CASSAVA ENTOMOLOGY

Activity 1. Arthropod taxonomic activities on CIAT commodity crops and others.

The IPDM project maintains a working collection of arthropod pests and their natural enemies associated with CIAT's mandate crops (cassava, beans, rice, tropical pastures and tropical fruits. This collection, now more than 20,000 specimens, also contains a limited number of species from other crops. The IPDM project also provides a service of identifying arthropod (primarily insects and arachnids) pests and natural enemies collected from these different crops. A database is maintained of all collections and this is made available to collaborating institutions, museums, universities and national research and extension programs. In return many of these institutions send us specimens for our collection.

During the past year numerous species were added to the collection. Active projects in biological control and host plant resistance require accurate and, hopefully, rapid identification of the pests and their potential natural enemies. The project maintains a biologist/taxonomist to manage this collection and its corresponding database, and when possible provide identification. CIAT has a limited capacity to do actual identifications. More often specimens that we collect or receive will need to be sent to the corresponding taxonomist that has specialized in a particular genus or family. We now have contact with approximately 50 taxonomists and several museums (British Museum, USDA, Beltsville Md.) that collaborate with us in providing identifications.

CIAT, through its commodity crop projects and the IPDM project encourage our collaborators in the different countries, especially in Latin America, to send us arthropod pests and their natural enemies collected from CIAT mandate and associated crops. In some cases we have provided them with collecting and shipping materials, as well as identifications of the specimens sent. Very often the specimens we collect or receive are new or unrecorded species and require the identification and naming of the species by the specialist taxonomist. This is often a time consuming process and may require several years before this information is made official through publication. An example of this is the current situation with cassava whitefly parasitoid natural enemies. Several are unrecorded species and we are awaiting official publication.

During the past year we initiated the collecting and identification of pests associated with tropical fruits in support of CIAT's activities in this area. At present, emphasis has been given to the fruit fly (Diptera: Tephritidae) complex that is associated with guava, plum, mango, papaya, zapallo (calabash) and others. Details of this study are reported as a separate activity in this document.

In addition, we continued the collecting and identification of homopterous species associated with the cassava crop as possible vectors of cassava frogskin disease. Attempts are now being made to establish a working colony of the major species identified. This research is reported as a separate activity.

The collection and identification of whiteflies associated with cassava, beans and numerous other crops was continued throughout the year. Phytophagous mite specimens associate with cassava and other crops were collected from Colombia and other countries (Haiti and Thailand).

Project 1 - Whiteflies

One of the activities of the CIAT convened "Systemwide Tropical Whitefly IPM Project" is to provide taxonomic support for whiteflies and their natural enemies collected from different cops and agroecosystems, primarily in Latin America, but may also include Africa and Asia. Project collaborators continue to send shipments of specimens collected for processing, monitoring and identification.

Objectives: Process and identify species collected in Peru, Brazil and other countries from different crops. The materials will be organized within the reference collection and registered in the data bank. Molecular techniques (RAPD-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, and permanent mounts are made in Canadian balsam (see CIAT PE-1 2002 Annual Report for more details). Parasitoids are sent to corresponding taxonomists.

Results: Samples were received for identification from Peru, Brazil and Africa. The original identification was made using the morphological key. A corresponding identification was made using molecular techniques with RAPD-PCR. This is a rapid and simple technique that is effective for determining differences between species.

The whitefly samples sent from Peru (Canete) were collected from several plant hosts, including sweet potato, cotton and weed species. These specimens were identified as *Bemisia afer*; using the RAPD-PRC test the same DNA banding pattern was obtained for all the specimens (using the OPA-04 primer). Seven bands of approximately 1018, 770, 720, 670, 625, 560 and 450 pb were observed in all of the samples **(Figure 1)**.

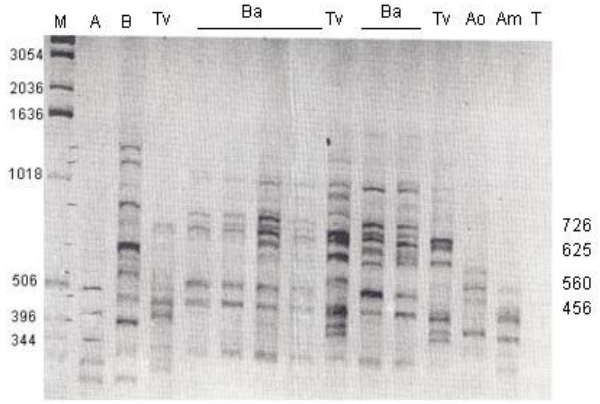

Figure 1. DNA Banding pattern obtained with the OPA-4 primer on whitefly samples from Peru and other sources. M=Marker 1 kb, line Ba (*Bemisia afer*). Other whiteflies include, Line: A (*B. tabaci* "A"); B (*B. tabaci* "B"); Tv (*Trialeurodes vaporariorum*); Tv (*T. variabilis*); Ao (*A. occidus*); Am (*A. malangae).*

Bemisia afer is an important species that is a potential danger for many crops in the Neotropics (see CIAT PE-1 2001 Annual Report for more details). *B. afer* has been recorded from many countries in Africa, the Middle East, Asia and Australia where it is reported feeding on a wide variety of plant hosts. It may be of African origin, where, in recent years, high populations have been found feeding on cassava. It is a "suspected" vector of cassava brown streak virus, but its possible role in virus transmission needs to be further researched and clarified. The dissemination of *B. afer* and the damage caused on different hosts needs to be monitored. It is considered a potential pest problem of cassava in the Americas and its potential as a virus vector in the Neotropics needs to be investigated.

Samples from Brazil. Whitefly samples from Brazil were sent by Alba R. Farias, the cassava entomologist at CNPMF/EMBRAPA in Cruz das Almas, Bahia. Specimens were collected from cassava in Jacaraci, Guajer and Licunio de Almeida in Bahia. All samples were identified as *Aleurothrixus aepim.* Identification was made using RAPD-PCR and banding patterns of samples from all three sites were identical and confirm the morphological identification. Four bands were detected at 400, 480, 700 and 800 pb **(Figure 2)**.

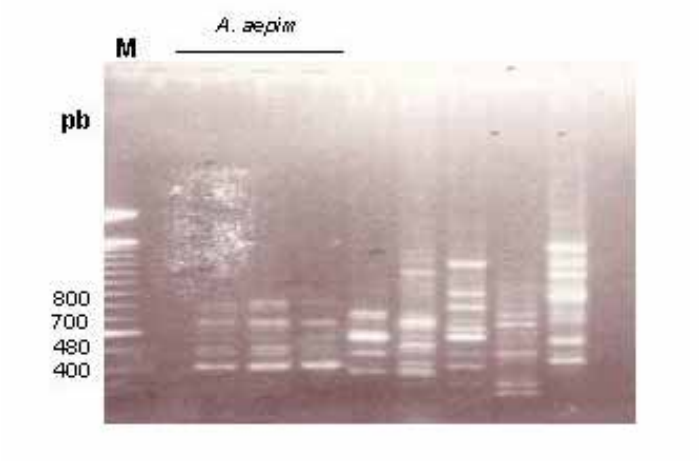

Figure 2. RAPD-PCR for whitefly samples from Bahia, Brazil.
M = Marker, lines 1-3 = *Aleurothrixus aepim* (Jacaraci, Guajer and Licinio de Almeida, Bahia); 4-8 = other whitefly species.

Samples from Africa. Whitefly samples from East Africa were collected from cassava and identified as *B. tabaci* using RAPD-PCR and the H9 and OPA-04 primer. These specimens were compared with *B. tabaci* being reared on cassava in the greenhouses at CIAT. Results show that the H9 primer gives a better definition of branding patterns. The samples from Africa gave bands of approximately 410, 590, 620, 810 and 1490 pb; the samples from Colombia showed bands of approximately 400, 520, 620, 750 and 980 pb **(Figure 3)**.

Included on the same gel were parasitoids of the cassava whitefly, *Aleurotrachelus socialis* to determine inter species differences. The parasitoid species were *Encarsia nigricephala, Eretmocerus* sp (Quindío, Colombia) and *Eretmocerus* sp (Tolima, Colombia). In this case the primer H16 was used, displaying differences in banding patterns between the three species. *Eretmocerus* sp (Quindío) gave three bands of 590, 870 and 1450 pb, while *Eretmocerus* sp

(Tolima) resulted in bands of 550, 700 and 890 pb. *E. nigricephala* shows band of 500 and 800 pb **(Figure 3)**.

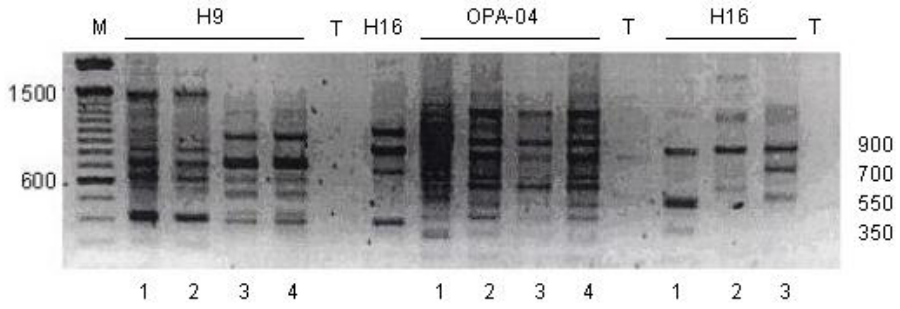

Figure 3. Banding patterns for whitefly and parasitoid species using primers H9, H16 and OPA-4, M+ marker 100 pb. Lines 1-2, *B. tabaci* (Africa); 3-4, *B. tabaci* (Colombia). On the same gel bands obtained with H16 primer. Line 1, *Encarsia nigricephala* (Valle); 2, *Eretmocerus* sp. (Quindío); 3, *Eretmocerus* sp (Tolima).

We need to use additional primers to achieve more banding patterns. However, these results help confirm that the *Eretmocerus* species from Tolima is different from the species in Quindío, even though morphologically they appear very similar.

Project 2 - Mite Identification

Phytophagous mites were collected from cassava at several locations in Colombia and from Haiti and Thailand. In addition to cassava collections were made from onion, guayacan, corn, coconut and ornamentals. These continuing collections, often accomplished during travels with other objectives, help us to determine geographical distribution, host plants, demographic conditions and provide taxonomic/morphological data for our database. Most collections are from cassava where 8 different species were collected from Colombia. *Oligonychus biharensis* was collected from Thailand on cassava and *Mononychellus caribbeanae* and *Eutetranychus banksi* were collected from cassava in Haiti.

The collection of the *Eriophyidae* mite from coconut on the Colombia Atlantic Coast may be of important significance. An *Eriophydae* mite is reported causing considerable damage in countries of Asia, especially Sri Lanka, and it is suspected that the species may be exotic and introduced from another region or continent. Efforts are underway to determine if the species in Sri Lanka is the same as what we find in Colombia.

Table 1. Phytophagous mites collected from cassava and other hosts from Colombia, Ecuador, Haiti and Thailand, during 2002-2003.

