Activity 1. Arthropod taxonomic activities on cassava and other crops.

Introduction

The IPDM project provides a service of identifying arthropod pests collected from different crops, but especially those crops related to CIAT's mandate and activities. These collections also include natural enemies related to crop pests, and much of this information is found in the subsequent activities in this report. A database is maintained of all collections and this is available to collaborating institutions and national research and extension programs.

One of the activities of the CIAT convened "Global Whitefly IPM Project" is to provide taxonomic support for whiteflies and their natural enemies collected from the different agroecosystems of Latin America (Neotropics). Project collaborators located in the numerous countries involved in the project (about 16 in Latin America) continue to send shipments of specimens collected for processing, monitoring and identification. These identifications are of vital importance for the development and implementation of IPM projects in these countries.

Collected specimens are conserved on microscope slides and documented in the whitefly database that is accessible through "Access." This service is also extended to parasitoids as well as other species collected from associated crops (i.e. crops associated with cassava) and made available to all collaborating institutions and countries. During the past year training in collecting, monitoring, and identification of whiteflies has been extended to scientists, students and collaborators from numerous institutions.

In addition to conventional morphological taxonomy techniques, during this year we have implemented in the cassava entomology laboratory, the application of molecular techniques based on PCR, especially for the identification of whiteflies and their parasitoids. These techniques offer a rapid, relatively low cost method for identifying critical species or complexes that are often morphologically indistinguishable during one of their life stages. These techniques or tools can help classify very small insects that often require fixation and microscopic mounting for identification.

In addition during the past year we initiated the collecting and identification of homopterous (Order: Homoptera) species associated with the cassava crop as possible vectors of Cassava Frogskin Disease. Studies were also initiated to collect and identify "fruit-flies" associated with various tropical fruit crops in a collaborative project with the Tropical Fruits Project.

Project I. Whiteflies

Objective: Process and identify whitefly species collected in Nicaragua, El Salvador, Brazil, Colombia, Ecuador (etc.) from several crops. The materials will be organized within the reference collection and registered in the data bank. Molecular techniques (PCR) will be used in the identification and personnel from national institutions will be trained.

Methodology: Whitefly samples are sent by collaborators in alcohol in vials. Permanent mounts are made in Canadian balsam; specimens are identified and stored in the collection at CIAT, and

registered in the database. Parasites are sent for identification to Dr. Gregory Evans (University of Florida) and Dr. Mike Rose (Montana State University).

Molecular techniques using DNA extraction, amplification (PCR) and RAPD's were used for identification of some whitefly species and two parasitoid species (*Eretmocerus mundus* and *E. eremicus*).

Training in these techniques was offered to ICA personnel (Turipaná) and postgraduate students from "La Escuela Politécnica del Ejército," Ecuador.

Results: Whitefly specimens sent from El Salvador, Nicaragua and Brazil were identified to species **(Table 1)**. Specimens were collected from numerous crops and at least six different whitefly species were identified.

Table 1. Whitefly species collected from several host in 5 countries (El Salvador, Brazil, Nicaragua, Panama and Colombia).

Country	Host	Species	No. of Samples
El Salvador	Pipian, Chile tomato, cucumber, squash, bean, eggplant, col, cowpea, radish, loroco, watermelon, sweet pepper, guisquil, cauliflower, soybean	*B. tabaci* (Gennadius)	60
El Salvador	Potato	*Trialeurodes vaporariorum* (Westwood)	2
Brazil	*Manihot esculenta*	*Aleurothrixus* sp. pos. aepim (Goeldii)	4
Nicaragua	Green pepper, tomato	*B. tabaci* (Gennadius)	17
Panamá	*Manihot esculenta*	*Trialeurodes variabilis* (Quaintance) *Aleurotrachelus socialis* Bondar.	2
Colombia	*Musa acuminata*	*Trialeurodes abutiloneus* Haldeman	1

The parasitoids collected from *B. tabaci* were identified by Dr. G. Evans. Two species *Encarsia tabacivor* and *E. nigricephala* were identified. We are waiting confirmation on other specimens sent to the two above-mentioned taxonomists.

Molecular Techniques: The technique of RAPD-PCR has been used to generate molecular markers that are useful in the identification of various groups of insects. The RAPDs-PCR for *B. tabaci* is with the primer OPC-04 (it showed polymorphism between the two populations, indicating a clear separation). These bands permitted distinguishing the two different biotypes for the *B. tabaci* population (which are morphologically identical). These amplified DNA fragments for sample A (biotype A) corresponding to 1636 pb, 890 pb and 469 pb, which are absent in the B samples (Biotype B). In this case two fragments at approximately 1327 pb and 1018 pb were observed; in addition similar bands appear for both biotypes **(Figure 1)**.

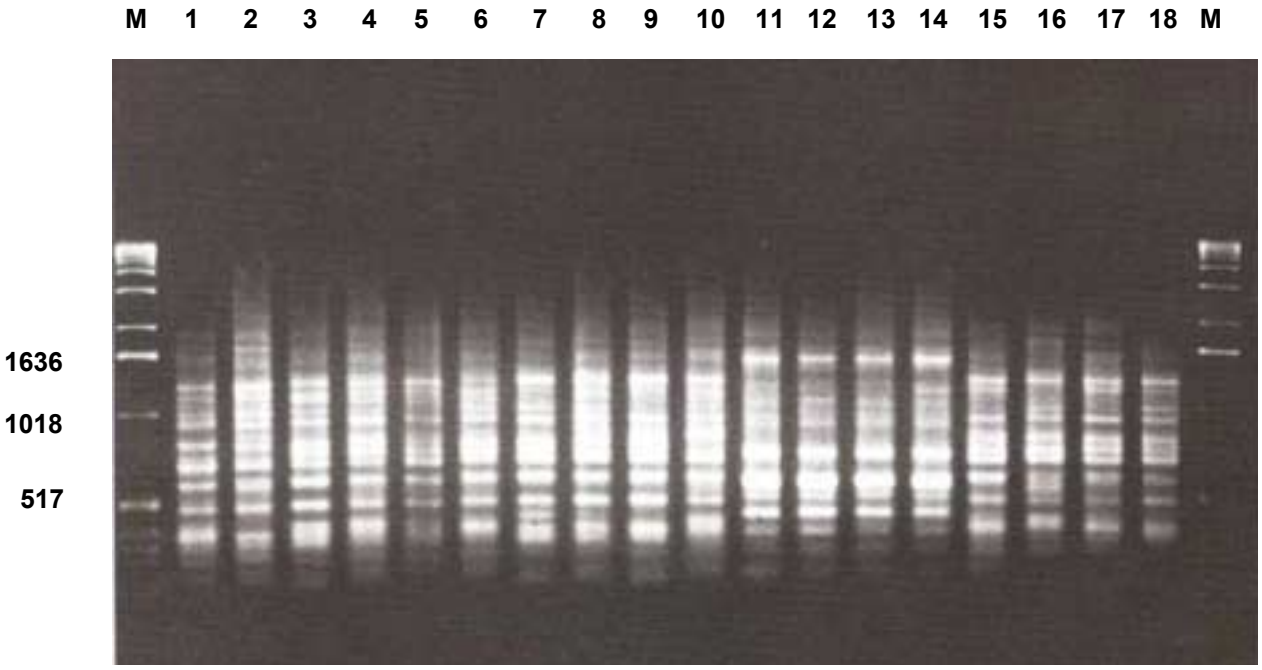

Figure 1. Identification of A & B biotypes of *B. tabaci* from RAPDs with primer OPC-04. Biotype B, lines 1-10 and 15-18, Biotype A, lines 11-14. M=Kb Marker.

This technique permits using dead insects, preserved in 70% alcohol, but must be dried before they are homogenized. Results are the same as when fresh individuals are used. This technique is relatively low cost and widely used, but has the drawback of not being easily reproducible and may be inconsistent. Therefore it is suggested that numerous replications be used, and to maintain reference populations as controls, since they can be contaminated by parasites or other organisms present in the insect (i.e. whitefly).

PCR's using the ITS primers (developed by J.L. Cenis, CIDA, Murcia, España) specific for the parasitoid *Eretmocerus*, produced 1 band that is used to differentiate the two populations collected. The bands present a molecular weight of approximately 700 pb for populations of *E. eremicus* and of 600 pb for *E. mundus* **(Figure 2)**. The use of specific markers as in the ITS (Internal Transcribed Spacer) case for *Eretmocerus* are costly and require time to determine their sequences, but they are very sensitive for accurate diagnosis. The species can be identified by size of the amplified product and visualized on the agarose gel.

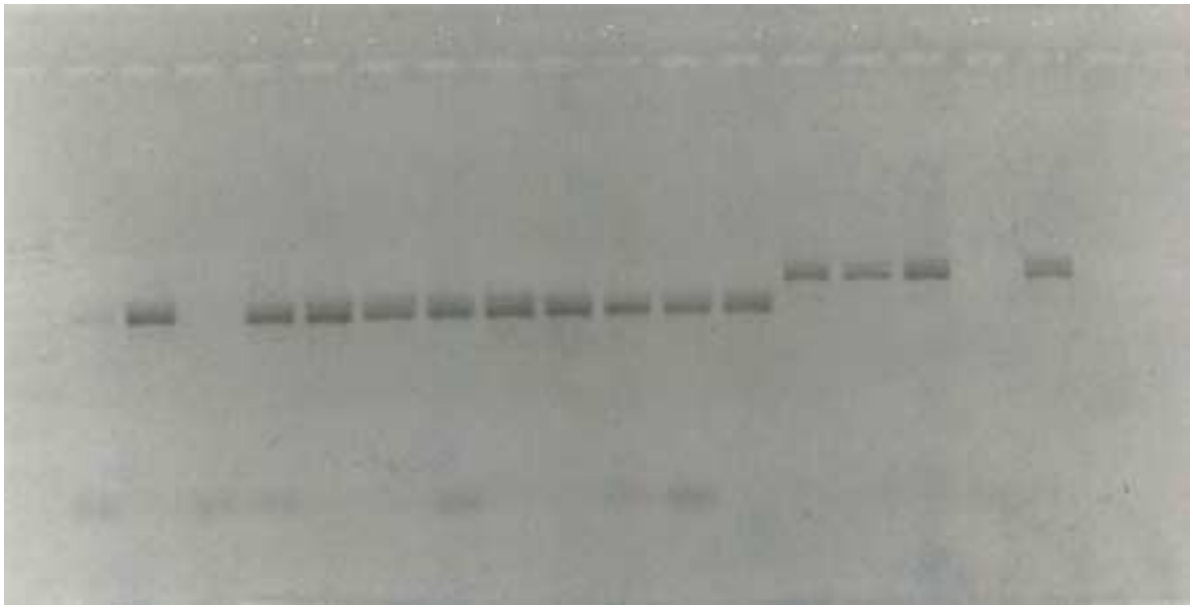

Figure 2. Band patterns generated by PCR with the sequence marker ITS for *Eretmocerus mundus* (lines 1-12), of ≈ 600 pb and *E. eremicus* (lines 1317) of ≈ 700 pb.

II. Homopterans/Frogskin Disease

The Insect Order Homoptera, is often referred to as "true bugs." It contains the families of leafhoppers, (Cicadellidae), plant hoppers (Fulgoridae, Delphacidae, Cixidae), treehoppers (Membracidae), spittlebugs or froghoppers (Cercopidae), as well as the whiteflies (Aleyrodidae). A characteristic that several of the species in this order have in common is their ability to transmit plant virus diseases or phytoplasms.

The cassava frogskin disease (CFSD) is causing considerable crop losses in Colombia and other countries and is hindering the movement and evaluation of germplasm. The long held assumption that CFSD was whitefly vectored, is presently being challenged. Other possible vectors among the aforementioned homopterans are now being considered, especially Cicadellidaes and Delphacidaes.

With the exception of whiteflies, other homopterans have not been identified as major pests of cassava, and their populations, when present are usually very low. A determined effort has been initiated to collect and identify the Homoptera species associated with cassava. Collections are being carried out at several different localities, especially where CFSD is prevalent. Numerous species have now been identified **(Table 2)**; the species *Scaphytopius* pos. *fuliginosus* (Cicadellidae) is most frequently collected and from several sites, including Valle del Cauca, Cauca and Tolima. Several species that have been collected, lack identification and will be sent to appropriate taxonomists.

These homopterans are most frequently found on cassava during the early morning hours and usually on young plants, 2 to 6 months. They are difficult to find on older plants. In addition, it has been observed that weedy cassava plots contain greater species diversity, indicating that many of these species may not be feeding on cassava, but rather on the associated weeds. Eventually, selected species will be reared under controlled conditions and vector pathogenicity tests carried out.

Table 2. Homopteran species collected from cassava plants at several locations in Colombia.

Department	Municipality	Site	Family	Species	Observations
Valle del Cauca	Palmira	CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus* Osborn	
Cauca	Santander de Quilichao	Hacienda Bariloche	Cicadellidae	*Scaphytopius* pos.*fuliginosus*	2 months field plot
			Delphacidae	1 species	
Cauca	Santander de Quilichao	Granja CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus*	Some plants with frogskin
Cauca	Santander de Quilichao	Granja CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus*	Weedy plot
				5 species unidentified	
				1 species	
			Delphacidae		
Quindío	La Tebaida		Cixiidae	1 species	Weedy plot
			Cicadellidae	5 species	
Risaralda	Morelia	Santa Rita	Cixiidae	1 species	Weedy plot
			Cicadellidae	4 species	
	Cerritos		Cixiidae	1 species	4 month field
			Cicadellidae	2 species	
Tolima	Espinal-Chicoral	Granja Nataima	Cicadellidae	*Scaphytopius* pos. *fuliginosus*	Some plants with frogskin
				1 species*	
	Gualanday		Cicadellidae	*Scaphytopius* pos.*fuliginosus*	Non-weedy plot
				1 species*	
	Ambalema	Vía Ambalema	Cicadellidae	1 species*	Weedy plot
	Espinal	San Francisco	Cicadellidae	1 species*	Weedy plot

* Similar Cicadellidae collected from the three sites (not identified, but appear to be an Empoasca).

Contributors: M. del P. Hernández, A.C. Bellotti.

Activity 2. Biological control of cassava whiteflies.

Introduction

Whiteflies are major pest of cassava throughout most of the tropical and subtropical regions of the world, as direct feeding pests and virus disease vectors. Eleven species of whiteflies have been identified feeding on cassava, eight of these are present in Colombia. *Aleurotrachelus socialis* is the predominant species in Northern South America (Colombia, Venezuela and Ecuador) where it can cause considerable crop loss due to its direct feeding on young cassava plants. *Aleurothrixus aepim* is the major species in Brazil, especially the Northeast (States of Bahia, Pernambuco and Ceara), where yield losses are reported. Additional species of importance are *Bemisia tuberculata* and *Trialeurodes variabilis*, both found throughout several regions of the Neotropics. *Bemisia afer* was recently introduced from East Africa into Peru and warrants considerable attention as it is an important pest in cassava in Africa.

Although *B. tabaci* has pan-tropical distribution, indications are that its populations are not uniform and may actually be a complex of species and biotypes. In Africa, moderate to high populations of *B. tabaci* are observed on cassava and it is the known vector of Africa Cassava Mosaic Disease (ACMD). In the Americas, however, high populations of *B. tabaci* on cassava are seldom, if ever, observed, and it is not presently known to transmit any virus diseases in the neotropics. However, its presence on cassava in the Americas, even if only in low populations is cause for concern as it has the potential to vector virus diseases, including ACMD, were it to be inadvertently introduced into the Americas. In surveys that we have performed over the past several years throughout numerous cassava growing regions of Colombia, Venezuela and Ecuador, we have not collected *B. tabaci* feeding on cassava from any site.

Whitefly populations may fluctuate considerably from one year or growing season to another; the direct cause for these population eruptions is not fully understood, although the overuse or misuse of pesticides may play an important role. Twenty to twenty five years ago high populations of *A. socialis* in Colombia were limited primarily to the Tolima Valley. In more recent years we have observed even higher populations in the Cauca Valley as well as other regions of Colombia. For example, as plantations size of cassava has increased in the Cauca Valley, *A. socialis* populations have also increased and in some cases it has been necessary to suspend cassava production in certain regions. It has also been observed that a change in planting pattern, where cassava is sown on a "staggered" basis, every 2 to 3 months, *A. socialis* populations may increase considerably, probably due to the continued presence of young cassava leaves, the preferred ovipositional sites for whitefly adults.

Control efforts for *A. socialis* on cassava at CIAT have traditionally concentrated on host plant resistance. In recent years added emphasis has also been given to biological control. The Colombia Ministry of Agriculture (MADR) through CORPOICA will release a cassava whitefly resistant variety. This variety, Nataima-31, was developed through a joint CIAT/CORPOICA collaboration over a 15 year period. The adoption of a new variety of cassava is a slow process and the variety may not be adapted to all cassava ecosystems. It is therefore necessary to develop alternative control methods, including, biological control and the use of selective pesticides. In recent Annual Reports (1999 to 2001) we have discussed the results of surveys to

detect natural enemies, especially parasitoids. We are also evaluating the role of fungal entomopathogens in whitefly IPM.

Objective: Determine the pathogenicity of several fungal hyphomycetes for control of the cassava whitefly *Aleurotrachelus socialis* under laboratory conditions.

Materials and Methods: Six entomopathogenic fungal isolates were selected from the CIAT "Ceparios;" these isolates had been previously collected from *A. socialis* in different cassava zones of Colombia **(Table 1)**. These isolates had been stored at -20°C in the CIAT collection, using the dry filter paper technique. They were reactivated by placing 10 to 20 adult *A. socialis* (from CIAT colony) in petri dishes with wet filter paper (distilled water) and the fungal entomopathogen. After 5 days, those adults with fungal mycelium present were isolated and placed on PDA.

Table 1. Isolates of native fungal entomopathogens collected in Colombia and evaluated for the whitefly, *Aleurotrachelus socialis* control in cassava.

Isolate	Fungal Entomopathogen	Host
CIAT 210	*Paecilomyces fumosoroseus*	*Trialeurodes vaporariorum*
CIAT 211	*Paecilomyces fumosoroseus*	*Trialeurodes vaporariorum*
CIAT 212	*Paecilomyces fumosoroseus*	*Trialeurodes vaporariorum*
CIAT 215	*Verticillium lecanii*	*Aleurotrachelus socialis*
CIAT 216	*Paecilomyces fumosoroseus*	*Aleurotrachelus socialis*
CIAT 217	*Beauveria bassiana*	*Aleurotrachelus socialis*

Once purified, the fungus was placed on a 0.5 % "insect agar" which consists of *A. socialis* adults that were collected from the field and the CIAT colony, and added to the PDA, previously sterilized **(Figure 1)**. 0.5 grams of macerated *A socialis* was added to 100 ml of agar, and autoclaved at 10 psi and 110°C for 10 minutes. The sterilized agar was poured into petri dishes and was sown with the 6 fungal isolates. A similar procedure was also carried out with *A. socialis* eggs and nymphs.

Evaluations of the isolates were accomplished on potted cassava plants (Var. CMC-40) infested with *A. socialis* adults, placed in nylon mesh cages in the greenhouse (30 ± 2°C and 50-60 % RH). Each plant was infested with 20-30 adults selected from the CIAT colony, and placed in small leaf cages located on cassava leaves, for a 24hr. period. After this period, the adults were removed. This procedure was also carried out at intervals of 4, 7, 14 and 23 days so that all development stages of the whitefly would be present upon fungal application.

The fungal pathogens were applied with a micro aspirator at 10 PSI. Spray coverage was evaluated using a hydrosensitive sheet of paper. The applied volume per treatment was 4.0 ± 0.5 ml per nymph. After application plants were placed in a growth room (28 ± 2°C and 80-90 % RH). Evaluations were made when adults emerged by counting nymphal skins, live and dead nymphs and dead nymphs with or without micosis. Leaves with nymphs and fungal application were removed and placed on moist filter paper for 4 to 5 days.

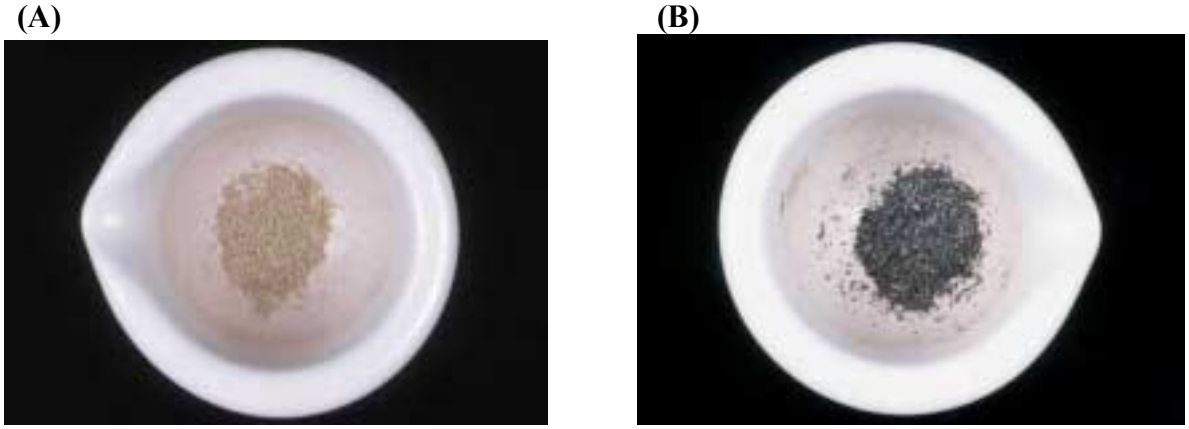

Figure 1. A. Adults of *Aleurotrachelus socialis* – B. Eggs and nymphs of *A. socialis*.

The experimental design utilized was completely randomized, with 10 replications per treatment, and each leaf cage was considered as an experimental unit. Controls consisted of sterile distilled water, and sterile distilled water plus tween 80 at 0.05%. Once the most susceptible whitefly stage to the fungus was determined, the most promising isolate was evaluated at concentrations of 1×10^3, 1×10^4, 1×10^5, 1×10^6, 5.0×10^6, 1×10^7 and 5.0×10^7 conc./ml.

Additionally 6 commercial products were evaluated at the recommended doses by each commercial source. Initially a quality control procedure was carried out for each product to determine product purity. Products were then tested for pathogenicity by applying them to whitefly eggs about to hatch, as this is the most susceptible stage.

Results: Evaluation of the six native isolates on the different development stages of *A. socialis* resulted in isolated CIAT 215 (*Verticillium lecanii*) being selected as the most promising **(Figure 2)**. Whitefly mortality was highest with this isolate, reaching 65.4%; isolate CIAT 217 (*Beauveria basssiana*) was next highest at 47.1% mortality. Mortality in the control treatments averaged 16%, which was lower than all of the isolates evaluated. This also indicates that the methodology utilized was adequate for evaluating the fungal entomopathogens on the different whitefly development stages.

Consequently, the isolate CIAT 215, having caused the highest mortality, was used to determine the whitefly stage most susceptible to fungal entomopathogens. It was observed that this isolate, *V. lecanii*, resulted in fungal mycelium growing on egg and nymphal stages of *A. socialis* **(Figure 4)**. When *V. lecanii* (CIAT 215) was applied to the egg and nymphal stages of *A. socialis*, mortality was above 50 % for all stages (egg and 4 nymphal stages) **(Figure 3)**. Mortality was highest when applied to the egg stage at 74 %, followed by 72 % for 2nd instar nymphs. Although these two differences were not significant, it was decided to use the egg stage to evaluate concentrations of the isolate CIAT 215. The commercial products were also evaluated using the egg stage.

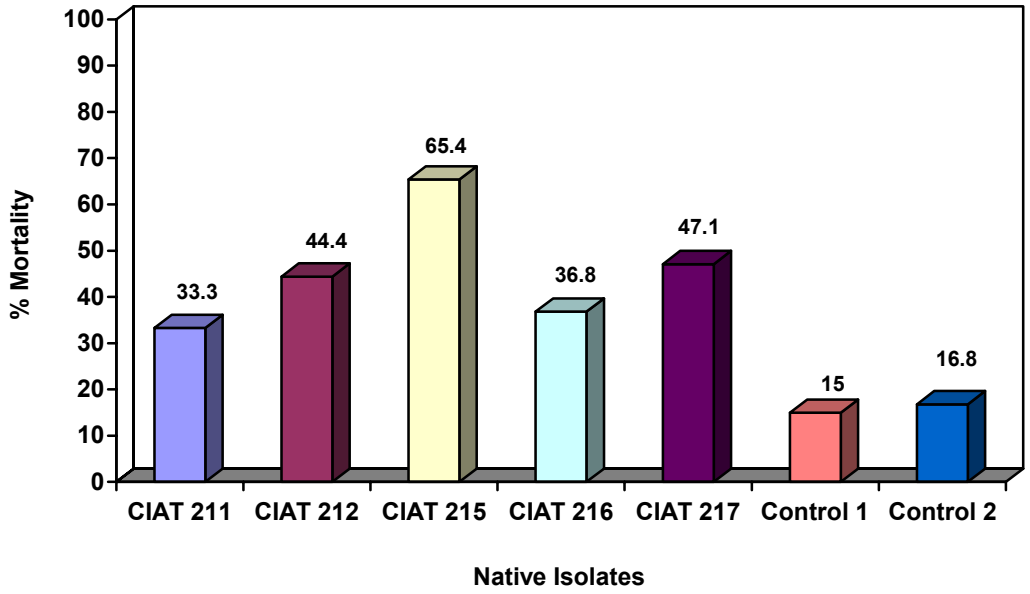

Figure 2. Percent mortality of *Aleurotrachelus socialis* eggs and nymphs with isolates of five native fungal entomopathogens.

