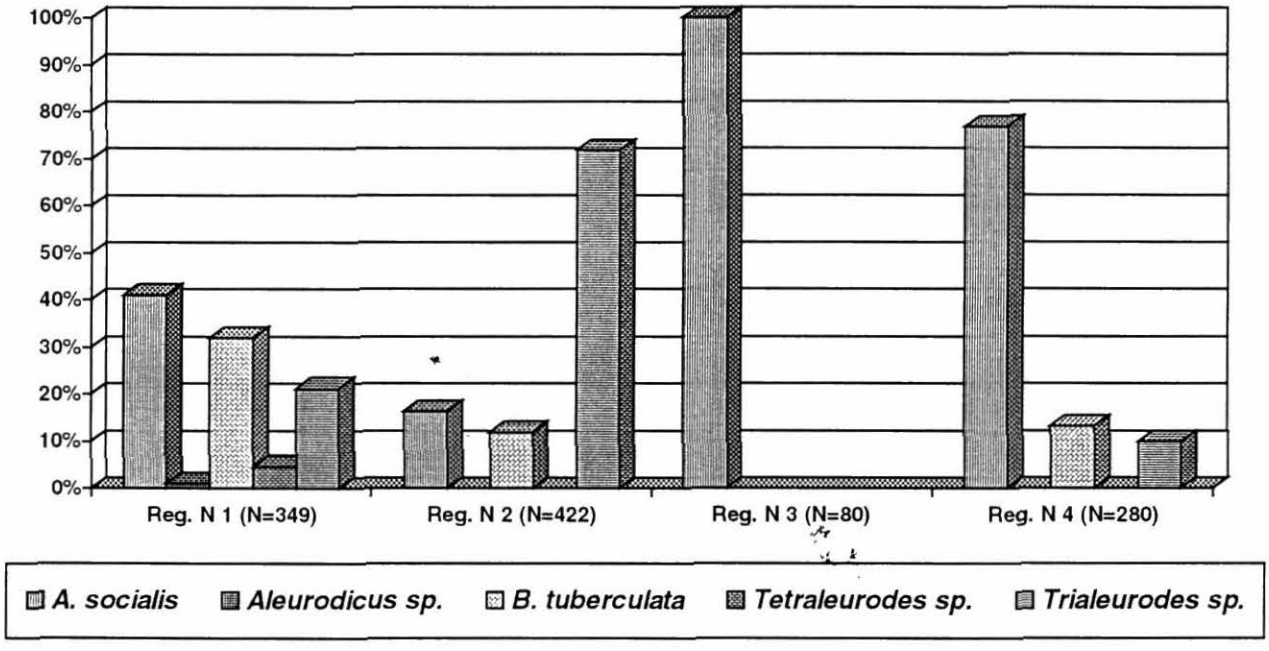


Figure 1.5. Whitefly species collected from cassava in four agroecoregions of Colombia (Reg. N1 = Caribbean; N2 = Andean; N3 = Interandean Cauca Valley; N4 = Interandean Magdalena Valley) (N = number of sites sampled).

Surveys in Ecuador resulted in the same five whitefly species being identified on cassava as found in Colombia (**Figure 1.6**). The Ecuador survey is from two regions, the Coastal Region and the Sierra. Data from the Ecuadorian coastal region differed from the Colombian coast in order of importance of the species collected. *Tetraleurodes* sp. was most frequently collected, followed by *A. socialis* and *Trialeurodes* sp. (**Figure 1.6**). *Aleurodicus* sp. and *B. tuberculata* were observed in low populations (<10%). In the Sierra region, *Trialeurodes* sp. was the predominant species collected. This latter observation corresponds to the Cauca Region in Colombia in m.a.s.l., where *Trialeurodes* sp. is also the predominant species (Annual Report PE1, 1999).

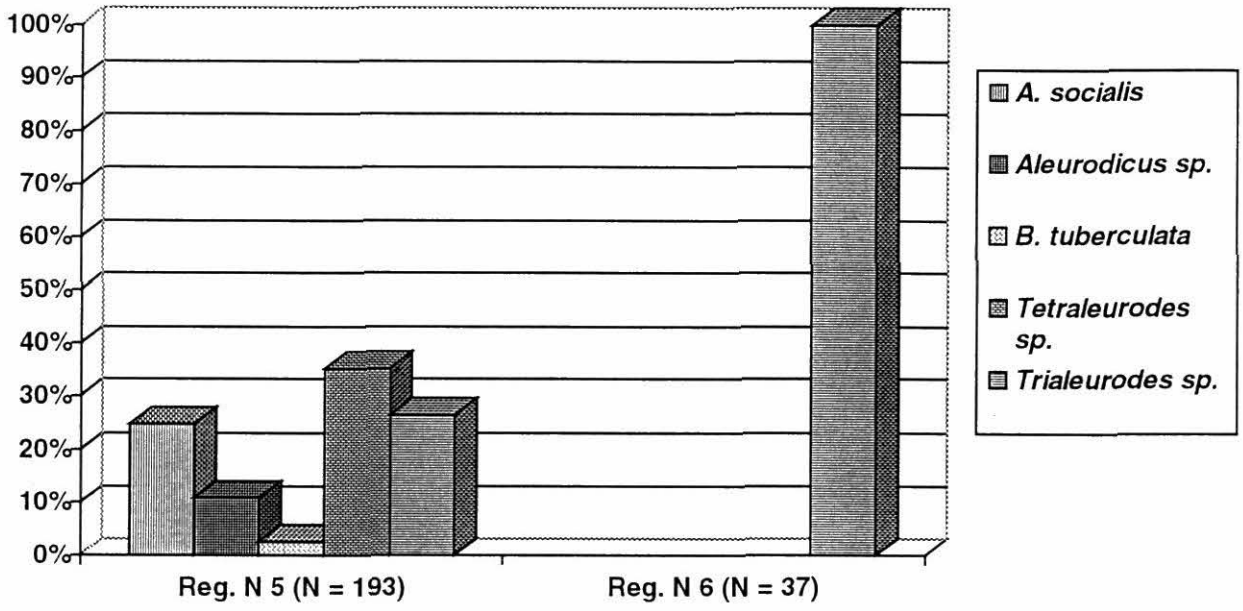


Figure 1.6. Whitefly species collected from cassava in two agroecoregions of Ecuador (Reg. N5 = Coastal Region; N6 = Sierra) (N = number of sites sampled).

Parasitoids

Surveys in Colombia have resulted in the identification of at least 10 parasitoid species associated with the whitefly species collected on cassava (**Table 1.2**). There are grouped into six genera, five families and two superfamilies. As can be observed in Table 1.2, several species are presently unidentified and in the hands of the aforementioned taxonomists. The Caribbean Region (N1) of Colombia shows the greatest species richness with nine species observed, *Eretmocerus* sp., *Encarsia* sp., *E. hispida, E. pergandiella, E. bellottii, E. sofia, E. luteola* Group, *E. strenua* Group and *Metaphycus* sp. (**Table 1.3**). In the Andean region (N2) *Eretmocerus* sp., *E. pergandiella, E. bellottii, E. hispida, Euderomphale* sp. and the hyperparasite *S. aleyrodis*. In the Cauca Valley, *Eretmocerus* sp. *Encarsia* sp., *E. hispida, E. bellottii, E. sofia and E. luteola* Groups were identified. The parasitoid *Amitus macgowni* was only collected from the Magdalena Valley (N4).

In Ecuador four parasitoid and one hyperparasite were collected. *Eretmocerus* sp. and *Encarsia* sp. were collected from both regions (N5 & N6) (**Table 1.2**). *Amitus* sp., *Euderomphale* sp. and the hyperparasite *S. aleyrodis*, collected from the Coastal Region (N5) were absent in the Sierra (N6).

A comparison of agroecoregion, whitefly species and parasitoid indicates that the greatest parasitoid species richness is found in association with *A. socialis* in three of the four regions sampled in Colombia (**Table 1.4**). In the Andean region one more parasitoid species was collected from *B. tuberculata* (4) than from *A. socialis* (3) and *Trialeurodes* sp. (3).

Table 1.2. Taxonomic classification of parasitoid species collected from cassava whiteflies from Colombia and Ecuador.

Order	Superfamily	Family	Genera	Species
Hymenoptera	Platygasteroidea or Proctotropoidea	Platygasteridae	*Amitus*	*macgowni*
"	Chalcidoidea	Aphelinidae	*Encarsia*	sp.
"	"	Aphelinidae	*Encarsia*	*hispida*
"	"	Aphelinidae	*Encarsia*	*bellottii*
"	"	Aphelinidae	*Encarsia*	*sofia*
"	"	Aphelinidae	*Encarsia*	*luteola* Group
"	"	Aphelinidae	*Encarsia*	*strenua* Group
"	"	Aphelinidae	*Eretmocerus*	sp.
"	"	Encyrtidae	*Metaphycus*	sp.
"	"	Eulophidae	*Euderomphale*	sp.
"	"	Signiphoridae	*Signiphora*	*aleyrodis*
"	Platygasteroidea Proctotropoidea	Platygasteridae	*Amitus*	sp.
"	Chalcidoidea	Aphelinidae	*Encarsia*	sp.
"	"	Aphelinidae	*Eretmocerus*	sp.
"	"	Eulophidae	*Euderomphale*	sp.
"	"	Signiphoridae	*Signiphora*	sp.

Table 1.3. Whitefly parasitoids collected from cassava from diverse agroecoregions of Colombia and Ecuador.

Species	*Reg. N1	*Reg. N2	*Reg. N3	*Reg. N4	**Reg. N5	**Reg. N6
Amitus sp.					X	
A. macgowni				X		
Eretmocerus sp.	X	X	X	X	X	X
Encarsia sp.	X		X	X	X	X
E. hispida	X	X	X			
E. pergandiella	X	X				
E. bellottii	X	X	X			
E. sofia	X		X			
E. luteola Group	X		X			
E. strenua Group	X					
Metaphycus sp.	X					
Euderomphale sp.		X			X	
S. aleyrodis		X		X	X	

Reg. N1 = Caribbean; Reg. N2 = Andean; Reg. N3 = Interandean Cauca Valley; Reg. N4 = Interandean Magdalena Valley; Reg. N5 = Costa, Ecuador; Reg. N6 = Sierra, Ecuador.

Table 1.4. The association between parasitoids and whitefly species in four agroecoregions of Colombia.

Region Natural	Parasitoids	Whiteflies				
		A. socialis	*Aleurodicus* sp.	*B. tuberculata*	*Tetraleurodes* sp.	*Trialeurodes* sp.
Caribe	*Encarsia* sp.	X	X	X		X
	Eretmocerus sp.	X		X	X	X
	E. hispida	X				
	E. pergandiella					X
	E. bellottii	X				
	E. luteola Gr.	X				X
	E. sofia	X		X		X
	E. strenua Gr.					X
	Metaphycus sp.			X		
Andina	*Eretmocerus* sp.	X		X		X
	E. hispida					X
	E. pergandiella			X		X
	E. bellottii	X				
	Euderomphale sp.			X		
	S. aleyrodis	X		X		
Valle Interandino del Cauca	*Encarsia* sp.	X				
	Eretmocerus sp.	X				
	E. hispida	X				
	E. bellottii	X				
	E. luteola Gr.	X				
	E. sofia	X				
Valle Interandino del Magdalena	*A. macgowni*	X				
	Encarsia sp.	X		X		
	Eretmocerus sp.	X		X		X
	S. aleyrodis	X				

The results from the two agroecoregions sampled in Ecuador show a similar trend (**Table 1.5**). Four parasitoid and one hyperparasitoid species was identified on *A. socialis*, all from the coastal agroecoregion. Three parasitoid species were identified from *Aleurodicus* sp., compared to only one from Colombia, and two from *Tetraleurodes* sp. vs. only one from Colombia. As can be observed in Table 1.5, all of the parasitoid species from Ecuador are still in the process of being identified by taxonomists.

Table 1.5. The association between parasitoids and whitefly species in four agroecoregions of Ecuador.

Region Natural	Parasitoids	Whiteflies				
		A. socialis	*Aleurodicus* sp.	*B. tuberculata*	*Tetraleurodes* sp.	*Trialeurodes* sp.
Costa	*Amitus* sp.	X				
	Encarsia sp.	X	X		X	
	Eretmocerus sp.	X	X		X	X
	Euderomphale sp.	X	X	X		
	S. aleyrodis				X	
Sierra	*Encarsia* sp.					X
	Eretmocerus sp.					X

In the Caribbean region of Colombia six parasitoid species were collected. Approximately 46% of the whitefly specimens collected were parasitized and 58% of those parasitized, were parasitized by *Eretmocerus* sp. The remaining 42% were parasitized by five species of *Encarsia* **(Figure 1.7)**. In the same region, four species of parasitoid were identified from *B. tuberculata;* less than 20% of the whitefly specimens collected were parasitized and *Eretmocerus* sp. and *Metaphycus* sp. accounted for about 38% and 35% of the parasitism respectively **(Figure 1.7B)**.

In the case of *Trialeurodes* sp. **(Figure 1.7C)**, six parasitoid species were identified and approximately 28% parasitism. *Encarsia* sp. and four other *Encarsia* species accounted for about 76% of the parasitism with *Eretmocerus* sp. for the remaining. *Tetraleurodes* sp., found in low population was only parasitized by *Eretmocerus* sp.; about 10.5% of the population was parasitized **(Figure 1.7D)**.

In the Andean Region three whitefly species (*A. socialis, B. tuberculata,* and *Trialeurodes* sp. were identified. Fifteen percent of *A. socialis*, 16% of *Trialeurodes* sp. and 34% of *B. tuberculata* were parasitized **(Figure 1.8)**. *E. bellottii* and *Eretmocerus* sp. were the parasitoid species most frequently observed parasitizing *A. socialis*. *E. pergandiella* accounted for more than 60% of *Trialeurodes* sp. parasitism and *Eretmocerus* sp. for more than 50% of *B. tuberculata* parasitism **(Figure 1.8A, B and C)**.

In the Cauca Valley Region, *A. socialis* was the predominant species and nearly 80% of the population was parasitized by six parasitoid species **(Figure 1.9)**. *E. hispida* accounted for nearly 60% of this parasitism.

In the Magdalena Valley, 67% of *A. socialis,* 49% of *B. tuberculata* and 25% of *Trialeurodes* sp. populations were parasitized. *Eretmocerus* sp. accounted for most of this parasitism, and was the only species collected from *Trialeurodes* sp. **(Figure 1.10A, B and C)**.

In Ecuador five whitefly species were collected from the two regions surveyed and the corresponding parasitoid complex has only been identified to genus. In the coastal region three parasitoid genera *Amitus, Encarsia* and *Eretmocerus* parasitized 67% of the *A. socialis* populations, more than 60% of these by *Eretmocerus*. 35% of the *Tetraleurodes* sp. populations was parasitized and nearly 80% of these by *Eretmocerus* sp. *Eretmocerus* sp. was only parasitoid found on *Trialeurodes* sp. (25% parasitism), and *B. tuberculata* (20%). *Aleurodicus* sp. was parasitized (38%) primarily by *Euderomphale* sp. (84%) **(Figure 1.11A to E)**.

In the Sierra region of Ecuador, more than 60% of *Trialeurodes* sp. population were parasitized by *Encarsia* sp. (90%) and *Eretmocerus* sp. (10%) **(Figure 1.12)**.

In regions of Colombia with low whitefly populations the predominant parasitoid was *Eretmocerus* sp. infesting *B. tuberculata, Trialeurodes* sp. and *Tetraleurodes* sp. and *E. pergandiella* on *Trialeurodes* sp.

In Ecuador, areas of low *A. socialis* populations, *Encarsia* species predominated, while *Euderomphale* sp. was found on *Aleurodicus* sp. and *Eretmocerus* on *Tetraleurodes* sp. and *Trialeurodes* sp.

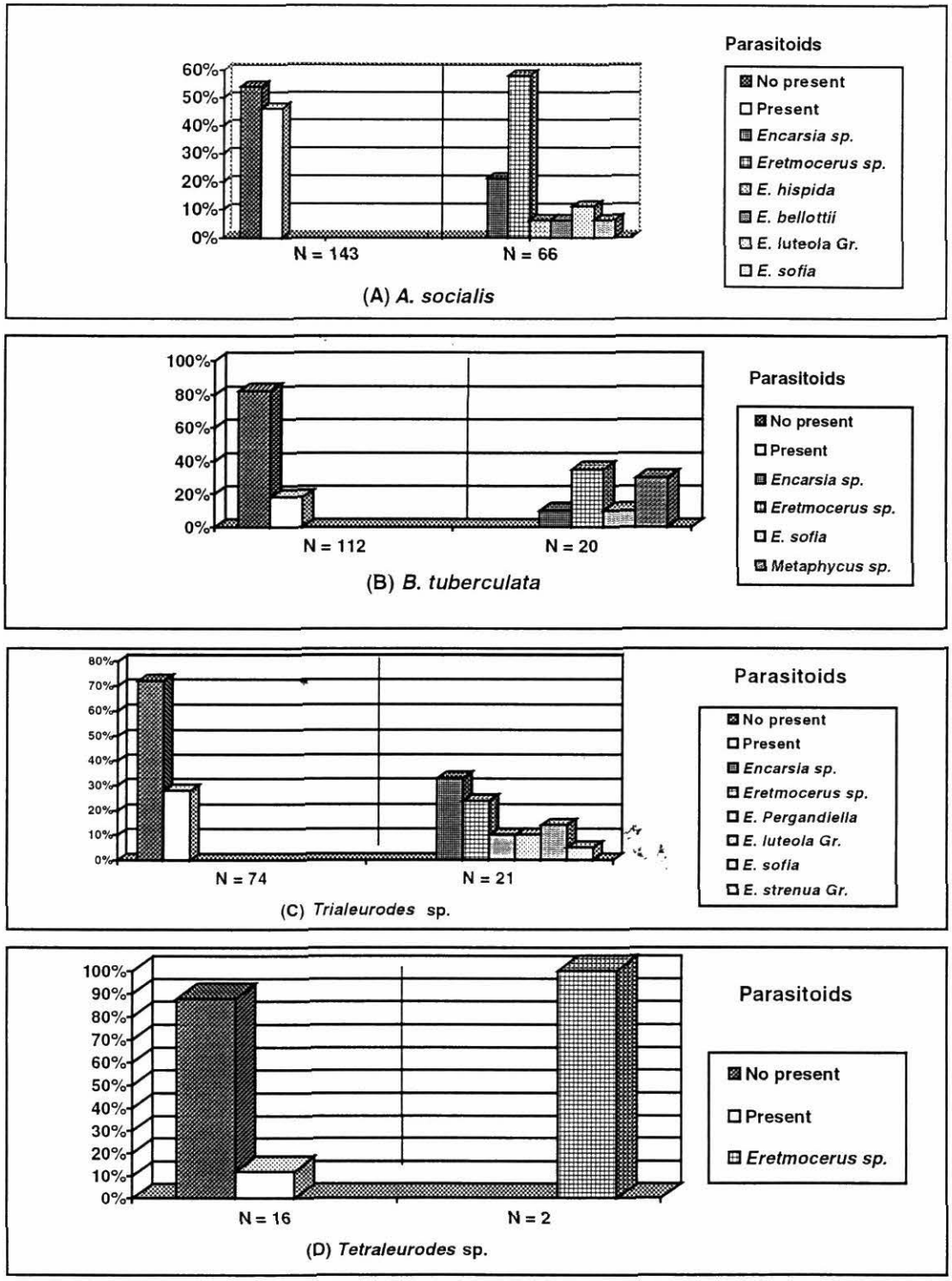


Figure 1.7. **Parasitoid frequency and parasitism rates on form whitefly species on cassava in the Caribbean region of Colombia.**

A. socialis, B. tuberculata, Tetraleurodes sp., and *Aleurodicus* sp. are usually distributed in low altitude regions, usually characterized by high temperatures and seasonally dry periods. *Trialeurodes* sp. is usually found in areas of moderate temperatures with constant rainy periods, throughout the year.

Eretmocerus parasitoids are often observed in areas with higher temperatures but due to the ample diversity in this genus it is difficult to predict adaptability tendencies. However *Euderomphale* sp. was found in conditions where there was low host density, a market seasonally dry period and high temperatures.

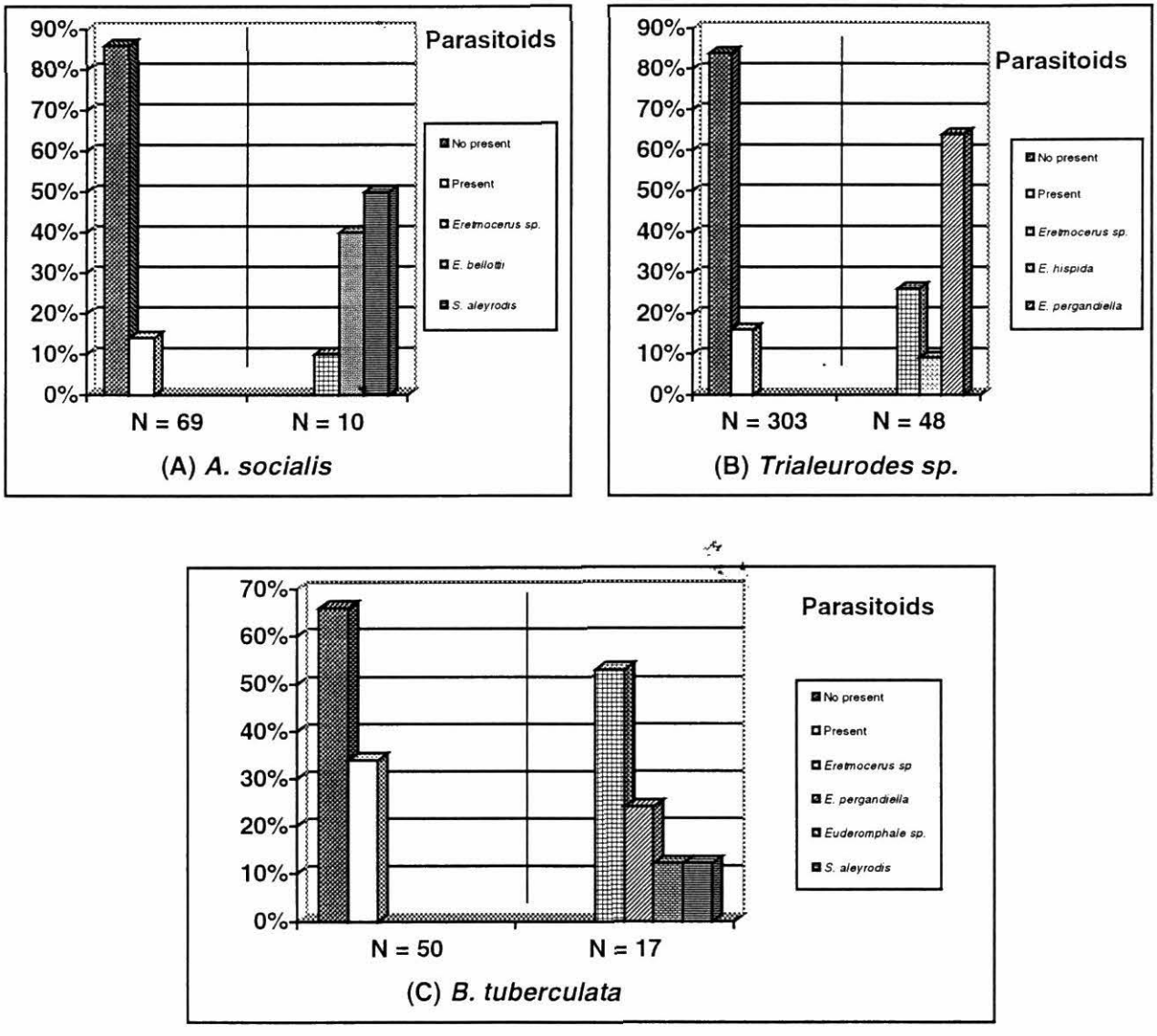


Figure 1.8. Parasitoid frequency and parasitism rates on three whitefly species on cassava in the Andean Region of Colombia.

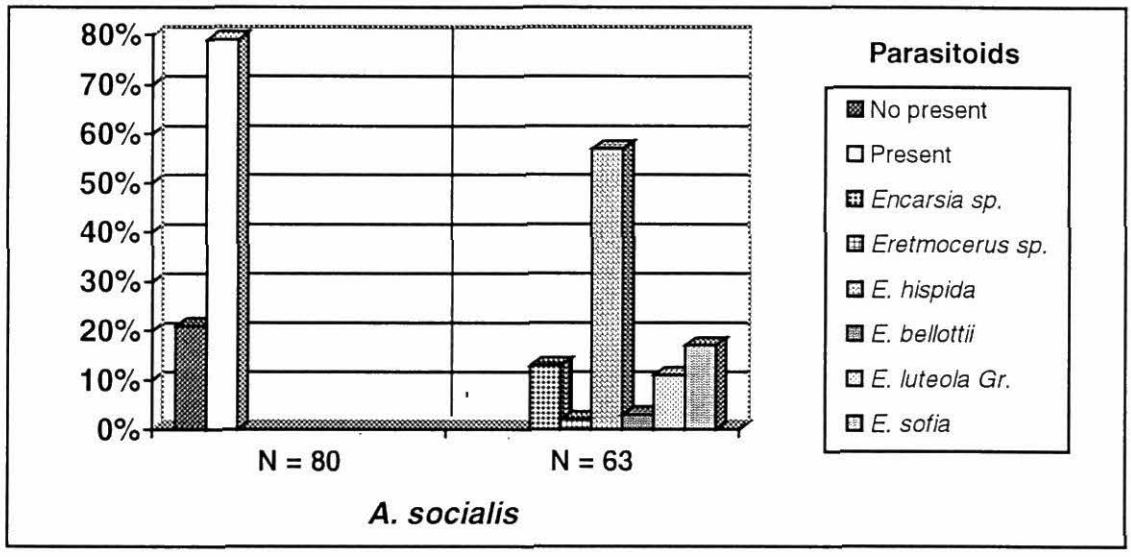


Figure 1.9. Parasitoid frequency and parasitism rates on *A. socialis* in the Cauca Valley of Colombia.

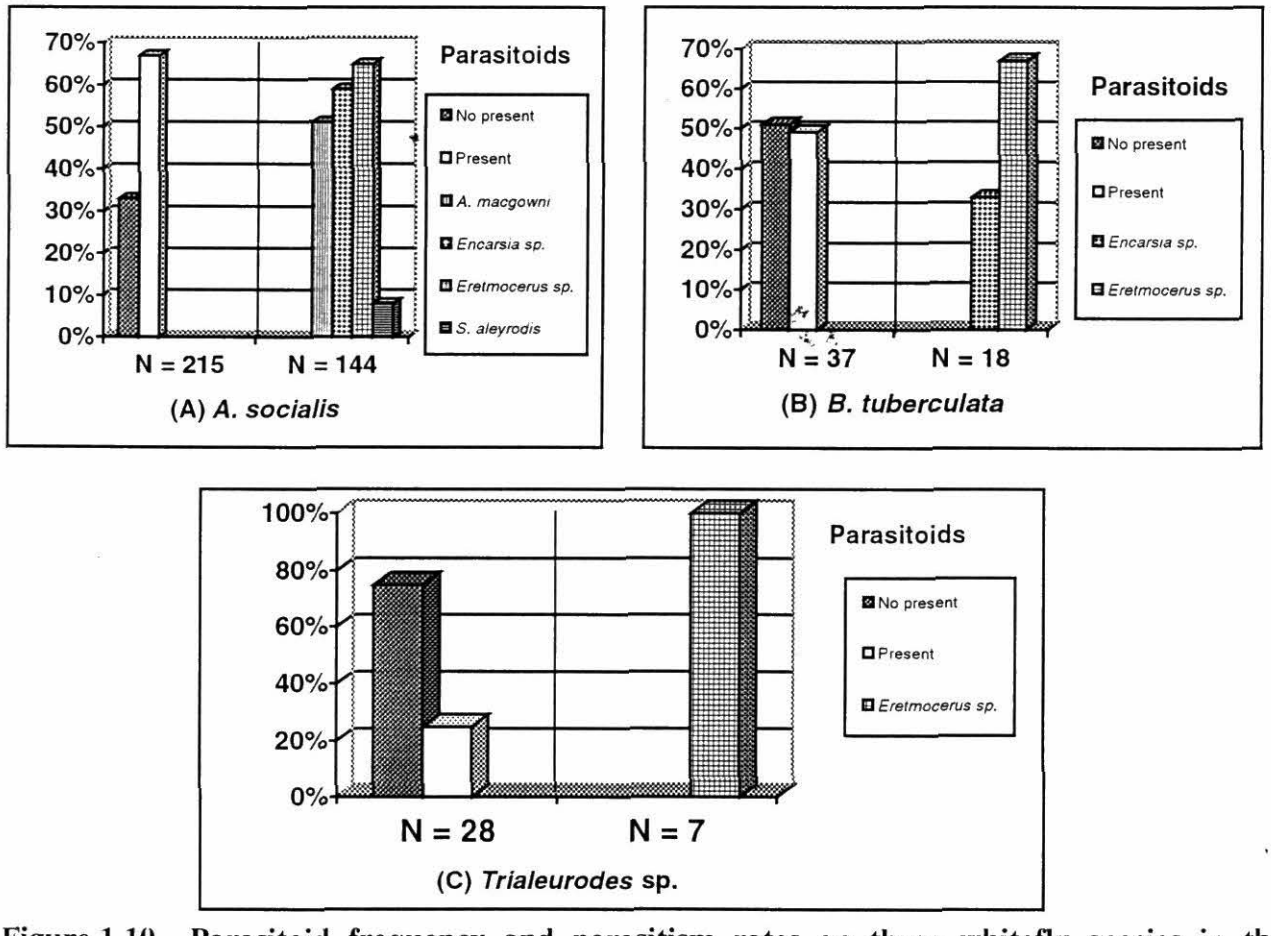
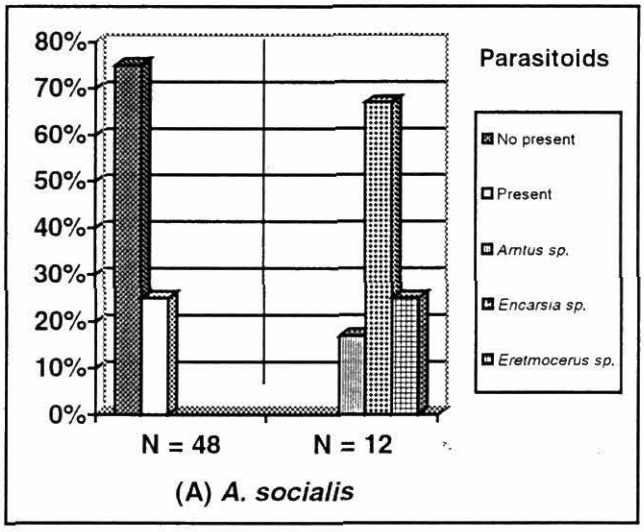
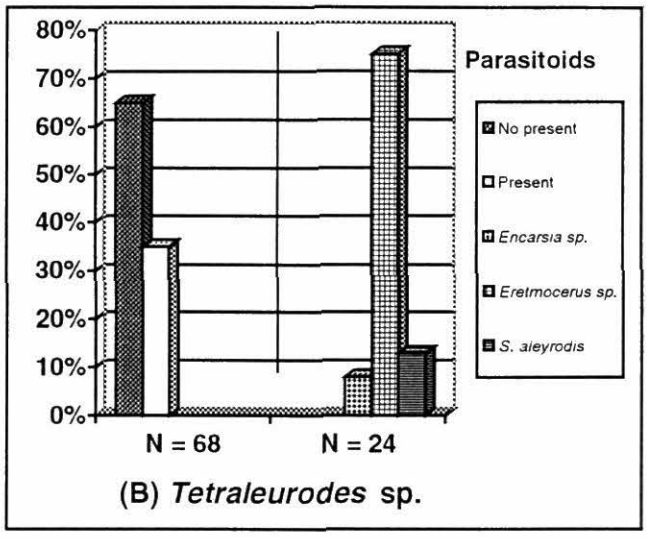
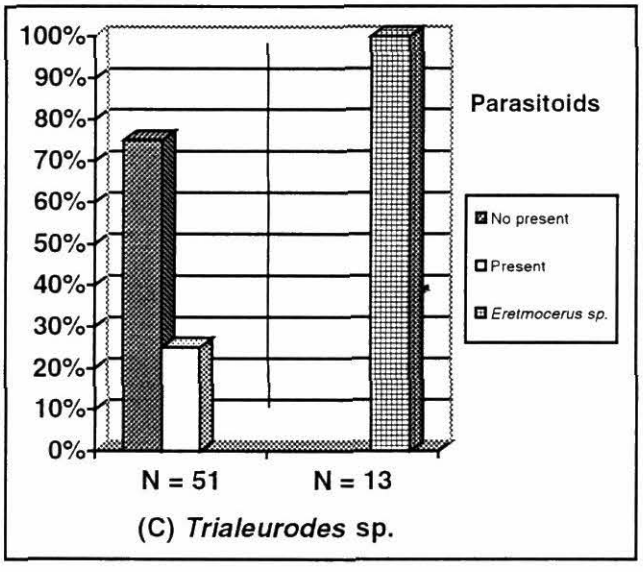
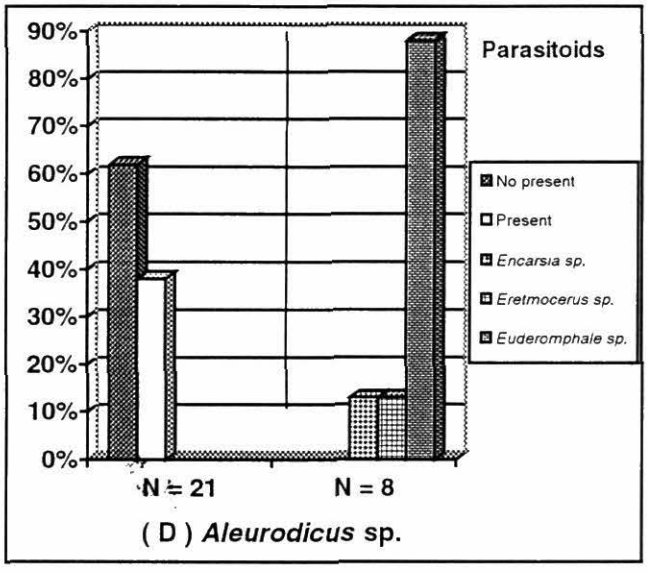


Figure 1.10. Parasitoid frequency and parasitism rates on three whitefly species in the Magdalena Valley of Colombia.









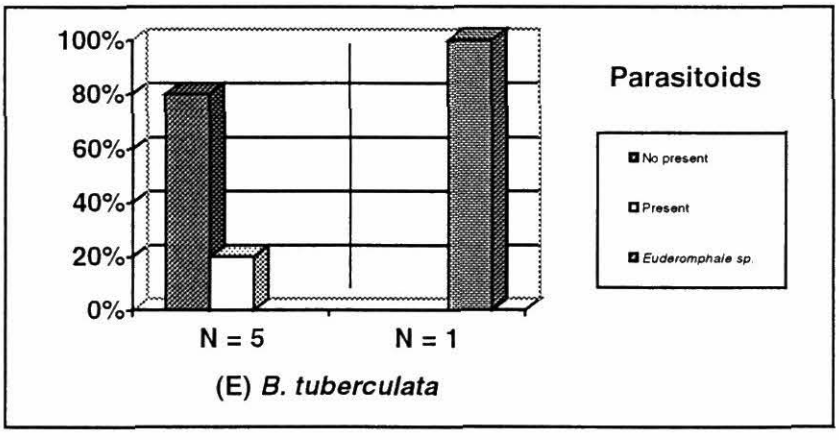


Figure 1.11. Parasitoid frequency and parasitism rates on fine whitefly species in the coastal region of Ecuador.

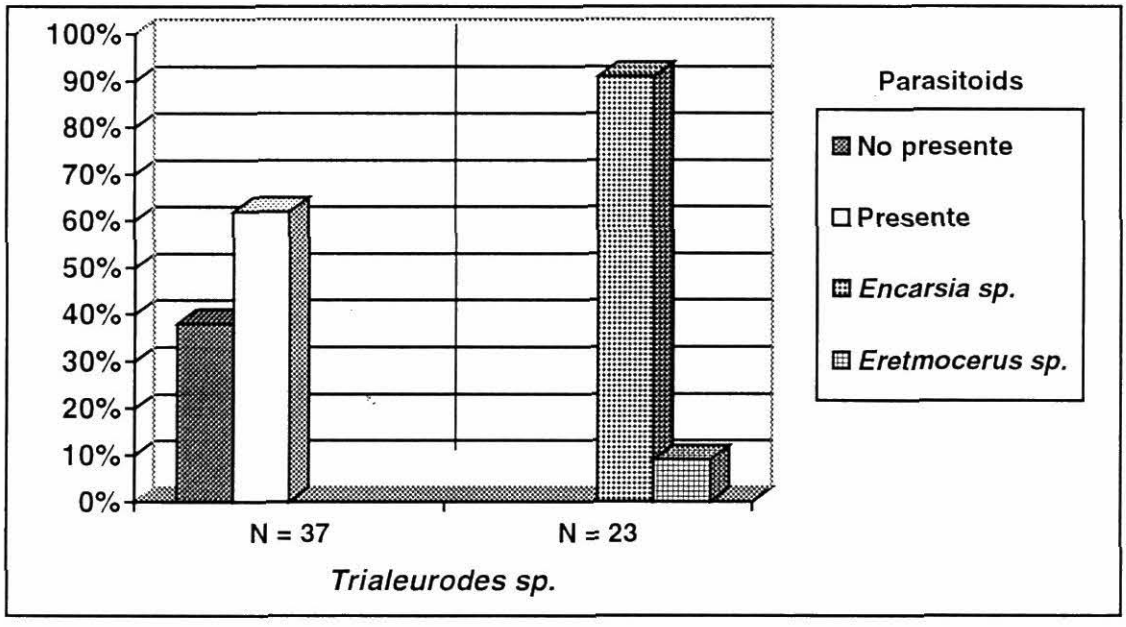


Figure 1.12. Parasitoid frequency and parasitism rates on *Trialeurodes* sp. in the Sierra region of Ecuador.

Activity 2. Biological control of mites with phytoseiid mite predators

Cassava IPM research at CIAT has given considerable emphasis to the biological control of mites of cassava and other crops. Since the early 1980; extensive evaluations of the natural enemy complex associated with cassava mites were carried out at more than 2400 sites from numerous countries. The principal target in most of these studies was the cassava green mite (CGM), *Mononychellus tanajoa*, this species causes major economic losses in cassava in the Americas, especially Brazil, and Africa, especially in seasonally dry, sub-humid, tropical agroecosystems. The search for natural enemies concentrated on mite predators of the family Phytoseiidae. An international campaign led by CIAT, IITA and CNPMF/EMPRAPA in Brazil was initiated to identify the key mite predators for introduction into Africa and Northeast Brazil.

Geographic regions in the Americas were identified and prioritized, using GIS support, to assist in targeting specific areas for exploration. Homologous maps based on agrometeorological data and microregional classification, comparing Africa and the Neotropics were prepared. Collecting zones were, therefore, usually chosen for their similitude to ecological homologues in Africa and Brazil. Between 1983 and 1990 forty (40) phytoseiid mite predator species associated with cassava and bodering crops were identified. The current predator mite reference collection held at CIAT conserves primarily those predators related to phytophagous mites found on cassava, but also contains numerous additional species found on associated or other crops. A data base has been designed that stores all of the information collected during surveys. This includes data on climate

and geography and identification of prey and predator species. This data base contains information collected on mites and their natural enemies since 1971. The following is a synopsis of this project.

Exploration for natural enemies was initiated in 1984 in several countries in Latin America. At each site visited, data was gathered on geographic location (longitude-latitude), climate, the presence of arthropod (mite and insect) pests, associated crops in the farming system, pesticide use, etc. Mite samples taken were placed in Lactophenol containing vials and slide mounted in the laboratory for identification. In addition, live specimens of predator mites were brought to CIAT for laboratory rearing and multiplication. These specimens, in a series of laboratory and field experiments, were evaluated for efficacy and many eventually chosen for shipment to Africa (via quarantine in Amsterdam, Holland), or Brazil. Each specimen is coded and corresponds to the field data collected.

The present data base uses Access (Microsoft/Windows). It is designed to offer easy access to pertinent data, to present and compare information in an orderly and classified manner, making it user friendly for collaborators and scientists that require this information.

Results

The present CIAT data base contains nearly 6000 entries from more than 2400 sites surveyed. The oldest sample is from 1971. Of the 6000 entries, 4299 have been identified by CIAT and other international taxonomists and the reference collection consists of 2368 microscope slides. Samples in the collection are from 31 countries, principally from the Americas, but also from other continents. 1576 entries are from Colombia, while other countries heavily sampled include Venezuela, Ecuador, Brazil and Cuba (**Figure 2.1**). Numerous other countries have been less sampled (**Figure 2.2**).

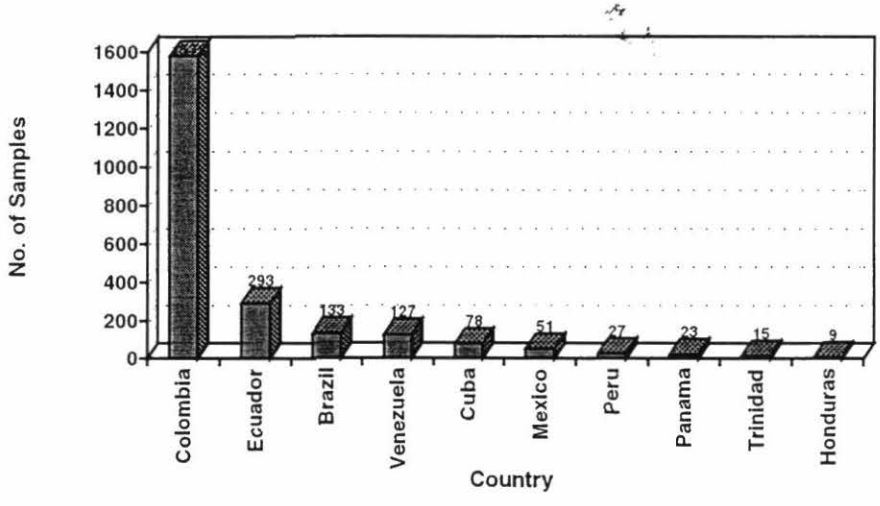


Figure 2.1. Samples collected for cassava mites and natural enemies from surveys in several Latin American countries.

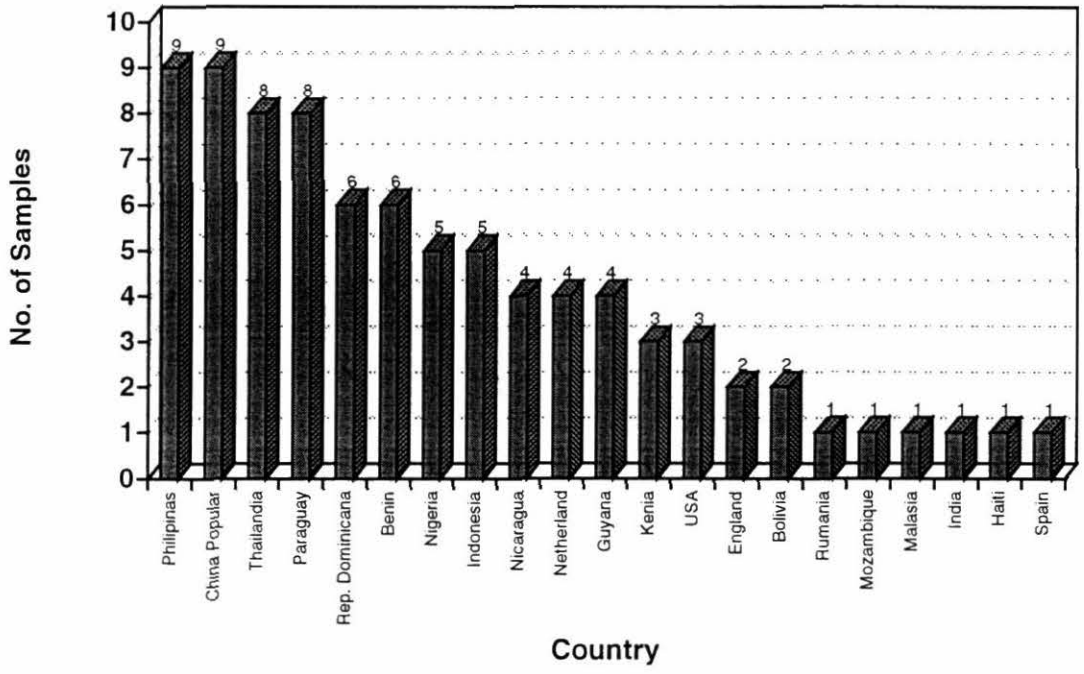


Figure 2.2. Samples collected for cassava and other crop mites and natural enemies from surveys in numerous countries.

During exploration and surveys, several other crops besides cassava were sampled. These include rice, orange, coffee, papaya, beans and a total of approximately 422 records on more than 38 hosts (**Figure 2.3**). Not all collections were made by CIAT personnel; about 14% have been made by NARs collaborators and other interested persons. The two families of mites most frequently observed during surveys were the Phytoseiidae, the predator mites and the Tetranychidae, the phytophagous mites found feeding on numerous hosts. Most specimens were collected from Colombia, Venezuela, Ecuador and Brazil (**Figure 2.4**), while other countries sampled include Cuba, Mexico, Peru, Panama, Benin, Paraguay, etc. (**Figure 2.5**). In addition to the *Tetranychidae,* other phytophagous mite families include the *Eriophyidae, Tarsonemidae* and *Tenuipalpidae*; additional predator families include *Ascidae, Stigmaeidae* and *Cheyletidae.*

Although specimens have been obtained from several hosts, the principal interest is in cassava, and the greatest number of samples from cassava are from Colombia, Ecuador, Venezuela and Brazil. Within the *Tetranychidae*, *M. tanajoa,* the cassava green mite and the associated *Phytoseiidae* complex were the primary target. A complex of *Tetranychidae* species were collected in the neotropics; *M. tanajoa*, the most frequently observed on cassava has 696 entries, *Olygonichus peruvianus* 393 entries, *M. caribbeanae* 332 entries, and *M. mcgregori* 134 entries (**Figure 2.6**). A total of 32 *Tetranychidae* species were found, including several of the genus *Tetranychus* (**Figure 2.6**).

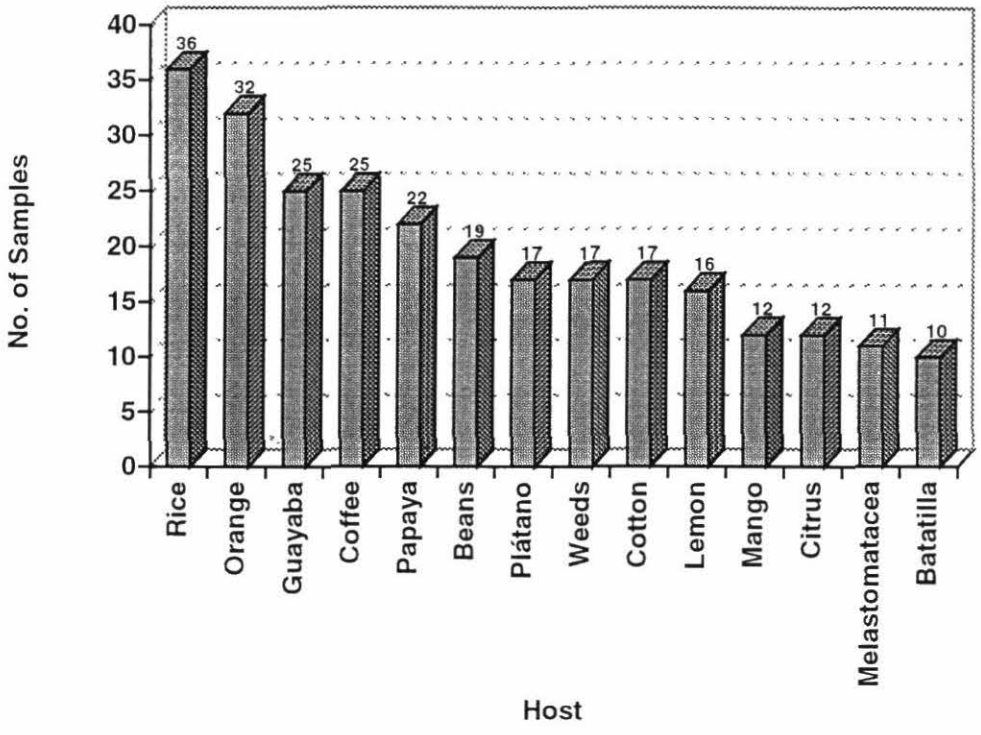


Figure 2.3. Hosts, other than cassava, surveyed and sampled for phytophagous mites and their natural enemies in Latin America and other regions.

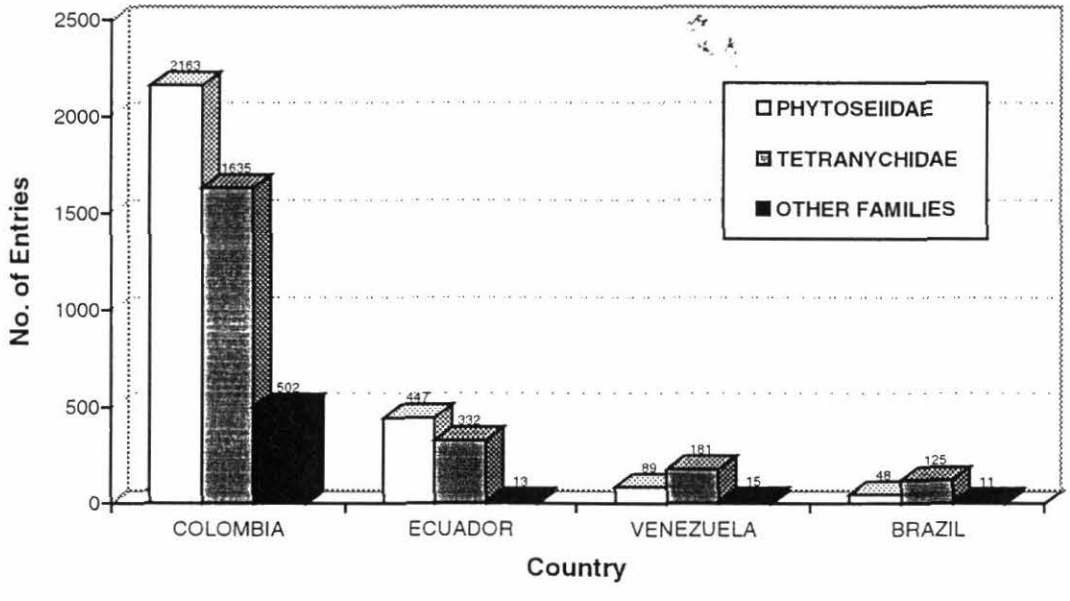


Figure 2.4. Countries registering the greatest number of phytophagous and phytoseiid mite families collected during surveys.

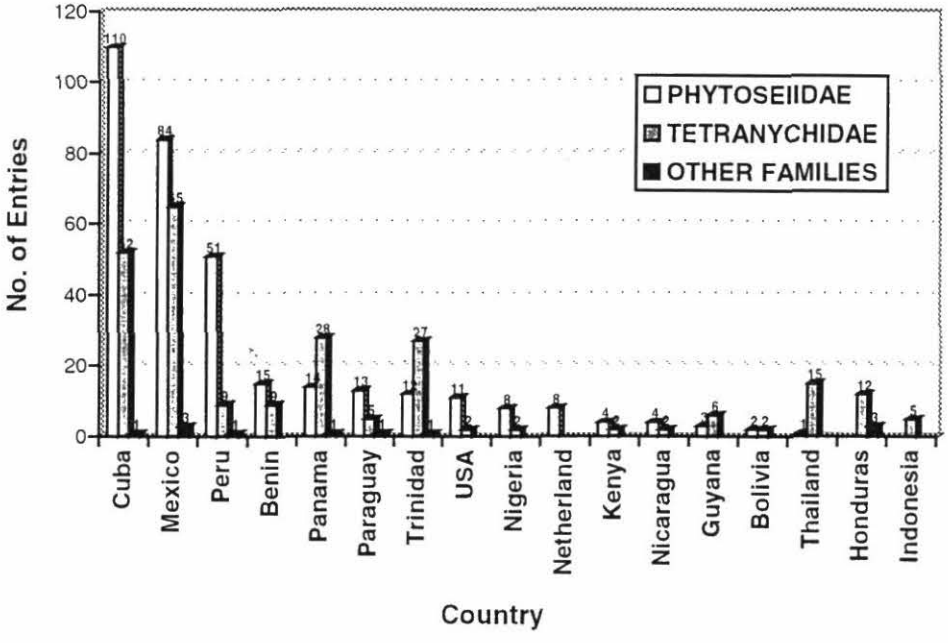


Figure 2.5. Number of records of *Tetranychidae* and phytoseiid mites identified from several countries surveyed.

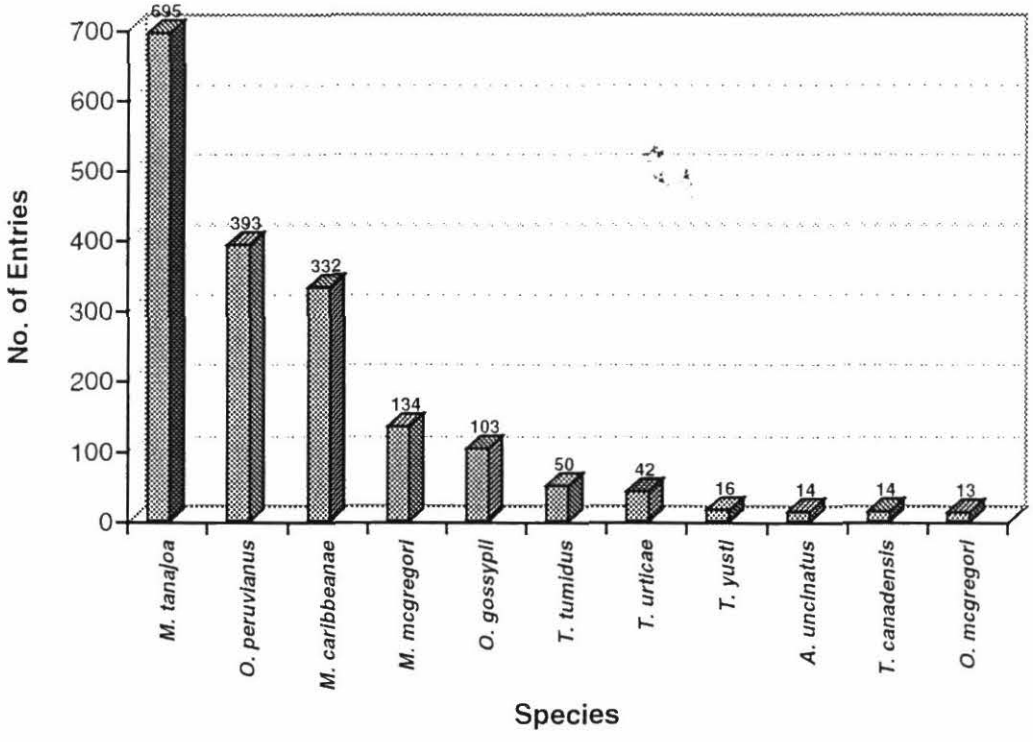


Figure 2.6. Species of *Tetranychidae* (phytophagous mites) collected from cassava in the Americas and number of records in collection.

In addition, 53 species of Phytoseiidae were found associated with cassava phytophagous mites. The newly recorded species *Typhlodromalus manihoti* was the most frequently reported and collected. The most important species, although not the most frequently observed is *T. aripo* **(Figure 2.7)**. This species has been the most successful to establish in Africa and has now disseminated to several countries where it is reported providing good control of *M. tanajoa.*

M. tanajoa has been collected during surveys from almost all countries in the Americas, although Colombia and Brazil have the greatest number of entries. As a result of these surveys, 17 new species of mites have been recorded. In addition about 25 new species of Phytoseiidae have also been recorded.

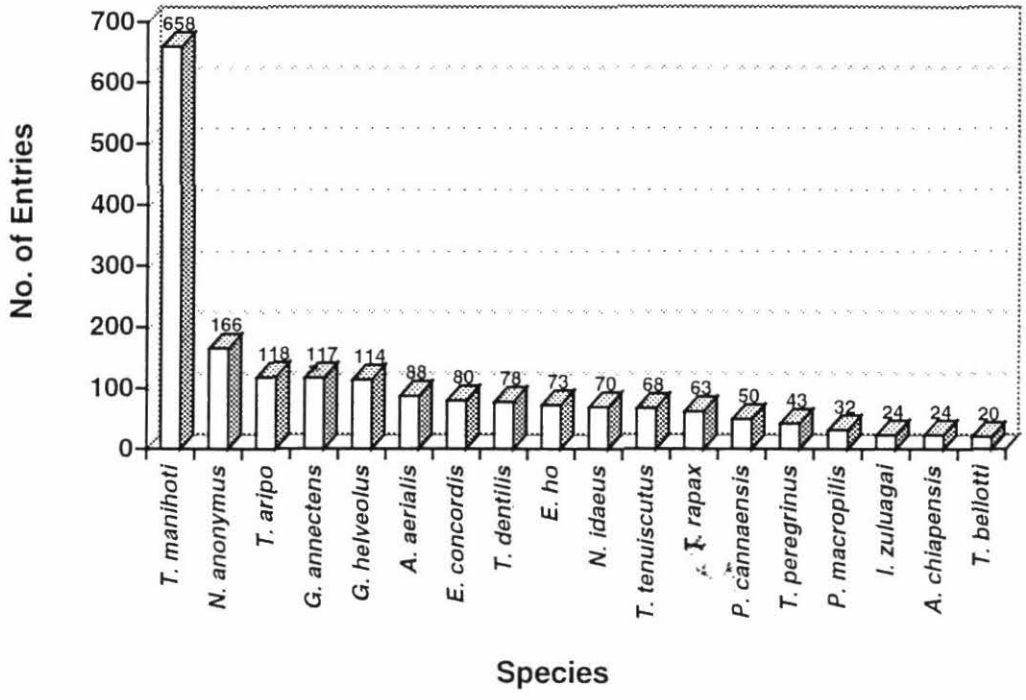


Figure 2.7. Phytoseiidae species most frequently collected from cassava mites in the neotropics (Americas).

Activity 3. Developing mass rearing methods for phytoseiidae mite predators

Phytophagous mites are major pests on numerous crops in tropical, subtropical and temperate regions. In many cases, numerous pesticides are used (and misused) to control mites. Because of their short life cycles, mite populations can increase rapidly, thereby requiring numerous and repeated pesticide applications. This not only increases production costs, but can also disrupt the biological control of secondary pests and increase environmental and health hazards.

Apple production is affected by mites, especially the species *Panonychus ulmi* (Kuch) in several areas, including Chile, Spain and the USA. In Spain for example an average of 12 applications of

up to 25 different pesticides per cycle is common, and is estimated to increase production costs by 15 to 20% of the crop value.

Biological control has been shown to be an effective and economically feasible alternative for reducing mite populations and damage in several fruits, including apples. The combination of CIAT's recent interest in fruit production and requests for assistance from the Chilean apple producers has given us (CIAT) the opportunity to explore possibilities in biological control of phytophagous mite on fruits. Our (CIAT's) already established expertise in this area (see previous section) gives us a strategic and comparative advantage for collaborative projects.

Biological control of *P. ulmi* through the employment of phytoseiid mite predators has been shown to be an affective in apples. Several phytoseiid species have been identified to predate on *P. ulmi.* Phytoseiids are especially effective because they are adaptive to different agroecosystems and have life cycles of shorter duration than the prey species. They have shown to be effective on Tetranychidae mites on several fruit crops including strawberry, citrus, grapes, etc. The selection of efficient predators depends on several factors or selection criteria; these include synchronization with the prey, climatic adaptation, high prey consumption, good searching capacity and ease to mass rear.

Mass rearing of phytoseiids has been a basic component of our mite biocontrol research and several techniques have been developed. The objective of a mass rearing program is to obtain, with minimal effort, space, and cost, the maximum number of high quality fertile female mite predators, in the shortest time possible.

The "Movimiento Agroecológico Chileno" (MACH), a Chilean NGO, is attempting to implement a biological control program for *P. ulmi* management on apples and other fruits. They requested CIAT's participation in training researchers in mite biocontrol and in developing methodologies for mass rearing, especially of the predator species *Neoseiulus californicus*, a species we have maintained in colony at CIAT.

The objective of this project is to develop an effective mass rearing method for the rapid multiplication of *N. californicus*. The strain that we use was collected in Manabí, Ecuador. The initial colony is maintained using the McMurtry & Scriven method, that has proven to be effective for laboratory colonies of selected Phytoseiidae species. This method consists of a metal tray, a 1cm flat, humid sponge; a laminated plexiglass plate is placed on the sponge and is encircled with Kleenex to serve as a barrier to predator escape. Loose cotton, placed on the plexiglass serves as a refuge for the predators. *Ricinus comunis* pollen, honey and *Tetranychus urticae* eggs are offered as food. The trays are maintained at 25°C and 70 $\pm$ 5° RH. *T. urticae* eggs are obtained from a mite colony maintained on beans and harvested mite infested leaves are "washed", "strained" and collected eggs refrigerated at 4°C.

Evaluations were done with three *N. californicus* female densities, three repetitions, and the addition of males to confirm female copulation. Densities were 50, 100 and 150 females; at the latter density, two treatments were included, one offering food twice a week and the other three times a week. Population counts were made beginning 20 days after initiation, and then every 8 days for 32 days. Counts were done by removing females from the tray units and transferring them

to another unit and leaving the immature until the following evaluation. The ANOVA model was used for the statistical analysis as well as the Fisher (LSD) for testing differences between treatments.

Results show significant differences between the initial female densities and the interaction of these densities and time of harvest (females removed) **(Table 3.1)**. This indicates that the initial population of females effects colony size.

The two highest densities (150 females with two different feeding regimes) produced significantly more offspring than the lower densities (100, 50 females) **(Table 3.2)**. The feeding regimes evaluated, 2 vs. 3 feedings per week, did not result in significant differences in offspring produced. These results indicate that two feedings, which requires less food and less effort, will give the same results in terms of offspring produced.