Sample	Country	Department	Municipality	Site	Host	Species
CIAT-2599	Colombia	Cauca	Caloto	La Robleda	Cassava	*M. tanajoa*
CIAT-2600	Colombia	Valle	Palmira	Palmaseca	Cassava	*M. tanajoa*
						M. caribbeanae
CIAT-2601	Colombia	Quindío	Armenia	Armenia	Cassava	*M. mcgregori*
						O. peruvianus
CIAT-2602	Colombia	Valle	Palmira	CIAT Invernadero	Cassava	*T. urticae*
CIAT-2603	Colombia	Atlántico	Malambo		Cassava	*T. tumidus*
						O. gosypii
						O. peruvianus
CIAT-2604	Colombia	Cordoba	Cga De Oro	El Copel	Cassava	*O. peruvianus*
						T. tumidus
CIAT-2605	Haiti		Pto. Principe	Double Harvest	Cassava	*M. caribbeanae**
						E. banksi
CIAT-2607	Colombia	Valle	Palmira	CIAT	Cassava	*T. urticae*
CIAT-2609	Ecuador			Tunguruagua	Onion	*Rhizoglyphus sp*
CIAT-2611	Colombia	Valle	Palmira	CIAT	Cassava	*M. tanajoa*
CIAT-2613	Colombia	Caldas		Santageda	Cassava	*O. punicae*
CIAT-2614	Thailand	Nakhon Rachasima		Huay Bong Tidi	Cassava	*O. biharensis*
CIAT-2615	Colombia	Valle	Palmira	CIAT	Guayacan	*Eotetranychus sp*
CIAT-2618	Colombia	Valle	Palmira	CIAT	Corn	*Eriophyidae'mites*
CIAT-2619	Colombia	Atlántico	Sto. Tomás		Coconut	*Eriophyidae'mites*
CIAT-2621	Colombia	Valle	Palmira	CIAT	Ornamentals	*Eriophyidae'mites*

* Mites infested with the Entomopathogens *Neozygites* sp.

M. = Mononychellus, O. = Oligonychus, E. = Eutetranychus, T.=Tetranychus

Contributors: María del Pilar Hernández, José María Guerrero, Anthony C. Bellotti.

Activity 2. Identification and characterization of some fruit fly species in the departments of Valle del Cauca, Tolima and Quindío.

CIAT's decision to add tropical fruits to its commodity portfolio has stirred interest in analyzing the possible arthropod pest problems that might be associated with regional fruit production. The Andean region is characterized by numerous agroecosystems and an impressive diversity of crops, and this is especially true of tropical fruits. Each fruit species will have a particular pest complex associated with it. At this time, since no specific fruit species has been designated as the priority species, it was decided not to concentrate on a particular species. There are, however certain groups of pests that consist of a species complex that can attack and damage numerous fruit species. The fruit fly complex is certainly an example of a pest that can damage numerous fruit species.

In Colombia, fruit flies are a serious problem and are found in nearly all of the fruit growing regions of the country. They are especially important and can cause considerably economic loss in the fruit export industry. In Latin America, about 20 fruit fly species have been reported causing losses calculated at about 25 million dollars per year.

The objectives of this initial study are to:

1. Establish a reference collection of fruit fly (*Anastrepha* spp) from the fruit growing regions of Valle del Cauca, Tolima and Quindío Departments of Colombia.
2. Sample and identify fruit hosts and the associated fruit fly species in the region.
3. Develop laboratory rearing methods to eventually study the biology and behavior of these species.
4. Initiate a literature search to establish a databank of present knowledge on fruit flies in the regions.

Literature Search

Fruit flies belong to the Order; Diptera and the Family: Tephritidae. Worldwide, approximately 4000 species have been described and 400 species are reported from the Americas (Núñez; 2000). In Colombia, the most important species belong to the genera *Anastrepha*, *Toxotrypana*, and *Ceratitis*. Of the three genera, *Anastrepha* is considered to be the most important economically, owing to the considerable damage it causes on different fruit species throughout the continent (Caraballo, 2001). The origin of this genus (*Anastrepha*) is the neotropics and it consists of more than 200 described species, of which, four are considered most important economically, *Anastrepha striata* Schiner, in guayaba; *A. fraterculus* (Wiedmann) in peach, mango, plum and others; *A. obliqua* (Macquart) in mango and plum, and *A. serpentina* (Wiedmann) in níspero (persimmon), caimito (star-apple) and other sapotaceous fruits. In addition two species, *Anastrepha pickeli* and *A. manihoti*, attack cassava fruits (and under certain conditions, cassava stems) but they are not considered as economically important.

Many *Anastrepha* species may be host specific; others will attack host plants within the same family. Examples of the latter include *A. grandis* attacking Cucurbitaceae, *A. oblique* attacking Anacardiaceae, *A. serpentina* on Sapotaceae, *A. striata* on Mirtaceae and *A. pallidipennis* on Passifloraceae. Generalist species such as *A. suspensa*, the Caribbean fruit fly, *A. fraterculus*, the

South American fruit fly and *A. ludens*, the Mexican fruit fly attack more than 60 diverse species. These species may also have numerous wild hosts that have not yet been identified. There are 15 *Anastrepha* species recorded in Colombia, usually found between 15 to 29ºC and from sea level to 2000 m.a.s.l. (Portilla, 1994).

Adult Tephritidos are about the size of a housefly and characterized by various colors, but predominantly yellow and translucent wings with longitudinal or transverse sports and bands. Adults live 1 to 3 months and females sexually mature in 3 to 4 days, copulating frequently (Portilla, 1994). Their biological development is influenced by humidity, temperature, light, native vegetation, pupation and ovipositional substrate and food availability.

Eggs of *Anastrepha* spp are usually a pale white, transparent and oviposited individually. A fully developed egg is opaque and the first instar larva is evident before hatching. The larvae are wedge-shaped with a rounded posterior. They are usually cream colored to yellow, but color can be influenced by food. There are three larval instars before pupation. Pupae are 1.4 to 1.8mm long and light straw colored to dark brown.

After copulating, females oviposit within the host fruit and the emerging larvae pass their instars feeding on the fruit pulp. The third instar larvae leave the fruit and pupates in the soil. Adults emerge within several days. The larval phase can vary from 13 to 28 days and pupae duration is 14 to 23 days. The preoviposition period is approximately 13 days; females can deposit 10 to 110 eggs per batch in fruit.

Field Sampling and Identification

Trips were made to fruit growing regions of the Department of Valle del Cauca, Tolima and Quindío. Ten municipalities were visited and fruit samples collected from 23 sites **(Table 1)**. Adult *Anastrepha* were also collected from the Department of Sucre, Sahún Municipality. Fruit samples were placed in paper bags and brought to the CIAT entomology laboratory where they were washed and submerged in a 0.4% hypochlorite solution for 1 minute. This prevents rapid fruit deterioration due to bacterial and fungal invasion. Fruits were then placed in a rearing chamber consisting of 27l x 18w x 15h cm plastic boxes with a screened top for aeration. Each box contained a layer of humid sterile soil **(Figures 3 and 4)**.

Figure 3

Figure 4

Figures 3 and 4. Larval development, pupation chambers for fruit flies (*Anastrepha* spp); chambers contain humid sterile soil for pupation.

Table 1. Sites sampled in the Department of Tolima, Valle del Cauca and Quindío (Colombia) for fruit fly (*Anastrepha* spp) infested fruit.

Department	Municipality	Locality	Host	Date
Tolima	Ibagué		*M. indica*	28 - VIII - 02
Tolima	Ibagué		*P. domestica*	28 - VIII - 02
Valle del Cauca	Cerrito	Sta. Elena	*P. guayava*	12 - IX - 02
Valle del Cauca	Bolívar	Plaza Vieja	*C. papaya*	24 - IX - 02
Valle del Cauca	Bolívar	San Fdo.	*P. guayava*	24 - IX - 02
Valle del Cauca	Bolívar	San Fdo.	*A. chirimoya*	24 - IX - 02
Valle del Cauca	Bolívar	San Fdo.	*M. indica*	24 - IX - 02
Valle del Cauca	Palmira	CIAT	*P. guayava*	02 - X - 02
Valle del Cauca	La Cumbre		*C. maxima*	02 - X - 02
Valle del Cauca	Candelaria	Cavasa	*C. papaya*	17 - X - 02
Valle del Cauca	Candelaria	Cavasa	*P. quadrangularis*	17 - X - 02
Valle del Cauca	Candelaria	Cavasa	*C. pubescens*	17 - X - 02
Valle del Cauca	Candelaria	Cavasa	*P. guayava*	17 - X - 02
Valle del Cauca	Candelaria	Cavasa	*A. muricata*	17 - X - 02
Valle del Cauca	Candelaria	Cavasa	*A. chirimoya*	17 - X - 02
Valle del Cauca	Candelaria	Cavasa	*M. indica*	17 - X - 02
Quindío	Montenegro	Varaya	*P. doméstica*	26 - IX - 02
Quindío	Montenegro	Varaya	*M. cordata*	26 - IX - 02
Quindío	Quimbaya	El Laurel	*P. guayava*	26 - IX - 02
Quindío	Quimbaya	Querman	*M. esculenta*	26 - IX - 02
Quindío	Circasia	La Cabaña	*P. guayava*	26 - IX - 02
Quindío	Circasia	Barcelona Baja	*P. guayava*	26 - IX - 02
Quindío	Armenia	La Primavera	*M. esculenta*	28 - IX - 02

Third instar fruit fly larvae upon emerging from the fruit will pupate in the soil; pupa were removed, washed in distilled water and placed in glass jars, also containing sterile soil, where adults emerged **(Figures 5 and 6)**. Adults were maintained on water plus bee honey solution for 2 to 3 days until the complete coloration for each species was attained. Those specimens separated for identification were placed in 60% alcohol; others were mounted on entomological pins and stored in the CIAT Arthropod Reference Collection.

Basically, three morphological characters are used in the identification of fruit flies; these are the thoracic design, the wing design and the female ovipositor. Based on these parameters, the wings and ovipositors of females were mounted to facilitate identification. This was done by removing the wing of each specimen and ovipositor and placing them on a glass slide with Hoyers media. Identification was done at the ICA (Instituto Colombiano Agropecuario) Laboratorio de Sanidad Vegetal in Palmira (Valle del Cauca).

Figure 5

Figure 6

Figures 5 and 6. Fruit flies pupae collected from development chambers are washed in distilled water and placed in glass jars for adult emergence.

Results: The fruits collected in Quindío and Valle del Cauca were mango, guava, papaya, cassava, chirimoya, plum, zapallo (calabash), sour-sop (guanábana), zapote (sapodilla), passion-flower (granadilla) and parayuela **(Table 2)**. 229 specimens were collected from these fruits and this resulted in six separate *Anastrepha* species (Table 2). The species *A. striata* was collected from guava in several localities in Quindío and Valle del Cauca. There are several other species reported from guava from these regions including *A. fraterculus*, *A. oblicua* and *A. ornate.* The fact that only *A. striata* was collected from guava may have something to do with the timming of the collections, September to October 2002. This supports the need to sample fruits throughout they year in order to determine if seasonality exists for the different *Anastrepha* species and the time of fruit infestation.

Table 2. *Anastrepha* (fruit fly) species collected from several hosts in Department of Tolima, Valle del Cauca and Quindío, Colombia (Sept. to Oct. 2002).

