CASSAVA ENTOMOLOGY

Activity 1. Arthropod taxonomic activities on CIAT commodity crops.

Taxonomy: The science dealing with the identification, naming and classification of organisms, is a vital component in a pest management program. Identification and classification is the basic approach to any scientific endeavor, and it is often critical to the success of pest management activities. An inaccurate identification of a pest organism can result in an acute loss of time and resources and delay the most appropriate response to pest attack and damage. An example of this is the misidentification between the cassava mealybug *Phenacoccus manihoti* and *P. herreni*; this delayed, by four years, the discovery of *P. manihoti's* key natural enemy, the parasitoid, *Apoanagyrus lopezi*, and its subsequent introduction into Africa. Accurate crop pest and natural enemy identification is an essential component in a pest and disease management program.

Insect systematics is considered, by some authors, to have begun with the work of Aristotle, who included the Entoma as a subdivision of the Anaima (invertebrates). Within the Entoma Aristotle place the Arthropoda (excluding Crustacea), Echinodermata, and Annelida (Gillott, 1980). For more than 2000 years hence, the identification and classification of arthropods, has been based primarily on external or morphological structures. Some use had also been made physiological, developmental, behavioral, and cytogenetic data.

In more recent years, the application of molecular techniques have played an increasingly influential role in the accurate identification of arthropod species and equally important, of biotypes. CIAT has recently implemented the use of molecular techniques, based on PCR, especially for the identification of whitefly species, their biotypes and natural enemies. These techniques can offer an accurate, rapid and relatively low cost method for identifying critical species or complexes.

The CIAT IPDM Project (PE-1) provides the service of identifying arthropod pests collected from various crops, but especially to CIAT's mandate crops and related activities. The project maintains a working collection of arthropod pests and their natural enemies for cassava, beans, rice, tropical pastures and tropical fruits, as well as those collected from related agroecosystems, such as vegetables, legumes, forest environments and others. A database containing information on the individual specimens accompanies the collection and this is made available to collaborating institutions, museums, universities and national research and extension programs. In addition, the project biologist/taxonomist maintains contact with more than 40 recognized taxonomists around the world and relies on their assistance in pest and natural enemy identification.

A major activity of the project is the CIAT convened "System wide Tropical Whitefly IPM Project (TWF/IPM)." CIAT provides taxonomic support for whiteflies and their natural enemies collected from different agroecosystems in the neotropics. Whitefly specimens from participating collaborators from numerous countries (approximately 15 in the Americas) are continuously being sent to us for identification. CIAT also maintain a capacity in molecular taxonomic techniques based on PCR for arthropod (especially whiteflies) identification. These techniques

offer a rapid method for the precise identification of critical species or species complexes (including biotypes) that are often morphologically indistinguishable during one of its life stages.

During the past year, numerous insect and mite species were added to the collection, which now numbers more than 20,000 specimens. The services provided, including access to the database, contributes to numerous CIAT and national program activities. During 2003-04 we continued collecting homopteran species that may be associated with Cassava Frogskin Disease. A description of these collections and identifications are reported in Activity 7. In addition surveys were initiated to determine the Chrysopidae species associated with cassava arthropod pests. The Chrysopidae constitute an important group of generalist predators that are often employed in biological control projects. The results of these surveys are reported in Activity 2. A description of some of the additional activities follows.

Project 1 – Whiteflies

Objective: Process and identify whitefly specimens collected in El Salvador and Mexico.

Methodology

El Salvador: CIAT's TWF/IPM project received 137 whitefly samples collected from the "Valle de Zapotitan" in El Salvador (C.A.). Samples were sent by Ing. Agr. Leopoldo Serrano Cervantes of the Departamento de Protección Vegetal (Facultad de Ciencias Agronómicas, Universidad de El Salvador) during December 2003 and processed during 2004.

Twenty, non-parasitized, pupae were selected from each sample; 10 of these were used to determine biotype (Virology Laboratory), and 20 were imbedded in microscope slides for morphological identification.

Results: *Bemisia tabaci* (Gennadius) was the whitefly species identified in 135 of the samples received. These were collected from 17 plant hosts **(Table 1)**. The specimens were mounted on 585 slides and included in the CIAT whitefly collection. Plant hosts included vegetables, legumes, melons, squashes and potato, indicating the wide host range of this pest species.

The two remaining samples were identified as *Aleurocybotus occiduus* Russell, collected from sorghum and maize in Chalatenango (Nueva Concepción, El Salvador).

México: Whitefly specimens were received from Tula, Tamaulipas, México and collected from *Lycopersicon esculentum* (Tomato) and *Menta piperita* (mint). Specimens were sent by Dr. Raul Díaz, INIFAB-CIR Sureste Campo Experimental Mococha (Merida, Yucatán, México). These specimens were identified as *Bemisia tabaci* and *Trialeurodes vaporariorum*.

Table 1. Plant hosts of the whitefly *Bemisia tabaci* collected in Valle de Zapotitan, El Salvador (December, 2003).