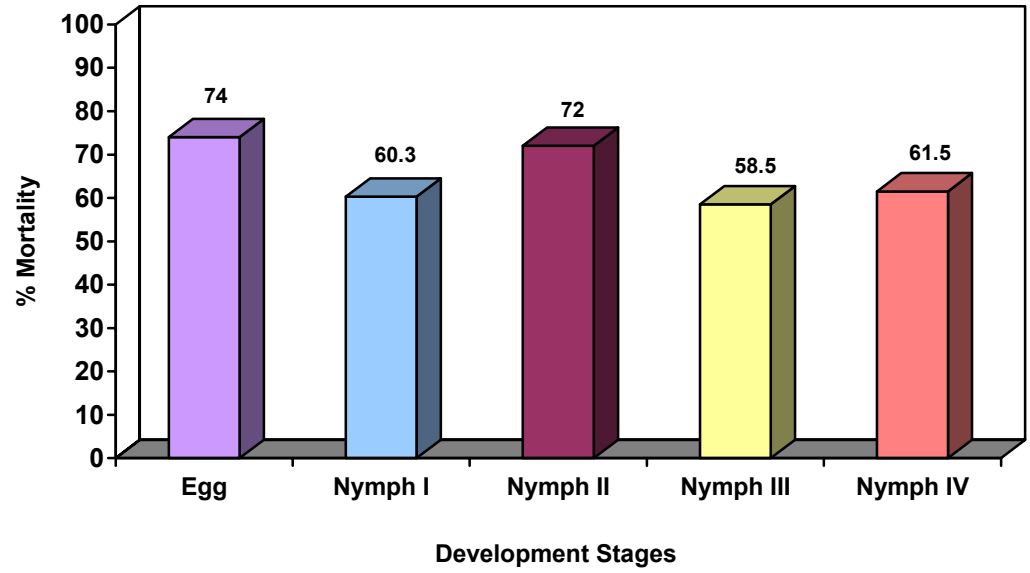

Figure 3. Percent mortality of *Aleurotrachelus socialis* eggs and nymphs due to applications of the fungal entomopathogen isolate CIAT 215 (*Verticillium lecanii*) in the greenhouse.

Results of applying different concentrations of isolate CIAT 215 to *A. socialis* eggs show that higher concentrations of the fungal entomopathogen will result in higher mortality of *A. socialis* **(Figure 5)**. The PROBIT test showed that the LC_{50} of isolate CIAT 215 is reached at a concentration of 1.4 x 10^7 conidia/ml with confidence limits of 3.6 x 10^5 – 1.5 x 10^9 conidia/ml. The LC_{90} was reached at a concentration of 2.3 x 10^{12} conidia/ml with confidence limits of 9.3 x 10^9 to 4.1 x 10^{21} **(Table 2)**.

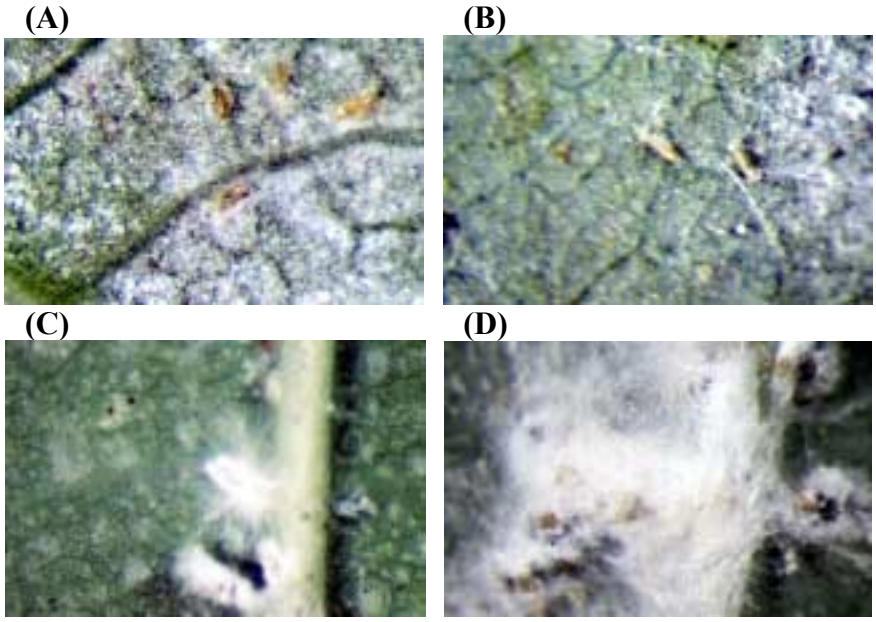

Figure 4. ***Verticillium lecanii*** **mycelium, present on life stages of** ***Aleurotrachelus socialis*** **(A) eggs; (B) 1st instar nymphs; (C) 2nd instar nymphs; (D) 3rd and 4th instar nymphs.**

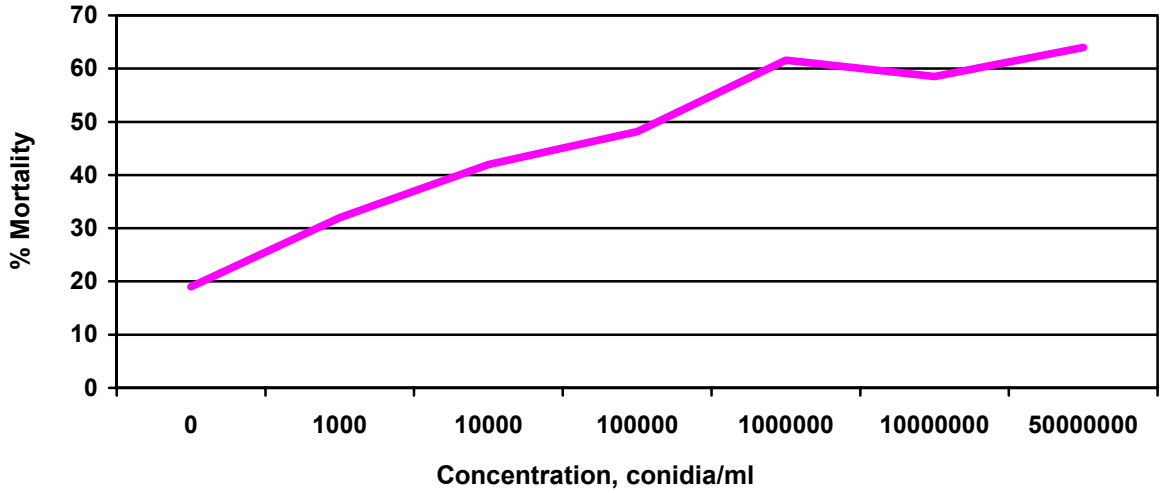

Figure 5. Egg (near hatch) mortality of ***Aleurotrachelus socialis*** **infested with several concentrations of the fungal entomopathogen isolate CIAT 215 (*Verticillium lecanii*).**

Table 2. Determination of LC_{50} and LC_{90} of the fungal entomopathogen isolate CIAT 215 (*Verticillium lecanii*).

N	CL_{50} (LC)*	CL_{90} (LC)	B ± EEM	X^2	P> X^2
2146	$1.4x10^7$ ($3.6x10^5$-$1.5x10^9$)	$2.3x10^{12}$ ($9.3x10^9$-$4.1x10^{21}$)	0.24±0.05	12.6	0.01

* Confidence limit at 95%

The evaluations of quality control for commercial formulated products show that these products did not contain the quantity of spores claimed on the label, rather at a lower concentration **(Table 3).** Viability tests show that only one product, *Paecilomyces fumosoroseus*, had a viability rating above 85%, the minimum percent that a formulated product should have, for a quick knock-down or kill in field applications **(Table 4)**. The purity test established the proportion of the biological agent in the formulated product, and also identifies any of the contaminants that might be present. In two products evaluated, there was no growth of the active ingredient, the fungal entomopathogen **(Table 4)**.

Table 3. Spore counts of several commercially formulated fungal entomopathogen products that were evaluated for *Aleurotrachelus socialis* control on cassava.

Product	Spore Counts Conidia/ml (Actual)	Spore Counts Conidia/ml (Ticketed)
Paecilomyces fumosoroseus	$6.6x10^8$	$2x10^{10}$
Verticillium lecanii	$8.1x10^6$	$2x10^7$
Beauveria bassiana	$1.9x10^8$	$2x10^9$
Verticillium lecanii	$1.3x10^8$	$2x10^{10}$
Verticillium lecanii	$9.0x10^7$	$2x10^{10}$
Verticillium lecanii	$2.0x10^7$	$2x10^9$

The pH can also influence fungal germination, with optimal range between 5.5 and 7.0. Only two products were in this range, both were *V. lecanii*. "Suspendibility" determines the time that a wettable powder requires to become suspended; some products suspension occurs rapidly, while others do not, or require more time, which could lead to occasional clogging of nozzle opening when applying the product **(Table 4)**.

Finally, each formulated commercial product was evaluated by applying it to *A. socialis* eggs that were near hatching. All commercial products evaluated resulted in whitefly mortalities below 50% **(Figure 6).** However the resulted mortalities on all products were significantly different than the control. Two products, *Beauveria bassiana* and *V. lecanii*, achieved 49.9% mortality while the control was 19.0%, indicating, at best, mediocre *A. socialis* control. It should be noted that these formulated products have not been recommended for *A. socialis* species on cassava, but rather are recommended for other species on other crops. This could be the reason for the lower mortality, results. These products could be tested on other development stages of *A. socialis* to determine if a higher mortality if feasible.

Results from these experiments indicate that the CIAT 215 isolate of *V. lecanii* has the potential to be commercially formulated and promoted or recommended for *A. socialis* control in cassava.

Table 4. Quality control of formulated commercial fungal entomopathogen products that were evaluated for *Aleurotrachelus socialis* control on cassava.

Product	Viability 24 h	Purity	PH	Suspensionability
Paecilomyces fumosoroseus	95%	98%	5.35	2 min.
Verticillium lecanii	35%	100%	5.54	4 min.
Beauveria bassiana	40%	100%	5.14	50 min.
Verticillium lecanii	22%	-	4.80	1.30 min.
Verticillium lecanii	13%	-	4.87	-
Verticillium lecanii	40%	40%	5.59	None

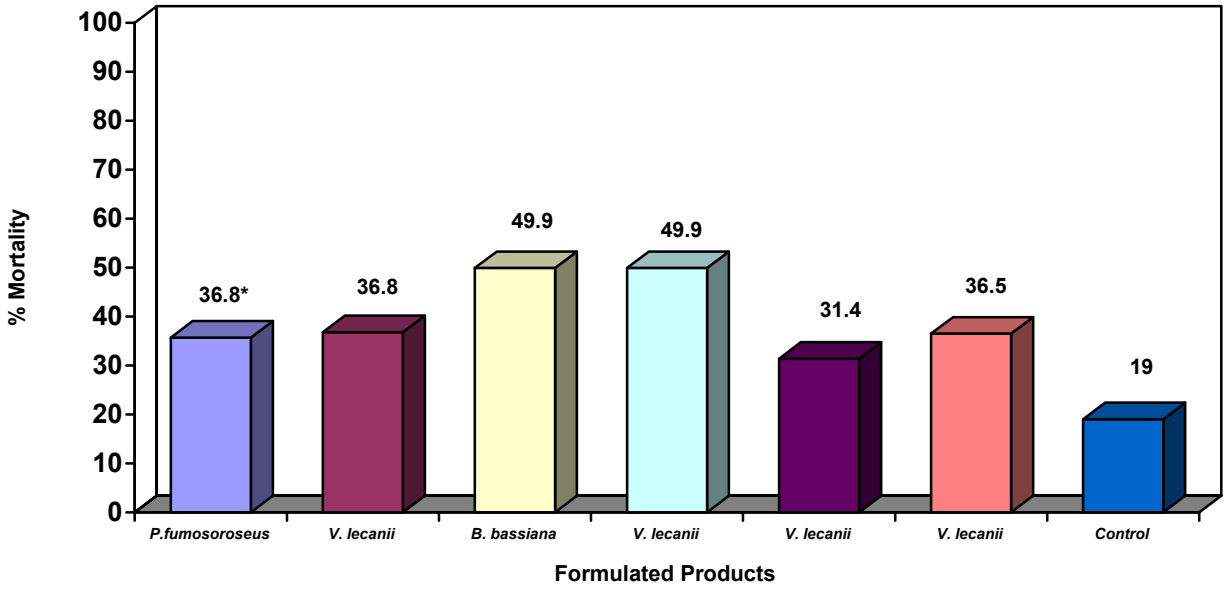

Figure 6. *Aleurotrachelus socialis* egg (near hatch) mortality with applications of formulated commercial fungal entomopathogen products.

Contributors: I. Aleán, A. Morales, C.M. Holguín, A.C. Bellotti.

Activity 3. Biological control of cassava whiteflies; surveys of cassava plantations in Yopal, Casanare, Colombia for parasitoid natural enemies.

On going surveys and explorations for natural enemies of cassava whiteflies have resulted in the identification of a considerable number of parasitoids, many of these new or unrecorded species. During 2002, in a previously unexplored area, the department of Casanare, Colombia, cassava fields heavily infested with whiteflies were surveyed for natural enemies.

Methodology: Field evaluations were done by randomly collecting leaves from whitefly infested fields and removing one-inch-square leaf sections and storing these in plastic vials to permit whitefly and parasitoid emergence. After emergence each inch-square leaf section was evaluated by removing emerged parasitoids and recording the number of pupae with parasitoid exit holes to determine percent parasitism. Parasitoids were placed in vials containing 70% alcohol and a preliminary identification to genus was made. Parasitoid specimens would be sent to appropriate taxonomists for precise identification to species.

Results: Results were obtained from 28 samples. The whitefly species *Aleurotrachelus socialis* represented 98.6% of the whitefly population, while the species *Bemisia tuberculata* represented the remaining 1.4%. A few individuals of *Trialeurodes variabilis* were also observed. A total of 3422 pupae of *A. socialis* were evaluated and only 333 or 9.7% were parasitized. A total of 49 pupae of B. tuberculata were collected and 19 or 38.9% were parasitized **(Table 1)**. This averaged out to only 10.1% of the total whitefly population parasitized. The predominance of *A. socialis* is consistent with survey results from other areas of Colombia. The low percentage of parasitism is also expected and indicates a possible reason for the high whitefly populations.

Numerous parasitoid species were observed and these belonged to three genera, *Encarsia*, *Eretmocerus* and *Amitus*. 41 individuals of the genus *Encarsia* were collected, 23 were *Eretmocerus* and 9 *Amitus*. Four "unidentified" or at least different, specimens were also collected. *Encarsia* sp was collected from 42.8% of the sample sites, *Eretmocerus* sp, from 39.3% and *Amitus* sp from 21.4%.

Casanare is part of the Colombian Llanos and these samples represent the first collections from this region and give added value to our understanding of the whitefly/parasitoid species association.

Table 1. Cassava whitefly parasitism from surveys in Yopal, Casanare (Llanos Orientales) of Colombia.

Whitefly Species	No. Pupae not Parasitized				No. Pupae Parasitized				Total Pupae in Sample	% Parasitism
	Total	Min.	Max.	Aver.	Total	Min.	Max	Aver.		
A. socialis	3089	21	283	110.3	333	1	30	11.9	3422	9.7
B. tuberculata	30	0	9	1.07	19	0	10	0.68	49	38.9
Total Parasitism	3119				352				3471	10.1

Contributors: B. Arias, A.C. Bellotti.

Activity 4. The establishment of an IPM program for cassava whiteflies in Valle del Cauca y Cauca departments in Colombia.

The shift from small cassava farms to larger plantations is often accompanied by an increase in phytosanitary problems, especially those associated with arthropod pests. The Cauca department is still characterized by small, 1 to 3 ha., cassava plantings, while in the Cauca Valley larger cassava plantations are becoming more common. The predominant pest in both areas is the cassava whitefly, especially the species *Aleurotrachelus socialis*. Small farmers are often resource limited and do not have access to agrochemicals, while larger farmers, with easy access to credit will often first resort to the use of insecticides for pest control. Surveys carried out with cassava farmers in both regions show that pest diversity is greater on smaller (under 10 ha.) plantations, while on larger plantations (> 10 ha.) there is less diversity, but populations of specific pests may be higher **(See PE-1 2001 Annual Report)**. An IPM program to combat cassava pests, especially whiteflies is being developed and is mostly financed through funds from the Colombian MADR.

The overall objective is to develop an IPM program for cassava pests, especially whiteflies, for both small and large cassava farmers; offering opportune, economic, efficient and sustainable management of cassava pests. The specific objectives include:

- Determine optimal cassava pest management practices with cassava producers in Valle del Cauca and Cauca.
- Identify selective chemical pesticides and biopesticides, determine doses and time of application for effective control with minimal effect on natural enemies.
- Carry out field trials to recommend to cassava farmers the use of effective natural enemies, including biopesticides, predators and parasitoids.
- Train farmers in cassava IPM practices, emphasizing the reduction of pesticide applications and the use of natural enemies.

Methodologies

I. Cassava producer surveys.

Visits have now been made to 102 cassava farmers in the regions. These visits surveyed farmer crop and pest management practices as well as farmer perceptions of their principal production constraints, including pest problems. In addition, during visits, cassava fields were also sampled to determine the presence of pests and diseases. Considerable data from the results of these surveys was reported in the PE-1 2001 Annual Report, and therefore will not be included in this year's report.

II. Evaluation of foliar applied chemical and biopesticides for whitefly (*A. socialis*) control in Jamundí, Valle del Cauca.

Over the past year, on cassava farms in Jamundí, several products with new or novel active ingredients were evaluated for whitefly control. The experimental design used was completely randomized blocks with seven treatments, one absolute control and four repetitions per

treatment. The treatments evaluated were Confidor (Imidacloprid), Oportune (Buprofezin), Eltra (Carbosulfan), Actara (Tiametoxan), Polo (Diafentiuron), Epingle (Piriproxifen), and Imidor (Imidacloprid 2), all applied at commercial dosage. The cassava variety was Reina (CM 6740-7). Evaluations were started when the first whitefly populations were observed, based on a populations scale **(Table 1)**, and then every 15 days hence until the crop reached 6 months.

Table 1. Population scale for *Aleurotrachelus socialis* on cassava.

Grade	Adults – Eggs	Nymphs - Pupae
1	Clean	Clean
2	1 – 50	1 –200
3	51 – 200	201 –500
4	201 – 500	501 – 2000
5	501 – 1000	2001 – 4000
6	>1000	>4000

At harvest, yield data was recorded from the two center rows of each treatment. Cassava market prices for small and large producers were noted at time of harvest and production costs, including the pesticide product, hand labor and other inputs such as irrigation (only large farmers). Larger producers, in general, had greater input costs than small farmers. With this data partial budgets were prepared and a marginal analysis was performed to calculate, cost per variable, total costs, total benefit, net benefit and cost-benefit-ratio.

III. Evaluation of imidacloprid for cassava whitefly control in Jamundí.

With the objective of delaying the build up of whitefly (*A. socialis*) populations and evaluate the efficiency of pesticide control, experiments were set up at the "Agrovelez" (a private enterprise) in Jamundí. The variety Reina was used to evaluate several dosages and application methods of imidacloprid, the active ingredient that gave the best results during preliminary experiments **(See PE-1 2001 Annual Report) (Figure 1)**. Two experiments, with 7 and 6 treatments, respectively were designed, using completely randomized blocks with one absolute control, one commercial control and four repetitions per treatment **(Tables 2 and 3)**.

Both experiments were carried out to harvest, recording yield, treatment costs, cassava root prices and including product efficiency in whitefly control (see previous experiment).

Table 2. Imidacloprid doses and form of application for cassava whitefly (*Aleurotrachelus socialis*) control in field trials (1st. trial).

Product	Doses/ha.	Form of Application
1 Confidor SC 350 (Concentrated suspension)	0.6 liters	Drench at planting*
2 Confidor SC 350 (Concentrated suspension)	0.8 liters	Drench at planting
3 Confidor SC 350 (Concentrated suspension)	0.2 liters	Drench at emergence
4 Gaucho FS 600 (Seed treatment)	0.4 liters	Stake treatment
5 Gaucho FS 600 (Seed treatment)	0.5 liters	Stake treatment
6 Confidor WG 70 (Granular application)	0.3 Kg	Drench at planting
7 Confidor WG 70 (Granular application)	0.4 Kg	Drench at planting
Commercial Control	0.2 liters	Drench at emergence
Absolute Control		

* Applied at the base of the plant.

Figure 1. Different forms of applying the pesticide imidacloprid to cassava in field.

Table 3. Imidacloprid doses and form of application for cassava whitefly (*Aleurotrachelus socialis*) control in field trials (2nd. trial).

Product	Doses/ha.	Form of Application
1 Confidor SC 350 (Concentrated Suspension)	0.6 liters	Drench at planting
2 Confidor SC 350 (Concentrated Suspension)	0.2 liters	Drench at planting
3 Confidor WG 70 (Granular Presentation)	0.3 Kg	Drench at emergence
4 Confidor WG 70 (Granular Presentation)	0.3 Kg	Stake treatment
5 Gaucho FS 600 (Seed treatment)	0.4 liters	Stake treatment
6 Confidor SC 350 (Seed treatment)	0.2 liters	Drench at planting
(Commercial Control)	0.2 liters	Drench at planting
Absolute Control		Drench at emergence

* Applied at the base of the plant.

Results

I. Evaluation of foliar applied chemical and biological pesticides.

Foliar applications of several pesticide showed that "tiametoxan" and "imidacloprid 1 and 2" were most efficient in reducing whitefly population **(Table 4)**. Results within the three treatments were not significantly different for adults, eggs and nymphs, but were significantly different when compared to other treatment and the control. Based on the 1 to 6 population scale **(Table 1)**, the three treatments (tiametoxan and imidacloprid 1 and 2) resulted in average populations of 2.66 for adults, 2.82 for eggs and 2.72 for nymphs, while in the control treatments

the population values were 3.65, 4.06 and 4.33 for adults, eggs and nymphs respectfully. All of the other treatments had values above 3.15.

Tale 4. The effect of foliar application of pesticides on the eggs, nymphs and adults of *Aleurotrachelus socialis* in Jamundí (Valle del Cauca).

Treatment	Adults[2]	Eggs	Nymphs
Control	3.65 a[1]	4.06 a	4.33 a
Carbosulfan	3.20 b	3.65 b	4.01 ab
Buprofezin	3.18 b	3.56 b	3.89 ab
Piriproxifen	3.17 b	3.55 b	3.85 ab
Diafentiuron	3.15 b	3.51 b	3.58 b
Imidacloprid 1	2.76 c	2.89 c	2.81 c
Imidacloprid 2	2.64 c	2.78 c	2.72 c
Tiametoxan	2.57 c	2.78 c	2.62 c

1 Duncan test: numbers followed by the same letter are not significantly different at the 5% level.

2 Based on the population scale, 1 = non-present; 2 = 1-200 individuals per leaf; 3 = 201-500 per leaf; 4 = 501 – 2000 per leaf; 5 = 2001-4000 per leaf; 6 = > 4000 per leaf.

Root yields were, in general, similar among the treatments, including the control **(Table 5)**. Also yields, in general, were low with the tiametoxan treatment highest at only 16.23 T/ha. Low yields could be due to the variety used in the experiment (Reina) or due to the presence of frogskin disease **(Table 5)** which was high in all of the treatments, including the control.

Table 5. Cassava yields and frogskin disease incidence in trials using foliar pesticide application to control the cassava whitefly, *Aleurotrachelus socialis* in Jamundí (Valle del Cauca).

Treatment	Yield Ton./ha.	Frogskin Incidence (%)
Imidacloprid 1	8.70	36.47
Buprofezin	10.96	32.26
Carbosulfan	10.30	20.62
Tiametoxan	16.23	17.71
Diafentiuron	7.41	36.02
Piriproxifen	9.35	24.53
Imidacloprid 2	11	36.28
Control	8.44	41.01

A cost/benefit analysis indicates that since larger farmers receive a higher price for their product, the C/B was above 1, and highest for the tiametoxan treatment with a C/B value of 2.09 **(Table 6)**. This means that the producer receives 1.09 pesos for each peso invested.

Table 6. Cost-Benefit ratio obtained by large cassava farmers ($330/kg) applying foliar pesticides to control of whiteflies (*Aleurotrachelus socialis*) at Jamundí; Valle del Cauca (values expressed in thousands of pesos Col./ha).

Treatment	Total Cost	Total Benefit	Net Benefit	C/B Ratio
Imidacloprid 1	2635.5	2871	235.5	1.09
Buprofezin	2602.7	3616.8	1014.1	1.39
Carbosulfan	2549.9	3399	849.1	1.33
Tiametoxan	2564.3	5355.9	2791.6	2.09
Diafentiuron	2601.9	2445.3	-156.6	0.94
Piriproxifen	2564.3	3085.5	521.2	1.20
Imidacloprid 2	2630.3	3630	999.7	1.38
Control	2396.3	2785.2	388.9	1.16

At the time of harvest the market price for the small farmer was $150 pesos per kilo, compared to $330 for larger farmers. Smaller farmers do not have the guaranteed market price that larger farmers have. Therefore the C/B ratio for smaller farmers was below 1 for all treatments, with the exception of tiametoxan where it was 1.34 to 1 **(Table 7).**

Table 7. Cost-Benefit ratio obtained by small farmers ($150/kg) applying foliar pesticide to control cassava whitefly (*Aleurotrachelus socialis*) at Jamundí; Valle del Cauca (values expressed in thousands of Col. pesos/ha).