Code	Host	Department	Municipality	Identification
01	*M. indica*	Tolima	Ibagué	*Anastrepha oblicua* Macquat 7♀ 6♂
02	*P. domestica*	Tolima	Ibagué	*Anastrepha fraterculus* Wiedmann 11♀ 10♂
03	*P. guayava*	Valle del Cauca	Cerrito	*Anastrepha striata* Schiner 14♀ 21♂
04	*P. guayava*	Valle del Cauca	Bolívar	*Anastrepha striata* Schiner 5♀ 7♂
05	*P. guayava*	Valle del Cauca	Palmira	*Anastrepha striata* Schiner 136♀ 155♂
06	*C. maxima*	Valle del Cauca	La Cumbre	*Anastrepha grandis* Trochez 2♀ 5♂
07	*P. guayava*	Valle del Cauca	Candelaria	*Anastrepha striata* Schiner 3♀ 5♂
08	*P. doméstica*	Quindío	Montenegro	*Anastrepha striata* Schiner Oblicua 2♀ 8♂
09	*M. cordata*	Quindío	Montenegro	*Anastrepha nunezae* Steyscal 20♀ 10♂
10	*P. guayava*	Quindío	Quimbaya	*Anastrepha striata* Schiner 1♀
11	*M. esculenta*	Quindío	Quimbaya	*Anastrepha pickeli* Lima 3♀ 4♂
12	*P. guayava*	Quindío	Circasia	*Anastrepha striata* Schiner 4♀ 7♂
13	*P. guayava*	Quindío	Circasia	*Anastrepha striata* Schiner 5♀ 7♂
14	*M. esculenta*	Quindío	Armenia	*Anastrepha pickeli* Lima 3♀ 4♂

The identifying morphological characteristics of four of the collected species are shown in figures 8 to 11.

A. *Anastrepha striata*

B. Ovipositor

C. Wing pattern

Figure 8. This species, known as the guava fruit fly, primarily attacks fruit of the Mirtaceae family but may also infest mango and sour orange (*Citrus aurantium*).

A. *Anastrepha grandis*

B. Ovipositor

C. Wing pattern

Figure 9. At present, *A. grandis* is not considered of major economic importance in Colombia as it mostly attacks cucurbitaceous (i.e. watermelon). It is considered as a quarantine pest in Argentina and Uruguay and may eventually have greater importance in Colombia.

A. *Anastrepha pickeli*

B. Ovipositor

C. Wing

Figure 10. This species has only been found attacking cassava fruits (and stems). When infesting cassava stems it can severe rotting due to the invasion of soft rot bacteria *Erwinia caratavora*. The latter causes a reduction in the quality of planting material (stem cuttings).

A. *Anastrepha nunezae*

B. Ovipositor

C. Wing

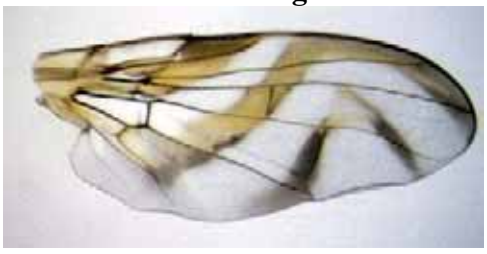

Figure 11. *A. nunezae* was found infesting zapote (*Quararibaea cordata*) especially between 900 to 1700 m.a.s.l.

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Activity 3. The identification and evaluation of homoptheran species as possible vectors of Cassava Frogskin Disease (CFSD).

Cassava frogskin disease (CFSD) probably originated in the Amazon region of South America and has now spread through several countries in the regions. It can cause considerable crop loss and hinders the movement of germplasm within and between countries. Its presence on the CIAT farm and adjacent cassava growing regions has affected our ability to evaluate germplasm and carry out research experiments. The epidemiology of CFSD is not sufficiently understood. CFSD dissemination by infected planting materials (stem cuttings) is well documented (Calvert and Thresh, 2001). Although previous studies have also indicated the involvement a whitefly (*Bemisia tuberculata*) vector, this is presently being questioned. Although CFSD has been described as a virus-like disease, the actual causal organism is still in doubt.

Some emphasis is presently being given to evaluating other homopterans, besides whiteflies, as possible vectors of CFSD. Emphasis is being given to the families of Cicadellidae and Delphacidae. Species within these two families are not considered as economic pests of cassava, however they are often observed, usually in low numbers, in cassava fields. Cicadellidae and Delphacidae species are known to be vectors of virus-like or phytoplasm like diseases. A revision of the CIAT cassava insect collection indicates few specimens of homopterans collected from cassava fields. It was therefore decided to initiate a determined effort to systematically survey and collect homopteran specimens from the different cassava growing regions of Colombia. Sampling is being done on cassava at different planting times, from crops being grown in distinct climatic conditions, and taking into account the presence or absence of CFSD.

Results: Homopterans have now been collected from nine Departments (States) and 17 localities in Colombia **(Table1)**. Between Sept. 2002 and Feb. 2003, collecting was done in Cauca, Quindío, Risaralda, Tolima, Córdoba, Valle del Cauca, Meta, Atlántico, Córdoba and Sucre. Observations during these collecting trips indicate that homopteran populations are very low in all of the sites surveyed. In some fields only 3 or 4 specimens were collected. It was also observed that heavily weeded cassava fields contained a greater diversity of species, and this was especially noticeable as the diversity of weed species also increased. These observations may also indicate that many or most of the specimens we are collecting from cassava may not necessarily be feeding on cassava and are present only because of the associated weed species. The latter point is important, as CFSD vectoring would only occur if the species actually feeds on cassava.

Collected species belong to three families, Cicadellidae, Cixiidae and Delphacidae. The Cicadellidae were collected in the greatest number and largest species diversity in almost all plots sampled. Cixiidae was the second family collected, but the species have not been identified. Delphacidae were collected only on the CIAT farm.

We have been able to identify some specimens to genus at CIAT based on our knowledge and available taxonomic keys. These include the Cicadellidae *Empoasca* sp, *Scaphytopius* sp. pos. *fuliginosus*, and *Stirellus bicolor*. These three genera are known vectors of viruses and phytoplasms on other crops. These species as well as most of the others that we have collected still require taxonomic verification and will be sent to the appropriate taxonomists.

Table 1. Homopteran species collected from cassava fields at several locations in Colombia.

Department	Municipality	Site	Family	Species	Observations
Valle del Cauca	Palmira	CIAT	Cicadellidae	**Scaphytopius sp. pos. fuliginosus*	
				Pos. Empoasca sp.	
			Cixiidae	1 sp. s.i.	
Cauca	Santander de Quilichao	Hacienda Bariloche	Cicadellidae	*Scaphytopius sp. pos. fuliginosus*	2 month field plot
			Delphacidae	1 sp. s.i.	
		Granja CIAT	Cicadellidae	*Scaphytopius sp. pos. fuliginosus*	Some plants with CFSD
			Cicadellidae	*Pos. Empoasca sp.*	
				5 spp, s.i.	
		Pescador	Cicadellidae	*Stirellus bicolor*	
				1 sp. s.i.	
Quindío	La Tebaida		Cicadellidae	5 spp.	Weedy plot
			Cixiidae	1 sp.	
	Armenia	La primavera	Cicadellidae	*Scaphytopius sp. pos. fuliginosus*	
				Pos. Empoasca sp.	
				Stirellus bicolor	
				1 sp. s.i.	
	Quimbaya	Vereda Querman	Cicadellidae	*Scaphytopius sp. pos. fuliginosus*	
				Scaphytopius sp. pos. fuliginosus	
				Stirellus bicolor	
Risaralda	Morelia	Santa Rita	Cicadellidae	3 spp. s.i.	
				Stirellus bicolor	
				1 sp. s.i.	
	Cerritos		Cicadellidae	2 spp. s.i.	
			Cixiidae	1 sp. s.i.	
Tolima	Chicoral	Granja Nataima	Cicadellidae	*Scaphytopius sp. pos. fuliginosus*	Some plants with CFSD
				Pos. Empoasca sp.	6 month cassava field
	Gualanday		Cicadellidae	*Scaphytopius sp. pos. fuliginosus*	7 month cassava field
				1 sp. s.i	
	Ambalema	Via Ambalema	Cicadellidae	*Pos. Empoasca sp.*	6 month cassava field
	Espinal	San Francisco	Cicadellidae	*Pos. Empoasca sp*	6-7 month cassava field
Meta	Villavicencio	Corpoica	Cicadellidae	1 sp. s.i.	
Atlántico	Pitalito		Cicadellidae	*Pos. Empoasca sp*	
Córdoba	Ciénaga de Oro		Cicadellidae	*Pos. Empoasca sp*	
Sucre	Corozal	Las Penas	Cicadellidae	*Pos. Empoasca sp*	Presence of CFSD

* *Scaphytopius*= (Platymetopius).

Rearing Homopterans. It has been decided, based on the evidence that we have now accumulated that *Scaphytopius sp* is a prime candidate as a vector of CFSD. This is based

primarily on the number of specimens we have collected and the frequency that we find this species in the different locations sampled **(Table 1)**. The initial attempt at establishing a colony of this species was not successful. It will be difficult, if not impossible, to maintain a *Scaphytopius* colony on cassava; beans (*P. vulgaris*) is a more receptive host and will probably be used for rearing this species. Once we have achieved a working colony, experiments will be designed and carried out to determine if this species can vector CFSD.

Identification of Species. A frequently collected species from many of the sampled sites was a Cicadellidae, probably of the genus *Empoasca*. An attempt was made to determine if all collected specimen were of the same species by utilizing a RAPD-PCR technique to determine differences by means of polymorphic bands. Tests were done on samples collected from the Departments of Atlántico (Pitalito), Córdoba (Ciénaga de Oro) and Sucre (Corozal). Preliminary results indicate differences between the three samples. The samples from Cordoba show bands approximately of 900, 680, 590 and 380 pb. The specimens from Sucre show four bands of 780, 650, 550 and 450 pb, while the specimens from Atlántico shows only one band of 560 pb. These samples will need to be re-examined to verify these results; therefore additional specimens will need to be collected.

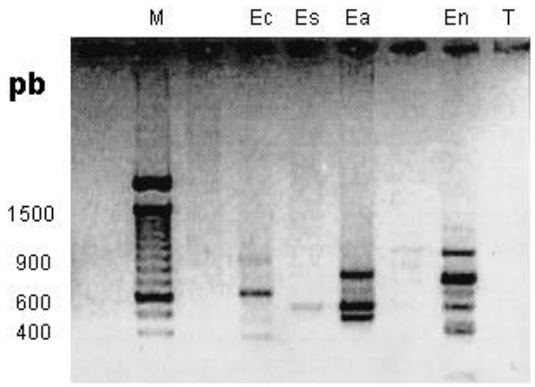

Figure 1. Band patterns obtained using RAPD-PCR with the primer H9 on samples of *Empoasca spp* from three cassava localities on the Colombian Atlantic Coast (M=marker, Lines EC=Córdoba; ES=Sucre; EA=Atlántico and EN=*Empoasca* from Guanábano, Valle

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Activity 4. Entomopathogenic nematodes: An alternative to Integrated Management of *Cyrtomenus bergi* Froeschner (Hemiptera:Cydnidae).

Objectives

1. To reisolate entomopathogenic native nematodes associated to *C. bergi* in three Colombian regions.
2. To evaluate virulence of *Steinernema* and *Heterorhabditis* species against *C. bergi* in laboratory.
3. To evaluate virulence of *Steinernema* and *Heterorhabditis* species against *C. bergi* in glasshouse.
4. To evaluate phenoloxidase isoenzimatic patterns from *C. bergi* inoculated with two entomopathogenic nematodes (EPNs).

Objective 1. To reisolate entomopathogenic native nematodes associated to *C. bergi* in three Colombian regions.

Methodology

Collection of soil samples: Soil samples were collected from three regions of Colombia during September–December of 2002. The sampling sites were selected because they were positive for nematodes according to survey done by Caicedo & Bellotti, 1996. The localities were Santander de Quilichao (Cauca), Santágueda (Caldas) y Pereira (Risaralda). Within a given site, a sample of ca. 1 Kg made up of a composite from three sub samples was taken. Each sub sample was obtained using a core cylinder of 10 cm of diameter and two depth 1-10 cm and 10-25 cm within an area of 10 m. Samples were placed in a plastic bag and transported in a cooler to the laboratory. At each site date, altitude and type of vegetation were recorded **(Table 1)**.