Table 3.1. Analysis of variance for female *Neoseiulus californicus* production using the McMurtry & Scriven method.

	G.L.	Average[2]	P
Rearing Unit	2	11148.1	0.7187
Initial Female Density	3	333445.9	0.0001**
Weeks to Harvest (Time)	3	68482.0	0.1278
Density/Time	9	114701.7	0.0051**

** Significant differences in initial density and density/time of harvest interaction.

Table 3.2. Analysis of variance for different densities of *Neoseiulus californicus* females using the McMurtry & Scriven method.

No. of Initial Females	N	Average[1]
150[2]	12	381.33a
150[3]	12	364.08a
100	12	85.75b
50	12	82.75b

1 LSD test, a = 0.05. Average followed by the same litter are no significantly different.
2 Food offered 3 times (Mon. Wed. Fri).
3 Food offered twice (Mon. & Thurs.).

Taking into account the number of female produced, results show that after two "harvest" (weeks), the numbers of females decrease **(Figure 3.1)**. This indicates that it is inefficient to maintain mass rearing colonies beyond two weeks, and it is preferable to initiate new colonies. Considering start-up time, this means that each unit should be maintained for approximately 1½ months.

It is also clear that start-up densities of 50 and 100 females is not recommended (for this species). This supports previous observations that have shown that initiating lab colonies with few individual results in slow population increases **(Figure 3.2)**. This method of rearing, therefore, results in an average of 360 females being produced each week, starting with 150 females with two feedings per week.

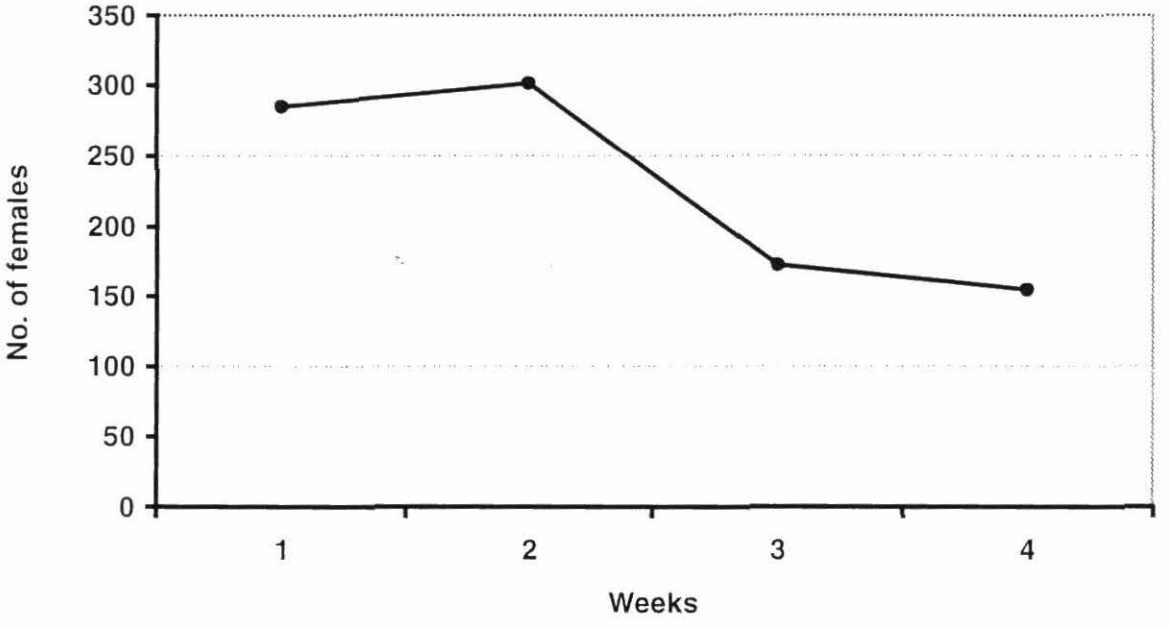


Figure 3.1. Average total number of females of *Neoseiulus californicus* harvested for four weeks (McMurtry & Scriven method).

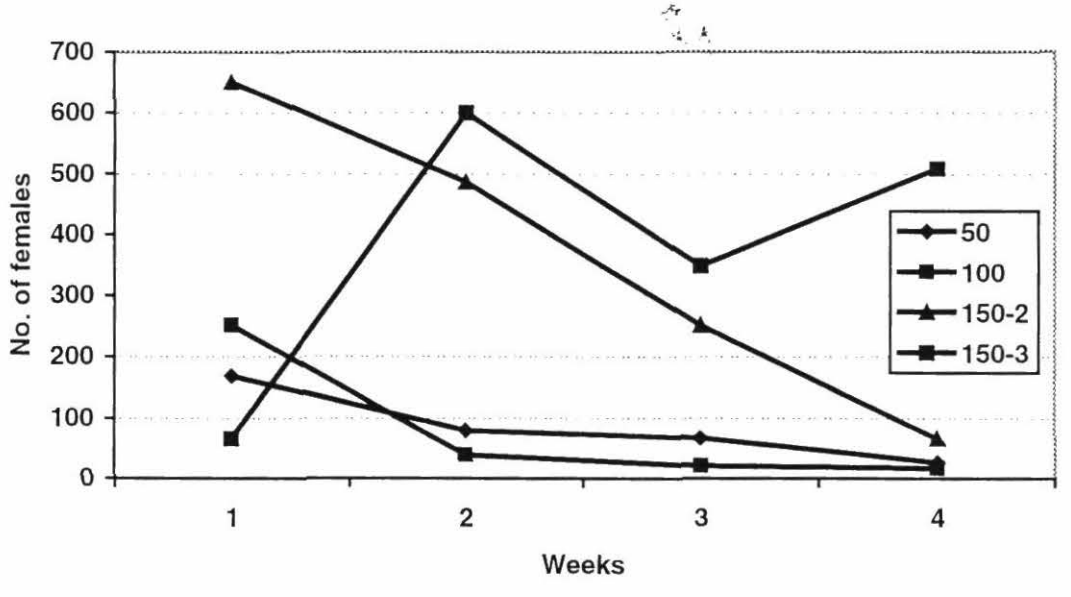


Figure 3.2. Numbers of *Neoseiulus californicus* females harvested often from weeks with different initial densities (McMurtry & Scriven method).

Activity 4. Potential of the fungal pathogen *Neozygites floridana* for the biological control of cassava green mite

Introduction

The cassava green mite, *Mononychellus tanajoa* was accidentally introduced into Africa in the early 1970's (Yaninek and Herren 1988) where it causes considerable crop loss, as well as in the semi-arid regions o NE Brazil. A major biological control effort was mounted to identify, evaluate and introduce key natural enemies into areas or regions where they were absent. Several years ago irregular levels of mortality were observed in green mite populations on cassava in CIAT (Alvarez et al. 1991) and in Northeast Brazil (Delalibera et al 1992), due to a fungus of the genus *Neozygites* sp. One of the first reports of this fungus infecting *Tetranychidae* was by Fisher (1951) in Florida. Mortality above 70% of *Panonÿcus citri* by a *Entomophthorales* species (probably *Neozygites*) were reported. The fungus was also found throughout the Florida peninsula, especially during certain times of the year (Van der Geest 1985). The fungus was projected as having considerable biological control potential.

Due to the taxonomic uncertainties of this type of fungus, it is often difficult to establish exact identification. The CIAT strains have been identified as *Neozygites floridana*; they were sent to Brazil for in vitro production (Leite et al 2000). There is some indication that this fungus may be sent to Africa for introduction into a biological control program for CGM. It is therefore important to determine fungal specificity, virulence and pathogenicity and to obtain more information on strains or races. One of the difficulties in working with this fungus, is its difficulty to maintain an in vitro culture. The CIAT strains have been stored since 1995 and it was decided to reactivate and multiply the fungus using an in-vivo method.

Methods

The fungus was reactivated using the Delalibera-Bohorquez method. The in-vivo method permits a continual supply of infected material for experimentation. Studies were done in an incubator chamber at 24°C and 12hour photophase. Isolates of *Neozygites* from different mite species (*Tetranychus urticae, M. tanajoa* and *M. caribbeanae*) were used. Cassava leaf discs (2cm diameter) were placed on a saturated polyethylene sponge in a 15cm (diameter) x 2cm (height) petri dish. Ten leaf discs are placed in each petri dish. A mummified mite placed in the center of each leaf disc was held in darkness for 12 to 15 hours at 24°C and high humidity (96%) to promote conidiogenesis.

Twenty five recently emerged *M. tanajoa* females were transferred to the border of the leaf disc, but removed from the site of conidia distribution. Each treatment consisted of 10 discs with *M. tanajoa*. The control consisted of 10 clean discs, without conidia. For the first three days, discs are sealed to encourage high relative humidity. Mites are transferred to new discs by placing the old discs individually over clean leaf discs, and waiting for the mites to move to the new disc as the old one dehydrates. Mite counts, distinguishing those that die from the fungus and those that drown, are made daily. Several different strains of the fungus were utilized in this experiment (**Table 4.1**).

Table 4.1. *Neozygites floridana* strains stored at CIAT and utilized for in-vivo multiplication.

Host	Source	No. Stored Vials	Fungal Generations	Last Reactivation
M. tanajoa	Benin, Africa	5	5	04/97
M. tanajoa	Cruz das Almas, Bahia, Brazil	7	3	08/96
M. tanajoa	Media Luna, Magdalena, Colombia	3	4	08/96
M. tanajoa	Piritiba, Brazil	5	4	04/98
M. tanajoa	Caruaru, Brazil	5	7	04/96
M. tanajoa	Pivijay	2	2	03/98
M. tanajoa	Santander de Quilichao	4	6	04/98
M. tanajoa	CIAT, Valle, Colombia	9	5	04/98
M. tanajoa	Risaralda, Colombia	1	1	07/98
T. urticae	CIAT, Valle, Colombia	5	4	08/96
T. urticae	Benin, Africa	4	7	08/96
M. caribbeanae	Ecuador	1	1	04/93

Results

In all evaluations with these strains, and in the three replications, no sporulation occurred. Mounted microscope slides resulted in an irregular formed conidia, indicating a possible storage effect on these structures. In previous experiments with these strains at CIAT, considerable conidia were observed **(Table 4.2).** Strains from which the highest number of mummies were obtained were TUCIAT 1 and 2, MtCIAT3, MtML, MtBenin, they have consistently obtained an average of 500 to 600 capiloconida per mummy.

It is possible, that with time, the viability of the different strains has decreased or been lost. A reactivation using in-vitro methodologies will be attempted, although the number of hyphal bodies is also low. In these evaluations, satisfactory growth of strains from *T. urticae* and was noted to be superior to that from strains from *M. tanajoa*. It is possible that since strains from *M. tanajoa* are more species specific than those from *T. urticae*, better controlled conditions are required, with a culture media higher or richer in nutrients and specific chemical supplements.

However, before considerable more costly investment is made, the priority of using this fungus in mite biocontrol should be determined. If necessary, CIAT strains should be reinforced with strains from Brazil and the USA.

Table 4.2. Average number of *Neozygites floridana* mummies from different strains, obtained each week utilizing an in-vivo culture method.

Host	Locality	Name	No. Mummies /Week
Tetranychus urticae	CIAT, Palmira, Valle (Colombia)	TuCIAT 1	168
T. urticae	CIAT, Palmira, Valle (Colombia)	TuCIAT 2	175
T. urticae	Avakpa, Benin	TuBenin	58
Mononychellus tanajoa	CIAT, Palmira, Valle (Colombia)	MtCIAT 1	82
M. tanajoa	CIAT, Palmira, Valle (Colombia)	MtCIAT 2	78
M. tanajoa	CIAT, Palmira, Valle (Colombia)	MtCIAT 3	110
M. tanajoa	Media Luna, Magdalena (Colombia)	MtML	130
M. tanajoa	Santander de Quilichao, Cauca (Colombia)	MtSQ	69
M. tanajoa	Avakpa, Benin	MtBenin	109
M. tanajoa	Cruz das Almas, Bahía (Brazil)	MtCDA 1	82
M. tanajoa	Cruz das Almas, Bahía (Brazil)	MtCDA 2	87
M. tanajoa	Caruaru, Pernambuco (Brazil)	MtCar	60
M. tanajoa	Piritiba, Bahía (Brazil)	MtPir	62

Activity 5. Biology of *Aleuroglandulus malangae*

Recent surveys in cassava fields in Colombia have resulted in the identification of the whitefly species *Aleuroglandulus malangae* being recorded for the first time on cassava. This species had been previously registered on ornamentals such *Colocasia* sp. but not on cassava. It was collected from cassava in the Córdoba and Atlántico departments of Colombia. *A. malangae* is of the Order Homoptera; Family, Aleyrodidae; subfamily; Aleyrodinae.

Studies on the biology of *A. malangae* were done in the laboratory with controlled temperature (24.5 ± 4°C) and humidity (RH 70 ± 30%). Adults of *A. malangae* were obtained from a *Colocasia* host and the initial biological studies are done on *Colocasia*. Adults were allowed to oviposit on circular portions of *Colocasia* leaf for a 24hr. period. These were placed in petri dishes with 1.2% agar, with the objective of maintaining constant humidity for longer leaf life. Observations were recorded daily, until adults emerged. In addition parasitized pupae of *A. malangae* were collected from the field, placed in small petri dishes and parasitoids allowed to emerge. A predator species was also collected from the field. All natural enemies were identified to species.

Biology of *A. malangae*

Eggs: Oviposition occurs mostly in groups but occasionally individual eggs are observed on the undersides of leaves. Eggs are elongated with a wider anterior end and adhere to the leaf surface by a short pedicle that firmly connects it to the plant time in an inclined position. Recently oviposited eggs are whitish-yellow in color; as egg development continues, cream colored, ovoid

spots develop on the upper half of the egg. Near hatching, two small red spots appear on each side of the anterior end. Egg development averages 8.0 days (**Table 5.1**). Eggs are 0.23mm length and 0.10mm at its widest part (**Table 5.2**).

There are four nymphal instars, with the forth instar the papal stage.

Nymph I: First instar translucent white nymphs are highly mobile. They are oval, slightly wider at the anterior end, with two red ocular spots, conspicuous legs without claws, and with a long setae and 17 pairs of marginal setae around the body; there is a large caudal (sc) pair of setae. Using mounted microscope slides, a pair of cephalic (sf) micro setae can be observed. The subdorsum is without complete compound or agglomerated pores. On the last abdominal segment, a vasiform orifice (ov) with a short, wide (In) lingula and a pair of setae in the distal (sc) part can be found (**Figure 5.1**). The average nymphal length is 0.31mm and 0.18mm width (**Table 5.2**). The duration of this nymphal stage is 4 days (**Table 5.1**).

Table 5.1. Average duration in days of the different developmental stages of the whitefly *Aleuroglandulus malangae*.

Stage	No. of Observations	Duration Days	Average Days
Egg	317	7,0 - 11,0	8,0
Nymph I	215	3,0 - 5,0	4,0
Nymph II	106	2,0 - 5,0	3,0
Nymph III	81	2,0 - 7,0	4,8
Nymph IV o Pupae	113	7,0 - 9,0	8,0

Table 5.2. Average size (length and width) of the developmental stages of the whitefly *A. malangae*.

Stage	No. of Observations	Length mm.	Width mm.	Average Length-Width mm.
Egg	50	0.12 – 0.25	0.02 - 0.13	0.23 - 0.10
Nymph I	66	0.24 – 0.33	0.09 - 0.20	0.31 - 0.18
Nymph II	30	0.41 – 0.47	0.26 - 0.31	0.45 - 0.28
Nymph III	40	0.61 – 0.81	0.43 - 0.52	0.68 - 0.45
Nymph IV o Pupae	47	0.86 – 1.29	0.60 - 1.26	1.20 - 0.84

Nymph II: Second instar nymphs are oval, whitish yellow, with a marginal wax crown (this is easily recognized when nymphs molt). The setae series, described in the first instar, disappears, leaving only an antenna (sa) and caudal (sc) pair that can be distinguished by its greater length. In mounted specimens, a uniformly crenulated margin (mcr) is observed. The red ocular spots and bucal apparatus are further developed than in the first instar. Dorsally, the abdominal segments are well defined by a series of depressions; the legs are vestigial, appearing as stumps (**Figure 5.2**). Nymphs are mostly immobile in this stage. The vasiform orifice is posteriorily narrow and is found further from the caudal margin. The lingula (ln) is present in a lobulated form at the distal end and a pair of setae are present. Second instar nymphs are 0.45 mm in length and 0.28 mm wide, and average duration is 3 days (**Tables 5.2 and 5.1**).

Nymph III: Upon molting the third instar nymph loses its waxy crown, is more ovoid then the previous instar, more translucent, a softer crenulated margin, the presence of caudal setae and pair of cephalic microsetae. As this instar continues to develop, a series of furrow or depressions appear on the sub-marginal region and the marginal waxy crown reappears. As it approaches its

next molt a hint of two pair of protuberances appears, one in the thoracic region and the other in the abdominal region. The abdominal segments are clearly visible. Ventrally, the legs and the bucal parts become more atrophied (**Figure 5.3**). Average length of this instar is 0.68 mm and average width 0.45 mm. Average duration is 4.8 days.

Nymph IV or Pupal Stage: Nymphs in the forth instar are brightly translucent, almost flat, the pair of protuberances are more pronounced in the thorax and less pronounced in the abdomen. The furrow or depressed areas in the submarginal region are more evident and the abdominal segments are less obvious. As this stage continues, development increases, the protuberances are transformed into two pair of well developed waxy tubes (hyaline); the pair found on the prothorax is larger and has a curved position that rests in the submarginal region. The other pair is found on the third abdominal segment and these are smaller. Each abdominal segment contains a pair of short setae, the caudal setae are medium sized. The vasiform orifice is triangular, distally bilobated, subcordate opercolum (opc), lobed lingula with a pair of large setae and a not well differentiated caudal furrow (**Figure 5.4**). Specimens mounted on microscope slides show two large simple, subcircular pores (ps) on the prothorax and a smaller pair on the third abdominal segment, caudal margin (mcd) and dentated tracheal combs. The pupae is 1.2mm in length and 0.8mm in width. Average duration of this stage is 8 days (**Tables 5.1 and 5.2**).

The egg to adult stage averages 27.8 days. Adults are small, with a yellowish body with white wings that do not completely rest over the abdomen. Males and females are approximately the same size.

Natural enemies collected include three parasitoids, *Encarsia guadeloupae* Viggiani; *Encarsia desantisi*: Viggiani, and *Encarsia hispida* De Santis. All were identified by Dr. Gregory Evans at the University of Florida. The predator *Nephaspis namolica* Gordon, was identified by Dr. R.G. Booth (IIE), London. This species is only known from Colombia.

Activity 6. Identification of whitefly species and their natural enemies and the organization of whitefly IPM reference collection.

One of the major activities of the Systemwide Whitefly IPM projects (See Output IV of this report) is the identification of whitefly species and their associated natural enemies, their geographical areas of importance and their associated hosts. Samples collected from the Americas (Latin America and the Caribbean) are processed at CIAT; Surveys and collections have been done in 13 countries from different agroecological regions. The correct identification of whitefly species and their related hosts are vital for the development and implementation of successful IPM programs.

Collaborating professionals in NAR's and other institutions in participating countries, have collected a considerable number of samples that have been sent to CIAT. These were processed and mounted for identification. They are presently available as permanently mounted slides, systematically organized and taxonomically classified within CIAT's Arthropod Reference Collection. Natural enemies that have been collected, principally in Colombia (see previous sections), are also held in this collection.

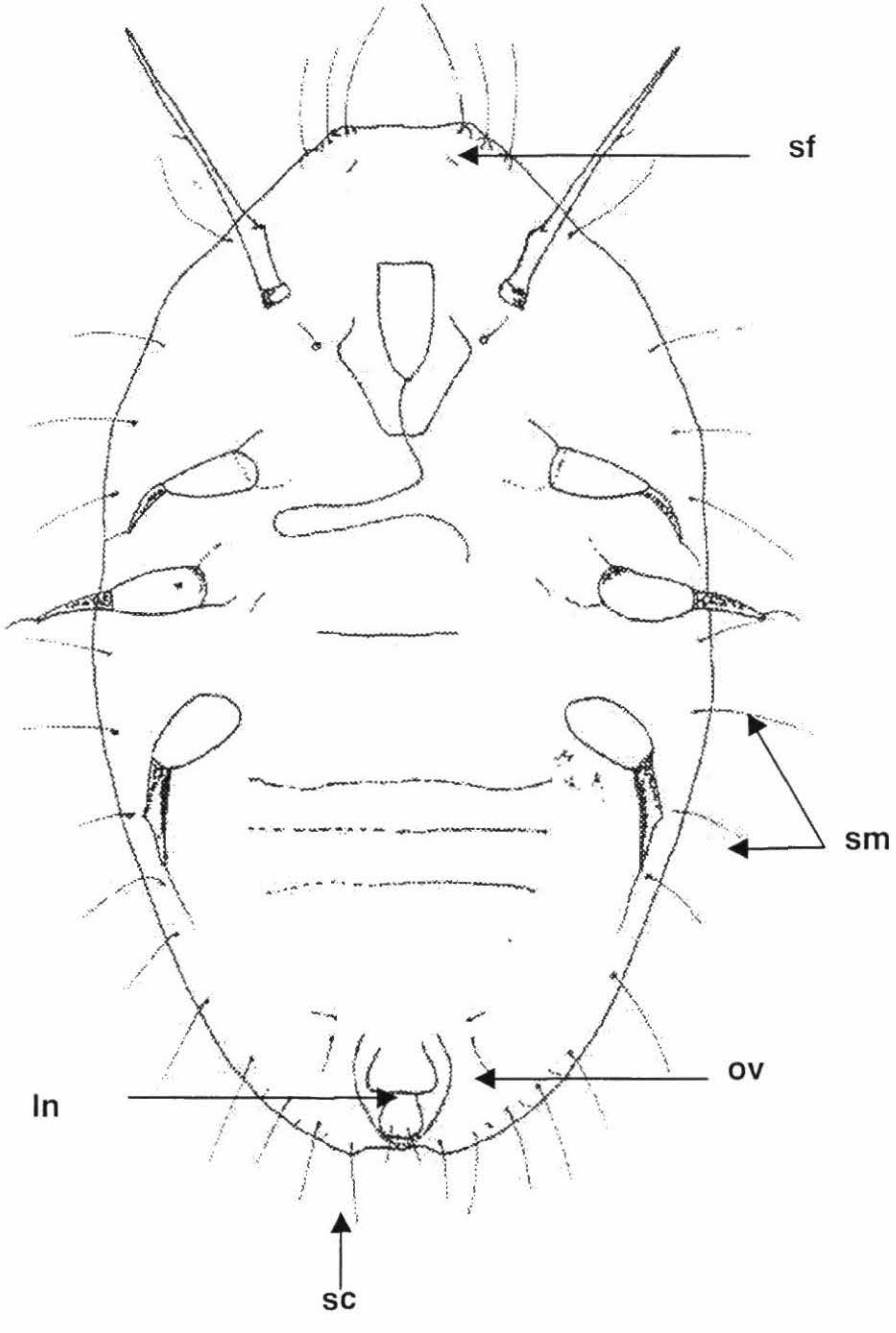


Figure 5.1. Nymph I of *A. malangae*.

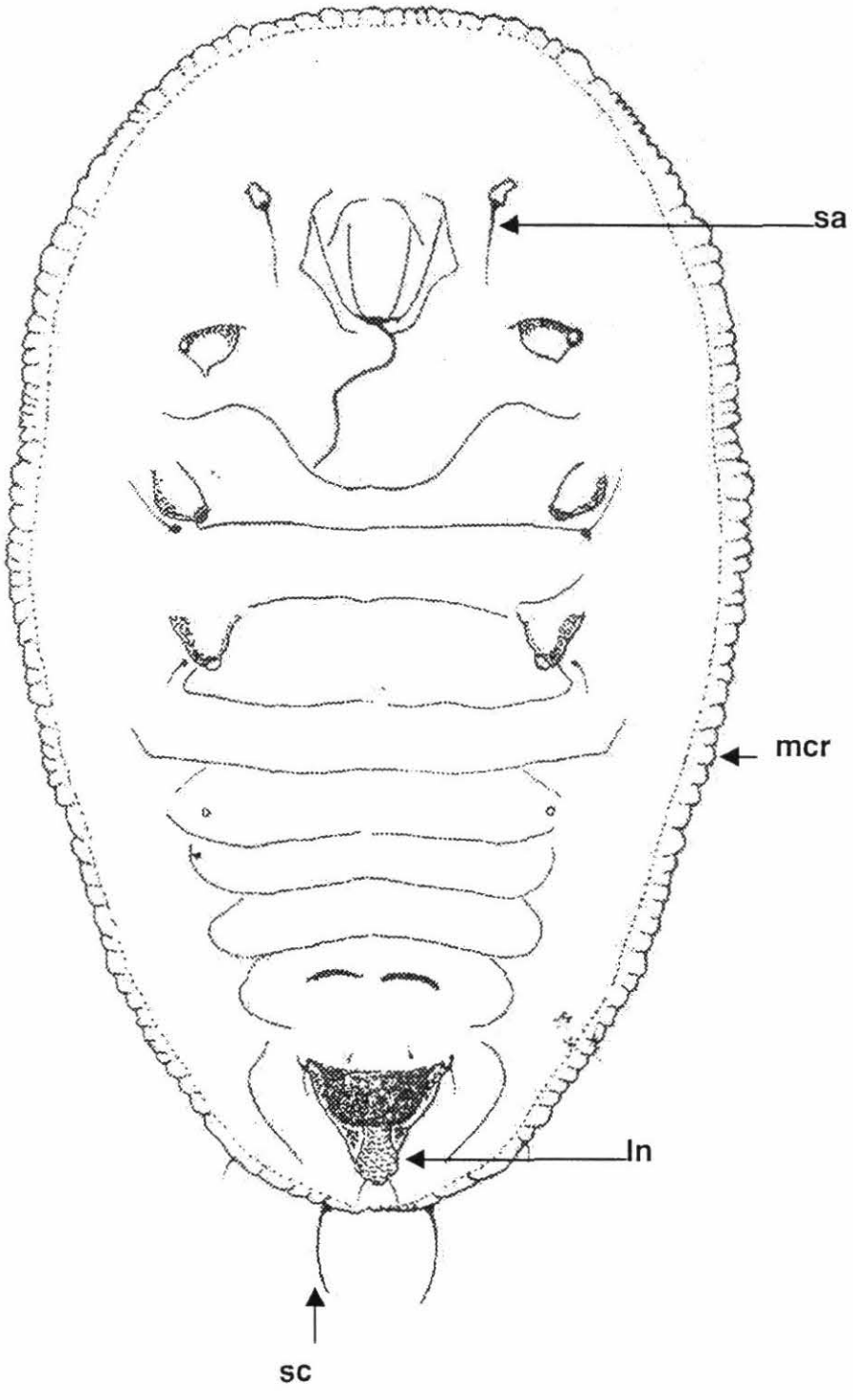


Figure 5.2. Nymph II of *A. malangae.*

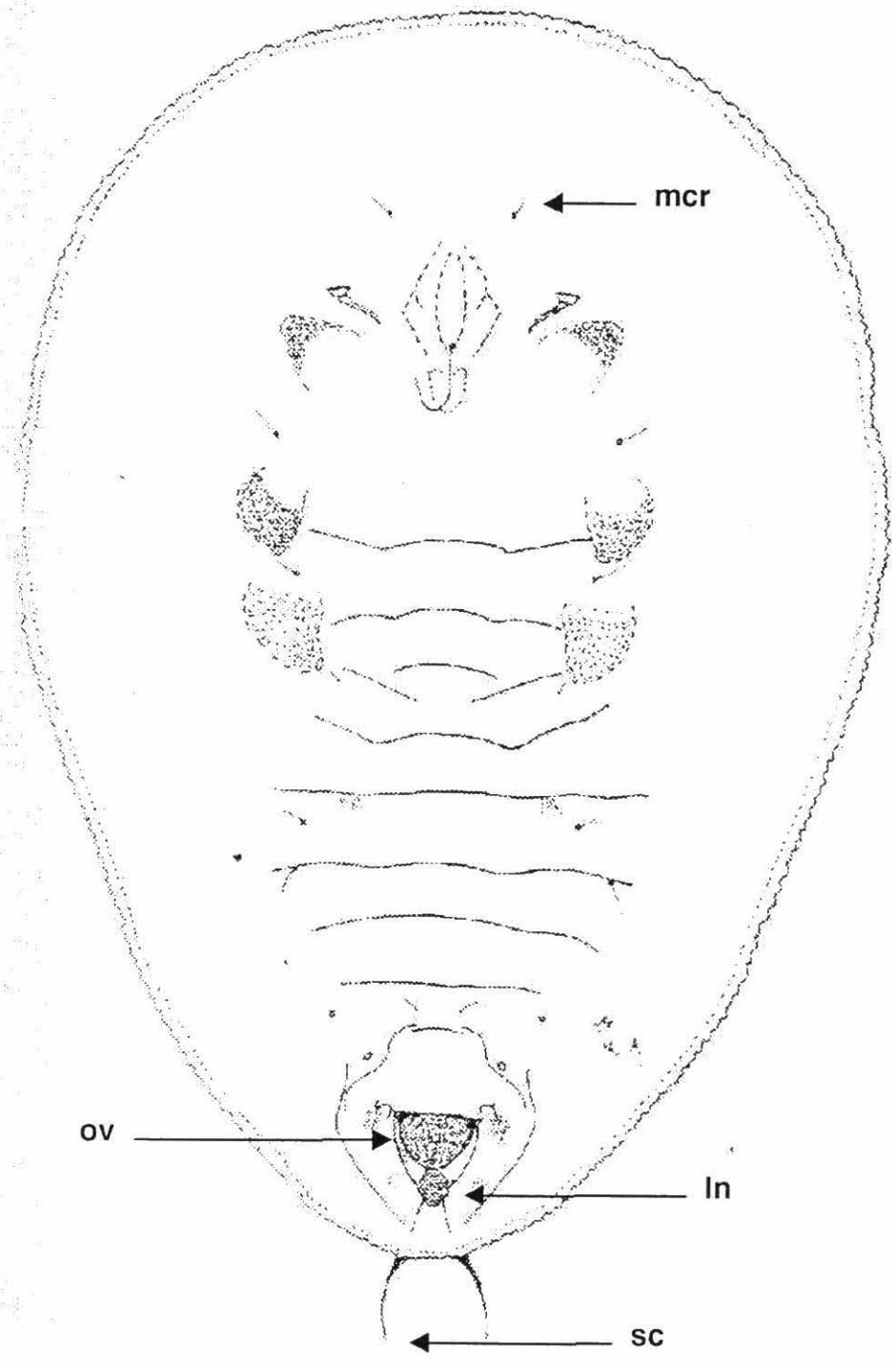


Figure 5.3. Nymph III of *A. malangae*.

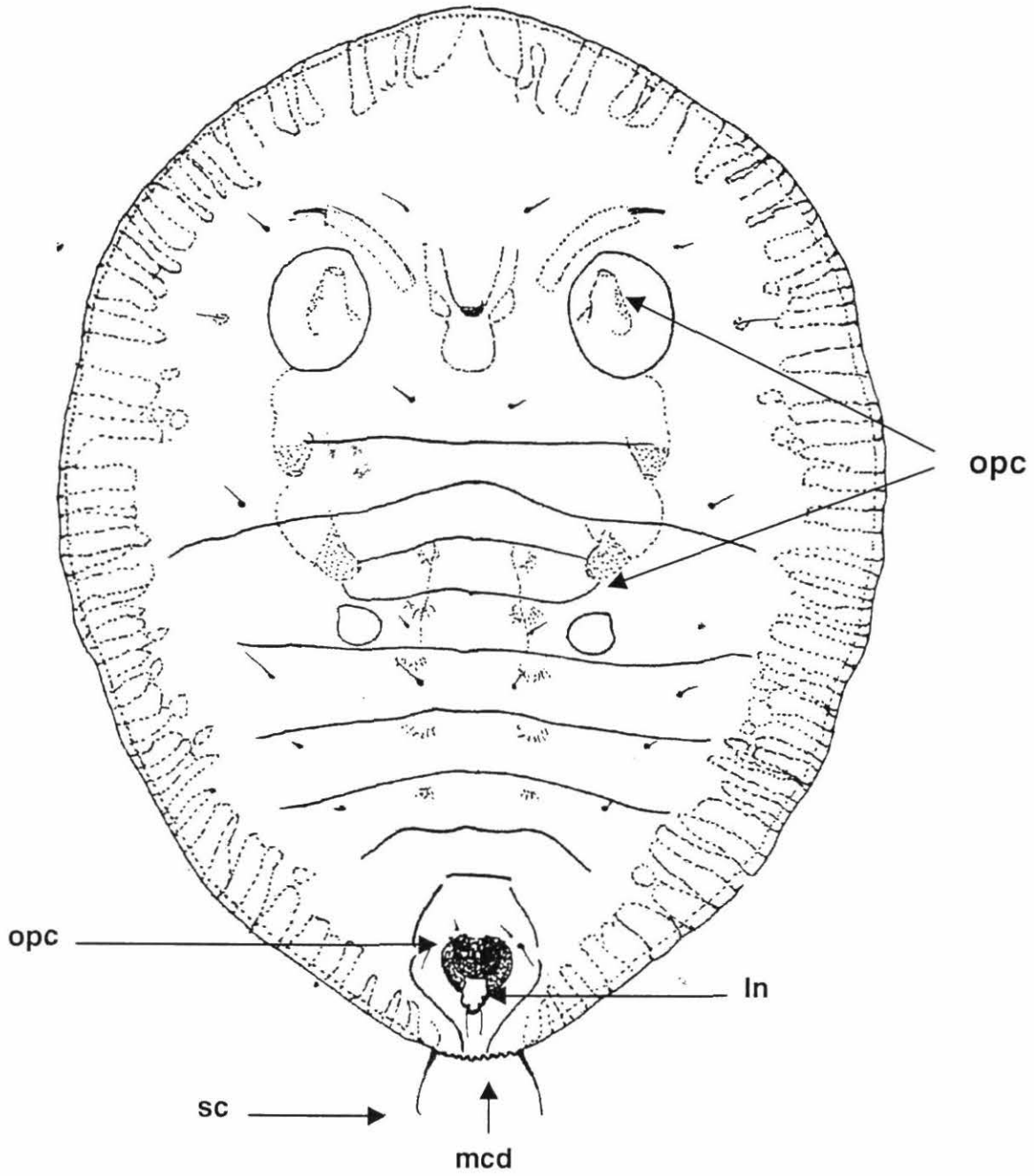


Figure 5.4. Nymph IV of pupae of *A. malangae*.

Results

Whitefly identification was done primarily at CIAT laboratories, but in close collaboration with Drs. Avas B. Hamon and Gregory Evans at the University of Florida. All samples (nymphs and pupae) were shipped to us by our collaborators in 70% alcohol. Once the diversity of each shipment was determined, pupae were than selected and slide mounted (posterior) for identification using Canada balsamic mounting media. Parasitoid preservation was in 70% alcohol or dry mounted according to taxonomic requirements. Forth (IV) instar whitefly nymphs (pupae) were mounted for identification purposes. The whitefly species identified were:

Bemisia tabaci (Gennadius)
Bemisia tuberculata Bondar
Trialeurodes vaporariorum (Westwood)
Trialeurodes sp. pos. variabilis (Quaintance)
Aleurodicus dispersus Russell
Aleurodicus sp. pos. dugesii Cockerell
Aleurotrachelus socialis Bondar

Thirteen countries in South and Central America and the Caribbean participated in the project and sent samples from different crops and plant hosts. The totals of samples sent from each country can be found in **Table 6.1**. El Salvador and Colombia represent the two countries with the highest number of samples received for processing. This table also indicates the numbers of permanently mounted slides being held in the CIAT Reference Collection. It is important to note that identification of some specimens are still awaiting confirmation by taxonomists.

Bemisia tabaci is the species with the widest host range (**Table 6.2**). It is important to note that *B. tabaci* was collected from *Manihot esculenta* in Colombia, Ecuador and the Dominican Republic.

Table 6.1. Countries represented and whitefly records in CIAT's Reference Collection.

Country	No. of Samples Sent	Host Plants	Species Whitefly Identified	No. of Mounted Slides
Argentina	6	2	1	36
Colombia	116	17	4	483
Costa Rica	41	9	2	175
El Salvador	167	14	2	353
Ecuador	33	9	2	229
USA	1	1	1	20
Guatemala	50	13	2	122
Honduras	29	6	2	127
México	2	2	1	16
Nicaragua	4	2	1	31
Panamá	49	13	3	181
Dominican Republic	25	7	2	143
Venezuela	8	5	1	64

Table 6.2. Host plants of *Bemisia tabaci* collected from South and Central America and the Caribbean.

Country	Host Plants
Argentina	Beans and soybeans
Colombia	Cotton, eggplant, brussel sprunts, snap bean melon, cucumber, watermelon, sorghum, tobacco, tomatoes, cassava and squash
Costa Rica	Chile, *Calopogonium*, *Eclipla alba*, beans, melon, cucumber and watermelon.
Ecuador	Eggplant, pumpkin, beans, weeds, melon, potato, watermelon, soybean, cassava, tomato.
USA	Melon
El Salvador	Gourd, chile, *Euphorbia*, beans, okra, cucumber, watermelon, *Physallis*, tomato, cabbage, loroco, pipian
Guatemala	Eggplant, chile, *Euphorbia*, beans, melon, okra, cucumber, watermelon, tobacco, tomato
Honduras	Chile, *Euphorbia*, beans, cucumber, tomato
Mexico	Eggplant and beans
Nicaragua	Beans and tomato
Panamá	Red pepper, melon, watermelon, tomato and squash
Dominican Republic	Eggplant, beans, melon, okra, cucumber, cassava and tomato
Venezuela	Pumpkin, melon, potato, cucumber and tomato

Activity 7. Biology of *Aleurotrachelus socialis*

There is conflicting information in the literature on some aspects of *A. socialis* biology. This is especially true for female ovipositional rates which are reported at 116 eggs per female throughout its 12 day adult duration.

A. socialis eruptions and extremely high populations, observed in recent years at CIAT and other localities, indicate that ovipositional rates may be higher than reported.

A small experiment was designed to try to measure more accurately female *A. socialis* oviposition. A 9x 1.5cm (diameter x height) plastic petri dish, with a perforated lid (to prevent moisture build up, which can cause adult whitefly mortality) was filled with agar to within 2mm of the top. Upon cooling, a cassava leaf portion (Var. MCol 1468) was placed on the agar. A pair of recently emerged whitefly adults was introduced into each petri dish, in the laboratory (25±2°C & 70±5%RH). Three hundred replicates of each treatment were evaluated for oviposition for 30 days.

Results

Females oviposited for a maximum of 18 days. Previous report indicated a 12-day adult duration. Maximum and minimum ovipositional rates varied considerably indicated by the high standard duration (**Table 7.1**). Accumulated oviposition reached a high of 244 eggs, an average of 181 and a minimum of 155 (**Figure 7.1**). These results show that *A. socialis* oviposition is higher than previously recorded (116 eggs per female) and partially accounts for the rapid population builds-up we observed in the field.

Table 7.1. Accumulated ovipositional potential of *A. socialis* females on cassava (Var. MCol 1468) under laboratory conditions.

Day	1	2	3	4	5	7	8	9	10	11	12	13	14	15	16	17	18
Average Oviposition	0.2	11.8	18.9	31.6	67.4	64.5	71.4	121	130	90.33	110.9	100.4	126.3	126.9	124	106.3	181
Maximum Oviposition	1	18	39	66	91	112	125	144	128	145	177	182	184	203	212	147	244
Minimum Oviposition	0	8	9	11	40	29	38	99	72	47	64	74	117	75	96	65	155
S.D	0.4	3.7	10.6	19.9	14.9	34.4	25.9	15.6	21.6	42.2	34.7	31.7	32.2	44	37	33.6	46.3
N	10	9	9	8	9	8	8	10	9	9	11	9	9	10	8	4	4

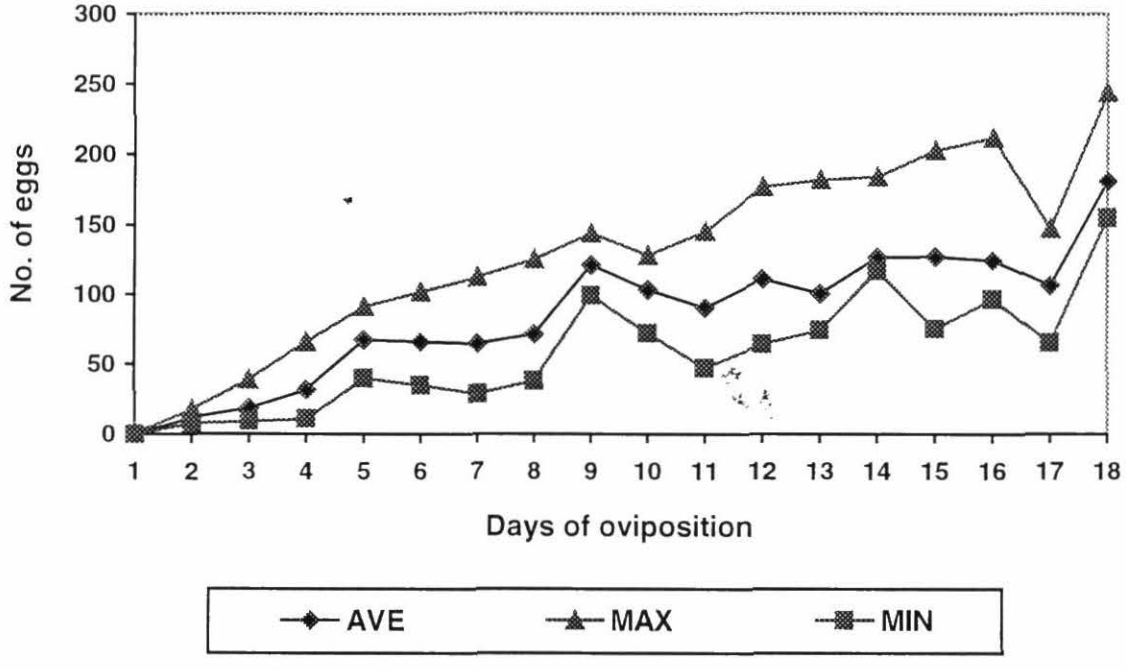


Figure 7.1. Accumulated oviposition of *Aleurotrachelus socialis* females on cassava varieties in the laboratory (26±2°C & 70±5%RH).

Activity 8. Production models for biopesticides

There is considerable potential for the use of biopesticides to replace chemical pesticides in cassava pest management. The effectiveness of the hornworm baculovirus and its successful implementation, especially on large plantations, exemplifies this possible trend. Entomopathogens have now been identified for numerous cassava pests, including mites, mealybugs, whiteflies, hornworms, burrower bugs, white grubs, and others. Further research is required to develop biopesticides and methodologies for their effective implementation.

A bottle neck in this process is the need for a finished, commercial, quality product that is available to cassava producers (as well as other crops) when the need arises. CIAT, as a research and development institution does not have the capacity, nor the role, of producing marketable biopesticides. This requires a link to, and collaboration with the biopesticide industry. Effective biopesticide use will become increasingly important, especially in cassava, where we are seeing an increase in larger plantations, where chemical pesticide use is an economically acceptable option. In addition, past research in farmers fields has shown that cassava yields can be reduced by more than 35% due to pest damage, especially, in seasonally dry areas.

CIAT (Project PE1) has entered into a collaborative agreement with local biopesticide companies to research, develop, evaluate and eventually market biopesticides for control of arthropod pests of cassava and other crops. CIAT's role in these collaborative efforts is primarily in the identification, research and evaluation of entomopathogens, to a limited degree in the development of biopesticides. Marketing aspects are almost solely the role of the private biopesticide companies, although it is planned that a share of profits will be reinvested in the research process.

The overall objective of this project, is to establish research and industrial production models for the development and use of entomopathogens for cassava and other crop pests.

The immediate objectives are twofold:

1. Establish a production model for the cassava hornworm baculovirus and its employment in IPM program with cassava producers **(Figure 8.1).**
2. Research and evaluate entomopathogens for control of pests of cassava and other crops, especially burrower bugs (*C. bergi*) and whiteflies **(Figure 8.2).**

The cassava hornworm, whiteflies and burrower bugs are major pests of cassava, causing considerable crop loss in Colombia (all three pests) and other countries (hornworm and whiteflies in Ecuador, Venezuela, Brazil, and others). The burrower bug, in addition to causing considerable crop damage in Colombia, is also reported as a pest in Venezuela and several countries in Central America (especially Panamá and Costa Rica). *C. bergi* also damages numerous other crops, such as onions, peanut, asparagus, maize, pasture grasses and potatoes. Whiteflies are a major pest of numerous crop, including numerous vegetable species, fruits, cotton, beans and other legumes. Chemical pesticide use is common, and often excessive, for all three pests. It has become increasingly necessary to develop alternatives to chemical pesticides to control these pests. The use of entomopathogens offers a more environmentally compatible alternative, but needs further development.

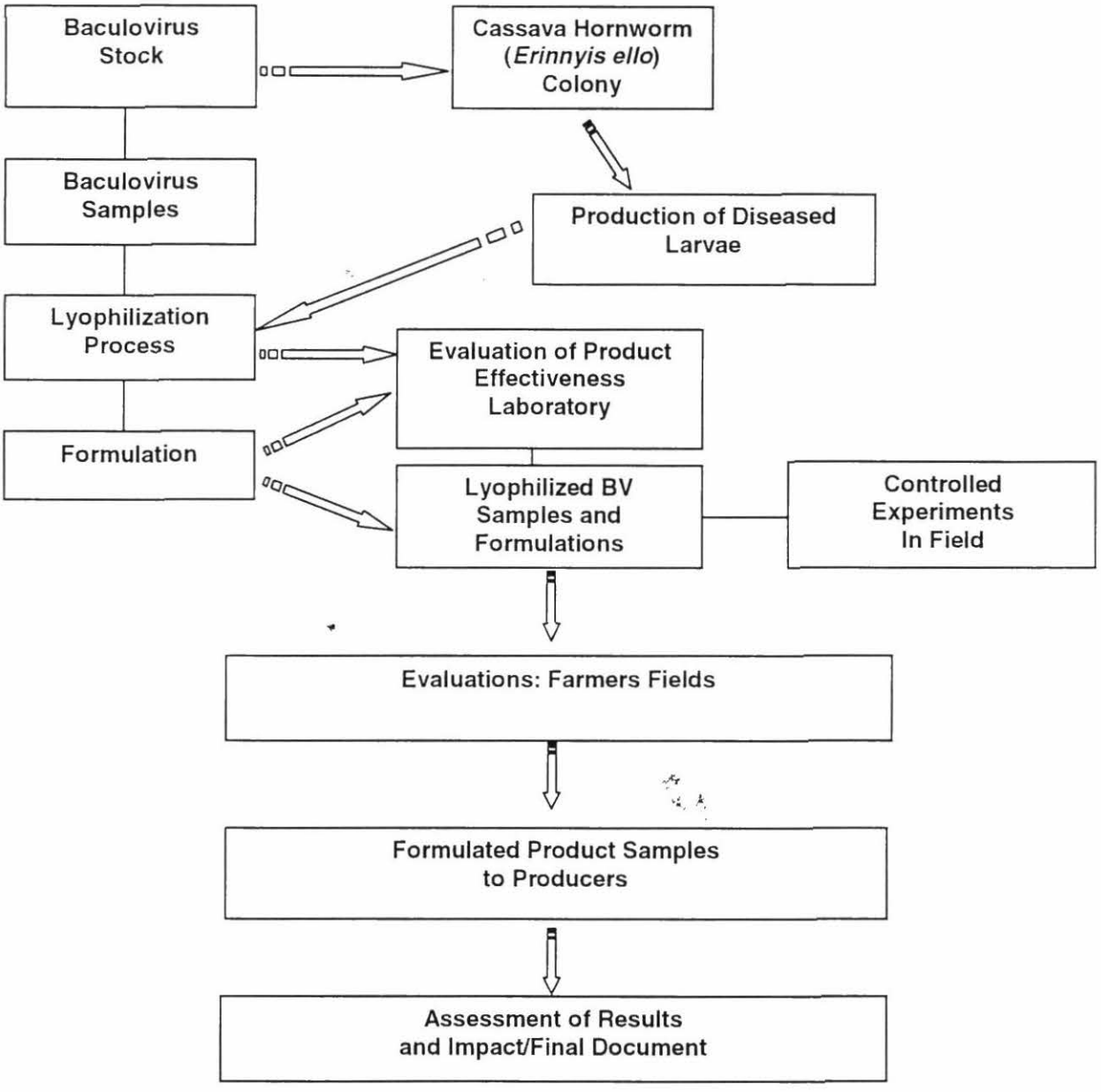


Figure 8.1. Sequential diagram for the commercial production of the cassava hornworm baculovirus.

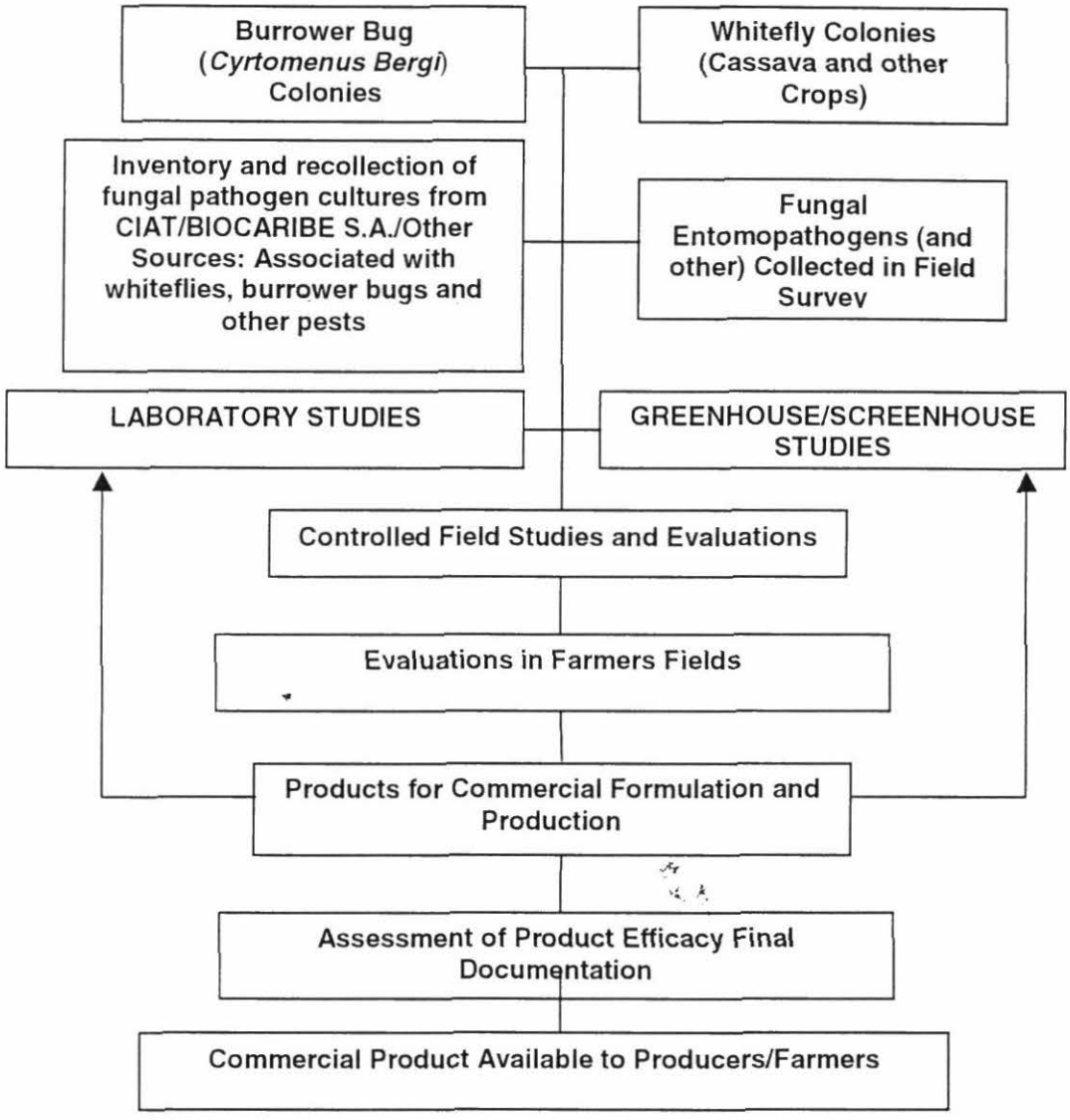


Figure 8.2. Sequential diagram for the development and commercial production of arthropod entomopathogens.

The hornworm baculovirus has proven to be effective in hornworm control and is ready for commercial production. Whitefly and burrower bug entomopathogens have been identified, and in limited cases commercial products exist (especially for whiteflies). However considerable research still needs to be done, including the identification of new or unrecorded pathogens and the evaluation of presently available products.

With the collaboration of BIOCARIBE S.A., a commercial biopesticide company, and considerable support from the Colombian Ministry of Agriculture, a collaborative plan has been developed, at least partially financed and put into operation to research, evaluate, develop and market commercial biopesticides. CIAT's Integrated Pest Management Project (PE-1), in collaboration with CLAYUCA and CIAT Project IP-3 (Cassava Germplasm Development), and BIOCARIBE S.A. are responsible for project execution.

Project Advances: *E. ello* baculovirus

A cassava hornworm colony has been reestablished at CIAT to provide a continual production of hornworm larvae for baculovirus production. A standardized "mother" solution of the baculovirus has been established. This consists of 170 grams of diseased larvae, liquefied in a blender with sterile distilled water, sifted through a fine mesh, and bring the solution up to 400 cc with additional sterile distilled water. This "mother" solution is maintained at 0 - 4°C. Several liters of this solution are now available. The solution is purified, homogenized and lyophilized at the BIOCARIBE S.A. Laboratories in Medellín. Products are presently being prepared for laboratory and field testing.

Project Advances: Arthropod entomopathogens

At this stage emphasis is being given to whiteflies and burrower bugs. Explorations and surveys are being carried out in several localities in Colombia, including Risaralda, Quindío, Caldas, Tolima for entomopathogens associated with *C. bergi*. Whitefly surveys, as described in another section of this report, are being done in several regions of Colombia and Ecuador (and eventually Venezuela). Cassava, as well as other crops are being surveyed for whitefly populations and their natural enemies, including entomopathogens. The objective of these surveys is to determine the presence of native entomopathogens that offer potential for evaluation and development as biopesticides.

Isolates of entomopathogens have already been field collected and laboratory cultured for *C. bergi* and whiteflies. *C. bergi* entomopathogens include *Metarrhizium anisopliae, Metarrhizium sp.* and *Beauveria bassiana, Paecelomyces* sp. and *Verticilium lecanni.* All of the aforementioned entomopathogens were collected from natural field populations of whiteflies and burrower bugs. During the 2000-2001 cycle, these entomopathogens will be laboratory and greenhouse evaluated. The most promising isolates will be further developed for field studies.

In addition, over the years of exploration and research, we have collected numerous isolates of several fungal entomopathogens. Many of these have been maintained in the CIAT IPM laboratory, or have been shared with other institutions such as CORPOICA and CENICAFE. This sharing and exchange of entomopathogens expands the possibility of identifying virulent strains for

potential product development. Seventy isolates of four fungal genera, *Metarrhizium, Beauveria, Verticillium* and *Paecelomyces*, collected from various agroecoregions of Colombia, were purified and added to the CIAT IPM entomopathogen stock collection, managed by the Forages Entomology personnel (see this Annual Report for more details on this collection). These isolates were collected from numerous arthropod hosts, mostly from cassava, and include whiteflies, hornworms, *C. bergi*, stemborers (*Chilomima clarkei*) and white grubs.

Laboratory and field experiments with commercial entomopathogen products for whitefly and *C. bergi* control has already been initiated. A colony of *C. bergi* has been reestablished on maize at CIAT to provide a continual pest supply for laboratory and greenhouse experiments. At present *M. anisopliae* isolates are being evaluated in the greenhouse, for eventual selection for field experiments.

Activity 9. Advances in the search for parasitoids of *Leptinotarsa undecemlineata*, in Valle del Cauca - Colombia.

Introduction

After almost 150 years of attempting to manage the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), it remains the key insect pest in most potato production areas of eastern United States and Canada (Radcliffe et al 1991). Suppression of the Colorado potato beetle (CPB) using insecticides has resulted in a series of ecological and environmental failures. The dependence on insecticide applications has created cycles of resistance, increased applications and environmental contamination. Sustainable CPB management must become less dependent on insecticide treatment to suppress CPB populations. A number of indigenous North American natural enemies exist, however, biotic mortality is insufficient to prevent CPB outbreaks. *Bacillus thuringiensis* (Bt)-based insecticides and genetically altered potato cultivars containing the Bt genes are available. However, the beetle's rapid development of resistance to a number of insecticides classes, suggests that Bt-potato cultivars will also select for resistant CPB populations.

The presumed center of origin for the genus *Leptinotarsa* ranges from northern South America to Mexico (Hsiao 1988, Jacques 1988). Previous explorations for natural enemies in Colombia have been relatively short term and have not concentrated on higher elevations (Ben Puttler, University of Missouri, formerly USDA-ARS, personal communication). Known natural enemies of *Leptinotarsa* spp. in Central and South America include an egg parasitoid, larval-pupal dipteran parasitoids, and several insect and mite predators. No larval hymenopteran parasitoid has been described. The egg parasitoid, *Edovum puttleri*, does not overwinter in the U.S.

We propose to search for natural enemies of *Leptinotarsa* spp. in mountainous areas of northern and western South America at elevations greater that 1500m, areas that are the origins of domesticated potatoes. Based upon previous collections in these regions (Puttler and Long 1983) we are confident that these areas contain undiscovered species of natural enemies or populations of known natural enemies (e.g. *E. puttleri*) that may be better adapted to North American climates. These studies are part of a collaborative agreement with Iowa St. University, Ames, Iowa, USA.

The objectives of this project are to:

1. Use GIS to determine areas of climatic similarity between North American distributions of *Leptinotarsa decemlineata* and South American climatic zones.
2. Determine the percentage of parasitism of *E. puttleri* though field collections using the information from Objective 1.
3. Determine the identity, geographic, and seasonal distribution of natural enemies of *Leptinotarsa* spp. in northern and western South America at elevations > 1500 m though field collections using the information from Objective 1.
4. Determine the activity at temperatures < 15°C of the egg parasitoid, *E. puttleri* collected from populations at elevations > 1500 m.

Methodology

Search for *L. undecemlineata*

"Turkeyberry" or "friegaplatos" is a weed fed upon by *Leptinotarsa* spp. in Colombia. In May 1999, plant samples were collected from Palmira, Colombia near a drainage ditch. One sample was sent to the Botanical Garden of New York for identification by Mike Nee. A second sample was placed in the herbarium of the International Center of Tropical Agriculture (CIAT), Palmira. This plant was identified as *Solanum torvum*. The Botanical Garden provided additional information on the distribution of this plant species in Colombia, including department, city and altitude of previous collections.

Peter Jones (GIS, CIAT) summarized the altitude of *S. torvum* collection sites in combination with a mapping program that uses both the monthly average temperature and the monthly mean of the daily temperature range, data on precipitation/month and day/night temperatures, to create distribution maps of this plant in Central and South America. Two groups of *S. torvum* that differ in altitude have been found, one group having the climate of Palmira and the other group having the climate of the department of Antioquia. A cladogram of the distribution of these two groups has been completed.

On November 1999 the search for *L. undecemlineata* in Colombia was begun according to the data provided by the GIS analysis plus local knowledge of CIAT workers.

Although turkeyberry and other solanaceas such as *Solanum nigrum* (Yerbamora), *S. pseudolulo* and *S. jaimaicense* were found in Bolo, 2 km from the urban perimeter of Palmira, *L. undecemlineata* was only found on *S. torvum*. Some individuals of both sexes were collected in trays with turkeyberry branches and were taken to the Entomology laboratory at CIAT to initiate rearing.

Field - collected adults were kept on branches of turkeyberry inserted in vials with water and covered with parafilm. These were placed in a plastic tray inside a cabinet at 25 °C, 70% RH and 12:12 LD. The branches were changed two or three times a week, depending on their vigor.

Because the females could not lay eggs on the branches, possibly because of the space, five methodologies were evaluated for obtaining eggs.

The methodology that gave the best success was when plants from Bolo and San Emigdio (7km from Palmira) were transplanted into pots with their own soil and placed in a glasshouse at CIAT. The different stages of beetles were separated by cages. Although the beetles remained on turkeyberry, the plants turned yellow before the female laid eggs. It was therefore decided to transplant plants to the field. This planting was done monthly in order to have a reserve for colony renewal.

The plants were sown at a distance of 1m from each other, forming little plots of 4 by 4 plants. Fertilizer (15 - 15 - 15, N-P-K) was applied at the time of sowing. When these plants were vigorous and 1 m tall, beetles were released to begin the rearing. Plants were enclosed in a black mucelina cage, 2 m high, 3 m wide and 3 m long. Beetles and cages were rotated to new plant plots as required.

Search for parasitoids of immatures stages of *L. undecemlineata*

In December 1999 egg masses of *L. undecemlineata* were found on the undersurface of turkeyberry leaves in Bolo-Palmira. These egg masses were collected in petri dishes and taken to the laboratory were they were placed in a rearing room kept at 25°C, 70% RH and 12:12 LD, provided by fluorescent light. The parasitoids that emerged were aspirated and transferred to a glass jar and supplied with cotton moistened with both water and honey water.

Rearing of *E. puttleri*

For the rearing of *E. puttleri* egg masses of *L. undecemlineata* from CIAT's field were used. Leaves of *S. torvum* with egg masses 1 -3 days old were cut and taken to the laboratory. The pieces of leaves were attached to a piece of paper and placed on the upper side of a square glass jar kept on its side. Three to four egg masses with 50 -80 eggs per mass, were placed in each jar. Cotton water and cotton honey were placed at the bottom of the jar and changed daily.

Adult parasitoids were collected with a pipette from egg masses of *L. undecemlineata* from Bolo-Palmira. Subsequently, approximately 30 - 50 parasitoids of mixed sex were released inside the glass recipient, and kept there 5 -7 days.