Treatment	Total Cost	Total Benefit	Net Benefit	C/B Ratio
Imidacloprid 1	1892.2	1305	-587.2	0.69
Buprofezin	1859.4	1644	-215.4	0.88
Carbosulfan	1806.6	1545	-261.6	0.86
Tiametoxan	1821.0	2434.5	613.5	1.34
Diafentiuron	1858.6	1111.5	-747.1	0.60
Piriproxifen	1821.0	1402.5	-418.5	0.77
Imidacloprid 2	1887.0	1650	-237.0	0.87
Control	1653.0	1266	-387.0	0.77

II. Evaluation of imidacloprid for cassava whitefly control.

Results show that all imidacloprid treatments were significantly different from the control **(Table 8)** for the three development stages, eggs, nymphs and adults. In general, all treatments retarded whitefly population appearance until 60 days. After 60 days two granular application one month apart were applied. This combination of a plant drench and stake treatment combined with the granular application maintained low whitefly populations **(Figure 2)**.

Table 8. The effects of applying imidacloprid on *Aleurotrachelus socialis* eggs, nymphs and adults, on cassava plants at Jamundí (Valle del Cauca).

Treatment	Doses	Adults	Eggs	Nymphs
1 SC – Drench at planting	0.6 liters	3.05 b	3.04 b	3.07 bc
2 SC – Drench at planting	0.8 liters	3.13 b	3.03 b	2.88 c
3 SC – Drench at emergence	0.2 liters	3.17 b	3.10 b	3.43 b
4 TS – Stake treatment	0.4 liters	3.15 b	3.30 b	3.34 bc
5 TS – Stake treatment	0.5 liters	3.19 b	3.23 b	3.14 bc
6 GR – Drench at planting	0.3 Kg	3.05 b	3.01 b	3.12 bc
7 GR – Drench at planting	0.4 Kg	3.02 b	3.11 b	3.18 bc
Commercial Control	0.2 liters	3.01 b	3.24 b	2.99 bc
Absolute Control		4.05 a	4.22 b	4.80 a

1. Duncan test: numbers followed by the same letter are not significantly different at the 5% level.
2. Based on population scale of 1 = none present; 2 = 1-200 individuals per leaf; 3 = 201-500 per leaf; 4 = 501 – 2000 per leaf; 5 = 2001-4000 per leaf; 6 = > 4000 per leaf.

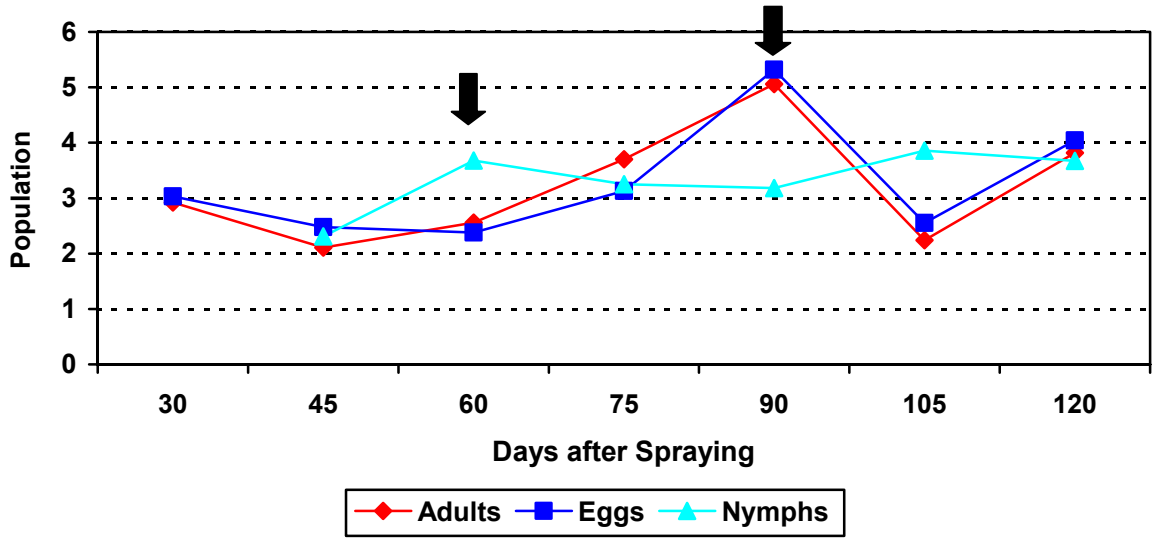

Figure 2. The effect of imidacloprid treatments over time on *Aleurotrachelus socialis* egg, nymph and adult populations (Valle del Cauca) in cassava.

The nymphal stage best indicates pesticide efficiency since egg and whitefly adult populations can vary due to adult migration from surrounding fields. Therefore nymphal populations were compared over time for each treatment and the control **(Figure 3).** All treatments reduced nymphal populations, but the lowest population was obtained using a concentrated suspension of imidacloprid as a drench at planting at the high dosage of 0.8 and 0.6 lt/ha. This is considered the most efficient treatment.

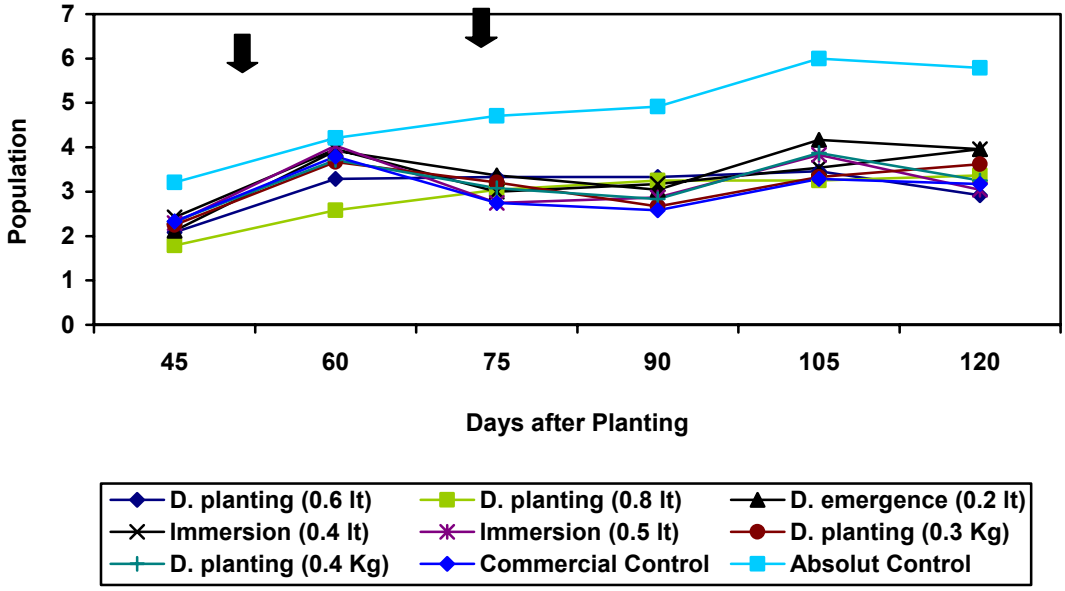

Figure 3. The effect of imidacloprid treatments over time on *Aleurotrachelus socialis* nymphs populations on cassava (Valle del Cauca).

Yields of all treatments were similar, producing an average of 10.3 T/ha; the same result as the previous experiment. Both of the control treatments had the lowest yields when compared to the pesticide treatments. All yields were adversely affected by the presence of frogskin disease **(Table 9)**.

Table 9. Cassava yields and frogskin incidence in imidacloprid treated cassava plots for whitefly (*A. socialis*) control at Jamundí (Valle del Cauca).

Treatment	Yield T/ha.	Frogskin Incidence (%)
1 SC – 0.6 lt – Drench at planting	10.85	22.36
2 SC – 0.8 lt – Drench at planting	11.6	25.61
3 SC – 0.2 lt – Drench at emergence.	9.37	17.63
4 TS – 0.4 lt – Immersion	8.55	38.44
5 TS – 0.5 lt – Immersion	9.67	22.81
6 Gr – 0.3 Kg – Drench at planting	10.01	22.46
7 Gr – 0.4 Kg – Drench at planting	11.84	22.32
Commercial Control	7.48	28.11
Absolute Control	4.75	19.90

The C/B ratio again showed that larger farmers benefit more from these inputs **(Table 10)**, indicating that the guaranteed higher prices that they received, makes chemical pest control more profitable. However it is more profitable to use only foliar applications than the combination of drench and granular described above. Both control treatments had values of C/B below 1, indicating that whitefly control is necessary for profitable cassava production in this region.

Table 10. Cost-Benefit ratio obtained by large cassava farmers ($330/Kg) applying different imidacloprid treatments to control whiteflies (*A. socialis*) at Jamundí, Valle del Cauca (values expressed in thousands of pesos per ha).

Treatment	Total Cost	Total Benefit	Net Benefit	C/B Ratio
1 SC – 0.6 lt – Drench at planting	2915.9	3580.5	664.6	1.23
2 SC – 0.8 lt – Drench at planting	3011.1	3831.3	820.2	1.27
3 SC – 0.2 lt – Drench at emergence	2725.5	3092.1	366.6	1.13
4 TS – 0.4 lt – Immersion	2773.9	2821.5	47.6	1.02
5 TS – 0.5 lt – Immersion	8221.8	3191.5	369.3	1.13
6 Gr – 0.3 Kg – Drench at planting	2643.8	3303.3	659.5	1.25
7 Gr – 0.4 Kg – Drench at planting	2810.3	3907.2	1096.9	1.39
Commercial Control	8720.3	2468.4	-251.9	0.91
Absolute Control	2396.3	1567.5	-828.8	0.65

The C/B for small farmers was below 1 for all treatments, although the lowest value was in the control plots **(Table 11)** again indicating the importance of whitefly control. These results indicate that for small farmers, it is not profitable to use chemicals or pesticides for whitefly control in cassava. However it should be taken into account that the most appropriate cassava variety was not used and that frogskin disease reduced yield considerably and this contributed to the low C/B ratio for small farmers.

Table 11. Cost-Benefit ratio obtained by small cassava farmers ($150/Kg) applying different imidacloprid treatments to control whitefly (*A. socialis*) at Jamundí, Valle del Cauca (values expressed in thousands of Col. pesos/ha).

Treatment	Total Cost	Total Benefit	Net Benefit	C/B Ratio
1 SC – 0.6 lt – Drench at planting	2172.6	1627.5	-545.1	0.75
2 SC – 0.8 lt – Drench at planting	2267.8	1741.5	-526.3	0.77
3 SC – 0.2 lt – Drench at emergence	1982.2	1405.5	-576.7	0.71
4 TS – 0.4 lt – Immersion	2030.6	1282.5	-748.1	0.63
5 TS – 0.5 lt – Immersion	2078.5	1450.5	-628	0.70
6 Gr – 0.3 Kg – Drench at planting	1900.5	1501.5	-399	0.79
7 Gr – 0.4 Kg – Drench at planting	2067	1776	-291	0.86
Commercial Control	1977	1122	-855	0.57
Absolute Control	1953	712.5	-940.5	0.43

In the second trial at Jamundí with imidacloprid applied at planting, the appearance of whiteflies was delayed for 45 days in all treatments. The control plots had the highest adult, egg and nymph populations with significant differences when compared to the treatments **(Table 12)**. In general, results from this trial were similar to the previous experiment **(Table 8)**.

Table 12. The effects of imidacloprid treatments on *Aleurotrachelus socialis* eggs, nymphs and adults (Trial 2).

Treatment	Doses	Adults	Eggs	Nymphs
1 SC – Drench at planting	0.6 liters	3.28 b	3.12 b	2.80 c
2 SC - Immersion	0.2 liters	3.28 b	3.20 b	3.12 bc
3 Gr – Drench at planting	0.3 Kg	3.38 b	3.42 b	3.18 bc
4 Gr – Stake treatment	0.3 Kg	3.18 b	3.09 b	3.11 bc
5 TS – Stake treatment	0.4 liters	3.29 b	3.16 b	3.14 bc
6 SC – Drench at planting	0.2 lt	3.59 b	3.51 b	3.58 b
Commercial Control	0.4 Kg	3.27 b	3.36 b	3.37 b
Absolute Control	0.2 liters	4.52 a	4.92 a	5.26 a

1. Duncan test: numbers followed by the same letter are not significantly different at the 5% level.
2. Based on population scale of 1 = none present; 2 = 1-200 individuals per leaf; 3 = 201-500 per leaf; 4 = 501 – 2000 per leaf; 5 = 2001-4000 per leaf; 6 = > 4000 per leaf.

Nymphal populations, over time, were similar for all treatments, ranging from 2 to 4 on the population scale, indicating a certain level of control **(Figure 4)**. The control plots gave similar results as in the previous trial, where whitefly nymphal populations ranged between 5 and 6 on the scale **(Figure 3 and 4)**. These results indicate that no control will lead to very high whitefly populations.

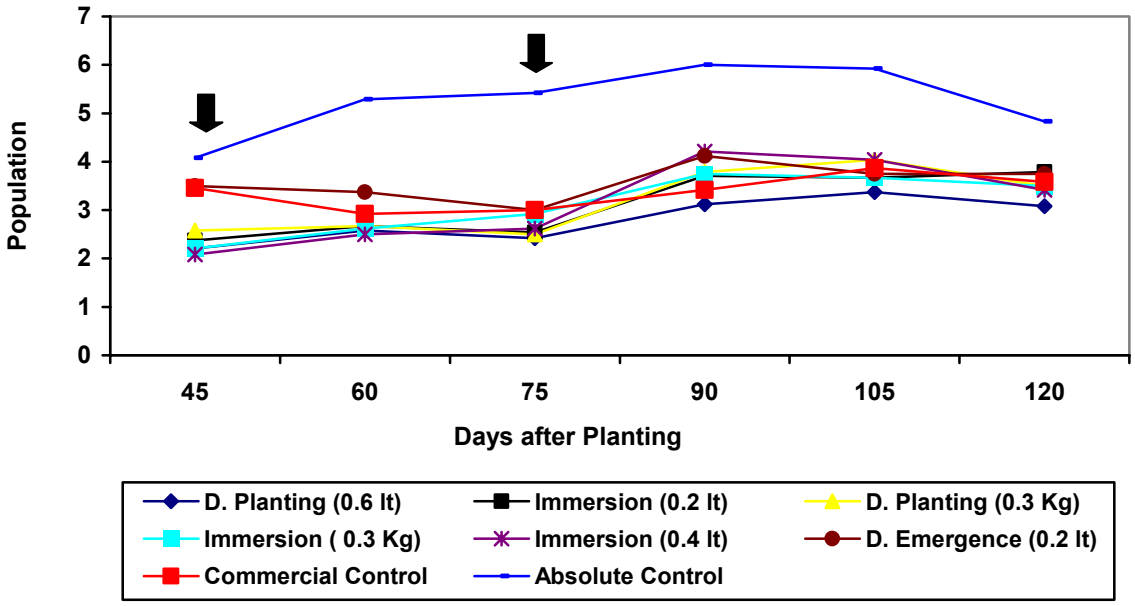

Figure 4. The effect of imidacloprid treatments over time on *Aleurotrachelus socialis* nymphs mortality (Trial # 2).

Yields in this trial were even lower than the previous trials, even though whitefly populations were similar for the 3 experiments **(Table 13)**. This dramatic decrease in yield is due primarily to the very high incidence of frogskin disease in this trial. Incidence levels ranged from 41 to 64% and averaging 49.7% across treatments.

Table 13. Cassava yields and frogskin disease incidence in imidacloprid treated plots for control of whiteflies (*A. socialis*) at Jamundí (Trial #2).

Treatment	Yield T/ha.	Frogskin Incidence (%)
1 SC – Drench at planting	8.56	44.74
2 SC - Immersion	4.73	48.95
3 Gr – Drench at planting	6.05	45.22
4 Gr – Stake treatment	6.61	44.91
5 TS – Stake treatment	6.76	41.04
6 SC – Drench at planting	6.13	64.65
Commercial Control	3.63	54.85
Absolute Control	4.25	53.38

Consequently the C/B ratio was lower, remaining below 1 for the treatments as well as the control plots, and for both large and small farmers **(Table 14 and 15)**. The variation in the incidence of frogskin disease in the three experiments in Jamundí indicates that there is no direct correlation between frogskin disease and whitefly populations. The incidence of frogskin was similar across treatments and experiments although higher in some trials.

Table 14. Cost-Benefit ratio obtained by large cassava farmers ($330/kg) applying different imidacloprid treatments to control whiteflies (*A. socialis*) at Jamundí (Trial #2) (values expressed in thousands of Col. pesos/ha).

Treatment	Total Cost	Total Benefit	Net Benefit	C/B Ratio
1 SC – Drench at planting	2915.9	2824.8	-91.11	0.97
2 SC - Immersion	2677.5	1560.9	-1116.61	0.58
3 Gr – Drench at planting	2643.8	1996.5	-647.31	0.76
4 Gr – Stake treatment	2595.8	2181.3	-414.51	0.84
5 TS – Stake treatment	2773.9	2230.8	-543.11	0.80
6 SC – Drench at planting	2725.5	2022.9	-702.61	0.74
Commercial Control	2720.3	1197.9	-1522.41	0.44
Absolute Control	2396.3	1402.5	-993.81	0.59

Table 15. Cost-Benefit ratio obtained by small cassava farmers ($150/kg) applying different imidacloprid treatments to control whiteflies (*A. socialis*) at Jamundí (Trial # 2) (values expressed in thousands of Col. pesos/ha).

Treatment	Total Cost	Total Benefit	Net Benefit	C/B Ratio
1 SC – Drench at planting	2172.6	1284.0	-888.6	0.59
2 SC - Immersion	1934.2	709.5	-1224.7	0.37
3 Gr – Drench at planting	1900.5	907.5	-993.0	0.48
4 Gr – Stake treatment	1852.5	991.5	-861.0	0.54
5 TS – Stake treatment	2030.6	1014	-1016.6	0.50
6 SC – Drench at planting	1982.2	919.5	-1062.7	0.46
Commercial Control	1977.0	544.5	-1432.5	0.28
Absolute Control	1653.0	637.5	-1015.5	0.39

Training cassava producers in IPM.

More than 200 persons, including farmers and technicians in the cassava growing regions of Cauca and Valle del Cauca have received training and information on cassava IPM, especially in the control of whiteflies. This has been accomplished through workshops, talks, conferences,

and field days, and has included information on pest behavior, damage, natural enemies and biological and chemical control of *A. socialis*.

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Activity 5. Evaluation of whitefly (*Aleurotrachelus socialis*) cassava clones for resistance to *Bemisia tabaci*.

Whiteflies are reported feeding on cassava in nearly all cassava growing regions of the tropics. Eleven species have now been identified globally. In the neotropics *Aleurotrachelus socialis* predominates in Northern South America, while *Aleurothrixus aepim* is the major species in Brazil. *Bemisia tabaci*, a pantropical species, predominates in Africa where it is the vector of Africa Cassava Mosaic Disease (ACMD). Until recently, *B. tabaci* biotypes found in the neotropics did not feed on cassava, and it has been speculated that the absence of ACMD in the Americas may be related to the inability of *B. tabaci* to colonize cassava. During the last decade a new biotype (B) of *B. tabaci* has been collected feeding on cassava in the neotropics.

The appearance of Biotype B is cause for concern as it is now considered that ACMD poses a more serious threat to cassava production in the Americas as most traditional varieties in the neotropics are highly susceptible to the disease. Therefore a project is now underway to identify possible *B. tabaci* resistance in cassava germplasm.

The CIAT cassava germplasm bank of more than 6000 accessions is continually being screened for resistance to arthropod pests, especially whiteflies. Over a period of more than 15 years several clones have been selected as sources of resistance to the whitefly species *Aleurotrachelus socialis*. The clone MEcu 72 has consistently expressed the highest levels of resistance. Additional cultivars expressing moderate to high levels of resistance in field trials include MEcu 64, MPer 335, MPer 415, MPer 317, MPer 216, MPer 221, MPer 265, MPer 266 and MPer 365. Whitefly resistance hybrids from a MEcu 72 x MBra 12 cross have been produced and evaluated; the progeny CG 489-31 is being released by the Colombian MADR to cassava producers in Nov. 2002.

The objectives of this research project is to determine if the *A. socialis* resistant sources will also express resistance to the B biotype of *Bemisia tabaci*.

Methodology: The stock of Biotype "B" of *B. tabaci* to initiate a colony on cassava came from the CIAT Bean Improvement Project (IP-1). *B. tabaci* adults, harvested from the bean colony, were allowed to first oviposit on poinsettia (*Euphorbia pulcherrima*). After five generations established on poinsettia, the colony was transferred to Jatropha (*Jatropha gossypiifolia*), where it has been established for 12 generations. The colony established on Jatropha was then transferred to both *Manihot esculenta* and *Manihot carthaginensis* **(Figure 1)**. The "B" biotype of *B. tabaci* colony has now been reared for two generations on *M. esculenta* (Var. MCol 2063) and 3 generations on *M. carthaginensis*. Colonies are maintained at CIAT in growth rooms under controlled conditions: 12 hrs. photoperiod, 25 ± 2°C and 50-80 % RH. This methodology was designed in order to gradually or progressively adapt *B. tabaci* from beans to *Manihot* species by passing it through related species of the Euphorbiaceae family.

Bean plants are held in the screen house for 17 days; poinsettia and Jatropha for 40 to 50 days and cassava 30 to 40 days, before being exposed to the *B. tabaci* colony. Plants are grown in 15cm diameter plastic pots, watered daily and receive no fertilizer nor pesticide during their development. Plants are placed in fine nylon meshed wooden cages (1m ht x 1m width), where

B. tabaci infestation takes place. Whitefly adults are harvested with a pipette suction devise, from the colony and released in the experimental cages. Fresh plants of each species are supplied for each of the colonies on a regular basis; usually no more than two generations of *B. tabaci* are reared on the same plant.

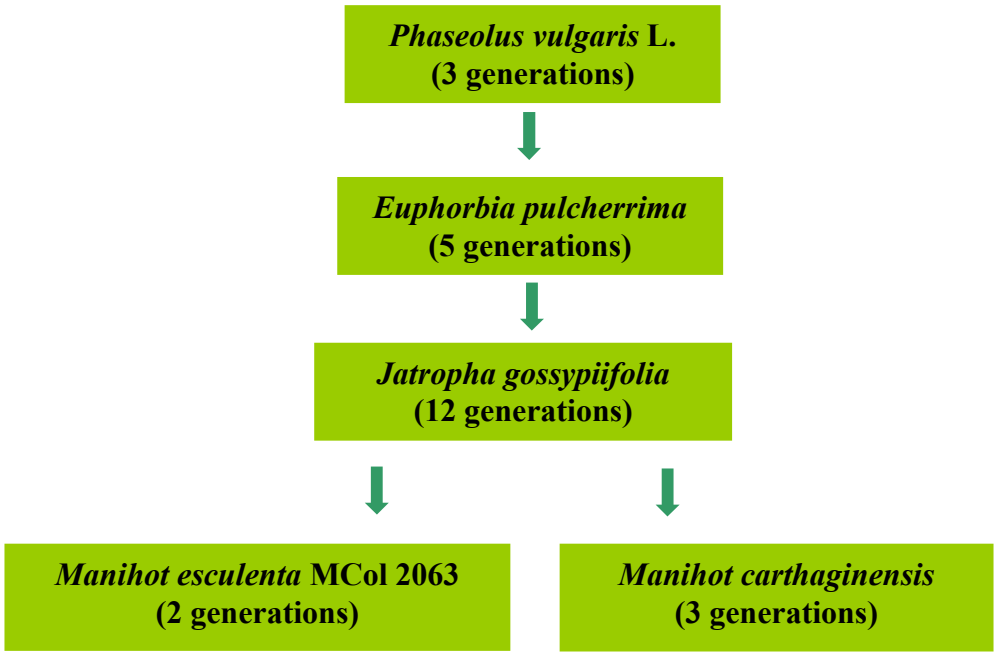

Figure 1. Plant species sequence for adapting the whitefly species *Bemisia tabaci*, B biotype, from beans (*P. vulgaris*) to *Manihot esculenta* and *M. carthaginensis.*

The cassava variety MCol 2063 was selected as a host plant because it is recognized as being very susceptible to whiteflies, especially to *A. socialis*. *M. carthaginensis*, also known as "yuca de Cartagena" is a wild species that grows naturally on the North Coast of Colombia and is being used in genetic improvement programs because of its high protein content, considerably higher than cultivated species of *M. esculenta.*

Preliminary studies were done with four wild species, *Manihot flabellifolia, M. peruviana, M. tristis* and *M. carthaginensis*, to evaluate their potential to host the B biotype of *B. tabaci*. *B. tabaci* adapted best to *M. carthaginensis* so this species was selected to establish a *B. tabaci* colony.

B. tabaci became established on MCol 2063 after 2 generations and on *M. carthaginensis* after 3 generations. The evaluation of cassava genotypes for resistance to *B. tabaci* was first done by exposing them to the *M. carthaginensis*, since this host showed the best adaptation. Every 3 months a molecular identification of the *B. tabaci* colony is carried out using RAPD-PCR to assure that contamination with the "A" biotype has not occurred.

The initial three *M. esculenta* genotypes selected to be evaluated are CMC-40 MEcu 72 and CG 489-34. CMC-40 is highly susceptible to *A. socialis*; the *A. socialis* colony is maintained on this genotype. MEcu 72 is highly resistant to *A. socialis*; evidenced by low populations, no damage symptoms and high nymphal mortality in laboratory studies. CG 489-34 is a hybrid progeny of a MEcu 72 x MBra 12 cross, displaying moderate levels of resistance to *A. socialis*.