Isolation of nematodes: The soil samples were processed within three days of collection. The 1 Kg soil sample was thoroughly mixed, ca 250 g cc of sub sampled was placed into a 300 cc plastic container, ten last instar larvae of the wax moth *Galleria mellonella* (L) were placed on the soil, and the container was covered with a lid and inverted (Bedding & Akhurst 1975; Kaya & Stock 1997). The containers were placed a plastic bag and held at room temperature 21-23 C for a period of 5-7 days. Dead larvae were collected and placed on humid chamber during one week and then transferred to White traps to collect the emerging Ijs (Kaya & Stock, 1997). The IJs were pooled from each sample and were used to infect fresh *G. mellonella* larvae to verify their pathogenicity and allow for progeny production for identification (Kaya & Stock, 1997). Soil samples that were positive for EPNs were analyzed by Physical soil Laboratory at CIAT for soil type, organic matter and pH.

Table 1. EPNs sampling sites from three regions of Colombian between September and December 2002.

Site	Locality	Department	Vegetation	Altitude	Sample No.	Date
Lagos de Brasilia	Santander de Quilichao	Cauca	Cassava	990	1	12-09-02
Finca Brasilia	Idem	Cauca	Cassava	990	2	12-09-02
La Agustina	Idem	Cauca	Cassava	990	1	12-09-02
La Chapa	Idem	Cauca	Cassava	990	1	12-09-02
Granja Motelindo	Santágueda	Caldas	Orange Figs Plantain	1050	1	1-10-02
La Colonia	Pereira	Risaralda	Onion Medicinal Mulberry	1900	1	3-10-02
La Florida	Pereira	Risaralda	Onion Cilantro Corn	1740	1	3-10-02
La Agustina	Santander de Quilichao	Cauca	Cassava	1340	1	1-12-02
El Pital	Idem	Cauca	Cassava	1500	1	1-12-02
La Independencia	Idem	Cauca	Cassava	1700	1	1-12-02
Cachimbal			Cassava	1370	1	1-12-02
Caloteño			Cassava	1500	1	1-12-02

Results: Entomopathogenic nematodes were recovered from 10 samples of the 193 collected in September-December of 2002. One Heterorhabditis species were recovered from one site, La Colonia, Risaralda, **(Figure 1 and Figure 2)**. The identification of the species is already in process by the taxonomist Patricia Stock, Arizona University.

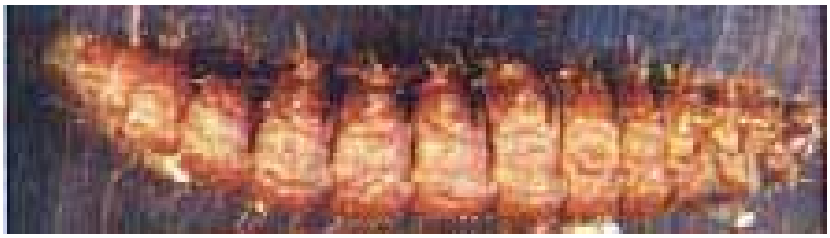

Figure 1. Larvae of *Galleria mellonella* infected with *Heterorhabditis sp*-CIAT. Photo by (Cristian Olaya, Microscopy Lab. Virology Unit, CIAT).

Figure 2. Male of *Heterorhabditis sp*-CIAT. Photo by (Cristian Olaya, Microscopy Lab. Virology Unit, CIAT).

Conclusions: It was an important finding of a native *Heterorhabditis sp*-CIAT in the same habitat of *C. bergi* with very few samples soil. It is well known that different species and strains exhibit differences in survival, infectivity and/or reproduction, which make them more or less suitable for particular control programs.

Objective 2. To evaluate virulence of Steinernema and Heterorhabditis species against *C. bergi* in laboratory.

Methodology: The bioassays were done at Management Integrate Pest and Diseases-Cassava Project laboratory with T 23°C and 70% HR.

Two different bioassay were tested:

a. Evaluation of six EPNs species against two *C. bergi* stages: fifth and adult with one EPNs dose, 5000 nematodes/ml.

b. Evaluation of three EPNs species against adult stage of *C. bergi* with five different doses.

Nematode Species

- Exotic
  Heterorhabditis bacteriophora-UK-Cenicafé (Hb)
  Steinernema riobrave –USA-CIAT (Sr)
- **Natives**
  Steinernema sp-SNI 0100-Cenicafé (SIN)
  Heterorhabditis sp-HNI 01980-Cenicafé (HNI)
  Steinernema feltiae cepa Villapinzón-UnalBogotá (Sf)
  Heterorhabditis sp-CIAT (HCIAT)

All nematodes were cultured in the last instar greater wax moth, *G. mellonella* L., larvae using the methods described by Kaya & Stock (1997). All nematodes were acclimated to room temperature for at least 24 h before inoculation.

Fifth and adult stages of *C. bergi* laboratory- reared, were exposed to 5000 infective juveniles per milliliter of each nematode species in a plastic cups containing 10 grams of sand (4% w/w) with one insect and one germinated corn seed (Caicedo & Bellotti, 1994). The experiment was replicated five times in randomized complete blocks with twelve replications. The control treatment was exposed to one milliliter of distilled water. Parasitism and mortality were recorded after 10 days and all insects were dissected under microscope-stereoscope.

In a second test, three species of nematodes were applied in lots of 2000, 4000, 6000, 8000 and 10.000 nematodes per milliliter against adult stage of *C. berg.* The experiment was replicated four times in randomized complete blocks. The evaluation time and method were the same as described previously.

The data were statistically analyzed by ANOVA (GLM) with mean separation by Duncan test and Probit analyses respectively.

Results: Of the six nematodes species evaluated, *Steinernema sp*- SNI 0100 was significantly the most efficient, causing 100% parasitism **(Figure 3)** but just 22% mortality **(Figure 4)** to the adult stage of *C. bergi* exposed to 5.000 nematodes. *Heterorhabditis sp*-HNI 0198 was the least effective, causing only 45% parasitism and 4% mortality. The adult stage was more susceptible than the fifth instar stage of *C. bergi* to all nematodes species. This result confirms that obtained with *S. carpocapsae* by Caicedo & Bellotti (1994).

When the adult stage of *C. bergi* was exposed to different doses of three nematodes species, no significant differences were observed between the lowest and the four highest doses (**Figure 3 and 4**). The results obtained were similar to the above mentioned, all three nematode species cause parasitism (65-100%) on the adult stage of *C. bergi* but they are not able to cause high mortality (3-40%).

These results suggest that it could be possible that *C. bergi* is showing an immune response against all six nematode species evaluated.

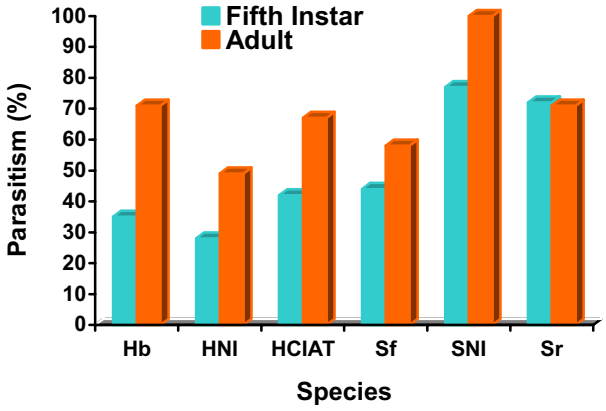

Figure 3. Parasitism of two *C. bergi* stages with six nematodes species.

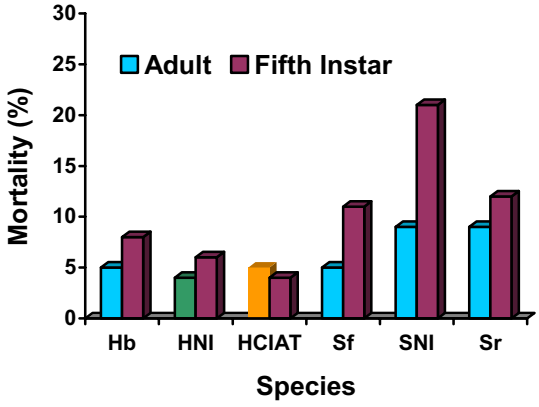

Figure 4. Mortality of two *C. bergi* stages with six nematodes species.

Conclusions: All of EPNs tested showed a good penetration on both stages of *C. bergi* with all doses evaluated, but they were not able to cause high mortality.

No significant differences in *C. bergi* parasitism and mortality were observed between the lowest and highest EPNs doses

Objective 3. Evaluate virulence of *Steinernema* and *Heterorhabditis* species against *C. bergi* in glasshouse.

Methodology: The ambiental conditions in the glasshouse were: T min 23°C; T max 34 C and HR min 60% and max 92%.

Three different bioassay were tested in the glasshouse:

a. Evaluation of three EPNs species against the adult stage of *C. bergi* with 1000 nematodes per milliliter.

b. Evaluation of two EPNs species against the adult stage of *C. bergi* with 25.000 nematodes per milliliter.

c. Evaluation of three EPNs species against the adult stage of *C. bergi* with 100.000 nematodes per milliliter.

Nematode Species

- **Exotic**
  Steinernema carpocapsae All strain-USA (Cenicafé)
  Steinernema riobrave –USA-CIAT
- **Natives**
  Steinernema sp-SNI 0100-Cenicafé
  Heterorhabditis sp-HNI 0198-Cenicafé
  Heterorhabditis sp-CIAT

In the first assay the adult stage laboratory-reared of *C. bergi* were exposed to 1000 infective juveniles per milliliter of each nematode species in a plastic cups containing 300 grams of sand (4%W/W) with one insect and one germinated corn seed. Treatments were arranged in a randomized complete block design with thirty replications. The control treatment was exposed to one milliliter of distilled water. Parasitism and mortality were recorded after 10 days and all insects were dissected under stereomicroscope.

In a second assay, two species of nematodes were applied at the rate of 25.000 nematodes per milliliter against adult stages of *C. bergi*. The experiment was replicated three times in randomized complete blocks with twelve replications.

In the last assay, three species of nematodes were evaluated at rate of 100.000 nematodes per milliliter against the adult stage of *C. bergi*. The experiment was replicated three times in randomized complete blocks with twelve replications. The arena, evaluation time and the evaluation procedure were done the same as described previously.

The data were statistical analyzed by Chi square and ANOVA (GLM).

Results: There were no significant differences among all the nematodes species and doses evaluated in the glasshouse against *C. bergi* adult. When it was exposed to 1000 nematodes of *S. carpocapsae, Steinernema sp* SNI 0100 and *Heterorhabditis sp* HNI0198 the parasitism was 21, 18 and 10% respectively and mortality was not observed **(Figure 5).** The parasitism and mortality caused by *S. carpocapsae* and *Heterorhabditis sp* HNI0198 increased with the dose; at 25.000 nematodes, 55 and 45% of parasitism and 29 and 9% of mortality respectively was observed **(Figure 6).** The adults exposed to 100.000 nematodes showed an increase in the mortality caused by, *Steinernema riobrave, Steinernema sp* SNI0100 and *Heterorhabditis sp*-CIAT, 33, 28 and 26% respectively **(Figure 7).**

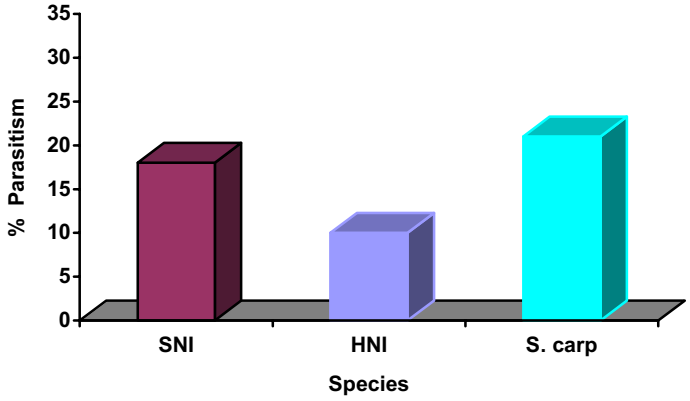

Figure 5. Parasitism of three species of EPNs species against *C. bergi* adult in glasshouse with 1000 nematodes.