Pieces of paper with egg masses were then transferred to plastic petri dishes for the emergence of the parasitoids. The emerged parasitoids were aspirated with a pipette and released in a new glass recipient. All phases of parasitoid rearing were performed with the same conditions described above.

Incidence of *E. puttleri* parasitism in the field

Knowing that *S. torvum* is found throughout the geographic valley of the Cauca River, the search for these plants were conducted during the rainy season from May to August 2000. The survey included the Panoramic Highway (department Valle del Cauca), including the townships of Yumbo (alt. 1000 m), Vijes (987 m), Yotoco (972 m), Río Frío (969 m), Bolívar (978 m), Roldanillo (965 m), Obando (939 m) and La Union (975 m).

Turkeyberry was found at several sites in these townships. Every plant was sampled and the egg masses of the beetles were transferred to glass vials and labeled with site and plant stratum (high, medium, and low). Eggs from each egg mass were separated with a brush into individual capsules. All eggs from the same mass were kept in a single petri dish. When these eggs eclosed, data were recorded on the number of beetle larvae, number and sex ratio parasitoids and number of nonviable eggs. Sampling was done according to the presence of the beetle in the field. To determine the sex ratio, each wasp was mounted on a slide and examined under the microscope.

The methodology described above injured some eggs so it was decided to keep the egg mass in petri dishes and await the emergence of larvae and parasitoids. Each petri dishes was reviewed daily and the larvae were isolated to avoid egg predation. Subsequently, the parasitoids were transferred to vials and sexed according to morphological characteristics.

Besides of the samples in the north of valley, the coffee zone was also sampled including some sites of the department of Caldas altitude 1550 m, in this site it was found adults beetles only. Sampling was tried in the department of Antioquia but because of security reasons, *L. uncecemlineata* was not found at the latter site.

Results and discussion

- *Solanum torvum* was found in sites such as pastures, ditches and abandoned areas.
- *S. torvum* and *S. jaimaicense* were the only solanaceous hosts found for *L. undecemlineata*.
- *L. undecemlineata* was best reared on *S. torvum* planted in the field. These conditions provided more space for insect and plant root development.
- According to a regional survey *S. torvum* was found from 950 - 1200 m altitude, but *L. undecemlineata* was most common at 950 - 1000 m.
- One egg parasitoid and one possible larval parasitoid (Chalcididae) of *L. undecemlineata* were found. The egg parasitoid was identified like *Edovum puttleri*.
- *E. puttleri* could be reared on egg masses of *L. undecemlineata*, cotton water and cotton honey provided in glass jar. All phases of parasitoid rearing were carried out at 25 °C, 70% RH, with a photoperiod of 12 :12 LD provided by fluorescent light.

According to the statistic analysis the variables that show significant differences were number of masses and percentage of larvae of *L. undecemlineata* according to the regions (North Valle and Palmira) **(Table 9.1)**. The mean number of mass was higher (1.31) in the North Valle than in Palmira (0.33) **(Figure 9.1)**.

The plants sampled in North Valle were from less accessible sites and several meters from roads and therefore protected from dust, an effect that could negatively effect egg emergence. Some egg masses were found covered in grime and no eggs emerged. Also, significant differences were found between strata (high, medium and low); the greatest number of egg masses were found in the highest strata with an average of 3.13 in North Valle **(Table 9.1)**. This may be because the beetle prefers to oviposit on young leaves.

Table 9.1. Descriptive statistics of the variables number of egg masses, eggs per mass, percentage of (larvae and non viable egg) of *Leptinotarsa undecemlineata* and percentage of parasitism of *Edovum puttleri*. In North Valle and Palmira Colombia, from May to August 2000.

		Number of Masses			Eggs per Mass			Percentage of Parasitism		
REGION	STRATA	N	MEAN	STDERR	N	MEAN	STDERR	N	MEAN	STDERR
North	1. High	15	3.13	0.81	13	70.33	8.86	13	48.68	6.21
Valle	2. Medium	15	0.67	0.23	6	56.17	12.87	6	29.98	10.67
	3. Low	15	0.13	0.13	1	100.00		1	22.00	
	ALL	45	1.31	0.34	20	67.56	7.10	20	41.73	5.44
Palmira	STRATA									
	1. High	11	0.64	0.15	7	67.43	18.91	7	47.74	10.10
	2. Medium	11	0.36	0.15	4	78.00	9.63	4	58.94	12.23
	3. Low	11	0.00	0.00	0	.	.	0	.	.
	ALL	33	0.33	0.08	11	71.27	12.21	11	51.81	7.62
ALL		78	0.90	0.20	31	68.88	6.19	31	45.31	4.44

		Percentage of Larvae			Percentage Non Viable Eggs		
REGION	STRATA	N	MEAN	STDERR	N	MEAN	STDERR
North	1. High	13	21.84	5.40	13	29.48	5.90
Valle	2. Medium	6	22.79	5.23	6	47.23	13.34
	3. Low	1	63.50	.	1	14.50	.
	ALL	20	24.21	4.29	20	34.06	5.73
Palmira	STRATA						
	1. High	7	0.64	0.48	7	51.62	9.82
	2. Medium	4	10.95	9.81	4	30.12	9.91
	3. Low	0	.	.	0	.	.
	ALL	11	4.39	3.61	11	43.80	7.63
ALL		31	17.17	3.47	31	37.51	4.58

An additional variable that showed significant differences was number of emerged larvae per region (**Table 9.2**). In North Valle the average larvae emerged was 24.2 and in Palmira 4.4 (**Figure 9.2**). There was a direct relationship between the number of egg masses and number of emerged larvae. Percent parasitism was equal in both regions (**Table 9.2**) with a global value of 45.3% (**Table 9.1**).

There was no significant differences in eggs per egg mass, or percentage of non viable eggs, between regions nor strata. This indicates that oviposition per *L. undecemlineata* female was similar for both regions and averaged 68.9 eggs (**Table 9.1**).

Table 9.2. Analysis of variance of samples of egg masses of *Leptinotarsa undecemlineata* in North Valle and Palmira, Colombia, from May to August 2000.

Source		Egg Masses	Eggs per Mass	% Parasitism	% Larvae	% Non Viable Eggs
Region	1	2.68 **	2.01	0.17	0.62**	0.009
Stratum	2	6.89 **	2.45	0.02	0.14	0.032
Region *Stratum	2	1.24 *	5.98	0.18	0.03	0.28
Error		0.35	4.26	0.07	0.05	0.09

** = Significant at 1% level. * = Significant at 5%.

L. undecemlineata has one principal host, *S. torvum*, a wild plant that, if frequently removed, can cause the search for additional hosts, perhaps of economic importance.

Edovum puttleri was found parasitizing eggs of *L. undecemlineata*, it is easy to rear, and has a total parasitism of 45.3%, which enhances its potential in an IPM program for the Colorado potato beetle *L. decemlineata*.

In addition a microhymenopteram of the Chalcidae family was observed parasitizing latter instars, however it was not possible to rear this parasite. A dipteran larvae parasite (Fam. Tachinidae) is reported from the coffee region. It is recommended that these parasitoids be further evaluated and the search for additional natural enemies continue. The parasitoid *E. puttleri* will be sent to the USDA for quarantine and will consequently be evaluated at different temperature grades (<15°C) to determine its potential in temperature zone winters.

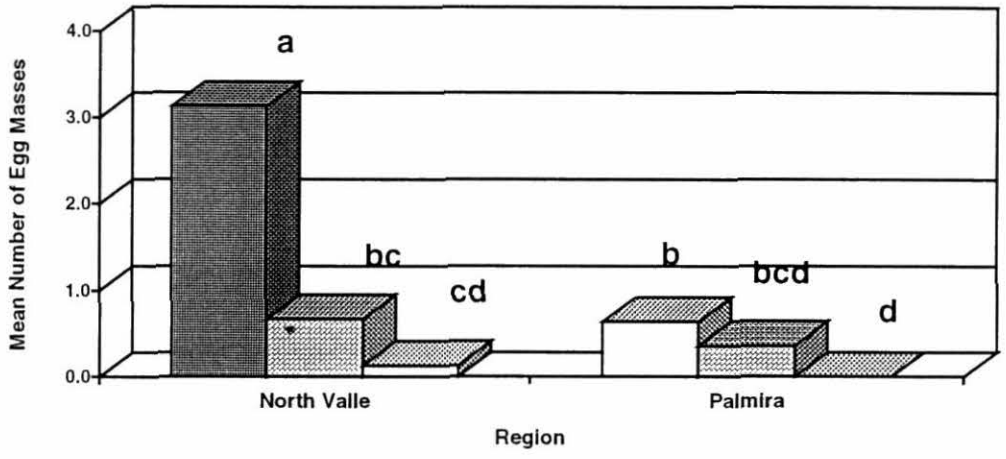


Figure 9.1. **Mean number of egg masses of *L. undecemlineata* in high, medium and low strata leaves in *S. torvum* in North Valle and Palmira.**

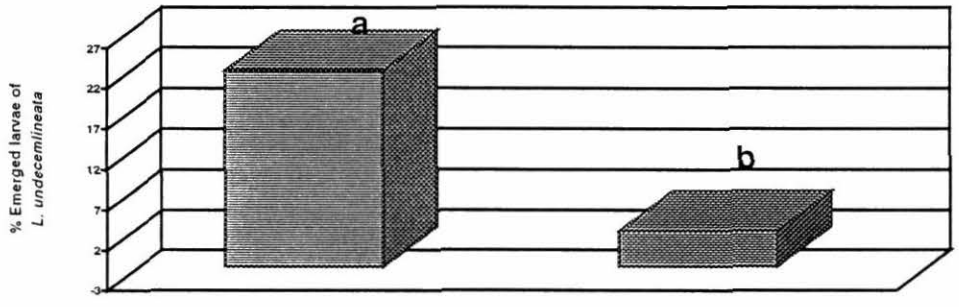


Figure 9.2. **Percentage of emerge larvae of *L. undecemlineata* on *S. torvum* in high, medium and low strata leaves in North Valle and Palmira.**

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Sub-Output 2. Plant Interactions of Cassava Mealybug and Whitefly for Reducing Populations.

Synthesis and identification of a contact kairomone mediating host recognition by a generalist parasitoid *Acerophagus coccois*.

Introduction

In South America, the cassava mealybug *Phenacoccus herreni* Cox & Williams (Homoptera: Pseudococcidae) is an important pest of cassava, *Manihot esculenta* Crantz (Euphorbiaceae), especially during drought, when insect population's increase (Bellotti *et al.*, 1983; Noronha, 1990). To control this mealybug, two encyrtid parasitoids *Acerophagus coccois* Smith and *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae) are being studied at the International Center for Tropical Agriculture (CIAT, its spanish acronym) located in Cali (Colombia). These parasitoids were released in semi-arid areas of the Brazilian states Bahia and Pernambuco in 1994 and 1995 (Smith & Bellotti, 1996; Bertschy, 1998). *A. coccois* is a generalist on different mealybug species whereas *A. vexans* is a specialist on *P. herreni* (CIAT, 1998).

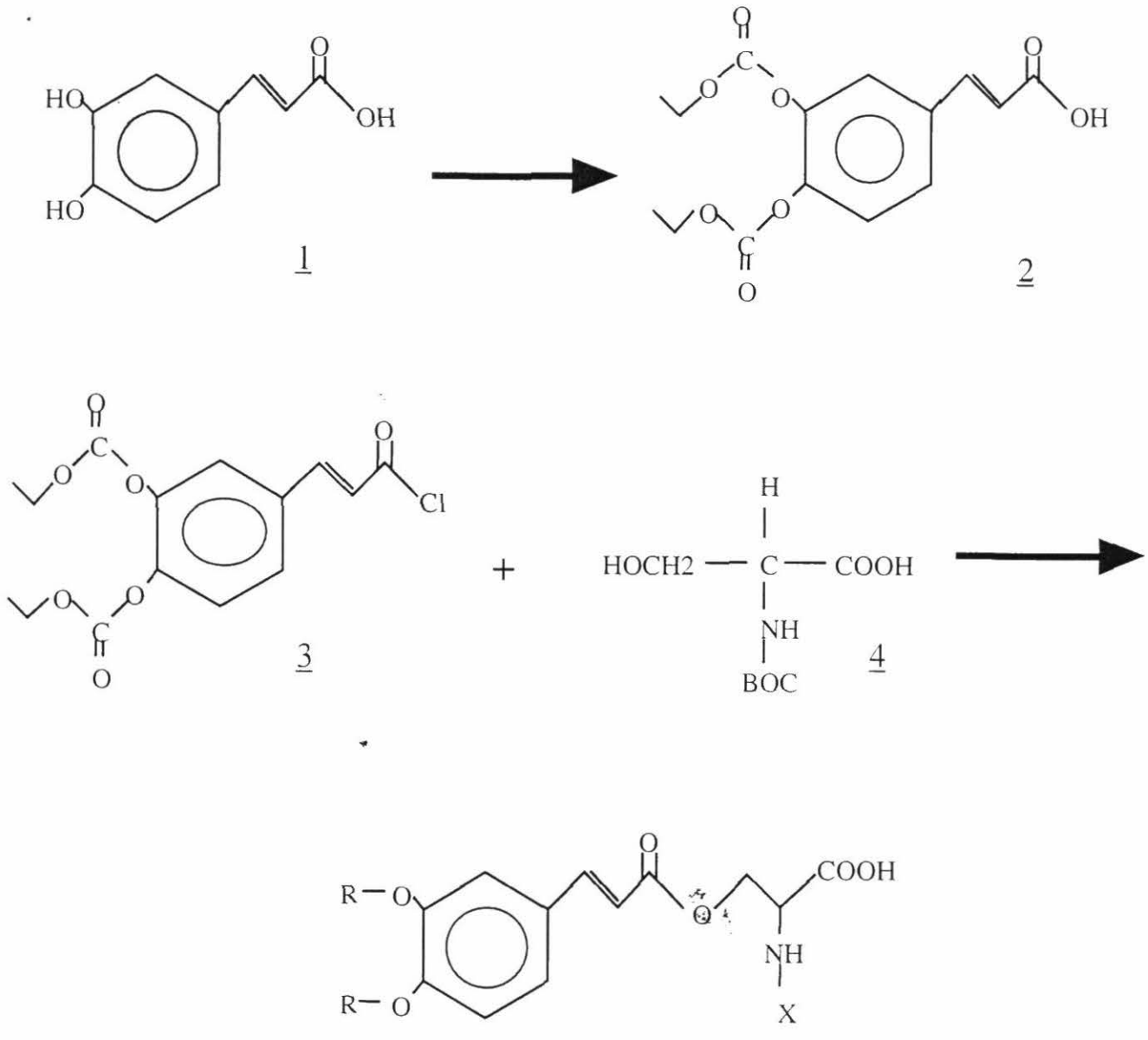
Previous works showed that *P. herreni* present on its surface body a phenolic compound constituted by a serine moiety and a caffeic acid moiety, and could more probably correspond to an O-caffeoylserine. It is not provided directly by the host plant but synthesized by the insect because it is found in artificial diet-reared insects, and it is detected in other mealybug species like *Icerya* sp. (Homoptera: Margarodidae), *Planococcus citri* Risso and *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae) (CIAT, 1999).

A caffeic acid ester was previously found in a Homoptera. O-caffeoyltyrosine, the ester of caffeic acid and tyrosine, was identified in the California red scale *Aonidiella aurantii* Maskell and mediates host recognition by the parasitic wasp *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae)(Hemiptera: Diaspididae)(Millar and Hare, 1993; Hare *et al.*, 1993).
Our objective in the present study was to confirm the preliminary detection of the O-caffeoylserine in *P. herreni* and to probe its influence on the host recognition behavior of *A. vexans* and *A. coccois*.

Activity 1. Synthesis and identification of the O-caffeoylserine from *P. herreni*

To confirm the structure of the putative O-caffeoylserine detected previously (CIAT, 1999), our objective was to synthesize this compound and verify by two chromatographic methods if the retention times were identical to those of the previous peak suspected as an O-caffeoylserine.

The methodology used to synthesize the O-caffeoylserine was that described by Millar & Hare (1993) (**Figure 1.1.**).



5: R = OCOOEt, X = BOC

6: R = OCOOEt, X = H

7: R = H, X = H

Figure 1.1. Synthesis scheme of the O-caffeoylserine.

The caffeic acid (1) was mixed with ethyl chloroformate to produce the 3,4-Diethoxycarbonyl-caffeic acid (2). The acid (2) was stirred with thionyl chloride to produce the 3,4-Diethoxycarbonyl-caffeoyl chloride (3). Then, the compound (3) was coupled with the N-t-BOC-(L)-serine to produce the compound (5). After stirring the compound (5) with a mixture of methylene chloride and trifluoroacetic acid, the dicarbonate was deposite to a TLC plate with a migration solvent of n-butanol-water-AcOH (4:1:1) to complete the reaction and obtain the O-caffeoylserine (7). The proton NMR (CD3OD) was readily interpreted as follows: 1H NMR (CD3OD): δ 7.43 (d, 1H, J=15.9 Hz, olefinic H), 6.78-7.06 (3H, m aromatic), 6.29 (d, 1H, J=15.8 Hz, olefinic H), 5.09 (br s, 1H), 4.10 (m, 1H), 3.79 (q, J=11.1 Hz, 2H). This compound was already synthesized and its NMR data given by Lin *et al.* (1999).

When the synthesized O-caffeoylserine was analysed by two chromatographic methods, we observed that this compound have a retention time of 66.2 min by autoanalysis of amino acid and 18.92 min by HPLC. These retention times corresponded exactly to those of the peak detected previously in the mealybug extracts and confirmed the identification of the O-caffeoylserine in *P. herreni*.

Activity 2. Biological study of the O-caffeoylserine

The synthetic and natural O-caffeoylserine were bioassayed. Bioassays were developed based upon the characteristic "druming and turning" behavior of wasps which characterized host recognition (Caïcedo A.M. & Calatayud P.-A., pers. obs.). This behavioral bout correspond to the fact that the female wasps investigate mealybugs or mealybug mimics by palpation with their antennae as they walk from one edge of the mealybug to the other. They then turn ≈90° and repeat this behavior one or more time. Bioassays were carried out using measured quantities of extracts and test chemicals applied to a cotton ball 2 mm in diameter. One treated ball was placed in a glass vial, and the solvent was allowed to evaporate. A female wasp, reared on *P. herreni*, was placed in the vial and the vial was stopped with cotton plug. Then, the wasp behavior was observed under dissecting microscope (6 x) for 15 min. For each experiment, the number of female wasp showing drums and turns were recorded.

For both parasitoid species, the extract of *P. herreni* surface in 70% ethanol was more attractive than the one in hexane, but no oviposition probe was observed (**Table 2.1.**). Moreover, this kind of ethanolic extract showed similar attractions to the natural host. No attraction was noted with the solvent alone, suggesting that the mediation of host recognition was really due to compound(s) present(s) in the ethanolic extract of *P. herreni* surface. Among the concentration of O-caffeoylserine tested, the range of concentrations between 3.75 pmol/µl to 0.03 nmol/µl was significantly more attractive to *A. coccois*. Furthermore, the female wasps of *A. coccois* were less attracted to the hydrolysis products of the O-caffeoylserine (resulting from the synthesis reaction) such as caffeic acid and serine and to the other compounds detected in *P. herreni* surface such as free amino acids than the synthetic O-caffeoylserine at different concentrations, confirming that the O-caffeoylserine mediates host recognition by *A. coccois*. Moreover, for this parasitoid species, the ester could also induce oviposition because among the wasp showing drums and turns at 0.03 nmol/µl of O-caffeoylserine, 24 % of them probed the cotton-ball with their ovipositors (data not shown). No oviposition probe was observed with the other concentrations of O-caffeoylserine tested. For *A. vexans*, whereas the synthetic O-caffeoylserine at 0.03 nmol/µl elicited more drums

and turns, no significant difference was obtained among all different concentrations of O-caffeoylserine tested. Furthermore, no significant difference in the percentage of wasps showing drums and turns was obtained between the synthetic O-caffeoylserine regardless of the concentration tested and the hydrolysis product of the ester (serine + caffeic acid). No attractive effect was noted with the other nitrogenous compounds analyzed in *P. herreni* surface such as free amino acids.
From all theses results, the biological activity of the O-caffeoylserine was more clearly evidenced with *A. coccois* than with *A. vexans*. This suggest that the O-caffeoylserine could be more implied in the medation of host recognition with the generalist parasitoid species *A. coccois* than with the specialist one *A. vexans*.

Table 2.1. Number of wasp females showing drums and turns (in %[a], n=25) according to the experiment.

Experiment	*A. coccois*	*A. vexans*
Natural host (*P. herreni*)	48bc	88c
Hexane	0a	0a
70% ethanol	0a	4a
Extract of P. herreni surface in hexane	8a	12ab
Extract of P. herreni surface in 70% ethanol	48bc	88c
0.3 nmol/µl of O-caffeoylserine in 70% ethanol	4a	16ab
0.03 nmol/µl of O-caffeoylserine in 70% ethanol	80d	40b
0.015 nmol/µl of O-caffeoylserine in 70% ethanol	80d	28b
3.75 pmol/µl of O-caffeoylserine in 70% ethanol	64cd	24b
0.03 nmol/µl of free serine and free caffeic acid in 70% ethanol	16a	12ab
Free amino acids in 70% ethanol[b]	20ab	0a

[a] % followed by different letters are significant differents according to a Chi-square test (column comparison),
[b] free amino acids analysed in the surface body of adult female of *P. herreni*: Asp (0.03 nmol/µl), Thr (0.01 nmol/µl), Asn (0.01 nmol/µl), Glu (0.08 nmol/µl), Gln (0.01 nmol/µl), Pro (0.03 nmol/µl), Gly (0.04 nmol/µl), Ala (0.14 nmol/µl), Val (0.02 nmol/µl), Met (0.01 nmol/µl), Ile (0.01 nmol/µl), Leu (0.01 nmol/µl), Tyr (0.02 nmol/µl), Phe (5 pmol/µl), Try (0.02 nmol/µl), Orn (5 pmol/µl), Lys (5 pmol/µl), His (0.01 nmole/µl), Arg (0.01 nmol/µl) in 70% ethanol.

Investigation of the pattern of occurrence and concentration of O-caffeoylserine and related compounds among hosts and nonhosts of *A. vexans* and *A. coccois*, may provide a better understanding of the process and evolution of host recognition by parasitoids species and suggest how such a process may be manipulated to improve the effectiveness of Hymenopteran parasitoids for biological control of pest insects.

Determine alternative mealybug and whitefly control methods other than biological control

Introduction

At present, proteinase inhibitors, lectins and α amylase inhibitors are the three classes of proteins considered to have the potential to function as chemical defensive factors in plant against attacking insect. Expression of insecticidal genes from the bacteria *Bacillus thuringiensis,* (Bt) in transgenic plants is now a proven technology in cotton and maize. Genetic transformation of cassava with Bt genes has been identified as desirable for protection against stemborers in cassava. It is well known that the success of toxic proteins used in plant protection depends on the nutritional mode of the

targeted insect. For Coleopterans and Lepidopterans species, which feed from crushed plant materials, the use of Bt, enzyme inhibitors and lectins are successful. To control Homopterous insects such as whiteflies, mealybugs and aphids, which feed directly on phloem sap, mainly lectins and Bt δ endototoxins are shown to be efficient.

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.

Activity 3. Screening of digestive enzymes in the gut of *Phenacoccus herreni* and *Aleurotrachelus socialis*

In order to identify other kind of enzyme inhibitors than proteinase or α amylase inhibitors more suitable to control mealybugs or whiteflies, the screening of digestive enzymes in the gut of *P. herreni* and *A. socialis* is first necessary. Fast semi-quantitative analysis of enzymatic activities present in different mealybug and whitefly gut tissues was performed using the API system (Bio-Mérieux, Marcy l'Etoile, FRA). Activities were detected by cleavage of a chromogenic substance (naphthyl derivatives) dispersed dry on a porous plastic microcup (≈ 100 µl). Enzymatic reactions were stopped by an SDS-based acid Tris buffer (Zym A) and visualised by a Fast blue BB solution (Zym B). The level of activity was determined by comparing the intensity of colour produced, with the colour scale provided with the kit (0-5, from ≤ 5 to ≥ 40 nmoles of substrate released). The kit used was the API-ZYM set for screening the presence of 19 different common enzymes.

Comparing enzyme activities found in digestive tracts to those found in the body after having extract the digestive tract, we may mention that the main activities exhibited by whole digestive tracts were alkaline phosphatase, esterase (C 4) and leucine aminopeptidase for *P. herreni*, and leucine aminopeptidase and ß glucosidase for *A. socialis* (**Figure 3.1.**). For the other enzyme activities revealed by API-ZYM system, it is difficult to affirm that they provided mainly from the digestive tract tissues because they exhibited higher or similar activities than tissues from the rest of the body. The confirmation of the main enzyme activities exhibited by whole digestive tracts of *P. herreni* and *A. socialis* is being investigated by electrophoresis methods.

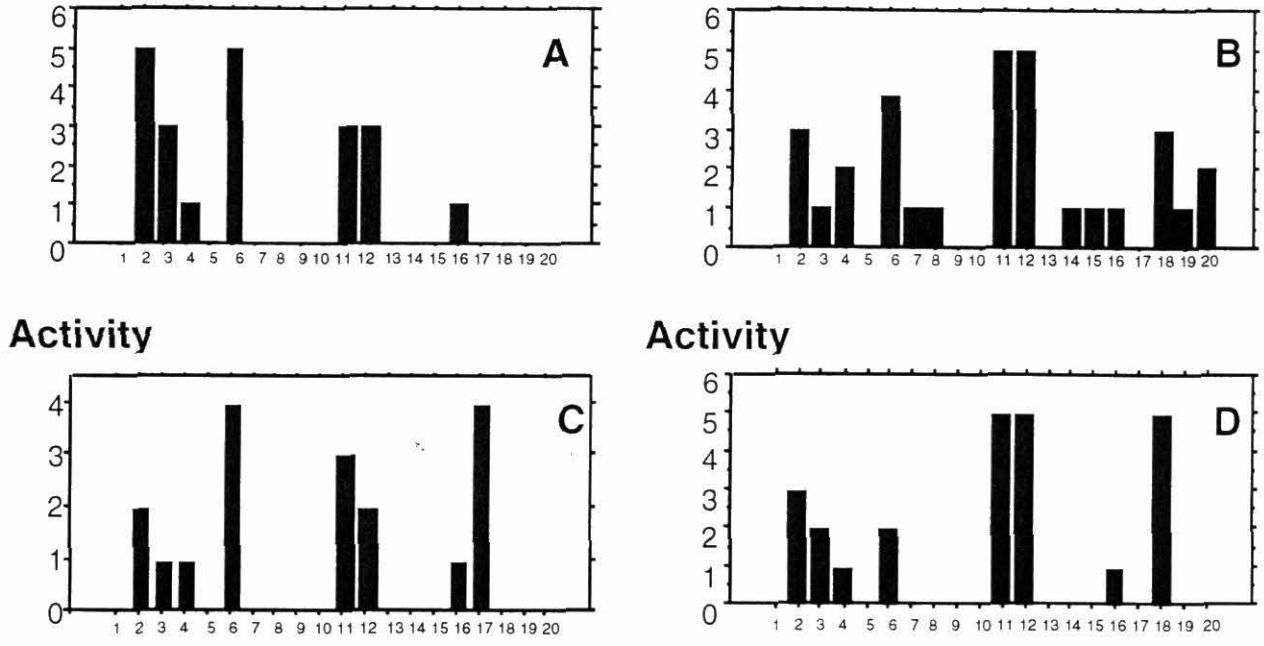


Figure 3.1. **Semi-quantitative analysis of enzyme activities of digestive tracts (A and C) and the rest of the body (B and D) from *P. herreni* (A and B) and *A. socialis*(C and D). All activities correspond to the section of gut or body after having removed the digestive tract as indicated (2 individual insects / substrate); ratings correspond to the liberation of 5, 10, 20, 30, or ≥ 40 nmoles of substrate per 4 h of incubation at 37°C (rates 1, 2, 3, 4 or 5 respectively, rate 0 = activity no detected).**

Enzymes tested: 1 control, 2 alkaline phosphatase, 3 esterase (C 4), 4 esterase lipase (C 8), 5 lipase (C 14), 6 leucine arylamidase, 7 valine arylamidase, 8 cystine arylamidase, 9 trypsin, 10 chymotrypsin, 11 acid phosphatase, 12 naphtol-AS-BI-phosphohydrolase, 13 α galactosidase, 14 β galactosidase, 15 β glucuronidase, 16 α glucosidase, 17 β glucosidase, 18 N-acetyl-β glucosaminidase, 19 α mannosidase, 20 α fucosidase.

Activity 4. Characterization of toxic proteins from the bacterial symbionts from entomophagous nematodes

Bacterial symbionts of entomophagous nematodes are members of the family Enterobacteriaceae and belong to the genera *Xenorhabdus* and *Photorhabdus*. It is well known that these bacteria live in a mutualistic association with nematodes and are released from the gut of the nematode upon invasion of the insect hemocoel by the nematodes.

The bacteria multiply and kill the host within 24 to 48 hours, and the nematodes feed on both the bacteria and the insect cadaver.

These bacteria can be cultivated away from the host and produce crystalline inclusion proteins, antifungal and bacterial compounds and secreted compounds with insecticidal toxicity characteristics directly into the growth medium.

These secreted compounds are active against a wide range of insects from different orders (Lepidoptera, Coleoptera and Dictyoptera) and are as patentable as the delta-endotoxins of Bt and therefore may provide useful alternatives to the deployment of Bt toxins in transgenic plants.
In 1992, it was found that native nematodes are naturally associated with *Cyrtomenus bergi* in three different locations of Colombia (Caïcedo, 1993). These nematodes were originally identified as three strains of *Heterorhabditis bacteriophora*, but their identification is now being revised by another taxonomist and could be related to new species.

Therefore assuming that the association symbiotic-nematode is specific, the possibility to identify new bacteria species from the Colombian native nematodes mentioned above is probable and the identification of new insecticide protein(s) from these bacteria is possible.

Activity 5. Molecular-based approach to the differentiation of mealybug (Homoptera: Pseudococcidae)

Phenacoccus manihoti, a species of mealybug causing severe distortion to cassava in Africa, was described by Matile-Ferrero (1977). *P. manihoti* has been introduced in the Afrotropical Region from the Neotropical Region in the 1970s. In Africa, it is present in Benin, Senegal, Congo, Zaïre, Togo, Gabon, Cameroun... and in South America, it is present only in restricted areas of Paraguay, Brazil and Bolivia. Another species of mealybug *Phenacoccus herreni*, causing distortion to cassava in South America, was described by Cox & Williams (1981). *P. herreni* is widely distributed in South America (Bolivia, Brazil, Colombia, French Guiana, Grenada, Guyana, Tobago, Venezuela), but it is not present in Africa.

These two mealybug species, specific to *Manihot* species, are morphologically similar and it is difficult to separate them because of the wide variation of morphological characters in both species. The consistent characters that distinguish them is that *P. manihoti* is pink and reproduces by thelytokous parthenogenesis and that *P. herreni* is yellow and bisexual.

The question arises as to whether or not *P. manihoti* is different to *P. herreni*?

In order to answer to this question, a Randomly Amplified Polymorphism Analysis (RAPD)-based approach for separating mealybug populations was developed.

The mealybug populations analysed were: *P. manihoti* from Congo and Paraguay, and *P. herreni* from Colombia and Brazil. Another Pseudococcid species *Phenacoccus madeirensis* Green from Colombia, polyphagous and known to be distinct to *P. manihoti* and *P. herreni* was also analyzed.

DNA was extracted from individuals that had been preserved in 70% alcohol and represent each population. RAPD was carried out using two primers, Operon H9 and H16. These had been used to distinguish *Bemisia tabaci* biotypes and whiteflies species.

From RAPD analysis using the primer H9 in Figure 5.1, *P. herreni* from Colombia was similar to *P. herreni* from Brazil, and *P. manihoti* from Congo similar to *P. manihoti* from Paraguay; whereas *P. herreni* was well distinguished to *P. manihoti* regardless of the geographic origin. Furthermore, *P. madeirensis* was very distinct to *P. herreni* and *P. manihoti*. Similar results were obtained with the second primer used, H16. The fact that the first two primer attempted were successful to distinguish the three species is further evidence that *P. herreni* and *P. manihoti* may well be more distantly related than previously thought. The validation of this suggestion is being investigated by mitochondrial 16S genes analysis. This will allow the degree of divergence between the populations to be better quantified.

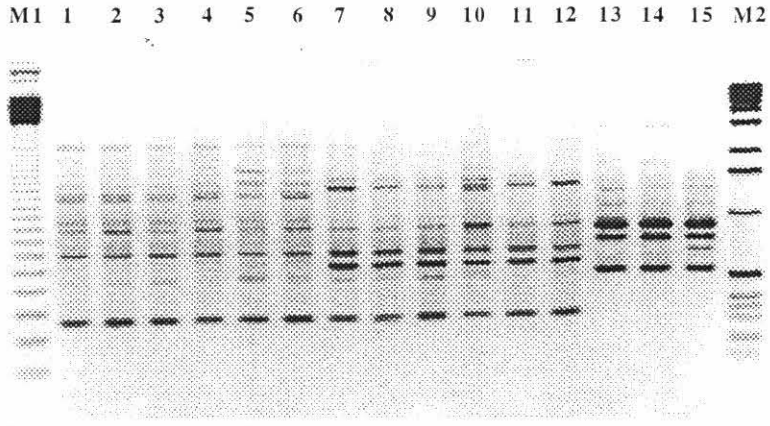


Figure 5.1. The RAPD products from individual mealybugs using the primer Operon H9. In lanes 1-3, *P. herreni* from Colombia; lanes 4-6, *P. herreni* from Brazil; lanes 7-9, *P. manihoti* from Congo; lanes 10-12, *P. manihoti* from Paraguay; lanes 13-15, *P. madeirensis* from Colombia; M1: 123 bp DNA ladder and M2: 1 Kb DNA ladder (BRL).

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

Activity 1. Diurnal activity pattern of *Aenasius vexans* and *Acerophagus coccois* females

Introduction

Most insects show specific daily patterns of behavioural activity and most parasitic hymenoptera are assumed to be day active (Godfray, 1994). High behavioural activity is a prerequisite for active movements of natural enemies to the host's habitat and its host. Locomotion is involved in host location (Welseloh, 1981) hence ultimately fundamental in parasitism efficiency. Further, understanding the behaviour of predators and parasitoids is critical to their successful use in biological control (Luck, 1990).

Aenasius vexans and *Acerophagus coccois* are two encyrtid parasitoids of cassava mealybug, *Phenacoccus herreni,* in Latin America. In order to study behaviours related to host location and make predictions on methods of field release, the diurnal activity pattern of these promising biological control agents was determined. Activity is not clearly defined, thus, for this bioassay, activity is defined as locomotory movement of an individual.

Materials and Methods

Every half hour from 8:00am to 16:30pm parasitoid activity was measured in a Petri dish containing a cassava leaf disc. The number of wasps performing one of the following activities, standing, walking and host handling, was recorded.

Results

Female parasitoids of *A. vexans* and *A. coccois* exhibited different patterns of activity during the observed period. *A. coccois* was constantly active throughout the photoperiod while *A. vexans* showed increasing activity starting around 10.30 and lasting until 16.00. Only 16.3 % of the parasitoids were active (**Figure 1.1**). *A. coccois* females remain equally active throughout the day. In average over the whole day, only 19.1 % of the parasitoids were active (**Figure 1.2**).

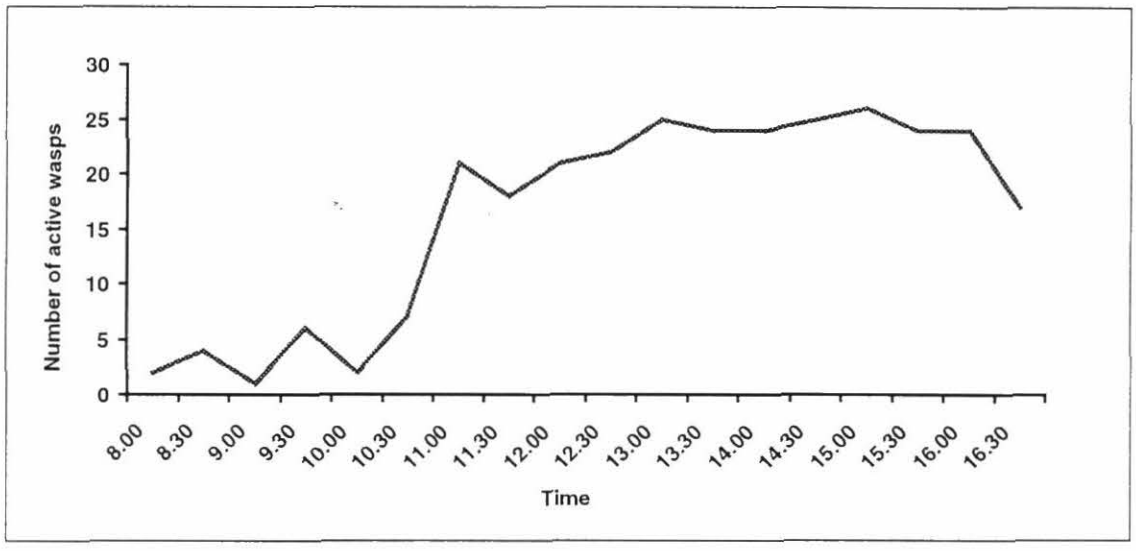

Figure 1.1. Diurnal activity pattern of the encyrtid parasitoid *Aenasius vexans.*

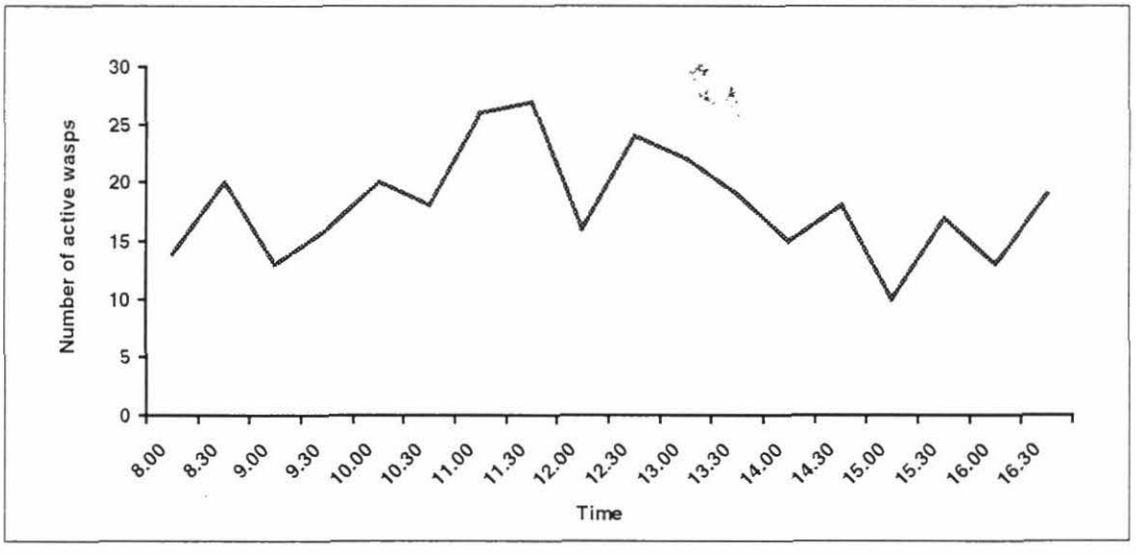

Figure 1.2. Diurnal activity pattern of the encyrtid parasitoid *Acerophagus coccois.*

Discussion

Results show that *A. vexans* females increased host searching activities dramatically 4 hours after the start of the photophase whereas *A. coccois* females remained constantly active thoughout the whole day. From the agricultural point of view, diurnal activity rhythms are an important element in the selection of time of release on natural enemies in biological control and may provide important clues for elucidating the temporal occurrence of biological events in the field, such as oviposition, resting, and other behaviours. For efficient field releases of *A. vexans* and *A. coccois* the hours of late morning may best be suited. This might decrease the wasp's tendency to disperse from the release point without looking for hosts.

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Contributor - Sub-output 3: Entomology

Brigitte Dorn, ETH.

Sub-output 4. Grass and legume genotypes with known reaction to pests and diseases, and to interaction with symbiont organisms are developed

Activity 1. Study the bioecology of spittlebug species in contrasting environments

Comparative biology of *Zulia* spittlebugs

Introduction

An inadequate understanding of the biology and behavior of most spittlebug species, plus a tendency to over generalize in those same aspects among species, has contributed to their ineffective management. Of the 15 species of spittlebugs associated with forage grasses in Colombia, only five have had their biology studied to any degree: *Aeneolamia lepidior*, *A. reducta*, *A. varia*, *Mahanarva* sp. nov., *Prosapia simulans* and *Zulia carbonaria*. To advance our understanding of the patterns of variation among taxa, we are examining the biology of three species in the genus *Zulia*: *Z. carbonaria*, *Z. pubescens* and *Zulia* sp. nov. With the exception of one study on *Z. carbonaria*, this genus has not yet been the focus of any biological or behavioral study and therefore aspects such as characterization and duration of the life stages, reproductive biology and oviposition sites are unknown and unavailable to guide advances in pest management.

Methods

Small-scale colonies were established to ensure availability of all life stages of the insect despite seasonality in the field. Source populations of *Z. carbonaria* and *Z. pubescens* were local in the department of Valle del Cauca while *Zulia* sp. nov. was collected on the Pacific coast in Nariño, its only known range in Colombia.

Methods were based on previous biological studies carried out by CIAT emphasizing morphological characterization of the life stages, duration of the life stages and reproductive biology. 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, and both sexes of the adults. Adult specimens were obtained from the field, nymphs were obtained from either the field or colony, and eggs were obtained 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 (**Figure 1.1**). Teneral adults (<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 eclosed first instars (<12 hours old) were placed singly in 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. The mean longevity of each life stage was based on 40 individuals.

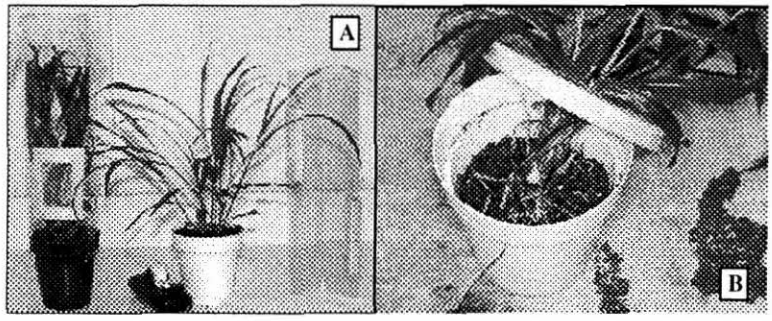

Figure 1.1. Pots employed to determine the longevity of adults (A) and nymphs (B).

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 duration of each of the four generalized developmental stages was based on 100 individuals.

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 **(Figure 1.2).** Each pot was infested with 2 females and 2 males from the colony and once they died eggs were recovered from four oviposition substrates: uncovered soil, soil covered by leaf litter, leaf litter and the plant surface.

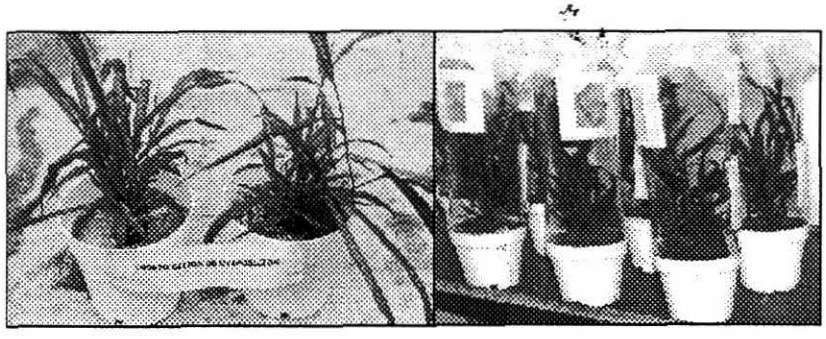

Figure 1.2. Pots for determining oviposition site preferences according to different oviposition substrates.

Results

Male and female *Z. carbonaria* were larger than *Z. pubescens* and *Zulia* sp. nov. in every morphological measure (width of head capsule and body; length of stylet, wing and body with and

without wings). Males of *Zulia* sp. nov. were the smallest life stage. With the exception of only a few measures, females were significantly larger than males for each species (**Table 1.1**).

Each species presented the four generalized developmental stages established for *A. varia* and other spittlebug species. In terms of size, *Zulia* sp. nov. eggs were smaller than the other two species during each developmental stage (**Table 1.2**). In phase S4, within 1-2 days of hatch, eggs of *Z. carbonaria* and *Z. pubescens* were 1.12 times longer than *Zulia* sp. nov. Despite significant differences in size between adult *Z. carbonaria* and *Z. pubescens*, no significant differences were detected in egg size with the exception of width in phase S2.

For *Z. carbonaria* and *Z. pubescens*, there were significant differences from each instar to the next in all parameters measured (width of head capsule, length of body, anterior wing pad and stylet) **(Table 1.3).** There were also significant differences among life stages between the two species for most measures, and these differences increased with instar. *Zulia* sp. nov. nymphs were not available for measurements.

Mean adult longevity was 19.6, 18.4 and 14.1 days, respectively, for *Z. carbonaria*, *Z. pubescens* and *Zulia* sp. nov. Longevity for *Zulia* sp. nov. was significantly less. By gender, longevity varied from 12.9-20.1 days among the three species **(Table 1.4).** No differences in longevity were detected between sexes of the same species.

Total duration of egg development varied from 14.3-17.4 days among the three species (**Table 1.5**), with *Z. carbonaria* > *Zulia* sp. nov. > *Z. pubescens*. Although S2 was the shortest stage for all species, S4 was the longest for *Z. carbonaria*, S3 for *Z. pubescens* and S1 for *Zulia* sp. nov., representing 38.9, 33.0 and 41.9%, respectively, of the total egg development time.

Total duration of nymph development varied from 38.0-42.6 days among the three species (**Table 1.6).** Duration was significantly longer in *Z. pubescens* given statistically longer stadia in instars I – IV. For each species, instar V was longer than other instars, representing 30.6, 33.1 y 30.7% of total nymphal development time for *Z. carbonaria*, *Z. pubescens* and *Zulia* sp. nov., respectively. For *Z. carbonaria* and *Z. pubescens*, there were no differences between instars I, II and III, but duration was incrementally longer in instars IV and V. For *Zulia* sp. nov., there were no differences among instars I – IV.

Table 1.1. Morphological characterization (mm) of *Zulia* adults by sex (mean±S.E., range, n=40).

Species	Sex	Head Capsule Width	Stylet Length	Body Length with Wings	Body Length without Wings	Anterior Wing Length	Body Width
Z. carbonaria	F	2.69±0.12 a	1.23±0.10 a	11.22±0.37 a	10.30±0.79 a	9.11±0.52 a	5.64±0.26 a
		(2.43-1.87)	(0.98-1.50)	(9.21-11.43)	(9.07-12.00)	(8.21-11.50)	(5.29-6.29)
	M	2.40±0.08 b	1.16±0.06 a	10.29±0.52 b	9.36±1.07 b	8.44±0.44 b	4.87±0.26 b
		(2.22-2.67)	(1.07-1.28)	(7.14-10.36)	(7.29-11.93)	(7.50-9.07)	(4.29-5.29)
Z. pubescens	F	2.25±0.10 d	1.03±0.07 c	8.98±0.54 d	8.57±1.03 dc	7.10±0.27 e	4.25±0.24 cd
		(1.96-2.46)	(0.89-1.28)	(7.79-10.64)	(7.07-11.07)	(6.29-7.50)	(4.14-5.36)
	M	2.14±0.06 d	0.97±0.06 d	8.74±0.50 dc	8.01±0.69 ed	7.13±0.34 d	4.41±0.26 d
		(1.99-2.25)	(0.84-1.07)	(8.93-10.71)	(7.00-10.00)	(6.57-8.36)	(4.00-5.36)
Zulia sp. Nov.	F	2.35±0.08 c	1.09±0.06 b	9.72±0.47 c	9.04±0.92 cb	7.75±0.40 c	4.65±0.30 c
		(2.16-2.49)	(0.98-1.24)	(8.93-10.71)	(6.93-10.93)	(7.14-9.21)	(4.00-5.21)
	M	2.08±0.08 f	1.05±0.06 bc	8.65±0.34 e	7.073±1.08 e	7.12±0.29 d	4.04±0.20 e
		(1.99-2.25)	(0.96-1.16)	(8.07-9.57)	(5.93-9.64)	(6.36-7.79)	(3.64-4.36)

Within columns, means followed by different letters are significantly different (P<0.05).

Table 1.2. Width and length (mm) of development stages of *Zulia* eggs (mean±S.E., range, n=68-100).

	S1		S2		S3		S4	
Species	Length	Width	Length	Width	Length	Width	Length	Width
Z. carbonaria	1.06±0.03 a	0.30±0.02 a	1.07±0.03 a	0.32±0.02 a	1.09±0.02a	0.33±0.02 a	1.12±0.03 a	0.38±0.02 a
	(1.00-1.13)	((0.24-0.34)	(0.99-1.13)	(0.27-0.37)	(1.01-1.14)	(0.29-0.39)	(1.06-1.20)	(0.34-0.43)
Z. pubescens	1.05±0.04 a	0.29±0.02 a	1.06±0.03 a	0.31±0.01 b	1.07±0.04a	0.33±0.02 a	1.12±0.04 a	0.37±0.02 a
	(0.97-1.17)	(0.24-0.30)	(1.00-1.17)	(0.27-0.33)	(1.00-1.19)	(0.29-0.36)	(1.00-1.24)	(0.34-0.41)
Zulia sp. nov.	0.95±0.04 b	0.28±0.01 b	0.97±0.03 b	0.29±0.01 c	0.97±0.03 b	0.32±0.02 b	1.00±0.03 b	0.34±0.01 b
	(0.84-1.01)	(0.24-0.31)	(0.86-1.03)	(0.27-0.31)	(0.91-1.04)	(0.29-0.34)	(0.93-1.07)	(0.31-0.36)

Within columns, means followed by different letters are significantly different (P<0.05).

Table 1.3. Morphological characterization (mm) of nymphal life stages of *Zulia* (mean, n=40).

Species	Instar	Head Capsule Width	Body Length	Anterior Wing Pad Length	Stylet Length
Z. carbonaria	I	0.39 a	1.57 a	-	0.32 a
	II	0.62 b	2.54 b	-	0.43 b
	III	0.97 c	4.01c	0.33 a	0.67 c
	IV	1.50 d	6.21 d	0.95 b	1.00 d
	Va	2.18 e	9.81 e	2.80 c	1.40 f
	Vb F	2.24 f	10.62 g	2.93 d	1.45 g
	Vb M	2.21 f	10.10 f	2.85 d	1.37 e
Z. pubescens	I	0.39 a	1.48 a	-	0.32 a
	II	0.58 b	2.89 b	-	0.40 b
	III	0.90 c	3.91 c	0.35 a	0.58 c
	IV	1.34 d	5.66 d	0.86 b	0.83 d
	Va	1.92 e	8.40 e	2.37 c	1.16 f
	Vb F	1.93 e	9.53 f	2.48 d	1.10 e
	Vb M	1.88 f	9.43 f	2.53 d	1.12 f

Within columns for each species, means followed by different letters are significantly different (P<0.05).

Table 1.4. Longevity (days) of *Zulia* adults by sex (mean, n=24-40).

Species	Sex	Longevity
Z. carbonaria	Female	20.4 a
	Male	18.4 a
Z. pubescens	Female	19.4 a
	Male	17.6 ab
Zulia sp. Nov.	Female	14.9 bc
	Male	12.9 bc

Within columns, means followed by different letters are significantly different (P<0.05).

Table 1.5. Duration (days) of *Zulia* eggs by development stage (mean±S.E., range, n=108-126).

Species	S1	S2	S3	S4	Total
Z. carbonaria	5.93±0.70 a (5-8)	1.07±0.25 a (1-2)	3.69±0.53 b (3-5)	6.77±0.54 a (5-8)	17.40±0.91 a (12-20)
Z. pubescens	4.14±0.35 b (4-5)	1.05±0.21 a (1-2)	4.73±0.44 a (4-5)	4.42±0.50 c (4-5)	14.34±0.51 c (13-16)
Zulia sp. nov.	6.11±0.59 a (5-8)	1.10±0.30 a (1-2)	2.92±0.78 c (2-4)	4.69±0.53 b (3-6)	14.57±1.49 b (10-18)

Within columns, means followed by different letters are significantly different (P<0.05).

Table 1.6. Duration (days) of *Zulia* nymphs by instar (mean, n=40).

Species	Instar I	II	III	IV	V	Total
Z. carbonaria	7.48 a	7.20 a	6.38 a	8.33 a	12.98 a	42.35 a
Z. pubescens	6.65 b	6.28 b	5.63 b	7.08 c	12.57 a	37.95 b
Zulia sp. nov.	7.96 a	7.13 a	6.71 a	7.79 b	13.08 a	42.67 a

Within columns, means followed by different letters are significantly different (P<0.05).

Based on these studies, the complete life cycle of *Z. carbonaria*, *Z. pubescens* and *Zulia* sp. nov. was 69.6, 61.5 and 64.4 days, respectively (**Table 1.7**).

Table 1.7. Life cycle summary for three *Zulia* species.

Life stage		Duration (days)		
		Zulia carbonaria	*Zulia pubescens*	*Zulia* sp. nov.
Egg	Sum	17.4	14.3	14.6
	S1	5.9	4.1	6.1
	S2	1.1	1.0	1.1
	S3	3.7	4.7	2.9
	S4	6.8	4.4	4.7
Nymph	Sum	42.4	38.0	42.7
	Instar I	7.5	6.6	8.0
	Instar II	7.2	6.3	7.1
	Instar III	6.4	5.6	6.7
	Instar IV	8.3	7.1	7.8
	Instar V	13.0	12.6	13.1
Adult	Half longevity[1]	9.8	9.2	7.1
	Female	20.4	19.4	14.9
Life cycle		69.6	61.5	64.4

[1] Mean longevity calculated from the Weibull distribution.

Each species demonstrated some degree of flexibility in oviposition preferences by using two or more oviposition substrates (**Figure 1.3**). However, there were marked differences among species in those preferences. Both *Z. carbonaria* and *Zulia* sp. nov. preferred uncovered soil, laying 72.7 and 73.5% of their eggs in that substrate. *Z. pubescens* preferred laying eggs on the plant surface where 59.2% of eggs were recovered.

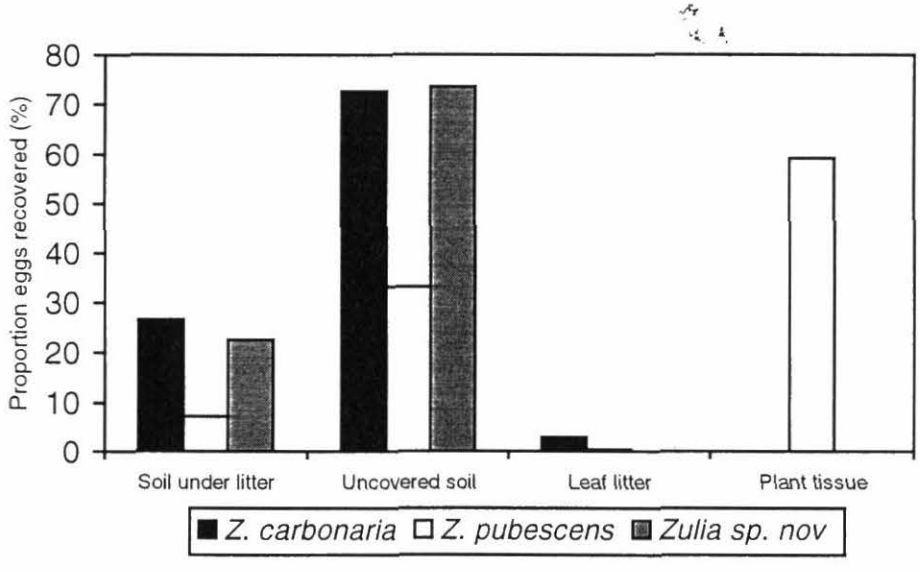

Figure 1.3. Oviposition site preferences determined in choice trials where eggs were recovered from four different substrates.

Discussion

In terms of size, adult spittlebugs from the genus *Zulia* exhibit the same sexual dimorphism as other genera studied to date (*Aeneolamia*, *Mahanarva*, *Prosapia*) in which females are larger than males in most body size measures. Reduction in the stylet length from Instar V to adult in *Z. carbonaria* and *Z. pubescens* is consistent with results from other species (*A. lepidior*, *A. reducta*, *Mahanarva* sp. nov., *Prosapia* sp. nov.). Eggs of all three species passed through the four generalized developmental stages expressed in other species (*A. lepidior*, *A. reducta*, *A. varia*, *Mahanarva* sp. nov., *Prosapia* sp. nov.). In terms of egg size, there appear to be general trends across genera where *Prosapia* = *Mahanarva* > *Zulia* > *Aeneolamia*.

The physical parameters measured for the nymphs demonstrate morphologically distinguishable instars. Like in other species, little overlap in head capsule width among instars makes this character highly diagnostic of instar within species. When supported by other physical characters such as degree of sclerotization and size and form of the wing pads, it is possible to accurately distinguish instars. Early and late instar V (Va, Vb) are distinguished by physical characters other than size, such as visibility of adult structures, similar to other species of spittlebug studied previously.

Adult longevity in *Zulia* is considerable longer than most previously studied species (*A. lepidior*, *A. reducta*, *A. varia*, *Mahanarva* sp. nov.) with the exception of *Prosapia* sp. nov. Mean longevity of adult *Z. carbonaria* was 7-8 days greater than those obtained in a previous study while duration of the egg stage was similar (Arango & Calderón 1981). Nymphal longevity for *Zulia* was greater than that obtained for *Aeneolamia* but similar to *Mahanarva* sp. nov. Unlike *A. lepidior* and *Mahanarva* sp. nov., for these species of *Zulia* instar V was the longest. Overall generation time varied from 61.5-69.6 days among species. The generation time calculated for *Z. carbonaria* (69.6) was similar to that determined in one previous study (72 days)

Consistent with other species, *Zulia* lays eggs in the soil, leaf litter and on the plant stem. Species-specific preferences vary widely, however. Two species prefer soil substrate like the genus *Aeneolamia*, while *Z. pubescens* prefers to lay eggs on the plant as is being demonstrated for *P. simulans* **(see Biology and habits of *Prosapia simulans* - Pag. 70).**

The methodologies established here have proven adequate for gathering biological information on previously unstudied spittlebug species. Continued studies will enable us to assess patterns of variation in spittlebug bioecology to help guide advances in spittlebug management.

Detection of the Central American forage and cane pest, *Prosapia simulans*, in South America

Introduction and Methods

Prosapia simulans is the most widely distributed species of grassland spittlebug, reported in the lowland tropics from Mexico to Panama. It is also a major pest of sugar cane in Central America. To our knowledge, this species and the genus *Prosapia* have never been reported in South America. Herein we report the first field detection of *P. simulans* in Colombia, quantitative measures of field abundance to make a preliminary assessment of population density and

persistence, and an additional record from museum specimens collected in Venezuela. A manuscript has been submitted that includes a summary of the literature on geographic distribution, bionomics and pest status; diagnostic characters to distinguish it from other grassland spittlebugs in northern South America; and a discussion of its possible mode of introduction and pest status potential.

Results

Six populations of *P. simulans* were discovered in the Cauca Valley in 1999-2000. All specimens were identified using characteristics of the male genitalia and compared with type specimens at the Natural History Museum (BMNH), London.

The first report was a single adult female obtained 2-VI-1999 during surveys of *Zulia carbonaria* populations in *Brachiaria dictyoneura* near Santander de Quilichao **(Table 1.8)**. Despite additional surveys in surrounding pastures and sugar cane fields, and weekly surveys in the same site ever since, no more individuals were recovered.

Table 1.8. Populations of *Prosapia simulans* detected in the Cauca Valley, Colombia.

Department	Municipality	Vereda	Elev. (m)	First	Host plants
Cauca	Santander de Quilichao	Santander de Quilichao	1060	2-VI-1999	Brachiaria dictyoneura
Valle del	Calima del Darien	Diamante la	1575	12-VI-2000	Brachiaria decumbens
Valle del	Calima del Darien	La Primavera	1621	12-VI-2000	Axonopus micay
Valle del	Yotoco	Cordobitas	1535	1-II-2000	Brachiaria decumbens
Valle del	El Cerrito	Santa Helena (a)	1155	2-VII-1999	Brachiaria decumbens
Valle del	El Cerrito	Santa Helena	1100	6-VII-2000	Brachiaria decumbens

Four additional populations were discovered subsequently **(Table 1.8)**. Populations at these sites were persistent because *P. simulans* was detected in various visits over several months. Sites varied over a broad elevational range (1060-1620 m) and with the exception of Santander de Quilichao, the dominant forage grass at each site was *Brachiaria decumbens*. The greatest populations detected were in Santa Helena. A survey on 4-IV-2000 estimated densities at 46.8 nymphs/m^2 (n = 10, 0.25m^2 quadrats) and 190 adults/50 sweeps (n = 4 series of 50 sweeps). Although economic thresholds based on quantitative yield loss data have never been established for grassland spittlebugs, these levels are considered highly damaging in Mexico where >30 nymphs/m^2 and >25 adults/50 sweeps are designated as "severe" infestations.

Host plants of *P. simulans* in these sites included *Axonopus micay*, *B. decumbens*, *B. dictyoneura*, *B. ruziziensis*, *Hyparrhenia rufa*, *Cynodon plectostachyus* and *Saccharum officinarum*. A single adult was found feeding on sugar cane in Cordobitas while a single nymph was reported by CENICAÑA on cane in Santa Helena.

An additional record of *P. simulans* in South America was discovered from museum specimens housed at CIAT's insect collection (2 specimens) and the Natural History Museum (7 specimens), London. This material was all collected 30-V-1980 by Gerardo Pérez Nieto from Venezuela, Bolivar State, La Vergareña, in pasture, calculated to be near 6.783°N, 63.559°W **(Figure 1.4)**. No

other South American specimens were found in the collections at Cornell University, the Universidad Nacional at Palmira (Cauca Valley) or the Universidad del Valle (Cauca Valley).