Experimental Procedures: HPR experiments with the three cassava genotypes were done in growth chambers under controlled temperature, humidity and photoperiod conditions. MEcu 72, CG 489-34 and CMC-40 plants were multiplied through vegetative cuttings and planted in 15cm diameter plastic pots. Plants were placed in a screen house. Experiments were initiated by selecting plants that possessed 4 to 6 true leaves and approximately 30 to 50 cm high. Biotype "B" *B. tabaci* recently emerged adults were harvested from the *M. carthaginensis* colony by using a pipette aspirator and a glass vial with a perforated lid and 40 pair were sexed. Each pair was placed within a 2.5cm diameter leaf-0clip cage **(Figure 2)** on a cassava leaf. Every 48 hours the adult pair was moved to new area of the leaf, until the female died. Fecundity was measured by counting eggs oviposited by each female during the 48 hr. period.

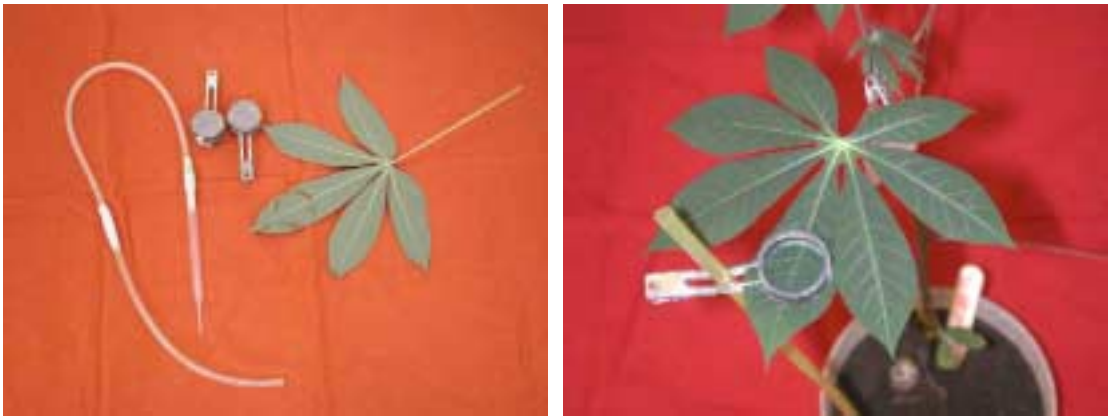

Figure 2. Methodologies for installing male and female biotype B of *Bemisia tabaci* on cassava (*Manihot esculenta*) leaves.

Experiments to determine development time, rate of survival and proportion of *B. tabaci* ("B") females developed were carried out under the previously described conditions. 40 adults *B. tabaci* "B" biotype were placed in the leaf cages on the leaf underside of each genotype. Adults were removed after 6 hours and 200 eggs were selected. Egg hatch and nymphal development or mortality were observed until the adult stage and the proportion of emerged females was noted **(Figure 3)**.

Demographic parameters were determined using a methodology described by Manzano (2000). Data on development time and survival of immatures were combined with experimental reproduction data "lxmx," to create life tables and calculate demographic parameters for *B. tabaci* "B." For each experiment the following parameters, defined by Price (1975) were calculated: net reproduction rate (Ro, this represents the number of females produced by each female in one generation), generational time (T = the average time span required between the

birth of the parents and the birth of their progeny. The intrinsic rate of increase of a population (r_m) for *B. tabaci* "B" is estimated using the equation proposed by Carey (1993):

$\sum \exp(-r_m x) lx mx = 1$

Where: x = age
Lx = age of specific survival
Mx = proportion of the females of the progeny of a female at age x.

To calculate the r_m values, the corrected age x + 0.5 was used (Carey, 1993).

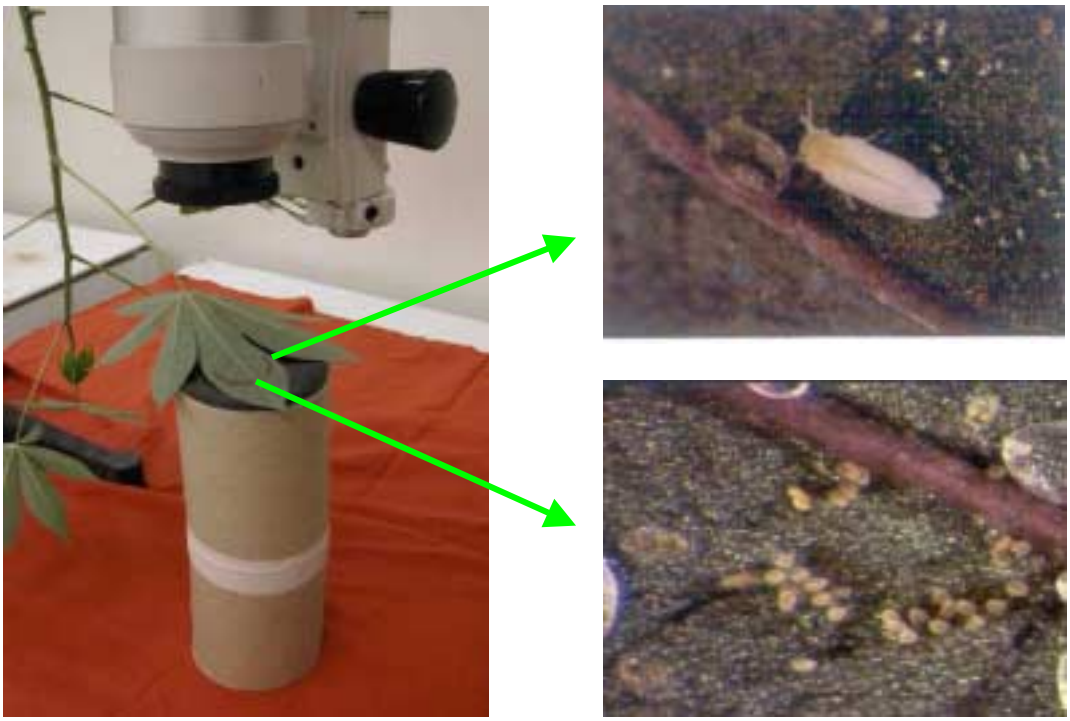

Figure 3. Techniques for recording egg and adult populations of biotype B of *Bemisia tabaci on cassava leaves.*

Data Analysis:

- Significant differences between the longevity values and fecundity on each cassava genotype were determined with an analysis of variance using the Kruskal-Wallis test.

- Values for oviposition rates were determined using an ANOVA analysis. For the three parameters, multiple comparisons were done using the Student-Newman-Keuls test.

- The differences in development time between cassava genotypes were evaluated with an analysis of variance using the Kruskal-Wallis test. Comparisons of survival rates were done using the x^2 test.

Results

A. Adaptation of *B. tabaci* "B" on several hosts.

Mean longevity of *B. tabaci* biotype "B" was highest on cassava when females originated from poinsettia (*E. pulcherrima*), when compared Jatropha (*J. gossypiifolia*) and beans (*P. vulgaris*). Average longevity differed significantly between the three hosts, except for the longevity values of *J. gossypiifolia* and *P. vulgaris* (Student Newman-Keuls $P < 0.05$, after Kruskal-Wallis $P < 0.0001$). Longevity of adults on cassava, originating from the 3 hosts is shown in Figure 4. Average fecundity was also significantly different between the three hosts (Kruskal-Wallis $P < 0.0001$), except for the values obtained between *E. pulcherrima* and *J. gossypiifolia* (Student Newman-Keuls $P < 0.05$) **(Table 1)**. Reproduction curves indicated by daily oviposition **(Figure 5)** resulted in higher oviposition on *J. gossypiifolia* although not over a long time period.

The average ovipositioned rate (eggs per female over two days) was higher for *J. gossypiifolia* (2.64) **(Table 1)**. Average ovipositional rate was significantly different between treatment (ANOVA, $P < 0.0001$); comparisons between females originating from the three hosts show no significant differences in average ovipositional rate (student Newman-Keuls $P < 0.05$).

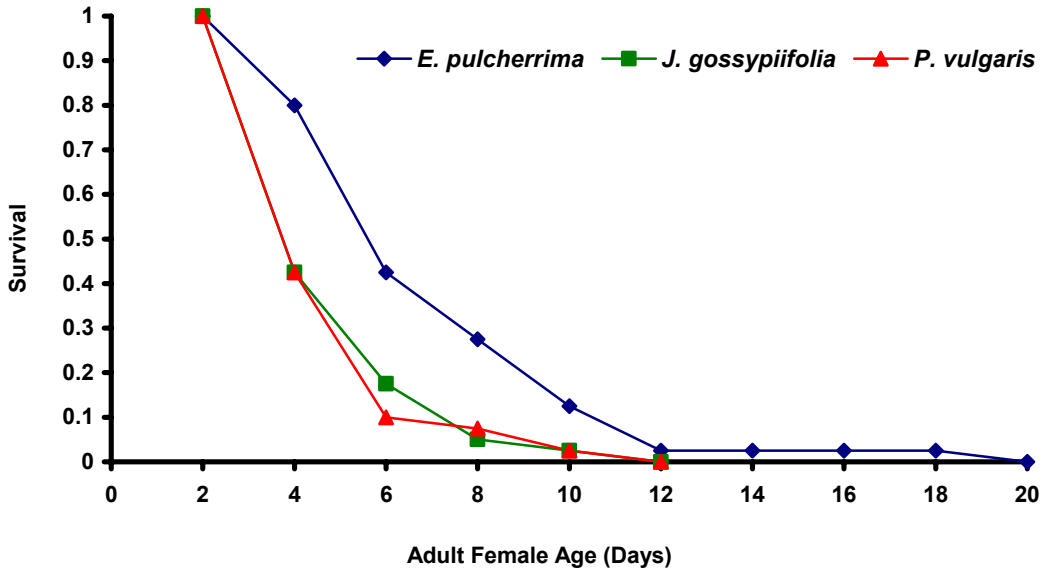

Figure 4. Survival curves of biotype B of *Bemisia tabaci* adult females, that originated from three separate hosts, (*Phaseolus vulgaris*, *Euphorbia pulcherrima* and *Jatropha gossypiifolia*).

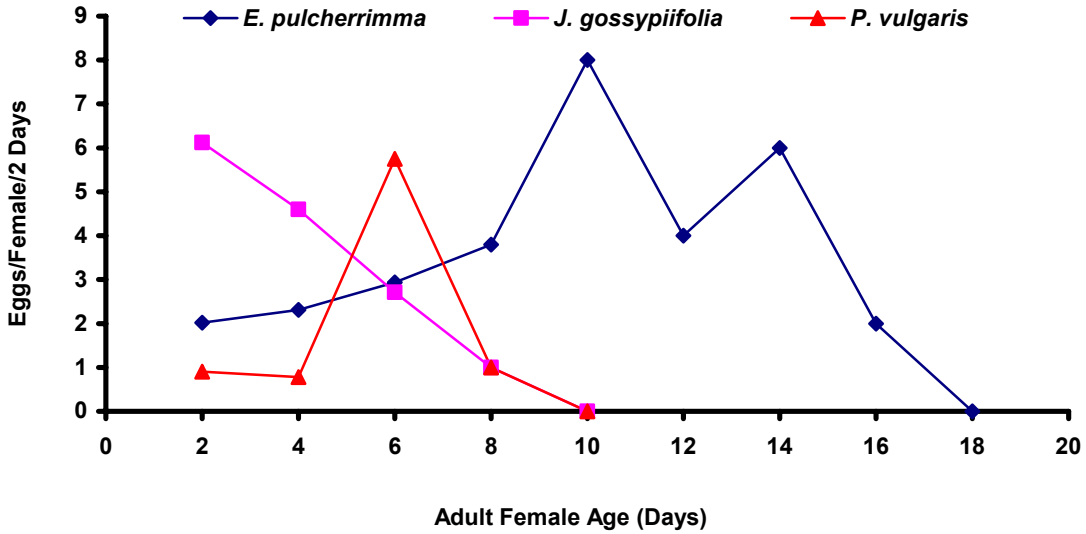

Figure 5. Reproduction curves of biotype B of *Bemisia tabaci* females that originated from three separate hosts (*Phaseolus vulgaris*, *Euphorbia pulcherrima* and *Jatropha gossypiifolia*).

Table 1. Mean longevity (d), mean fecundity and rate of oviposition (eggs/female/2 days) of biotype B of *Bemisia tabaci* on cassava from females originating from 3 host populations.

Parameter	*J. gossypiifolia*	*E. pulcherrimma*	*P. vulgaris*
Mean Longevity*	3.25 a	5.6 b	3.1 a
Range	2-10	2-18	2-10
# Insects	40	40	40
Mean Fecundity*	8.6 a	7.65 a	1.82 b
Range	1-41	1-48	1-19
Mean Rate Oviposition ϕ	2.64 a	1.36 b	0.58 c
Range	0.5-8	0.4-3	0.5-3.5

Figures followed by different letters across columns indicate significant differences.

* Kruskal-Wallis P< 0.0001, Student-Newman-Keuls method P< 0.05.

ϕ One-way ANOVA P< 0.0001, Student-Newman-Keuls method P< 0.05.

Development time for progeny from females from *E. pulcherrima* (50 days) and *P. vulgaris* (49.5) were similar; the lowest development time was from progeny from *J. gossypiifolia* females (44.4 days). The rate of survival for immatures was significantly different for *J. gossypiifolia* with respect to the other two hosts. The proportion of females was equal for all three hosts (50%) Table 2.

Discussion: The intrinsic rate of growth (r_m) of *B. tabaci* "B" originating from each of the hosts and developing on *M. esculenta* (MCol 2063), permits determining that *J. gossypiifolia* was best adapted in that the *B. tabaci* "B" population had the highest intrinsic rate of growth, exceeding *E.*

pulcherrima by 8.3% and *P. vulgaris* by 58.3%. In addition it presented the lowest generational time, 44.76 days from generation to generation **(Table 2)**.

Survivorship of the immature states of *B. tabaci* "B" was 27.5% on *M. esculenta/J. gossypiifolia* relationship, considerably higher than the other two hosts. When this is expressed together with other demographic parameters **(Table 2)** the adaptation advantage that *B. tabaci* "B" has when population originate on *J. gossypiifolia* is obvious when compared to the other hosts.

Table 2. Demographic parameters from biotype B of *Bemisia tabaci* on *Manihot esculenta*; adult females originating from three separate plant host species.

Parameter	*J. gossypiifolia*	*E. pulcherrima*	*P. vulgaris*
Development time (d)	44.41	50.60	49.50
Rate of survival (%)	27.50	3.00	2.00
Proportion females (%)	50.90	50.00	50.00
Intrinsic rate of increase (r_m)	0.048	0.044	0.020
Net reproductive rate (Ro) ∑lxmx	8.63	11.60	1.82
Generation time (T)	44.76	56.03	51.30

B. Evaluation of resistance/tolerance of cassava genotypes *B. tabaci* "B."

The low intrinsic rate of increase values (r_m) and the demographic values of *B. tabaci* "B" on *M. esculenta* (MCol 2063) indicate that population is low on this host and that there is not a significant increase in one generation to another. Because of these results, and additional host was sought to adapt populations of *B. tabaci* "B" to *M. esculenta*. *M carthaginensis*, because it is closely related to *M. esculenta* was chosen as the population source for evaluation of *M. esculenta* genotypes (complete data on *M. carthaginensis* not yet available) **(Figure 6)**.

Preliminary results indicate that adult longevity on the three genotypes, CMC-40, MEcu 72 and CG 489-34 ranged from 2 to 20 days, but was longest on MEcu 72 **(Figure 7)**. Average longevity was not significantly different between genotypes (Kruskal-Wallis, P=0.0809). The fecundity range for the three genotypes was 1 to 40 eggs per female over 2 days. The average fecundity was significantly different between genotypes (Kruskal-Wallis, $P < 0.0001$), except for MEcu 72 values (6.3) and CG 489-34 (5.07) (Student-Newman-Keuls, $P < 0.05$) **(Figure 8)**. Oviposition was lowest on CMC-40.

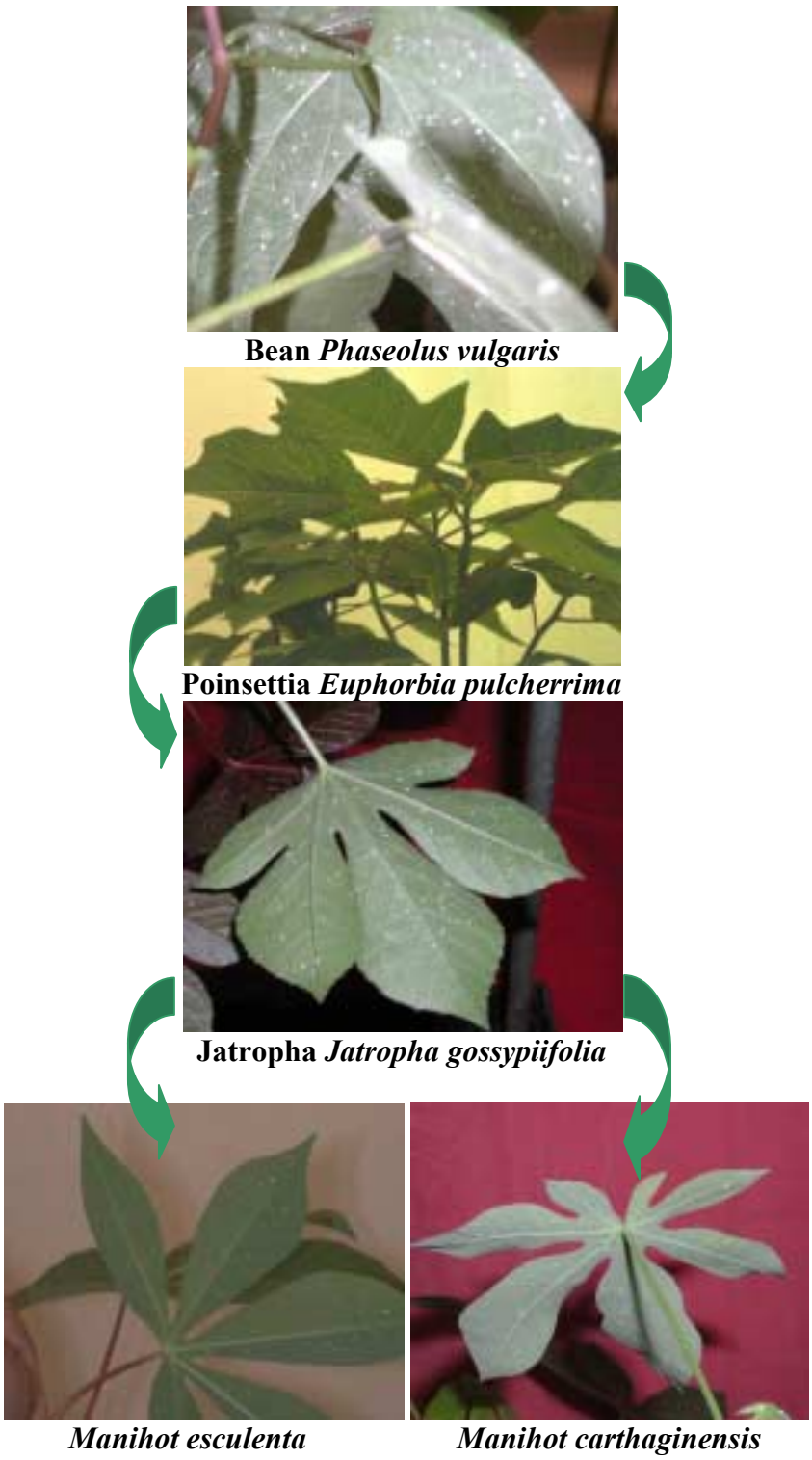

Figure 6. Photo scheme of plant species sequence for adapting *Bemisia tabaci*, biotype B from beans to *Manihot esculenta* and *M. carthaginensis.*

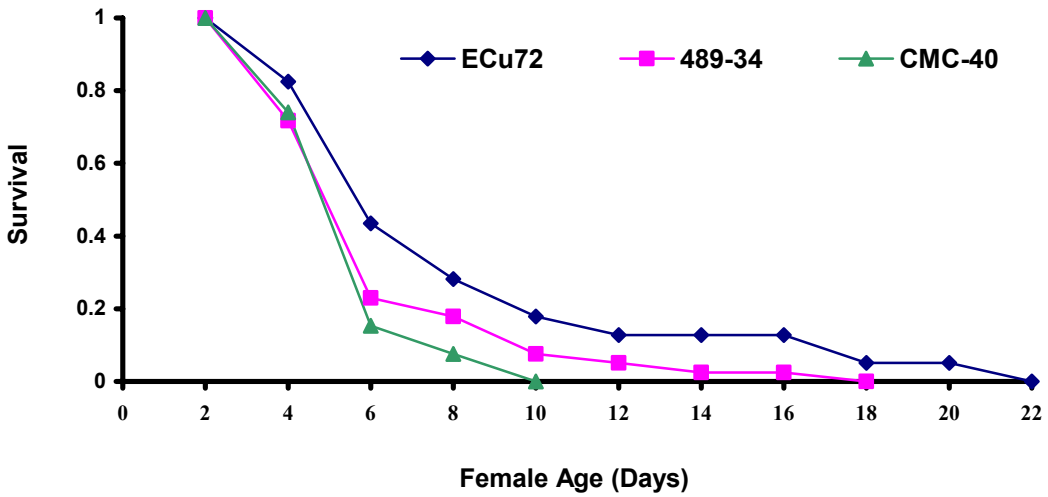

Figure 7. Survival curves of biotype B of *Bemisia tabaci* on three cassava genotypes: MEcu 72, CG 489-34 and CMC-40.

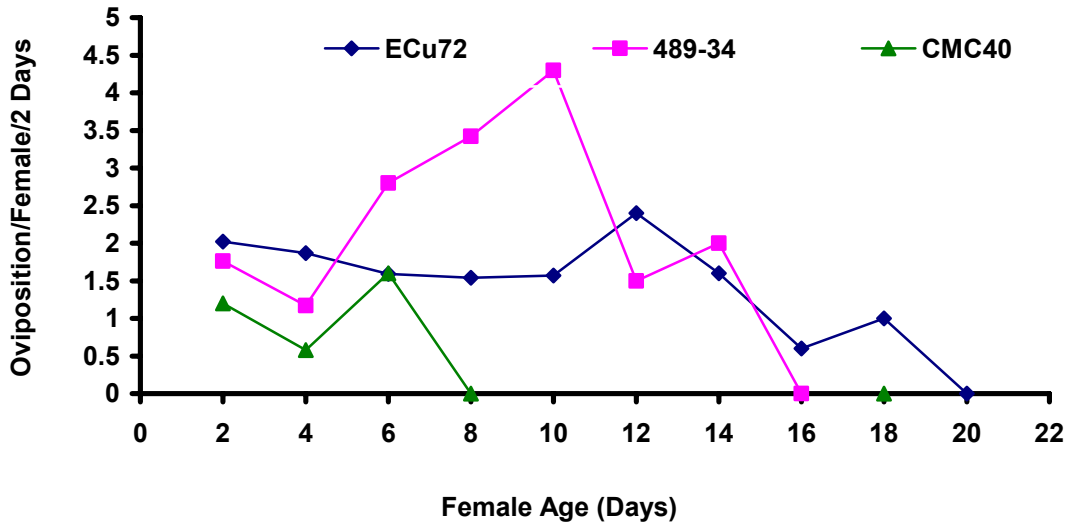

Figure 8. Reproduction curves of biotype B of *Bemisia tabaci* on three genotypes of cassava: MEcu 72, CMC-40 and CG 489-34.

The average ovipositional rate (eggs/female/2 days) increased from 0.49 in CMC-40 to 0.89 in MEcu 72. The average rate of oviposition was significantly different for the 3 genotypes (One-Way ANOVA $P < 0.0001$), except for the comparison between MEcu 72 and CG 489-34 (Student-Newman-Keuls, $P < 0.05$) **(Table 3)**.

Table 3. Mean longevity (days), Mean fecundity (eggs) and mean rate of oviposition (eggs/female/2 days) of biotype B of *Bemisia tabaci* on three cassava genotypes.

Parameter	CG 489-34	CMC-40	MEcu-72
Mean longevity *	5.07a	3.9a	6.3a
Range	2-16	2-8	2-20
# Insects	39	39	39
Mean fecundity *	4.35a	1.89b	5.61a
Range	1-24	1-12	1-40
Mean Rate of oviposition ϕ	0.86a	0.49b	0.89a
Range	0.25-1.56	0.25-2.75	0.25-3.8

Figures followed by different letters across columns indicate significant differences.
* Kruskal-Wallis P< 0.0001, Student-Newman-Keuls method P< 0.05.
ϕ One-Way ANOVA P< 0.0001, Student-Newman-Keuls method P< 0.05.

Development time for MEcu 72 was 55.1 days, **(Table 4)** indicating a very low level of adaptation for this *B. tabaci* "B" on this genotype. On genotypes CMC-40 and CG 489-34 *B. tabaci* "B" did not complete its cycle of egg to adult, only permitting nymphal development to the third instar **(Figure 9)**.