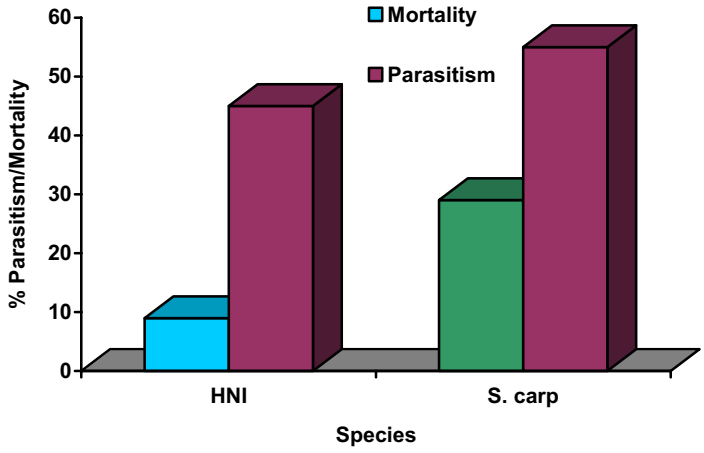

Figure 6. Parasitism and Mortality of two EPNs species against *C. bergi* adult with 25.000 nematodes in glasshouse

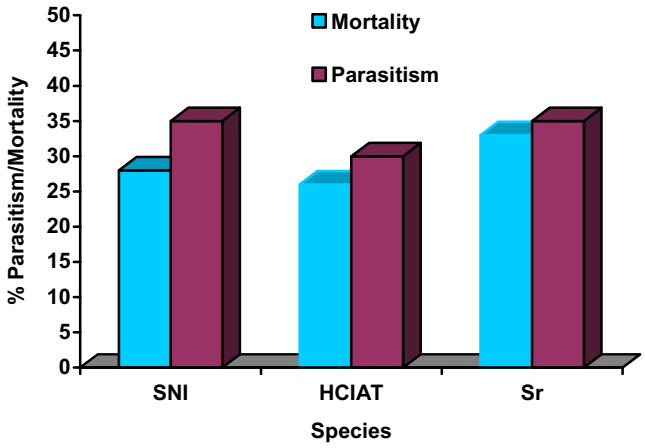

Figure 7. Parasitism and Mortality of three EPNs species against *C. bergi* adult stage in glasshouse with 100.000 nematodes

Conclusions: Significant differences in *C. bergi* parasitism and mortality were not observed between EPNs species evaluated in the glasshouse.

An increase EPNs virulence was observed when nematode doses were increased.

It will be necessary to continue these studies on nematode behavior and virulence against *C. bergi* before field assays can be performed.

Objective 4. Evaluate phenoloxidase (PO) isoenzimatic patterns from *C. bergi* inoculated with two entomopathogenic nematodes (EPNs).

Methodology: When an insect is wounded, a darkly pigmented region appears in the injured area. This is due to the proteolytic activation of a zymogen, prophenylphenoloxidase PPO by the activated phenoloxidase PO, and subsequent formation of melanin. Melanin is often deposited around encapsulated objects, in hemocyte nodules, and at sites of fungal infection of cuticle. Melanization appears to be important in defense reactions of insects through its action in wound healing and pathogen sequestration (Guillespie et al, 1997).

Thus, the possibilities of a link between the low virulence of all the EPNs species evaluated against *C. bergi* and the phenoloxidase activity evaluated.

Experiments were conducted in two laboratories, MIP-Cassava program at CIAT and Chemical laboratory of Caldas University.

Nematodes Species: The nematodes species used in this assay, *Steinernema sp*-SNI198-Cenicafé and *Heterorhabditis sp*-CIAT were cultured in the last instar greater wax moth, *G. mellonella* L., larvae using the methods described by Kaya & Stock (1997). All nematodes were

acclimated to room temperature for at least 24 h before inoculation. *C. bergi* adults were taken from laboratory mass rear.

Three treatments were designed with live and dead nematodes and trypsin. Ten adults of *C. bergi* were injected with 10.000 live nematodes/10ul of each species and placed in a sand filled plastic cup (10 g with 4% w/w). Another ten insects were injected the same way but with dead nematodes. The nematodes were killed in hot water for ten minutes (98 C). Two periods of infection were tested with each nematode, 24 and 48 hours. Two concentrations of trypsin were injected, 1300 and 130 unities of activity. All the control treatments were injected only with distilled water. The last treatment was evaluated at 24 hours only.

Processing Samples: The insects were macerated with liquid nitrogen and 20 mg of sample diluted with 100ul of distilled water were centrifuged to 14.000 rpm for 10 minutes. Fast System was filled with 3-4 ul of supernatant. Separation bands were done by isoelectroenfoque (IEF pH 3-9)**.**

Results: A typical pattern of activity of phenoloxidase in *C. bergi* adults when they were injected with live nematodes of each species is presented in Figure 8.

A similar pattern was observed when the bugs were injected with dead nematodes is presented in Figure 9. When a foraging invading organism is too large to be phogocytosed, it becomes encapsulated by multiple layers of hemocytes and/or a melanin coat. The latter reaction was observed in all the nematodes species that penetrating *C. bergi* stages (**Figure 10**).

Two types of encapsulation are distinguished in insects: cellular encapsulation, mainly described in Lepidoptera, and melanotic (humoral) encapsulation more typical for Diptera and now observed specifically in *C. bergi*, (Hemiptera: Cydnidae). Melanotic encapsulation, which is always associated with PO activity, cellular encapsulation can occur without any sign of melanization. This does not exclude the possibility that components of the activating pathway other than PO may be required in some way (Guillespie et al 1997; Jarosz, 1998).

Differences were observed in the proteolytic activity among bugs injected with two trypsin concentrations and distilled water (**Figure 11**). This confirms a possible ability of *C. bergi* to defend itself from foreign organisms or substances as observed with the PO activity mentioned above.

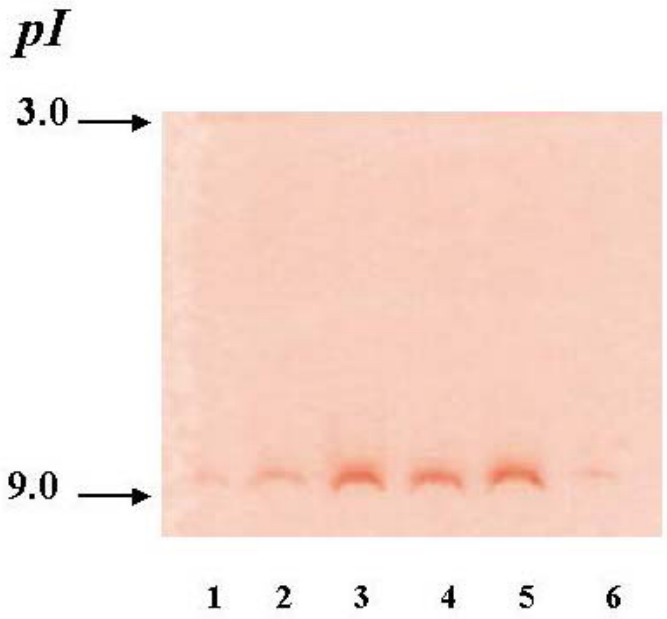

Figure 8. **Phenoloxidases of *C. bergi* infected with live nematodes. 1 y 6 control (24h); 2. SNI (24h); 3. SNI (48h); 4. HCIAT (24h); 5. HCIAT (48h).**

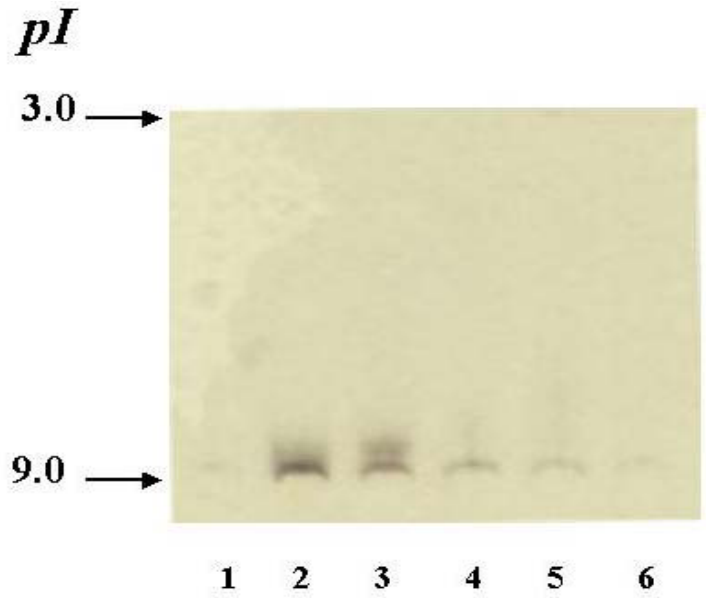

Figure 9. **Phenoloxidases of *C. bergi* infected with death nematodes. 1 y 6 control; 2. SNI (24h); 3. SNI (40h); 4. HCIAT (24h); 5. HCIAT (40h).**

Figure 10. IJs melanizaded on *C. bergi* stages after 10 days of inoculation. Photo by (Cristian Olaya, Microscopy Lab. Virology Unit, CIAT).

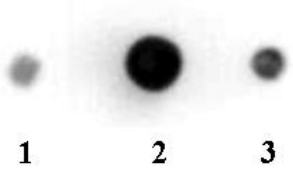

Figure 11. Phenoloxidase activity blot of *C. bergi* injected with tripsin. 1. Control (ADE); 2. C1 (1300 units of tripsin activity); 3. C2 (130 units of tripsin activity).

Conclusions: It could be due to phenoloxidase activation as insect's immune response by *C. bergi*.

It will be necessary to evaluate more EPNs species against *C. bergi* to further explain this behavior before field evaluation.

Low EPNs's virulence was observed in all of the bioassays done in the lab and glasshouse conditions on two C. bergi stages.

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Contributors: Ana Milena Caicedo V., Anthony C. Bellotti, Paul-André Calatayud, Juan Carlos López N., Arnubio Valencia, Patricia Stock, Diego Fernando Múnera, Arturo Carabalí, María Elena Cuellar, María Paulina Quintero, Harold Trujillo.

Activity 5. Development and formulation of biological pesticide for integrated pest management in cassava.

As cassava production increases in Colombia, it is expected that the control or management of arthropod pest problems will play an important role in increasing yields and stabilizing production. Two of the major economic pests include the cassava hornworm (*Erinnyis ello)* and the burrower bug (*Cyrtomenus bergi*) (see PE-1 Annual Reports 2001 and 2002, CIAT, for additional information).

The cassava hornworm can reduce cassava yields by as much as 70% if repeated attacks occur. Although numerous natural enemies (more than 30 species) have been recorded, they are not capable of maintaining hornworm populations below economic injury levels. The mass migratory behavior of *E. ello* adults renders natural biological control ineffective. A baculovirus (granulosis virus) of *E. ello* has proven to be the most manageable and effective of the natural enemy complex associated with *E. ello*. In general, baculovirus application is relatively easy, economically favorable and environmentally sustainable. Cyclical *E. ello* attacks on the Colombia North Coast and other areas have facilitated the need for a commercial baculovirus product that is readily available to cassava producers.