P. simulans can be separated from the other 17 species associated with wild and cultivated graminoids in Colombia and Ecuador by dorsal color pattern: dark brown to black with one transverse band across the center of the pronotum and two across the tegmen **(Figure 1.4).** As the only known member of the genus in South America, *P. simulans* is also distinguished by the genus definition of Fennah (1949, 1953) and supporting male genitalia characters discussed by Hamilton (1977).

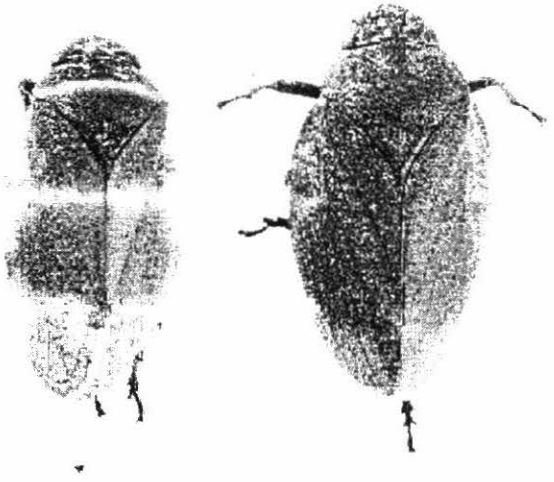

Figure 1.4. ***Prosapia simulans*, Central American spittlebug newly reported from Colombia (Cauca Valley) and South America. Male (left) and female.**

In Mexico and Central America, there is significant variation in the color and form of the transverse bands, ranging from yellow to pink/red to orange, broad to narrow, and distinct to completely obscured particularly in females. The Colombian populations displayed a particular subset of this color and pattern variation. Of 18 males examined, all had narrow pale yellow tegminal bands with some reduction of the posterior band. The color of the venter was predominantly pink (55%), but some individuals were yellowish brown (28%) or intermediate (17%). Background tegmen color was usually brown (94%) but sometimes black (6%). Unlike *P. simulans* from Costa Rica, males from the Colombian populations had black subgenital plates with black patches on the lateral sides of the abdominal sternites. Of 10 females examined, all had both tegminal bands greatly reduced to barely evident on a black background. Female venters were black with red (90%) to yellowish brown (10%) markings.

Discussion

The populations of *P. simulans* detected in *B. decumbens* are persistent, at economically damaging levels, and cover a broad elevational range (1000-1600 m). Wider surveys should be carried out to identify the distribution of *P. simulans* and monitor its spread in pastures and cane plantations of

the Cauca Valley. At the spittlebug densities detected in this study, milk and beef cattle production will be negatively affected and the persistence of improved *B. decumbens* pastures will be compromised. Spread or introduction of this species to lowland regions of Colombia such as the Caribbean coast or the extensive eastern Llanos could have severe economic implications.

Up to now, *P. simulans* has not been reported on sugar cane beyond the observations noted above. Nevertheless, because the evidence suggests that *P. simulans* was introduced, cane producers should consider this species a potential threat. The menace may be heightened now as management shifts from preharvest burning to green production by the year 2005; elimination of burning may increase the susceptibility of sugar cane to this new insect pest. In Central America, this species is an injurious pest of cane in Honduras and Nicaragua. Up to now, the Cauca Valley of Colombia has been distinguished for the lack of spittlebug pests whereas essentially all other cane-producing regions of Central and South America have experienced major spittlebug pest problems.

Although the occurrence of *P. simulans* in Colombia and Venezuela could be attributed to low endemic populations only recently detected, we believe this is unlikely because *P. simulans* is an aposematic and economically important pest species. Furthermore, the Cauca Valley and Venezuela have been under relatively high surveillance over the last 20 years due to CIAT's activities and extensive fieldwork on grassland spittlebugs conducted in the 1950's across Venezuela. Our proposition is that *P. simulans* has been slowly invading from its known southern range in Panama and has advanced into the Cauca Valley from the Pacific Coast. Unfortunately, there are no known collections of cercopids from the remote areas of Chocó and the Darien to test this mode of introduction. Human-mediated introduction is one explanation for the Venezuelan reports. *Prosapia simulans* lays a majority of eggs on the plant stem (**see Detection of the Central American forage and cane pest, *Prosapia simulans,* in South America - Pag. 67**), therefore arrival with infested vegetative material is a possibility .

Finally, these observations highlight the need for care in transfer of vegetative and soil materials associated with cercopid host plants. There is some other anecdotal evidence for regional introductions of grassland spittlebugs such as *Z. carbonaria* from the Cauca Valley into the Colombian Amazon, and an isolated report of the Central Brazil species *Notozulia entreriana* in the Colombian Llanos **(see Identity and distribution of spittlebugs associated with graminoids of Colombia and Ecuador - Pag. 85).** One well-documented case is *Lepyronia coleoptrata* (Homoptera: Aphrophoridae), a Palearctic spittlebug with immigrant status in the United States (Hoebeke & Hamilton 1983). With the increasing movement of vegetative material throughout the Caribbean Basin and northward insect range expansion due to warming trends, sugar cane and forage grass production in the southern United States, like the Cauca Valley, would be threatened by the arrival of new spittlebug pests. The southeast United States already suffers from the native *Prosapia bicincta*, a damaging pest of forage grass, turf grass and ornamentals.

Biology and habits of *Prosapia simulans*

Introduction

The Central American forage and cane pest, *Prosapia simulans*, was recently reported for the first time in Colombia and South America **(see Detection of the Central American forage and cane**

pest, *Prosapia simulans*, in South America - Pag. 67). It is urgent to carry out a preliminary diagnosis of this pest in the Cauca Valley because several persistent populations over a broad elevational range (1100-1621 m) have been detected, some at economically damaging levels in pastures of *Brachiaria decumbens*. Moreover, this species poses a threat to sugar cane production especially with the future prohibition of burning (2005). This change in cultural practice is known to affect the status of insect pests in cane, and in Brazil it is known to have promoted previously unimportant spittlebug species to high pest status.

A literature review has shown that there is little known about the biology and ecology of this species despite being one of the most widely distributed spittlebugs in America, occurring from Mexico to Panama. To gather biological information relevant to this species, and relevant to the conditions in Cauca Valley of Colombia, we launched studies on its bioecology.

Methods

A small colony of *P. simulans* was established to provide insects for study and overcome seasonality of field populations. Descriptive studies on biology were carried out according to previously established methodologies **(see Comparative biology of *Zulia* spittlebugs - Pag. 61)** focusing on three aspects: morphological characterization of the life stages, duration of the life stages and reproductive biology. The following is a summary of results obtained to date.

Results

For adults, mean lengths of six morphological measures were greater in females than males with the exception of the posterior wing **(Table 1.9)**. For eggs, mean length and width increased with development phase **(Table 1.10)**

Table 1.9. Morphological characterization (mm) of *P. simulans* adults by sex (mean±S.E., range, n=40).

Sex	Head Capsule Width	Stylet Length	Body Length with Wings	Body Length without Wings	Anterior Wing Length	Body Width
F	2.31±0.06 a	0.98±0.07 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)
M	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)

Within columns, means followed by different letters are significantly different (P<0.05).

Table 1.10. 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)

Mean longevity of adults (n=80) was 16.5 days. No statistical difference was detected between males (15.3 days, n=40) and females (17.9 days, n=40).

The duration of egg development differed between two collection sites, Santa Helena (El Cerrito, 1155 m elev.) and Cordobitas (Yotoco, 1535 m elev.). These two sites represented low and high elevation zones of the Cauca Valley. Mean egg development time in the lowland site was 50.5 days, significantly longer than the upland site with 18.0 days (**Table 1.11**). Eggs from the lowland site had an extended S1 and S2 stage, representing 71.5 and 19.0% of the total development time, versus 38.4 and 11.8% in the highland site. An extended S2 phase is evidence for egg diapause, however such an extended S1 stage has not been documented in grassland spittlebug before. Both stages may relate to an egg quiescence associated with unfavorable dry conditions.

Table 1.11. Duration (days) of *P. simulans* eggs by development stage and locality (mean±S.E., range, n=16-66).

	Development stage				
Locality	S1	S2	S3	S4	Total
Lowland: Santa Helena	36.14±4.52 (33-50)	9.60±7.30 (2-28)	3.63±1.15 (1-5)	5.57±1.02 (3-7)	50.53±3.58 a (45-57)
Upland: Cordobitas	6.90±1.09 (6-13)	2.13±1.69 (1-9)	3.98±0.77 (2-5)	5.18±0.58 (4-7)	17.99±1.27 (16-23) b

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

During development, *P. simulans* eggs lacked certain externally visible features in particular development stages, namely appearance of a red pigment spot in S2 and red eye and abdominal spots in S3. Pigment spots were not visible until S4. For this reason it was hard to distinguish eggs of S1 and S2 (S2 is distinguished from S3 by a rupture in the chorion) and therefore an extended phase may have been erroneously attributed to S1.

Mean development time for nymphs was 45.6 days (**Table 1.12**). Instar V was the longest, representing 28.8% of total development time, followed by instars II and IV, and then instars I and II.

Table 1.12. Duration of *Zulia* nymphs by instar (days) (n=40-55).

	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).

From these studies, the life cycle of *P. simulans* is approximately 72.5 days (18.0+45.6+8.9, egg+nymph+1/2 adult).

Prosapia simulans laid a majority, 82.6%, of eggs on the host plant stem, particularly the lower third. No eggs were recovered from the leaf litter while 3.6 and 13.8% were recovered from bare soil and soil under leaf litter, respectively.

Discussion

The methodology used here proved effective in rapidly assessing the biology of this previously unknown species. In common with most other grassland spittlebug studied to date, *P. simulans* is

sexually dimorphic with adult females larger than males, instars can be reliably distinguished with certain morphological measures, and eggs increase in size with development. Observations on an extended S2 stage is evidence for egg diapause, common in most species.

Total life cycle of 72.5 days is longer than that reported from two Central American studies (58.4 and 58.0 days) and is comparable to *Z. carbonaria*, a species encountered in many of the same field sites in Colombia. The strong preference (82.6%) for oviposition on the plant surface is distinct from other Colombian species studied to date (*A. lepidior*, *A. reducta*, *A. varia*, *Mahanarva* sp. nov., *Z. carbonaria*, *Zulia* sp. nov.), with the exception of *Z. pubescens*, also found in the same field sites in Colombia, that lays 59.2% of eggs on the same substrate **(see Comparative biology of *Zulia* spittlebugs - Pag. 61).**

Characterization of substrate communication in adult spittlebugs

Introduction

Substrate communication is a well-known mate recognition behavior in leafhoppers, planthoppers and treehoppers that has also demonstrated taxonomic utility for species differentiation. Until recently, this form of communication has not been examined in the froghoppers, or adult spittlebugs, and thereby represents a poorly understood yet fundamental aspect of behavior important to our basic understanding of this pest group. In 1998 and 1999 we developed recording and analysis methodologies to describe substrate communication for the first time in two spittlebug species, *Aeneolamia varia* and *Zulia carbonaria*. Structure of the male courtship calls were significantly different between these two taxa. To further characterize substrate communication in this insect family and gauge differences among species, male courtship calls were described in three new Colombian species: *Prosapia simulans*, *Zulia pubescens* and *Zulia* sp. nov.

Methods

All recordings were done with adult males obtained directly from the field or from small-scale colonies established at CIAT to support other biological studies. Only individual males were used in order to avoid disruption from responding females or interfering males. For recordings, males were placed on a stem of a preferred host plant with three leaves. This arrangement gave adults sufficient space and opportunity to feed and walk. *P. simulans* was offered *Brachiaria decumbens* as a host plant, *Z. carbonaria B. ruziziensis* and *Zulia* sp. nov. *B. mutica*.

Recordings were captured according to previously established methodologies. A ceramic crystal phonograph cartridge in contact with the plant stem converted vibrations into electrical signals followed by amplification and storage in a computer. Data were analyzed with software specialized for processing and analyzing sound files (CoolEdit 2000, Syntrillium Software Corporation). The following physical parameters were measured: call frequency, call duration, pulse duration and frequency of pulse repetition (FPR). Pulse was defined as the minimal unit of repetition within the call. If multiple calls were obtained for the same individual, these parameters were averaged to give an individual mean. Individual males were considered the units of repetition.

Results

A total of 11 recordings from 7 individuals was obtained for *P. simulans*, 1-2 calls per individual. Mean call duration was 5.41 sec. with a frequency of 456.22 Hz. Pulses were simple, with duration 141.40 msec. and frequency of pulse repetition (FPR) 13.36 pulses/sec. **(Figure 1.5).**

A total of 19 recordings was obtained from 10 individuals of *Z. pubescens*, 1-3 calls per individual. Mean call duration was 21.61 sec., much longer than any of the four other species studied to date. In certain occasions calls consisted of three well-defined phrases **(Figure 1.6).** Mean call frequency was 376.10 Hz with pulse duration 147.44 msec. and FPR 4.98 pulses/sec. Unlike other species, pulses were not simple; they consisted of one large subpulse followed by 3 small subpulses.

A total of 32 recordings from 9 individuals was obtained for *Zulia* sp. nov., 1-14 calls per individual. Mean call duration was 5.97 sec. with a frequency of 419.94 Hz. Pulses were simple, with duration of 63.90 msec. and FPR 8.12 pulses/sec. **(Figure 1.7)**

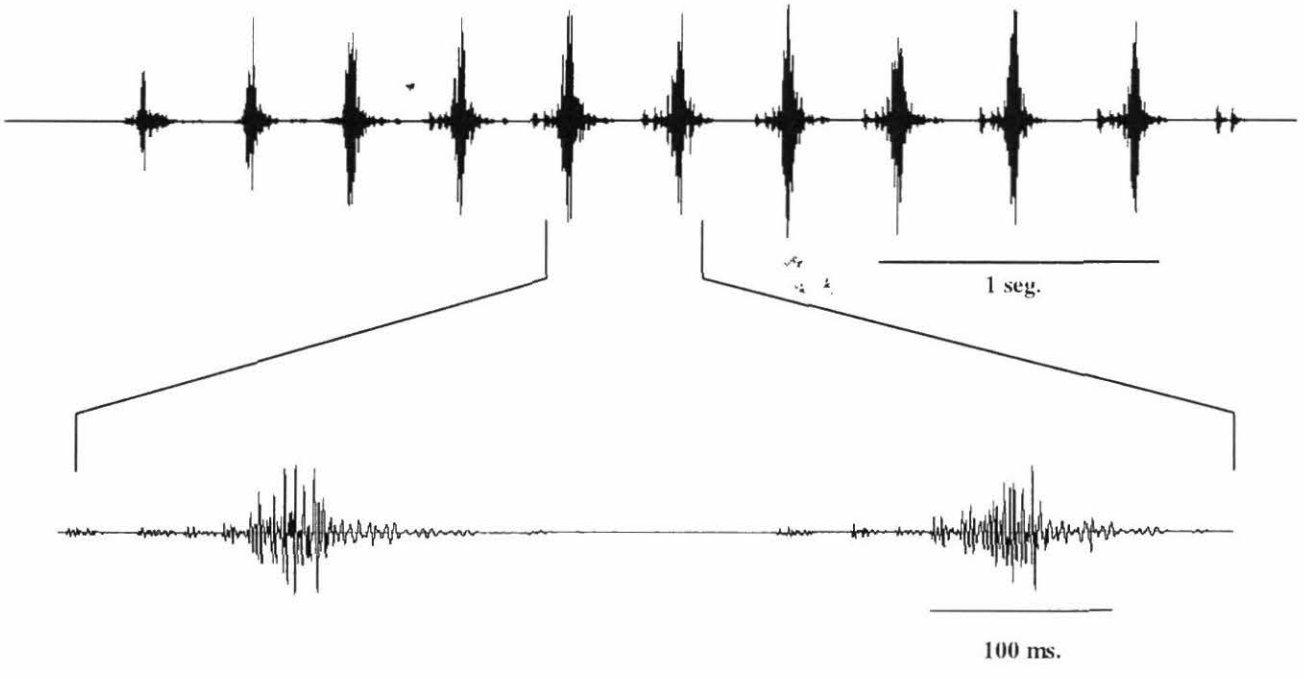

Figure 1. 5. Courtship call of male *P. simulans* (above) with details of two pulses (below).

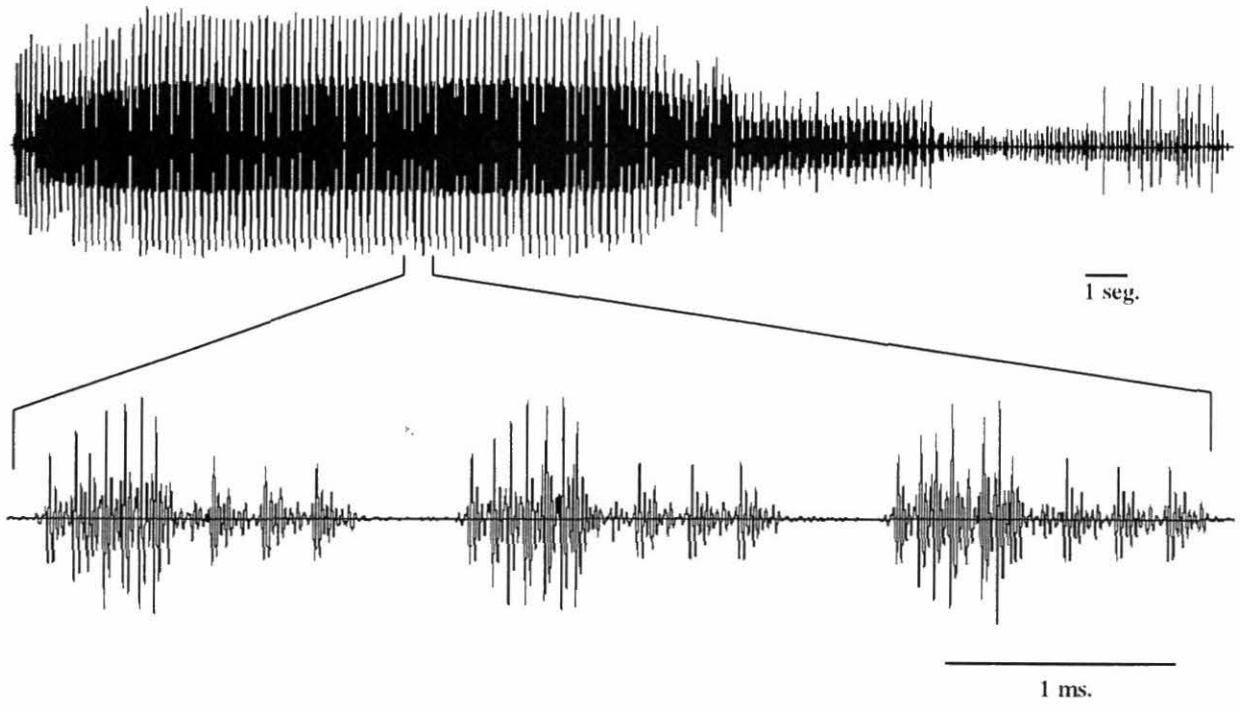

Figure 1.6. Courtship call of male *Z. pubescens* (above) with details of three pulses (below).

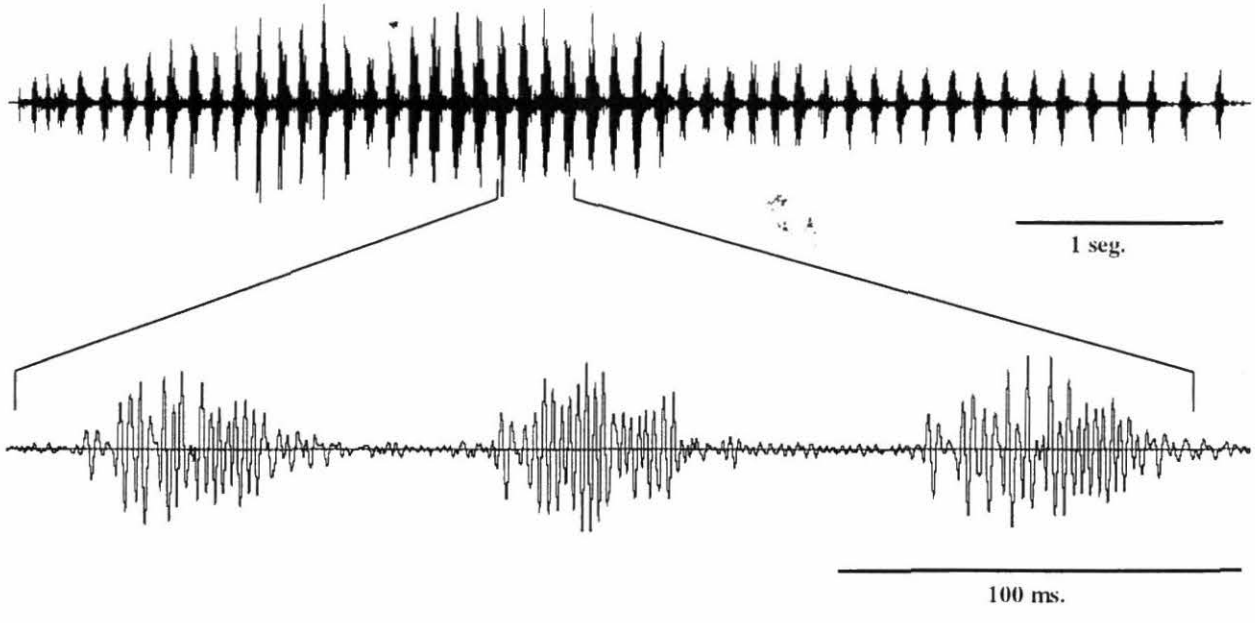

Figure 1.7. Courtship call of male *Zulia* sp. nov. (above) with details of three pulses (below).

There were differences among these three species in terms of all four parameters of call structure (**Table 1.13**). FRP was different for all three while call frequency, call duration, and pulse duration each had an indistinguishable species pair.

Table 1.13. Summary (mean±S.E., range) of male courtship call structure in 5 spittlebug species (data for *Z. carbonaria* and *A. varia* obtained from CIAT 1999).

Parameter	*A. varia*	*Z. Carbonaria*	*P. simulans*	*Z. pubescens*	*Zulia* sp. nov.
Call duration	3.43±0.08	9.50±0.59	5.41±0.41 b	21.61±1.95 a	5.97±0.38 b
(sec.)	(3.35-3.51)	(8.91-10.09)	(5.00-5.82)	(19.66-23.56)	(5.59-6.35)
Call frequency	425.2±37.3	317.4±23.2	456.2±24.7 bc	376.1±19.4 a	419.9±18.8 ab
(Hz)	(387.9-462.4)	(293.9-340.9)	(431.56-480.91)	(359.2-393.0)	(401.1-438.8)
Pulse duration	22.43	57.16	141.7±8.0 b	147.4± 4.2 b	63.90±1.30 a
(msec.)	-	-	(133.72-149.58)	(143.05-151.39)	(62.60-65.20)
FPR	42.16±0.56	10.50±0.37	13.36±0.90 c	4.98±0.13a	8.12±0.25 b
(pulses/sec.)	(41.16-42.72)	(10.13-10.87)	(12.46-14.26)	(4.85-5.11)	(7.87-8.37)
N	5		7	10	8

For each parameter, means followed by different letters are significantly different (P<0.05); *A. varia* and *Z. carbonaria* were not included in the statistical analysis.

Discussion

Among the five species evaluated to date, the call structure of male courtship calls vary significantly in terms of all parameters measured. Whether these parameters vary with genus as well will depend on obtaining results from more species. In Colombia three species from each of three genera (*Aeneolamia, Mahanarva, Zulia*) are available for study to gather more information on the behavioral relevance of substrate communication. Additional studies will allow us to further characterize the behavior in the family Cercopidae, assess its utility as a taxonomic tool, and offer more characters to suggest patterns of relatedness among species.

Population dynamics and phenology of spittlebugs in the Cauca Valley

Introduction

Spittlebug pest problems are apparently increasing in the hillsides and interandean regions of Colombia such as the Cauca Valley. This area has a bimodal precipitation pattern, thereby representing an environment for studying spittlebug seasonality that is distinct from previously studied lowland sites of the highly seasonal Caribbean coast, intermediate seasonal Orinoquia Piedmont, and the continuously humid Amazonian Piedmont. Moreover, the spittlebug complex has not yet been studied in the Cauca Valley. In this report we summarize second-year results from detailed population surveys of the spittlebug complex in the Cauca Valley.

Methods

Methods were the same as those used in previous population studies. The study farm (Las Palmas) was situated in Santander de Quilichao, Cauca, where surveys were initiated in January 1999 in pastures of *Brachiaria dictyoneura* associated with the legume *Centrosema* sp. Three 0.5 ha plots were established in separate pastures and divided into four subplots 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. Each nymph was determined to instar, each adult was

determined to sex and species, and all natural enemies were identified. Surveys were performed weekly. The following results are for the period January to May 2000.

Results

With the exception of one female, *Prosapia simulans*, **(see Detection of the Central American forage and cane pest, *Prosapia simulans*, in South America - Pag. 67)**, all adults collected in 1999 and 2000 were *Zulia carbonaria*. A total of 1062 nymphs and 550 adults were sampled over the period January-May. Total abundance of nymphs and adults varied 6.0 and 2.3 times, respectively between the plot of lowest (Plot 2) and highest (Plot 1) abundance.

Population fluctuation curves showed peaks that were less synchronous than 1999 **(Figures 1.8, 1.9).** Regardless, since all nymphs were determined to instar, it was possible to interpret population peaks as discrete generations based on recruitment from one life stage to the next. Although there was overlap in generations, an initial assessment was made of cumulative insect-days to calculate when each generation of nymphs and adults had accumulated 50% abundance **(Table 1.14)**. Mean time between subsequent generations varied from 29.2-55.8 across the three plots. At the farm level (summed plots) generation time was calculated as 47.4 days. These calculations do not correspond well with generation time estimates (69.6 days) determined from screenhouse studies on the life cycle of *Z. carbonaria* **(see Comparative biology of *Zulia* spittlebugs - Pag. 61).** This incongruence supports the idea that the documented population peaks represent overlapping generations that did not give rise to the generation immediately following.

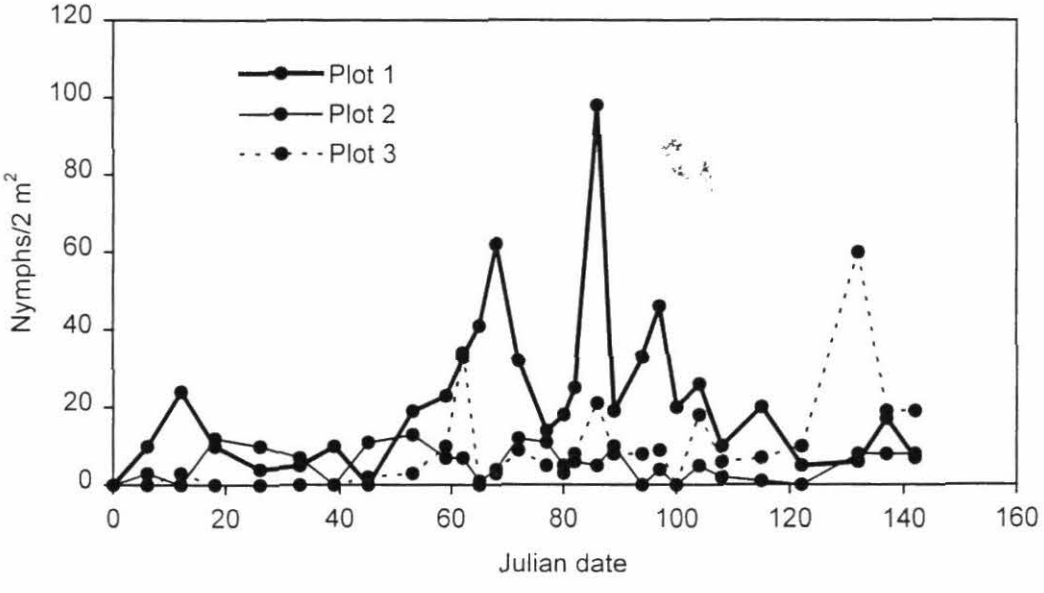

Figure 1.8. Fluctuation curves of *Z. carbonaria* nymphs in three plots of *B. dictyoneura*, Cauca, January-May, 2000.

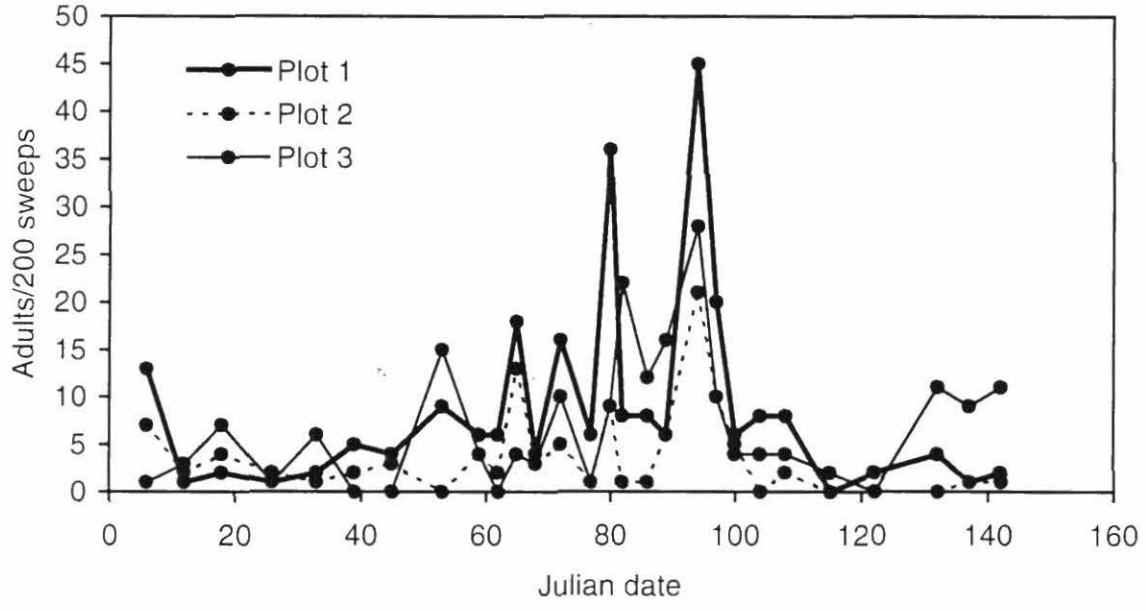

Figure 1.9. Fluctuation curves of *Z. carbonaria* adults in three plots of *B. dictyoneura*, Cauca, January-May, 2000.

Table 1.14. Population phenology of *Z. carbonaria* in Cauca expressed in terms of julian date of accumulation of 50% abundance for sequential generations.

Life Stage	Generation	Plot 1	Plot 2	Plot 3	Summed Plots
Nymph	1	15.6	23.5	-	26.6
Adult	1	34.9	21.1	-	39.3
Nymph	2	66.0	54.7	60.3	63.9
Adult	2	73.3	67.8	56.8	69.5
Nymph	3	89.9	77.4	84.6	90.3
Adult	3	95.0	88.1	90.9	94.3
Mean generation time[1]		55.8	49.7	29.2	47.4

[1] Mean of time between subsequent nymph generations and adult generations (n=2 or 4)

The incidence of natural enemies was low compared to 1999. From January-May 2000 only 6 larvae of *Salpingogaster nigra* (Diptera: Syrphidae, predaceous on spittlebug nymphs) were sampled in spittle mass surveys, and 6 parasitic mites (Acari: Erythraeidae) were found on sampled adults. In 1999, a total of 74 larvae, 40 pupae and 9 adult *S. nigra* were sampled, as well as 79 parasitic mites.

Discussion

Future analyses will examine the correlation between population phenology and certain climatic variables such as rainfall. This site will be included in a comparative study along with Meta and Sucre to assess how well arrival of the early wet season generation can be predicted based on

rainfall patterns at the end of the dry season **(see Documentation of first generation population phenology in two lowland sites - Pag. 79)**. The apparent overlap of generations documented in early 2000 indicates a weaker seasonality of *Z. carbonaria* populations than expected. This may be caused by lack of a severe dry season that is expected to promote a synchronous population development upon return of the wet seasons rains. Alternatively, the diapause syndrome of *Z. carbonaria* may be distinct from other species studied to date and thereby lead to a weak correspondence between environmental seasonality and population phenology.

Documentation of first generation population phenology in two lowland sites

Introduction

In regions with seasonal precipitation, spittlebug management may depend on suppression of the initial outbreaks before the adults colonize new areas or lay eggs that contribute to future generations. Effectively targeting control tactics therefore requires that we predict when and where the early season foci occur on the farm level. Given the correspondence between spittlebug abundance and the favorable conditions of the wet season, it may be possible to predict the arrival of the first generation of nymphs based on the precipitation patterns of the early wet season together with information on the determinants of diapause termination.

Methods

We documented the early season population dynamics of the spittlebug complex in three contrasting sites: the Caribbean coast (Corozal, Sucre), Orinoquia Piedmont (La Libertad, Meta), and the Interandean Region (Santander de Quilichao, Cauca). The data obtained from 2000 and 2001 will complement previous results from 1997 and 1998 giving repetitions over four seasonal cycles. The survey methodology in 2000 was identical to that of other population studies **(see Population dynamics and phenology of spittlebugs in the Cauca Valley - Pag. 76)** only the survey period was limited to the early wet season, beginning one week after the first major rainfall and continuing for two months. Surveys were performed twice weekly with the collaboration of Universidad de Sucre (Sucre) and CORPOICA C.I. La Libertad (Meta). Results from Sucre and Meta follow while results from Cauca are described in **(see Population dynamics and phenology of spittlebugs in the Cauca Valley – Pag. 76).**

Results

In Meta, 64 nymphs were sampled, but none were detected in Plots 2 and 3. The date (julian) of first detection was 108, with the first generation arriving at peak abundance (50% accumulated insect days) on day 117 **(Table 1.15, Figure 1.10)**.

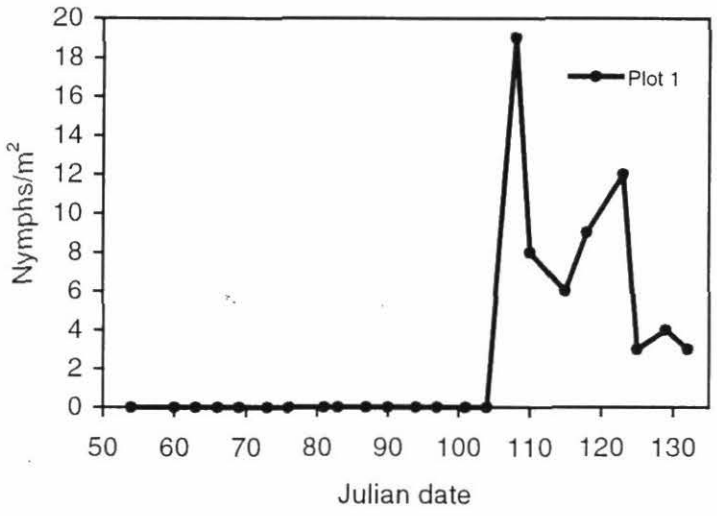

Figure 1.10. Population fluctuation of first generation spittlebug nymphs in Meta, 2000.

Species composition of the 566 sampled adults was 75.4% *A. varia*, 22.1% *A. reducta* and 2.5% *Z. pubescens*. Both *Aeneolamia* species were detected in all three plots while *Z. pubescens* was detected only in Plot 1. Across the three plots the proportion of *A. varia* ranged from 70.0-95.4% and *A. reducta* from 4.8-26.8% **(Table 1.16).** The date of first detection of adults and the date of peak abundance varied by only 3 days across plots **(Figure 1.11).** For summed plots, the first generation adults peaked day 121, only 4 days after nymphs.

In Sucre, 93 nymphs were sampled. Dates of first detection across plots varied from 132-143 while dates of peak abundance varied from 145-151 **(Table 1.15, Figure 1.12)**. Species composition of the 2953 sampled adults was 100% *A. reducta*. The date of first detection of adults was the same for each plot while the date of peak abundance varied from 155-161, or 10-13 days after the respective nymphal peak **(Figure 1.13).** For summed plots, the first generation adults peaked day 157, only 4 days after nymphs.

Table 1.16. Adult abundance and species composition in three survey sites, Meta.

	Plot 1		Plot 2		Plot 3	
Species	Number	Proportion	Number	Proportion	Number	Proportion
A. varia	305	70.0%	62	95.4%	60	92.3%
A. reducta	117	26.8%	3	4.8%	5	7.7%
Z. pubescens	14	3.2%	0	0.0%	0	0.0%

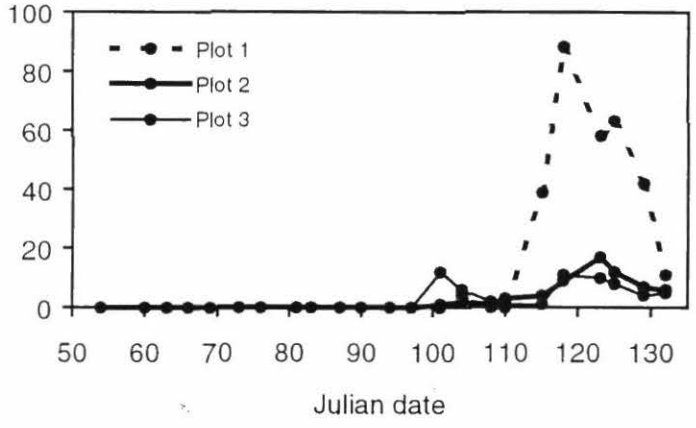

Figure 1.11. Population fluctuation of first generation spittlebug adults in Meta, 2000.

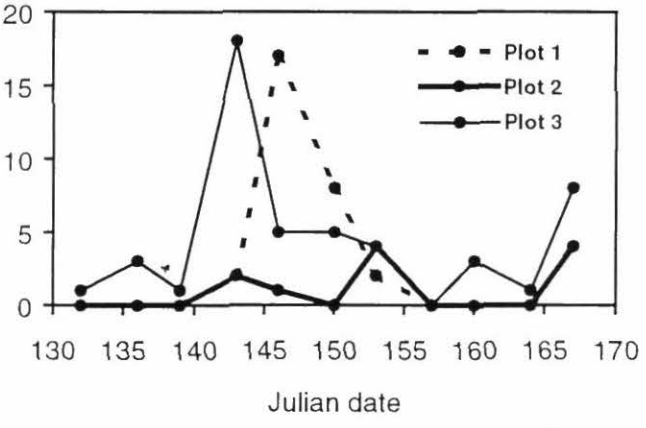

Figure 1.12. Population fluctuation of first generation *A. reducta* nymphs in Sucre, 2000.

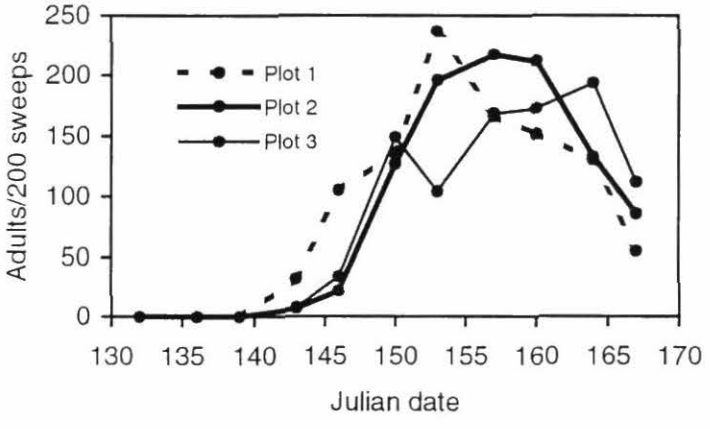

Figure 1.13. Population fluctuation of first generation *A. reducta* adults in Sucre, 2000.

Discussion

Precipitation data is being gathered from these sites to complement the early season population fluctuation curves. The combined data from 1997, 1998, 2000 and 2001 will be analyzed as four repetitions for Meta, Sucre and Cauca to test whether arrival of first generation spittlebugs is correlated with rainfall at the beginning of the wet season. The targeting of control tactics will depend on our ability to predict the arrival of early season outbreaks based on rainfall patterns and complemented by new information being gathered on the determinants of egg diapause **(see Preoviposition determinants of egg diapause - Pag. 82).**

Preoviposition determinants of egg diapause

Introduction

Egg diapause permits synchrony between spittlebug populations and the favorable conditions of the wet season. It has been shown in some species that diapause incidence (i.e. the proportion of eggs that are not immediately developing) is lowest at the beginning of the wet season and highest at the end when late season females lay diapausing eggs that survive the harsh conditions of the dry season. An explanation for seasonal changes in diapause is hindered by little information on preovipositional cues that serve as token stimuli to the insect. In temperate zones photoperiod and temperature play important roles but in tropical areas other factors such as plant quality may be more important in the regulation of diapause induction. It is the immature that normally perceives the environmental cues that regulate diapause induction. In this phase of our continuing studies on the diapause in *A. varia* we assess the role of drought stress on the nymphal host plant.

Methods

Plants of *Brachiaria ruziziensis* were established in pots with a proliferation of surface roots necessary for nymph development. Each pot was infested with 20 *A. varia* eggs about to hatch. Four treatment combinations were established based on two factors: surface microclimate (with or without aluminum lids that maintain dark and humid soil surface conditions) and water stress (full field capacity or watering every other day at ¼ field capacity). It was predicted that poor host plant quality or extreme conditions would promote diapause induction.

The adults that emerged from each treatment were placed in separate large petri dishes for oviposition in moist filter paper. After 72 hours eggs were disinfected, incubated (27°C, 100% RH, total darkness) and then scored for eclosion twice weekly. The effect of experimental conditions on diapause was measured as the proportion of eggs in diapause and time to eclosion. Nymph mortality, plant dry weight and quality were measured to confirm an effect of treatments on the host plants. Each treatment repetition consisted of 10 pots in a randomized block design with initiation date as blocks.

Results

To date two repetitions have been completed. Preliminary results show nymph survival ranging from 22-35% among treatments, diapause incidence 0–0.2%, and time to eclosion for nondiapause

and diapause eggs 19.7-20.2 and 39.9-40.1 days, respectively **(Table 1.17).** Of 4476 evaluated eggs, only 6 were diapausing.

Table 1.17. Influence of host plant quality on nymph mortality and egg diapause in *A. varia*.

Factor	No water Stress		Water Stress	
	With Lid	Without Lid	With Lid	Without Lid
Nymph survival	29%	22%	35%	23%
Eggs evaluated	1648	822	996	1010
Diapause incidence	0.24%	0.24%	0%	0%
Time to eclosion nondiapause	20.2	19.7	20.1	19.7
Time to eclosion diapause	40.1	39.9	-	-

Discussion

After two repetitions results showed no effect of these experimental adverse conditions on diapause. We are concerned that the experimental insect population is not appropriate for these studies. There is an extremely strong selection pressure in the CIAT colony against diapause because eggs that are not immediately developing are discarded and do not contribute to subsequent generations. Moreover, replenishment of the colony with field individuals has switched from Meta to Caquetá, from a seasonally dry site where diapause is advantageous to a continuously humid site where diapause may be a selective disadvantage. To address this dilemma, we are planning additional experiments using eggs obtained from females captured in the field in Meta.

Seasonal changes in the incidence and duration of egg diapause

Introduction

Interpretation of the population fluctuations and phenology of spittlebugs depends on an understanding of egg diapause, which synchronizes the insect life cycle with seasonal environmental changes such as precipitation patterns. It has been shown in some species of spittlebugs that diapause incidence (i.e. the proportion of eggs that are not immediately developing) is lowest at the beginning of the wet season and highest at the end when late season females lay diapausing eggs that survive the harsh conditions of the dry season. We are examining seasonal changes in diapause incidence in three contrasting sites of Colombia to complement studies on population dynamics.

Methods

One year of field sampling began in January 2000 in three sites where ongoing surveys are documenting population fluctuations of different spittlebug species: Cauca (*Zulia carbonaria*), Meta (*Aeneolamia varia*) and Sucre (*Aeneolamia reducta*). This work was done in collaboration with CORPOICA C.I. La Libertad and the Universidad del Sucre. In three previously established study plots in each regions **(see Populations dynamics and phenology of spittlebugs in the Cauca Valley, Pag. 76 and Documentation of first generation population phenology in two lowland sites, Pag. 79)**, egg collections were made every 2 weeks. Two samples of field caught females were placed in separate large petri dishes for oviposition on moist filter paper. Given

differences in availability according to site and date, groups consisted of 1-5 individuals for Cauca and 1-25 for Meta and Sucre. After three days, eggs were sent by express mail to CIAT, disinfected (2-3% solution of sodium hypochlorite for 3 min and rinsed thoroughly with distilled water) and incubated (27°C, 100 % RH, total darkness). Twice weekly the groups were evaluated for egg eclosion, and eggs that eclosed after 30 days were considered diapausing.

Results

Of the total 18,753 viable eggs evaluated from January-August, only a very small proportion (0.18%) was diapausing: 0.27% of the total collected in Cauca, 0.06% in Meta and 1.39% in Sucre **(Table 1.18).** Over 16 collections in Cauca (n=1445 eggs), diapausing eggs were detected on only two sequential dates representing 2.1 and 5.7% of the total eggs. Over 9 collections in Meta (n=9408 eggs), diapausing eggs were detected only once (0.16%), and over 4 collections in Sucre (n=7900 eggs) a very small proportion of diapausing eggs (0.05 – 3.16%) was detected each date.

Mean time to eclosion for nondiapause eggs of *Z. carbonaria*, *A. varia* and *A reducta* was 18.2±2.6, 17.2±1.8, and 18.0±1.2 days, respectively. For diapausing eggs, mean eclosion was 48.2±12.5, 39.1 (6 eggs eclosed the same day) and 51.2±13.2 days.

Discussion

In Cauca and Sucre, dates with diapausing eggs correspond to brief dry periods in the middle of the wet season ("veranillo de San Juán"), which may have prompted diapause induction in the small proportion of eggs. Meta did not experience a veranillo nor did eggs from that region show more than a barely detectable level of diapause. Over the study period completed to date, all three regions were in the rainy season during which high proportions of immediately developing eggs are predicted. We expect to document an increasing proportion of diapausing eggs laid by females at the end of the wet season when egg dormancy should be advantageous for dry season survival.

Table 1.18. Incidence of egg diapause in field populations of three spittlebug species.

Cauca: *Z. carbonaria*		Meta: *A. varia*		Sucre: *A. reducta*	
Collection Date	Proportion Diapause (%)	Collection Date	Proportion Diapause (%)	Collection Date	Proportion Diapause (%)
28 March	0	27 April	0	9 June	0.05
7 April	0	11 May	0	30 June	0.12
12 April	0	25 May	0	14 July	3.16
5 may	0	8 June	0	28 July	0.85
15 May	0	23 June	0		
17 May	0	5 July	0.16		
27 May	0	18 July	0		
5 June	0	1 August	0		
9 June	0	16 August	0		
16 June	0				
23 June	0				
2 July	2.08				
13 July	5.66				
25 July	0				
3 August	0				
21 August	0				

Activity 2. Diagnosis of spittlebug for elaborating IPM components

Identity and distribution of spittlebugs associated with graminoids of Colombia and Ecuador

Introduction

Variation in the biology, habitat and taxonomy of neotropical spittlebugs seriously compromises their effective management given the tendency to overgeneralize the diverse insect/host/habitat associations. Despite certain broad generalities, there is considerable bioecological variation in aspects such as duration of the life stages, oviposition sites and number of generations per year. In addition, cercopids are pests in diverse habitats because of their wide geographic (southeast U.S. to northern Argentina), altitudinal (0-3000 m elev.), habitat management (intensive to extensive grazing systems) and host plant (essentially all economically important genera of forage grasses, sugar cane, and occasionally other graminoid crops such as rice and turfgrass) range. These dimensions have implications for pest status and the tailoring of control strategies to particular sites.

Grassland spittlebugs are also a taxonomically diverse group. In the Neotropics there are dozens of native species associated with wild and cultivated graminoids, representing 11 genera: *Aeneolamia*, *Deois*, *Isozulia*, *Kanaima*, *Mahanarva*, *Maxantonia*, *Notozulia*, *Prosapia*, *Sphenorhina*, *Tunaima* and *Zulia*. Relevant species in the genera *Monecphora*, *Phytozamia* and *Tomaspis* have been transferred to other genera. All these taxa belong to the subfamily Tomaspidinae, tribe Tomaspidini (sensu Fennah 1968).

Despite their economic importance, the taxonomy of this group is not very advanced. The complex presents a high degree of intraspecific variation and interspecific convergence that complicates species differentiation. In addition, very few cercopid species have descriptions of male genitalia, a key character for determination of genus and species. Published reports and studies on grassland spittlebugs in Colombia and Ecuador are scarce, documenting 7 species for Colombia (*Aeneolamia bogotensis, A. lepidior, A. varia, Sphenorhina rubra, Zulia birubromaculata, Z. carbonaria, Z. pubescens*) and 5 for Ecuador (*Isozulia minor, Mahanarva andigena, M. phantastica, S. rubra, Z. pubescens*).

Designing an effective IPM program for this pest group will depend on precise species determinations. Recent studies are demonstrating that the expression of host plant resistance, for instance, depends on the particular spittlebug species. It is therefore critical that management tactics consider the spittlebug/habitat/host relationships in detail.

The present study was undertaken to assess and summarize the diversity, classification and distribution of spittlebugs associated with wild and cultivated graminoids of Colombia and Ecuador. This work is considered timely because broadened research on this pest in the last four years by various regional collaborators has uncovered new species, distribution and host plant records, plus nomenclature clarifications and changes that should be disseminated as an updated taxonomic foundation.

Methods

Distribution information was obtained from fieldwork (1996-2000) and revision of museum collections (1999-2000). Distribution data were collected from visits to four institutions: The Natural History Museum (London, UK) (BMNH), Cornell University (Ithaca, US) (CU), CIAT's taxonomic reference collection (Cali, Colombia) (CIAT) and the Universidad Nacional at Palmira (Palmira, Colombia) (UNP). All adult specimens were identified to species and in the majority of cases this could be confirmed through examination of type specimens at BMNH. Certain characters of the male genitalia formed the basis for determinations while color and size served as secondary supporting characters.

Information from museum collections was complemented by reports and observations from the field. In particular, these included studies carried out over the last four years by CIAT and various national collaborators (Universidad de la Amazonia, Universidad de Sucre, CORPOICA C.I. Turipaná, La Libertad, Macagual, El Mira) in the Colombian departments of Caquetá, Cauca, Córdoba, Meta, Nariño, Sucre and Valle del Cauca.

The analysis of spittlebug diversity and distribution in Ecuador was more preliminary. No museums in Ecuador were visited and few specimens were available in the four collections examined. Information from field observations was obtained during a trip to Puyo (Prov. Pastaza) in collaboration with SESA (Servicio Ecuatoriano de Sanidad Vegetal) and from material sent to CIAT for identification from three Ecuadorian entities.
Analysis was limited to cercopids associated with wild or cultivated graminoid hosts. This subgroup of species was determined by host record information in the collection data of museum specimens or from the literature. Geographic distribution data and host plant data were limited to the examined specimens; because of taxonomic errors, it was decided not to include data from published observations (the few exceptions are highlighted).

Results

From the four institutions, 2651 mounted specimens were examined from Colombia and 85 from Ecuador. Approximately 271, 99, 22 and 20 Colombian distribution records were obtained from CIAT, UNP, CU and BMNH, respectively, but only 5, 0, 5 and 17 for Ecuador. Museum and field data were acquired for 21 of the 32 Colombian departments and 9 of the 20 Ecuadorian provinces.

The presence of 15 species from 6 genera in Colombia and 9 species from 4 genera in Ecuador was confirmed for a total of 18 species from 7 genera: *Aeneolamia*, *Isozulia*, *Mahanarva*, *Notozulia*, *Prosapia*, *Sphenorhina* and *Zulia* **(Table 2.1, 2.2 and 2.3).** *Isozulia* was not reported in Colombia while *Aeneolamia*, *Notozulia* and *Prosapia* were not reported for Ecuador. All of these genera are known from the literature as graminoid pests, however *Notozulia* was reported for the first time for Colombia and *Prosapia* for the first time in South America **(see Detection of the Central American forage and cane pest, *Prosapia simulans,* in South America - Pag. 67).**

Table 2.1. Diversity of spittlebugs associated with graminoids of Colombia and Ecuador.

Species	Country	
	Colombia	Ecuador
Aeneolamia bogotensis (Distant) *(Tomaspis)*	X	-
Aeneolamia lepidior (Fowler) *(Tomaspis)*	X	-
Aeneolamia reducta (Lallemand) *(Monecphora)*	X	-
Aeneolamia varia (Fabricius) *(Cercopis)*	X	-
Isozulia astralis (Distant) *(Tomaspis)*	-	X
Isozulia minor (christenseni) Fennah	-	X
Mahanarva andigena (Jacobi) *(Tomaspis)*	X	X
Mahanarva phantastica (Breddin) *(Tomaspis)*	X	X
Mahanarva sp. Nov.	X	X
Notozulia entreriana (Berg) *(Tomaspis)*	X	-
Prosapia simulans (Walker) *(Sphenorhina)*	X	-
Sphenorhina rubra (L.) *(Cicada)*	X	X
Sphenorhina sp. 1	X	-
Sphenorhina sp. 2	-	X
Zulia birubromaculata (Lallemand) *(Monecphora)*	X	-
Zulia carbonaria (Lallemand) *(Monecphora)*	X	-
Zulia pubescens (Fabricius) *(Cercopis)*	X	X
Zulia sp. Nov.	X	X

Seven new species reports were confirmed from Colombia: *M. andigena*, *M. phantastica*, *Mahanarva* sp. nov., *N. entreriana*, *P. simulans*, *Sphenorhina* sp. 1 and *Zulia* sp. nov. There were four new reports for Ecuador: *I. astralis*, *Mahanarva* sp. nov., *Sphenorhina* sp. 2 and *Zulia* sp. nov. The species list for Ecuador includes *I. minor* (*christenseni*) and *M. phantastica*, which were not examined in this study but were both originally described from Ecuadorian specimens. Six species were found in both countries: *M. andigena*, *M. phantastica*, *Mahanarva* sp. nov., *S. rubra*, *Z. pubescens* and *Zulia* sp. nov. Two species are confirmed as undescribed: *Mahanarva* sp. nov. (Amazonian Piedmont of Colombia and Ecuador, Coastal Ecuador) and *Zulia* sp. nov. (Pacific Coast of Colombia and Ecuador).

Table 2.2. Diversity and distribution of spittlebugs associated with graminoids in Ecuador

Species	Ecuadorian Provinces	Geographic Zone	
		Amazonia	Coast
I. astralis	Pastaza	X	
I. minor (christenseni)[1]	Napo	X	
M. andigena	Chimborazo, Esmeraldas, Guajas, Pastaza, Tungurahua	X	X
M. phantastica[1]	Tungurahua		
Mahanarva sp. Nov.	Napo, Pichincha, Sucumbios	X	X
S. rubra	Napo	X	
Sphenorhina sp. 2	Pastaza	X	
Z. pubescens	Cotopaxi, Napo, Pastaza, Pichincha, Sucumbios, Tungurahua	X	
Zulia sp. nov.	Esmeraldas, Pichincha		X

[1] Specimens not examined in this study but location cited in the literature

Table 2.3. Diversity and distribution of spittlebugs associated with graminoids in Colombia.

Species	Colombian Department																				
	Ama	Ant	Atl	Bol	Boy	Cal	Caq	Cas	Cau	Ces	Cór	Cun	Mag	Met	Nar	Qui	Ris	San	Suc	Tol	Val
A. bogotensis	-	-	-	-	-	-	-	-	-	-	-	X	-	X	-	-	-	-	-	-	-
A. lepidior	-	X	X	X	-	X	-	-	X[1]	X	X	-	X	-	-	-	-	X	X	-	X
A. reducta	-	-	X	X	-	-	-	-	-	X	X	-	X	X	-	-	-	X	X	X	-
A. varia	-	-	-	-	-	-	X	X	-	-	-	-	-	X	-	-	-	-	-	-	-
M. andigena	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-
M. phantastica	-	X	-	-	-	X	-	-	X[2]	-	-	-	-	-	-	X	-	-	-	X[2]	X
Mahanarva sp. Nov.	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N. entreriana	-	-	-	-	-	-	-	-	-	-	-	-	-	X[2]	-	-	-	-	-	-	-
P. simulans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
S. rubra	-	-	-	-	-	-	-	-	-	-	-	-	-	X[2]	-	-	-	-	-	-	-
Sphenorhina sp. 1	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-
Z. birubromaculata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X[2]	-	-	-	-	-	X
Z. carbonaria	-	X	-	-	-	X	X	-	X	-	-	-	-	-	-	-	X[2]	-	-	-	X
Z. pubescens	X[2]	X	-	-	X[1]	-	X	-	X	-	-	-	-	X	X	-	-	X[2]	-	X	X
Zulia sp. nov.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-

[1] Gorgona Island
[2] Only one record

One major change in nomenclature was confirmed. The species previously known in Colombia as *Z. colombiana* is actually *Z. carbonaria*. Furthermore, *Z. colombiana* is a junior synonym of *Z. pubescens* and should therefore be retired from usage. Secondly, although certain Colombian specimens in CIAT were labeled as *A. flavilatera*, the presence of this species in Colombia could not be confirmed. It is thought that these specimens were confused with similar morphotypes of *A. varia*. *A. flavilatera* is known from Venezuela to Surinam.

Compared to other neotropical regions, Colombia has a relatively high spittlebug diversity. Costa Rica has 8 species from 3 genera reported, Venezuela 9 species from 5 genera and Brazil 16 species from 7 genera. Colombia shares *A. lepidior*, *A. reducta* and *P. simulans* with Costa Rica; *A. bogotensis*, *A. lepidior*, *A. reducta*, *A. varia*, *P. simulans* and *S. rubra* with Venezuela; and *N. entreriana* and *S. rubra* with Brazil. More detailed distribution surveys from Ecuador should significantly increase the number of species reported for this country. In a single visit to the Amazonian region (Puyo, Prov. Pastaza) a very high local diversity was encountered: 4 species from 4 genera (*I. astralis*, *M. andigena*, *Sphenorhina* sp. 2 and *Z. pubescens*)

Distribution data for Colombia indicate that the spittlebug complex varies in general terms among ecoregions. In the lowland tropics, *A. lepidior* and *A. reducta* are most important in the Caribbean Coast, *M. andigena* and *Zulia* sp. nov on the Pacific Coast, and *A. reducta* and *A. varia* in the Eastern Llanos. In the interandean regions *P. simulans*, *Z. carbonaria* and *Z. pubescens* predominate in the Cauca River Valley while *A. reducta*, *Z. carbonaria* and *Z. pubescens* are most common in the Upper and Central Magdalena River Valley. The predominant species in the Andean zone are *M. phantastica* and *Z. pubescens*, while *A. varia* and *Z. pubescens* are most important in the Amazonian Piedmont. The Colombian departments with the most diverse fauna (Cauca, Meta, Valle del Cauca) correspond to the regions where collection activity has probably been the highest due to the presence of CIAT and CORPOICA. It is therefore critical that further distributional surveys be carried out in other regions, particularly the 11 departments where no records were uncovered.

Spittlebugs were confirmed from 27 host plants in Colombia and Ecuador: *Andropogon gayanus*, *Axonopus compressus*, *A. micay*, *A. scoparius*, *Brachiaria plantaginea*, *B. brizantha*, *B. decumbens*, *B. dictyoneura*, *B. humidicola*, *B. mutica*, *Bothriochloa pertusa*, *Bothriochloa* sp., *Calopogonium* sp., *Cestrum* sp., *Cynodon plectostachys*, *Cynodon* sp., *Dichanthium aristatum*, *Dichromena ciliata*, *Digitaria decumbens*, *Homolepsis aturensis*, *Hyparrhenia rufa*, *Melinis minutiflora*, *Oryza sativa*, *Panicum maximum*, *Pennisetum clandestinum*, *Saccharum officinarum* and *Sorghum halepense*.

Discussion

Despite their economic importance in Colombia and other Latin American countries, new taxonomic, distribution, host plant and taxonomic information has been obtained. Correct taxonomic determinations and placing of voucher specimens are critical for augmenting the impact of research. Descriptions of male genitalia are important to distinguish species, however at the geographic level it should be possible to develop keys to sympatric species based on overall body size and color pattern.

Prosapia simulans in South America, *N. entreriana* in the Eastern Llanos and *Z. carbonaria* in the Amazonian Piedmont could represent invasions or range expansion from Central America, Brazil and Cauca Valley, respectively. Evidence suggests that introductions of exotic species constitute a risk for forage grass and sugar cane production in the new habitats. Care in transfer of vegetative host plant material is merited.

More detailed inventories and distributional surveys are also required, particularly in key regions such as the Chocó, Pacific Coast and Amazonia of Colombia. The summary for Ecuador is considered only a preliminary assessment. Information on the geographic distribution and identity of grassland spittlebugs in Colombia and other regions will serve to monitor range expansion and new species introductions. The determinants of distribution of grassland spittlebugs are poorly understood. Broadened distribution surveys and evaluation of museum material could lead to more detailed analysis of geographic range. With the aid of GIS software such as Flora-Map, range can be interpreted in terms of certain climatic variables such as temperature and precipitation and thereby used to construct probability maps of occurrence for assessing areas at risk for range expansion, outbreaks or introductions.

Results indicate that distribution varies depending on species and that different geographic regions support a distinct complex. Spittlebug management strategies should therefore be formulated according to the species composition of the local complex since there is significant variation among species in terms of biology and ecology.

Evaluation of an artificial diet for maintenance of spittlebug adults

Introduction

This activity contributes to the development of bioassays for the evaluation of proteins with potential insecticidal properties that could be incorporated into *Brachiaria* through genetic transformation. As a fundamental first step, we are investigating artificial diets for the maintenance of spittlebug adults. This diet will enable screening of potential proteins for insecticidal effects before the process of transformation. Potential factors include lectins, which are known to have deleterious effects on other sap-sucking Homoptera.