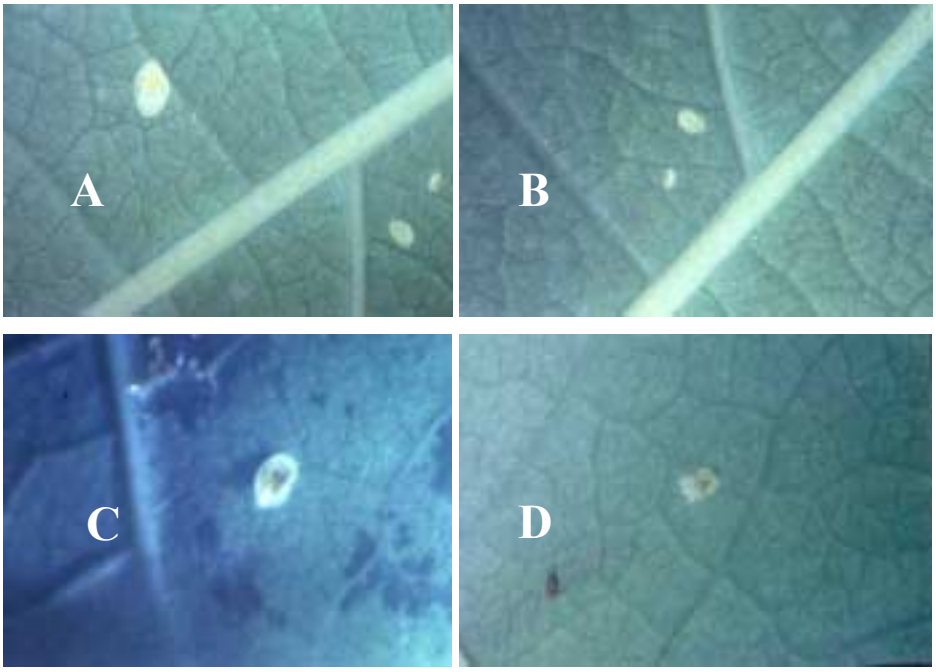

Figure 9. Development stages of biotype B of *Bemisia tabaci*: A and B. N1, N2 and N3 (30 days) on MEcu 72; C. N2 and eggs (30 days) on CG 489-34, and D. N2 (30 days) on CMC-40.

These preliminary results indicate that none of the three cassava genotypes are adequate hosts for *B. tabaci* "B" as it only reached the adult stage on MEcu 72 with a low survival of only 3% **(Table 4)**.

In addition, since *B. tabaci* "B" females oviposited on all 3 genotypes, oviposition is not a good indication of adaptability or host acceptance since nymphal survival did not occur on two genotypes and was very low on the third. It should also be noted that MEcu 72 is resistant to *A. socialis*, CG 489-34 is moderately resistant and CMC-40 is susceptible. Results expressed in these experiments, although not conclusive, are different.

Table 4. Demographic parameters of biotype B of *Bemisia tabaci* on genotype MEcu 72.

Parameter	MEcu 72
Development time (d)	55.1
Rate of survival (%)	3
Proportion of females (%)	33
Intrinsic rate of increase (r_m)	0.2958345
Net reproduction rate (Ro) ∑lxmx	5.61
Generation time	58.33

Contributors: A. Carabalí, A.C. Bellotti.

Activity 6. Biological control of cassava mites: A taxonomic key to the identification of phytoseiid predatory mites associated with cassava phytophagous mites.

Research into the control of cassava mites at CIAT has had two main thrusts: host plant resistance and biological control. The cassava green mite (CGM), *Mononychellus tanajoa*, is the most important species and is reported causing yield losses in the Americas and Africa, especially in seasonally dry regions of the lowland tropics (Ezulike, et al., 1981; Byrne, et al., 1983; Yaninek and Animashaun, 1987; Braun, et al., 1989). *M. tanajoa* is native to the Neotropics and it was first reported from Brazil in 1938. It first appeared in Africa (Uganda) in 1971 and by 1985 it had spread across most of the cassava belt, occurring in 27 countries (Yaninek and Herren, 1988; Herren and Neuenschwander, 1991; Skovgård, et al., 1993) and causing estimated root yield losses of 13-80%.

Extensive survey of cassava fields and experimental data indicate that although CGM is present throughout much of the lowland Neotropics, severe outbreaks causing significant yield loses are rare, except in parts of Brazil. From 1983-1990 extensive evaluations of the natural enemy complex associated with cassava mites were carried out at 2400 sites in 14 countries of the Americas (Byrne et al., 1983; Bellotti, et al., 1987). This led to the identification of the phytoseiid predator complex associated with cassava mites. Collecting zones were usually chosen for their similarity to ecological homologues in Africa and Brazil. Of the 87 species collected and stored, 25 are new or unrecorded species; 76% (66 species) were collected from cassava (Melo, 2002). The CIAT/Brazil collection is now organized into a true reference collection with accompanying database and can be used readily for species description or redescription, where types and paratypes may be found.

The most important biological control agents of cassava mites belong to the family Phytoseiidae (Hog, 1982). Phytoseiids have been used very effectively for mite control in greenhouses, and in general, are better than other predators for reducing pest mite populations (Bellotti, et al., 1985).

Since 1984 numerous phytoseiid species have been sent from Colombia and Brazil to Africa, and three of these species (*Typhlodromalus manihoti*, *T. aripo*, and *Neoseiulus idaeus*) from Brazil have become established in Africa (Yaninek, et al., 1991; 1993; Bellotti, et al., 1999). *T. aripo* appears to be the most promising of the three; it disperses rapidly and is now found in 14 African countries. Field evaluations show that *T. aripo* reduces CGM populations by 35-60%, resulting in a cassava root dry matter increase of 30-37%.

The first activities of a biological control program is to accurately identify the pest species and its natural enemies. This usually requires taxonomic specialists. In order to make an accurate classification, a taxonomic key is required of all the known species; this will facilitate the most efficient use of the natural enemy complex associated with the pest species (Cave, 1995). Biological control specialists realize the importance of taxonomy in the introduction, conservation and multiplication of natural enemies. This collaboration with taxonomists begins during the exploratory phase, and facilitates the search for the correct natural enemies for optimal results in a biological control program (Moraes, 1987).

The major limitation in phytoseiid species identification is their diversity (More than 1500 species of this family are known), their size and the difficulty in finding taxonomic keys for each species and genus. It is for this reason that a taxonomic key for the Phytoseiidae associated with cassava mites was developed through a CIAT-Brazil (Department of Entomology, Fitopathology and Agriculture Zoology, ESALQ-University of Sao Paulo) collaborative project. The development of this key will facilitate the identification of phytoseiid mite natural enemies, for researchers around the globe.

Researchers from CIAT (Elsa Liliana Melo) and Brazil (Gilberto de Moraes and Antonio C. Lofego) have been collaborating on this task since 1998 and the key is now ready for publication. This includes all 53 of the phytoseiid species found in the Americas **(Table 1).** The classification system used comprises the Order Parasitiformes, which describes females of the Phytoseiidae family; only females are used for the identification. This family is divided into 3 subfamilies, Amblyseiinae, Phytoseiinae and Typhlodrominae, which are differentiated by the presence or absence of setas. The genus-species are grouped within each of the sub-families **(Table 2).**

A detailed description accompanies each species, based on size and structures. This permits a rapid and accurate identification; a drawing of each species is included in the taxonomic key; Figure 1 is an example of one of these species.

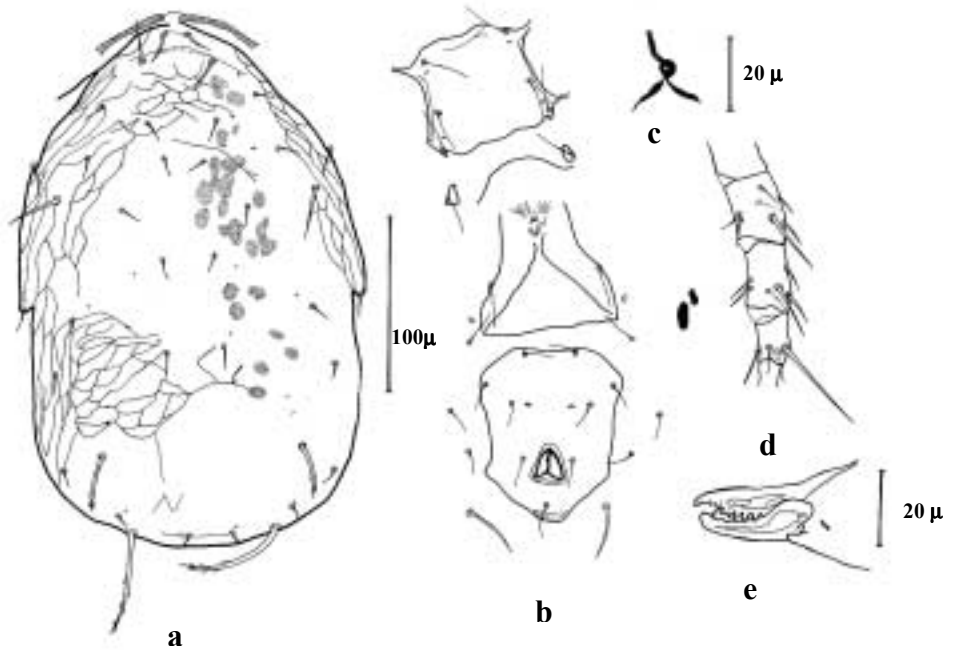

Figure 1. A description of the phytoseiid species *Typhlodromips mangleae*,
1a. Dorsal shield with typical setation (number, distribution and size);
1b. Ventral shields;
1c. Spermatheca calyx;
1d. Leg IV (macrosetae);
1e. Chelicera (form, size and number of teeth) (measurements given in microns µ).

Table 1. Phytoseiidae species associated with the cassava green mite, *Mononychellus tanajoa,* included in the taxonomic identification key and collected from cassava in several countries in the Americas.

Species	Country where Collected
Amblyseius aerialis (Muma)	Brazil, Colombia, Cuba
Amblyseius chiapensis DeLeon	Brazil, Colombia
Amblyseius coffeae DeLeon	Colombia
Amblyseius herbicolus (Chant)	Brazil, Colombia
Amblyseius largoensis (Muma)	Brazil, Mexico
Amblyseius tamatavensis Blommers	Cuba
Amblyseius vasiformis Moraes & Mesa	Ecuador
Euseius alatus DeLeon	Brazil, Colombia
Euseius casearíae DeLeon	Colombia
Euseius citrifolius Denmark & Muma	Brazil
Euseius concordis (Chant)	Brazil, Colombia, Paraguay
Euseius ho (DeLeon)	Brazil, Colombia, Ecuador, Peru
Euseius naindaimei (Chant & Baker)	Colombia
Euseius sibelius (DeLeon)	Brazil, Colombia
Euseius vivax (Chant & Baker)	Cuba, Mexico, Nicaragua
Galendrominus (Galendrominus) alveolaris (DeLeon)	Colombia
Galendromus (Galensromus) annectens (DeLeon)	Brazil, Colombia, Mexico
Galendromus (Galendromus) helveolus (Chant)	Colombia, Ecuador, Mexico, Panama
Galendromus (Galendromus) longipilus (Nesbitt)	Colombia
Galendromus (Galendromus) pilosus (Chant)	Mexico
Iphiseiodis zuluagai Denmark & Muma	Brazil, Colombia, Panama
Metaseiulus (Metaseiulus) neoflumenis Moraes & Kreiter	Panama
Neoseiulus anonymus (Chant & Baker)	Cuba, Brazil, Colombia, Mexico, Peru
Neoseiulus bellottii (Moraes & Mesa)	Brazil, Colombia
Neoseiulus californicus (McGregor)	Colombia, Ecuador
Neoseiulus idaeus Denmark & Muma	Brazil, Colombia, Paraguay, Venezuela
Neoseiulus neoaurescens (Moraes & Mesa)	Paraguay
Neoseiulus paraibensis (Moraes & McMurtry)	Colombia, Cuba
Neoseiulus tunus (DeLeon)	Colombia
Paraphytoseius multidentatus Swirski & Shechter	Colombia
Phytoseiulus fragariae Denmark & Schicha	Colombia
Phytoseiulus macropilis (Banks	Brazil, Colombia, Mexico, Peru
Phytoseius averrhoae DeLeon	Ecuador
Phytoseius purseglovei DeLeon	Colombia
Proprioseiopsis caliensis (Moraes & Mesa)	Colombia
Proprioseiopsis cannaensis (Muma)	Brazil, Colombia, Cuba, Paraguay
Proprioseiopsis mexicanus (Garman)	Brazil, Colombia, Cuba, Panama
Proprioseiopsis neotropicus (Ehara)	Colombia
Proprioseiopsis ovatus (Garman)	Ecuador
Proprioseius mirandai DeLeon	Cuba, Mexico
Ricoseius loxocheles (De Leon)	Ecuador
Typhlodromalus aripo DeLeon	Brazil, Colombia, Ecuador, Paraguay
Typhlodromalus limonicus (Garman & McGregor)	Brazil, Colombia, Trinidad
Typhlodromalus manihoti (Moraes)	Bolivia, Brazil, Colombia, Cuba, Ecuador, Nicaragua, Paraguay, Peru, Surinam, Trinidad, Venezuela
Typhlodromalus peregrinus (Muma)	Brazil, Colombia, Ecuador, Guyana, Mexico
Typhlodromalus tenuiscutus (McMurtry & Moraes)	Colombia, Ecuador, Panama
Typhlodromalus villacarmelensis (Moraes)	Colombia
Typhlodromina subtropica Muma & Denmark	Colombia
Typhlodromina tropica (Chant)	Colombia
Typhlodromips dentilis (DeLeon)	Colombia, Cuba
Typhlodromips gonzalezi (Moraes & Mesa)	Colombia
Typhlodromips mangleae DeLeon	Colombia
Typhlodromus (Antoseius) transvaalensis (Nesbit)	Colombia

Table 2. Phytoseiidae species collected from cassava and grouped by Subfamily; included in taxonomic identification key.

Subfamily	Species	
Amblyseiinae	*Amblyseius aerialis*	*Neoseiulus tunus*
	Amblyseius chiapensis	*Neoseiulus paraibensis*
	Amblyseius coffeae	*Paraphytoseius multidentatus*
	Amblyseius herbicolus	*Phytoseiulus fragariae*
	Amblyseius largoensis	*Phytoseiulus macropilis*
	Amblyseius tamatanensis	*Proprioseiopsis caliensis*
	Amblyseius vasiformis	*Proprioseiopsis cannaensis*
	Euseius alatus	*Proprioseiopsis mexicanus*
	Euseius casearíae	*Proprioseiopsis neotropicus*
	Euseius citrifolius	*Proprioseiopsis ovatus*
	Euseius concordis	*Proprioseius mirandai*
	Euseius ho	*Ricoseius loxocheles*
	Euseius naindaimei	*Typhlodromalus aripo*
	Euseius sibelius	*Typhlodromalus limonicus*
	Euseius vivax	*Typhlodromalus manihoti*
	Iphiseiodis zuluagai	*Typhlodromalus peregrinus*
	Neoseiulus anonymus	*Typhlodromalus tenuiscutus*
	Neoseiulus bellotti	*Typhlodromalus villacarmelensis*
	Neoseiulus californicus	*Typhlodromips dentilis*
	Neoseiulus idaeus	*Typhlodromips gonzalezi*
	Neoseiulus neoaurescens	*Typhlodromips mangleae*
Phytoseiinae	*Phytoseius averrhoae*	
	Phytoseius purseglovei	
Typhlodrominae	*Galendromimus alveolaris*	
	Galendromus (Galendromus) annectens	
	Galendromus (Galendromus) helveolus	
	Galendromus (Galendromus) longipilus	
	Galendromus (Galendromus) pilosus	
	Metaseiulus (Metaseiulus) neoflumenis	
	Typhlodromina subtropica	
	Typhlodromina tropica	
	Typhlodromus (Antoseius) transvaalensis	

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Contributors: E.L. Melo, G. de Moraes, A.C. Bellotti.

Activity 7. Phytophagous mite identification, cassava and other crops.

For more than 25 years CIAT has collected and maintained a collection of phytophagous mites. This collection is updated with new additions every year and information stored in a database is available to collaborators around the globe (i.e. recent request from The Colombia Federation of Rice Growers (FEDEARROZ), the Instituto Colombiano Agropecuario (ICA), Instituto Nacional de Investigaciones Agropecuarias (INIA), Venezuela, El Instituto de Investigación Agropecuaria de Panamá (IDIAP) and Exportadores de Bananos del Ecuador (DOLE).

By constantly adding to the collection and database, through our surveys and travels to other countries and regions, we are able to map more accurately the distribution of these pests and their natural enemies. This is valuable information for IPM, Biological Control or Germplasm Development Programs. During 2002, phytophagous mites were collected from cassava, rice, uchuva, sorghum, banana and cratylia from Colombia, Panamá, Ecuador and Venezuela **(Table 1)**.

Table 1. Phytophagous mite species collected from cassava and other host during 2001-2002 and added to CIAT collection.

Sample	Country	Dept.	Site	Host	Species
2567	Colombia	Atlántico	Polonuevo	Cassava	*Tetranychus tumidus* *Oligonychus gossypi**
2568	Colombia	Atlántico	Pitalito	Cassava	*Oligonychus gossypi** *O. peruvianus**
2569	Colombia	Atlántico	Baranoa	Cassava	*Mononychellus tanajoa***** *M. caribbeanae**
2574	Colombia	Valle	Palmira, CIAT	Rice	*Schizotetranychus paezi*
2576	Colombia	Cundinamarca	Granada	Uchuva	*Eriophyidae's mites* *Tarsonemidae's mites*
2577	Colombia	Casanare	Nunchia	Rice	*S. paezi*
2580	Colombia	Tolima	CORPOICA, Nataima	Cassava	*M. tanajoa* *M. mcgregori*
2581	Colombia	Valle	Palmaseca	Cassava	*T. urticae*
2583	Venezuela	Anzoategui	Frailes	Cassava	*M. caribbeanae*
2584	Venezuela	Anzoategui	Pariaguan	Cassava	*M. tanajoa* *M. caribbeanae*
2585	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2586	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2587	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2588	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2589	Venezuela	Anzoategui	El Tigre, INIA	Cratylia	*M. planki*
2590	Venezuela	Anzoategui	El Tigre, INIA	Sorghum	*O. grypus*
2591	Venezuela	Cogedes	Tinoco	Cassava	*M. tanajoa*
2592	Colombia	Valle	Palmaseca	Cassava	*M. tanajoa***
2593	Ecuador	El oro	Pasaje	Banana	*Tetranychys sp.*
2594	Panamá	Cocle	Anton	Cassava	*M. caribbeanae***
2595	Panamá	Herrera	La Asunción	Cassava	*M. tanajoa*
2596	Panamá	Chiriqui	Siogui abajo	Cassava	Calacarus guerreroi
2597	Colombia	Cauca	Quilichao, CIAT	Cassava	*M. tanajoa* *M. mcgregori*
2598	Colombia	Valle	Palmira, CIAT	Banana	*O. yothersi*

* *Neozygites* pathogen infesting tetranychid mites.
** High incidence of *Neozygites fungus.*

Contributors: J.M. Guerrero, A.C. Bellotti.

Activity 8. Development and formulation of biological pesticides for integrated pest management in cassava.

In recent years the cultivated cassava area in Colombia has increased from 184,472 hectares in 1996 to 211,618 ha in 1999. This data indicates that cassava production and its products, especially in starch and animal feed are playing an increasingly important role in the country. As production increases there is a tendency towards an increase in plantation size in some regions of the country. This has subsequently led to increased problems with certain cassava pests, including the cassava hornworm (*Erinnyis ello*) and burrower bugs (*Cyrtomenus bergi*).

Cassava hornworm attacks can reduce yields by as much as 70% if repeated attacks occur. More than 30 natural enemies of this pest have been identified, including parasites, predators and entomopathogens. A baculovirus (granulosis virus) of *E. ello* has proven to be the most effective biocontrol agent (See 2001 PE-1 Annual Report). In general baculovirus application is relatively easy, economically viable and ecologically sustainable. Since the cassava hornworm is a migratory pests, the release of parasites or predators is very difficult to synchronize with population buildups and the use of chemical pesticides is both costly and ineffective, often leading to increased frequency of hornworm attacks. The use of the baculovirus has been successfully implemented in Brazil and Venezuela but no commercial product was available in Colombia. Severe hornworm attacks have occurred cyclically on the Colombia North Coast (Departments of Sucre, Córdoba, Bolívar and Magdalena) over the past several years, causing considerable loss in yield and root quality.

The cassava burrower bug (*C. bergi*), a multi host pest, continues to cause considerable damage to cassava roots in the central regions of Colombia (i.e. the coffee zone) as well as several other countries (i.e. Panamá, Costa Rica, Cuba; **see this Annual Report, Activity No. 10).** *C. bergi* is one of the most important soil pests of cassava as it directly attacks the edible root parenchyma. In the department of Quindío, it is estimated that about 1500 ha have been removed from cassava production due to *C. bergi* presence and damage. In addition, in recent years, *C. bergi* is reported causing severe damage to other crop species such as onion, peanut and coriander. Control of *C. bergi* in these crops requires considerable pesticide use, with at best, only marginal results and this is not only costly, but environmentally damaging and a danger to human health.

Research at CIAT to control *C. bergi*, in recent years has turned to the use of fungal entomopathogens and entomopathogenic nematodes. Research activities have also emphasized the need to obtain a more complete knowledge of pest biology, ecology and behavior. Several entomopathogenic fungi and nematodes have been identified and some have proven highly effective in laboratory and greenhouse studies. As in the case of the cassava hornworm, there is an immediate need to formulate and develop commercial biopesticides that will be made available to both small and large cassava producers.

To meet this need CIAT and BIOCARIBE S.A., a commercial biopesticide company, have developed a collaborative relationship with the objective to research, evaluate, formulate and develop entomopathogens for commercial purposes, to combat cassava pest problems. A partnership has also been formed with the "Universidad de Antioquia" and their "Grupo

Interdisciplinario de Estudios Moleculares" (GIEM), to identify, isolate, purify and formulate biopesticides, including botanical insecticides.

The present project aims to establish industrial production models for biological pesticides that will offer farmers (Colombia and other countries) easily manageable, efficient and economically attractive bio or botanical pesticides. Emphasis is initially given to cassava pests, such as the cassava hornworm and burrower bug. These tools, it is assumed, will serve as models for future products and control of pests of other crops.

Methodologies

I. The cassava hornworm *E. ello*.

The project to develop a biopesticide of the baculovirus to control *E. ello* was initiated in 2000. BIOCARIBE has the responsibility to develop a commercial product while CIAT does the laboratory and field testing. The project is well advanced; the baculovirus has been evaluated in the laboratory and field and a formulized product has been developed (**See PE-1 Annual Report 2000-2001**). The project is now positioned to enter into Phase II; Figure 1 presents a description of the procedures and activities involved.

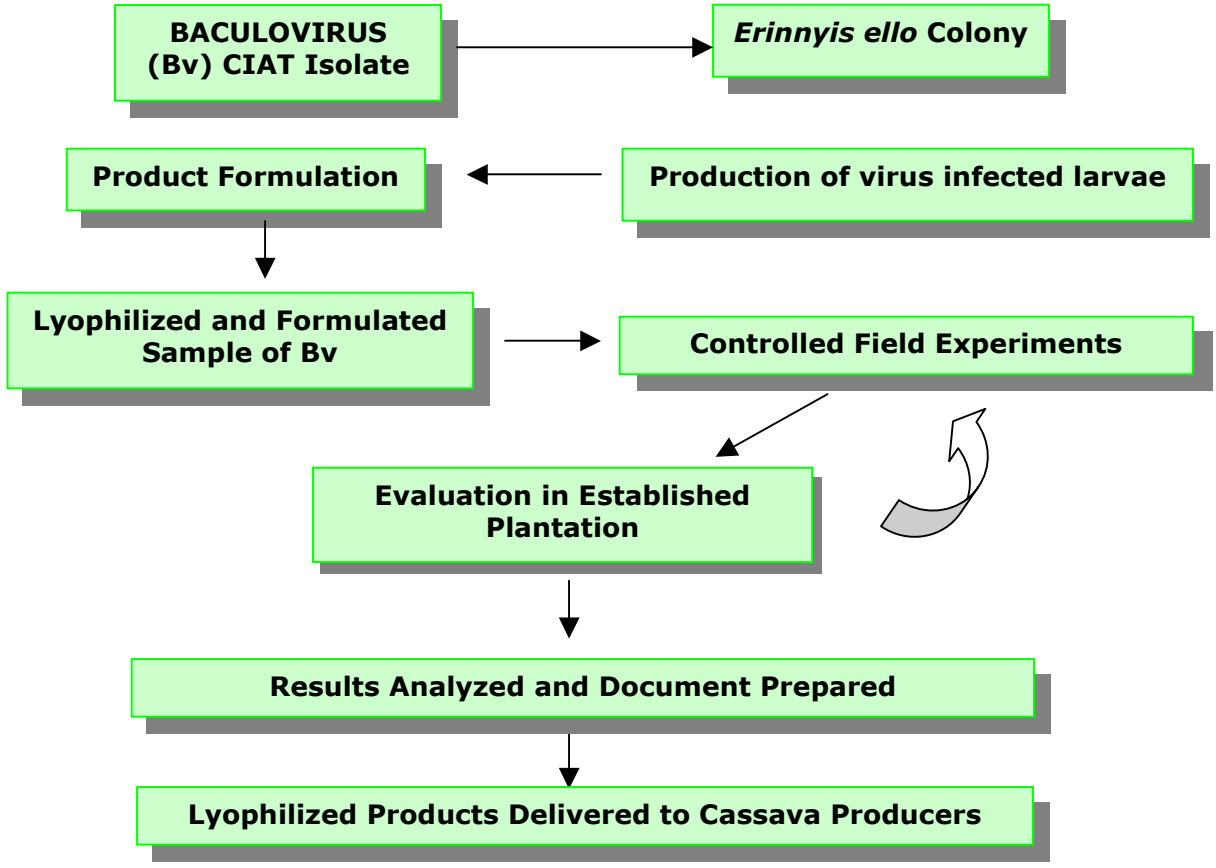

Figure 1. Procedures and activities involved in the commercialization of cassava hornworm baculovirus.