A formulated baculovirus biopesticide has been developed by the Colombia company "BIOTROPICAL" (formerly BIOCARIBE) in a collaborative project with CIAT **(Figure 1)**. This product has been approved by ICA/MADR for commercial release and is now available to cassava producers as a wettable powder.

Figure 1. A commercially available baculovirus, formulated by "Biotropical" for cassava hornworm (*Erinnyis ello*). Note credits to MADR and CIAT.

Field trials to evaluate the efficacy of this product were carried out at two locations in Colombia, San Luis in the Department of Tolima and in the Department of Risaralda. During natural hornworm attacks, the baculovirus (Trade name Bio Virus) was applied at doses of 300 grams per hectare. In Tolima, hornworm mortality reached 93% and in Risaralda, it was 85% **(Figure 2)**.

BIOTROPICAL has increased production of the baculovirus and the product is now available to cassava producers in several regions of Colombia including the Atlantic Coast and the Llanos Orientales. Initial results indicate that the product is very efficient in suppressing and controlling hornworm populations. Farmers have been trained in the use of the baculovirus biopesticide through field days and training courses, especially in collaboration with CLAYUCA **(Figure 3)**.

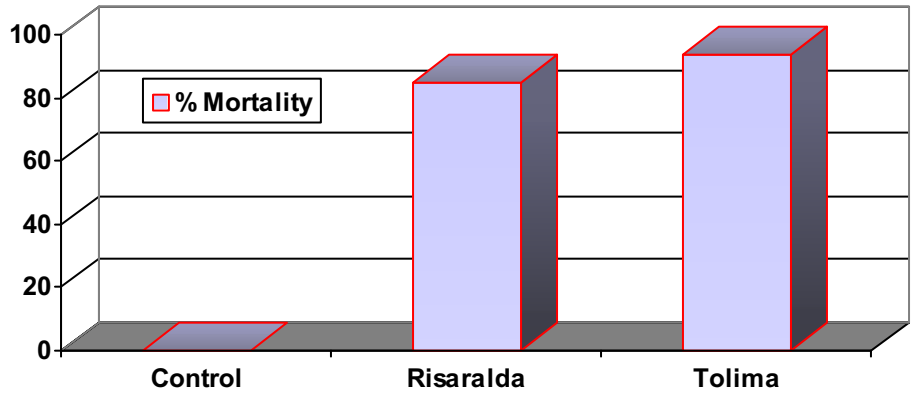

Figure 2. Cassava hornworm (*E. ello*) mortality due to field applications (300 grams/ha) of the *E. ello* baculovirus (Biovirus: BIOTROPICAL) at two locations in Colombia.

Figure 3. Farmer training in the use of biopesticides to control cassava pests such as the hornworm (*E. ello*) and burrow bug (*C. bergi*).

Publications and pamphlets on product use are available and have been distributed and several newspaper articles on this product have appeared.

EL TIEMPO SÁBADO 23 DE AGOSTO DE 2003 1-7

TIERRAS & GANADOS

INSUMOS / CONTRA EL GUSANO CACHÓN Y EL CHINCHE DE LA VIRUELA

Bioplaguicidas para la yuca

Luego de 15 años de investigación, el Ciat encontró la solución al problema de dos plagas que 'han hecho de las suyas' en las 11,9 millones de hectáreas yuqueras del país.

La yuca es hoy el centro de atracción de la industria de alimentos balanceados para la avicultura. ¿La razón? La harina de sus hojas y raíces puede reemplazar totalmente a los cereales sin afectar los rendimientos de las aves, tanto de carne como ponedoras.

"En la alimentación de pollos de engorde y aves de postura, las harinas de raíces y follaje (hojas) de yuca proporcionan una fuente de energía, proteína y pigmentos naturales", dice un documento de Fenavi (el gremio avícola).

En la economía agrícola es tan importante que está contemplada dentro de los planes de reactivación agrícola trazados para este año por el Ministerio de Agricultura, bien sea como sembrado principal o asociado con maíz.

"Es un cultivo de pequeños productores campesinos quienes, para el tratamiento de plagas y enfermedades, atienden cualquier recomendación del vecino", dice Anthony Bellotti, líder del proyecto de yuca del Centro Internacional de Agricultura Tropical (Ciat).

El comentario del científico obedece, específicamente, a dos plagas que atacan de forma severa a las 1,9 millones de hectáreas sembradas anualmente en Colombia: el chinche de la viruela y el gusano cachón.

Precisamente, sobre éstas, Bellotti mostró el resultado de 20 años de investigación en el Ciat: los hongos *Metharizium* y *Beauveirus*, específicos para el control de estas dos plagas.

MATAS DE YUCA sanas, sin problemas de plagas o enfermedades, es lo que busca el Ciat.

El Chinche es la plaga más temida por los cultivadores, ya que al vivir bajo suelo, es difícil percatar el daño que le están haciendo a los tubérculos de la mata.

Además, ha ocasionado el abandono de las tierras dedicadas a la yuca y el cambio de los sitios de siembra.

Por lo anterior, en alianza con el Ministerio de Agricultura y una empresa agroindustrial de Medellín, el Ciat concluyó su investigación, que dio como resultado una variedad del hongo llamado *Metharizium* que ataca exclusivamente a esta plaga.

"El insumo ya está formulado, tiene una etiqueta comercial y la empresa antioqueña Biocaribe lo lanzará al mercado durante este segundo semestre con el nombre comercial de Bioriziun", anunció el científico.

EL GUSANO CACHÓN

Los entomólogos (quienes estudian los insectos) conocen al gusano cachón como *Erinys ellose*. Es una larva de gran tamaño, de color verde o castaño, que consume vorazmente las hojas hasta dejar las plantas totalmente defoliadas, con un efecto tan devastador, que en tres días puede acabar con un cultivo.

Los yuqueros, regularmente, controlan al gusano con insecticidas químicos (como Carbaril o Malatbion), mientras que otros han propuesto los insecticidas biológicos formulados con el hongo *Bacillus thuringiensis* y con la avispa llamada *Trichogramma*, que parasita los huevos y larvas del gusano.

El Ciat, por su parte, propone el hongo *Baculovirus*, específico para esta plaga, como su resultado de la investigación de 15 años, lo mismo que un insecticida que el mismo agricultor puede preparar.

"El primero es un producto que ya se consigue en el mercado colombiano con el nombre de Bioviruas.

"Para el segundo, basta recolectar los gusanos (a los que les encanta colgarse de las ramas de yuca), licuarlos disuelto en agua, pasar 'la colada' por un cedazo y aplicarla sobre las hojas de los cultivos.

"Éste es un plaguicida casero altamente efectivo, aunque no es muy agradable utilizar la licuadora de la casa para hacer jugos de gusanos", concluyó el ingeniero agrónomo, Carlos Julio Herrera, quien es asistente de investigación del proyecto de yuca del Ciat.

Figure 4. (Photo of Newspaper article and field day).

Cassava Burrower Bug

The cassava burrow bug, *Cyrtomenus bergi*, causes direct damage to cassava roots and is a serious pest in Colombia and several other countries in the region (Panama and Costa Rica). It attacks cassava roots by inserting its thin, strong stylet through the root peal and into the parenchyma, where it feeds. In so doing, it acts as a vector of several root rot pathogens (*Fusarium, Phytophthora*, etc.) that cause black to brown root rot lesions on the fleshy, white parenchyma. This damage greatly reduces the commercial value of the root and can cause cassava fields and plantings to be abandoned. As a soil pest, spending most of its life cycle below ground, it can be very difficult to control. The use of toxic soil pesticides can reduce root damage, but they are costly and environmentally hazardous. *C. bergi* is a multi-host pest, attacking and damaging several other corps including onion, peanut and asparagus. During the past year severe attacks of *C. bergi* were recorded on the maize crop in the Cauca Valley **(Figure 5)**. Large patches or fields of maize can be severely damaged causing 100% plant loss.

Figure 5. A commercial maize field with *C. bergi* damage in the Cauca Valley, Colombia (2003).

Approximately 35 isolates of fungal entomopathogens have been evaluated for *C. bergi* biological control (see CIAT PE-1 Annual Report 2002 for additional information). One isolate of the fungal entomopathogen *Metarhizium annisopliae* has resulted in a very good biocide activity, especially in laboratory and greenhouse studies. It has been evaluated at different contractions and formulations to give optimal control of *C. bergi*. BIOTROPICAL is preparing a commercial product for release in the near future **(Figure 6).**

Figure 6. Two isolates of preformulated *Metarhizium annisopliae* in powder form for *Cyrtomenus bergi* control. *C. bergi* attacked by *M. annisopliae* and BIOTROPICAL product label. Note credits to MADR and CIAT.

Contributor: Carlos Julio Herrera, Anthony C. Bellotti.

Activity 6. Preliminary and basic studies of the whitefly predator *Chrysoperla carnea* (Stephens) (Neuroptera: Crysopidae).

Biological control has been a major component in the strategy to control cassava pests. Traditionally farmers have employed applications of pesticides for whitefly control across several crops. Pesticide use on cassava, in general, has been minimal. However, for whitefly control, cassava farmer have often resisted to the use of toxic pesticides. It has been documented that the use of pesticides to control whiteflies can dramatically reduce the effectiveness of natural biological control, often resulting in increasingly higher whitefly populations. These tactics may have contributed to the high populations of whiteflies observed in recent years on cassava in several regions of Colombia, including the CIAT farm.

Research in biological control of whiteflies, has more recently, concentrated primarily on the identification and evaluation of parasitoids (see PE-1 Annual Reports, 2000, 2001, 2002; CIAT). Field studies and observations have also indicated that predators may play an important role in regulating whitefly populations. Predators in general, are less studied than parasitoids; it is often difficult to accurately measure the impact that predators have on insect population dynamics in field situations. One of the predators most often observed feeding on cassava whiteflies (especially the species *Aleurotrachelus socialis*) are crysopids (Neuroptera: Crysopidae). Crysopids are generalist predators, feeding on the eggs and immatures of numerous arthropod species. In some areas, they have been studied, mass reared commercially and released into different cropping systems. The objective of this laboratory study is to determine the efficiency of the predator *C. carnea* on the different instars of *A. socialis*.

Methodology: Studies were carried out in growth chambers and the greenhouse at CIAT (Temp 26°C and 67.5% RH). Whitefly adults and immatures were obtained from the *A. socialis* colony in the greenhouse (Var. CMC 40; Temp. 27±2°C, 60-70% RH). Adult *C. carnea* used in these studies were obtained from a commercial biocontrol company located in Palmira, Valle. The experimental design was completely randomized with five treatments and eight repetitions within each treatment. Each treatment corresponded to an *A. socialis* stage (egg, 3 nymphal instars, and pupae). Four male and four female *C. carnea* were released into each repetition/treatment. The experimental unit consisted of 500 cc plastic bottles with 2% nutrient agar. Cassava leaf discs containing 100 individuals of each developmental stage were placed on the agar in each plastic bottle. Adult *C. carnea* were released into each plastic bottle and consumption of the *A. socialis* development stages was recorded every four hours. A second experiment following a similar methodology as previously described (with 10 repetitions of each treatment) evaluated the consumption of *A. socialis* developmental stages by *C. carnea* larvae.