Methods

The diets evaluated in these preliminary studies were based on a published recipe prepared for *Aeneolamia varia saccharina* (Hagley 1967). Adult *A. varia* from CIAT's colony were presented with 500 µl liquid diet in parafilm sachets (3 x 3.5 cm) while housed in large petri dishes (15 cm diameter, 2 cm tall). In a first phase, longevity of adults on the original artificial diet was compared to longevity of adults feeding on *Brachiaria ruziziensis* stems (with bases in small vial of water) in the same petri dish environment (5 repetitions).

In the second phase, five alternative diets were prepared and evaluated against the original diet. Alterations were made to reduce costs, simplify preparation, and increase effectiveness. In the modified diets 2, 3, 4 and 5, yeast extract and casein hydrolysate replaced the various amino acids components of the original diet. Ribofavin was reduced from 43 to 0.25 ml/100 ml and B_{12} was

replaced by P-aminobenzoic acid in diets 3, 4 and 5. Wesson salt was replaced by individual salt components in diets 2, 3 and 4, but diet 5 had Wesson salts plus individual salt components. $MgCl_2$ and KH_2PO_4 were added to diets 4 and 5. Each modified diet was compared with the original diet on separate study dates.

For all experiments, 2 adult males and 2 adult females were evaluated per petri dish. Mean adult longevity was calculated with a Weibull distribution and compared between treatments.

Results

Adult *A. varia* effectively acquired diet through the parafilm sachets. Mean adult longevity under experimental petri dish conditions and plant stems was similar to results obtained for adults kept on potted plants under acetate cages. Although statistical analyses have not yet been performed, mean adult longevity with the original diet was three days longer than the control of plant stems (**Figure 2.1**).

Effectiveness of the original diet decreased with time: only one batch was made and mean adult longevity decreased with subsequent trials of modified diets (**Figure 2.1**). None of the modification diets therefore surpassed the original diet in effectiveness at maintaining *A. varia* adults.

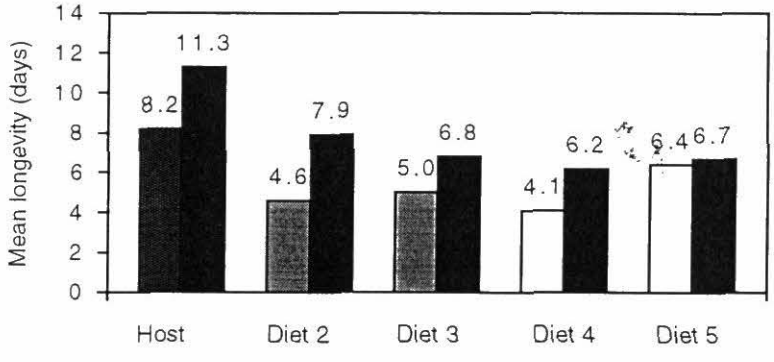

Figure 2.1. Mean longevity of adult *A. varia* comparing an original artificial diet (black bars) to the host plant and modified diets.

Discussion

Based on these preliminary results, the evaluation methodology is appropriate for assessing the longevity of *A. varia* adults on artificial diets. A diet originally published in 1967 equals or surpasses the host plant in maintaining adults under these experimental conditions. Thus far, diets modified to reduce costs and simplify preparation do not work as well as the original diet. Overall,

this diet and this methodology appears show promise for developing a bioassay for plant and fungus factors of interest to *Brachiaria* transformation. New modifications of the original diet will be sought to overcome the aforementioned limitations. An additional limitation has been the precipitation of product, which should be overcome to avoid loss of active ingredients of the diet or of the extracts being evaluated. A new experimental design will be used to evaluate several diets simultaneously, including a host plant control.

Identity, incidence and maintenance of spittlebug fungal entomopathogens

Introduction

Very few natural enemies of spittlebugs have been assessed for their potential as agents of biological control. Of the five different classes of spittlebug natural enemies in Colombia (parasitic flies, mites, nematodes; predaceous flies; fungal pathogens), fungal entomopathogens are the most diverse and widespread. As a component of IPM they have had no success in forage grasses, and have achieved only marginal, highly variable or poorly documented success in sugar cane. One limitation is that past studies have focused on a narrow genetic diversity of isolates, largely limited to the species *Metarhizium anisopliae*. Ongoing studies on the spittlebug complex in contrasting regions of Colombia have allowed us to collect, isolate, propagate and store a diverse collection of fungal entomopathogens obtained from a broad range of spittlebug species and habitats. This ceparium is designed to serve as a source of pathogenic material for studies that focus on advancing the use of fungal entomopathogens as components for the integrated management of spittlebugs in pastures and cane fields.

Methods

Spittlebug nymphs and adults with evidence of mycosis were obtained during visits to the field. Fungal entomopathogens were isolated using two methods. If specimens were covered in mycelium or spores, and external contamination was limited, a sample was taken directly with a dissection needle for inoculation of culture medium in a petri dish. In cases where the insect was highly contaminated with little evidence of fungus, the specimen was sterilized in a test tube by vigorously agitating for 2-3 min in a solution of sodium hypochlorite (2%), rinsed 2-3 times with sterile distilled water, dried on sterile paper towel under a laminar flow hood, and divided into pieces for inoculation.

The culture medium for both isolation methods was Sabureaud agar modified with yeast extract (1%) and lactic acid (1%). Two to three days after inoculation, once the colonies measured about 1 cm diameter, the most promising were reisolated in culture medium and repeated as necessary to obtain a pure culture.

To prepare purified fungus for storage, 20-25 pieces (1 cm^2) of sterile filter paper were laid on modified Sabureaud agar in a petri dish. A small piece of the colony was taken from the vegetative growth zone and placed on each paper. After incubation and growth for 20-25 d, the pieces of filter paper were removed from the medium and dried in a new sterile petri dish under incubation for an additional 15-20 d. The dried paper and their fungus colonies were placed in labeled glycine envelopes for freezer storage in plastic boxes (–20°C).

Copies of isolates were periodically sent to the Collection of Entomopathogenic Fungal Cultures (ARSEF-USDA), Ithaca, USA for taxonomic identification by Richard Humber.

Results

The ceparium includes a total of 75 strains that have been isolated, propagated and placed under catalogued storage **(Table 2.4).** Of these, 14, 11, 40, and 10 were acquired during 1997, 1998, 1999 and 2000 respectively. A total of 71 have been obtained from spittlebugs, two from whiteflies (*Trialeurodes vaporariorum*), one from a planthopper (*Tagosodes orizicolus*) and one from a leaf miner. With collaboration of the ARSEF-USDA, 44 isolates have been identified to genus and 23 to species.

The strains isolated from spittlebugs belong to 10 different genera and 12 different species of fungus. *Metarhizium* is the most common genus with 16 isolates, all identified as *M. anisopliae*. *Fusarium* is represented by 15 isolates, none yet identified to species. *Paecilomyces* is represented by six isolates and is the most diverse genus with three species identified (*P. crustaceus*, *P. farinosis*, *P. lilacinus*), and one undetermined. The seven other fungus genera are *Aspergillus*, *Beauveria* (*B. bassiana*), *Curvularia*, *Dactylella*, *Penicillium*, *Sporothrix*, and *Trichoderma* (*T. viridae*).

Strains were isolated from both nymphal (11 isolates) and adult (60 isolates) life stages. Hosts include 4 genera and 7 species of Colombian spittlebugs: *A. reducta*, *A. varia*, *Mahanarva andigena*, *Mahanarva* sp. nov.? *P. simulans*, *Z. carbonaria*, and *Z. pubescens*. Source regions include six Colombian departments (Caquetá, Cauca, Meta, Nariño, Sucre, Valle del Cauca) representing the Pacific Coast, Caribbean Coast, Amazonian Piedmont, Orinoquia Piedmont, and Interandean Region.

Discussion

The CIAT ceparium constitutes the largest collection of fungal entomopathogens isolated from grassland spittlebugs with the exception of a ceparium in Brazil that includes approximately 90 strains (CENARGEN, EMBRAPA, Brasilia). Based on the known fungal taxa, host taxa, and host ecoregions represented, the CIAT ceparium is a highly diverse collection of pathogenic material. This germplasm collection is a critical tool and resource for research on developing fungal entomopathogens as biological control agents of spittlebugs in major agroecosystems. In general, only a small number of strains of *M. anisopliae* and *B. bassiana* have ever been considered for the biological control of spittlebugs. It is anticipated that screening of this collection will identify isolates of *M. anisopliae*, *B. bassiana* and other fungal species with higher virulence than previously evaluated strains, and also identify those with enhanced quality attributes such as increased tolerance to solar radiation, low humidity and low water quality.

Table 2.4. Collection of fungal entomopathogens isolated from grassland spittlebugs (Homoptera: Cercopidae).

Isolate[1]		Host[2]	Isolate[1]		Host[2]
Accession	Species	Species	Accession	Species	Species
CIAT 001	*Metarhizium anisopliae*	*Z. pubescens*	CIAT 037	*Dactylella* sp.	*A. varia*
CIAT 002	*Metarhizium anisopliae*	*Z. pubescens*	CIAT 038	*Fusarium* sp.	*Z. pubescens*
CIAT 003	*Metarhizium anisopliae*	*A. varia*	CIAT 039	*Fusarium* sp.	*Z. pubescens*
CIAT 004	*Fusarium* sp.	*Z. pubescens*	CIAT 040	*Fusarium* sp.	*Z. pubescens*
CIAT 005	*Metarhizium anisopliae*	*Mahanarva* sp. n.	CIAT 041	undet.	*Z. pubescens*
CIAT 006	*Metarhizium anisopliae*	*Mahanarva* sp. n.	CIAT 042	*Metarhizium anisopliae*	*Z. carbonaria*
CIAT 007	*Metarhizium anisopliae*	*Z. pubescens*	CIAT 043	*Paecilomyces lilacinus*	*Z. carbonaria*
CIAT 008	*Metarhizium anisopliae*	undet.	CIAT 044	*Beauveria bassiana*	*Z. carbonaria*
CIAT 009	*Paecilomyces farinosis*	undet.	CIAT 045	undet.	*M. andigena*
CIAT 010	*Metarhizium anisopliae*	undet.	CIAT 046	*Fusarium* sp.	*M. andigena*
CIAT 011	*Paecilomyces* sp.	undet.	CIAT 047	undet.	*Z. carbonaria*
CIAT 012	*Paecilomyces lilacinus*	*Z. pubescens*	CIAT 048	undet.	*Z. carbonaria*
CIAT 013	*Sporothrix* sp.	undet.	CIAT 049	*Fusarium* sp.	*M. andigena*
CIAT 014	*Metarhizium anisopliae*	*A. varia*	CIAT 050	*Fusarium* sp.	*Z. carbonaria*
CIAT 015	*Metarhizium anisopliae*	*A. varia*	CIAT 051	undet.	*Z. carbonaria*
CIAT 016	*Trichoderma viridae*	*A. varia*	CIAT 052	*Paecilomyces crustaceus*	*Z. carbonaria*
CIAT 017	*Metarhizium anisopliae*	*A. varia*	CIAT 053	*Metarhizium anisopliae*	*Z. carbonaria*
CIAT 018	*Metarhizium anisopliae*	*A. varia*	CIAT 054	undet.	*A. varia*
CIAT 019	*Metarhizium anisopliae*	*A. varia*	CIAT 055	undet.	*A. varia*
CIAT 020	*Fusarium* sp.	*Z. carbonaria*	CIAT 056	undet.	*Z. pubescens*
CIAT 021	*Fusarium* sp.	*Z. carbonaria*	CIAT 057	undet.	Undet.
CIAT 022	*Fusarium* sp.	*Z. carbonaria*	CIAT 058	undet.	*Z. pubescens*
CIAT 023	*Fusarium* sp.	*Z. carbonaria*	CIAT 059	undet.	*A. varia*
CIAT 024	*Fusarium* sp.	*Z. carbonaria*	CIAT 060	undet.	*A. varia*
CIAT 025	*Fusarium* sp.	*Z. carbonaria*	CIAT 061	undet.	*A. varia*
CIAT 026	*Metarhizium anisopliae*	*Z. carbonaria*	CIAT 062	undet.	*A. varia*
CIAT 027	*Penicillium* sp.	*Z. carbonaria*	CIAT 066	undet.	*Z. pubescens*
CIAT 028	*Fusarium* sp.	*Z. carbonaria*	CIAT 067	undet.	*Z. pubescens*
CIAT 029	*Penicillium* sp.	*Z. carbonaria*	CIAT 068	undet.	*Z. pubescens*
CIAT 030	*Metarhizium anisopliae*	*T. orizicolus*[3]	CIAT 069	undet.	Leaf miner[3]
CIAT 031	*Undet.*	*A. reducta*	CIAT 070	undet.	*T. vaporariorum*[3]
CIAT 032	*Curvularia* sp.	*Z. carbonaria*	CIAT 071	undet.	*T. vaporariorum*[3]
CIAT 033	*Undet.*	*Z. pubescens*	CIAT 072	undet.	*Z. pubescens*
CIAT 034	*Aspergillus* sp.	*A. varia*	CIAT 073	undet.	*Z. carbonaria*
CIAT 035	Undet.	*A. varia*	CIAT 074	undet.	*P. simulans*
CIAT 036	Undet.	*A. varia*	CIAT 075	undet.	Undet.

[1] Identifications made by Richard Humber, ARSEF-USDA, Ithaca, USA

[2] It is usually not possible to determine species of nymphal hosts

[3] Non-spittlebug host

Screening fungal entomopathogens for virulence to spittlebug adults

Introduction

Fungal entomopathogens currently demonstrate more potential for spittlebug management than any other class of natural enemy. Despite reports of high virulence in the laboratory, however, effectiveness in the field (pastures) has never been demonstrated. Focus on a narrow diversity of isolates, lack of consideration of insect-pathogen interactions, poor formulation and application technologies, and inadequate field evaluation methodologies have compromised successful deployment. Exploiting and assessing this diversity for biological control depends on a dependable and rapid methodology for quantifying virulence in the laboratory and screening the collection of isolates. The following is a summary of investigations into a screening methodology for spittlebug adults and results of virulence screening of a diverse array of isolates.

Methods

Evaluation units were 30-day old plants (7-10 stems) of *Brachiaria ruziziensis* (CIAT 654) in pots (13 cm diameter) covered by acetate cylinders (15 cm diameter x 40 cm tall). These plants were infested with 10 adult tenerals (< 24 hours old) of *Aeneolamia varia* obtained from CIAT's colony. Two to three hours after infestation plants were sprayed with 5 ml of a concentrated conidial suspension (10^8 con /ml) with an airbrush and compressor (10 PSI). Ten repetitions (pots) were performed for each evaluated isolate, and every block (evaluation date) included a control consisting of water plus tween (0.05%). After spraying, plants and insects were maintained in a growth chamber (27°C ± 2°C, RH 80% ± 10%). The effectiveness of the treatments 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.

The following results pertain to 46 isolates evaluated with this methodology during 1999 and 2000, 28 of which were evaluated since last year. Of this group, 33 corresponded to *Metarhizium anisopliae*, 7 to unidentified species of *Fusarium*, 1 to *Paecilomyces farinosis*, 1 to *Paecilomyces lilacinus* and 4 undetermined. All isolates evaluated were previously reactivated on adults of *A. varia*; 35 were multisporic and 11 monosporic isolates.

Results

Overall mortality in the control was 25.1%, consistent with results from the previous year and an acceptable level for gauging efficiency. This evaluation method appears to be effective and appropriate for quantifying virulence against adults of *A. varia*.

Absolute adult mortality ranged broadly from 10.6–95.1% **(Figure 2.2).** Analysis of variance showed significant differences among isolates in virulence (P<0.0001) **(Table 2.5).** Of the 46 total isolates, 17 obtained mortality scores >50%, 14 > 60%, 9 >70%, 3 >80% and 1 >90%. CIAT 054 was the most virulent, killing 95.1% of *A. varia* adults over the 5-day evaluation period. This strain, and the second most effective, CIAT 055, have not yet been identified but probably belong to the genus *Metarhizium*.

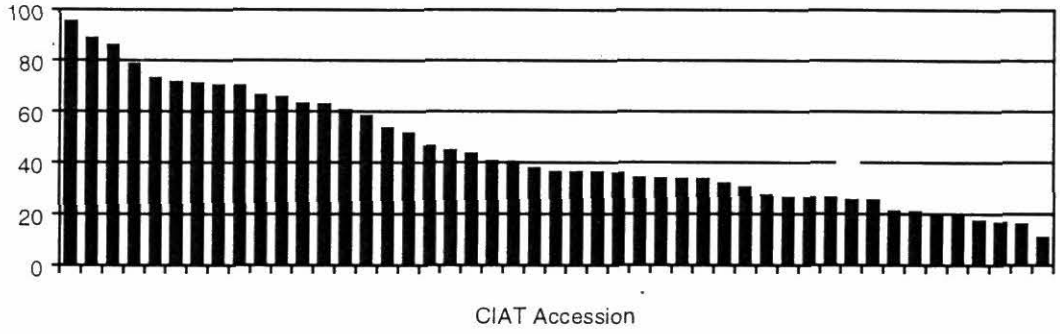

Figure 2.2. **Virulence of 46 isolates of fungal entomopathogens to *A. varia* adults. The control was water plus tween.**

Table 2.5. **Identification, origin and virulence (percent mortality) of select Colombian isolates of fungal entomopathogens on *Aeneolamia varia* adults.**

			Host		
Accession	Species	Virulence[1]	Species	Life stage	Origin
CIAT 054	undet.	95.1 a	*Aeneolamia varia*	Adult	CIAT, Valle
CIAT 055	undet.	88.6 ab	*Aeneolamia varia*	Nymph	CIAT, Valle
CIAT 007-C	*M. anisopliae*	85.8 abc	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 003	*M. anisopliae*	78.5 abcd	*Aeneolamia varia*	Adult	Albania, Caquetá
CIAT O53	*M. anisopliae*	73.0 bcde	*Zulia carbonaria*	Adult	Quilichao, Cauca
CIAT 042	*M. anisopliae*	71.6 bcedf	*Zulia carbonaria*	Adult	Quilichao, Cauca
CIAT 007	*M. anisopliae*	71.1 bcedf	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 002-B	*M. anisopliae*	70.3 bcedf	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 010	*M. anisopliae*	70.0 bcedf	undet.	Nymph	Florencia, Caquetá
CIAT 007-B	*M. anisopliae*	66.3 cdefg	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 001	*M. anisopliae*	65.5 cdefg	*Zulia pubescens*	Adult	Florencia, Caquetá
CIAT 007-A	*M. anisopliae*	62.9 edfgh	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT O09	*P. lilacinus*	62.8 edfgh	undet.	Nymph	Florencia, Caquetá
CIAT 002-A	*M. anisopliae*	60.4 edfghi	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 018	*M. anisopliae*	58.3 edfghij	*Aeneolamia varia*	Adult	CIAT, Valle
CIAT 025	*Fusarium sp.*	53.4 dfghij	*Zulia carbonaria*	Adult	Quilichao, Cauca
CIAT 006	*M. anisopliae*	51.4 fghijkl	*Mahanarva* sp. nov.	Adult	Albania, Caquetá
Control[2]		25.1 m			

[1] Means followed by different letters are significantly different (P<0.05).
[2] Control of water plus tween (0.05%).

Although 79% of the isolates achieving >60% mortality were *Metarhizium*, this genus was also the most represented in the evaluation group, comprising 72% of the total isolates evaluated. The most virulent isolates of other genera were *Paecilomyces lilacinus* (CIAT 009) at 62.8% and *Fusarium* sp. (CIAT 025) at 53.5%. All isolates in the most virulent group (>60% mortality) originally came

from the Interandean Region (departments of Cauca and Valle) and the Amazonian Piedmont (department of Caquetá).

Discussion

The evaluated isolates demonstrate a broad range of virulence against *A. varia* adults. The most effective strains also represent a broad host and geographic range of Colombia. Although strains of *Paecilomyces* and *Fusarium* were not as effective as *Metarhizium*, these taxa should not be ignored since they were relatively under represented in the screened population. Moreover, different taxa may have other biological attributes that are desirable once the isolates are brought to the field. Virulence to other spittlebug species and life stages, growth characteristics for propagation, and tolerance to adverse field conditions are some of the many factors important to suppressing pest populations in the field.

The next phase of this investigation will address variation in virulence across different spittlebug species and life stages. Based on the results of the present studies, five isolates have now been chosen for the next studies. These include the three overall most virulent isolates (CIAT 055, CIAT 054, CIAT 007-C: *M. anisopliae* and two *Metarhizium* sp.) and the best *Paecilomyces* (CIAT 009) and *Fusarium* (CIAT 025) isolates.

Evaluation methodology for measuring virulence of fungal entomopathogens to spittlebug nymphs

Introduction

Advances in spittlebug management will depend on better knowledge of the nymphal life stage, which has been traditionally underemphasized relative to adults. For instance, nymphs are more difficult to survey in the field and manage in the lab, consequently CIAT's fungal entomopathogen collection has very few strains isolated from the immatures. Yet because nymphs account for about 70% of the generation time, requiring 5-7 weeks to complete development, there is a broader window of opportunity for certain management tactics. A rapid and reliable methodology now exists for screening fungal entomopathogens for virulence to spittlebug adults **(see Screening fungal entomopathogens for virulence to spittlebug adults - Pag. 95).** In order to (1) obtain more information about the effectiveness of isolates in the laboratory before advancing to a field phase, and (2) to gauge variation in virulence between life stages, a methodology was developed and evaluated for screening fungal entomopathogens for virulence to spittlebug nymphs.

Methods

Evaluation units were the same small-scale PVC tubes (1.5" diameter) now standard for host plant resistance screening. At 6 wk after planting with *Brachiaria ruziziensis* (CIAT 654), sufficient surface roots were 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. Concentrated conidial suspensions (10^8 conidias/ml) were prepared for four select isolates of *Metarhizium* (CIAT 005,

CIAT 053, CIAT 054, CIAT 055) and one of *Paecilomyces* (CIAT 012) in sterile water with tween (0.05%). Each of these isolates was recently reactivated in adults of *A. varia*. Applications were made with an airbrush and compressor (10 PSI) at a volume of 1 ml for substrate and nymph applications and <1 ml for direct egg applications.

Four experimental treatments were evaluated: application to eggs (in petri dishes, followed by infestation), application to substrate (in PVC unit before egg infestation), application to eggs and substrate, and application to nymphs (in PVC units 4 days after infestation). Each treatment had a corresponding control with water plus tween (0.05%). There were ten repetitions of each treatment. The units were fertilized with urea (2 g/l water) before infestation and 15 day later. Half of the repetitions were evaluated for surviving nymphs 15 days after infestation and the other half at 34 days (3-4 days before adult emergence in the control treatments).

Results

Mean mortality in the control nymphs was 24.6 and 40.3% at the early (15-day) and late (34-day) evaluation periods, respectively **(Table 2.6).** The high mortality in the late evaluation is probably attributed to an overly heavy infestation level on the susceptible *B. ruziziensis* host.

Although only five isolates were evaluated, remarkably high nymph mortality was achieved. When data were averaged across the early and late evaluation periods, the three most virulent treatment/isolate combinations achieved 84.3 (CIAT 055, egg+substrate application), 83.1 (CIAT 054, egg application) and 83.1% (CIAT 053, egg application) mortality. These three isolates are thereby considered equally promising for inclusion in future control trials.

Table 2.6. Virulence (% mortality) of different isolate/treatment combinations summed across the early and late evaluation periods to nymphs of *A. varia*.

Accession	Treatment	Virulence	Accession	Treatment	Virulence
CIAT 055	eggs+substrate	84.33	CIAT 055	Substrate	65.00
CIAT 054	eggs	83.11	CIAT 005	Substrate	64.69
CIAT 053	eggs	83.07	CIAT 005	Nymphs	63.70
CIAT 005	egg+substrate	80.00	CIAT 012	Nymphs	55.94
CIAT 053	nymphs	80.00	Control	egg+substrate	41.73
CIAT 054	egg+substrate	79.64	Control	Substrate	34.33
CIAT 053	egg+substrate	77.67	CIAT 012	Substrate	32.00
CIAT 055	nymphs	76.69	Control	Nymphs	28.89
CIAT 053	substrate	75.00	CIAT 012	egg+substrate	25.69
CIAT 055	eggs	73.78	CIAT 054	Substrate	22.00
CIAT 054	nymphs	71.85	Control	Eggs	20.00
CIAT 005	eggs	70.19	CIAT 012	Eggs	19.39

For the early evaluation period, virulence varied from 19.4-87.1% for the egg application, 22.0-54.0% for the substrate application, 34.5-80.0% for egg+substrate, and 55.3-68.7% for the nymph application (4-day) **(Figure 2.3).** Although complementary results were obtained from the late evaluation **(Figure 2.4)**, the high nymphal mortality in the control relative to the treatments makes interpretation difficult.

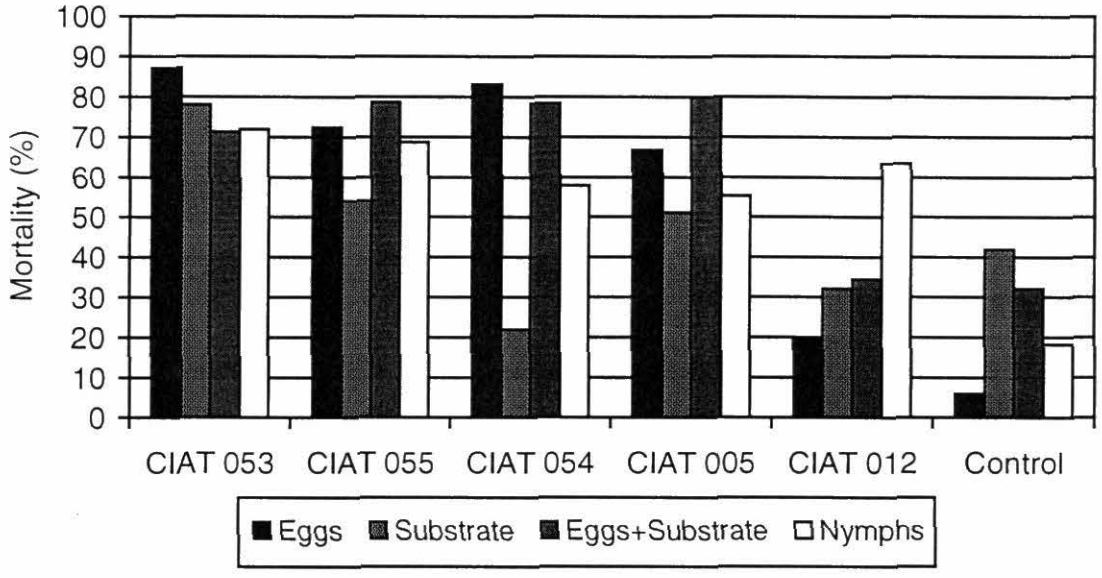

Figure 2.3. **Virulence (% mortality) of five fungal entomopathogen isolates and four application treatments to nymphs of *A. varia* 15 days after application to eggs or substrate.**

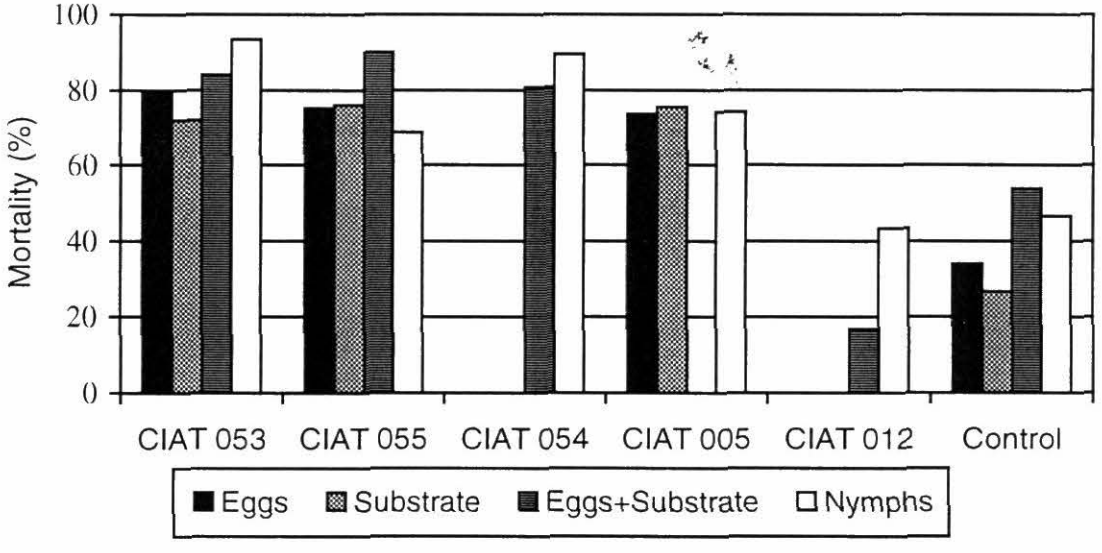

Figure 2.4. **Virulence (% mortality) of five fungal entomopathogen isolates and four application treatments to nymphs of *A. varia* 34 days after application to eggs or substrate.**

Among the four *Metarhizium* isolates averaged across the early and late evaluation periods, the egg application was highest for two isolates, while the combined egg+substrate application was highest for the other two. These results indicate that eggs about to hatch may be a more susceptible life stage than nymphs, and that direct application is more effective than application to the surrounding soil substrate.

Discussion

Although analysis of these data is incomplete, the results suggest certain adjustments in this methodology for future studies. Egg infestation levels will be reduced to seven (vs. ten) to promote higher survivability of the control nymphs through the late evaluation period. Second, to begin to elucidate the role of the spittle mass as a shelter from conidia, additional 5-day and 10-day post egg infestation treatments will be added. It is suspected that direct egg application will be the most effective treatment for screening virulence to spittlebug nymphs.

Evidence gathered in these experiments indicates that application of entomopathogens during the late egg stage is equally or more effective than application after egg eclosion. It is likely that when applied directly to the eggs, mortality is enhanced because emerging nymphs (1) more rapidly encounter conidia as they search for feeding sites, (2) are more susceptible to conidia establishment compared to early first instars that have already established spittle masses, and (3) are more rapidly affected by conidia than later instars due to smaller size and less protective integument. When the application is post eclosion, an effect of mortality might be delayed until later instars for the same reasons of differential susceptibility due to size and rate of contact with conidia.

These results indicate that in more seasonal environments, where early wet season emergence is relatively synchronous, predicating approximate time of egg eclosion will help target application of entomopathogen products. In less seasonal environments, where there is little population synchrony, entomopathogen strains should be sought that persist in the environment and thereby promote secondary contact between conidia and susceptible nymphal or adult life stages.

Recovery and maintenance of fungal and bacterial isolates entomopathogenic to cassava pests

Introduction

Developing biorational options for pest management in cassava will depend on the manipulation of natural enemies such as pathogens. Some fungal and bacterial pathogens of cassava insects have been examined in past studies at CIAT, but the material has been neglected and poorly maintained over the periods of research inactivity. In parallel with development of a new collection of fungal entomopathogens of spittlebugs in forage grasses, the objectives of this study were to (1) recover pathogen material relevant to cassava production and (2) integrate new material from recent field collections.

Methods

Efforts were made to recover a series of isolates used in prior studies at CIAT but neglected over a period of a few to several years during which they were stored under inappropriate conditions. All

fungal material was inoculated onto Sabureaud agar modified with yeast extract (1%) and lactic acid (1%) and propagated as necessary to discard contaminants and recover pure material which was then stored on sterile filter paper at -20°C **(see Identity, incidence and maintenance of spittlebug fungal entomopathogens - Pag. 92)**. A total of 34 isolates were put through this recovery process. Secondly, new strains were isolated from material recently collected in the field according to previously established methodology **(see Identity, incidence and maintenance of spittlebug fungal entomopathogens, Pag. 92).**

Results

Of the 32 fungal and 2 bacterial isolates put through the recovery process, 21 fungal and 2 bacterial isolates were deemed viable and recovered **(Table 2.7).** Many of these are currently being propagated for inclusion in the ceparium. Bacteria were the major contaminant of the fungal material. In four cases the strain could not be recovered because growth of fungal contaminants such as *Aspergillus* and *Penicilium* masked or suppressed the entomopathogenic strain. In the remaining five cases the material was deemed unviable because repeated inoculations of different culture media could not produce growth. This consequence is normal when fungal material is stored for an extended period without occasional reactivation on culture medium.

A total of 26 new isolates of fungal entomopathogens were obtained from specimens recently collected in the Colombian departments of Cauca, Risaralda and Tolima. Of these 8 belong to the burrower bug *Cyrtomenus bergi*, 17 to the stem borer *Chilomima klarkei*, and one to an unidentified white grub (Scarabaeidae).

Discussion

Only 21 of 32 fungal isolates were recovered after the extended period of neglect and poor storage conditions. It is therefore critical that this material be well-maintained to stay available as a resource for promoting biological control in cassava and other crops. The value of this material was further compromised because the relevant collection data was insufficient and unreliable. For instance, 19 fungal and 2 bacterial isolates were made on the same date (July 1994), from the same host (*C. bergi*) at the same site (Popayan), but no other data is available to ascertain whether these should be maintained as separate isolates.

A major challenge in maintaining the strength of resources such as the entomopathogen ceparium and the insect taxonomy collection is achieving continuity of management in the face of short term project structure and loss of core funding. Like most germplasm collections, maintenance is an ongoing investment of time and resources such as in the case of fungal entomopathogens where it is desirable to reactivate material on culture medium approximately every year. In addition, high quality functioning of this ceparium requires collection norms that ensure registry of all relevant field data.

Table 2.7. Host, origin and recovery status of fungal and bacterial isolates entomopathogenic to different cassava insects.

	Origin				Origin		
Host species	Dept.	Mcpo.	Status	Host species	Dept.	Mcpo.	Status
Aleurotrachellus socialis	undet.	undet.	I	*Cyrtomenus bergi*	Cauca	Popayan	I
Aleurotrachellus socialis	undet.	undet.	I	*Cyrtomenus bergi*	Cauca	Popayan	I
Aleurotrachellus socialis	undet.	undet.	I	*Cyrtomenus bergi*	Cauca	Popayan	I
Corinus sp.	Valle	La cumbre	D	*Cyrtomenus bergi*	Cauca	Popayan	C
Cyrtomenus bergi	undet.	undet.	D	*Cyrtomenus bergi*	Cauca	Popayan	D
Cyrtomenus bergi	undet.	undet.	I	*Cyrtomenus bergi*	Cauca	Popayan	I
Cyrtomenus bergi	undet.	undet.	I	*Cyrtomenus bergi*	Cauca	Popayan	I
Cyrtomenus bergi	Cauca	Popayan	D	*Cyrtomenus bergi*	Cauca	Popayan	C
Cyrtomenus bergi	Cauca	Popayan	S	*Erinnyis ello*	undet.	undet.	D
Cyrtomenus bergi	Cauca	Popayan	I	*Galeria mellonella*	Valle	Pradera	D
Cyrtomenus bergi	Cauca	Popayan	C	*Galeria mellonella*	Valle	Pradera	D
Cyrtomenus bergi	Cauca	Popayan	S	*Trialeurodes vaporariorum*	Valle	Pradera	I
Cyrtomenus bergi	Cauca	Popayan	D	*Trialeurodes vaporariorum*	Valle	Pradera	D
Cyrtomenus bergi	Cauca	Popayan	S	*Trialeurodes vaporariorum*	Valle	Pradera	I
Cyrtomenus bergi[2]	Cauca	Popayan	D	Tarantula	Valle	Palmira	I
Cyrtomenus bergi[2]	Cauca	Popayan	D	undet.	Valle	Palmira	I
Cyrtomenus bergi	Cauca	Popayan	S	undet.	undet.	undet.	D
Cyrtomenus bergi	Cauca	Popayan	S	undet.	undet.	undet.	D
Cyrtomenus bergi	Cauca	Popayan	I	undet.	undet.	undet.	C
Cyrtomenus bergi	Cauca	Popayan					

[1] Status: C = Contaminated, D = Drying, I = Inoculated onto culture medium, S = Sterile

[2] Unknown bacteria.

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Sub-Output 5. Disease Complexes Described, Characterized and Analyzed

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

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 980 bp. Restriction digestion with *Alu*I, *Msp*I, *Cfo*I, and *Dra*I of the product amplified for the ITS region showed different restriction patterns, which corresponded to the species tested. In this study, 10 *Phytophthora* species, obtained from different crops (**Table 1.1**), were identified by molecular techniques, based on ITS rDNA sequences.

Table 1.1. List of *Phytophthora* isolates obtained from different crops.

Isolate No.	Specie	Host
1	*Phytophthora cactorum*	*Fragaria*
44	*Phytophthora capsici*	*Manihot esculenta*
3	*Phytophthora capsici*	*Capsicum annuum*
4	*Phytophthora cinnamomi*	*Calluna*
5	*Phytophthora citricola*	*Medicago sativa*
6	*Phytophthora drechsleri/cryptogea*	*Asparagus officinalis*
7	*Phytophthora erythroseptica*	*Lycopersium esculentum*
8	*Phytophthora capsisi*	*Gerbera*
9	*Phytophthora megasperma*	*Rubus ideaw*
P12	*Phytophthora vignae*	*Manihot esculenta*
P4	*Phytophthora palmivora*	*Manihot esculenta*
11	*Phytophthora nicotianae*	*Nicotiana tabacum*

PCR-RFLP restriction patterns of rDNA

Amplification of the ITS region and the 5.8S ribosomal gene in all *Phytophthora* isolates generated fragments of equal size (980 bp) with ITS 1 and ITS 4 primers. From the 10 restriction enzymes evaluated, four—*Alu*I, *Msp*I, *Dra*I, and *Cfo*I—showed different band patterns that clearly differentiated the species (**Figures 1.1A, B, C, D, and 1.2**).

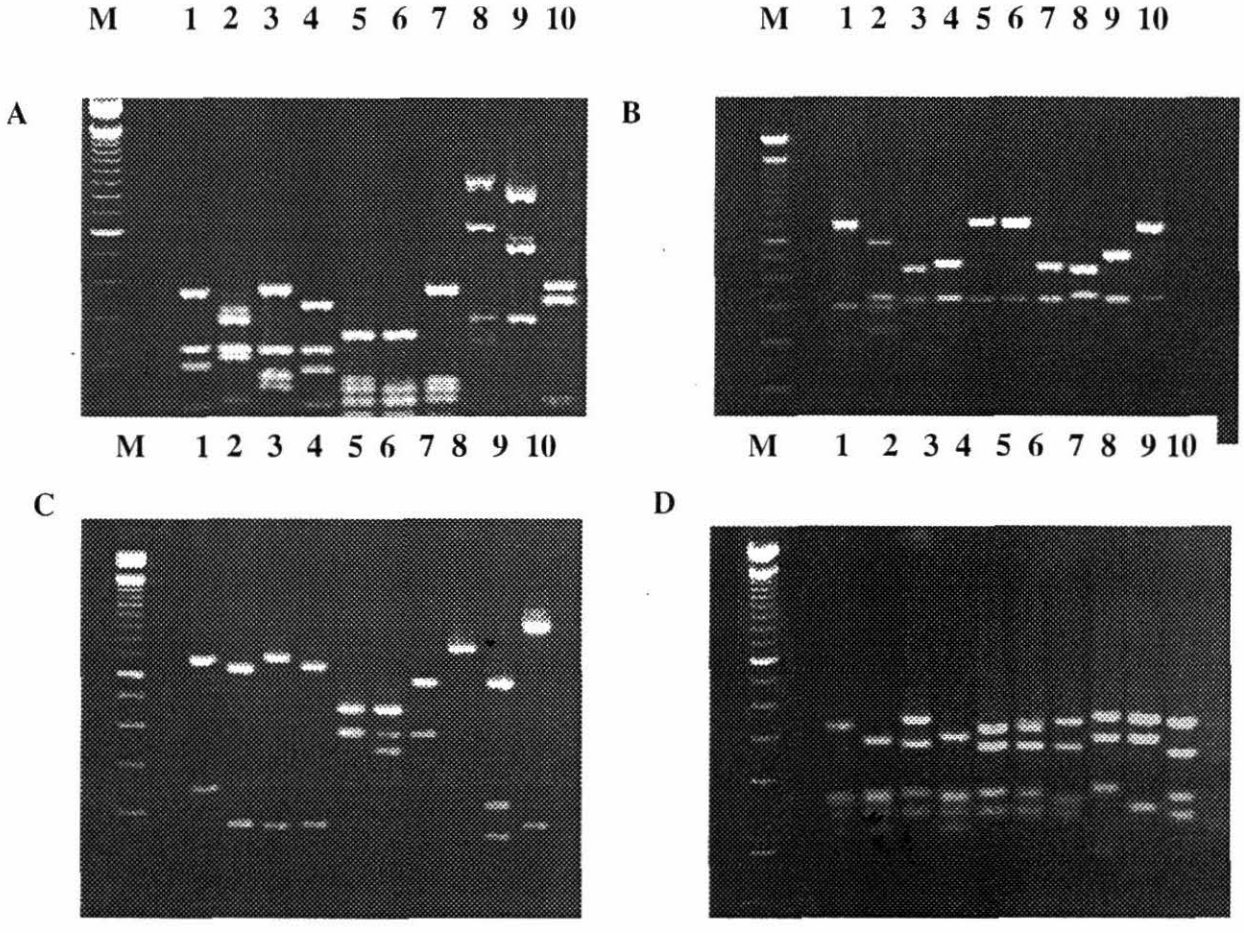


Figure 1.1. RFLP profiles observed in different isolates using the enzymes *Msp*I (A), *Alu*I (B), *Dra*I (C), *Cfo*I (D). Lane M= 100bp ladder (Gibco BRL) marker; lanes 1-8 *Phytophthora* spp.; lane 9= *Pythium* sp.; lane 10= *Phytophthora* sp.

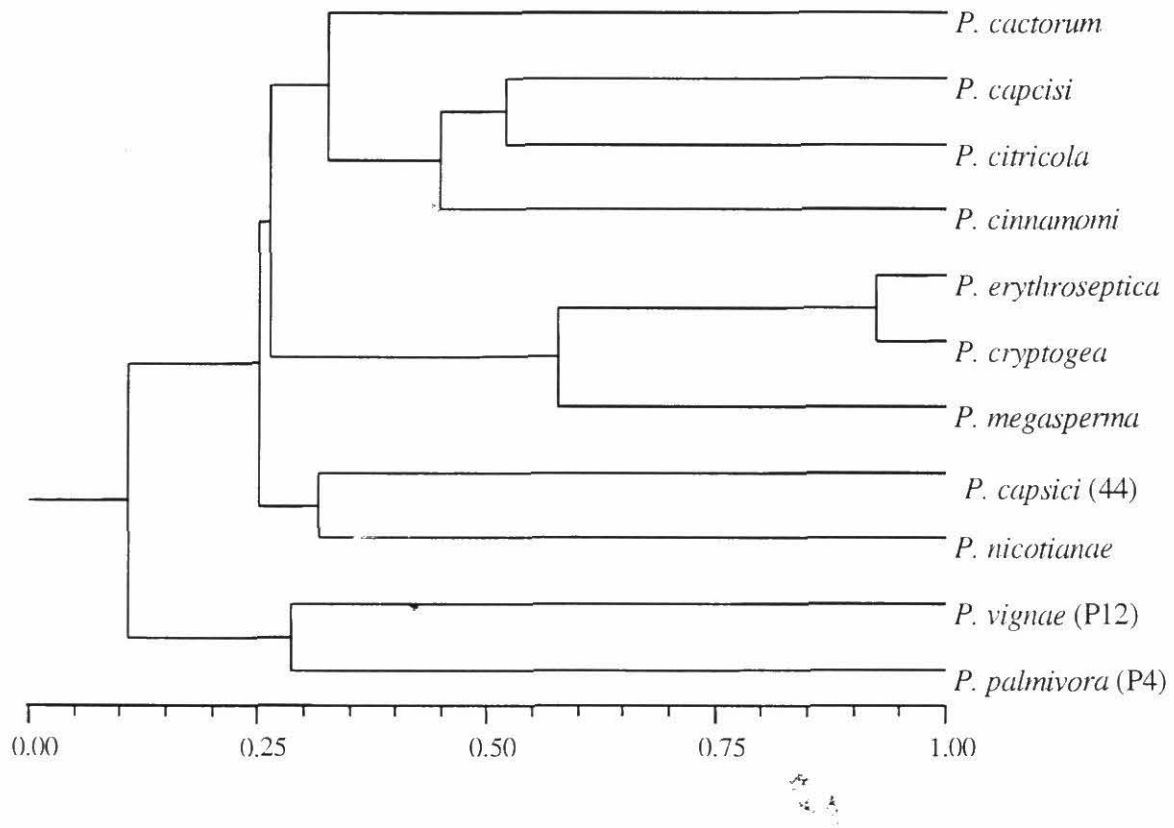


Figure 1.2. **Phenogram of *Phytophthora* species based on restriction patterns of the PCR-ITS products, using the enzymes *Alu*I, *Msp*I, *Dra*I. And *Cfo*I, The phenogram was constructed using the UPGMA method and DICE index, of the NTTSYS-pc package.**

Activity 2. Identifying *Phytophthora capsici* and a second-generation interspecific hybrid by isoenzyme analysis

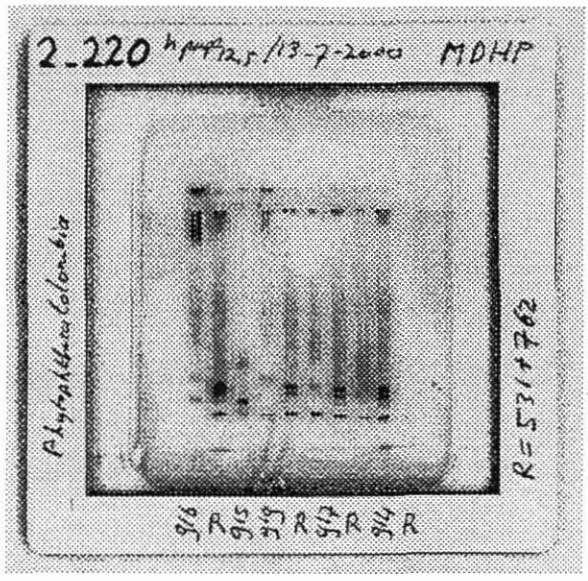
Distinguishing *Phytophthora* species according to cultural characters is difficult: many morphological characters overlap and a high level of phenotypical plasticity exists. Moreover, some isolates fail to develop sexual structures, making identification impossible. To find evidence for the hypothesis that some difficult-to-identify *Phytophthora* isolates in fact belong to two taxa, the possibilities of isozyme analysis were explored. This technique proved very successful in solving taxonomic problems (Oudemans et al. 1991; Man in 't Veld et al. 1998).

Isozyme analysis has the advantage over other techniques (e.g., ITS and mtDNA) in that it can detect sexual crosses between different species, particularly if dimeric enzymes are used. An extra band is created (the so-called heterodimeric band) when outcrossing has occurred (Plant Protection Service 1998, Annual Report). Because no theoretical basis exists on which to predict those enzymes that have diagnostic value, a random set of enzymes was explored.

A collaborative work was initiated between CIAT and the Plant Protection Service (PPS) at Wageningen, Netherlands. In a first study, five isolates of *Phytophthora* were characterized by isoenzyme analysis (**Figure 2.1**). By extracting with sand, different enzymes were obtained from fungal mycelia grown during 4 days in a Tryptone soya broth medium. After conservation at -80°C, the isozymes were visible by electrophoresis. Phenylmethylsulfonylfluoride (PMSF), a serine protease inhibitor, and glycerol were used as buffer solutions. Two enzyme activities, malate dehydrogenase peroxisomal (MDHP) and malate dehydrogenase (MDH), were useful for identifying the isolates 44 and Ají as *Phytophthora capsici.*

In a second study, the following isolates were identified: P4 as *P. palmivora* and P3700 as *P. cryptogea*. These results demonstrated that isozyme analysis provides an alternative for identifying *Phytophthora* species. Only the strain obtained from asparagus showed to be a second-generation interspecific *Phytophthora* hybrid.

A



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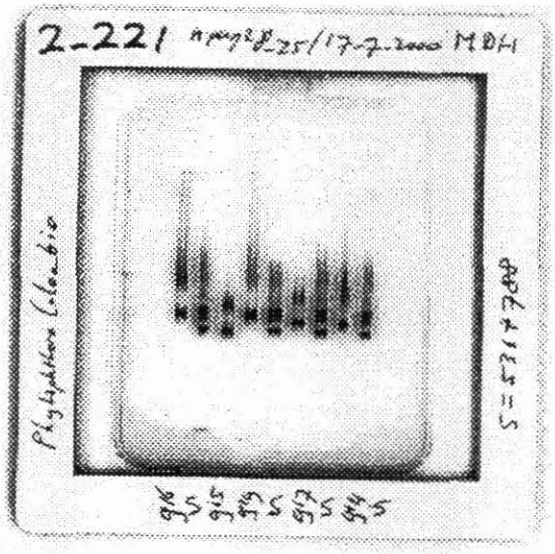

Figure 2.1. **Identifying two isolates of *Phytophthora capsici* obtained from cassava and hot pepper at Colombia. Other isolates are included as control. Lane 916 = isolate Gerbera (*P. cryptogea*) from *Gerbera jamesonii*; lane 915 = isolate P12 (*P. vignae*) from *Manihot esculenta*; lane 919 = *P. cryptogea/drechsleri*; lane 917 = isolate Ají (*P. capsici*) from *Capsicum annuum*; lane 914 = isolate 44 (*P. capsici*) from *Manihot esculenta*; R = reference ladder. (A) Malate dehydrogenase peroxisomal (MDHP); (B) malate dehydrogenase (MDH).**

Activity 3. Molecular characterization of *Phytophthora* and *Pythium* isolates obtained from cassava at different locations

Phytophtora and *Pythium* isolates were analyzed, using random amplified microsatellites (RAMS) and random amplified polymorphic DNA (RAPD), to detect variability in the fungi. **Table 3.1** lists 18 isolates that were obtained from infected tissue and soil samples from different host plants.

DNA isolation technique

DNA was isolated as described by Moller et al. (1992) and with modifications by Bardin et al. (1997). The protocol included mycelium disruption, two phenol/chloroform (1:1 v/v) extractions and one chloroform/isoamylic alcohol (24:1 v/v) extraction, and DNA precipitation in a volume of 100% isopropanol. The isolated DNA was resuspended in 25 µL of TE (10 mM Tris/HCL buffer (pH 8.0) containing 1 mM EDTA.

Table 3.1. List of isolates used in this study, several of their important morphological characteristics, and their pathogenicity.

Isolate No.	Identi-fication	Host	Geographical Origin	Source	Remarks[a]
1	5D	Cassava	Caicedonia, Valle	Rhizosphere	Growth fast. Pathogenic in roots. *Pythium.* Oospores pleurotic. Chlamydospores present.
2	5G	Cassava	Caicedonia, Valle	Rhizosphere	Pathogenic in roots and sprouts. *Pythium.* Growth above 35° C. Sporangia elongated.
3	3FE	Oil palm	Tumaco, Valle	Root	Chlamydospores present. *Pythium helicoides.*
4	51A	Cassava	Santander de Quilichao, Cauca	Rhizosphere	Growth slow. Many hyphal swellings. Pathogenic in roots and sprouts. *Pythium.*
5	27A	Cassava	Montenegro, Quindío	Rhizosphere	Growth fast. Pathogenic in roots and sprouts. *Pythium.*
6	10A	Cassava	Caicedonia, Valle	Rhizosphere	Growth slow. Chlamydospores present. Pathogenic in roots and sprouts. *Pythium.*
7	P12	Cassava	Brazil		Sporangia semi-papillated and ovoid.Oospores absent. Highly pathogenic in roots and sprouts. *Phytophthora vignae.*
8	T7II	Cassava	Caicedonia, Valle	Rhizosphere	Pathogenic in roots and sprouts. *Pythium.*
9	2B	Cassava	Caicedonia, Valle	Rhizosphere	Pathogenic in roots. *Pythium.*
10	69	Cassava	Buenaventura, Valle		Fast growth. Chlamydospores present. Highly pathogenic in roots and sprouts. *Pythium.* Sporangia papillated. Clamydospores.
11	44	Cassava	Barcelona, Quindío	Rhizosphere	Pathogenic in roots and sprouts. *Phytophthora capsici*
12	43	Cassava	Santander de Quilichao, Cauca	Rhizosphere	Oospores aplerotic. *Pythium.*
13	MTR-6	Cassava	Mitú, Vaupés		Growth slowly. *Pythium.* Sporangia elongated. Oospores aplerotic.
14	T7III	Cassava	Caicedonia, Valle	Rhizosphere	Chlamydospores abundant. Pathogenic in roots. *Pythium ultimum* or *Pythium irregulare.*
15	P4	Cassava	Colombia		Growth slowly. Sporangia and chlamydospores present Pathogenic in roots and sprouts. *Phytophthora palmivora.*
16	META	Cassava	Meta		Sporangia with elongated neck. Oospores markedly aplerotic. *Pythium.*
17	AJI	Hot pepper	Palmira, valle	Stem	Sporangia papillated. Clamydospores. *Phytophthora capsici*
18	16A	Cassava	Santander de Quilichao, Cauca	Root	Only pathogenic in roots. Chlamydospores large. Sporangia. Hyphal swellings present. *Pythium.*

[a] Morphology was studied on Cherry Agar Medium incubated at 22° C in the dark. Colonies were also studied in water cultures. Sporangia were induced by seeds of sweet pepper and soil extract. MEKA was used to identify of *Phytophthora* species. Pathogenicity was determined by inoculating whole cassava roots or sprouts from young plants of the cassava varieties M Bra 12, M Col 1505, HMC-1 and M Tai 8.

Reactions with RAMS

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. After the cycles, the reaction was ended with a 7-min extension at 72°C.

Reactions with RAPD

The thermal profile used for the primers designed for *Phytophthora* genetic studies was 95°C initial denaturation for 3 min; 44 cycles of denaturation at 95°C for 30 s, alignment at 57°C for 30 s, extension at 72°C for 2 min; and a final extension at 72°C for 10 min.

In both reactions, 5 ng of genomic DNA was used and the amplified products were loaded on 1.5% agarose gel, containing 0.5 mg/mL of ethidium bromide. Four RAMS microsatellites and eight RAPD primers yielded clear, intensive, reproducible, and polymorphic patterns (**Figures 3.1A, B**).

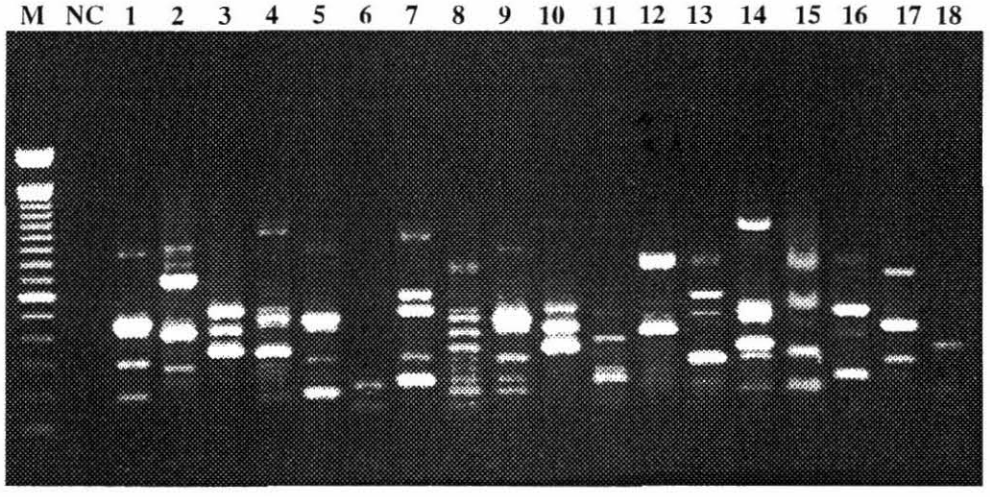

Figure 3.1A. PCR-RAPD product from individual isolates obtained with PNIC-1 primer.

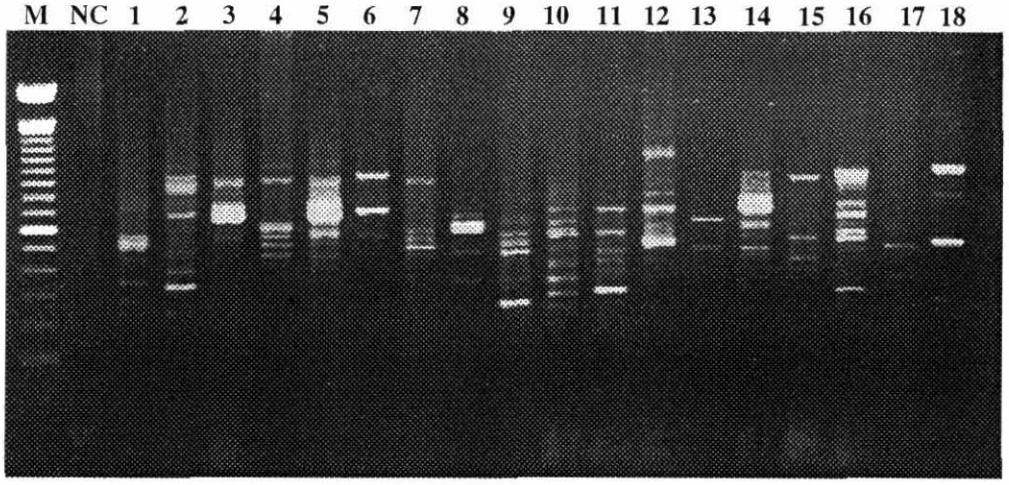

Figure 3.1B. RAMS profiles observed in different isolates, using the ACA primer.

Activity 4. Pathogenic and molecular characterization of brazilian isolates of *Sphaceloma manihoticola*

Introduction

Superelongation, caused by the fungus *Sphaceloma manihoticola*, sexual stage *Elsinoe brasiliensis*, is a major cassava disease in Central America, Colombia, Brazil, and Venezuela. Typical symptoms include weak stems; dieback; defoliation; necrotic leaf spots; cankers on leaf veins, petioles, and stems; leaf and stem distortion; and internode elongation in severely affected plants. Crop losses can be as high as 80%. By using molecular techniques, we aimed to determine (1) the pathogenic variability of fungal isolates from central-southern Brazil, and (2) their genetic variability.

Materials and Methods

Twenty isolates were collected in central-southern Brazil from cassava plants affected with superelongation disease and from *Euphorbia heterophylla*, another Euphorbiaceae species. Collection sites were Paranavaí (Paraná); Campo Grande (Mato Grosso do Sul); and Campos Novos, Assis, Platina, Cândido Mota, Echaporã, Conchal, and Campinas (all in São Paulo) (**Table 4.1**). Isolates were conserved at 4° C in inclined vials containing natural PDA.

Pathogenicity analysis. The cassava varieties M Bra 703 (susceptible) and M Bra 12 (resistant) were inoculated with these isolates and incubated at 30°C and 100% relative humidity for 5 days, then at 30°C and 98% until evaluations at 7, 14, and 28 days after inoculation. An experimental

design with eight replicates was used, where the main plots were varieties and subplots were isolates, and the experimental unit two plants. Isolates were clustered into four groups according to disease severity on the two inoculated varieties, using the Ward minimum variance analysis. Area under the disease progress curve (AUDPC) was also calculated.

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

Amplifying rDNA by PCR. The internal transcribed spacer (ITS) region of the gene 5.8S from ribosomal DNA was amplified, using a thermocycler (MJ Research, Watertown, MA), adjusted to the following program: (1) 95°C for 3 min; (2) 57°C for 30 s; (3) 72°C for 2 min; (4) 95°C for 30 s; (5) 24 cycles of steps 2 to 4; (6) 50°C for 30 s; and (7) 72°C for 10 min. The amplified segments were analyzed by electrophoresis in a gel comprising 1.5% agarose, TBE 0.5× buffer (Trizma-base, boric acid, EDTA, pH 8.0), and ethidium bromide at 10 mg mL^{-1}.

The electrophoresis chamber was maintained at a constant 90 V for 90 min. A 100-bp marker was included in each gel. A photo was taken under ultraviolet light, using an Eagle eye II image analyzer (Stratagene, La Jolla, CA).

Table 4.1. Origins of *Sphaceloma manihoticola* isolates used to study the fungus's genetic and pathogenic diversity. The fungus causes superelongation disease in cassava (*Manihot esculenta* Crantz).

Isolate	Germplasm	Plant part	Geographic origin[a]
1	*Euphorbia heterophylla*	Stem	Campos Novos, SP
2	Fibra	Leaf	Campos Novos, SP
3	IAC-13	Leaf	Campos Novos, SP
4p	IAC-13	Petiole	Paranavaí, PR
5	IAC-13	Leaf	Assis, SP
6	*Euphorbia heterophylla*	Stem	Conchal, SP
7h	Roxinha	Leaf	Platina, SP
7p	Roxinha	Petiole	Platina, SP
8	IAC-13	Leaf	Candido Mota, SP
9	IAC-12	Leaf	Conchal, SP
10	B. S. Catarina	Leaf	Conchal, SP
11p	Fibra	Petiole	Conchal, SP
12	IAC-13	Leaf	Conchal, SP
13h	Roxinha	Leaf	Echaporá, SP
14	Roxinha	Leaf	Campos Novos, SP
15	B. S. Catarina	Leaf	Campos Novos, SP
Bh	Unknown	Leaf	Campo Grande, MS
25h	F 1047	Leaf	Campinas, SP
26h	Clone under selection	Leaf	Campinas, SP

[a] Brazilian states: SP = São Paulo; PR = Paraná; MS = Mato Grosso do Sul.

Restriction enzymes. For restriction analysis, 11 enzymes were used. a sample of 15 µl of the product from the pcr reaction was taken, and 2 µl of a buffer of 10× enzyme and 1 µl of restriction enzyme were added. The suspension was incubated for 16 h at an average temperature of 37°c, after which 2 µl of running buffer was added. A gel, comprising 1.5% agarose, tbe 1× buffer, and ethidium bromide at 10 mg mL^{-1}, was then placed in an electrophoresis chamber for 2 h at a constant 90 V.

Random amplified polymorphic DNA (RAPD). The RAPD technique was identical to that described for the PCR but, instead of using ITS primers, 12 decaprimers that randomly amplified genome fragments of DNA samples of *S. manihoticola* were used.