II. The cassava burrower bug *C. bergi*.

Laboratory colonies of *C. bergi* have been established, using maize seed as the food source. *C. bergi* was collected from several localities and colonies established at BIOCARIBE in Medellín, as well as at CIAT. Numerous fungal entomopathogens have been collected from the field populations of *C. bergi*, or have been obtained from other sources (CENICAFE and CIAT's ceparia). During Phase II of this project, numerous fungal entomopathogens were evaluated in the laboratory **(See PE-1 Annual Report 2001)**. Identification of the pathogens collected was done by the Cornell University in a joint collaborative project. It was decided during Phase I that isolates of *Metarhizium annisopliae* offered the best potential for control of *C. bergi*. It should be noted that this project also involves the search for fungal entomopathogens to control cassava whiteflies, they are parallel parts of the same project. Field studies with entomopathogens for *C. bergi* control were set up in Quindío, the Colombian coffee zone where *C. bergi* is an endemic pest. Studies with whiteflies using entomopathogens were set up in Valle del Cauca and Tolima. Once field evaluations are complete, this project will enter into the product formulation and development phase, which is the responsibility of BIOCARIBE. Figure 2 presents a description of the procedures and activities during Phase II of this project.

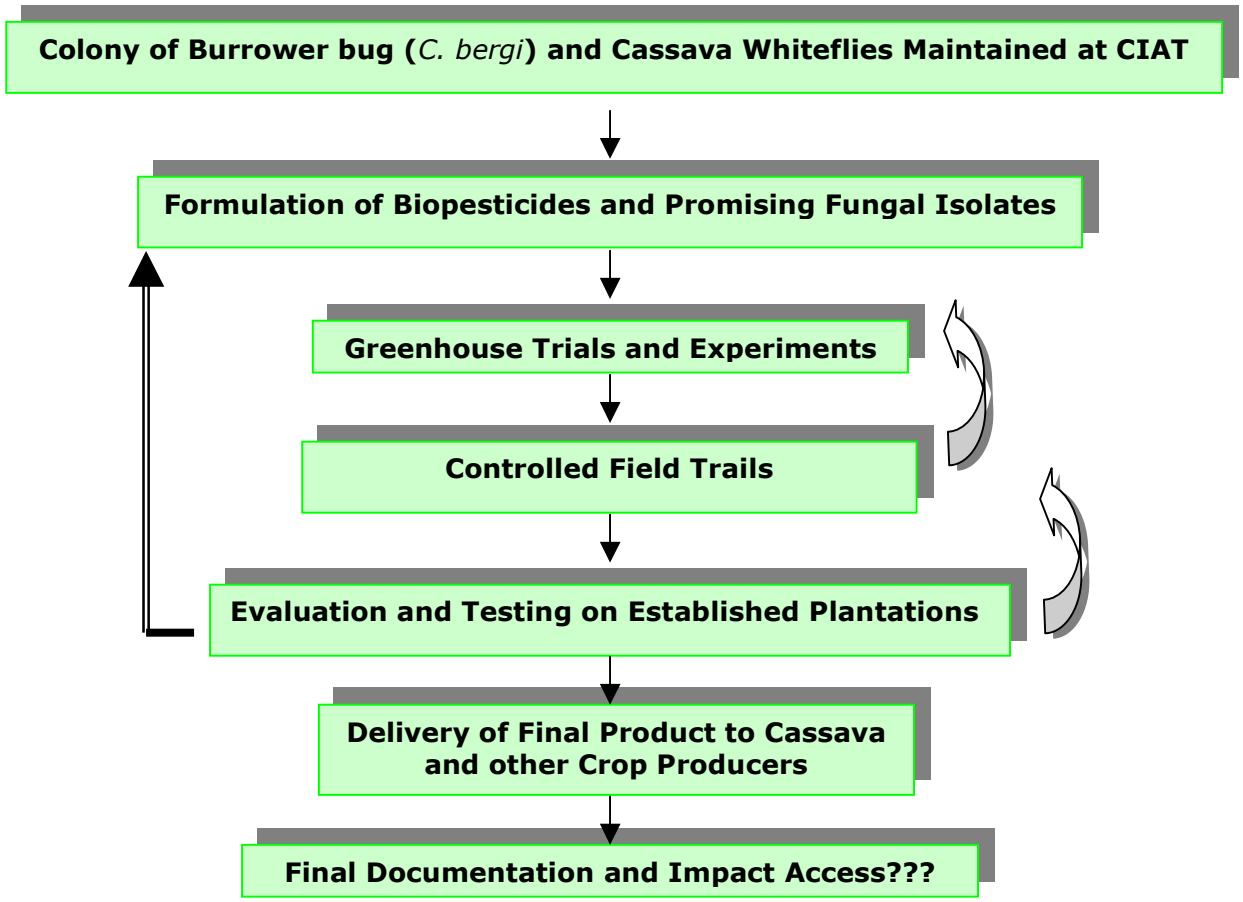

Figure 2. Procedures and activities involved in the commercialization of burrower bug (*Cyrtomenus bergi*) and cassava whitefly biopesticides.

Results

I. The cassava hornworm

A formulated biopesticide was developed by BIOCARIBE (PBv) and this was evaluated at three concentrations (0.003, 0.0045 and 0.006% BvL). These were evaluated on first to third instars of *E. ello* with very satisfactory results. The dry powder formulation resulted in larval mortalities above 90%, 72 hours after application, while the vegetable oil (neem) formulation resulted in mortalities above 80%. With both formulations mortality was initiated during the first 24 hours after applications **(Figure 3)**.

Figure 3. Mortality of third instar larvae of the cassava hornworm (*E. ello*) due to formulated baculovirus.

During the past years several regions of Colombia have been visited where hornworm attacks have occurred and workshops have been organized to familiarize cassava producers in the handling and applications of this biopesticide. In the Municipality of San Angel, Magdalena, more than 200 farmers of the local UMATA (Jan. 14-15, 2002) attended a workshop on the use of the Baculovirus as part of a cassava IPM program.

In Tolima a workshop was organized in the Municipality of San Luis, where 20 farmers of the local UMATA and the Department Secretary of Agriculture attended. During this workshop the formulated Baculovirus Product **(Figure 4)** was applied to a severe hornworm infestation that was occurring at the time on a 4-month cassava plantation. The Baculovirus was applied at 300 gr./ha with *E. ello* larvae mostly in the first three instars, approximately 20 larvae per plant density. The final evaluation, done with the farmer, resulted in a 91.3% mortality or control. The control fields where no application was made resulted in no larval mortality. These results confirm the effectiveness of the baculovirus.

Figure 4. Baculovirus formulated product for cassava hornworm control. Note credits to CIAT and MADR.

Workshops and conferences have been carried out at several additional localities including in Antioquia, Risaralda, Roldanillo, Cauca, Tolima, Sucre and Santander, as well as several conferences that were held at CIAT with groups visiting from different regions of the country.

BIOCARIBE has now been officially licensed by ICA (MADR) as the producer of Baculovirus and has initiated a marketing campaign and product distribution. As an example of the need and international appeal for this product, BIOCARIBE has been contracted by entities in Mexico, to supply the Baculovirus to control *E. ello* in that country.

II. The cassava burrower bug, *C. bergi*.

Numerous isolates of *M. anisopliae* ere evaluated in the laboratory for control of *C. bergi*; three were chosen as promising; CIAT 224, CIAT 230 and CIAT 240. These isolates were sent to Cornell University for positive identification and identified as *M. annisopliae*. These isolates were sent to BIOCARIBE for industrial multiplication and formulation **(Figure 5)**. Exploratory evaluations of two isolates were done in the laboratory at CIAT using CIAT 230 and CIAT 224. Mortality tests were performed with 8 concentrations of each isolate, in order to determine the optimal doses for highest mortality. Evaluations were done in unsterilized soil, with *C. bergi* feeding on maize. *C. bergi* instars were emerged in solutions of the different concentrations for each isolate. Isolate CIAT 230 was the most effective with mortality reaching 70%, 19 days after application **(Figure 6)**. Fungal parasitized insects were covered with black spots, with a green superficial sporulation **(Figure 7)**. After death, the *C. bergi* stages were placed on an agar plate to verify that the fungus present was *M. annisopliae*. Results were positive **(Figure 8)**. Additional experiments to verify these results and to further evaluate these fungal pathogens are presently underway.

Figure 5. Preformulated *M. annisopliae* in powder form of two isolates CIAT 230 and CIAT 224.

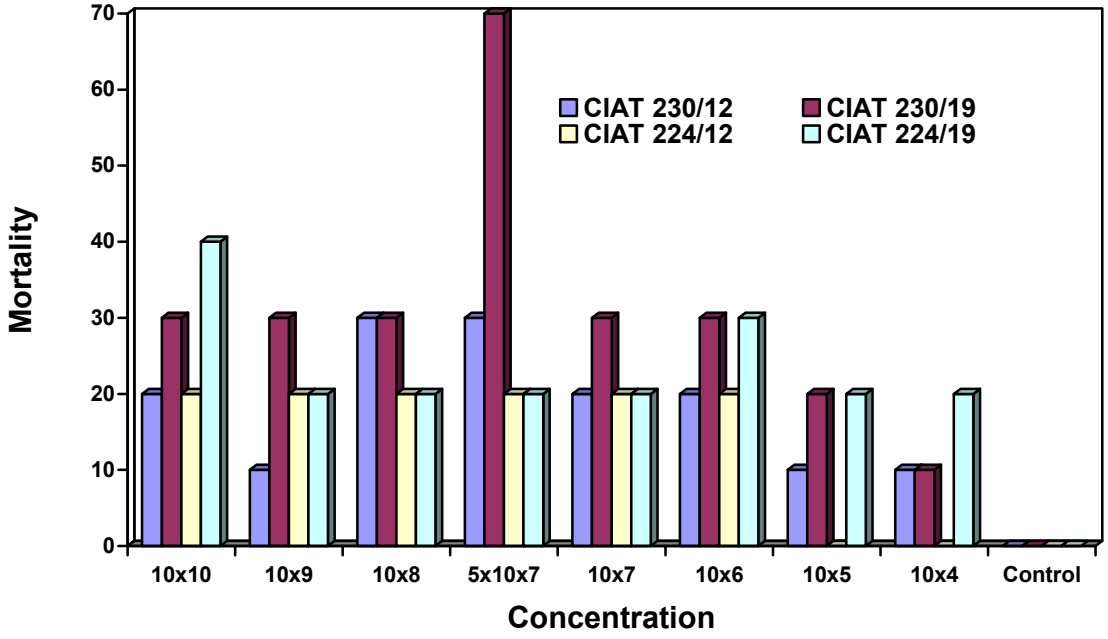

Figure 6. Mortality of *C. bergi,* the cassava borrower bug, caused by applications of *Metarhizium annisopliae* in the soil at different concentrations.

Figure 7. ***Cyrtomenus bergi*** **individuals parasitized by the fungal entomopathogen** ***M. annisopliae*** **showing the initial and final phases of green fungal growth.**

Figure 8. ***M. annisopliae*** **isolated from parasitized** ***C. bergi*****.**

Evaluation of these products in commercial crops has already been initiated. In the coffee growing regions (Pereira), *C. bergi* is a major pest problem in commercial onion fields. Damage is so severe that pesticides are applied every 20 days to control *C. bergi*. If no control is executed, farmers will lose 80 to 100% of their crop. It is hopped that any technology developed to control *C. bergi* will also be transferable to other crops such as corianda, beans and groundnut, and that the fungal entomopathogen products will be able to replace the chemical pesticides being presently used.

During the month of June 2002, collaboration with ICA/Sucre (Sincelejo) was initiated (Dr. Alvaro Mestra) to set up a series of field trials with cassava farmers in the region, where severe attacks of *C. bergi* are occurring. The isolates of *M. annisopliae* CIAT 224 and CIAT 230 at different concentrations are being evaluated **(Figure 9)**.

Julio 25/2002.

CIAT e ICA aúnan esfuerzos:

Control biológico al Chinche de la yuca

Por MARIA JOSÉ CAMPOS
El Universal

SINCELEJO

El Centro Internacional de Agricultura Tropical, CIAT, y el Instituto Colombiano Agropecuario, ICA, iniciaron ayer la siembra de un proyecto piloto en Colombia, para el control biológico del Chinche de la yuca de la raíz de la yuca.

El programa piloto se implementará en la vereda El Palmar de Tolú, con la asistencia y apoyo del Comité de Investigaciones Agrícola Local, constituido con pequeños agricultores de aquella localidad e impulsado por el ICA, informó Alvaro Mestra, coordinador de Sanidad Vegetal del Instituto.

Para la puesta en marcha del proyecto, el Centro Internacional envió al investigador Carlos Julio Herrera que implementará en Tolú, el control botánico con la utilización de una leguminosa, que actuará como repelente y la siembra de dos cepas del hongo metarrizium.

Del programa se estableció que se aplicará en dos fases y en diferentes épocas del año, con el objeto de evaluar los resultados y aplicarlos en otras partes del Departamento y el País, en donde la plaga en mención ha hecho serios estragos.

El Centro Internacional está interesado en aplicar el control biológico en el campo, luego de los magníficos resultados obtenidos en laboratorio, explicó Mestra Guerra.

El piloto se aplicará hasta finales de la actual vigencia, para analizar detalladamente los resultados de la aplicación de

EL PROGRAMA PILOTO de control biológico que aplicará el CIAT y el ICA en Tolú, es el primero de este tipo que se implementará en Colombia contra el Chinche de la viruela de la raíz de la yuca.

campo.

La visita de representantes del CIAT, permitirá además evaluar el tratamiento experimental de las semillas con productos químicos.

De acuerdo con los datos suministrados por Sanidad Vegetal del ICA, durante la vigencia inmediatamente anterior, la plaga que ataca la viruela de la raíz de la yuca, dejó pérdidas en más del 60% de la siembra, por lo que los pequeños y medianos labriegos se han visto afectados notablemente al producir.

"Lo que sucede es que el Chinche se alimenta de pastos y maíz pero al no encontrar cultivos naturales acceso con la raíz de la yuca", aseveró el funcionario del ICA.

La plaga se ha extendido dejando graves consecuencias en más de 11 veredas de Tolúviejo, Tolú y San Onofre y en otros departamentos como en Córdoba, concretamente en Ciénaga de Oro, Cauca y el Llano.

Prevención contra el Picudo

Entre tanto, en la mañana de ayer en San Pedro, se reunieron los miembros del Consejo Asesor para el Manejo Integral de Plagas del Algodón.

Durante la actividad se dieron a conocer y se concertaron acciones para continuar con el proceso de disminución de las consecuencias dejadas por el Picudo el año pasado.

"En la cosecha de la vigencia anterior sólo se hicieron aplicaciones correspondientes al 8.5 en comparación con los años anteriores en los que las aplicaciones iban de 4.5 a 5", explicó David Antonio Bertel, director regional del ICA.

Por tanto, en la medida en que se disminuye la utilización de agroquímicos, se contamina menos el ambiente, se minimizan los riesgos para la salud y los costos de producción disminuyen.

La convocatoria en la que cuya presencia el ICA, los gremios, gerentes de cooperativas, agricultores y Umata, entre otros, permitió determinar las fechas de siembra, los procesos de participación de las autoridades sanitarias y las barreras climáticas.

Figure 9. Newspaper report (Diario El Universal), on the CIAT-ICA. Collaborative field studies to develop tools for control of the burrower bug, *C. bergi*.

III. Trials with extracts of *Crotalaria juncea* to control *C. bergi*.

Research done by CIAT Cassava IPM project during the 1980's showed that intercropping cassava with sunne-hemp, *C. juncea*, would reduce *C. bergi* damage from 67% down to 3-4%. However this intercropping scheme, also reduced cassava yields by 25 to 35% and consequently this technology was never fully accepted by cassava farmers, although occasionally some would still use the technique. *C. juncea* is also an excellent green manure and is used as an organic fertilizer and as a possible animal feed.

The relationship between *C. juncea* and *C. bergi* behavior is not well understood; however research on this plant family Fabacea, indicate the presence of several compounds, known as alkaloids, and these compounds have shown acute hepatic toxicity, fetus toxicity and several other muscular disorders or lowering of blood pressure.

The Interdisciplinary Group of Molecular Studies (GIEM) at the University of Antioquia in collaboration with CIAT have shown interest in evaluating *C. juncea* potential as a botanical pesticide. They have developed preliminary trials in the laboratory using *Drosophila melanogasters* as the model pest organism. They are testing crude extracts from Crotalaria that is being grown at CIAT on *D. melanogasters.*

Initial results indicate biological action is only manifested in *C. juncea* seed; other parts of the plant (i.e. leaves, stem, roots) did not give positive results with respect to insecticidal activity. However repellant action has been noted in extracts from other plant parts. Extracts do not show insecticidal action on *D. melanogasters* adults as no mortality resulted, however an effect was observed in the change from pupae to adult at the highest concentrations of crotalaria extract (above 1000 ppm) **(Table 1**). As can be observed with concentrations of 700 ppm less than 30% of the pupae developed to adult stage and at 7500 ppm, 100% of pupae failed to reach adult stage.

Table 1. Evaluations of the biocidal activity of seed extracts of Crotalaria *juncea* on Diosophila melanogasters.

Concentration	No. Pupae	No. Adults	% Inhibition (pupae-adult)
Control (0)	176	170	0.0
50	156	154	0.0
100	198	193	0.0
400	161	170	11.0
700	255	70	72.55
1000	17	7	58.80
5000	0	0	100
7500	51	0	100
10000	0	0	100

Due to these preliminary results, a bioassay was carried out at CIAT to determine the effects of Crotalaria extract on *C. bergi*. The artificial diet that was developed to rear *C. bergi* (See this Report and PE-1 2001 Annual Report) was utilized. First instars *C. bergi* were fed on the artificial diet containing different concentrations of the Crotalaria extracts. A control treatment consisted on only the artificial diet. Each experimental unit consisted of 10 *C. bergi* individuals of the same age in two replications. Observations every 5 days up to 20 days (4 observations).

Beginning with the first observation, 5 days after application *C. bergi* mortality occurred **(Table 2)** even at the lower concentrations. Highest mortality occurred at the higher concentrations reaching 100% at 20 days after application. With these preliminary results we were able to determine that the seeds of *C. bergi* contain extracts with insecticidal activity. These results also indicate the need to continue with this research so a joint project between CIAT, the University of Antioquia, and BIOCARIBE was submitted to MADR to continue into the next phase of this research.

Table 2. Evaluations of the biocide activity of *Crotalaria juncea* seed extracts on the cassava burrower bug *C. bergi* (1st instar).

Concentration (ppm)	Mortality			
	5 DAA*	10 DAA	15 DAA	20 DAA
10.000	32.4**	66.5	91.5	100
7.000	58.2	91.5	100	100
5.000	57.7	91.5	100	100
3.000	18.3	66.5	100	100
1.000	0	74.7	83	100
Control (0)	0	33	33	33

* DAA: Days after applied.
** % Mortality of 2 repetitions.

During these preliminary experiments we could also note that Crotalaria extracts caused *C. bergi* developmental and behavioral differences. *C. bergi* that fed on the diet containing extracts was much smaller in size or there were delays in molting to the next instar and mortality often occurred.

Contributors: C.J. Herrera, BIOCARIBE (Guillermo León Hernández), A.C. Bellotti.

Activity 9. Evaluation of the effect of cassava hornworm baculovirus on the effectiveness and behavior of *E. ello* egg parasites.

There is a large complex of natural enemies associated with the cassava hornworm (*Erinnyis ello*). Two microhymenopteran, *Trichogramma* sp and *Telenomus* sp are important egg parasites and arguably play a role in reducing hornworm populations. We have consistently recommended the use of a naturally occurring baculovirus of *E. ello* as an effective control and a commercial product has been formulated and is available to cassava producers (see previous activity).

During a natural hornworm outbreak at Santander de Quilichao during My-June 2002, concentrations of the baculovirus were applied to cassava fields on plants containing numerous recently oviposited *E. ello* eggs. An evaluation of egg parasitism was made by randomly collecting eggs from the field and placing them in a multicelled clear plastic grid **(Figure 1)** where egg eclosion could be easily observed. Evaluations of parasitized eggs were also made in the field. One evaluation was made prior to baculovirus application and a second on the day of application in the field. Two applications of the baculovirus were made (May 22 and 25) using a micronizer sprayer and 5cc of the baculovirus (30% base solution) per liter of water. Egg parasitism evaluations were then carried out on June 12 and July 24. No egg parasites were released into these fields.

Figure 1. Multicelled plastic grid to quantify *E. ello* egg parasitism.

Results: In the first evaluation (May 20) with the grid technique egg parasitism reached 45%; all the parasites were *Trichogramma* sp. During the second evaluation (May 22), also prior to the baculovirus application, egg parasitism had reached 72.4%, 40.6% due to *Trichogramma* sp and 31.8% due to *Telenomus* sp. The field evaluation on May 22, combined parasitism was 68.4%, 57.8% due to *Trichogramma* and 11% to *Telenomus* **(Table 1)**.

Table 1. Cassava hornworm egg parasitism caused by the micro hymenopteran *Trichogramma* sp and *Telenomus* sp before and after applications of the *E. ello* baculovirus, at Santander de Quilichao, Colombia (May to July 2002).

Date of Evaluation **	*Multicelled Plate			Field Evaluation		
	Parasitism Total %	Parasitism *Trichogramma* %	Parasitism *Telenomus* %	Parasitism Total %	Parasitism *Trichogramma* %	Parasitism *Telenomus* %
20-05-02	45.0	45.0	-	-	-	-
22-05-02	72.4	40.6	31.8	68.4	57.8	11.0
12-06-02	88.3	49.5	34.1	75.8	53.7	22.2
24-07-02	-	-	-	96.0	56.5	39.5

* Multicelled clear plastic container.

** Baculovirus applied on May 22 and 25, 2002.

The third and forth evaluations, both made after the application of the baculovirus, indicate an increase in egg parasitism. On June 12, approximately 3 weeks after the baculovirus application, egg parasitism, using the grid technique, reached 83.3% (49.5% *Trichogramma* and 34.1% *Telenomus* sp). The field evaluations were similar, 75.8% parasitism (53.7% *Trichogramma* sp and 22.2% *Telenomus* sp). The forth evaluation, two months after Bv application (July 24), involved only the field evaluation, and this resulted in very high egg parasitism, 96.0% (56.5% *Trichogramma* sp and 39.5% *Telenomus* sp).

These results indicate that a baculovirus application has no obvious effect on naturally occurring egg parasitism and can be used in an IPM program without detrimental effects to natural control. Very few hornworm larvae were observed in the field, indicating that the baculovirus may have also contributed to suppressing *E. ello* populations.

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Activity 10. Integrated management of the cassava burrower bug (*Cyrtomenus bergi*) in Panamá; a collaborative project.

Introduction

During the past several years the area in cassava production in Panamá has increased from 1393 ha. in 1994-95 to 2874 ha. in 1998-99 (MIDA, 1999). Much of this production is destined for the export market where product (root) quality is of highest importance. The cassava burrower bug causes direct damage to cassava roots, causing brown to black rotting lesions on the white edible root parenchyma. This considerably reduces the commercial value of the root, especially for the export market.

C. bergi was first detected in Panamá in 1983 in cassava plantations of the Asentamiento Campesino "Manitos Ocueñas" in Ocú, Herrera Providence. Yield losses of over 70% were reported (Aguilar et al., 1989). The geographic distribution of *C. bergi* includes reports from Cuba, Honduras, Costa Rica, Colombia, Trinidad, Venezuela, Brazil, Argentina and Panamá.

Damage to cassava roots can occur throughout the growing cycle of the crop, but usually it is not detected until harvest when the root is peeled and the brown to black lesions are easily visible. The pest is a generalist feeder with numerous hosts; its biology, ecology, behavior and damage have been described in detail (Riis 1991; Bellotti et al., 1999; Bellotti, 2002).

The increased demand for cassava in the fresh market, combined with opportunities in the international market, has promoted the need of evaluating and identifying new germplasm tolerant to *C. bergi* attack, but with culinary characteristics required in the fresh consumption market.

In addition the Panamá Institute for Agricultural Research (IDIAP), has undertaken the search for new alternatives in the management of this pest. Emphasis is given to biological and botanical control, the use of tolerant varieties, and the avoidance of pesticide use. IDIAP has requested CIAT' s participation in a collaborative project to evaluate and study pest behavior and possible control methods. Previous studies coordinated by IDIAP as part of a project with FIAFOR (Foundation for Agricultural and Forestry Research of Panamá) on the biology and behavior of *C. bergi* were organized by Ing. José Antonio Aguilar.

Methodologies

I. Germplasm evaluation.

In vitro germplasm from CIAT catalogued as tolerant to *C. bergi* attack was sent to Panamá and planted in *C. bergi* infested fields. A total of 12 varieties were evaluated, 9 from CIAT, 1 from Costa Rica and 2 local varieties **(Table 1).** The experimental design was randomized blocks with four repetitions; each experimental block was 7 x 7 plants. Cassava stakes were treated with an insecticide and fungicide and planted at 1m x 1m, weeds were controlled and a 15-30-8 fertilizer applied.

Table 1. *Cyrtomenus bergi* tolerant and susceptible cassava germplasm evaluated at La Asunción, Panamá.

Cassava Varieties	Origin
MCol 1 185	CIAT*
MCol 707A	CIAT*
MCol 707B	CIAT*
MCub 8	CIAT*
MCub 51	CIAT*
MPer 597	CIAT*
MBra 675	CIAT*
MCub 32	CIAT*
MCol 1389	CIAT*
Brasileña	Local**
Valencia	Costa Rica**
Colombiana	Colombiana**

* Germplasm identified as tolerant to *C. bergi*.
** Susceptible germplasm.

II. Population dynamics.

Population dynamics of *C. bergi* were studied on the local variety, Brasileña. Periodic random samples are made in each plot beginning at 3 months of plant age and continued each month until harvest (3 plants from each plot were sampled). The percentage infestation in the field was estimated using the following formula:

$$\% \text{ Infestation} = \frac{\text{Number of plants infested}}{\text{Total number of plants}} \times 100$$

Estimates of level of damage of cassava roots were done by randomly sampling three plants, roots were peeled and a damage scale was employed to measure the number of root lesions **(Table 2)**. To determine *C. bergi* field populations, a 30 cm deep by 40 cm diameter soil sample was excavated and examined for *C. bergi* presence.