Instar preference for consumption by *C. carnea* was determined by placing cassava leaf lobes containing the targeted whitefly instars on a humid cotton bed in petri dishes (150 x 25mm). Third instar larvae of *C. carnea* were introduced into the center of the petri dish that contained all the aforementioned developmental stages. This methodology proved to be impractical as it caused excessive *C. carnea* larval mortality (probably due to entrapment in the humid cotton). Therefore, a second method was employed using potted cassava plants with small leaf cages, each infested with 20 whitefly adults. After 24 hours the adults were removed and the areas of

infestation marked. This procedure was carried out at 4, 7, 14 and 23 days to have all the *A. socialis* developmental stages available when *C. carnea* larvae were introduced.

To measure *C. carnea* oviposition the experimental unit consisted of a carton cylinder 8cm in diameter and 10cm long, the interior lined with white paper and a mesh top to allow aeration. A male and female *C. carnea* adult was introduced into each chamber (four days after emergence when oviposition is initiated). Daily, a cotton ball humidified with a commercial feeding media, was attached to the muslin mesh. Oviposition was evaluated every 24 hours.

Results and Discussion: No significant differences were found in the consumption of different instars of *A. socialis* by *C. carnea* **(Figure 1)**. No significant differences in consumption were observed between the nymphal and pupal stage. However, egg consumption was significantly different from that of nymphs and pupae **(Figure 1)**. Egg and nymphal consumption was measured by recording the time required for 50% consumption of the prey stage being offered. *C. carnea* adult required 80 hours to consume 50% of these nymphal instars and pupae and 77 hours to consume 50% of the eggs offered.

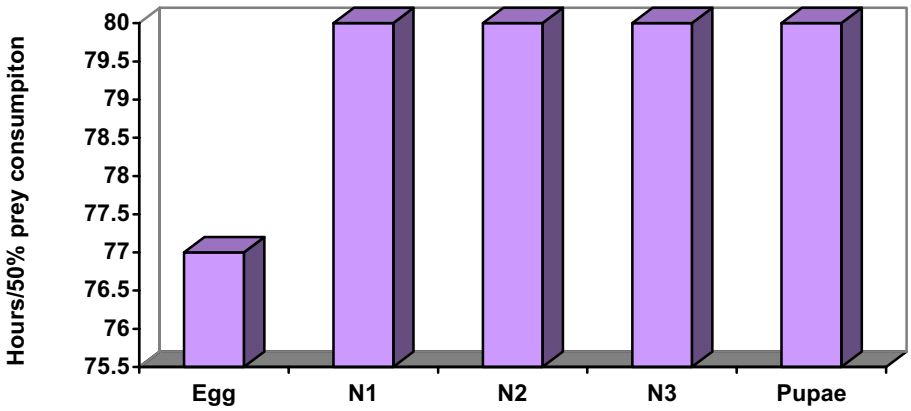

Figure 1. Consumption of *A. socialis* immatures by *C. carnea* (Neuroptera: Crysopidae) adults in laboratory studies (Duncan Multiple Comparison test at 0.05%).

Female *C. carnea* are slightly more voracious feeders of *A. socialis* immatures than are males. There was a significant difference in time required for females 78 hours) to consume 50% of the prey stage than males (80 hours) **(Figure 2)**.

The results for larval feeding of *C. carnea* were different from those of adult feeding. There resulted significant differences for *C. carnea* larval feeding on the different *A. socialis* prey instars **(Figure 3)**. *C. carnea* preferred feeding on first and second instars. 50% consumption of first instar nymphs occurred in about 30 hours compared to about 70 hours for second instar nymphs, 78 hours for third instar and 80 hours for forth instar. 50% of egg consumption occurred at about 75 hours **(Figure 3)**. It was also observed that most adult feeding was nocturnal, supporting evidence that the Crysopidae family is primarily nocturnal feeders (Hogan, 1970).

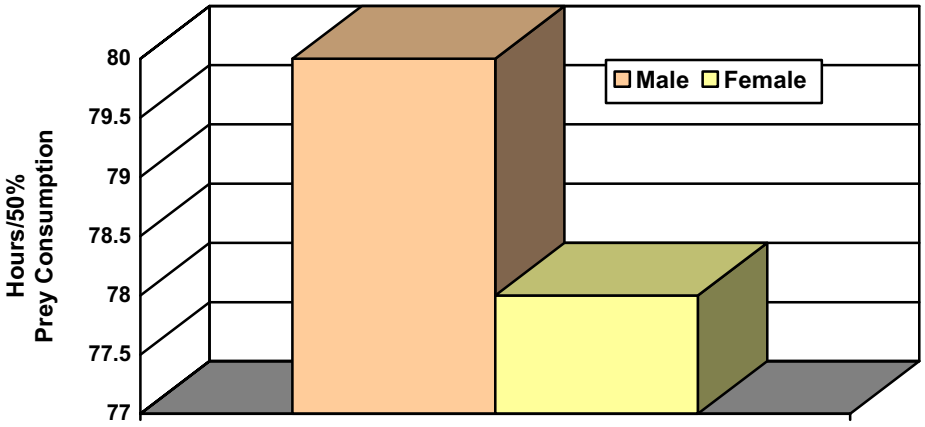

Figure 2. Consumption of *A. socialis* immatures by male and female *C. carnea* adults in laboratory studies.

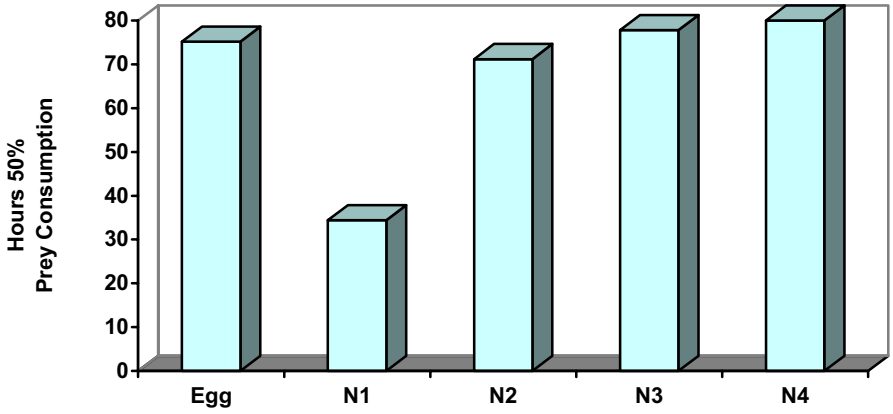

Figure 3. Consumption of *A. socialis* immatures by *C. carnea* larvae in laboratory studies (Duncan Multiple Comparison test at 0.05%).

It was observed that oviposition was initiated on the 4th day that adult *C. carnea* were introduced into the experimental units. Females lived on the average of 27 days but oviposition occurred primarily between the 4th to 12th day **(Figure 4)**. Between the 6th and 7th day oviposition peaked at a 19.5 average, while the overall average was 14.0 eggs per day during the 8 day period. Each *C. carnea* female oviposited an average of 112 eggs during its ovipositional period. This is considered low and may have been negatively influenced by the artificial diet that was offered.

In general, *C. carnea* larvae appear to be more efficient predators than adults. However, field releases are more easily achieved with adults. *C. carnea* displays a significant preference for *A. socialis* first instar larvae.

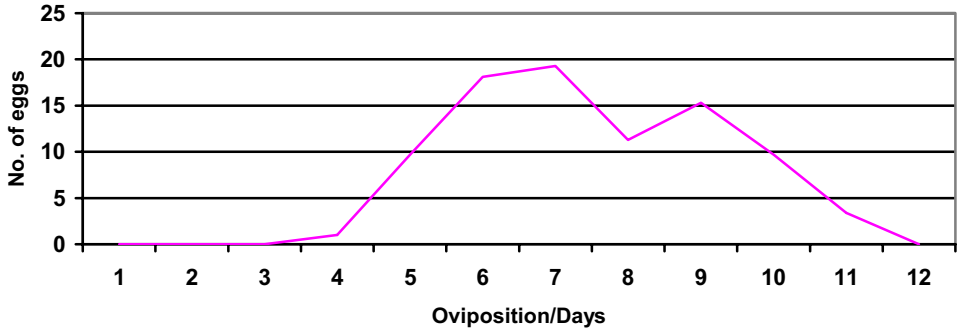

Figure 4. Oviposition of *C. carnea* adult females feeding on an artificial diet in laboratory studies.

Contributor: Claudia María Holguín, Luis Fernando Giraldo, Anthony C. Bellotti.

Activity 7. The evaluation of genetically modified cassava on the development and survival of the cassava hornworm, *Erynnis ello.*

The technology of genetic transformation by introducing gene sequences through bombardment of DNA particles has permitted the development of transgenic cassava varieties. This technology offers a potential tool to combat economically important and difficult to control cassava arthropod pests. The use of the *Bacillus turiginensis* (Bt) gene to produce crop resistance to insect pests, especially lepidopterans, has been highly successful and is well documented. This technology has been used successfully in crops such as maize, soybeans, cotton and others.

There are several lepidopteran pests of cassava, but the two most important are the cassava hornworm, *Erynnis ello* and the stemborer, *Chilomina clarkei*. Both pests are difficult to control; the former because of its migratory behavior and the latter because once it penetrates the cassava stem it is well protected from biological and chemical control measures. To control *C. clarkei*, CIAT has initiated research based on introducing insect-resistant Bt genes through *Agrobacterium*-mediated transformation into cassava embryonic tissue to develop resistant cultivars. Previous research has shown that commercial biopesticides containing *B. turiginensis* is effective in controlling *E. ello*. However, the availability of these biopesticides to small cassava farmers is unreliable, costly, and proper timing of application for most effective control is difficult to achieve.

A study was developed to evaluate the effectiveness of the BT gene in transgenic cassava to control the cassava hornworm. This research is being carried out in collaboration with the biotechnology project (SB-2). Results on the studies of the effect of Bt genes in transgenic cassava on *C. clarkei* development are reported in the SB2 Annual Report.

Methodology: Two cassava varieties, TMS L.55 and ICA Costeña were genetically modified with the BT gene, and were compared to two controls CMC 40 and TMS L.55, that were not genetically modified. The bioassay was carried out by removing young cassava leaves from the aforementioned plants and placing them in petri-dishes. Leaves were changed/replaced on a daily basis. First instar *E. ello* larvae were introduced into the experimental unit and evaluations on weight increment and mortality/survival were carried out over an 11-day period.

Results: A marked increase in *E. ello* larval weight occurred when feeding on CMC 40, in comparison with the three other treatments (Transgenic ICA Costeña and Transgenic and non-transgenic TMS-L.55 **(Figure 1 and 2)**. This difference was also observed in the amount of leaf tissue consumed. The dramatic larval weight increase when feeding on CMC 40 was expected as this variety is susceptible to most insects, including *E. ello*. ICA Costeña is also susceptible to *E. ello*, therefore the significantly reduced weight gain probably can be attributed to the presence of the Bt gene. These results, however, are inconclusive as non-transgenic ICA Costeña was not included in the treatments.