Data analysis. The electrophoretic patterns of DNA were quantified, using as criterion the presence or absence of bands. To estimate genetic relationships between isolates, a phylogenetic tree was constructed, using the UPGMA method with the SAHN and TREE options of the NTSYS-pc 2.01 (F.J. Rohlf, Exeter Software, New York).

Results

Symptoms appeared on both cassava cultivars M Bra 703 (susceptible in the field) and M Bra 12 (resistant). Cultivar M Bra 703 was susceptible to 68.4% of the isolates of the pathogen, intermediately resistant to 26.3%, and tolerant of 5.3%. Cultivar M Bra 12 was tolerant of 36.8% of the isolates, intermediately resistant to 36.8%, and susceptible to 26.4% **(Figure 4.1)**.

The ITS region was amplified with primers ITS4 and ITS5. For all isolates, a homogeneous band of about 645 bp **(Figure 4.2)** was observed. The product generated by the PCR technique was digested with the endonucleases *Cfo*I, *Msp*I, *Hinf*I, *Hae*III, and *Taq*I, presenting similar patterns of bands for all isolates and for each enzyme **(Figures 4.3a, b)**. Twelve primers were evaluated, of which OPA-01, OPA-02, and OPA-03 were selected because they showed reproducible bands in most isolates **(Figure 4.4)**. Polymorphism with these single primers differentiated the isolates **(Figure 4.5)**.

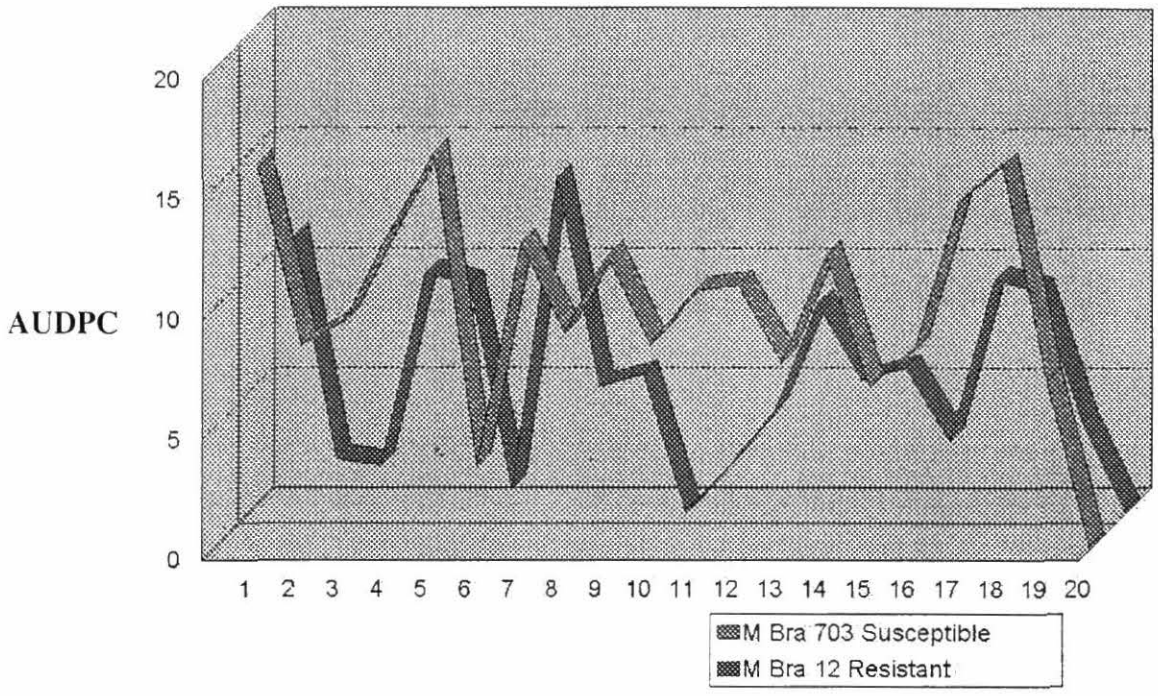

Figure 4.1. Two varieties of cassava (*Manihot esculenta* Crantz) are compared for their susceptibility to superelongation disease. AUDPC = area under the disease progress curve.

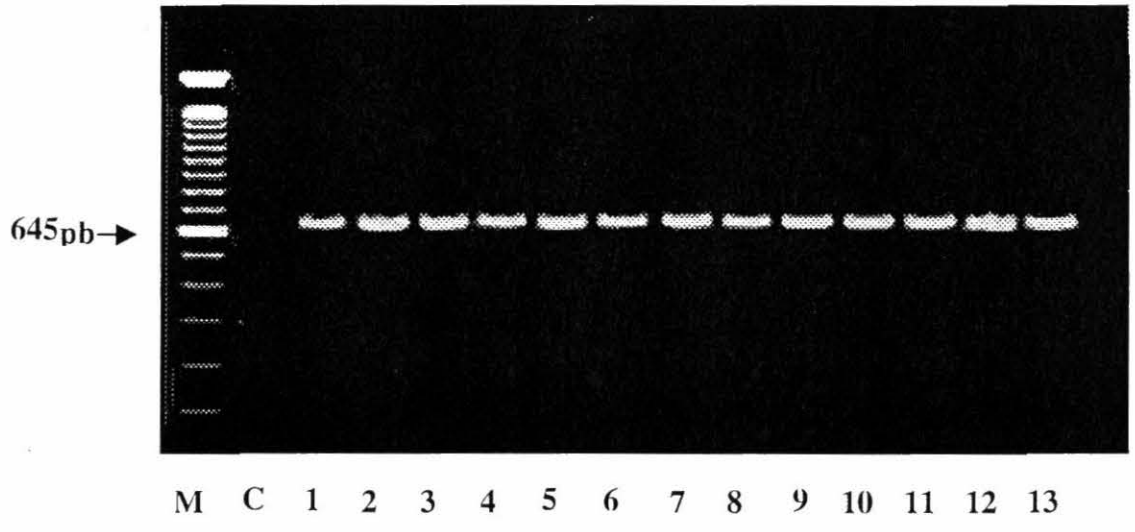

Figure 4.2. Amplification of the ITS4 and ITS5 regions of ribosomal DNA from the fungus *Sphaceloma manihoticola*. M = marker (100 bp); C = control; lanes 1 to 13 = isolates from Campos Novos, Paranavaí, Assis, Platina, Candido Mota, Conchal, and Echaporá, Brazil; lane 13 = Campos Novos.

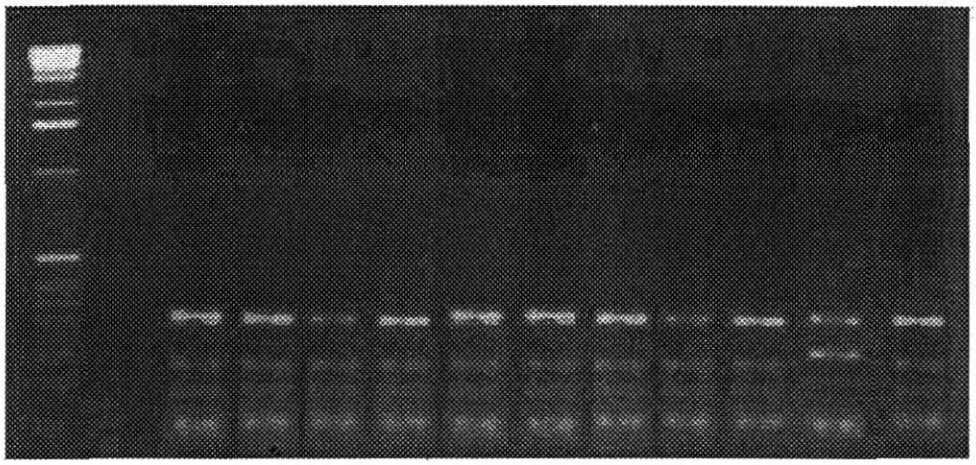

Figure 4.3a. Electrophoresis of ITS fragments digested with the endonuclease *Msp*I. M = marker (100 Bp); C = control; lanes 1 to 11 = isolates from Campos Novos, Paranavaí, Candido Mota, Conchal, and Echaporá, Brazil.

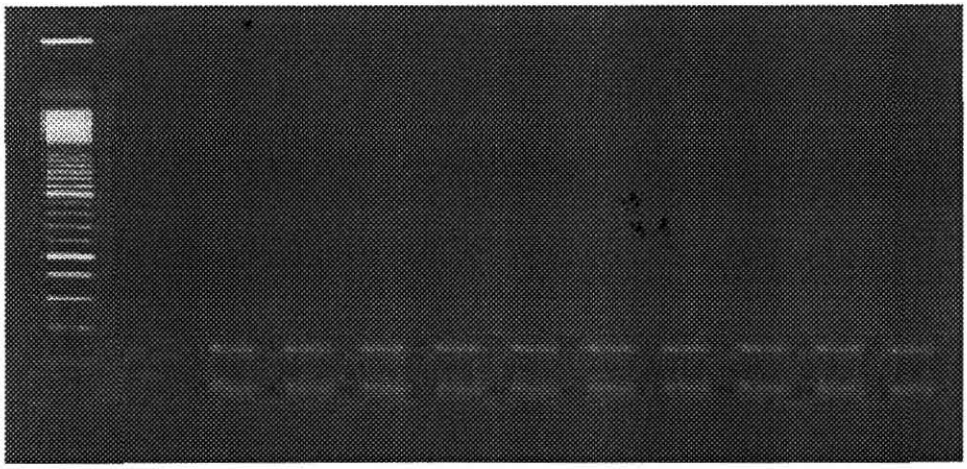

Figure 4.3b. Electrophoresis of ITS fragments digested with the endonuclease *Cfo*I. M = marker (100 bp); C = control; lanes 1 to 11 = isolates from Campos Novos, Paranavaí, Candido Mota, Conchal, and Echaporá, Brazil.

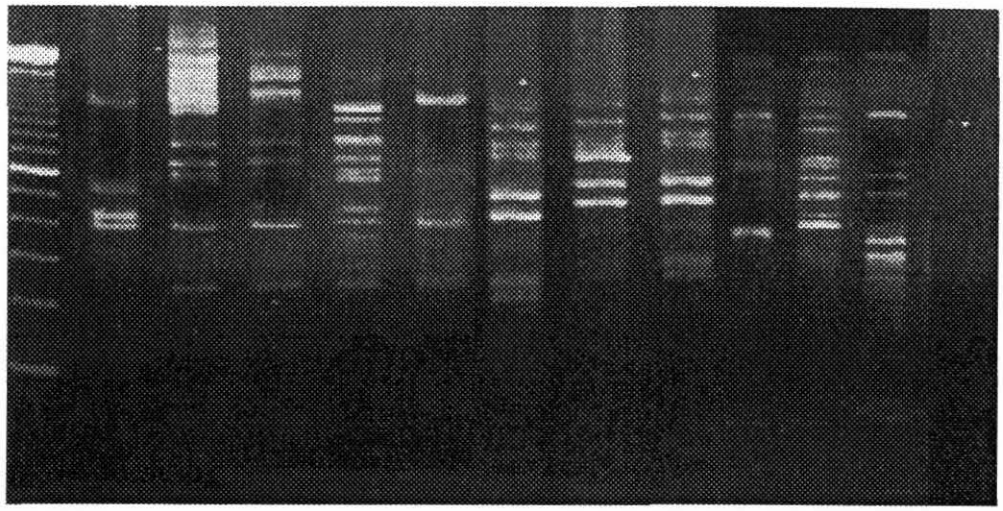

Figure 4.4. Patterns of bands obtained with the primers OPA-01 (lanes 1 to 4), OPA-02 (lanes 5 to 7), and OPA-03 (lanes 8 to 11). Isolates were collected from Campos Novos, Paranavaí, Conchal, and Platina, Brazil. M = marker (100 bp); C = control.

In the dendrogram constructed by RAPDs the isolates are seen to separate into five groups with a similarity level of 0.6. One primer group is formed by the isolates 1 and 6, which originated from *Euphorbia heterophylla* (Euphorbiaceae), collected in the State of São Paulo. This plant species colonizes cassava crops and is host to the pathogen.

The second group is formed by isolates from the States of São Paulo, Paraná, and Mato Grosso do Sul. The isolates 4p and 3, obtained from the variety IAC-13 in Paranavaí (Paraná) and Campos Novos (São Paulo), demonstrated their similarity. The isolates BH of Campo Grande (Mato Grosso do Sul) and 26H from Campinas (São Paulo) separate from this group, presenting a third genetic line.

A fourth group is formed by the isolates 12, 15, 13H, 14, and 25H, found on different varieties and collected from various municipalities of the State of São Paulo. The last, fifth, group is formed by the single and different isolate 10 from Conchal, also of the State of São Paulo.

Within some of the different analyzed groups, isolates diverged little from each other, for example, 1 and 6, 4p and 3, 9 and 11p, 7H and7p. However, other isolates showed smaller similarity indexes: 8 and 5, 13H and 14, 10 and 25H.

The results obtained with RAPDs demonstrate polymorphism among isolates, indicating the pathogen's genetic diversity.

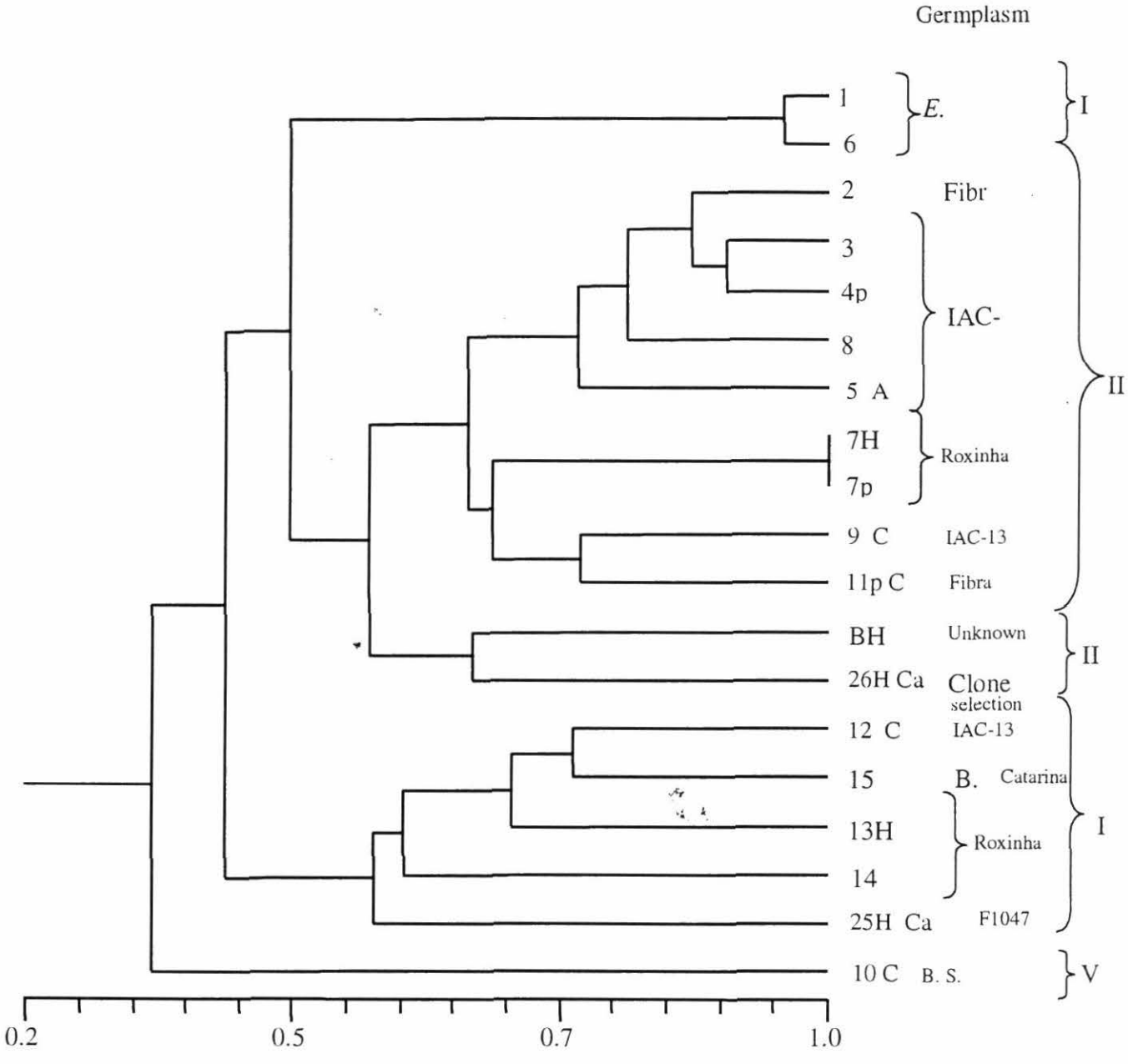

Figure 4.5. **Dendrogram of 20 isolates of *Sphaceloma manihoticola*, a fungus that causes superelongation disease in cassava (*Manihot esculenta* Crantz). CN = Campos Novos (São Paulo); P = Paranavaí (Paraná); A = Assis (São Paulo); C = Conchal (São Paulo); Pl = Platina (São Paulo); CM = Candido Mota (São Paulo); Ech = Echaporá (São Paulo); CG = Mato Grosso do Sul (São Paulo); Ca = Campinas (São Paulo). (RAPSs).**

Identification of Virulence phenotypes of *Sphaceloma manihoticola* from different regions in Brazil.

Virulence variation was determined by inoculating through wounding sprouts of 15 cassava genotype differentials. The inocula were six selected isolates from different regions of Brazil. The inoculated sprouts were incubated for 5 days at 95% relative humidity and 27°C, then transferred to the greenhouse and observed for symptom development for 7, 14, 21, and 28 days after inoculation.

Based on the 15 cassava genotype differentials, 6 isolates were grouped into 5 pathotypes. 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 are in highly susceptible groups and the most susceptible is M Bra 237. The most tolerant genotype was CM 2177-2. (**Table 4.2**). Some correlation between sample source, geographical origin, virulence variation, and DNA polymorphism was also observed.

Table 4.2. Characterization of *S. manihoticola* by virulence, RAPD and RAMS.

Isolate	Origin at Brazil	Genotype susceptible[a]	% Infection	AUDPC[b]	Severity group[c]	RAPD Group	RAMS Group
Bh	Campo Grande, MS	7, 8, 9, 11	26	46.47	S	III	III
1	Campos Novos, SP	4, 8, 9, 11	26	43.31	I	I	I
4p	Paranavai, PR	1, 2, 3, 4, 5, 7, 8, 9, 10, 11	66	55.12	HS	II	III
5	Assis, SP	6, 7, 8, 9	60	50.40	R	II	III
7h	Platina, SP	6, 7 ,8, 9, 10, 11, 12, 13, 14, 15	66	55.91	HS	II	I
25h	Campinas, SP	2, 3, 4, 5	73	53.10	HS	IV	III
Control		-		24.95	HR		

a. Inoculated cassava varieties:

1 = M Bra 703	2 = M Bra 97	3 = M Bra 12	4 = M Bra 917	5 = M Bra 886
6 = Brasileira Juan 1	7 = M Nga 2	8 = M Bra 1044	9 = M Bra 237	10 = M Bra 1045
11 = M Col 2215	12 = C 6915-1	13 = CM 3306-9	14 = CM 3306-4	15 = CM 2177-2

b. Area below the curve of the progress of the disease.

c. Groups of susceptibility of the inoculated cassava varieties: HS = highly susceptible; S = susceptible; I = intermediate; R = resistant; HR = highly resistant.

References:

Alvarez E; Molina ML. 2000. Characterizing the *Sphaceloma* fungus, causal agent of superlongation disease in cassava. Plant Dis 84(4): 423–428.

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

Zeigler RS; Alvarez E; Lozano JC. 1983. Characteristics of cassava resistance to superelongation disease (*Elsinoe brasiliensis*). Trop Pest Manage 29(2): 148–158.

Activity 5. Molecular fingerprinting of *Sphaceloma manihoticola*, using amplified fragment length polymorphism (AFLP) and random amplified microsatellite (RAMS)

Amplified fragment length polymorphism (AFLP) of *Sphaceloma manihoticola*

The AFLP technique is based on the amplification of subsets of genomic restriction fragments, using PCR. DNA is cut with restriction enzymes, and double-stranded adapters are ligated to the ends of the DNA-fragments to generate template DNA for amplification. The sequence of the adapters and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments. Selective nucleotides are included at the 3' ends of the PCR primer, which therefore can only prime DNA synthesis from a subset of the restriction sites. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotides will be amplified (Vos et al. 1995)

The study aimed to develop an efficient DNA fingerprinting protocol for *Sphaceloma manihoticola* and to use AFLP markers for analyzing the genetic diversity in single-spore cultures of *Sphaceloma manihoticola*. Seven isolates from different zones in central-southern Brazil and Colombia were evaluated with two combinations of primers: EAC/MA and EAC/MC, which were selected for being able to detect a high number of polymorphic bands (**Figure 5.1**).

The primer sequence complementary to the *Eco*RI end of the DNA template, E + AC (5'-GACTGCGTACCAATTCAC-3'), was used in combination with the *Mse*I primer M + C (5'-GATGAGTCCTGAGTAAC-3'). Another *Eco*RI primer, E + AC (5'-GACTGCGTACCAATTCAC-3') was used in 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). The AFLP-DNA fingerprinting protocol (Gibco BRL, AFLP Analysis System for Microorganisms; Microorganism Primer Kit) was optimized for *Sphaceloma manihoticola*.

The RAMS technique, originally described by Zietkiewicz et al. (1994), can be applied to fungi. The technique combines most of the benefits of the RAPD and microsatellite analyses, and is therefore promising for studies on genetic variation. In RAMS analysis, the DNA between the distal ends of two closely located microsatellites is amplified and the resulting PCR products are separated electrophoretically (Hantula et al. 1997). In this study, we tested the homogeneity of *S. manihoticola* species by studying isolates from different host plants.

Genetic variation was considerably high when comparing variation among different *S. manihoticola* isolates. Results are shown in **Figure 5.2**, where the patterns obtained with the ACA primer are presented. Similar results were obtained with other primers.

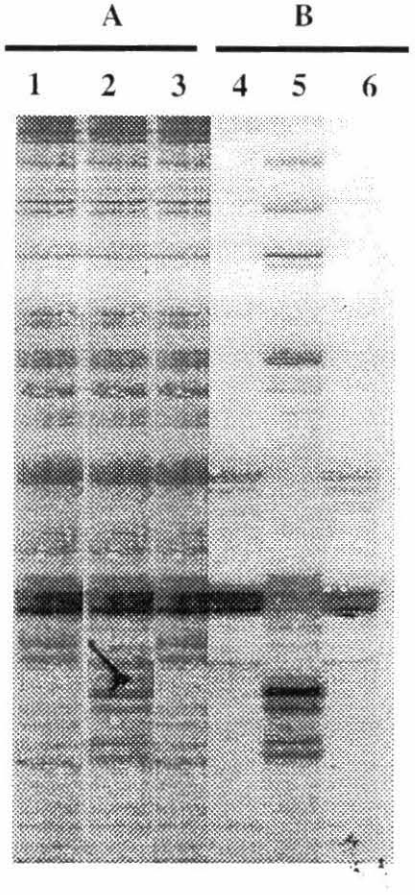

Figure 5.1. Illustration of the principle of *Eco*RI-*Mse*I fragments. AFLP fingerprints of *Sphaceloma manihoticola* DNA were made, using primer combinations with a single selective base for the *Eco*RI primers and two selective bases for the *Mse*I primer. Panels A and B refer to the primer combinations EAC/MA and EAC/MC, respectively.

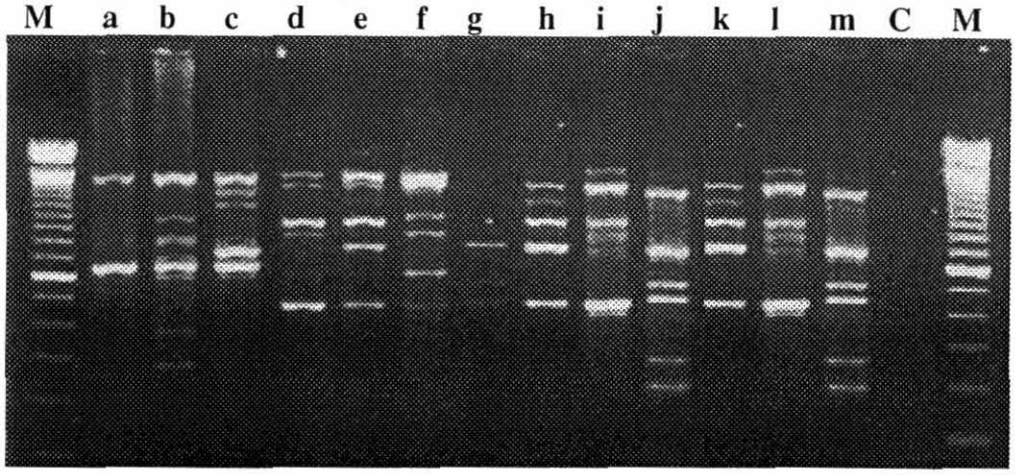

Figure 5.2. **Patterns of bands obtained with the ACA primer. Isolates were collected from (a to g) Campos Novos, Paranavaí, Conchal, and Platina, Brazil, and (h to m) Puerto López, Granada, and Carimagua, Colombia. C = control; M = arker (100 bp).**

The patterns of the amplified products obtained with the three primers showed variation within *Sphaceloma*. Ten polymorphic bands were detected in patterns obtained with the CCA primer. Four of these were polymorphic. The area below 550 pb consisted of two banding patterns. Two polymorphic markers were observed in the area between 800-1000 pb (**Figure 5.3**). The area within 200-500 pb contained a same-size marker for all isolates but with differences in the intensity of bands. In general, the four primers evaluated showed specific band patterns for the species tested.

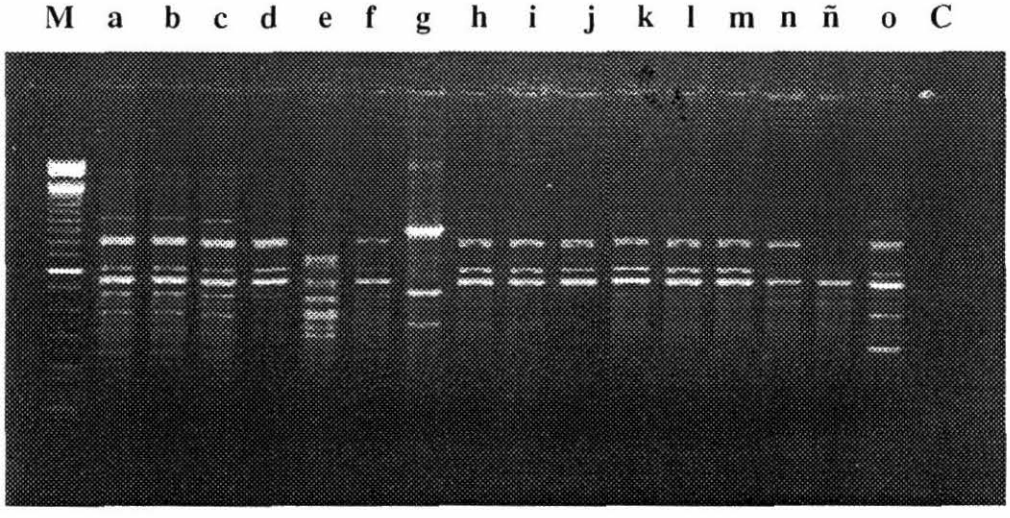

Figure 5.3. **RAMS profiles observed within the isolates of *Sphaceloma manihoticola* with CCA primer. Isolates were collected from (a to g) Campos Novos, Paranavaí, Conchal, and Platina, Brazil, and (h to o) Puerto López, Granada, and Carimagua, Colombia. C = control; M = marker (100 bp).**

The RAMS technique showed polymorphisms between isolates of the same municipality, between municipalities, and between countries, which indicates that genetic diversity of the pathogen exists. Four genetic lineages were observed, the first containing a Brazilian (São Paulo) isolate from *E. heterophylla*. The second lineage comprising Brazilian (São Paulo) isolates from *Manihot esculenta* for which differences were found between the regions Echaporá, Conchal, Candido Mota, Campos Novos, and Platinia.

The third lineage is also homogenous, comprising mainly isolates from Carimagua, Puerto López, Granada, and ICA—La Libertad (Meta, Colombia).

The fourth lineage, containing isolates from Colombia and Brazil, is heterogeneous and differentiates between the Departments of Cauca and Bolívar and the States of São Paulo, Mato Grosso, and Paraná. By analyzing a dendrogram **(Figure 5.4)**, the isolates from each country can be separated into three groups: (1) 2Sm, 3Sm, 7HSm, and 7pSm; (2) 14Sm and 15Sm; and (3) 9Sm, 12Sm, 11pSm, and 13H. The isolates 1Sm and 9Sm form an independent group; isolate 10Sm groups with isolate 1 from different municipalities in Colombia.

For the two isolates 136 and 41, from the Department of Meta, Colombia, two groups can be formed, with one isolate each. Isolates from Assis (São Paulo) and Paranavaí (Paraná) are similar, and are related to isolate 82 from Santander de Quilichao (Cauca, Colombia) and Carmen de Bolívar (Bolívar, Colombia).

Isolate 26H from the municipality Campinas (São Paulo) is similar to the isolate BH of Campo Grande (Mato Grosso do Sul). Similar isolates were found originating from different municipalities and countries, thus suggesting movement of planting materials between municipalities and therefore of the pathogen's migration and interchange of genes.

References

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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.

Zietkiewicz E; Rafalski A; Labuda D. 1994. Genome fingerprinting simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176-183.

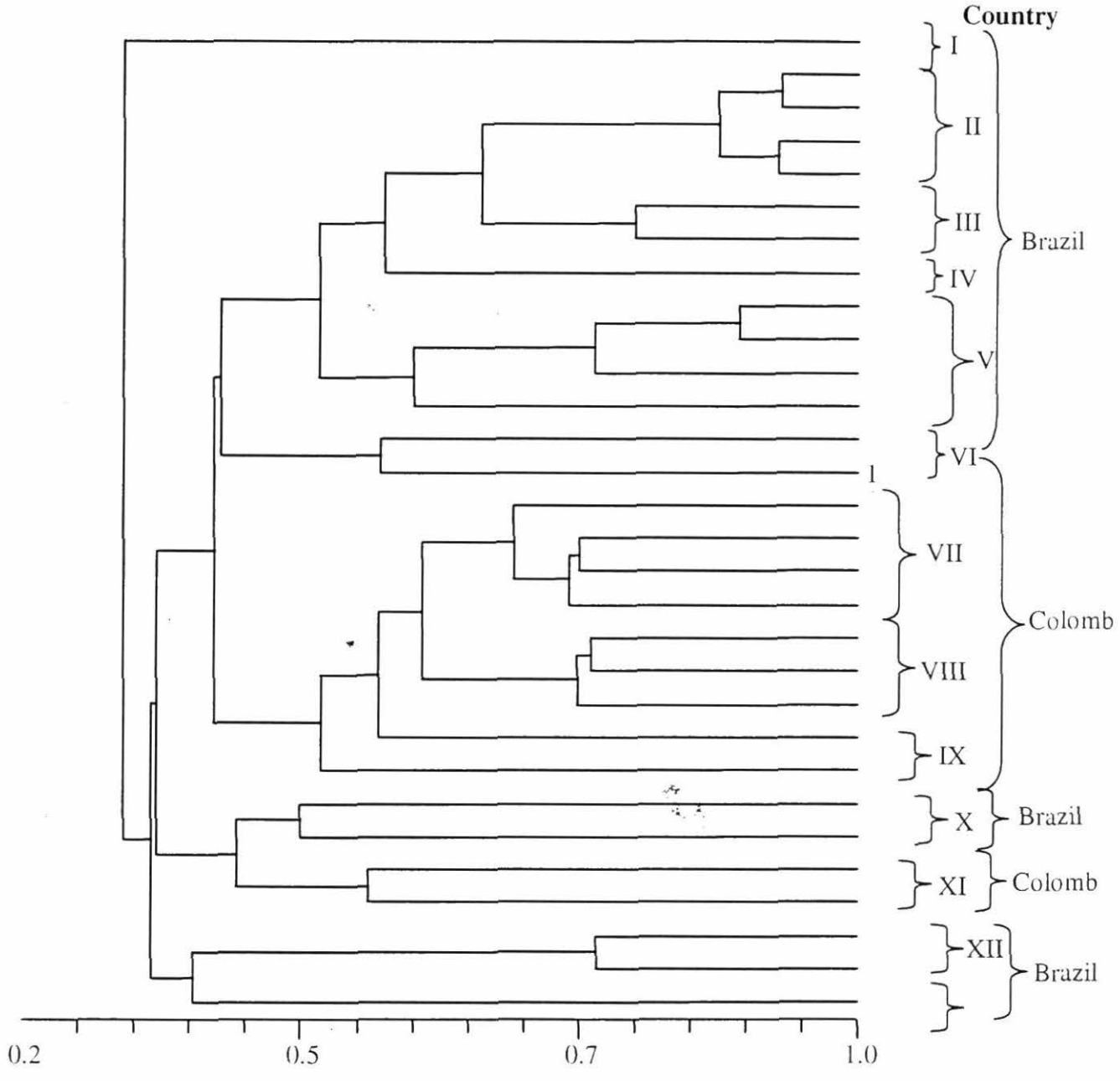

Figure 5.4. **Dendrogram of 19 isolates of *Sphaceloma manihoticola*, a fungus that causes superelongation disease in cassava (*Manihot esculenta* Crantz), using random amplified microsatellites (RAMS).**

Activity 6. Genetic diversity and pathogenicity of *Sphaerotheca pannosa* var. *rosae* causing powdery mildew of roses in Colombia

Introduction

Powdery mildew, a disease caused by the fungus *Sphaerotheca pannosa* (Wallr. Fr.) Lév. var. *rosae* Woronichin, is a major constraint to rose production. In Colombia, this disease is widely distributed, affecting the quality of *Rosa* spp. grown in the greenhouse and causing serious economic losses. The molecular and pathogenic characterization of isolates will generate information on the pathogen's genetic structure, thereby helping to make the most appropriate and durable crop management decisions.

Materials and Methods

Collecting isolates. Isolates were obtained from cultivars Aalsmeer Gold, Charlotte, Classy, Konfetti, Livia, and Tineke, all grown on farms located in the municipalities of Suesca, Zipaquirá, Gachancipá, Cota, and Madrid in the Department of Cundinamarca. This geographical region, where most export roses are cultivated, is known as the Sabana de Bogotá.

Analyzing pathogenicity. Pathogenicity was evaluated weekly by counting the number of colonies of *S. pannosa* var. *rosae* found on the first three true leaves of each inoculated plant. Four evaluations were carried out. The area under the disease progress curve (AUDPC) was calculated for each isolate. The data obtained were analyzed by clustering, using the Ward technique, based on minimum variance.

Extracting DNA. DNA was extracted from conidia stored in gelatin capsules, using Chelex 100, as described by Walsh et al. (1991). With the help of a stereoscope, a constant small quantity of conidia was taken for each isolate and deposited in a 1.5-mL Eppendorf tube. To each sample, 5% Chelex 100 resin was added and the suspension vigorously shaken in a vortex for 5 s, then incubated at 56°C for 2 h, and mixed again in the vortex for 5 s. The tubes were placed in a hot water bath at 98°C for exactly 8 min, then agitated once more for 5 s, and later centrifuged at 15,000 rpm for 5 min. The supernatant was transferred to another tube for amplification.

Amplifying rDNA by PCR. The reaction was performed in a final volume of 25 µL, containing 0.2 mM of each nucleotide, 3 mM $MgCl_2$; and 0.5 mM of each of the primers ITS 1 and ITS 2, 1× buffer for the enzyme *Taq* polymerase, diluting everything in sterilized distilled water. Amplification was performed in a thermocycler (MJ Research, Watertown, MA), using the following thermal profile: (1) preliminary denaturation at 94°C for 1 min; (2) a 30-s denaturation at 94°C; (3) a 30-s coupling at 55°C; (4) a 1-min extension at 72°C; (5) 45 cycles of steps 2 to 4; and (6) a final extension of 8 min at 72° C. The amplified products were separated by electrophoresis in 1.5% agar gel (w/v) in a TBE 0.5× buffer for 5 h at a constant 70V. A Gibco-BRL marker of 100 bp was placed in each gel. The gels were stained with ethidium bromide at 10 mg/mL^{-1}, then photographed under ultraviolet light, using an Eagle Eye II image analyzer (Stratagene, La Jolla, CA).

Restriction of amplified fragments (ITS-RFLP). Six restriction enzymes were tested for analysis. A 15-µL sample of PCR-amplified product was digested with a mixture of 1 µL restriction enzyme and 2 µL of 10× buffer. The suspension was incubated for 16 h at 37°C. The samples were submitted to electrophoresis for 4 h in 1.5% agarose gels at a constant 70 V, and detected by staining with ethidium bromide.

Results

Differences among isolates infecting flower buds showed pathogen specialization toward specific plant tissues. Incidence of isolates in cvs. Konfetti and Livia fluctuated between 5 and 73 colonies per plant. An average of 11 leaflets per plant were infected, indicating that the inoculum was infectious and conditions optimal. Cultivar Livia and an unidentified cultivar from Palmira proved the most susceptible, whereas cvs. Classy and Charlotte were the most resistant (**Figure 6.1**). For the variable pathogenicity, interaction was observed between isolate, cultivar, and origin. Results indicated that an interaction existed between host cultivar and pathogen.

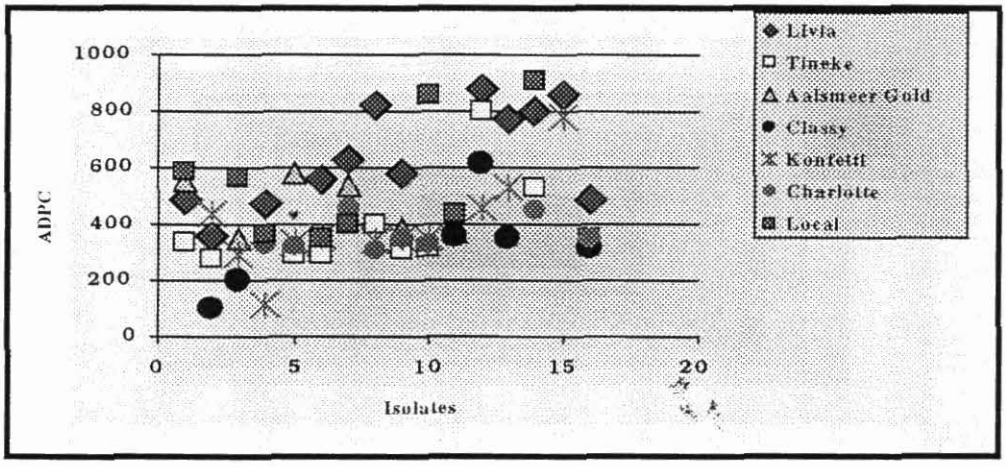

Figure 6.1. Pathogenic diversity detected in a population of *Sphaerotheca pannosa* var. *rosae* on inoculated plants in a growth chamber. Isolate origin: Sp1-Sp5 = Madrid; Sp6-Sp8 = Zipaquirá; Sp9 and Sp10 = Cota; Sp11-Sp15 = Suesca; and Sp16 = Gachancipá.

Isolate Sp6 did not cause significant damage in cv. Tineke, suggesting the existence of two races or pathotypes within the population analyzed.

The amplification of the ITS region and the 5.8S ribosomal gene in analyzed isolates of *S. pannosa* var. *rosae* generated fragments of equal size (296 bp) with the ITS 1 and ITS 2 primers (**Figure 6.2**).

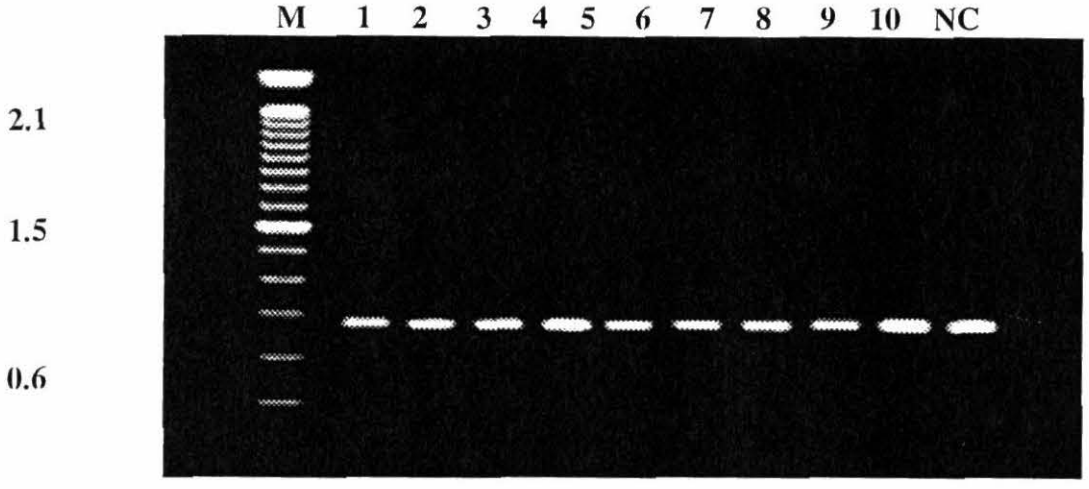

Figure 6.2. **Amplification of the ITS region (ITS1 and ITS2) of ribosomal DNA of the fungus *Sphaerotheca pannosa* var. *rosae*. M = marker of molecular size 100 pb (value in kilobases); lanes 2 to 11 = isolates of *S. pannosa* var. *rosae*; lane NC = negative control.**

Of six restriction enzymes known to digest the amplified product of *S. pannosa* var. *rosae* isolates, enzymes *Alu*I and *Hind*I totally digested the amplified DNA product; with two constant bands obtained per isolate (**Figure 6.3**).

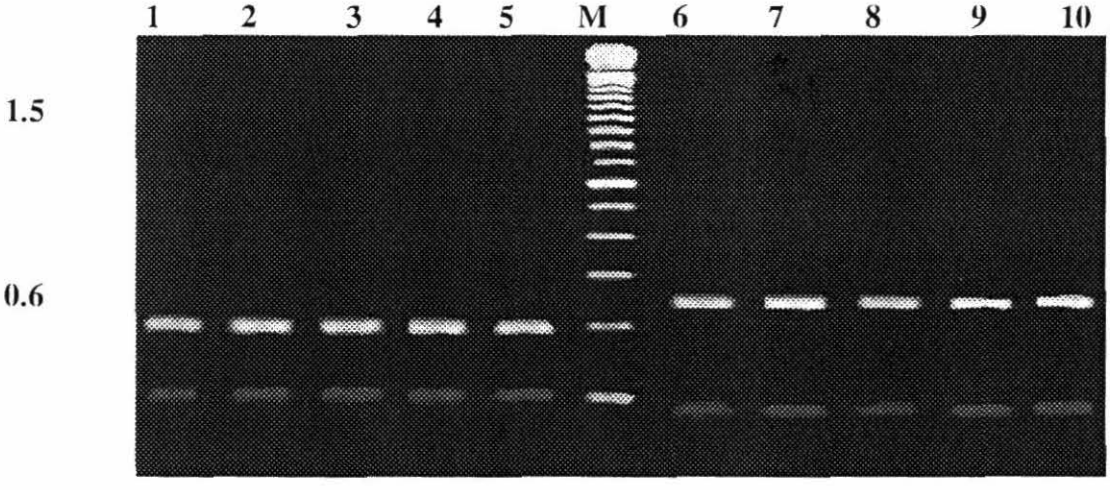

Figure 6.3. **Electrophoresis of fragments of ITS digested with the endonucleases *Alu*I and *Hind*I. Lanes 1 to 5 = isolates digested with *Alu*I; M = marker of molecular size 100 pb (value in kilobases); lanes 1 to 5 = isolates digested with *Hind*I.**

Polymorphism was detected in 16 isolates from five flower farms in the Sabana de Bogotá. Twenty-eight primers were examined, of which the 12 most polymorphic primers were used for statistical analysis. The primer OPO-02 reflected the degree of polymorphism that was detected in the species *S. pannosa* var. *rosae* **(Figure 6.4)**.

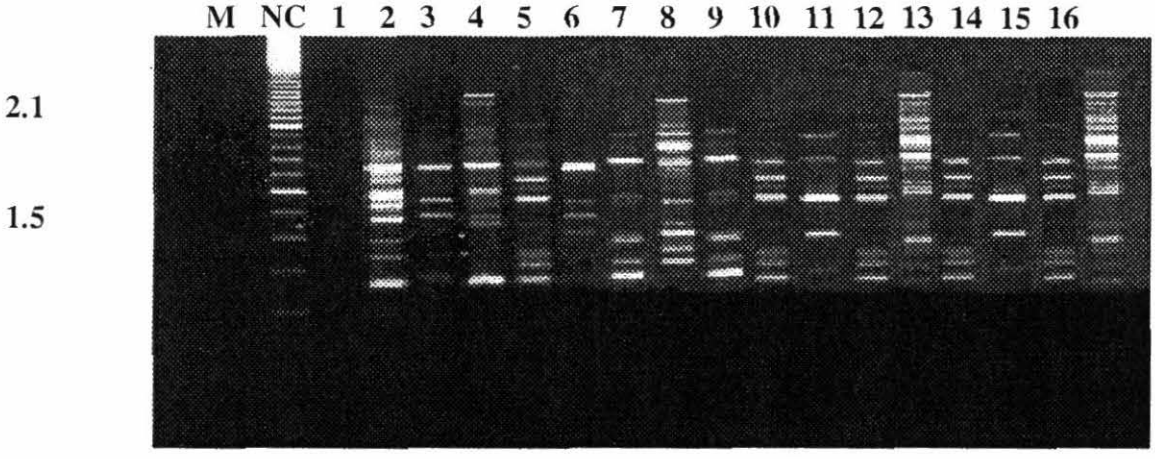

Figure 6.4. Amplification of random polymorphic fragments of DNA of 16 isolates of *S. pannosa* var. *rosae*. Lane M = marker of molecular size 100 pb (value in kilobases); NC = negative control; lanes 1 to 16 = molecular bands of the isolates.

ACA primer, a microsatellite-type primer that evaluates a highly specific region of the genome, also showed polymorphism **(Figure 6.5)**.

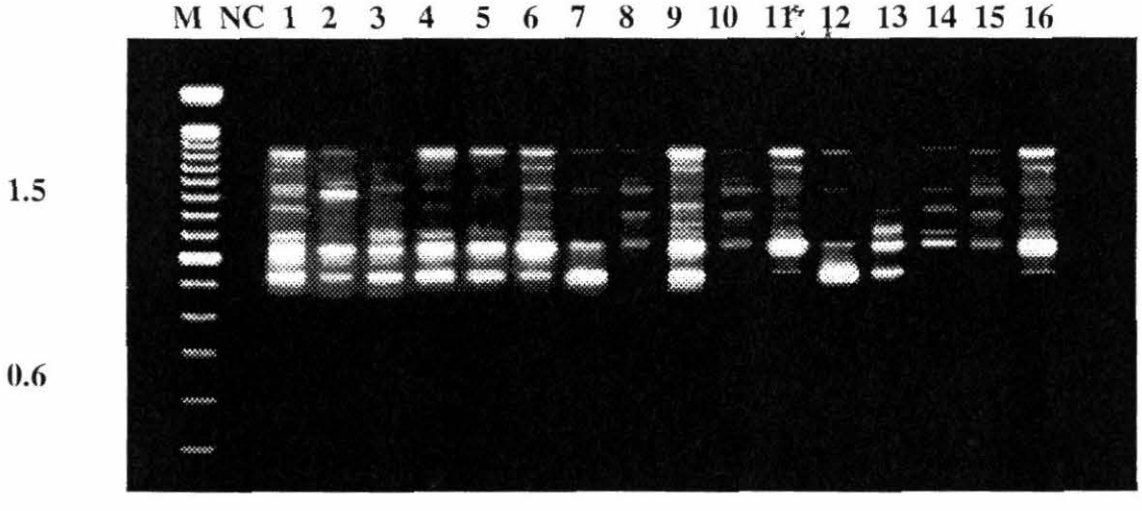

Figure 6.5. Patterns of bands obtained with the ACA microsatellite. M = marker of molecular size 100 pb (value in kilobases); NC = negative control; lanes 1to16 = isolates of *S. pannosa* var *rosae*.

A total of 143 bands were read and a dendrogram constructed, based on 70 polymorphic bands (**Figure 6.6**). This statistical analysis showed the population of 16 isolates clustering into three genetic groups (Dice similarity index of 0.60). A correlation coefficient of +0.79 between origin, genetic variability, and pathogenic diversity was determined (r = +0.63). The correlation between pathogenicity and conidium width was +0.52.

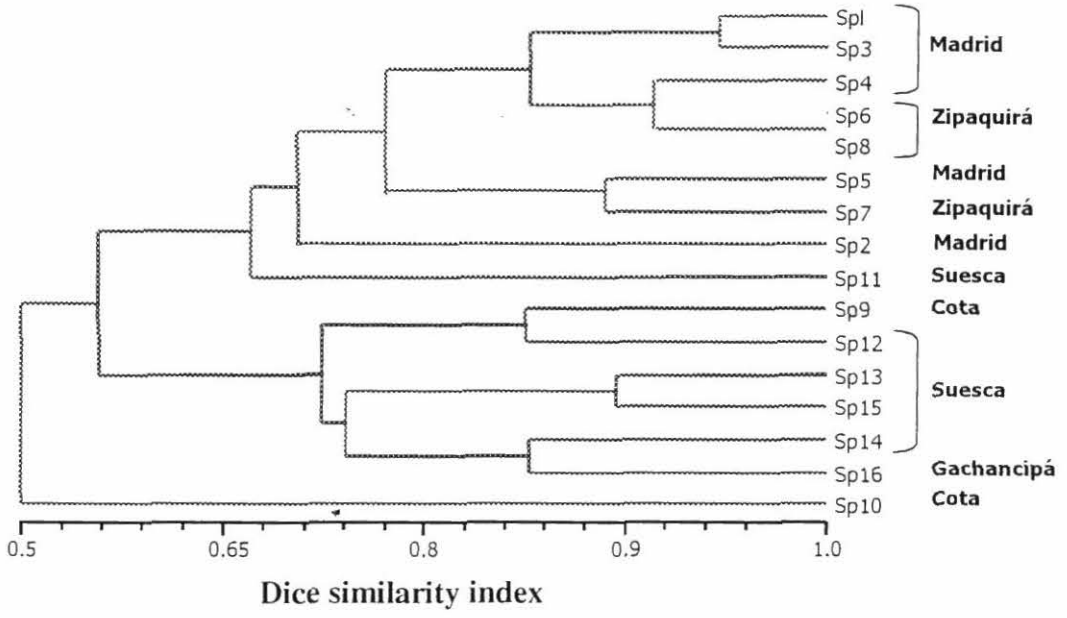

Figure 6.6. Phenogram from a hierarchical cluster analysis of data.

Conclusions

The pathogenic and molecular analyses revealed considerable genetic diversity in the populations of *S. pannosa var rosae* evaluated. The relationship between origin and genetic diversity suggests that different disease management practices will be needed according to the farm and environment. The genetic variability observed with the analyzed primers proves that the pathogen is able to mutate and, depending on the environment in which it is found, can adopt several forms by which to survive in different tissues of the rose plant.

Activity 7. Genetic analysis of the fungus (*Ceratocystis paradoxa* complex), causing bud rot disease in oil palm

Forty-two isolates of *C. paradoxa* and related fungi, most from Colombia, were examined and placed into the culture collection of CIAT. Summary information on these isolates is given in **(Table 7.1)**, which includes morphological features and mating systems of the isolates (self-sterility or self-fertility, and mating type where known). Twenty-one of the isolates were used for DNA sequencing of the internal transcribed spacer regions (ITS 1 and ITS 2) of the nuclear rDNA. These data were analyzed, using "phylogenetic analysis using parsimony" (PAUP), and the result of that analysis is shown in Figure 7.1.

Table 7.1. Isolates of the *Ceratocystis paradoxa* complex (causing bud rot disease in oil palm) examined for their mating systems and conidial morphology.

Species	Isolate Numbers	Host	Location	Mating Systems	Conidia in Chains or Single	Conidia Rough or Smooth Walled
Ceratocystis paradoxa, sensu stricto	#C053, CPT- 006	Palm	Villavicencio	Self-sterile	Chains	Smooth
	#C954,CPT,007	Palm	Villavicencio	Self-sterile	Chains	Smooth
	#C955, CPT-033	Palm	Villavicencio	Self-sterile	Chains	Smooth
	#C956, CPT-010	Palm	Villavicencio	Self-sterile	Chains	Smooth
	#C957, CPT-009	Palm	Villavicencio	Self-sterile	Chains	Smooth
	#C958, CPT-002, CIAT TH058	Palm	Villavicencio	Self-sterile, MAT-2	Chains	Smooth
	#C959, CPT-011	Palm	Villavicencio	Self-sterile	Chains	Smooth
(Sweet smell)	#C1001, CBS 601.70	*Ananas comosus*	Brazil	Self-sterile	Chains	Smooth
	#C1003, CBS 374.83	*Phoenix caraeiensis*	Spain	Self-sterile	Chains	Smooth
	#C1025, C009	From Bud	Colombia	Self-sterile	Chains	Smooth
	#C1026, C001	From Root	Colombia	Self-sterile	Chains	Smooth
	#C1027, C003	From Insect	Colombia	Self-sterile	Chains	Smooth
	#C1028, C004	From Soil	Colombia	Self-sterile	Chains	Smooth
	#C1029, C006	From Bud	Colombia	Self-sterile	Chains	Smooth
	#C1030, C007	From Stipe	Colombia	Self-sterile	Chains	Smooth
	#C1031, E005	From Soil	Colombia	Self-sterile	Chains	Smooth
	#C1032, CPT-004R		Villavicencio, Colombia	Self-sterile	Chains	Smooth
	#C1090, E4, 03F		Ecuador	Self-sterile, Mat-1	Chains	Smooth
	#C1091, E103B, 035		Ecuador	Self-sterile	Chains	Smooth
	#C1092, Tumaco 4, 068		Tumaco, Colombia	Self-sterile	Chains	Smooth
	#C1093, Tumaco g0A, 044		Tumaco, Colombia	Self-sterile	Chains	Smooth
	#C1094, Cp-T-020, VI-13-9F		Costa Caribe, Colombia	Self-sterile, MAT-2	Chains	Smooth
	C1107, CIAT, TH 038, E33A		South America	Heterothallic, MAT-1	Chains	Smooth
	#C1161, 2	From rachis	Tumaco,	Self-sterile	Chains	Smooth

Species	Isolate Numbers	Host	Location	Mating Systems	Conidia in Chains or Single	Conidia Rough or Smooth Walled
			Colombia			
	#C1162, 3	From a spear leaf	Llanos, Colombia	Self-sterile	Chains	Smooth
	#C1163, 4	From a spear leaf	Llanos, Colombia	Self-sterile	Chains	Smooth
	#C1164, 5	From a rachis	Ecuador	Self-sterile	Chains	Smooth
	#C1165, 6	Meristem	Ecuador	Self-sterile	Chains	Smooth
	#C1166, 7	Spear leaf	Ecuador	Self-sterile	Chains	Smooth
	#C1167, 8		Magdalena Medio, Colombia	Self-sterile	Chains	Smooth
	#C1168, 9		Magdalena Medio, Colombia	Self-sterile	Chains	Smooth
	#C1169, 10	From a stem	Magdalena Medio, Colombia	Self-sterile	Chains	Smooth
	#C1170, 11	From a bud	Brazil	Self-sterile	Chains	Smooth
	#C1171, 12	From a bud	Brazil	Self-sterile	Chains	Smooth
	#C11712, 13	From a root	Brazil	Self-sterile	Chains	Smooth
C. paradoxa, Banana form?	#C915, UFV-171	*Musa* sp.	Brazil Belen	Self-sterile	Chains	Smooth
	#C907, CMW 1546	*Musa* sp.	?, From Collection NZ	Self-sterile	Chains	Smooth
Possibly *C. paradoxa* (Holotype of *Hughesiella euricoi*)	#C1002,CBS 893.70, IMI 96739, DAOM 75211, IMUR 640	Air	Brasil	Self-sterile	Chains	Smooth
Undescribed Species (Cucumber Smell)	#C914, UFV-170	*Cocus nucifera*	Brazil Maranhao	Self-sterile (SS) Progeny 1:1 for SF:SS (SF = self-fertile)	Chains	Smooth
(Cucumber Smell)	#C1021	*Elaeis guineensis*	Magdalena Medio, Colombia	Self-sterile, Progeny all SF	Chains	Smooth
C. radicicola	#C869, CMW3186, CBS114.47	*Phoenix dactylifera*	California, USA	Probably self-sterile, Progeny SS	Single	Rough
	#C870, CMW3191, CBS146.59, IMI36479	*Phoenix dactylifera*	California, USA	Probably self-sterile, Progeny SS	Single	Rough

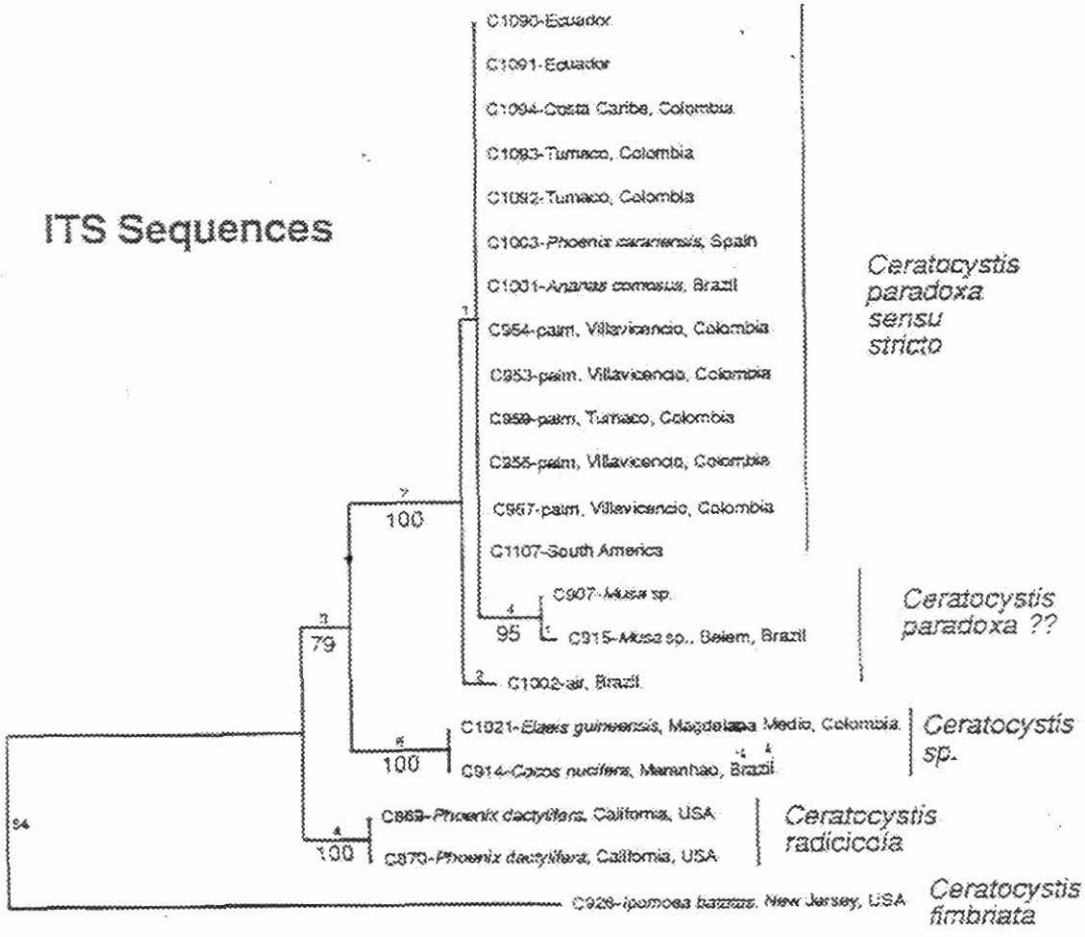

Figure 7.1. One of four most parsimonious trees of 82 steps based on 537 aligned bases from the ITS-1, ITS-2 and 5.8s rDNA sequences of *Ceratocystis paradoxa* and relatives. The tree is rooted to *C. fimbriata*. Numbers above branches indicate number of base substitutions, and numbers below branches are bootstrap values. CI= 0.939, HI=0.061, RI= 0.909.

Morphological comparisons, mating systems, and phylogenetic analysis show strong support for the delineation of five different taxa in this complex:

1. The species primarily responsible for disease in oil palm is *Ceratocystis paradoxa* in the strict sense. This species forms thick-walled, pigmented conidia (chlamydospores or aleuriospores) in chains on the top of conidiophores, and the spore walls are smooth. Thirteen isolates of *C. paradoxa* from oil palm, date palm, and pineapple proved to have identical ITS sequences. We crossed many of the oil palm isolates and found them to be interfertile and heterothallic. Crossing of four isolates revealed two arbitrarily assigned mating types (MAT-1 and MAT-2) and resulted in perithecia and ascospores meeting the description of *C. paradoxa*.

2. Two isolates from banana are morphologically similar to the other *C. paradoxa* isolates but differ in their ITS sequences by four or five base substitutions, suggesting that they are a separate lineage, perhaps specialized to *Musa* species. More banana isolates should be tested.

3. An isolate from air in Brazil had an ITS sequence that differed from the *C. paradoxa* isolates by three base substitutions. This isolate had single conidia at the top of conidiophores rather than chains of conidia. This isolate is from the holotype of *Hughesiella euricoi*, which was synonymized with *C. paradoxa* by Nag Raj and Kendrick. However, it appears to be a distinct species.

4. Two strains differed substantially from *C. paradoxa* in their ITS sequence, had a cucumber- like smell, were self-fertile, and produced rough-walled rather than smooth-walled chlamydospores. Thus, this species is clearly distinct from *C. paradoxa* and should be described as new. In ITS sequence and morphology, this species is intermediate between *C. paradoxa* and *C. radicicola*. Progeny of a selfing from the oil palm isolate from Colombia were all self-fertile. In contrast, progeny of a selfing from the coconut palm isolate from Brazil segregated 1:1 for self-fertility : self-sterility, consistent with the model for unidirectional mating type switching found in most species of *Ceratocystis*.