Table 2. *C. bergi* root damage evaluation scale associated with numbers of root lesions.

Damage Level	% Lesions
0	**No spots**
1	0-6
2	7-12
3	13-25
4	26-50
5	51-75
6	76-100

III. Associated crops.

An experiment was designed to determine the value of associated or intercropping of cassava with several legumes **(Table 3)**. The cassava variety was Brasileña and planting density was 10,000 plants/ha; *Crotalaria juncea*, planted at 40 kg/ha; *Mucuna* was planted on cassava hills 1 m apart and 40 cm between plants; *Vigna unguiculata* at two rows 0.4 m separated; Frijolillo 25 kg/ha. in the center of the cassava row and 1 m between rows of frijolillo; *Arachis pintoi* was

sown vegetatively at 15 to 20 cm between cassava rows separated 1m between rows and 0.4 m between plants.

Table 3. Cropping systems of cassava and legumes evaluate for control of *C. bergi*.

Production System
Cassava Monoculture
Cassava + *Crotalaria juncea*
Cassava + *Mucuna*
Cassava + Bean *Vigna unguiculata*
Cassava + Frijolillo Cleome
Cassava + Forage Peanut *Arachis pintoi*

Results

I. Germplasm.

All cassava varieties evaluated were shown to be susceptible to *C. bergi*. The number of plants damaged of each variety ranged from 32.8% of Valencia to 78.5% of Brasileña. More than 60% of all the CIAT varieties were damaged. Damage intensity expressed as damage rating was between 4 and 6 (on a 0 t 6 scale), indicating from 26 to 100 lesions per root **(Table 4)**. These results indicate that none of the varieties are resistant nor tolerant to *C. bergi* attack, and runs counter to results previously obtained in Manizales, Colombia. This could be attributed to a higher pest pressure in these trials.

Table 4. Evaluation of tolerant and susceptible cassava germplasm for *C. bergi* damage.

Germplasm	(Kg/25)	Yield	% Damage	Damage Intensity
Colombiana	54	21.6	61.8	5
MBra 675	53.8	21.5	67.8	5
Brasileña	47.9	19.1	78.5	6
MCol 707 A	42	16.8	66.4	5
MCol 707 B	39.1	15.6	73	5
MCol 1185	37.3	14.9	71.2	5
MPer 597	34.5	13.8	78.3	6
MCub 32	31.5	12.6	61.5	5
MCub 8	27.9	11.1	71.7	5
MCub 51	26.7	10.7	70.7	5
Valencia	22.5	9	32.8	4
MCol 1389	21.7	8.7	38.1	4

II. Population dynamics.

Monthly evaluations measured *C. bergi* presence and estimated damage levels. A progressive increase in the % infestation and % of root area damaged was observed over time. Beginning at 3 months of crop development, a 33.3 % infestation level and 3.3 % root surface damage was observed. During the 4th and 5th months, infestation levels increased to 66.6% and 100 % of plants infested at 6 months **(Table 5)**. The percent of area of the root parenchyma damage increased from 5.5, 12, 73.3% at 4, 5 and 6 months respectively **(Figure 1)**.

Table 5. Variables evaluated in studies on the population dynamics and damage caused by *C. bergi* to cassava roots.

Locality	Variables of Study	Monthly Evaluations								
		1*	2*	3*	4*	5*	6*	7	Total	Average
	Plant height (m)	0.6	1.2	1.5	1.9	2.1	2.1		9.73	1.6
	Root length (cm)	31.3	43	48.7	49	66	37.6		275.8	45.9
	No. Total roots	3.3	9	9.3	8	10	10		49.6	8.2
	No. Infected roots	0.3	0.6	2.6	6	7.6	8		25.3	4.2
	% Infestations									
	Total plants	33.3	66.6	66.6	100	100	100		466.6	77.7
	Damage intensity (Esc. CIAT)	1	1	2	5	4	5		18	4
	% Root area infected	3.3	5.5	12	73.3	50	69		213.1	35.5
LA ASUNCIÓN	No. of total *C. bergi*	0	1	2.6	7.6	1.6	4.6		17.6	2.9
(Sr. Juan Campos)	No. Females	0	1	1.6	5.6	1.3	1		10.6	1.7
	No. Males	0	0	0.6	0.3	0.3	0		1.3	0.2
	No. Nymphs	0	0	0.3	1.6	0	3.6		5.6	0.9
	No. Dead *C. bergi*	0	0	0	0.3	0	0		0.3	0.06
	No. Adults	0	0	0	0.3	0	0		0.3	0.06
	No. Nymphs	0	0	0	0	0	0		0	0
	Plant age/attack (months)	0.8	2	3	6	2	4		17.8	2.9
	Sample depth (cm)	30	30	30	30	30	30			30
	Plantation age (months)	3	4	5	6	7	8			

* The values presented correspond to an average of 3 evaluations.

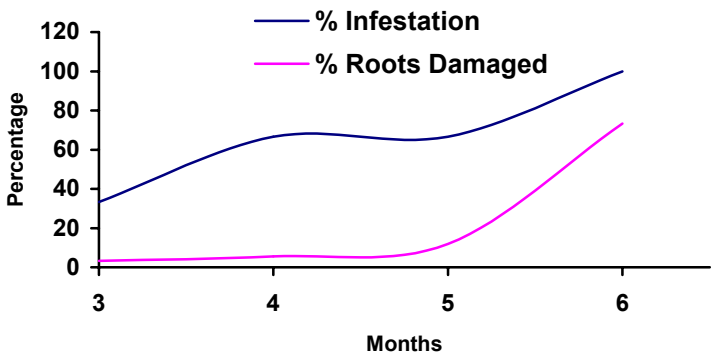

Figure 1. Percent root damage and infestation by *C. bergi*.

III. Associated crops.

The lowest infestation level was observed in cassava intercropped with *Mucuna* with 35% of the plants infested and only 7.9% of the root parenchyma damaged. However this intercrop system also resulted in the lowest cassava root yields. This was probably due to the competition between the crops; both were planted at the same time. The highest infestation level was the cassava-Cleome intercropping system, the cassava-Arachis and cassava monoculture with infestation values of 8, 65 and 65% respectively. These same systems resulted in damage intensity levels of 26.5, 24.6 and 15.6 % respectively **(Table 6).**

Table 6. Evaluation of *C. bergi* damage and infestation in relation to different legume intercroppings.

Production System	Root Yield	Total Root Yield	% Infestation	% Root Damage	Damage Intensity
Cassava Monoculture	37.3	14.9	65	15.6	3
Cassava + Crotalaria	27.3	10.9	50	20.7	3
Cassava + *Mucuna*	13.5	5.4	35	7.9	2
Cassava + Bean (Vigna)	35.8	14.3	60	18	3
Cassava + Cleome	28.4	11.3	80	24	3
Cassava + A. pintoi	31.5	12.6	65	26.5	4

The intercropping with crotalaria, which has given very good results in Colombia, had a 50% infestation level, but a 20.7% root damage.

Future Activities

- Evaluation of biological pesticides in field plantings.
- Periodic trips to Panamá by CIAT scientists (two visits have already been made).
- The continued installing of experiments on behavior and management of *C. bergi*.
- Training of personnel in Panamá and at CIAT in biological control.

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Activity 11. Influence of HCN on the burrowing bug *Cyrtomenus bergi* (Cydnidae, Hemiptera) in artificial diets.

HCN toxicity to the burrowing bug *Cyrtomenus bergi* (Cydnidae, Hemiptera)

Introduction

The burrowing bug, *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae), is considered to be one of the most important soil borne pests, especially in Colombia, Panama and Costa Rica. It causes severe damages to cassava roots, as well as roots of other cultivated crops such as onions, coffee, sugarcane, potatoes, peanuts, maize (Bellotti et al., 1999). The nymphs and adults of this insect cause damage to the fleshy roots of cassava by inserting their strong stylet to feed. The wounds left in the roots by the stylet are good opportunities for soil-borne pathogens to invade which therefore makes the roots commercially unacceptable. Controlling *C. bergi* is difficult because of its polyphagous nature and its adaptation to the soil environment. Pesticide applications are costly, environmentally hazardous, and not always effective (Bellotti et al., 1999).

A recent screening of cassava germplasm indicates that HPR (Host Plant Resistance) may be also available. Cassava is characterized by the presence of cyanide compounds in leaves, stems and tubers (Arihantana & Buckle, 1986; Ezeala & Okoro, 1986; Pancoro & Hughes, 1992) mainly linamarin (Butler et al., 1965), a cyanogenic glucoside which is hydrolyzed into glucose and free HCN after tissue damage (Conn, 1980). This hydrolysis, called cyanogenesis, occurs also after insertion of the strong burrower bug stylet into the roots. Furthermore, laboratory studies have shown that adults and nymphs of *C. bergi* that fed on a high-HCN clone had increased mortality (Bellotti & Riis, 1994). Nevertheless, further research is required as results are not conclusive, because attempting to test HCN as toxic factor to *C. bergi* in artificial conditions have failed, due to the fact that an artificial diet was not available for this insect species.

Since the 1950's, the use of artificial diets has provided a better knowledge of the nutritional requirements of various insect species (Singh, 1977; Cohen, 2001). In plant resistance studies, these artificial diets have been very widely used to bioassay the activity of allelochemicals against various insect pests and have played a particularly important role in the bioassay of individual factors involved as resistance mechanisms. Therefore, when studying plant resistance, artificial diets of known chemical composition (holidic diet) have been shown to be indispensable tool.

The purpose of our study was to develop a bioassay using a holidic liquid diet for testing HCN contents on newly hatched larvae and adults of *C. bergi* survivals.

Material and Methods

Insects. A stock culture of burrowing bug, from a population of Pereira (Colombia), was maintained in the laboratory on germinated seeds of maize at 25°C, 80% r.h. and L12:D12 photoperiod. Egg masses from the soil were collected and incubated at 25°C until eclosion. The newly hatched larvae and adults from the mass rearing were used for the experiments.

Plants. Maize seeds (cv Icacikuani 110) were treated with chloride hypochloride (3%) and then placed in a plastic box under water-humidified filter paper during 3 days at 25°C for germination. Germinated seeds were used for insects rearing.

Artificial diets. The standard medium used, was diet derived from Ap3 (Calatayud, 2000), except for sucrose and cholesteryl benzoate, which were adjusted to 6 g/100mL and 5 mg/100mL respectively. For testing HCN influence on insect survivals, several HCN concentrations from 500 to 10,000 ppm were used. For all diets, pH was adjusted to 7.0 with potassium hydroxide, and the medium was then filter-sterilized (0.22µm Millipore units). Pieces of foam rubber (1cm x 1cm) were soaked into the liquid diet and enclosed in sterile Parafilm sachets, constituting artificial seeds. Then, artificial seeds were placed individually upon about 10 cm3 of sterile soil into a plastic cup (height, 4.5 cm; diameter, 4 cm) closed with corresponding lids, constituting the rearing unit.

Rearing experiments. Germinated seeds of maize, used as a control, and artificial seeds mentioned above containing modified-Ap3 diet were tested. Natural or artificial seeds were placed individually in the aforementioned rearing unit. One insect was reared in each rearing unit. For each rearing treatment 50 neonates were tested. Biological performances were estimated by recording mortality during the recorded developmental time, by measuring the length of adults and by weighing them.

Toxicity tests. KCN, as HCN source in the diet, was tested for toxicity following the protocol used for an aphid species by Rahbé & Febvay (1993). Based on the aforementioned control diet, various concentrations of HCN from 500 to 10,000 ppm or mg L-1 were tested. Neonate larvae and adults were previously reared during 5 days on artificial seeds containing the control liquid diet for rearing adaptation. One insect was deposited in each rearing unit. For each developmental stage, three groups of 10 insects were deposited at day 0 with artificial seeds containing diets at various HCN concentrations from 0 to 1,500 ppm for neonates and 0 to 10,000 ppm for adults; and mortality at day 4 (sufficient time to detect toxicity; Cortés M.L., personal observations) was recorded.

All experiments were conducted at 25°C, 80% r.h. and L12: D12 photoperiod.

Data analysis. Chi-square test was used to compare the difference in survivorship to adulthood between insects reared on maize seeds than those reared on modified-Ap3 diet. Mann Whitney's *U*-test was used to compare the difference in biological performances (length, weight of the adults and developmental time). These statistics were completed using the Statview software (Abacus concept, USA).

Each HCN concentration yields three mortality percentages (day 4), allowing to calculate toxicity indices. The Log(C) and probit (%) transformations were used to calculate either LC50 or LC20, together with their respective confidence intervals at 5% level (Bliss, 1935). These calculation and statistical analyses of these indices were completed using a MacIntosh program from Febvay & Rahbé (1991).

Results and Discussion

Rearing experiments. With this diet and rearing technique, we reared *C. bergi* from newly hatched larvae to adults **(Table 1)**. The duration of the larval period ranged from 88 to 135 days, with an average of 101 days. This period was much longer than observed on germinated seeds in similar conditions (72 days). Although the biological performances (adult length and weight) on the artificial diet were significantly reduced, they were not as much reduced when compared with that obtained on maize (see **Table 1**). Survivorship to adulthood on modified-Ap3 diet was lower (18%) than on natural seeds (72%). This reduction in survival of diet-reared insects appeared independent of diet performance *per se* and was mainly induced by some drastic problems of fungal contamination of the diet (M. L. Cortés, personal observations). Although the modified-Ap3 diet and rearing unit were changed biweekly to limit fungal contaminations, higher fungal contamination inducing mortalities of the insects occurred on this kind of artificial diets as compared to maize seeds.

Table 1. Biological performances of *C. bergi* on different diets. Adult lengths and weights were measured at day corresponding to developmental time on each diet.

Diet	% Adults (n)	Length (mm, mean[1] ± SE)	Weight (mg, mean[1] ± SE)	Developmental Time (days, mean[1] ± SE)
Maize	72 (36) b	9.0 ± 0.1 b	59.0 ± 1.3 b	72.4 ± 1.6 a
Ap3	18 (9) a	8.1 ± 0.2 a	45.4 ± 2.4 a	100.7 ± 6.3 b

[1] Within a column, means followed by different letters are significantly different ($P < 0.05$; Chi-square test for % adults comparison and Mann Whitney's *U*-test for length, weight and developmental time comparisons).

In addition to their reduced developmental performance, diet-reared insects were not able to oviposit viable eggs. All these results indicated clearly that this technique and diet used is not suitable to rear *C. bergi* artificially, but they are useful to test potentially active molecules acting as toxic to the burrower bug. In fact, it is possible to perform acute toxicity tests during periods shorter than those used here, and therefore to obtain reliable mortality data on modified Ap3 (90% survival on the first 40 days of development in the present test).

Toxicity assays. Mortalities were recorded for HCN concentrations ranged from 800 ppm to 1,500 ppm for newly hatched larvae, and from 2,000 to 10,000 ppm for adults **(Table 2)**. This result indicated clearly that newly hatched larvae are more sensitive to HCN in the diet than adults probably because the adults developed a more efficient detoxification system. Therefore, the both corrected LC20 and LC50 on day 4 are higher for adults, amounting to 2,764 and 4,781 ppm respectively, as compared to the values registered for neonates (1,016 and 1,101 ppm respectively). At the end of the tests, each HCN diet was analyzed to verify if the total HCN content input was not reduced by volatile characteristic of this compound in solution (L. Riis, personal observations). In all cases, the total HCN content was unchanged (data not shown) and confirmed the validity of the LC20 and LC50 values. The concentrations reported for high HCN cassava varieties are ranged between 272 and 1,066 ppm (dry weight) of cassava root parenchyma (Wheatley et al., 1992; Riis et al., 1995). Therefore, some of them containing about 1,000 ppm of HCN in root parenchyma are toxic to *C. bergi* newly hatched larvae according to the both LC20 and LC50 values **(Table 2)**. In contrast, most of the high-HCN cassava varieties are not toxic to *C. bergi* adults (see both LC20 and LC50 values in Table 2). Nevertheless, it was registered higher-HCN cassava clones

containing 4,200 ppm of root parenchyma (T. Sánchez, personal communication) which should be toxic to *C. bergi* adults.

Table 2. Biological data (mortality in % [means ± SE], LC_{20} and LC_{50}) for HCN tests performed at two burrowing bug developmental stages.

HCN Concentration (ppm)	Mortality (% Without Correction)	
	Neonates	Adults
0	0	0
500	0	0
800	0	0
1,000	16.7 ± 3.3	0
1,100	33.3 ± 3.3	0
1,200	76.7 ± 3.3	0
1,250	93.3 ± 3.3	0
1,500	100	0
2,000	100	13.3 ± 3.3
5,000	100	30.0 ± 5.8
6,000	100	55.0 ± 5.0
8,000	100	86.7 ± 3.3
10,000	100	100
LC_{20} [confidence interval, p=0.05] (ppm)	1,016 [892 – 1,157]	2,764 [2,056 – 3,714]
LC_{50} [confidence interval, p=0.05] (ppm)	1,101 [1,026 – 1,183]	4,781 [3,959 – 5,774]

In conclusion, although the artificial diet and rearing unit used are not suitable to rear *C. bergi* artificially, they are useful to test active molecules such as HCN for toxicity. In this context, it is the first time in our knowledge that a holidic liquid medium was used to test active molecules on a Cydnidae species. It was clearly demonstrated that HCN level in the diet amounting 1,000 ppm is toxic to *C. bergi* newly hatched larvae, confirming that high-HCN cassava varieties are toxic to *C. bergi* as evoqued by Bellotti & Riis (1994) and Riis et al. (1995).

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Activity 12. Identification of toxic protein to *Phenacoccus herreni.*

Toxic proteins in plants are considered to have the potential to function as chemical defensive factors against attacking insect. These proteins should be considered as important factors in plant-insect interactions when developing host plant resistance programs. In fact, such proteins representing direct gene products could most directly lend themselves to genetic engineering manipulations for crop improvement.

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

Leaves of *Jatropha gossipiifolia* (Euphorbiaceae) showed a strong toxicity to *P. herreni* (CIAT, Annual Report, 1999). In fact, after 24 hours of infestation on this plant resulted in 95% mortality and after 48 hours, 100% mortality of *P. herreni*. Toxicity was evidenced only in extracts of young leaves of *J. gossipiifolia*. Furthermore, when this extract was boiled the toxicity disappeared suggesting that protein(s) are involved (CIAT, Annual Report, 2001).

Therefore, the purpose of this activity was to purify the protein for identification. The first step of this purification was to precipitate the toxic protein by the common salting out technique using increasing concentrations of ammonium sulfate under low temperature conditions (Deutscher, 1990; Harrison, 1993; Jakoby, 1971; Janson & Lars, 1989). This technique consist of separating by precipitation, relating to their size and molecular weight the toxic protein, to the rest of protein pool not involved in the toxicity in the crude extract. Five distinct fractions were obtained: 0 – 20%, 20-40%, 40 – 60%, 60 – 80% and 80 – 100%. Each fraction was dried and introduced into the standard medium derived from A0 of Febvay et al. (1988), except for sucrose, which was adjusted to 200 g/L, for toxicity evaluation. All diets were enclosed in sterile Parafilm sachets and stretched on the top of a standard film box (black; height, 5 cm; diameter, 3.2 cm), which constituted the rearing unit. Groups of 60-80 neonate larvae were placed directly in experimental rearing units. After 48 hours, higher mortality was recorded with the 60-80% fraction (**Figure 1**), suggesting by the high ammonium sulfate concentration able to precipitate the protein, that the toxic protein should have a low molecular weight. KCN was used as positive control for toxicity.

Additionally, the purification procedure will be improved by using a Bio-Rad Rotofor system based on the isolectric point separation. A preliminary use of this system was unsuccessful due to some loss of toxicity after purification indicating that probably the toxic protein should be an enzyme. The improvement of this technique to purify well the protein is in course.

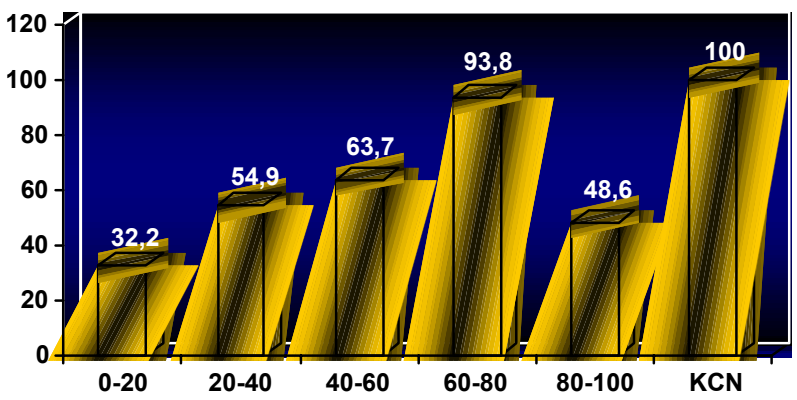

Figure 1. Neonate mortalities recorded on different diets after 48 h, containing extracts obtained by five distinct concentrations of ammonium sulfate: 0-20%, 20-40%, 40-60%, 60-80% and 80-100%, and containing KCN as positive toxicity control.

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Donors: French Ministry of Education, IRD, CIAT (logistic contribution).

Activity 13. Studies on the natural resistance of four wild *Manihot* species (Manihot spp) to three arthropod pests (*Mononychellus tanajoa, Aleurotrachelus socialis* and *Phenacoccus herreni*), under greenhouse conditions.

Cassava arthropod pests have been shown to significantly reduce root yield through both direct (the cassava root) and indirect (leaves and stems) feeding. Control of cassava pests relies mostly on a combination of host plant resistance (HPR) and biological control (BC). These two complementary systems can be low cost to the farmer and environmentally sound as well as effective in reducing damage in an integrated pest management (IPM) program. There is a large complex of arthropod pests attacking cassava, especially in the neotropics, the regions of origin of the cassava crop.

The objective of this research is to evaluate four species of wild Manihot as a potential source of resistance to three of the major pests of cassava, mites (*Mononychellus tanajoa*), whiteflies (*Aleurotrachelus socialis*) and mealybugs (*Phenacoccus herreni*). Mites, whiteflies and mealybugs cause significant yield losses in cassava in the Americas, Africa and Asia.

This research is divided into two parts; the first consists in the acquisition and establishment of vegetative materials of the *Manihot* species. Different methodologies are used, including rooting techniques, soil-sand mixtures, soil source (site or location). The second part consists of infesting the different wild species, as well as control genotypes with the aforementioned arthropods, and carrying out an evaluation of infestation levels (population dynamics, behavior and damage.

Introduction

Manihot spp (Euphorbiaceae) is a genus native to the neotropics with a wide range of habitats, that extend from the south of Arizona (USA) to Argentina (Rogers and Appan, 1973). Species within this group are perennials and vary from short-stemmed bushes to 10 to 12 meter high trees. The majority of the species have tuberous roots and some can accumulate large quantities of starch, as is the case with cassava (*Manihot esculenta*), an important tropical crop that is the major calorie source for more than 500 million persons (Allem, 1992; Best and Henry, 1992).

In spite of cassava's importance, certain aspects such as crop origin and its phytogenetic relationship with other species within the genus have not been well clarified. Several different sites have been proposed as the center of origin within the neotropics, with diverse evidence; the South of Mexico and Guatemala (Rogers, 1963; Renvoise, 1972), the Caribbean Coast of Colombia and Venezuela (Saur, 1952), and the Amazon Basin of Brazil (Decandalle, 1967; Nassar, 1978; Allem, 1987, 1994) are areas proposed to be the center of origin and domestication in accordance with linguistic, anthological, archaeological, taxonomic and geographic information.

From a morphological point of view, independent taxonomic studies indicate diverse wild taxa of the genus as most closely related to cassava, in general dividing between species native to North and Central America and species of South America origin. Rogers and Appan (1973) propose *M. aesculifolia*, the Central American species closest to cassava. Allem (1987) disagrees and proposes *M. tristis*, native of South America as the closest relative to cassava. He has also reported that in collecting trips in Brazil (1992, 1994), he has found two wild morphological variations of the

cultivated species; a glabrous *M. esculenta* subspecies *flabellifolia*, and a pubescent one, *M. esculenta* subspecies *peruviana*.

It has only been in recent years that wild *Manihot* species have been incorporated into characterization, conservation and use by phytogenetic resources. Although considerable variability and heterozygosity is assumed in the wild species, owing to their wide geographic distribution cross polinization and the presence of different characteristics that would be desirable in the crop, studies have not been done to confirm these attributes. At CIAT, the small collection of wild germplasm suffers from complete documentation and is poorly represented geographically. There does not exist, at present, a regular characterization and evaluation of wild germplasm (Roa, 1997).

Bertran (1993) proposes that *M. aesculifolia* is the species closest to cassava and that *M. carthaginensis*, a species distributed on the Caribbean Colombo-Venezuelan coast, is one of the closest parents to the cultivated species. It is clear that a sufficient genetic diversity or variation needs to be maintained of these wild species to insure future adaptation, and methods should be developed that permits estimating the diversity present in a species (Roa, 1997).

Multiplication of wild *Manihot* species.

The species selected for this phase of the project were *M. carthaginensis*, *M. esculenta* subsp. *flabellifolia*, *M. esculenta* subsp. p*eruviana*, and *M. tristis*. These were selected for various reasons, including their relationship to the cultivated species, their centers of origin or domestication in relation to cassava, similarity to cassava in morphological characteristics and in vivo or in vitro availability **(Table 1)**.

Table 1. Distribution and ecology of *Manihot* species included in the insect resistance study.