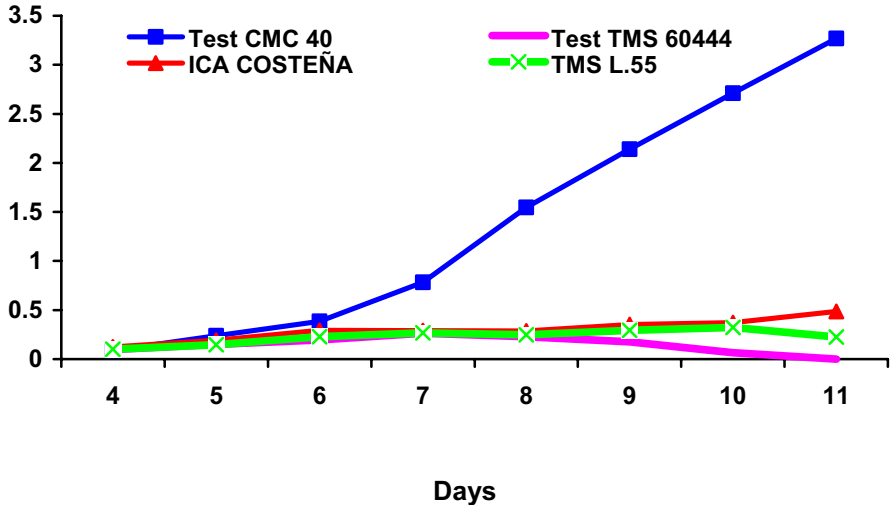

Figure 1. ***Erynnis ello*** **larval weight increments over time when feeding non-transgenic CMC 40 and TMS 60444 vs. feeding on transgenic (Bt) ICA Costeña and TMS-L.55.**

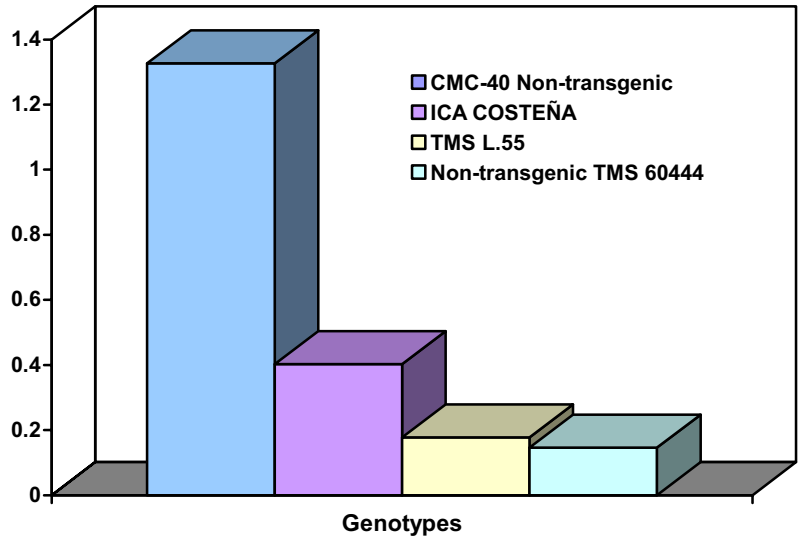

Figure 2. Average weight gain in grams of *Erynnis ello* first instar larvae feeding on non-transgenic CMC 40 and TMS 60444 vs. larvae feeding on transgenic ICA Costeña and TMS L-55 (AOV probability at 0.05 level).

The results with transgenic vs. non-transgenic TMS 60444 is most interesting and unexpected. Larval weight gain was equally low for both treatments **(Figure 2)** indicating that resistance to *E. ello* may exist in this cassava variety. Field observations and laboratory and field evaluations of cassava genotypes at CIAT over a period of 30 years has not previously indicated the presence of *E. ello* resistance in cassava germplasm.

The experiment was repeated using rooted five-week old potted plants of CMC 40 and transgenic and non-transgenic TMS 60444. The plants were placed in large cages and infested with third instar *E. ello* larvae. Results were even more dramatic as all larvae feeding on transgenic and non-transgenic TMS 60444 died within 72 hours after initiating feeding. Those larvae placed on CMC 40 continued feeding until plants were completed defoliated.

TMS varieties were bought to CIAT from IITA in Nigeria, and are resistant to Africa Cassava Mosaic Disease (ACMD). The origin of these varieties is an interspecific cross between *Manihot esculenta* (cultivated cassava) and *M. glasiovi*, a wild species. The resistance to ACMD comes from *M. glasiovi,* originating from crosses that were carried out during the 1930's. It is therefore possible, and quite feasible, that the *E. ello* resistance on display in TMS L.55 also originated from *M. glasiovi*. It has long been speculated that the wild *Manihot* species might contain resistant genes to numerous cassava pests and some preliminary research has been carried out (see Activity 6.9 CIAT IP-3 Annual Report 2003).

Continued research is underway to further explore the resistance to *E. ello* being observed in the genotype TMS L.55.

Contributor: Carlos Julio Herrera, Anthony C. Bellotti.

Activity 8. Publications, book chapters, posters, conferences, training and consultancies.

Publications

CALATAYUD P.-A.; POLANÍA M.A.; GUILLAUD J.; MÚNERA D.F.; HAMON J.C.; BELLOTTI A.C. 2002. Role of single amino acids in phagostimulation, growth, and development of the cassava mealybug *Phenacoccus herreni.* Entomol Exp Appl 104/2-3:363-367.

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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. Journal of Economic Entomology.

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TRUJILLO, H., ARIAS, B., GUERRERO, J., HERNANDEZ, P., BELLOTTI, A., J. E. PEÑA. Survey of parasitoids of whiteflies (Homoptera: Aleyrodidae) in cassava growing regions of Colombia and Ecuador. Florida Entomologist.

CORTÉS, M.L., T. SÁNCHEZ, L. RIIS, A.C. BELLOTTI, P.-A. CALATAYUD. A bioassay to test HCN toxicity to the burrowing bug *Cyrtomenus bergi*. Entomologia Experimentalis et Applicata 108.

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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.

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

Posters

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Book Chapter

Bento, J.M.S., G.J. de Moraes, A.P. de Mattos, J.F. Warumby e A.C. Bellotti. 2002. Controle biológico da cochonilha da mandioca no nordeste do Brasil. ***In:*** Controle Biológico No Brasil. Eds. J.R. P. Parra, P.S.M. Botelho, B.S. Corrêa-Ferreira, J.M.S. Bento. Editora Manole Ltda. 2002. Sao Paulo, Brasil, pp 395-408.

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Burbano, M., M. 2003. Multiplicación de material de especies silvestres y domesticadas del género Manihot y estudio de su resistencia natural a tres plagas de cultivo (*Mononychellus tanajoa, Aleurotrachelus socialis,* y *Phenacoccus herreni*) en condiciones controladas de temperatura y humedad relativa. Thesis (Biologist). Universidad del Valle, Facultad de Ciencias, Cali, Colombia. 107 p.

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Carabalí, A. 2003. Evaluación del potencial de resistencia/tolerancia de diferentes genotipos de yuca *M. esculenta* Crantz al biotipo B de *B. tabaci* de mosca blanca *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). Thesis MsC. Sciences-Biology, Universidad del Valle, Cali, CO.

Gómez S., M.J. 2003. Caracterización de genotipos de yuca (*Manihot esculenta* Crantz) por su resistencia a mosca blanca *Aleurotrachelus socialis* Bondar (Homóptera: Aleyrodidae). (Agronomist Engineer). Universidad Nacional de Colombia, Palmira, CO.

Training and Consultancy Services offered during 2003

Organizer	Place	Date	Participants	Received by	Service
MADR – CIAT	Santander de Quilichao (Cauca)	August 2002	9	Farmers	Integrated pest management practices for the whitefly and frogskin disease
ESPE (Escuela Superior Politécnica del Ejército de Quito-Ecuador/CIAT)	CIAT	30 Aug.-13 Sept. 2002	17	ESPE (Escuela Superior Politécnica del Ejército de Quito)	Biological control; integrated pest management (IPM) in cassava and beans; conferences combined with laboratory, field and greenhouse studies, and practical techniques; visits to biopesticide laboratories (Laboratorios Laverlam and Productos Biológicos PERKINS)
CLAYUCA-MADR-CIAT	Bucaramanga	3-6 Sept. 2002	40	Technicians, professionals, and local cassava farmers	Intensive training course in modern cassava production, processing, and usage systems in Colombia
MADR-CIAT	Suárez (Cauca)	19 Nov. 2002	30	Technicians and local farmers	Workshop on pest management in cassava
Universidad de Caldas	CIAT	27 Nov. 2002	34	Students	Biological control; integrated pest management (IPM) in cassava; visits to laboratories, field plots, and greenhouses
MADR-CIAT	Popayán (Cauca)	29 Nov. 2002	40	Technicians, professionals, and local farmers	Cassava crop management

Organizer	Place	Date	Participants	Received by	Service
ICA-Bogotá	CIAT	4-9 Dec. 2002	1	Alcibíades Suárez	Mites and taxonomy of whiteflies
CIAT	CIAT	20-26 February 2003	5	María P. Quintero, Ana M. Caicedo, Irina Aleán, Cristian Olaya, Elsa L. Melo	Workshop on Identification of Entomoparasitic Nematodes: General Management Issues
CLAYUCA	Puerto Asís-Putumayo	4-8 March 2003	36	Technicians, professionals, local farmers, UMATA officials, officials of the Secretary of Agriculture, NGO officials	Seminar on Integrated Pest Management
CORPOICA-Tibaitatá	Bogotá	17-21 March 2003	1	Dr. Juan Alberto Arias, Corpoica researcher	Workshop on Baculovirus Purification Techniques
CIAT	CIAT	18 February 2003	4	Professor and postgraduate students in science-related areas of the Universidad del Valle	Taxonomy and identification of whiteflies
ICA and Fundación Huairasachac	Puerto Asís, Putumayo	10-12 April 2003	25	Agronomist, zootechnicians, and extension workers of NGOs	Integrated pasture management
Universidad de Caldas	CIAT	21 April 2003	6	Postgraduate students in entomology	Biological control applied to cassava
Universidad de Caldas	CIAT	15 May 2003	25	Agronomy students	Cassava entomology and pest control
CIAT/ Dr.Ralf- Udo Ehler, Kiel University, Germany)	CIAT	16-20 June 2003	20	Scientists/research assistants/students of the CIAT IPM Unit and other external entities	Use of entomopathogenic nematodes
CIAT, Rural Agroenterprises Development Project Agroenterprises	CIAT	7-10 July 2003	3	Visitors from Honduras, government officials	Biological control applied to cassava
CIAT Human Resource Development Fund	CIAT	7-11 July 2003	1	Research assistant of the CIAT IPM Unit, Carlos Julio Herrera	How to design and manage successful research projects

Organizer	Place	Date	Participants	Received by	Service
CIAT	Cali - XXX Congreso Socolen	17-19 July 2003	8	Researchers of the CIAT IPM Unit	Presentation of papers/posters:
Universidad de Antioquia	CIAT	15 August 2003	15	Students of the University's Biology Institute	Microbiological Control of Cassava Pests
CIAT	CIAT	27 August 2003	1	Edwin Iquize, Visitor from Bolivia	Training in sampling techniques to analyze the incidence and population fluctuations of the spittlebug and soil arthropods
CIAT	CIAT	28 August 2003	1	Edwin Iquize, Visitor from Bolivia	Visit to CIAT's experiment station in Santander de Quilichao. Training in sampling techniques
ESPE (Escuela Superior Politécnica del ejército de Quito-Ecuador); CIAT	CIAT - IPDM Unit	17-19 Sep. 2003	3	ESPE (Escuela Superior Politécnica del Ejército de Quito-Ecuador)	Integrated management of the whitefly, the cassava hornworm, entomopathogens and mites
The Egyptian International Centre for Agriculture	The Egyptian International Centre for Agriculture	10 July 25 Sept., 2003	1	María del Pilar Hernández de IPM-Entom. Yuca	Training Course on Integrated Pest Management

Collaborators:
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Donor Institutions
USAID
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DFID
Ministerio de Agricultura y Desarrollo Rural – MADR, Colombia
IRD - France
Federal Ministry for Economic Co-operation and Development (BMZ)

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IRD, France
CNPMF, EMBRAPA, Brazil
IAC, Sao Paulo, Brazil
USDA, Beltsville MD, Fort Pierce, FL - 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, Bogotá, 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
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