Ceratocystis radicicola has been reported only by the original authors of the species, from date palm in southern California. Although morphologically similar to *C. paradoxa* and occurring on similar hosts, the ITS sequences clearly separate these species. The two isolates from Brazil were each self-fertile, but recovered single ascospore progeny were self-sterile. This species is reported to be heterothallic with two mating types, that is, to have a mating system similar to *C. paradoxa sensu stricto*, but we were unable to confirm this in our crossing experiments.

Activity 8. Plant disease diagnosis

Introduction

The Diagnostic Service provides technical and/or biological information to the staff at headquarters, Extension Services, National Inspection Services, and private companies. The main purpose of this research section is to diagnose problems based on submissions and samples sent in from the field. It is most important that the Pathology Section's Diagnostic Service is familiar with the latest scientific technology and developments, and is able to apply up-to-date techniques.

Cooperation with extension and national inspection services also increases phytosanitary activities, for which the private sector has become a major client.

This year, the Service received samples for bacteriological and fungal diagnoses. Relevant data from bacteria that have been isolated and identified are presented in table 8.1. The Diagnostic Service continued work on identifying fungal microorganisms involved in crop disease and adapting techniques to meet specific needs. The Service was financed by private industry.

Later in the year, three trainees from the Danish Government Institute of Seed Pathology and the Universidad Nacional de Colombia visited our laboratory for further training in detecting and identifying bacterial pathogens.

Table 8.1. Disease-causing bacterial and fungal pathogens identified on different crops by the Diagnostic Service of the CIAT Cassava Pathology Section.

Host	Symptoms	Isolation Methodology	Result
Fescue (*Festuca* spp.)	Necrotic leaf lesions	PDA 27° C	*Curvularia* sp.
Fescue (*Festuca* spp.)	Necrosis and drying (brown color) of the leaves	Selective culture medium for *Pythiaceae*	*Pythiaceae*
Heliconia	Brown Wilting	Selective culture medium for Bacteria	*Ralstonia solanacearum*
Hot pepper (*Capsicum frutescens*)	Wilting and necrotic stems	Selective culture medium for *Pythiaceae*	*Phytophthora capsici*
Oil palm (*Elaeis guineensis*)	Bud rot and wilting	Directly, 13 different culture medium. Baiting with *Bouganvilla* and cassava. Inoculation of *Carica papaya* fruits	-
Orquid (*Dendrobium*)	Leaves with circular water soaked lesion leaf spots, light green to brown or black, necrotic stems	Potato dextrose agar (PDA) at 25°C during 5 days	*Cercospora dendrobii*
Orquid (*Dendrobium*)	Leaves and stems showing Necrotic and a chlorotic spots and stem	Nutrient agar (NA) and yeast dextrose calcium carbonate agar at 30 °C, individual colonies on King B medium	*Pseudomonas cattleyae*
Orquid (*Dendrobium*)	Leaves and stem to covered by black mycelium	Direct observation	*Capnodium* spp.
Orquid (*Dendrobium*)	Petals and stems covered by black mycelium and other fungal structures	PDA at 24 °C during 5 days	*Cladosporium* spp.
Orquid (*Dendrobium*)	Necrotic stems and root rot	V8-juice Agar, PDA, Water Agar and NA at 28 °C during 7 days	*Fusarium proliferatum*
Sweet pepper (*Capsicum annuum*)	Dry rot of the basal part of the stem	Directly on PDA	*Sclerotium rolfsii*
Water melon (*Cucumis melo*)	Angular leaf spots	Directly on PDA	*Alternaria* spp.

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Sub-output 6. Pest and Disease Complexes Described and Analyzed.

Activity 1. The role of the whitefly *B. tuberculata* as a vector of CFSD

The whitefly *Bemisia tuberculata* has been reported as the vector of cassava frogskin disease (CFSD). There is both field evidence and greenhouse transmission studies. The rate of transmission is extremely low and has led to the conclusion the *B. tuberculata* is a very inefficient vector of CFSD. The level of transmission was so low that one could question if this is indeed the vector of CFSD. This year it was decided to repeat the transmission studies and to quantify the transmission of CFSD.

To understand the efficiency of transmission, three experimental designs were attempted. The first consisted of allowing *B. tuberculata* to feed for 24 hours on infected plants of the line SM 909-25. Then 100 whiteflies per plant were transferred to healthy Secundina by placing 20 whiteflies in each of 5 small plastic cages that were clipped onto the undersides of the leaves. The inoculation period was for seven days. This experiment was repeated twice and there was no transmission of CFSD. This indicates that the period of acquisition may be more than one day or that there is a latent period before the insects can transmit the disease.

The second method of transmission consisted of using *B. tuberculata* that had been reared on the CFSD infected plants of the line SM 909-25. Each cage contained 10 healthy plants of the variety Secundina, and approximately 500 insects were used for the inoculation. After an eight day period of inoculation, the whiteflies were eliminated and the plants were observed for mosaic symptoms on the leaves. This was repeated six times and none of the plants developed symptoms. Because the insects were reared on infected plants, this experimental design eliminates the problem with the short acquisition time or a latent period. Still there was no transmission and indicates that it is difficult to develop standard conditions favorable for the transmission of CFSD.

The third experimental design puts significantly more disease pressure on the healthy plants. This method also used whiteflies that had been reared on CFSD infected cassava. A total of four different sources of the disease were used in separate trials including the lines SM 909-25 and CM 5460-10. To maximize disease pressure, both healthy and disease plants were place in the same cage along with approximately 2000 insects. The plants with the insects were maintained for an inoculation period of 38 days. In all the trials a total of 92 plants were used and seven (8%) developed symptoms of CFSD (**Table 1.1**). There was some indication that more disease pressure caused higher levels of infection. In the trial with 20 healthy plants, none of them developed CFSD. In the two trials that had only 4 healthy and 4 diseased plants, two of the plants or 25% developed the disease.

Why are the transmission rates so low? It is possible that the insect has resistance to the virus. This would be similar to the case of *T. orizicolus* and RHBV in which the challenge is to maintain highly viruliferous colonies by selective crosses of proven vectors. Is the vector just inefficient or can the conditions be improved to increase the rate of transmission. These experiments will continue in order to better understand disease transmission as well as the causal agent of CFSD.

Table 1.1. *B. tuberculata* transmission experiments for the transmission of CFSD.

Experiment Number	Source of CFSD	Number of Diseased and Healthy Plants	Number of Plants that Developed CFSD
1	CM 5460-10	4 diseased & 4 healthy plants	1
2	CM 5460-10	4 diseased & 4 healthy plants	1
3	CMD 80, 86, & 5	4 diseased & 12 healthy plants	0
4	CMD 80, 86, & 5	6 diseased & 6 healthy plants	1
5	SM 909-25	6 diseased & 6 healthy plants	2
6	SM 909-25	6 diseased & 10 healthy plants	1
7	SM 909-25	6 diseased & 20 healthy plants	0

Activity 2. Characterization of the causal agent of CFSD

There is evidence that a reo-like virus is associated with CFSD. The attempts to clone the causal agent using dsRNA extractions from infected plants, has resulted in many different cDNA clones. Although most of them are cassava ribosomal genes, some appear to be viruses. Examples include a viral RNA dependent RNA polymerase gene, but it appears to be more of a mycovirus than a plant virus. One clone has similarity with the rice ragged stunt virus RNA 5, but this sequence appears to be incorporated into the gene of cassava. The efforts to use dsRNA in the investigation of the causal agent of CFSD continued, but this year the emphasis was on experimenting with different virus extraction techniques.

From earlier studies, it is known that it is difficult to purify the causal agent of CFSD. Part of the problem is cassava. It contains many substances that make it difficult to purify even those viruses that are well characterized and relatively stable such as cassava common mosaic virus.

Purification of potential viruses were made from expanding leaves of cassava varieties that have develop the mosaic leaf symptoms when they are affected with CFSD. The most successful method attempted were the extractions that use the solvent Freon 113. The preparations were run on sucrose gradients. There were some promising bands in the gradients and these were concentrated by centrifugation. When viewed in the electron microscope, some fractions contained virus-like particles of approximately 70 nm. In polyacrylamide gels, these are four principal proteins. When subject to RNA extraction, there were some small RNA species but none were the size expected for a viral genome. Similar extractions were made from the roots of CFSD infected plants, but all the bands in the gradients were found in both healthy and infected plants. The bands found in the healthy roots are similar size to those found in leaves and roots of plants affected by CFSD. At this time, more experiments are needed to clarify if the virus-like particles are consistently associated with CFSD, but it probable that they are only some forms of plant structure.

An extraction procedure scaled down for very small weights was used on *B. tuberculata* that had been reared on CFSD infected plants. This also yielded bands that were fairly high in the gradient, and that were in both the whiteflies fed on healthy and infected plants. In some purifications, there was a second very thin band near the bottom of the gradients. This fraction contained virus-like particles that are approximately 70 nm in diameter and the preparations appeared free of contaminants. The extractions of these particles are not consistent but this is considered to be the most promising line of research to purify virus-like particles that are candidates for the causal agents of CFSD.

Activity 3. Identifying cassava germplasm that is resistant to CFSD

Cassava frogskin disease is highly destructive because it affects the roots. It can cause drastic reductions in both yield and quality. The roots affected with CFSD tend to have much less starch and are more fibrous. This is the fifth cycle (year) of an experiment to select germplasm resistant to CFSD. The starting materials were the 630 lines of the cassava core collection. Each of the cassava plants was inoculated with CFSD by grafting with affected stem cutting of the line CM 5460-10. Most clones have passed through four or five cycles (each cycle is one year), and the cassava lines with moderate or severe root symptoms have been eliminated from the experiment.

In April 1999, 142 cassava lines that have consistent been rated as tolerant or negative for symptoms of CFSD were planted. This year the climatic conditions were highly favorable for the expression of CFSD symptoms. In the 1999 harvest of 152 lines only 9 were rejected because of moderate or severe symptoms of CFSD. This year with the conditions favorable for the expression of the symptoms 17 of 143 lines were eliminated from the experiment because of CFSD (**Table 3.1.**). Four of the lines died due to other factors. There were 126 lines representing approximate 20% of the core collect which appear to have a significant level of resistance or tolerance to infection with CFSD.

Although there were 122 clones that were still showing resistance to CFSD, many were not in good shape. The core collection is a group of cassava that represents the larger gene bank of over 5000 clones. These come from all sorts of environments and many are not adapted to the growing conditions in acid soils with high pest and disease pressure that is characteristic of the Cauca region of Colombia.

Table 3.1. Lines of the cassava core collection that were evaluated for their reaction to cassava frogskin disease in April 2000.

Lines Evaluated	Total 143	%
Lines with severe symptoms	8	5.6
Lines with moderate symptoms	9	6.3
Lines with light symptoms	55	38.5
Lines showing no symptoms	67	46.9

[1] In four of the lines all the plants were dead.

Each plant was rated for vigor, plant height, root weight, number of commercial stem cuttings, and weight of the stem cuttings. These agronomic traits were used to reduce the number of lines that were planted in April 2000 to 67 lines. The idea is to select lines that grow well in the agroecosystems of Colombia. Similar efforts will be needed for the other countries in which CFSD is an emerging disease.

The resistance to CFSD is not immunity to the causal agent. Fifty of the lines harvested in April 2000 that were either negative or showing only mild root symptoms, were tested for the presence of CFSD by grafting to the indicator variety Secundina. Forty seven of these were able to transmit CFSD to Secundina and this indicates that these varieties are tolerant to the pathogen.

This study has demonstrated that there is widespread resistance to CFSD. Multilocational trials are going to take years to complete and to maximize the value will include studies on the epidemiology

of CFSD. Since these trials are going to be planted in several areas, the cassava must be clean planting material. Since the rate of infection is slow and the subsequence analysis of resistance will take at least five years. Susceptible varieties will be interplanted with the resistant ones and the disease spread will be carefully monitored.

The is sufficient evidence that a pool of germplasm with resistance to CFSD has been identified. This resistance is widespread in the cassava germplasm, and it needs to be systematically incorporated into cassava breeding efforts.

Activity 4. Molecular characterization of potexviruses infecting cassava

This activity has become a joint activity with a private company that is developing a set of diagnostic oligonucleotide primers for the detection of potexviruses. These primers are supposed to be able to amplify a region of all potexviruses. The collaboration is beneficial to the company because they get to test their diagnostic primers for three additional potexviruses. If successful, they will provide CIAT with the ability to clone a portion of the genome of cassava X virus and cassava Colombian symptomless virus. This is especially important for CCSpV because it is extremely difficult to purify and maintain sufficient quantities of antisera for diagnostic purposes.

Activity 5. Using molecular techniques to analyze whitefly species and biotypes in Latin America

When identifying and describing insect taxa, morphology has historically been used to separate species. Among many groups of insects, however, morphological characters can vary with respect to environmental factors within a single species, or be so convergent and cryptic among closely related species, to be of limited usefulness. Under such conditions studies of their biology and molecular profiles become essential to defining species and characterizing populations. At a molecular level, protein and DNA polymorphisms can be combined with studies of biological characteristics by using one of four experimental or technological approaches: electrophoresis of allozymes, analysis of randomly amplified polymorphic DNAs (RAPDs), and nucleic acid sequence comparisons of nuclear or mitochondrial DNA markers. Herein, we review the application of molecular approaches to characterizing whitefly (*Bemisia tabaci*) populations and biotypes in Latin America.

DNA sequence comparisons can be made between individuals or populations using PCR generated sequences from several markers in either the nuclear or mitochondrial genome. The mitochondrial genome offers some advantages because it is maternally inherited and non-recombining and contains known and predictable gene sequences for which general insect primers have been designed. Some sequences vary enough to be useful for population level analyses (eg: the control region, parts of cytochrome oxidases I-III) while others evolve slowly enough to be more appropriate to analyses above the species level (eg: conserved parts of cytochrome oxidase I (COI), 12S and parts of the 16S r-DNA). However, as with some nuclear markers, comparisons between mitochondrial sequences may reflect local gene evolution and not population or species evolution. Nuclear data offer a wide range of neutral markers but can be particularly difficult to design PCR primers for specific targets in poorly known or unknown genomes (eg: introns). Thus, many studies have used variable regions surrounded by relatively conserved sequences (eg: internal transcribed

spacers or ITS of the rDNA genes) for population/species analyses, or more conserved sequences (eg: 18S rDNA) for higher order studies. An alternative approach to specific nuclear markers involves the use of short, random sequence PCR primers that anneal randomly in the genome and produce characteristic banding patterns of collections of PCR products when run on an agarose gel (RAPDs). Advantages here include the low cost and capability of processing large numbers of samples without the necessity of cloning or sequencing PCR products as well as no requirement for detailed sequence knowledge about the genome. Disadvantages include the fact that repeatability can be difficult, and the polymorphisms may be hard to distinguish from PCR artifacts. That is, template preparation and amplification conditions must be very consistent and tightly controlled. Interpretation of negative data (absence of bands) may also have numerous explanations.

A regional view of whitefly populations

Most of the samples of that were determined to be in the *B. tabaci* complex by morphological methods were classified as either biotype A or B. In general about 15% of the samples could not be amplified. Only 23% of the samples from Venezuela could be amplified. This may demonstrate the importance of the proper sample handling. Approximately 5% of the samples also had RAPD PCR products that could not be identified. These may well be either different species or unique biotypes. While this level of uncertainty did not detract from the survey, those unidentified samples are a source of potentially different whitefly populations that merit further study.

Activity 6. Developing sequence characterized amplified region (SCAR) to identify whiteflies in the Bemisia complex

RAPDs are produced by using short oligonucleotides, generally of ten base pairs. This allows the generation of many amplified PCR products using just one primer. The lack of specificity of the short oligonucleotides can lead to results that are difficult to interpret because of similar size PCR products. Also shorter oligonucleotides are even more sensitive to single base changes. RAPD markers can be converted in a sequence characterized amplified region (SCAR). This specific marker utilizes a pair of oligonucleotides generally 20-25 base in length and amplifies a single region of a genome. This makes the interpretation of the results much simpler than RAPDs. Instead of a group of bands with a degree of variability, a SCAR produces single bands in the target organism.

The SCAR for the *B. tabaci* biotype B was developed by cloning the RAPD PCR products of the primer H9. The cDNA clones were analyzed and sequenced. The primers were designed and tested. One set of primers only amplified *B. tabaci* biotype B (**Figure 6.1.**). This set of primers did not amplify any tested population of *B. tabaci* biotype A or *T. vaporariorum*. Further testing is needed to confirm that these primers amplify this PCR product in geographically separated populations of *B. tabaci* biotype B. Given the lack of diversity in the evolutionary studies, it is expected that this SCAR will be useful to rapidly identify all population of biotype B.

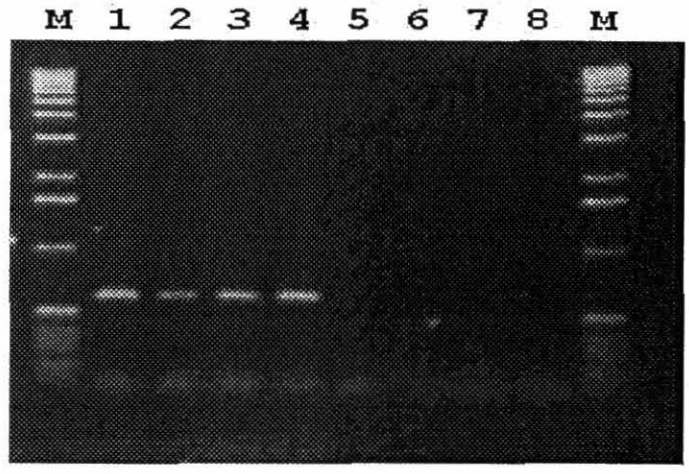

Figure 6.1. A SCAR specific to *B. tabaci* biotype B. Lines 1- 4 are *B. tabaci* biotype B; lines 5-8 are biotype A; M is a 1kb ladder.

The current research is directed to developing SCARs for *B. tabaci* biotype A and *T. vaporariorum.* Each set of primers will amplify bands of different sizes. This will allow the primers to be mixed together and in a single PCR reaction distinguish between the whiteflies. This will make more extensive surveys possible and the technique will be more consistent in all the laboratories that need the diagnostic capability to rapidly identify whitefly pests. .

Activity 7. Using molecular techniques to analyze whitefly species and biotypes in Latin America

Molecular methodologies were used in this study for evolutionary analysis and surveys of whitefly distribution. Both methods yield very different types of information. For evolutionary studies, a part of the DNA from each individual whitefly was cloned and sequenced. This limits the numbers but yields detailed and useful information. To further evolutionary studies, one can analyze additional whitefly species and include genes such as the COI to compliment the existing knowledge.

The molecular method of RAPDs proved essential in distinguishing between the *B. tabaci* biotype A and B. This method only depends on extracting DNA and using PCR to amplify a pattern of DNA species. The major problem with this method is the interpretation of results. Already one SCAR was developed for *B. tabaci* biotype B, and we are making progress in the development of SCARs for *B. tabaci* biotype A and *T. vaporarorium.* Specific unambiguous results of one amplified band for each species or biotype of whitefly would simplify the interpretation of results and make the method widely applicable.

Activity 8. Characterization of citrus viruses: Analyzing populations of citrus tristeza virus

The characterization of citrus tristeza virus (CTV) is a collaboration between CORPOICA and CIAT. The first phase of the project has been the development of the diagnostic capacity to identify and characterize strains of CTV. Dr. Ronald Brlansky and Richard Lee of the U. of Florida have provided technical support.

One of the methods to characterize the severity of CTV strains is using serological methods. Two commercially available antisera were used to characterize 55 isolates of CTV from three regions of Colombia. Isolates of CTV were collected from Valle, Quindio and Magdalena. One antiserum was able to detect all CTV isolates and the other MCA13 is a monoclonal antibody that is specific for severe strains of CTV. **(Table 8.1.)**

The Valle is the only region in which not all the trees tested were positive for CTV. Still CTV is widespread and most of the isolates are severe strains. Quindio is the region with the lowest percentage of severe CTV isolates in contrast to Magdalena where nearly all of the CTV isolates are severe.

Table 8.1. Serological characterization of citrus tristeza isolates using one antisera that detect all isolates and another that only detects severe isolates.

Region	Number of samples	General CTV antisera	MCA 13 antisera
Valle	21	76 % positive	67 % positive
Quindio	16	100 % positive	56 % positive
Magdalena	18	100 % positive	94 % positive

The regions of Tolima and the Llanos need to be included in the survey. A biological analysis of the strains is being developed and will complement the serological assay. The coat proteins of six of the CTV isolates have been cloned and are awaiting the analysis of the sequence data. Worldwide there are many isolates for which there is molecular data on the coat protein and this information will be useful in understanding the complex of CTV isolates in Colombia. The second phase of the project will be the identification of appropriate isolates to be used in to cross protect citrus from severe isolates of CTV.

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Sub-output 7. Pest and Disease Complex Described and Analyzed

Activity 1. Virulence characterization of pathogen diversity of *Phaeoisariopsis griseola* in Africa

Introduction

Use of host resistance is an effective strategy for poor resource farmers against angular leaf spot (ALS) caused by *Phaeoisariopsis griseola*. But occurrence of pathogen variability in the pathogen can adversely affect the effectiveness of resistance. On-going research based on virulence and molecular characterization of African isolates show occurrence of Mesoamerican, Andean and Afro-Andean pathogen groups. Most countries where ALS is a problem, information about the genetic structure of *P. griseola* and distribution is inadequate. To design and develop durable resistance it is important to characterize pathogen variability and its distribution.

Methods

Isolates characterized came from important bean growing areas in Tanzania (southern highlands and northern region) and Ethiopia (Jima) not previously covered. A total of 35 isolates were characterized using virulence on the basis of a set of 12 bean angular leaf spot differentials (6 Andean and 6 Mesoamerican).

Results and Discussion

Twenty-three isolates from southern Tanzania belonged to 14 races, three from northern Tanzania belonged to three races while, seven from Ethiopia belonged to 7 races an indication of wide pathogenic variation common of *P. griseola* (**Table 1.1.**). About a half of all isolates characterized gave reactions associated with meso-American pathogen group, while another half gave reaction associated with Afro-Andean group. Two gave reactions associated with the Andean pathogen. It is interesting to note that two isolates infected Mex 54 (invoking an intermediate reaction). Mex 54 is the only differential, which continues to maintain resistance to almost all races that have been identified so far in Africa. While meso-American races occur both in Latin America and Africa, there is a very distinct group of Mesoamerican races especially those that attack Mex 54,which are unique to Latin America but hardly found in Africa or vice versa.

Table 1.1. Virulence diversity of *P. griseola* in Tanzania and Ethiopia.

			Phenotypic reaction on differential cultivars[x]											
			Andean							Mesoamerican				
Isolate Identification	Origin[y]	Race	A	B	C	D	E	F	G	H	I	J	K	L
JM5	ETH	22-0		b	c		e							
UYL-1	TZ	63-0	a	b	c	d	e	f						
JM4	ETH	35-2	a	b				f		h				
UYL-9	TZ	62-5		b	c	d	e	f	g		i			
JM3	ETH	47-3	a	b	c	d		f	g	h				
JM1	ETH	40-6				d		f		h	i			
JM2	ETH	63-6	a	b	c	d	e	f		h	i			
UY-ILOMB	TZ	63-36	a	b	c	d	e	f			i			l
UY-CAL-1	TZ	63-36	a	b	c	d	e	f			i			l
UY-M54	TZ	63-37	a	b	c	d	e	f	g		i			l
UYL-11	TZ	63-37	a	b	c	d	e	f	g		i			l
Se3	TZ	31-7	a	b	c	d	e		g	h	i			
UY-AX-37	TZ	62-37		b	c	d	e	f	g		i			l
UY	TZ	63-38	a	b	c	d	e	f		h	i			l
Areka	ETH	47-35	a	b	c	d		f		h	i			l
UYL-18	TZ	62-22		b	c	d	e	f		h	i		k	
Se1	TZ	63-7	a	b	c	d	e	f	g	h	i			
UYL-14	TZ	63-7	a	b	c	d	e	f	g	h	i			
UYL-7	TZ	63-7	a	b	c	d	e	f	g	h	i			
Se2	TZ	31-23	a	b	c	d	e		g	h	i		k	
UYL-13	TZ	62-53		b	c	d	e	f	g		i		k	l
UYL-2	TZ	47-39	a	b	c	d		f	g	h	i			l
UYL-12	TZ	63-39	a	b	c	d	e	f	g	h	i			l
UYL-17	TZ	63-39	a	b	c	d	e	f	g	h	i			l
UYL-3	TZ	63-39	a	b	c	d	e	f	g	h	i			l
UYL-5	TZ	63-39	a	b	c	d	e	f	g	h	i			l
UYL-6	TZ	63-39	a	b	c	d	e	f	g	h	i			l
JM6	ETH	63-39	a	b	c	d	e	f	g	h	i			l
UYL-8	TZ	63-39	a	b	c	d	e	f	g	h	i			l
UYL-10	TZ	63-47	a	b	c	d	e	f	g	h	i	j		l
UYL-16	TZ	63-55	a	b	c	d	e	f	g	h	i		k	l
UYL-19	TZ	63-55	a	b	c	d	e	f	g	h	i		k	l
UYL-21	TZ	63-63	a	b	c	d	e	f	g	h	i	j	k	l

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

Activity 2. Pathogen population structure of *Phaeoisariopsis griseola* in varietal mixtures

Introduction

Phaeoisariopsis griseola is a highly variable pathogen. Studies in Latin America have shown that *P. griseola* isolates from the Andean varieties are virulent on Andean and not on Mesoamerican varieties, supporting the notion that the fungus has co-evolved with its common bean host. A similar situation seems to occur in Africa, except that, there also occurs an Andean pathogen sub-group (Afro-Andean), which attacks some of the Mesoamerican varieties. In Africa, this is thought to be due to the growing together of diverse germplasm belonging to the two Phaseolus genepools. In some regions, beans are grown as varietal mixtures but little is known of *P. griseola* pathogen

population structure. Understanding the latter in these production systems is important in formulating management strategies for ALS in common beans.

Methods

Components of two varietal mixtures, one from Kisoro district in southwest Uganda, and another from Rwandan germplasm collected under the Seed of Hope (SOH) programme were initially characterized for their seed and plant characteristics. Components of the Kisoro mixture consisted mainly of indeterminate, medium seed sizes and a variety of seed colors. The SOH materials consisted of mixture of determinate and semi-climber, large-, medium- and small-seeded types and a variety of seed colors. Fifteen components of each mixture were grown (as a mixture) in the field in Senge at Kawanda. CAL 96, K20 and MCM 5001 all released varieties in Uganda were included.

DNA extraction was done on single spore cultures of isolates obtained from almost all components grown. Good amplification was obtained and isolate differentiation was done using four microsatellite primers [(CA)n; (CGA)n; (GT)n; and (TG)n].

Results and Discussion

All cultivars had some infection but variation occurred in severity. Isolate differentiation using the (GT)n microsatellite primer is shown on **Figure 2.1**. A dendogram exhibiting similarities of isolates based on combined data from all four primers used is shown on **Figure 2.2.** Result from the latter and also from a multiple correspondence analysis show differentiation of isolates into four groups. The two isolates (3KW and 30KW) in one group came from components of the Kisoro varietal mixture while 5 (12KW, 15KW, 31KW, 32KW and 35KW) out of 7 isolates in another group came from components of the SOH mixture. No pattern could be read with the other two groups. It was apparent that there are differences between and within groups. However, this variation needs to be correlated with variation in virulence to establish pathogen diversity patterns under conditions of such and similar germplasm diversity. This may in turn influence sampling techniques used in pathogen diversity studies in Africa and strategies in developing resistance under varietal mixtures conditions.

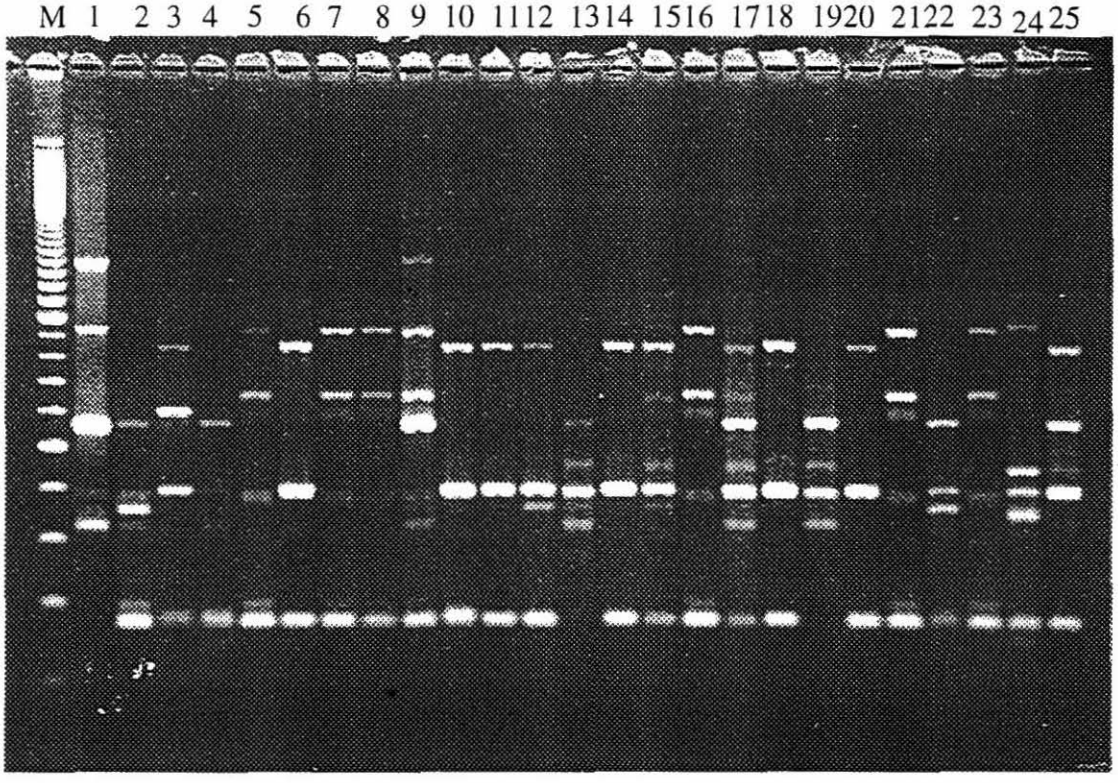

Figure 2.1. **A picture of 28 isolates of *Phaeoisariopsis griseola* isolated from components of two varietal mixtures and three released varieties based on microsatellites using the (GT)n primer.**

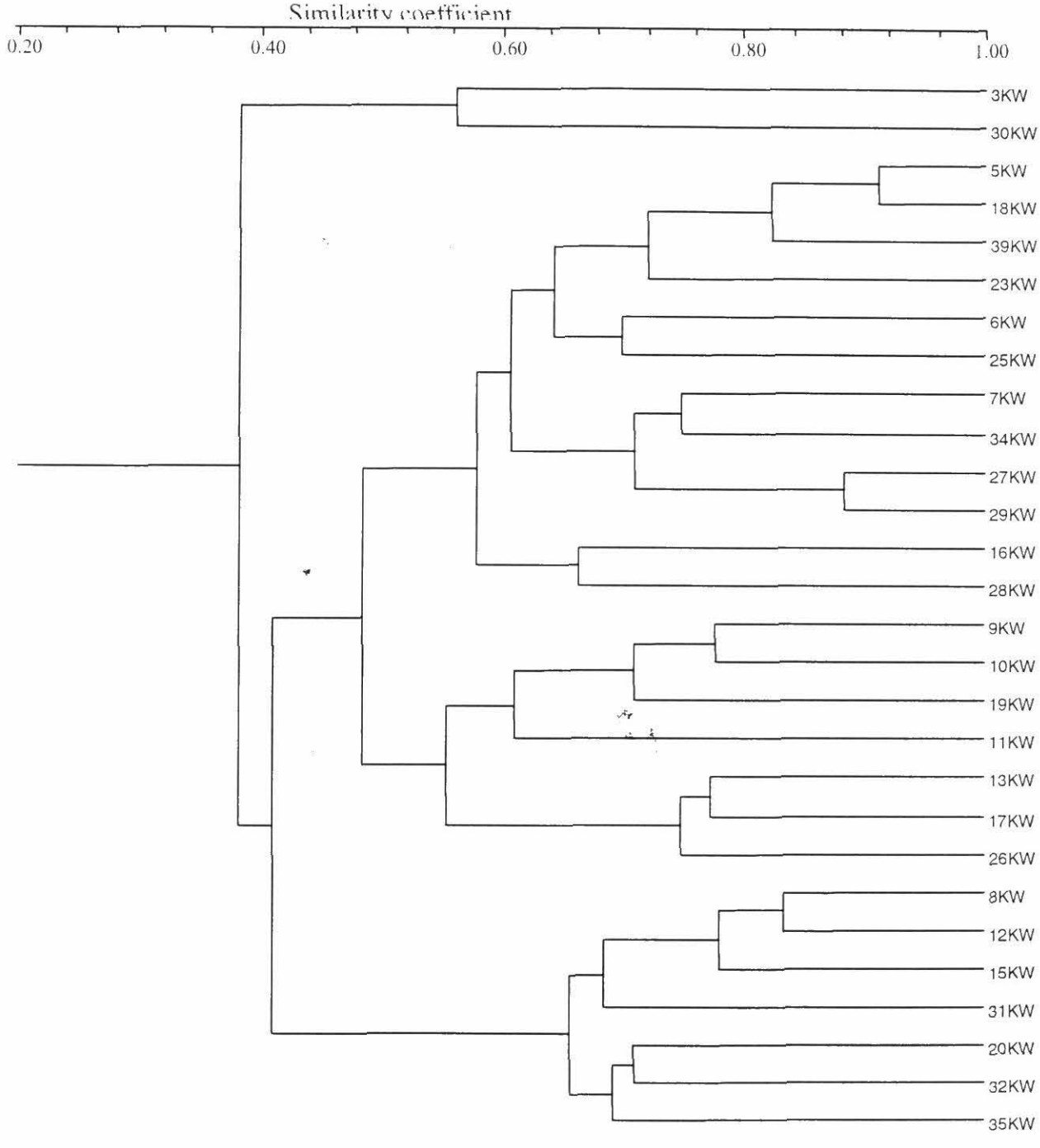

Figure 2.2. Dendogram of 28 isolates of *Phaeoisariopsis griseola* isolated from components of two varietal mixtures and three released varieties based on microsatellites using 4 microsatellite primers.

Activity 3. Epidemiology of bean root rots: Characterization of Fusarium spp associated with bean roots in Uganda.

Introduction

Some of the management efforts against soil-borne diseases are directed at inoculum levels, which can influence disease incidence and severity. Elucidation of the identity of the disease complex (*Pythium* and *Fusarium* spp.) and interactions between them with biotic and a biotic factors are essential in understanding appropriate management strategies. This requires development and use of diagnostic tools for identification and quantification of the components of the pathogen complex.

Methods: Under a DFID supported collaborative project between National Agricultural Research Organization (NARO), (Uganda); Horticultural Research International (HRI) and Natural Research International (NRI) UK and CIAT, studies were initiated to characterize *Pythium* and *Fusarium* species associated with root rot diseases in southwest Uganda where root rots have increasingly become important. Several plant and soil samples were collected from the districts of Kabale and Kisoro district in southwest and Mbale in Eastern Uganda. Initial characterization of was based on cultural and morphological characteristics following isolation on semi-selective media and preliminary molecular characteristics for Fusarium.

Results and Discussion

Pythium spp: A total of 47 isolates have been characterized using morphological structures (sporangium presence, form, and size; size, wall thickness, and the plerotic conditions of the oospore; oogonium location, ornamentation, size, and shape; and the number, origin and morphology of the antheridia) to determine the predominant *Pythium* species in Uganda, and also categorize *Pythium* isolates that are difficult to identify using morphological features.

Results from the morphological characterization indicate that *Pythium* species so far identified include *P. sylvaticum* (5), *P. oligandrum* (3), *P. rostratum* (1), HS group (3) from Mbale; *P. sylvaticum* (2), HS group (1), *P. spinosum* (2), *P. ultimum* (1) from Kabale; *P. irregulare* (1), *P. echinulatum* (1), suspected *P. salpingophorum* (1) in Kisoro. Twenty-two isolates that were difficult to identify using morphological structures were categorized in three groups. The isolates are to be further characterized using pathogenicity and molecular methods. *Pythium* species whose DNA base sequences and primers are known will be the basis for molecular characterization.

Fusarium spp: Results from cultural characteristics suggested that most of about 100 Fusarium cultures collected were *F. oxysporum*. Most failed to grow on NSB (semi-selective for *Fusarium solani*). A few grew on NSB and some of these exhibited *F. solani* f.sp *phaseoli* characteristics.
Two methods of DNA extraction were compared; the SDS-phenol method and direct boiling of spores at 96°C for 5 min. Both methods yielded amplifiable DNA, though boiling spores was a very quick method and could facilitate DNA extraction from many fungal samples in a short time. Two primer pairs F. SOLITS IF/5R and 442/444 were able to discriminate clearly between *F. solani* and *F. oxysporum,* respectively. Discrimination using RFLP was also attempted using 2 restriction enzymes: *Hae*III and *Msp*I. Though both could easily differentiate between *F. oxysporum* and *F. solani*, *Hae*III was a better choice because it was able to digest both *F. solani* and *F. oxysporum*. *Msp*I only produced a digestion product from *F. solani* and not from *F. oxysporum*

Contributors – Sub-output 7: Phytopathology

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

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

Activity 1. Response of cassava to hot-water treatment

Introduction

In diseased plant propagation material, heat treatment can inactivate or kill many pathogens, fungi, bacteria, viruses, and MLOs through their mycelia and spores, present inside and outside cassava asexual seed.

Stem cuttings of three cassava varieties used in Colombia (M Per 183, HMC-1, and Manzana, the last a variety grown locally in the Department of Quindío) were submitted to four hot-water treatments. These were (1) immersion for 49 min in water at 49°C, (2) immersion for 10 min in water at 49°C, followed by 24 h at ambient temperature, and another 5 h at 49°C; (3) immersion for 5 h at 49°C; and (4) no treatment. The cuttings were then planted into 4-inch-diameter pots, and germination, plant height and incidence of CBB at 1 month were evaluated.

Because the three varieties were obtained from a highly infested cassava field with CBB the effect of thermotherapy on this disease was evaluated in a greenhouse. Incidence of CBB was 36% for (4) (**Table 1.1**). Plants from cassava stem cuttings treated by immersion during five hours in hot water did not present any symptoms of CBB. The same treatment but only 49 minutes reduced CBB incidence significantly (7%). Evaluation of the effect of heat treatment on other economically important diseases (i.e., FSD, and Phytophthora root rot), transmitted by stem cuttings, is under way. Observations on bud germination revealed that hot-water treatment for 49 min at 49°C did not harm the cassava plants. As suspected, germination of HMC-1 and M Per 183 was not affected by the five-hour treatment applying a pre-treatment. Plants averaged 45% higher in treatments (1) than in (2) and (3). The increased growth for (2) compared to (3) was probably due to the induction of stress enzymes which help the plant to survive at adverse conditions.

In the 1997/98, 1998/99, and 1999/2000 seasons, the effect of thermotherapy on cassava varieties was investigated in Cauca, Valle del Cauca, and Quindío, Colombia. Hot-water treatment at 49°C for 49 min did not reduce the germination rate of cassava plantings. Higher temperature, even for short periods, can kill cassava cuttings. The effects of hot-water treatments on different diseases were not determined for lack of high and uniform disease pressure. No differences were observed between an automatic regulated hot-water bath and a simple system, using an oil drum and firewood.

Table 1.1. Effect of thermotherapy on the germination of cassava stem cuttings infected with cassava bacterial blight (CBB) planted in the greenhouse under conditions favorable for disease expression. Water temperature was maintained at a constant 49°C.

	Duration of the Treatment		Variety			Treatment		
Variety	Pre-treatment	Treatment	Germination (%)	Plant Height (cm)	Incidence of CBB (%)	Germination (%)	Plant height (cm)	Incidence of CBB (%)
HMC-1	0	0	100	13.1	30			
M Per 183	0	0	84	4.7	28			
Manzana	0	0	90	10.2	50	91	9.4	36
HMC-1	0	49 min.	100	9.5	0			
M Per 183	0	49 min.	90	6.5	10			
Manzana	0	49 min.	90	11.3	10	93	9.1	7
HMC-1	0	5 hours	48	3.8	0			
M Per 183	0	5 hours	30	1.3	0			
Manzana	0	5 hours	40	5.2	0	39	3.4	0
HMC-1	10 min.	5 hours	90	8.6	0			
M Per 183	10 min.	5 hours	90	4.9	0			
Manzana	10 min.	5 hours	65	7.1	0	82	6.9	0

[a] Ten days after planting.

Activity 2. Evaluation of different management components to control Phytophthora Root Rot in the field at Cauca, Valle and Quindío.

Department of Valle del Caucă

The following Root rot control methods were evaluated under field conditions at Caicedonia (Valle del Cauca).

Two potassium sources, two thermotherapy treatments of stem cuttings at 49° C for 49 min, biological root rot control with *Trichoderma* spp. and Micobiol® (mixture of *Trichoderma* spp., *Bacillus thuringiensis, Beauveria bassiana, Metarhizium anisople, Paecilomyces lilacinus,Paecilomyces fumosoroseus, Nomureae rileyi, Entomophthora muscae, Hirsutella thompsonii* and *Verticillium lecanii*). The cassava genotypes ICA Catumare, M Col 1505 and M Per 183, were used in this study, and evaluated for resistance to *Phytophthora* root rots.

MCOL 1505 did not show necrotic stem symptoms, but plant establishment was only 65%. Plant height increased with the application of *Trichoderma* spp. and potassium nitrate (KNO_3). KCl applications increased stem rot, while *Trichoderma* spp. and KNO_3, reduced the damage. Stem cutting thermotherapy, *Trichoderma*, KNO_3 and MCOL 1505 significantly reduced stem rot **(Table 2.1)**. The local variety HMC 1 performed better than the other evaluated genotypes.

Table 2.1. Effect of different control practices on cassava performance and stem rot ' disease in a field trial established at Valle del Cauca.

Treatment	Performance (%)	Height (m)[b]	Severity of stem rot	Incidence of stem rot (% plants)[c]		
				1	2	3
Cuttings treatment						
Water bath (49° for 49 min)	94	1.23	1.5	55	36	9
Water heated by fire wood (49° for 49 min)	92	1.28	1.8	42	39	19
Traditional management						
Micobiol[d]	96	1.24	1.8	37	45	18
Trichoderma spp ($1x10^4$ con/ml)	96	1.39	1.5	58	31	11
Traditional management						
KCl (20 g/plant)	96	1.16	2.0	31	35	34
KNO_3 (20 g/plant)	88	1.51	1.4	73	17	9
Traditional management						
ICA Catumare	98	1.28	1.8	52	21	27
M Col 1505	65	1.14	1.0	100	0	0
M Per 183	88	1.29	1.7	40	48	12
Traditional management						
Farmer management	100	1.28	1.7	46	35	19

[a] Ten months after planting.
[b] Average of plant height
[c] Evaluation scale. 1: plant without stem rot; 2: plant with some stem rot; 3: plants with all stem rot.
[d] Biological product based on fungi for disease and pest control (immersion for 30 min in 6 Kg/Lt suspension and application of 50 ml per plant).

Department of Quindío

Different control practices were evaluated for their effect on crop yield, bacterial blight and root rot at Montenegro (Quindío). Main results are presented in **Table 2.2.** Yields were higher for CIAT control practices such as immersion of stakes in a suspension of the biocontrol agent *Trichoderma* spp. (isolate 14PDA-4) and its frequent application on plants. The application of potassium sulfate and potassium chloride was also beneficial for crop yield compared to the local practices used by farmers. The IPM package proposed by CIAT resulted in aproxymately 20% increased yield compared to the local practice of the farmer.

Table 2.2. Effect of different control practices on cassava performance, root rot (RR) and bacterial blight (CBB) at Montenegro (Quindío, Colombia).

Control practice	Plant height average (m)	Root Yield (ton/ha)	Number of stakes/ plant	CBB Incidence (% affected plants)	CBB Severity[1]	RR Incidence (% affected plants)	RR Severity (Tons. Affected roots/ha)
Variety HMC-1							
Hot water treatment[3]	1.73	62 a[2]	36 a	21 a	89	2.0 a	3.7 a
Biocontrol agent							
Trichoderma[4]	1.89	63 a	36 a	16 a	89	2.0 a	1.8 a
Micobiol5	2.31	60 a	37 a	12 a	56	1.3 a	0.2 a
Ridomil (metalaxyl)	1.91	70 a	39 a	16 a	89	1.7 a	1.0 a
Potassium chloride (KCl)	1.90	70 a	37 a	18 a	100	2.0 a	0.3 a
Potassium sulphate (K_2SO_4)	1.90	80 a	38 a	24 a	100	2.0 a	1.1 a
Local commercial varieties							
Manzana	1.93	41 a	36 a	21 a	100	2.0 a	7.1 a
HMC-1	1.86	51 a	37 a	22 a	100	1.8 a	6.1 a

1 Value between 0 and 3 where 0 = without symptoms and 3 = highly infected.

2 Duncan's Multiple Range Test, Alpha= 0.05.

3 Oil drum and firewood, water temperature 49° C during 49 minutes.

4 Isolate 14PDA-4.

5 *Trichoderma, Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Paecilomyces fumosoroseus, Hirsutella thompsonii, Bacillus thurigiensis*

Second planting cycle

Two experiments were established at San Jerónimo and Mondomito (Santander de Quilichao, Cauca), to evaluate the effect of some crop management practices on control of *Phytophthora* spp. The fertilizers Agropremix® (see nutrient content above), potassium chloride (200 Kg/ha K_2O), potassium sulfate (200 Kg/ha K_2O), stem cuttings thermotherapy, *Trichoderma* isolates 14PDA-4 and 19TSM-3A (1 x 10^4 conidia/ml), stem cutting selection and the elite genotype CM 6740-7 were evaluated, and compared with the farmer's crop management strategy of incorporating chicken manure (250 g/plant) into the soil. In San Jerónimo, 500 Kg/ha of calcium and magnesium lime were incorporated, as traditional farmer's practice. Fertilizer treatments were applied 35 days after planting in San Jerónimo and at planting in Mondomito. The variety planted was Verdecita (M Col 1505), the stem cuttings were obtained from San Jerónimo, where the disease was detected in a previous crop. Germination was not affected by thermotherapy (**Table 2.3**).

Table 2.3. Germination of cassava stakes after different treatments n two farms at Santander de Quilichao (Cauca).

Treatment	Stake Germination (%)	
	San Jerónimo	Mondomito
Fertilizers		
Agropremix (30 g/plant)	96.7	98.3
K_2SO_4 (36 g/plant)[b]	85.0	100.0
KCl (30 g/plant)[b]	80.0	98.3
Thermoterapy	92.5	98.3
Biological control		
Trichoderma 14PDA-4	83.3	93.3
Trichoderma 19TSM-3A	92.5	-[a]
Cuttings selection	75.0	96.7
Genotype CM 6740-7	91.7	98.3
Control (chicken manure)	85.0	98.3

[a] Not evaluated
[b] Equivalent to 18 g/plant of K_2O

Three field experiments were conducted at the farms El Jardín (La Tebaida, Quindío), Las Mercedes and Guayaquil (Montenegro, Quindío), to evaluate the effect of some crop management practices on *Phytophthora* control. The following treatments were evaluated:

1. Fertilization with KCl (30 g/plant) and K_2SO_4 (36 g/plant), compared with farmer fertilization (El Jardín and Las Mercedes: 35 g/plant of Ammonium sulfate: Borax mixture in 50:1.5 rate; Guayaquil: 50 g/plant of 10-30-10). Fertilizer treatments were applied 45 days after planting.

2. Stem treatment of cuttings with thermotherapy (49°C for 49 min), chemical treatment by immersion for 5 minutes in Orthocide® (Captan, 4 g/L of commercial product) and Ridomil® (Metalaxyl, 3 g/L of commercial product) and immersion in Longlife® at 4% (Ascorbic acid).

3. Biological control of *Phytophthora* root rot, was acomplished, by immersion of stakes for 10 min in a *Trichoderma* suspension (1 x 10^4 conidia/mL), with the isolates 19 TSM-3A and 41 PDA-3A. 100 mL/plant of fungal suspension was also used as a drench near the stakes.

4. Varietal resistance using the genotypes HMC-1, Ica Catumare, M Per 183 and the local variety Chiroza (M Col 2066).

Germination of heat treated stakes at Quindío was low (26.7 and 68.3% for each experiment respectively), compared with the same treatment at Cauca (98.3%, **Table 2.4**). Higher temperature, even for a short period can kill cassava cuttings.

Table 2.4. Germination percentage of plants established at three farms at Quindío department.

Treatment	Location		
	El Jardín	Las Mercedes	Guayaquil
Fertilization			
KCl (30 g/plant)	88.3	66.6	96.7
$K2SO_4$ (36 g/plant)	91.7	95.0	95.0
Farmer control[a]	91.7	85.0	88.3
Control without fertilization	93.3	93.3	86.7
Cuttings treatment			
Thermoterapy (49°C for 49 min)	40.0	26.7	68.3
Orthocide (4g/lt) + Ridomil (3g/lt)	100.0	96.7	73.3
Lonlife 4% (Ascorbic acid)	-	-	96.7
Biological control			
Trichoderma (19 TSM-3A)	88.3	98.3	95.0
Trichoderma (41 PDA-3A)	96.7	71.7	90.0
Varietal resistance			
HMC-1	100.0	100.0	91.7
Ica Catumare	88.3	86.7	89.8
M Per 183	93.3	95.0	-
Chiroza	93.3	83.3	70.0

[a] mmonium sulfate + Borax (50:1.5) :35 g/plant at El Jardín and Las Mercedes; 10-30-10 (NPK): 50 g/plant at Guayaquil

Some 35-day-old plants, affected by *Xanthomonas axonopodis* pv. *manihotis* were observed in the following treatments. KCl, farmer control, *Trichoderma*, chemical treatment and in the genotypes M PER 183 and HMC-1 at Las Mercedes and El Jardín, where CBB incidence.

Activity 3. Controlling powdery mildew of roses in Colombia, using a plant extract and foliar fertilizers

Introduction

Sphaerotheca pannosa var. *rosae* (Wallr.) Lév., the causal agent of powdery mildew, is a major pathogen of roses in Colombia. Disease symptoms develop quickly, affecting flower quality and causing significant economic losses. Control measures include foliar applications of fungicides to reduce pathogenic inocula. Because fungicide-resistant *Sphaerotheca* isolates can survive for several years in the field, the risk of building up a resistant population by continuously applying fungicides is very high (Reuveni et al. 1994). 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. 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.

Materials and Methods

Greenhouse evaluation. Pathogen-infected plants of different rose varieties were treated with foliar applications of fertilizers and extract from *Swinglea glutinosa*.

Plant materials. The following cultivars were used to evaluate the effectiveness of test treatments: Aalsmeer Gold, Charlotte, Classy, Konfetti, Livia, and Tineke. These plants were propagated by rooting cuttings, conserving the leaves of the two upper buds, in trays located within a greenhouse. After about 1 month, seedlings were transplanted to pots, 5 inches in diameter, and left to grow in a screenhouse.

Inoculation. Plants were inoculated by spraying conidia with a spore spreader driven by a ventilator with a plastic cylinder and a metal grid at the outlet. Leaves infected with powdery mildew were placed at this outlet and the ventilator formed a constant airflow that carried the conidia toward plant leaves. Two plants per treatment were inoculated in a greenhouse with temperatures ranging between 20°C and 27°C and relative humidity between 70% and 90%.

Swinglea extract and fertilizers. To prepare this extract, healthy leaves were cut from *Swinglea glutinosa* shrubs established at CIAT and applied at the rate of 100 g *Swinglea* leaves per liter of distilled water. The extract was obtained by first liquefying the leaves, then straining the mixture through six layers of gauze and centrifuging at 8000 rpm for 40 min. Finally, 2 mL/liter of Inex-A were added to the solution as dispersal agent and adherent.

The following fertilizers were tested: potassium monophosphate (KH_2PO_4), potassium diphosphate (K_2HPO_4), sodium phosphate (Na_3PO_4), and monoammonium phosphate ($NH_4H_2PO_4$). Treatments with Elosal (elementary sulfur at 80%) and sterilized, distilled water were included as checks.

Applications. Both sides of the leaves were sprayed with a 1-L pump. The foliage was completely wetted with the product. Other treatments were applied in sufficient amounts to soak, without overwetting, the foliage.

Assessing treatment effectiveness. Evaluations were performed on (1) the first four true leaves (tri- or pentafoliolate) per stem, (2) two stems per plant, (3) stems with the highest number of infected leaves, and (4) leaves with very small colonies of the fungus and recorded as infected. During the evaluation, another trial was conducted in which the plants were first treated with foliar fertilizers and *Swinglea* extract, then inoculated with the fungus 2 h after treatment. The treatments were also evaluated for their effectiveness in reducing the severity of black spot, a disease, caused by *Diplocarpon rosae* Wolfe that occurs in association with powdery mildew under test conditions in Palmira.

Residual and phytotoxic effects. An experiment was carried out to determine the residual and phytotoxic effect of the treatments on plants of different rose cultivars. Each of several products was applied to a pair of healthy, leaf-spot-free plants and assessed on a daily basis. Two applications were made, a week apart.

Results

Effects on powdery mildew. The treatments in which the incidence of powdery mildew was lowest in rose plants were *Swinglea* extract, Elosal, and KH_2PO_4 (**Table 3.1**). The treatment with Na_3PO_4 showed the highest disease incidence, after the check treatment with water.

When KH_2PO_4 fertilizer was applied to plants of cv. Tineke, these did not become infected by powdery mildew, unlike the plants of other cultivars, which showed symptoms. Resistance was therefore induced by KH_2PO_4 in this rose genotype. After 2 weeks, the KH_2PO_4 continued to have an effect on the fungus. In contrast, 25% of the check's leaf area was infected.

Phytotoxic effects. Residual or phytotoxic effects were observed after applications. The results can be summarized as follows:

1. Plants treated with sterilized distilled water, *Swinglea* extract, and fertilizer $NH_4H_2PO_4$ did not present residual effects until after the third application of the extract, when a white residue was observed on leaves, possibly caused by applications of Omite or higher concentrations of the extract. By centrifuging the extract and thus separating the residues from the supernatant, we prevented the formation of spots on leaves after application. This procedure did not reduce the extract's effectiveness in controlling powdery mildew of roses.
2. With Elosal, an opalescent white residue was observed on about 90% of the foliage of treated plants.
3. The fertilizer KH_2PO_4 caused spattering of white residue on about 90% of the foliage.
4. With fertilizer K_2HPO_4, the leaves appear moist, and 3 days after application, white spots appeared on about 10% of the foliage.
5. For 3 days, after Na_3PO_4 was applied, leaves looked oily, then leaf apices showed burns.

Effects on black spot. Table 3.1 indicates that the K_2HPO_4 treatment was the most effective for controlling black spot, reducing by 98% the leaf area infected (average of four leaves), compared with the check treated with sterilized water. This fertilizer was more effective than Elosal, although differences were not statistically significant.

Table 3.1. Effectiveness of fertilizers and a plant extract in controlling powdery mildew (*Sphaerotheca pannosa* var. *rosae*) and black spot (*Diplocarpon rosae*) in seven rose cultivars. Applications of Elosal and water were included as checks [a].

Treatment		Leaf Area Infected by *S. pannosa var. rosae* (%)		Leaf Area Infected by *D. rosae* (%)	
Active Ingredient or Product	Concentration (g/liter)	First Leaf	Average of 4 Leaves[b]	First Leaf	Average of 4 Leaves[b]
Sterilized distilled water	-	22.3 a	10.9 a	13.6 a	9.9 a
Elosal	3 ml/liter	0.8 b	0.2 b	3.7 ab	1.4 ab
Swinglea extract	100	3.9 b	1.6 b	3.2 ab	4.1 ab
KH_2PO_4	13.60	5.9 ab	3.2 ab	9.5 ab	2.7 ab
K_2HPO_4	17.40	9.3 ab	2.7 b	0.7 b	0.2 b
Na_3PO_4	16.05	13.5 ab	6.8 ab	7.6 ab	1.9 ab
$NH_4H_2PO_4$	11.45	6.4 ab	3.7 ab	9.3 ab	5.6 ab
Average		8.9	4.2	6.8	3.7

[a] Averages followed by the same letter do not differ significantly (LSD, $\alpha = 5\%$).
[b] First four true leaves (tri- or pentafoliolate).

Discussion

Controlling powdery mildew of roses with chemical fungicides is difficult and expensive under conditions that favor the disease. Alternative control methods based on fertilizers and plant extracts should therefore be developed.

Foliage application of an extract from *Swinglea glutinosa* reduced the number of leaves affected by powdery mildew of roses. Pastor-Corrales (1991) reported the inhibitory effect of this extract on the causal agent of anthracnose (*Colletrotrichum lindemuthianum*) and Ascochyta blight in beans. Future tests should be conducted on flowers grown in greenhouses to assess the effectiveness of this biofungicide.

Plants of cultivar Tineke, treated with KH_2PO_4, did not show disease symptoms, suggesting that resistance was induced by this fertilizer. Reuveni et al. (1993, 1994, 1995a, b) had already observed this response in roses and cucumber. Because of its preventive capacity, the induction of resistance has high potential in disease control, allowing for a more effective management of powdery mildew of roses. For the immediate term, a product can be developed to induce disease resistance in roses, or a biofungicide developed to help control diseases. Future research should evaluate the effect of foliar fertilizers and *Swinglea* extract on other economically important diseases such as downy mildew of roses, caused by *Peronospora sparsa*.

Activity 4. Participatory disease and crop management in the Colombian northeast Amazon

Ash (200 g/plant), organic matter (200 g/plant) from dead leaves taken from forest soil, and a mixture of both at a 1:1 ratio were evaluated, employing farmer participatory research methodologies, for their effects on the yield of four native cassava varieties. The cassava was grown in *chagras*, which are small plots of slash-and-burn agriculture in rain forest. Cassava is planted with other crops. Farmers were women from two Tukano indigenous communities at Mitú (Vaupés). The same treatments and chemical fertilizers (Di-ammonium phosphate at 2.5 g/plant and potassium chloride at 2.5 g/plant) were evaluated for the control of *Phytophthora vignae* under greenhouse conditions at CIAT. Young stems of M Bra 1044 were inoculated with the isolate P12, using the wounding method.

Table 4.1 shows that ash and organic matter increased the yield of native cassava in Mitú. Some of the farmers observed root rot reduction with the ash treatments. Under greenhouse conditions, two treatments—ash alone and ash mixed with organic matter—were the most effective methods for controlling *P. vignae*.

Table 4.1. Effect of organic matter from dead leaves collected from forest soil and ash on the yield kg/plant) of four native cassava varieties in two indigenous communities from Mitú (Vaupés, Colombia) and on severity of Phytophthora rot in young stems.

	Community						
	Seima Cachivera			Puerto Palomas			
	Variety			Variety			Greenhouse
Treatment	Algodón	Borugo	Average	Abiyú	Lapa	Average	Disease Lesion (cm^2)[a]
Ash	0.65	0.70	0.68	0.51	0.72	0.62	0.99
Ash + Organic matter	0.60	0.74	0.67	0.64	0.79	0.72	1.69
Organic matter	0.63	0.66	0.65	0.63	0.72	0.67	4.14
Control	0.49	0.68	0.59	-	0.46	0.46	6.52
Chemical fertilizer	-	-	-	-	-	-	6.20

[a] Lesion caused by *Phytophthora vignae* on young stems.

Contributors – Sub-output 1: Phytopathology

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Sub-output 2. Germplasm with Relevant Traits Developed and Widely Disseminated in Africa.

Activity 1. Germplasm to address African production constraints: Evaluation of lines for tolerance to BSM in Uyole and Mulungu

Introduction

To help solve the beans stem maggot problem, bean germplasm lines and others from the CIAT breeding program were distributed to several collaborating NARS in the network for evaluation and further promotion to farmers.

Methods

Selections were based on tolerance to BSM and yield.

Results and Discussion

Results that have been received so far indicate several of the materials had superior performance against the pest and some of them combine this attribute with high yield. At both Kisii (western Kenya) and Mulungu (Kivu province Congo DR) the top performers were CIAT bred lines (**Table 1.1**). These two entries are of medium seed size and have purple and cream seed colours respectively. Other entries such as G 22501 and G 23333 (originally from Burundi) and G 22258 (from Rwanda) and selected through the core collections, showed adaptation and good performance at Mulungu which is ecologically similar. Some of these materials have shown good performance also in Kenya, Malawi, Tanzania and other places. The TBF lines will now be multiplied and disseminated more widely through IPM promotion activities.

Table 1.1. Performance of BSM tolerant lines at Mulungu, Congo DR.

Entry Name	Origin	% BSM Mortality	Rank		Grain yld in kg/ha	Rank		Good Performance at other Locations
GR 13-P	CIAT Br.	15.1 cde	6	(1)[a]	2235.2 a	1	(1)[a]	KYA, TZA
G 22501	BND	6.0 e	1	(6)	2102.4 ab	2	(6)	TZA, MWI,
GR 13-C	CIATBr.	13.1 cde	5	(8)	1989.6 abc	3	(7)	KYA, TZA
G 11727	PER	16.0 bcde	8		1794.4 abcd	4		
G 8047	KYA	28.7 ab	15	(2)	1739.2 abcd	5	(4)	KYA, TZA
G 22258	RWD	17.7 bcde	10		1721.6 abcd	6		RSA,TZA
G 5625	MEX	16.2 bcde	9	(4)	1568.8 abcde	7	(2)	KYA, TZA
IKI	?	18.3 bcde	11		1499.2 abcde	8		TZA
PAD 3	CIATBr.	7.5 de	2		1335.2 abcdef	9		MWI,
G 23333	BND	10.9 cde	4		1086.4 bcdef	10		
ZPv 292	UGA	19.6 bcd	13		974.4 cdef	11		ZBA, TZA
G 11746	PER	10.1 cde	3		875.2 def	12		TZA
G15430	ZBA	18.8 bcde	12		618.0 ef	13		
Beshbesh	CIAT Br.	21.8 abc	14		428.2 ef	14		ETH
G 23070	MWI	34.9 a	16	(7)	307.4 f	15	(5)	KYA
G13910	ECD	13.5 cde	5		282.4 f	16		

[a] Performance ranking in Kisii, western Kenya

Sub-output 3. Sustainable Bean Production Systems.