	M. brachyloba	***M. carthaginensis***	***M. esculenta* subsp. *flabellifolia***	***M. esculenta* subsp. *peruviana***	***M. tristis***
Distribution	Bolivia, Brazil, Colombia, Ecuador, Costa Rica, Guyana, Perú, Venezuela[1]	Colombia, Venezuela, Brazil, Dominican Republic, Trinidad y Tobago	Brazil, Venezuela, Surinam, Guyana[2]	Brazil, Peru[2]	Brazil, Venezuela, Surinam[1]
Ecology	Common in zones of secondary growth and under slight shade around river banks[1]	Xeric forests and growing in limestone, in open areas and costal zones[1]	Dry semi-deciduous forests (Campo Cerrado, Brazil) and disturbed Amazon forest[2]	Disturbed Amazonian Forests[2]	Grown on poor rock or granite soils[1]

[1] Rogers and Appan, 1973.
[2] Allem, 1994.

Numerous plantings of the above mentioned species were made in an attempt to multiply and maintain adequate number of plants within each species to evaluate against the target arthropod species. At least 12 attempts were made over the past 9 months to get good plant establishment. Stem cuttings were first sown in propagation chambers or in pots with mixtures of sand and soil

(3:1 proportion). Few cuttings germinated. In some cases fungicides and rooting hormones were added to the soil mixtures. Attempts were also made to root stem cuttings in liquid (water) solution, but this resulted in a high percentage of stem rotting causing plant mortality. Rotting and high plant mortality also occurred when plant shoots were placed in sand-soil mixtures or in distilled water. The addition of an antifungal, anti rotting agent (Banrot) did not prevent rotting and high mortality. The success rate for establishing a consistent supply of these five wild Manihot species was very low **(Table 2)**. Some success was achieved with *M. carthaginensis* and *M. tristis*, but the percent survival was low.

More recently, the last batch of *M. esculenta* subsp. *flabellifolia* and *M. peruviana* received from plantings in Santander and in-vitro multiplied materials have germinated and progressing well. 100% of these plants have survived **(Table 2)**.

In conclusion of this phase I, 44 genotypes of Manihot, including wild, domesticated and cultivated; 17 of these did not survive, 13 presented very low levels of survival (< 25%) and three genotypes between 25 and 50% and only 1 at 50 to 75%. In the latter plantings (Sept 14, 2002) 10 genotypes had survival rates between 75-100% but these were recently planted and their long term survival is not yet proven. Our experience up to this point indicates that very high humidity is detrimental to the establishment (rooting and survival) of these wild *Manihot* species.

Due to the difficulty in establishing sufficient wild species, it has not been feasible to establish colonies of mites, whiteflies and mealybugs on wild *Manihot*. There is no assurance that we will be able to accomplish this establishment in the near future. It is recommended that the pest species be established for 5 generations on wild *Manihot* before evaluating germplasm. Give the life cycle of each of the arthropod species this calculates to 2 months for mites, four months for mealybugs and nearly 6 months for whiteflies. The lack of establishment of the wild Manihot species has deterred initiating the second part of this project. Hopefully these problems will be overcome in the near future.

Table 2. Plant survival of *Manihot* species evaluated in insect resistance study.

Species	Genotype	No. Plants Sown	No. Plants Survived	% Survival
Manihot carthaginensis	30-1	30	1	3.33
Manihot carthaginensis	30-4	30	0	0
Manihot carthaginensis	30-5	30	1	3.33
Manihot carthaginensis	31-1	30	2	6.66
Manihot carthaginensis	37-8	48	3	6.25
Manihot carthaginensis	160-5	220	2	0.90
Manihot carthaginensis	160-4	160	16	10
Manihot flabellifolia	180-2	21	0	0
Manihot flabellifolia	213-7	355	0	0
Manihot flabellifolia	225-2	105	0	0
Manihot flabellifolia	230-2	110	4	3.63
Manihot peruviana	240-3	106	5	4.71
Manihot peruviana	241-3	161	0	0
Manihot peruviana	248-1	85	0	0
Manihot peruviana	254-1	42	0	0
Manihot peruviana	266-4	25	0	0
Manihot peruviana	269-1	13	0	0
Manihot tristis	130-3	365	1	0.27
Manihot tristis	132-36	326	27	8.28
Manihot tristis	144-2	30	0	0
Flores	Domesticated	30	17	56.66
Santa Catalina	Domesticated	27	13	48.14
Ibacaba	Domesticated	23	1	4.34
Siringa	Domesticated	18	4	22.22
Lapa Blanca	Domesticated	26	20	76.92
Yuca de agua	Domesticated	14	0	0
Inayá	Domesticated	10	0	0
Nupara	Domesticated	20	15	75
Pintadillo	Domesticated	15	0	0
Tresmesina dulce	Domesticated	4	0	0
Abeja	Domesticated	9	2	22.22
Dulce Cucura	Domesticated	7	0	0
Wasoco	Domesticated	9	0	0
Pupuña	Domesticated	22	0	0
Manihot flabellifolia	439	27	27	100
Manihot flabellifolia	443	23	23	100
Manihot esculenta sub. *Flabellifolia*	444-002	10	10	100
Manihot peruviana	414	17	17	100
Manihot peruviana	417-003	7	7	100
Manihot peruviana	417-005	28	28	100
Manihot esculenta	MBra 12	12	12	100
Manihot esculenta	CM 7395	17	17	100
Manihot esculenta	CMC 40	15	8	53.3
Manihot esculenta	MEcu 72	15	15	100

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Activity 14. Integrated control of subterranean pests in South America.

Introduction

This project started on April 1. Due to the short period this annual report informs mostly about the recent activities that were conducted to install the project.

Preliminary Activities

The project was originally designed for the Department Cauca, however, security problems made it necessary to identify alternative survey zones. Previously existing relations with SENA Armenia and the University of Caldas, Manizales, suggested conducting surveys in the coffee zone (Quindío, Risaralda, Caldas) where guerrilla activities are not frequent and where cassava has become one of the most important crops. Unfortunately, SENA Armenia was not a reliable partner so that we had to identify and interview farmers by ourselves. We also intensified contacts with University of Caldas, Universidad del Valle, CORPOICA (Rionegro) and the University of Bogotá.

The difficult financial situation made it necessary to apply for more funding. In May and June we submitted three proposals to SENA, Colciencias and the German Eiselen Foundation.

Preliminary field surveys in Quindío, where large-scale farmers dominate, revealed that white grubs and burrower bugs were almost not present. However, farmer interviews indicated that these pests appear every year, but they are well controlled through the application of insecticides. One farmer applied successfully entomopathogenic fungi against soil pests. Small-scale farmers in Risaralda recognize white grubs and *C. bergi* are recognized as important pests. A great abundance of soil pests were found in cassava (Pereira) and onion (Florida) fields. Due to the dry season soil pests were rare in Caldas (Granja Montelindo). However, eight undescribed species were collected. Due to the necessity to collect insects for rearing and carry out of bioassays. Routine pest surveys and their natural control agents were established in Pereira (cassava and pasture) and in Florida (onion and grassland). It is planned to include some farms in Quindío next year if additional funding makes the extension of field surveys possible.

The increasing traveling costs made it necessary to include less remote sites such as the North of Cauca. Security in that part of Cauca seems to permit field surveys. These are conducted in cassava and pasture at CIAT's experimental station in Santander de Quilichao, Caldono and Pescador. Since July routine surveys are being conducted in North of Cauca and in two zones close to Pereira.

Establishment of Activities

On August 26/27 (2002) we organized a meeting with all project partners: The University del Valle (James Montoya), University of Bogotá (Miguel Serrano), University of Caldas (Fernando Vallejo) and the Investigation Centre in Rionegro (Martha Londoño). The objective was to coordinate and synchronize project objectives and activities. There was an agreement on following issues:

- Study of actual pest complexes and associated natural enemies.
- The selected crops are: Pasture kikuyo (*Pennisetum clandestinum*) and potato (*Solanum tuberosum*) in the North and East of Antioquia, the same crops in Cundinamarca; cassava, pasture and onion (*Allium fistulosum*) in Risaralda, and cassava and pasture in the North of Cauca.
- An preliminary field survey will provide information on the ideal experiment size
- The pests will be collected in a sample area of one square meter. This methodology is considered as more reliable for diversity studies than monoliths (30x30 cm)
- Synchronization of white grub sampling techniques
- Synchronization of collection of natural control agents
- Carry out experiments on economic damage under controlled conditions.

Completed Activities

- Accommodation of a lab for white grubs colonies at Quindío, CIAT. At this moment (10/09/02) there are about 850 white grubs present after a peak of more than 1200 four weeks previous. This rate of decrease is within the normal range due to diseases and handling.
- Collection of white grubs in Caldono for identification in cassava fields and in forest ecosystems. Forests were included to compare cultivated fields with a less disturbed environment. 600 specimen were collected in the forest: Dominant species: *Phyllophaga menetriesi*, *Cyclocephala* spp., *Anomala* spp. Also 600 specimen were collected in cassava: Dominant genera: *Phyllophaga*, *Plectris*.
- About 600 specimens collected in cassava fields in Pereira: *P. menetriesi, Cyclocephala.*
- Shipment from Panama (ca. 50 specimen): Dominance of *Phyllophaga* and *Leucothyreus* sp., some *Anomalini.*
- Farmer interviews in Quindío, Risaralda, Cauca. Up to now we have interviewed more than 80 farmers. The aim is to interview about 60 farmers in each region, i.e. about 200 interviews.
- Isolation of 15 entomopathogenic fungi and 5 bacteria strains. No native nematodes have yet been isolated from the samples in Pereira and Cauca.

Goals to achieve by the end of 2002

- Interviews with farmers to document traditional knowledge, pesticide use, cultural practices and crop species affected.
- Pests' species and biological control agents identified.
- Use of light and pheromone traps.
- Maintain white grub and *C. bergi* colonies.
- Routine of screening of entomopathogens established.

Theses Underway

- Maria Paulina Quintero (Univalle): Studies of pathogenicity of nematodes on *Phyllophaga menetriesi.*
- Lina María Serna: (Universidad de Caldas): Basic knowledge of white grubs, *C. bergi* and their natural enemies (Florida).

- Nelly Villegas (Universidad de Caldas): Basic knowledge of white grubs, *C. bergi* and their natural enemies (Pereira).
- César Zuluaga (Universidad de Bogotá): Basic knowledge of white grubs, *C. bergi* and their natural enemies (Cundinamarca).

Planned Theses

- Lisa Struck (U de Hanover): Influence of Crotalaria on the behavior of *C. bergi* (initiates in November 2002).
- Juliana Jaramillo (M.Sc., Hanover): Pathogenicity of entomopathogenic fungi against *C. bergi* and white grubs in semi-controlled experiments (initiates on September 23, 2002).
- Ana Milena Caicedo (M.Sc., Hanover): Pathogenicity of entomopathogenic nematodes against *C. bergi* and white grubs in semi-controlled experiments (initiates in April 2003).

Contributors: A. Gaigl.

Activity 15. Integrated Pest and Disease Management Web Site.

The Web site of the Integrated Pest and Disease Management was launched in April 2002. The web site offers immediate access to information and research activities in pests and diseases of cassava, beans and tropical pastures and links with other CIAT projects. In addition the Web site provides a description of the IPDM project and information on project highlights, technology products, publications, services, research themes, the IPDM Annual Reports and announces training and conference events.

Characteristics of the Web Site

Under the URL http://www.ciat.cgiar.org/ipm/index.htm
The web site is offered to a variety of users that include:

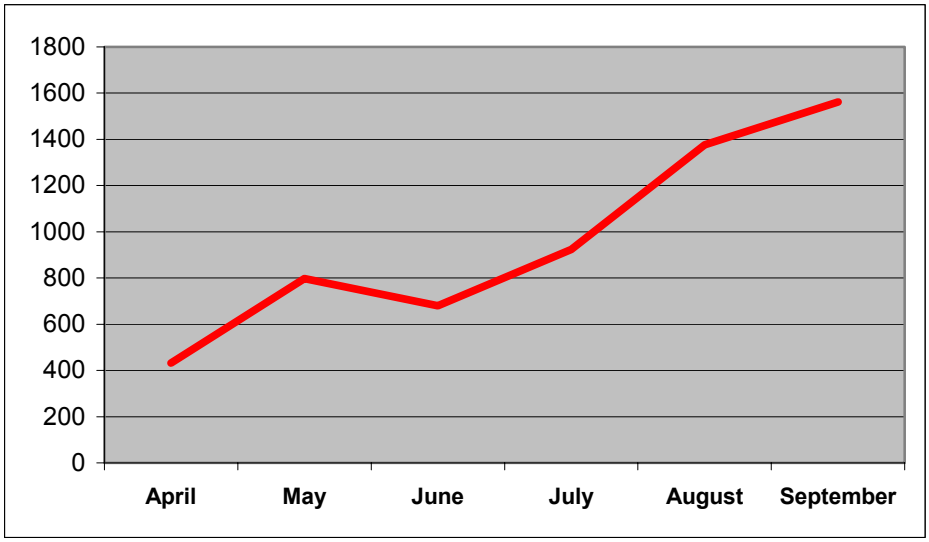
- Universities
- Research Institutions
- National Agricultural and Biological Research Organizations
- Non Governmental Organizations (NGO's)
- National Extension Services
- The Scientific Community in general
- Donor Agencies
- Biological Societies and Congresses

The Web site offers numerous services, products and information that includes:

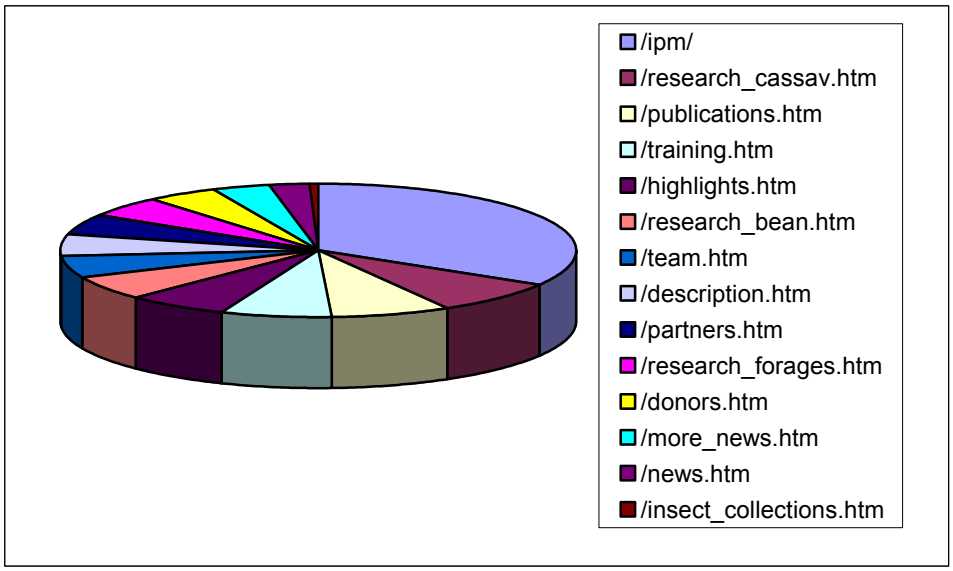
- An IPM data base
- CIAT, IPDM project Annual Reports
- Information on basic knowledge of cassava pests and diseases
- Information and research on bean pests and diseases in Africa
- Direct links to other CIAT projects that include IP-3, CLAYUCA, SN-3, and several other CIAT projects
- Information and research on bioecology of spittlebugs
- A catalog of electronic and print products providing information on tools, technologies and research methods
- A list of CIAT and other publication on IPDM and related themes
- A description of CIAT IPDM project (PE-1)
- A link to CIAT arthropod collection
- Up coming events such as conferences and congresses related to IPM

First Impact Results through Web Statistics

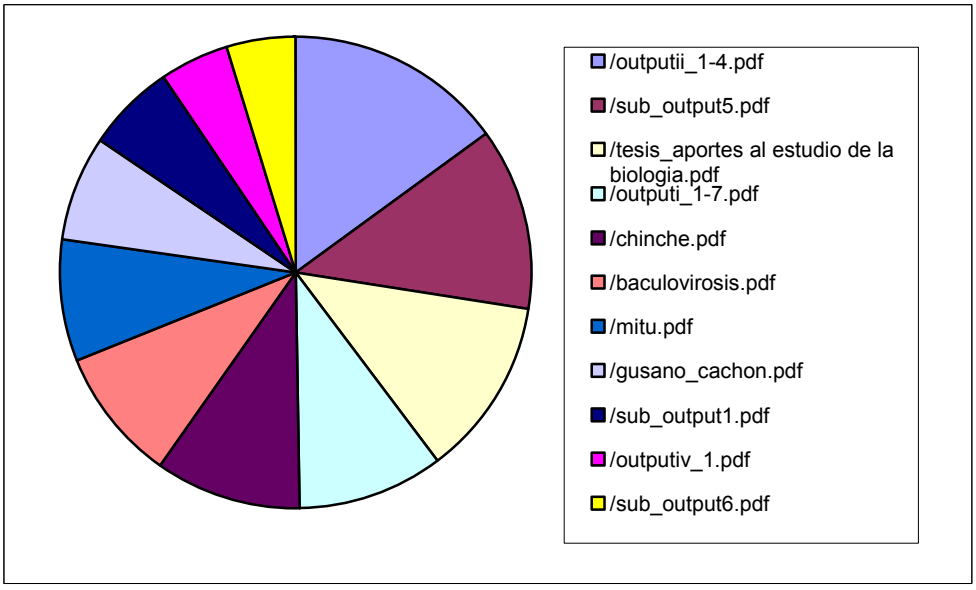
Hits and Downloads



The number of visits to the web site has steadily increased from about 400 in April to nearly 1600 in September. The most frequently visited pages include general IPM, cassava research publications, highlights, training, bean and forage research.



The most popular downloads are output research, thesis and information and research on specific pests and diseases, such as burrower bugs and hornworms (baculovirus).



Perspectives

The information provided on the web site is updated on a continual basis but at least once every month. It is projected that research themes and downloaded information will expand and that new linkages within and outside CIAT will be formed. Information is offered in both English and Spanish, and it is planned to translate more information into Spanish as resources permit.

Activity 16. Publications, book chapters, posters, conferences, training and consultancies.

Publications

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ALVAREZ, J.A., A. ACOSTA, A.C. BELLOTTI and A.R. BRAUN. Pathogenicity of a fungus associated with *Tetranychus urticae* Koch and *Mononychellus tanajoa* (Bondar), mite pests of cassava, *Manihot esculenta* Crantz.

RIIS, L., A.C. BELLOTTI, M. BONIERBALE, and G. O'BRIEN. Cyanogenic Potential in Cassava, its influence on a Generalist Insect Herbivore. Submitted to Ecological Entomology.

CASTILLO, J.A., A.C. BELLOTTI, and L. SMITH. Whiteflies (Homoptera: Aleyrodidae) encountered in cassava (*Manihot esculenta* Crantz) in Colombia: Geographical, altitudinal and climatic distribution. Submitted, Environmental Entomology.

RIIS, L., A.C. BELLOTTI, O. CASTAÑO. In field damage on cassava clones of high and low cyanogenci potential due to a generalist insect herbivore. Submitted to International Journal of Pest Management.

RIIS, L., B. ARIAS, and A.C. BELLOTTI. Bionomics and populations growth statics of the subterranean burrower bug *Cyrtomenus bergi* on Different Host Plants. Submitted to Entomologia Experimentalis et Applicata.

DORN, B., L. MATTIACCI, A.C. BELLOTTI and S. DORN. Diurnal activity and host-habitat location of mass-conditioned parasitoids of the cassava mealybugs.

Posters

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Training and Consultancy Services offered during 2002

Organizer	Place	Date	Participants	Received by	Service
CIAT-MADR	Municipality of San Angel (Magdalena)	14-17 January	20	Farmers	Training on how to handle the cassava hornworm
University of Puerto Rico	CIAT	19-25 February	1	Dr. Alberto Pantoja	Training on how to raise Phytoseiidae mites
CVC – Assistant Directorate of Environmental Heritage	CVC Tuluá	19 March	80	CVC staff, technicians, cassava producers	Biological control in cassava crops
CIAT/Ministry of Agriculture Development Institute, Secretary of Agriculture of Tolima	San Luis (Tolima)	21 March	25	Technicians, cassava producers, farmers	Pest management in cassava, with emphasis on whiteflies and the cassava hornworm
CIAT	CIAT	18 April	35	Students of entomology and plant pathology, Universidad de Caldas	Microbiological management of cassava-associated pests
INTEP	Roldanillo (Valle)	23 April	80	Students, technicians, farmers	Integrated pest management in cassava
MADR	La Balsa (Cauca)	25 April	21	Technicians, professionals, and local cassava farmers	Workshop and field day on integrated pest management in cassava, with emphasis on whiteflies, cassava burrowing bug, and the cassava hornworm
CLAYUCA	Municipality of El Tigre, State of Anzoategui; Municipality of San Carlos, State of Cojedes; Municipality of Maracaibo, State of Zulia (Venezuela)	5-16 May	150	Professionals and technicians of INIA, UNILLEZ University, U.C.V. (Maracay), and FONACIT	Integrated insect management in cassava Physiological and plant health problems in the field
MADR	Ibagué (Tolima)	17-20 June	17	Professionals, technicians, farmers	Workshop and field day on integrated pest management in cassava, with emphasis on the use of baculovirus (formulated) to control the cassava hornworm
MADR	Sincelejo-Tolú (Sucre)	27 June	18	Professionals, technicians, farmers	Biological management of the cassava burrowing bug
CLAYUCA	CIAT	27 June	35	Professionals, technicians, farmers	Biological management of the cassava burrowing bug and the cassava hornworm

Organizer	Place	Date	Participants	Received by	Service
MADR	Mondomo (Cauca)	2 July	18	Professionals, technicians, farmers	Workshop and field days on integrated pest management in cassava
CIAT (Human Resource Development Fund)	XXIX SOCOLEN Congress (Montería)	16-19 July	310	Professionals interested in agricultural information	Development of an artificial holidic diet for Cyrtomenus bergi, Froeschner (Hemiptera: Cydnidae)
FIAFOR-IDIAP Project	Panama	21-27 July	70	José A. Aguilar, members of the 'Reverendo Domínguez Basterra' Cooperative (Ocu) and of 'La Solución' Farming Cooperative (Sioguí), IDIAP researchers	Trials to evaluate the field application of biological agents in Ocú to control the cassava burrowing bug
CIAT	Bogotá	5-9 August	17	Elsa Liliana Melo	II Course on Entomoparasitic Nematodes
FIAFOR	CIAT	12-24 August	1	José A. Aguilar	Integrated management to control the cassava burrowing bug
CIAT	Universidad Nacional de Medellín	27 August	3	Students	Informative talk on the biological control of mites and entomoparasitic nematodes

Varieties released this year

Cassava variety NATAIMA-31 released by CORPOICA in collaboration with CIAT as high yielding whitefly resistant variety.

Collaborators

Anthony C. Bellotti, Josefina Martínez, Bernardo Arias, José María Guerrero, María del Pilar Hernández, María Elena Cuéllar, Elsa Liliana Melo, Adriana Bohórquez, Carlos Julio Herrera, Carolina Ramírez, Ana Milena Caicedo, Claudia María Holguín, Harold Trujillo (Colciencias Visitor Research), Diego Fernando Múnera, María Luisa Cortés, Irina Alean (Student, Universidad Javeriana, Bogotá), Maritza Burbano (Student, Universidad del Valle, Cali), Miller Gómez (Student, Universidad Nacional, Palmira), Mónica Lucía Marín (Student Practice, Universidad de Caldas, Manizales), Arturo Carabalí (Student, Universidad del Valle, Cali), Carlos Ñañes, Gerardino Pérez, Rodrigo Zúñiga, Rómulo Riascos, Adriano Muñoz, Carmen Elisa Mendoza, Gustavo Trujillo.

Project staff - CIAT
IRD (formerly ORSTOM)
Paul-André Calatayud

Donor Institutions
USAID
USDA
MFAT – New Zealand Ministry of Foreign Affairs
DFID
Ministerio de Agricultura y Desarrollo Rural – MADR, Colombia
IRD - France
Federal Ministry for Economic Co-operation and Development (BMZ)
CIAT (strategic findings)

Collaborators: Other Institutions
CLAYUCA (Dr. Bernardo Ospina)
CENICAFE, Chinchiná, Colombia – Juan Carlos López, Alex Bustillo, Gabriel Cadena
Universidad de Caldas, Manizales, Colombia – Arnubio J. Valencia
University of California Davis, Davis, USA – Patricia Stock
University of Florida, Gainsville, USA – Jorge Peña, Gregory Evans
Systematic laboratory in Livingston, Montana, USA – Mike Rose
BIOCARIBE S.A., Medellín, Colombia – Guillermo León Hernández
ETH, Zurich, Switzerland – Silvia Dorn
FIAFOR - José Antonio Aguilar

Collaborating Institutions
INRA-INSA, Laboratoire de Biologie Appliquée, Villeurbanne, France
IRD, France
CNPMF, EMBRAPA, Brazil
IAC, Sao Paulo, Brazil
USDA, USA
Cornell University, USA
Crop and Food Research Institute, New Zealand
British Museum
INIA – Instituto Nacional de Investigación Agrícola – Anzoátegui, Venezuela
Ministerio de Agricultura y Desarrollo Rural, MADR, Colombia
CORPOICA, Nataima, Colombia
Universidad Nacional, Palmira, Colombia
Universidad de Antioquia, Medellín, Colombia

Linkages with Other CIAT Projects and with CIAT's Partner Institutions
IPRA, based at CIAT, Colombia
Instituto Agronómico de Campinas (IAC), Brazil
Instituto de Investigaciones de Viandas Tropicales – INIVIT, Cuba
Universidad Nacional de Colombia, Sede Palmira, Colombia
EMBRAPA, Cruz das Almas, Brazil
Escuela Politécnica del Ejército (ESPE), Ecuador