Activity 1. Collaborate with other IARCs and Advanced Research Institutes to develop IPM components to reduce crop losses from pests

Introduction

New partnerships developed with the Institute of Arable Crops Research (IACR) -Rothamsted, UK and the ICIPE to do basic research to improve our understanding of the interaction insect plant interactions relating to bean stem maggots, aphids and other bean pests and their natural enemies.

Method

Because of common interest in developing sustainable strategies for small scale farmers, we have initiated an informal collaboration to pull our complementary strengths to address our common objective.

Results and Discussion

After initial electronic discussions we developed a proposal on the "*Development of a push-pull strategy utilising indigenous host and non-host plants to improve management of bean insect pests for smallholder farmers in eastern Africa* " in collaboration with NARS scientists. The proposal has passed the initial screening by DFID CPP and a project memorandum in under consideration. Under this arrangement, CIAT will undertake studies on interactions between the bean plant and its companion crops as it affects pest and natural enemy interactions, ICIPE and IACR-Rothamsted will undertake studies to understand the chemical communication systems underlying the interactions. The research will contribute to IPM for bean pests.

Activity 2. Efficient methods for systems improvement: Understanding bean stem maggot ecology in Tanzania

Introduction

Population patterns and species dominance among bean stem maggots are believed to be changing. *O. spencerella* is gaining dominance in Ethiopia, an area where it was previously unknown. BSM ecology is also not well understood, leading to difficulties in predicting populations and likely attacks.

Methods

Beans were planted on weekly basis through the year from November 1999 and BSM species composition was monitored.

Results

O. spencerella was dominant through the year, with a small and brief reversal in species dominance in early to mid May (**Figure 2.1**). BSM populations dropped sharply in late February to early March, just before the rains and planting of beans started. The populations picked up again in late April to early May. This period of population depression corresponds to the bean planting period and explains why BSM is less serious at Selian in the March planted crop while the late planted crop is more severely attacked. The population dynamics appear to have remained the same, as (Swaine 1968) and (Wallace 1939) observed the same trend. However there appears to be shift in species composition with *O. spencerella* gaining complete dominance over *O. phaseoli* as previously strong reversals in species dominance were not observed. Factors influencing the population change are under investigation.

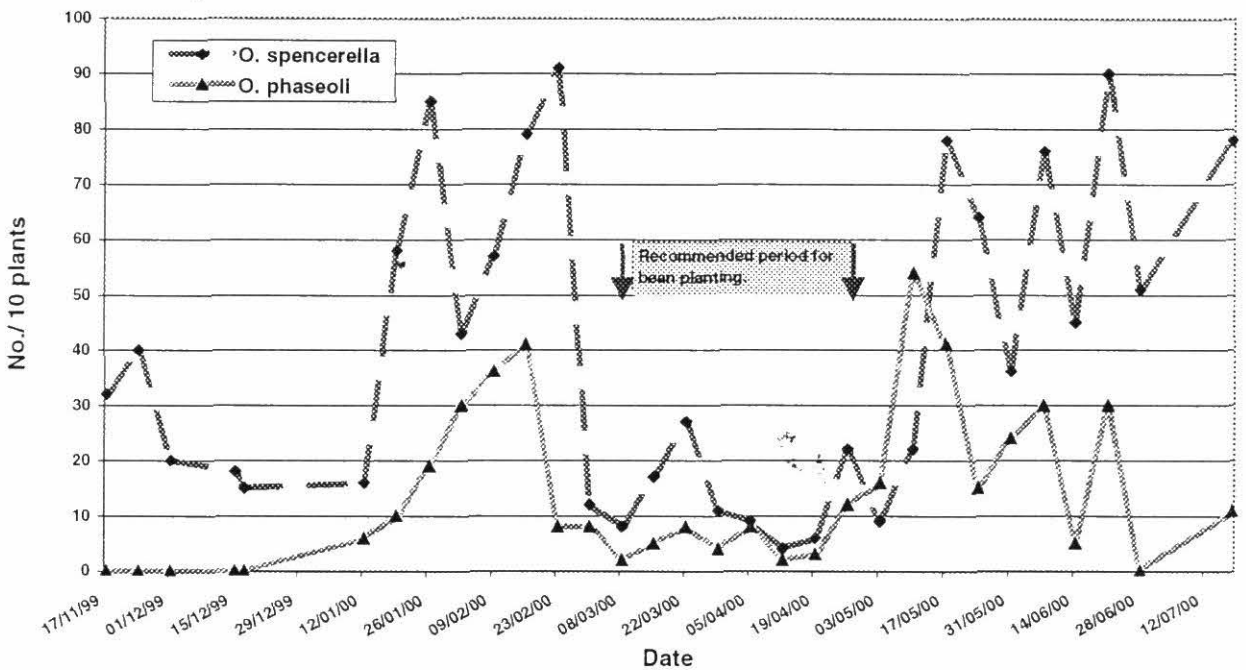

Figure 2.1. Bean stem maggot population dynamics at Selian 1999/2000.

References:

Swaine G. 1968. Studies on the biology and control of pests of seed beans (*Phaseolus vulgaris*) in northern Tanzania. Bull. Ent. Res. 59:323-338.

Wallace G.B. 1939. French bean diseases and bean fly in East Africa. E. Afr. Agric. J. 5: 170-175.

Activity 3. Effect of companion crops on pest and natural enemy populations in bean intercrops

Introduction

To examine and understand the multiple interactions among beans and various companion crops, bean pests and natural enemies with a view to identifying combinations that would lead to pest reductions and damage to beans.

Methods

Eight crop varieties that are commonly grown with beans were selected for a study of their effect on bean colonization by pests (aphids and bean stem maggots, whiteflies and thrips). The companion crops (cowpeas, soybeans, Kale (sukumawiki), bell pepper, onion, tomatoes, maize and sorghum) were planted before the planting of beans. Pest colonization was monitored with traps and other sampling procedures.

Results and Discussion

Tomatoes appeared to be attractive to BSM and white flies, the populations of these pests were highest in this intercrop with beans. Cowpeas appeared to attract BSM into the crop and beans planted with cowpeas were more heavily infested than beans alone. The other companion crops, soybean, pepper, maize, sorghum and onion appeared to have no effect on BSM, but the maize/bean intercrop had less BSM and white flies and thrips. This could be due to the shading effect of the maize over the bean canopy (this will be investigated in future trails). Intercropping with cale or pepper reduced white fly and thrips populations. Kale is known to have high levels of isothiocyanates that confuse certain aphids in their host location. The effect of such volatiles was not apparent in this trial. This study is in its initial stages and data reported here are only preliminary.

Table 3.1. Influence of companion crops on pest activity in bean intercrops.

	Pest Activity				
Companion Crop	% BSM Infestation	BSM/Plant	Whitefly /Trap	Aphids/Trap	Thrips per Trap
Cowpea	93.3 a	3.7 b	4.7 bc	3.0 b	27.7 a
Soybean	86.7 a	2.5 bc	5.0 bc	0.7 b	16.3 abc
Maize	60.0 b	1.7 c	1.2 d	0.3 b	3.3 d
Sorghum	86.7 a	2.9 bc	4.1 bcd	2.7 b	13.7 bcd
Tomatoes	100.0 a	5.5 a	8.8 a	2.0 b	7.0 cd
Pepper	86.7 a	2.9 bc	2.9 cd	1.3 b	7.7 cd
Onions	73.3 ab	3.4 b	8.0 a	3.7 ab	15.7 abcd
Kale	73.3 ab	2.1 c	3.0 cd	7.3 a	8.7 cd
Beans untr. Sole	86.7 a	2.5 bc	8.3 a	1.3 b	17.3 abc
Beans trt. Sole	73.3 ab	2.3 bc	6.4 ab	3.7 ab	26.0 ab
$LSD_{.05}$	25.8	1.5	3.3	4.2	12.5

Means in the same column followed by different letters are different at the 5 % level.

Activity 4. Scaling up IPM for bean pests in northern Tanzania through a decentralized system: Bean foliage beetle (*Ootheca* spp.) IPM promotion with the extension service

Introduction

IPM strategies for bean pests have been developed by CIAT and the Bean Networks at pilot sites but scaling up to other areas has been slow. National extension systems need to be strengthened to disseminate IPM strategies more widely to farmers. This year we initiated work to identify and develop strategies for scaling up IPM and improve adoption among small-scale bean growers in northern Tanzania.

Method

IPM strategies for bean foliage beetles were developed with in collaboration with farmers from Hai district so when the district extension office requested help in scaling up to other areas we sought participation of the farmers once again. In a stakeholders meeting, the management strategies were reviewed and dissemination pathways were discussed. Each group (farmers and extension officers representing a village) considered their resources and opportunities and identified appropriate dissemination pathways to suit their circumstances. The dissemination pathways selected by different villages in a rank order were: 1. on-farm demonstrations, 2. demonstrations in schools, 3. training through farmer research groups, 4. distribution of extension information leaflets about the problem and its management, and 5. awareness creation seminars and field tours. Each group discussed the pest and the available options for management with their respective village farmer' research groups, who modified or added to them, as they found necessary. The farmer research groups implemented the demonstrations with the assistance of the local village extension officer. We monitored farmers' perception of the IPM strategies and the promotion process used with questionnaire surveys during field days.

Results and Discussion

IPM strategies selected at the stakeholders meeting were: 1. adjustment of planting time (delayed planting) of beans to avoid peaks of BFB infestation, and 2. the application of botanical pesticides (neem seed oil and neem seed powder). The farmers added traditional technologies such as: 1. fermented cow urine ("Mkojo"), 2. fermented liquid effluence from the cow shed (a mixture of urine and feces = "Mfori"), 3. kerosene and soap mixed with water, and 4. ashes. These treatments were evaluated together in the participatory demonstrations. **Figure 4.1** shows the performance of the different treatments, depending on the village. The dissemination strategies were seminars and distribution of extension leaflets to create awareness about the pest, and field demonstrations of the management strategies. The dissemination centres were schools, community training centres and farms at vantage locations within the village.

All treatments worked better that the control. The effect of cow urine lasted longer and delayed re-infestation better than the others (**Figure 4.1**). To publicize the technologies further the groups held a series of field days, and invited the local communities to view and discuss the demonstrations. Ashes, neem oil and fermented cow urine were the most preferred treatments for

BFB control by participants at the field days (**Figure 4.2**). About the dissemination process, the community preferred: 1. more seminars for awareness creation, 2. improvement of the extension system (empowering of the extension service to deliver), 3. dissemination through the mass media to reach more farmers. In addition the non-participating farmers were strongly in favour of more demonstrations while members of the FRGs were in favour of dissemination through the mass media and distribution of extension leaflets (**Figure 4.3**). Also, the large farmers (> 1 acre of beans) selected group training as their preferred option for IPM dissemination while the small farmers preferred demonstrations (**Figure 4.4**). It appeared from further discussion that small farmers had less time for regular group activities as they have limited access to external labour and are therefore fully occupied by farm and other activities.

The selection of different dissemination pathways by the large and small farmers suggests that these groups should be targeted with different approaches. In a post -season monitoring and evaluation exercise, the farmers cautioned that multiple strategies should be used rather a single one as different strategies target different categories of farmers. There is now an increased demand for IPM promotion to reach more farmers. The extension service plans to develop mass media (radio and newspaper) messages on *Ootheca* management for a wider audience. We are also seeking funds from DFID to disseminate bean pests IPM more widely across the eastern and southern Africa region.

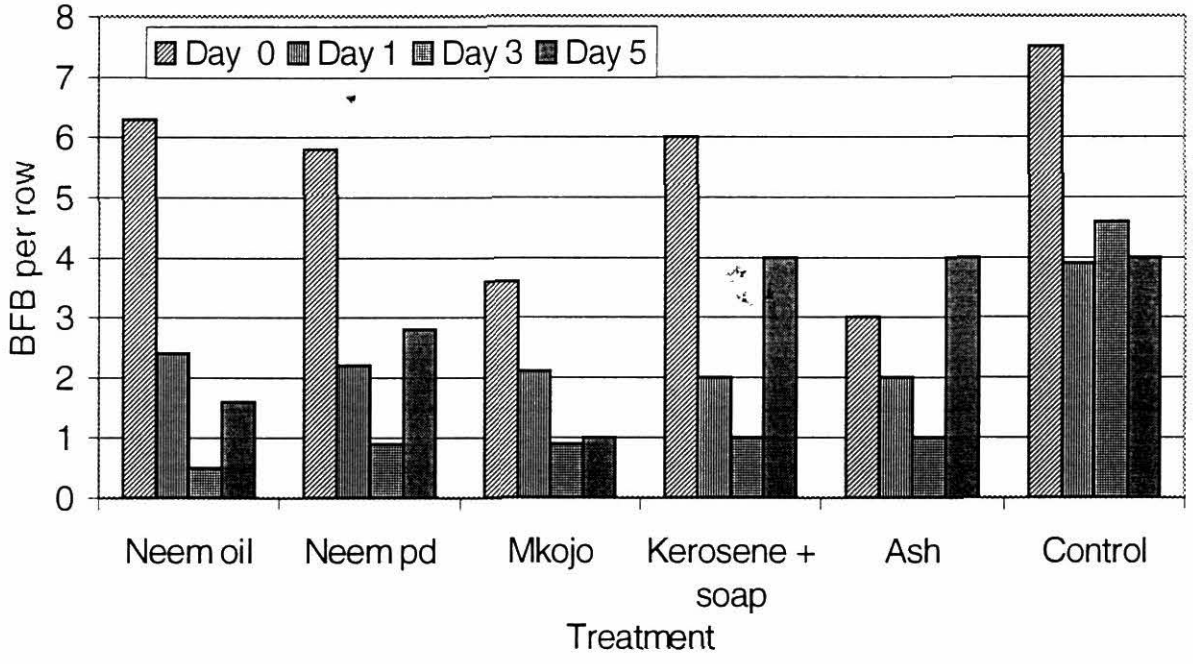

Figure 4.1. Performance of neem and other traditional products against bean foliage beetles farmers' fields at Hai.

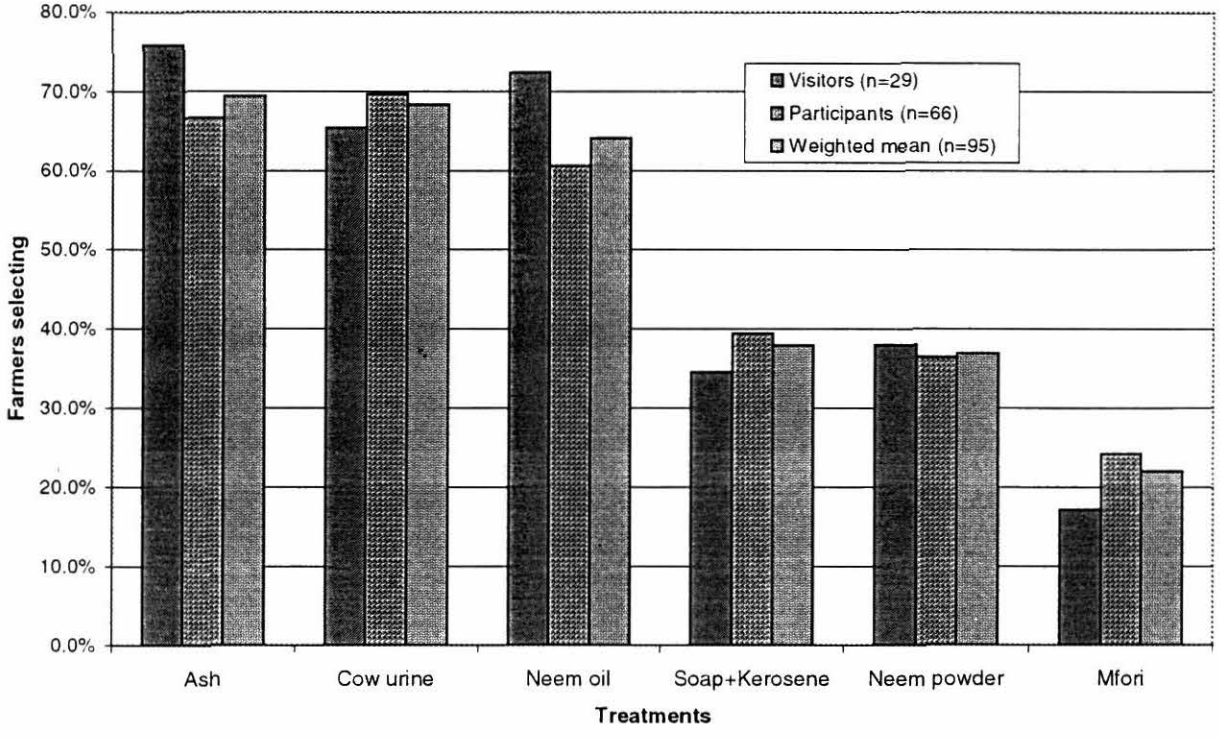

Figure 4.2. Preferences shown by members and non-members of FRGs for the BFB management strategies.

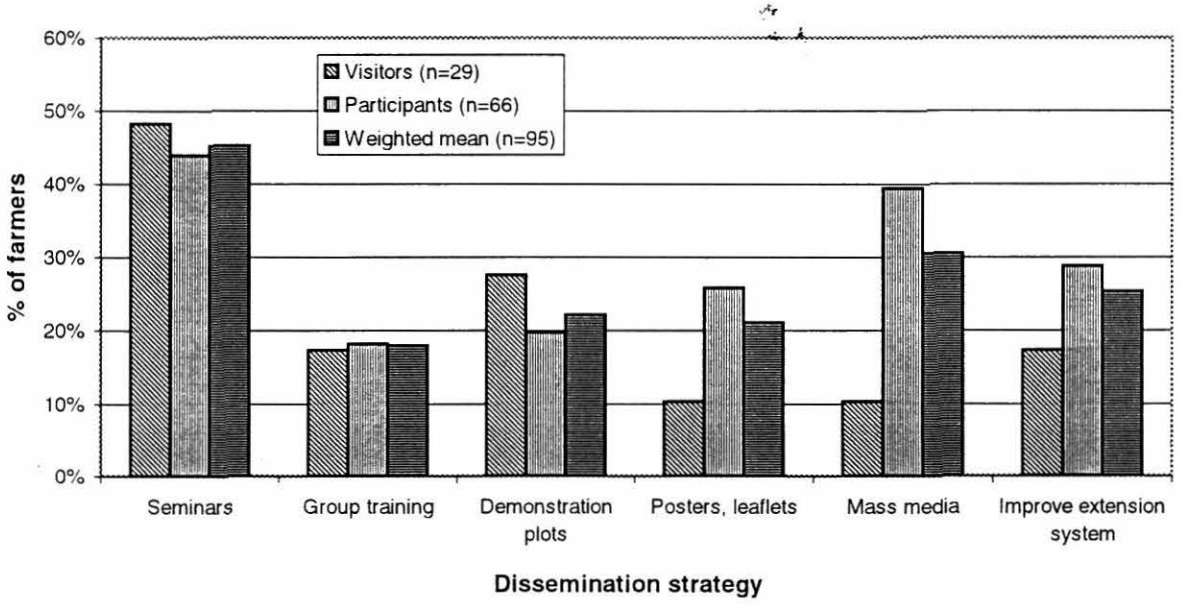

Figure 4.3. Selection of IPM dissemination channels by members and non-members of Farmers' research group at Hai (Kilimanjaro region).

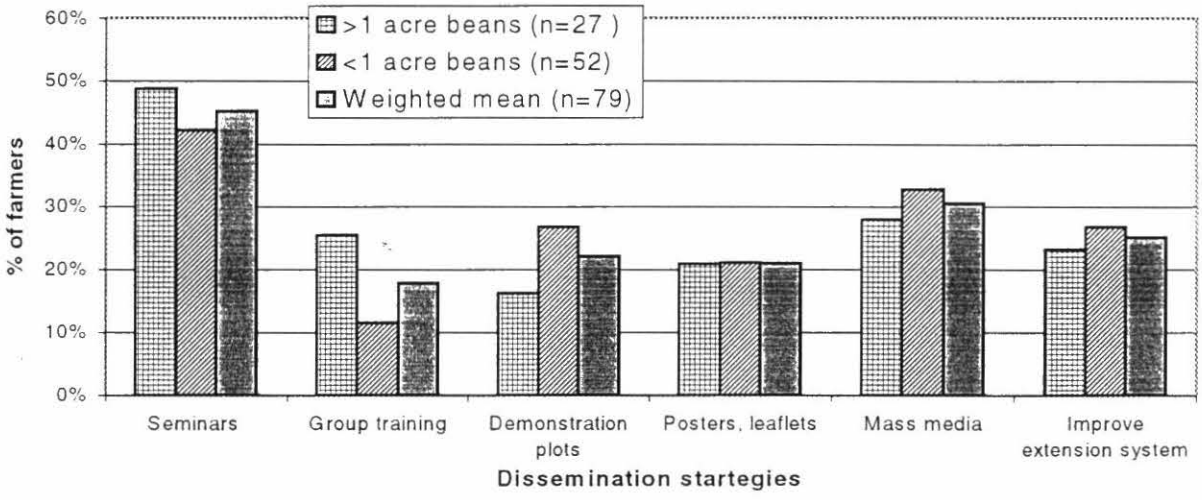

Figure 4.4. Selection of IPM dissemination channels by large and small farmers at Hai (Kilimanjaro region).

Contributors – Sub-outputs 2-3: Entomology

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Mr Jackson Lyimo, Field Assistant (Left in August 2000)
Mr Hendry Mziray, Research Assistant (Joined in October 2000)
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Mr Abdalla Gamba Security Guard
Mr Meseiki Laizer, Security Guard

Staff at the Arusha Base

Dr Pyindji Mukishi, ECABREN Coordinator
Ms. Ursula Hollenweger, SDC Research Associate

Sub-output 4. Pest and Disease Management Components and IPM Strategies and Tactic Developed.

Activity 1. Interactions between Fusarium wilt and bean stem maggot and nematodes

Introduction

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *phaseoli* is an important disease on some popular climbing bean varieties such G 2333 (Umubano) in the Great Lakes Region. Some of the few resistant bush varieties to root rots are also susceptible. This vascular organism invades the plant via the root system and is likely to be influenced by infection and damage by bean stem maggot and nematodes (*Meloidogyne* and *Pratylenchus* spp). Considering the widespread prevalence and importance of BSM and in some areas nematodes, knowledge on their interaction with fusarium wilt is useful in developing management strategies against the disease.

Methodology

Interaction between fusarium wilt and BSM, and nematodes were determined using a variety G 2333 known to be very susceptible to wilt. Assessments were done over two seasons in on-farm and on-station trials at PNL-Mulungu in DRC Planting was done at weekly intervals over three weeks. Treatments included the control of BSM and nematodes by use endosulphan and furadan respectively and observing the effects on fusarium wilt.

Results and Discussion

Incidence and severity of fusarium wilt were significantly reduced in plots where BSM was controlled particularly during the 1st and 3rd planting dates. A similar but not significant effect was observed with the control of root knot nematodes. This implies that the presence of BSM and to some extent nematodes results in the increase in incidence and severity of fusarium wilt. This means therefore that in the development of management practices to control Fusarium wilt, the possible influence of BSM and nematodes have to be considered.

Contributors – Sub-output 4: Phytophathology

R. Buruchara , NARO, HRI and NRI
Kijana Ruhebuza (PNL, Mulungu)

Sub-output 5. Pest and Disease Management Components and IPM Strategies.

Activity 1. CIAT campus effort to minimize the presence of cassava frogskin disease

Despite several warnings, little was done to control the spread of CFSD within the CIAT campus. When the incidence of this disease is low, the spread is very slow but the rate of spread increases as the incidence is greater. The expression of symptoms is variable with the most important factor being temperature. This disease is more of a problem in years with more rain and lower average temperatures.

In the CIAT germplasm, the incidence of CFSD has been increasing for many years. Since the disease affects the yield and root quality, it is interfering with experiments and with the exchange of cassava germplasm within Colombia. An action plan is now being implemented to reduce the level of CFSD in the CIAT germplasm. Cassava frogskin disease (CFSD) is a problem in the cassava germplasm in the CIAT farm, and the principal method of dissemination is infected stem cuttings.

The teams working with cassava have agreed to act together to reduce to the lowest level possible the incidence of CFSD on the CIAT campus.

1. All cassava stem cuttings that are imported or exported from the CIAT farm must pass through the certification process.
2. All cassava plantings on the CIAT campus must be approved.
3. Secundina is currently being grown at and is available for certification of cassava germplasm.
4. At harvest the roots of the plant must be inspected for CFSD and those plants with symptoms will be destroyed. If the material is especially valuable, it can be treated by chemotherapy and cleaned by meristem tip culture.

In general the plan is functioning well but there will need to be some compromises and adjustments in the way research is done in cassava. Also it was decided to attempt to reduce the incidence of the whitefly *Aleurotrachelus socialis*. The control strategy is to synchronize planting of cassava and break the cycle of whitefly reproduction. This will begin in April of 2001, and there will be one month with almost no cassava on the CIAT farm. Then the cassava will be planted during a 4-6 week period. This will be the only planting date for cassava grown on the CIAT campus.

These control measure are being utilized to rapidly reduce the incidence of CFSD in the CIAT germplasm. By the middle of 2001, it is expected that the levels of CFSD on the CIAT campus will be very low. Also it is expected that there will be lower pest pressure on the cassava trails and germplasm grown on the CIAT campus.

Activity 2. Certification of cassava germplasm

With the increased levels of CFSD in CIAT materials, it has become more important to certify that the materials that are used in experiments both on the CIAT campus and in other parts of Colombia are free of CFSD. Grafting to the indicator varieties is the most secure method for the detection of

CFSD. To assure constant supplies of the indicator landrace Secundina, it has been planted in isolated areas where cassava has not traditionally been grown. At the beginning of 2000, there was a shortage of Secundina for the certification of cassava. There are now sufficient materials for the needs of cassava certification of the CIAT materials.

Activity 3. Development of citrus certification program

This effort is in the beginning phase of building capacity jointly with Corpoica and the University of Florida. In addition implementing diagnostic assays for citrus tristeza virus, we are developing the ability to detect other citrus problems. Citrus blight is a disease of unknown etiology, and we are collaborating in testing diagnostic reagents and in a project to identify the cause. Citrus leprosis is a problem in Brazil, Venezuela and Panama. This is a complex of a virus and a mite and detection of the virus needs to be part of a certification program. The development of citrus psorosis virus is also underway. This capacity build is important because it is adding diagnostic techniques not readily available in the region and increases CIAT's visibility in a new area of research and technology transfer.

Contributors – Sub-output 5: Virology

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223707

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

Activity 1. Group training of extension workers, technicians and farmers and students

Training Offered

CIAT. Palmira. December 9-18. Técnicas moleculares aplicadas a la identificación de la resistencia a enfermedades de diferentes cultivos. Course to researchers from different universities and institutes from Colombia. Financing: Agricultural Ministry of Colombia.

Pereira, Risaralda. April 3-6, 2000. Training to multipliers of technology transfer. Technicians from SENA Armenia, Comité de Cafeteros de Risaralda, Secretaría de Agricultura de Risaralda, UMATAs (Risaralda, Quindío and Norte del Valle). Farmers from Quindío, Risaralda and North of Valle del Cauca.

Aguazul, Casanare. May 30 - June 2, 2000. Technicians from Fundación CEMILLA, Secretaría de Agricultura y Desarrollo Rural de Agua Azul, UMATAs Casanare. Training in cassava disease integrated management.

CIAT. September, 2000. Dr. Hanne Nielsen of the collaborative project with the Danish Government Institute of Seed Pathology (DGISP), Copenhagen, Denmark. Training in Molecular characterization of bacterial and fungal pathogens.

CIAT. October, 2000. María del Socorro Balcázar, Project SB-2, CIAT. Training in molecular characterization of *Xanthomonas campestris*.

Mitú, Vaupés. October 17-20, 2000. Training of extension workers and farmers from CRIC and Secretaria de Agricultura on participative research and crop protection.

Training Received

Participation in training courses of research assitants: José Luis Claroz, Michigan State University (USA), and John B. Loke, Plant Protection Service, Wageningen, (Netherlands), Danish Government Institute of Seed Pathology (Denmark), and Scottish Crop Research Institute (Scotland).

Presentations for University Groups

Llano, G.A., Claroz, J.L. and Mejía, J.F. Diagnóstico, caracterización y control de enfermedades de yuca y flores. To Biology students from Universidad Pedagógica de Bogotá. May 27.

Claroz, J.L., Llano, G.A.,. and Loke, J.B. Técnicas moleculares aplicadas a fitopatología; aislamiento de *Phytophthora* spp.; Investigación participativa para el control de pudriciones de yuca

en comunidades indígenas de Mitú (Colombia). To Agroecological Engineering students from Universidad de la Amazonia de Florencia (Caquetá). September 22.

Loke, J.B. Caracterización molecular y patogénica de *Sphaerotheca pannosa* var. *rosae* de Colombia. To students from Universidad San Buenaventura. Oct 13.

Contributors – OUTPUT 3: Phytopathology

Elizabeth Alvarez
Germán Llano
John B. Loke
José Luis Claroz
Juan Bernardo Pérez
Herney Rengifo
Carmenza Durán

223708

OUTPUT IV. GLOBAL IPM NETWORKS AND KNOWLEDGE SYSTEMS DEVELOPED

Sub-output 1. Sustainable Integrated Management of Whiteflies as Pests and Vectors of Plant Viruses in the Tropics.

In 1996, The CGIAR Whitefly IPM Task Force defined a Goal, Project Purpose, Outputs and Activities for the Whitefly IPM Project. The Outputs and Activities **(Figure 1)** contemplate the formation of a research NETWORK for whiteflies (WFs) and whitefly-transmitted viruses (WTVs) in the Tropics; extensive DIAGNOSIS and characterization of the WF/WTV problem; BASIC research on pest and disease dynamics; testing and integration of IPM strategies and tactics; TRAINING; and project IMPACT assessment.

In 1997, Danida granted funds for Phase 1, a start-up phase, of the Project. Phase 1 work concentrated on the formation of a pan-tropical research network for WFs and WTVs in the Tropics, and the extensive diagnosis and characterization of the WF/WTV problem in Latin America and Africa (Outputs 1 and 2, **Figure 1; Figure 2**).

The specific objectives of the Phase 1 work were to organize professionals working on whitefly problems in the Tropics, through formal but also informal networks and, within the framework of those networks, to improve characterization of the whitefly problem in the Tropics, in order to fundament a sound research agenda as well as select critical geographical areas (hot spots) to target intensive research activities and IPM component testing for Phase 2 work (Outputs 3 and 4, **Figure 1**).

Strengthening the Whitefly Network in the Tropics

During Phase 1 we successfully consolidated the Coordination Team for the Whitefly IPM Project. The Coordination Team reflects the structure of the Project which is based on the eco-regional approach to our work, and the nature of the problems being addressed, as originally defined by the Whitefly IPM Task Force in 1996: 1) whiteflies as pests in the tropical highlands; 2) whiteflies as virus vectors in mixed cropping systems of the tropical lowlands, and 3) whiteflies as vectors of virus and pests in cassava **(Figure 2).**

Pamela Anderson (CIAT) Coordinator, Whitefly IPM Project
Richard Markham (IITA) Coordinator, Systemwide Program on IPM

Sub-project Coordinators:

Sub-project 1: Cesar Cardona (CIAT) South American sub-project on Whiteflies as Pests in the Tropical Highlands

Sub-project 2: Francisco Morales (CIAT) Central America, Caribbean and Mexican sub-project on Whiteflies as Virus Vectors in Mixed Cropping Systems

Sub-project 3: Lisbeth Riis (ICIPE) Eastern and Southern Africa sub-project on Whiteflies as Virus Vectors in Mixed Cropping Systems

Sub-project 4: Peter Hanson (AVRDC) SE Asia sub-project on Whiteflies as Virus Vectors in Mixed Cropping Systems

Sub-project 5: James Legg (IITA) Sub-saharan Africa sub-project on Whiteflies as Virus Vectors in Cassava and Sweetpotato

Sub-project 6: Anthony Bellotti (CIAT) South American sub-project on Whiteflies as Pests in Cassava

During Phase 1, Danida funded Sub-projects 1, 2, 3, and 5, in Latin America and Africa. That funding, which allowed us to initiate the Whitefly IPM Project and begin establishing the network, was the basis for bringing other Donor Partners into the Project. We obtained additional support from: the Australian Centre for International Agricultural Research (ACIAR) to support Sub-project 4; the US Agency for International Development (USAID-Office for Foreign Disaster Assistance) and the UK Department for International Development (DFID) to support Sub-project 5; USAID and the New Zealand Ministry of Foreign Affairs and Trade (MFAT) for Sub-project 6; the US Department of Agriculture-Agricultural Research Service (USDA-ARS) to support the coordination and some basic research in epidemiology, and the Ford Foundation (PRGA Small Grants) to support pilot site Farmer Participatory Research (**Figure 1**).

As a result of the combined resources from all Donor Partners, we have established a formal pan-tropical network that includes:

5 International Agricultural Research Centers (CIAT, ICIPE, IITA, AVRDC, CIP)
10 Basic Research Organizations (in Australia, Germany, New Zealand, UK, USA)
54 NARS institutions in 30 countries across the Tropics (13 in Latin America, 10 in Africa, 8 in Asia) - all involved in the extensive survey activities

We were also able to do informal networking by sharing literature, advice, and the protocols that we developed, as well as providing diagnoses of biological samples and training, on a limited basis, to NARS scientists who were not formally part of the Project.

It was clear from the formal and informal networking activities during Phase 1 that the principal requests from the NARS scientists are increased access to: literature, technical information and resources, and Project results. Progress was made in this direction during Phase 1, through the development of a World Wide Web Site for the Whitefly IPM Project, which includes a searchable database of the tropical literature on whiteflies, a Directory of Professionals working on Whiteflies in the Tropics, Project results from Phase 1, and WWW links to other technical information on WF/WTVs (**Figure 3**). We expect the first version of the Web Site will go on-line by the end of 2000.

The details of research results from Phase 1 will be presented in a 50-chapter book, which will be ready in early 2001. The following are several highlights which complement whitefly and geminivirus results reported earlier in this Annual Report.

Hot Spot Target Areas for Continued Whitefly Research

Based on results from Phase I extensive diagnostic surveys, the following hot spots were identified as target areas for basic research activities, as well as the IPM component testing (**Figure 4**). These are areas in which the pest and disease problems are severe and where the logistics (strong NARS collaboration, infrastructure, access to target areas, etc.) will enable successful completion of collaborative research activities.

Sub-project 1:

1) Tenerife/Pradera, Colombia
2) Valle de Chota, Ecuador
3) Sumapaz, Colombia
4) Nariño, Colombia

Sub-project 2:

1) Zapotitán Valley, El Salvador
2) Monjas, Guatemala
3) Azua, Dominican Republic
4) Los Mochis, Sinaloa, Mexico

Sub-project 3:

1) Gezira, Sudan
2) Arusha, Tanzania
3) Kampala, Uganda
4) Kibwezi, Kenya

Sub-project 4:

1) Kagera, Tanzania
2) Mwanza, Tanzania
3) Oyo, Nigeria
4) Mpigi, Uganda

Basic Research on Pest and Disease Dynamics

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is the principal vector of tropical plant viruses, and therefore the object of study in Sub-projects 2, 3 and 4. *B. tabaci* transmits at least 50 plant viruses, primarily geminiviruses, which cause serious epidemics in crops throughout the Tropics. The viruses are transmitted as *B. tabaci* feeds and moves from plant to plant. Thus whitefly vector management becomes an important part of IPM for disease management.

Whiteflies spread viruses both within fields and among fields across the landscape. In crops that are good reproductive host plants, *Bemisia tabaci* multiplies rapidly. *Bemisia* is also polyphagous, i.e. feeding on many crop plants. During Phase 1 we determined that there are serious whitefly-transmitted viruses infecting tomatoes, beans, cassava, sweet potato, peppers, melons, eggplant, cotton and tobacco. *Bemisia*'s ability to increase rapidly and its polyphagous feeding behavior

accelerates the spread of the disease within the field and to neighboring fields, resulting in epidemic events across the landscape.

During Phase 1, in a research project with Harvard University, a mathematical model that captures the dynamics of a pathosystem with one whitefly species and one virus in one crop, was computer verified (**Figure 5**). The model serves to pinpoint those components of the system that can be manipulated by the farmer to minimize the crop damage caused by the whitefly-transmitted plant viruses within their fields. Once field validated, this model can be used to give practical guidence on which IPM strategies and components - among the many available IPM tactics - should be prioritized for further study and testing.

During Phase 1, we also made progress on the development of a Geographical Information System (GIS) for whitefly/geminivirus problems in Latin America (**Figure 6**). This GIS will serve as the basis for regional epidemiological studies. With such a model in hand, we will be able to address the whitefly problem on a larger geographical scale by exploring the effects of various IPM "area-wide management" options, including policy options. The landscape model will aid in the development of such strategies by indicating which management options will have the largest impact regionally.

IPM Component Research

***Trialeurodes vaporariorum* as pest in the tropical highlands.** The Phase 1 survey revealed that the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), is by far the most important whitefly affecting annual crops as a direct pest in Andean highlands of Colombia and Ecuador. The survey also indicated that this whitefly has become the target of insecticide sprays even in areas where there is no need to spray. This in turn has raised other insects to major pest status. Insecticide abuse has created a serious imbalance that has accelerated the development of insecticide resistance and increased crop production costs. The combination of these factors tends to undermine the sustainability of prevailing cropping systems in the region. The problem is much more serious on beans and tomatoes than on any other crop, and is affecting the welfare and economy of small farmers throughout the highlands.

Phase 1 work has also identified several promising IPM tactics for managing *T. vaporariorum* in highland beans and tomatoes, specifically: replacement of broad-spectrum, toxic insecticides with more selective insecticides, with insecticide applications based on the use of simple action thresholds which have been developed (more rational chemical control); use of biological control agents, specifically the whitefly parasitoid *Amitus* and the entomopathogen *Verticillium*; "leaf roguing" of whitefly-infested plants (physical control), and incorporation of crop residues and manipulation of cropping dates (cultural controls).

Together with local farmers groups and extension agents, these tactics are ready to be tested in the target areas and developed into IPM systems. These systems are expected to have application throughout the Andean highlands.

***Bemisia tabaci* as virus vector in mixed cropping systems.** Use of synthetic insecticides continues to be the primary tactic used by farmers in their attempts to manage whiteflies. At the same time, Phase 1 analysis concluded that the bulk of IPM research and field trials is focused on alternatives to synthetic pesticides. Latin America has been suffering production crises for the past decade, as a result of the whitefly-transmitted geminiviruses. Thus, many alternative tactics are being explored. In Central America, Mexico and the Caribbean, at least 29 botanical insecticides (with special emphasis on the use of *Azadiracta indica*, NEEM) and five soaps have been looked at as alternative insecticides. Potential biological control agents (native fauna) of *Bemisia tabaci* cited within the regional literature or identified during the Phase 1 survey include 20 wasp parasitoids, 10 predators, and six fungal entomopathogens. Physical control tactics widely practiced include the use of screen-protected seedbeds, tunnels, yellow sticky traps, and living barriers of sorghum, maize and sunflowers. The cultural control practices cited are numerous: crop rotation, ground cover, plastic mulches, mixed cropping, high planting density, roguing, weed management, trap crops, high phosphate fertilizer, pruning, planting dates, irrigation cut-off dates, destruction of crop residues, and separation of seedbeds from field plantings. Legal controls involve quarantine measures, zoning of crops, regulated planting and harvesting dates, and crop-free periods. And, several studies on adoption of IPM tactics by producers indicate willingness on the part of the producers (60-100% adoption for some tactics) to implement IPM alternatives.

We also discovered that the IPM response was highly localized within the Central American, Caribbean and Mexican region, and related to the research preferences of the local researchers. The challenge is to prioritize, test and integrate those IPM tactics that will have the greatest impact on reducing damage from the whitefly-transmitted viruses, for subsequent dissemination and testing throughout the region.

In both Sub-project 2 and 3, Phase 2 basic research will focus on the *Bemisia*-transmitted geminivirues infecting tomatoes. In Project 2, 3 and 4, it is the geminiviruses infecting tomatoes which farmers, NARS scientists and regional networks identify as their most serious problem. In all three eco-regions, we have the same insect, transmitting the same virus to the same host. Thus, the Bemisia-geminivirus-tomato pathosystem will serve as a model system allowing comparative work between the regions. The damage and insecticide response is not yet as serious in Eastern Africa as it is in Latin America. Advances in IPM systems developed by farmer-researcher teams in Latin American will be shared with Sub-project 3 in hopes of preventing the pesticide treadmill phenomena in Eastern Africa.

Sub-project 5. *Bemisia tabaci* as virus vector in cassava and sweetpotato. Current control efforts for both cassava mosaic disease (CMD) and sweet potato virus disease (SPVD) depend almost entirely on the deployment of virus-resistant varieties. This has resulted in major control successes, most notably in areas affected by the CMD epidemic in East Africa. However, difficulties in multiplication and distribution of CMD-resistant varieties, along with perceived inferiority in the quality of these varieties have resulted in distribution to a relatively small proportion of areas affected by CMD. Resistant varieties are the cornerstone of cassava IPM; efforts in this area will continue.

However, complementary approaches to disease management remain largely untested. Preliminary work during Phase 1 indicates that research on the use of varietal mixtures merits further research attention, as does the potential for intercropping cassava with non-*Bemisia* host plants.

Similarly, while phytosanitation, for the planting of virus-free cassava stock, has long been recommended as a CMD control technique, there are still no clear and simple guidelines on how to incorporate phytosanitation into an IPM system. During Phase 2, an important target will be the development of robust guidelines for the use of virus-free cassava stock of both susceptible and resistant varieties under contrasting conditions of CMD epidemiology.

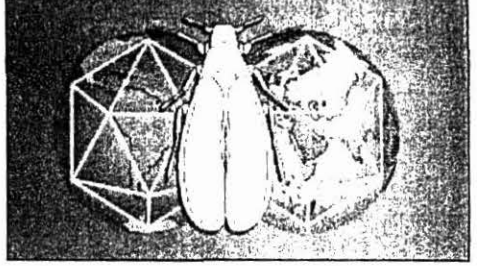
There is currently no IPM approach for the control of CMD and SPVD, which involves a vector management component. The Phase 1 surveys identified that the wasp *Encarsia sophia* is the principal parasitoid of *Bemisia tabaci* in cassava, and that this parasitoid is widely distributed. The biology and ecology of *E. sophia* is already well-studied. Therefore, natural enemy conservation and enhancement may be a viable IPM tactic for vector management in the cassava/sweet potato systems.

We are currently planning and fund-raising for Phase 2 (2001-2003) of the CGIAR Whitefly IPM Project.

Contributor – Sub-output 1: Entomology

Pamela Anderson

Work Breakdown Structure: Formal Collaborations



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

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

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USAID USAID - OFDA
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Figure 1.

Sub-Projects and Linkages for CGIAR Whitefly IPM Project

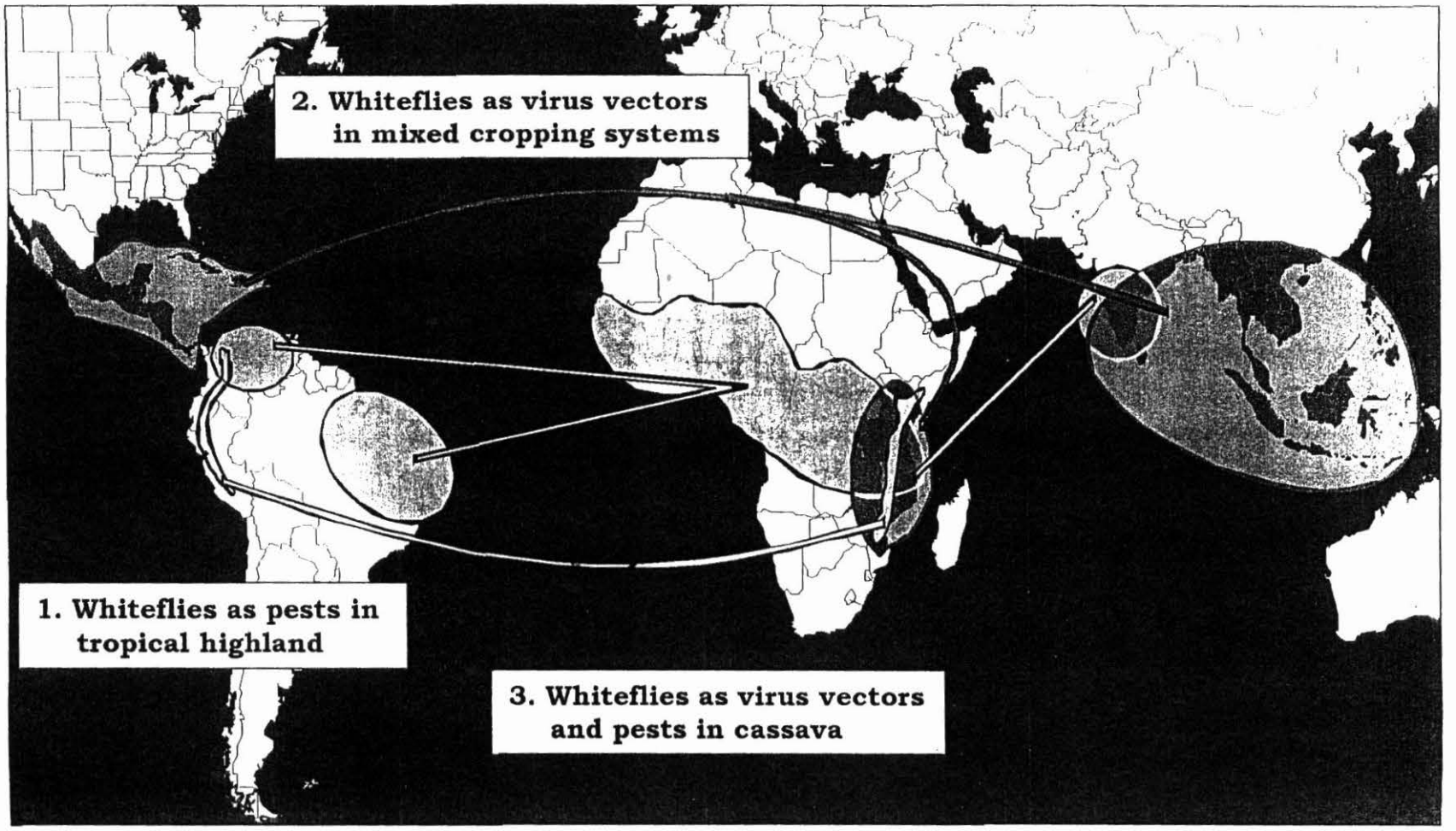
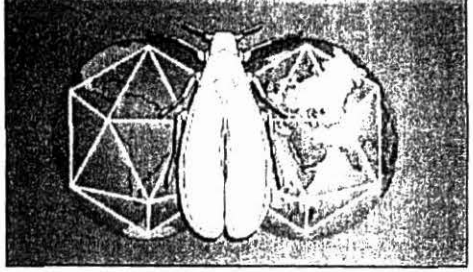
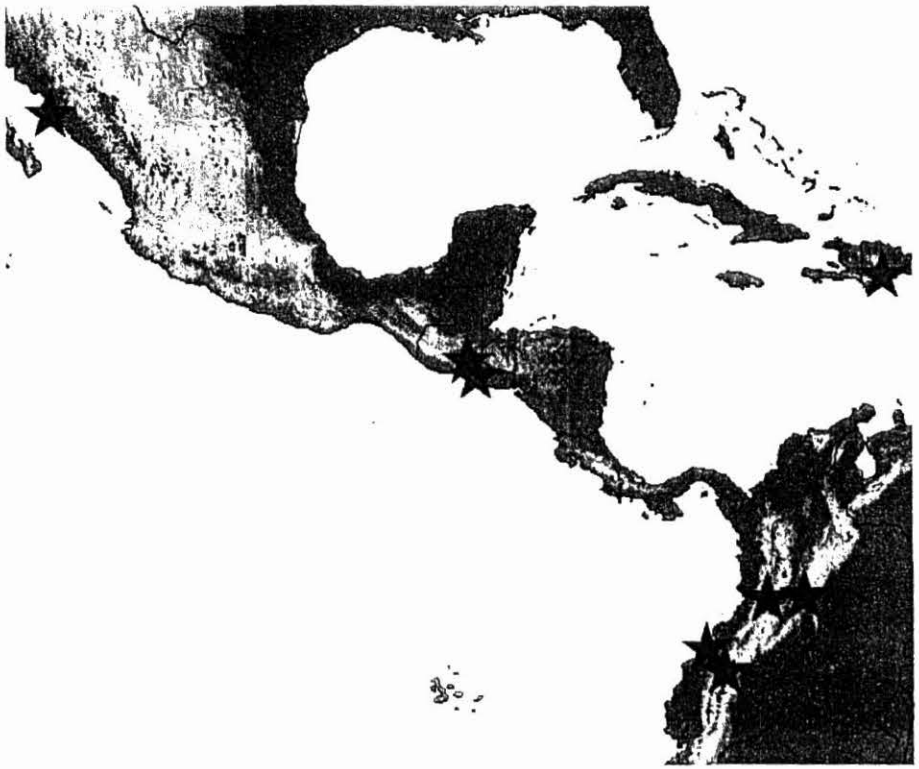


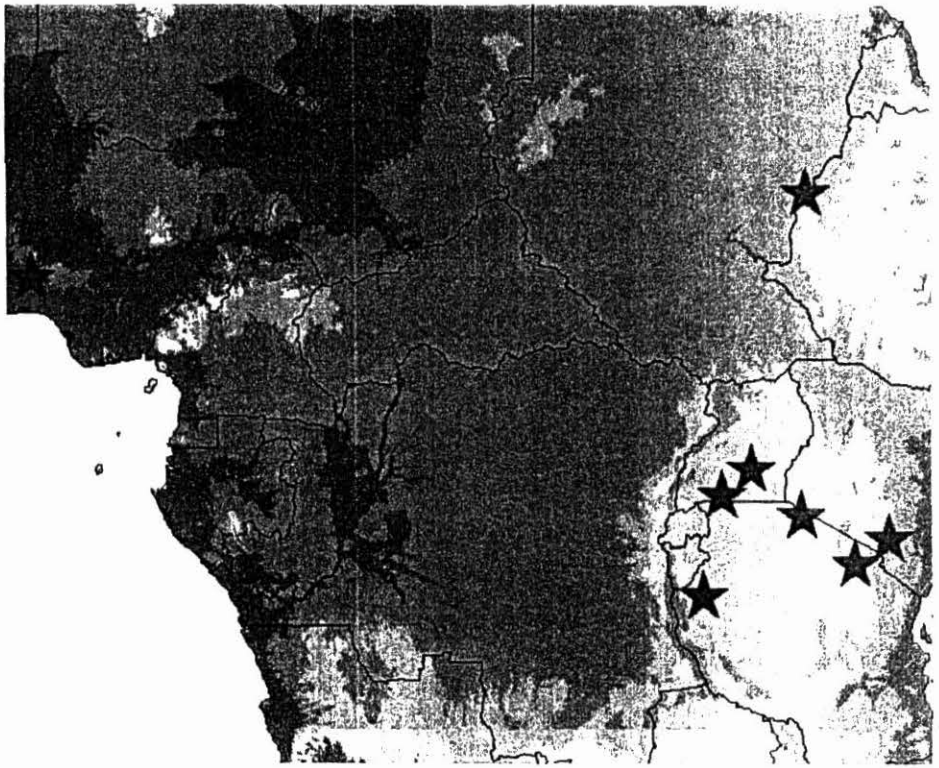
Figure 2.

Critical Target Sites - Hot Spots for Continued Whitefly and Geminivirus Research





LATIN AMERICA



AFRICA

Figure 4.

Whitefly Task Force

Research Projects

Project Partners

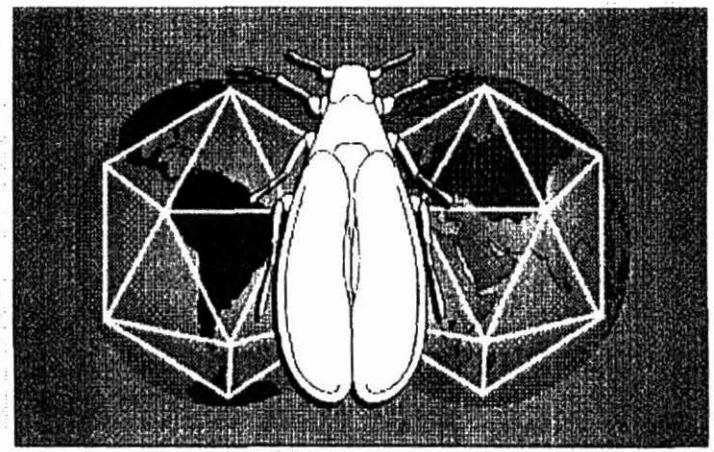
Methodology Guide

Project Results

Documentation

SP-IPM

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



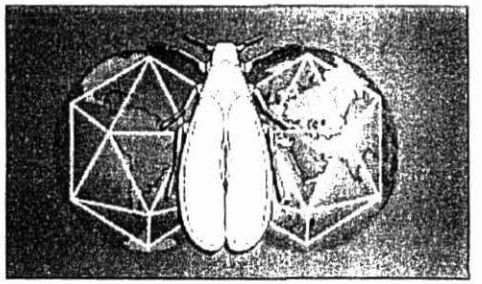
Welcome to the Whitefly IPM Project of the Consultative Group on International Agricultural Research (CGIAR). This project is part of the CGIAR System-Wide Programme on Integrated Pest Management (SP-IPM).

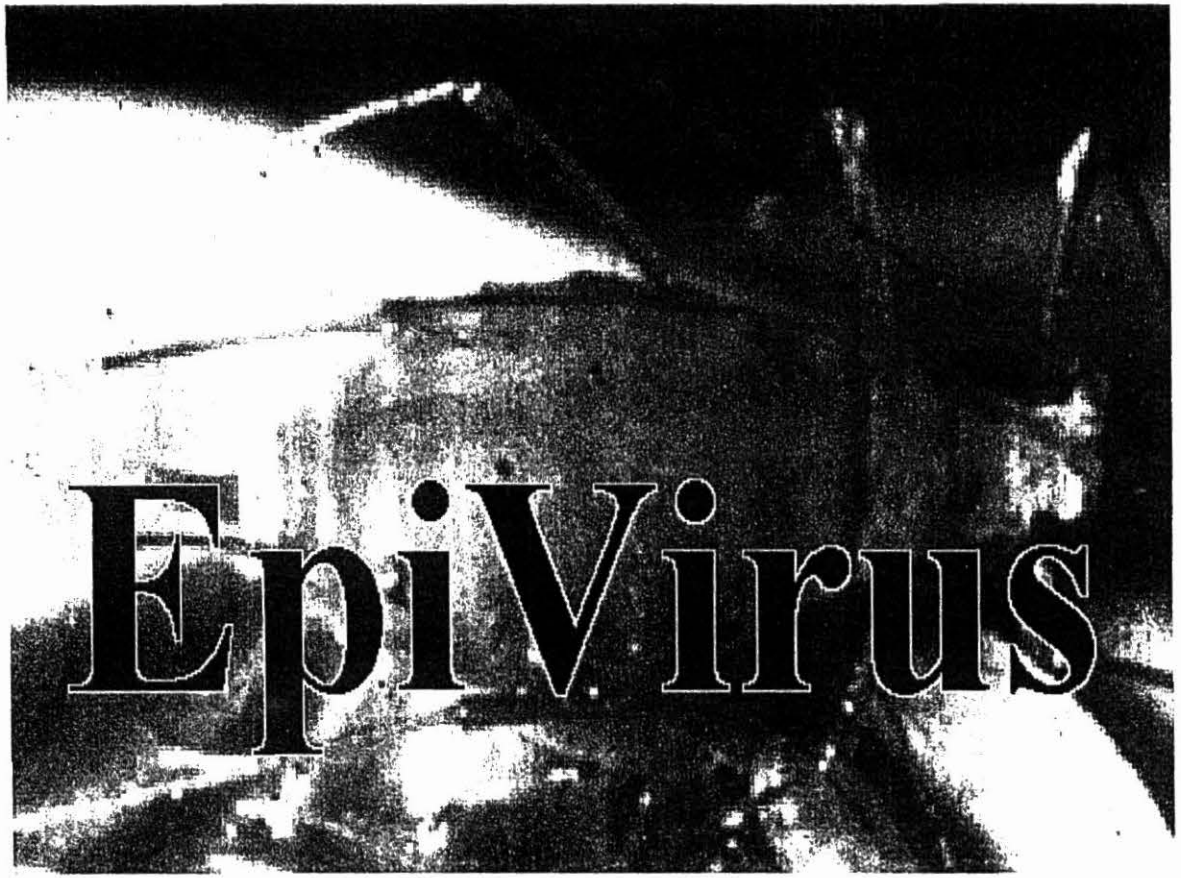
Contact **What's New**

Whitefly Task Force / Research Projects / Project Partners / Methodology Guide / Project Results / Documentation

Figure 3.

Pest and Disease Dynamics
Field-Level Analysis

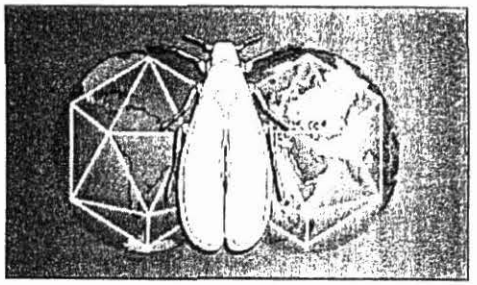


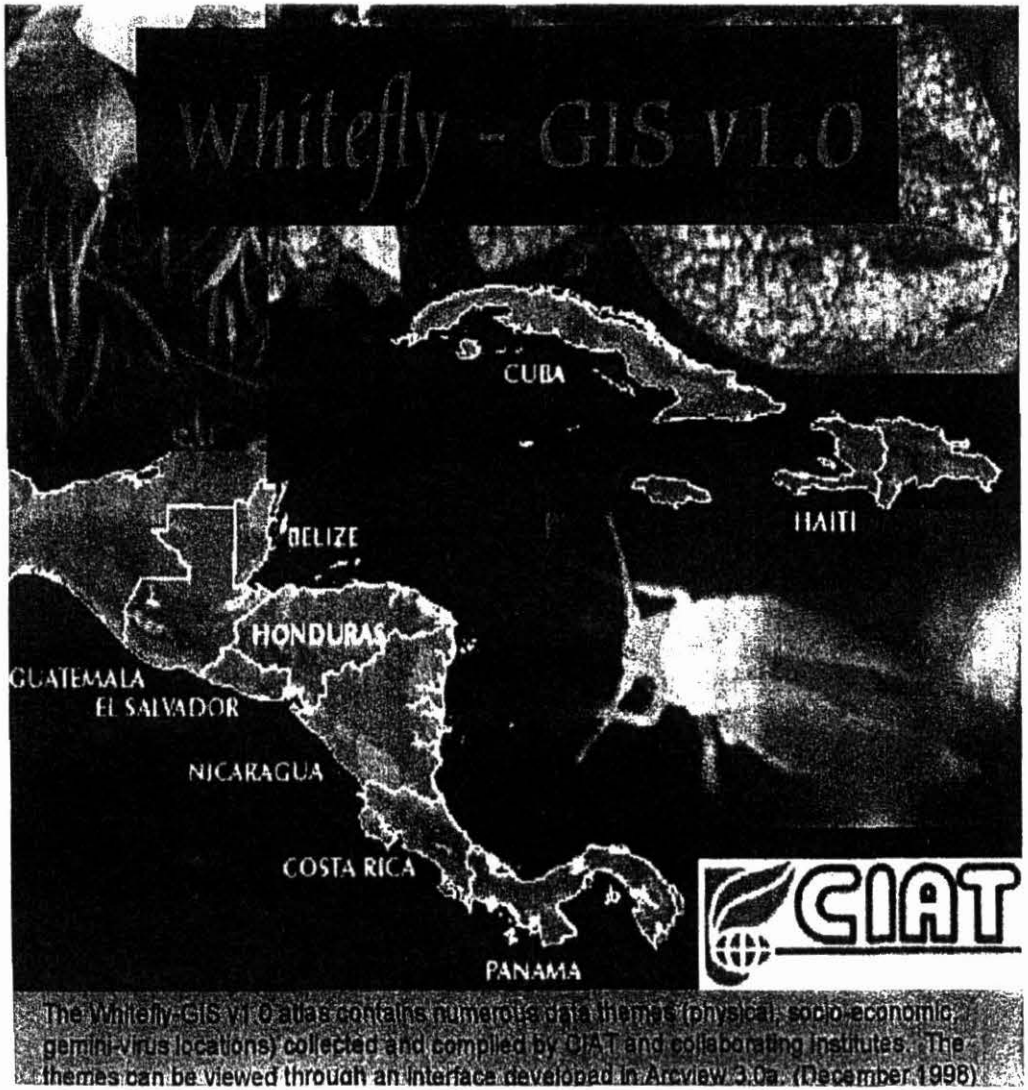


Research Tools
Decision Support
Tools - For
Producers

Figure 5.

Pest and Disease Dynamics Regional Analysis





The Whitefly-GIS v1.0 atlas contains numerous data themes (physical, socio-economic, gemini-virus locations) collected and compiled by CIAT and collaborating institutes. The themes can be viewed through an interface developed in Arcview 3.0a. (December 1998)

Figure 6.

Research Tools
Decision Support Tools - For Communities And Policy Makers

Sub-output 2. Preparation of Internet Ready IPM Documents.

Ecoport is an internet venture to allow access to information on plant pathogens and pests. It is jointly sponsored by University of Florida, FAO, and Smithsonian Museum of Natural History. Access to the site is free. This type of effort with over 75,000 records and 40 taxonomic keys depends on expects around the world volunteering their time. This is the type of effort that is making more information available without cost to anyone with internet access. Many of our partners have internet access and we are collaborating to place information on the pathogens of cassava and rice into the database.

Contributors – Sub-output 2: Virology

Lee Calvert
Ivan Lozano
Maritza Cuervo
Natalia Villareal.

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Linkages with Other CIAT Projects and with CIAT's Partner Institutions

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