Crop	Scientific Name
Squash	*Cucúrbita moschata*
Eggplant	*Solanum melongena*
Broccoli	*Brassica oleracea*
Sunnehemp	*Crotaliaria guatemalensis*
Sweet pepper	*Capsicum annum*
Beans	*Phaseolus vulgaris*
Guisquil	*Sechium edule*
Mint	*Menta piperita*
Loroco	*Fernaldia pandurata*
Melon	*Cucumis melo*
Okra	*Hibiscus esculentus*
Potato	*Solanum tuberosum*
Cucumber	*Cucumis sativus*
Pipian (Squash)	*Cucúrbita mixta*
Cabbage	*Brassica oleracea capitara*
Watermelon	*Citrullus lanatus*
Tomato	*Lycopersicon esculentum*

Project 2 - Mites

Phytophagous mites are an important pest of cassava and other CIAT related crops. Mite collections from cassava have been carried out for nearly 30 years on cassava and the CIAT collections consists of more than 10,000 specimens mounted on microscope slides. These extensive collections provide a unique description of the complex of phytophagous mites associated with cassava, their geographic distribution, alternate hosts and natural enemies. All the aforementioned information is contained in the CIAT maintained database.

During 2003-2004 mite collections were carried out in numerous sites in Colombia and Ecuador on cassava, avocado, coffee, citrus, guanábana (custard-apple), mango, coconut, guava, madroño, sweet potato, rice, ornamentals, and others **(Table 2)**. Many of these identifications were done at the request of national institutions such as ICA (El Instituto Colombiano Agropecuario), CENICAFE (El Centro Nacional de Investigaciones del Café), INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuaria), Estación Experimental Portoviejo, Ecuador. CIAT's expertise in acarology, especially in the identification of phytophagous mites and the mite predators, Phytoseiidae, is recognized in many countries in the Americas.

As can be observed in Table 2, an impressive diversity of phytophagous mites were collected from numerous hosts. Samples #2634 and 2635, with the species *Tetranychus urticae* and *Mononychellus tanajoa* respectively (both collected from cassava) contained the presence of the entomopathogen fungus, *Neozygites* sp. This fungus is often observed as an important natural enemy of cassava mites.

The mite *Steneotarsonemus spinki* Smiley (Fam. Tarsonemidae) is reported causing damage to the rice crop in Cuba (Ramos et al, 2001), and more recently in Costa Rica. This species has not yet been reported in Colombia but it is important to monitor rice fields throughout the country to

identify species within this family. Samples # 2653 from Santa Roca (Villavicencio) and #2654, from CIAT, Palmira are identified as Tarsonemidos, found in the panicle of sparkled rice grains. Taxonomic observations indicate that these mites are *Tarsonemus bilobatus*, a species reported as mostly feeding on fungus spores.

Table 2. Phytophagous mites collected from cassava and other hosts from Colombia and Ecuador.

Sample	Country	Department	Municipality	Site	Host	Species
2622	Colombia	Valle	Ginebra	Ginebra	Guava	*Eriophyidae' mites*
2623	Colombia	Tolima	Espinal	Toluva	Basil	*Poliphagotharsonemus latus*
2627	Colombia	Valle	Cali	Cali	Chiminango	*Eotetranychus sp*
2628	Colombia	Atlántico	Sto. Tomas	El Esfuerzo	Coconut	*Eriophyidae'mites*
2629	Colombia	Caldas	Palestina	Montelindo	Avocado	*Allonychus braziliensis*
2631	Ecuador	Manabí	Portoviejo	Teodomira	Cassava	*Mononychellus caribbeanae* *Oligonychus peruvianus*
2632	Ecuador	Manabí	Portoviejo	E.E.INIA	Cassava	*Mononychellus caribbeanae*
2633	Colombia	Valle	Palmira	CIAT	Sweet potato	*Tetranychus ludeni*
2634	Colombia	Valle	Palmira	CIAT	Cassava	*Tetranychus urticae**
2635	Colombia	Valle	Palmira	CIAT	Cassava	*Mononychellus tanajoa**
2636	Ecuador	Pichincha	Quinche	Chivan	Rose	*Eotetranychus sp*
2637	Colombia	Valle	Palmira	CIAT	*Mutinga sp*	*Tetranychus urticae*
2638	Colombia	Valle	Palmira	Potrerillo	Madroño	*Tuckerella sp*
2639	Colombia	Caldas	Chinchiná	CENICAFE	*Mirabilis sp*	*Poliphagotharsonemus latus*
2640	Colombia	Caldas	Chinchiná	CENICAFE	Pasture	*Phyllocoptes sp*
2641	Colombia	Caldas	Chinchiná	CENICAFE	Coffee	*Oligonychus yothersi*
2642	Colombia	Caldas	Palestina	Montelindo	Citrus (orange)	*Oligonychus gossypi*
2643	Colombia	Caldas	Palestina	Montelindo	Avocado	*Allunychus braziliensis*
2644	Colombia	Caldas	Palestina	Montelindo	Plantain	*Oligonychus punicae*
2646	Colombia	Atlántico	Sabanalarga	Jalapa	Sweet potato	*Tetranychus ludeni*
2647	Colombia	Magdalena	Tamalameque	Tamalameque	Cassava	*Mononychellus tanajoa*
2648	Colombia	Magdalena	Ciénaga	Ciénaga	Cassava	*Mononychellus tanajoa*
2650	Colombia	Valle	Cali	Cali	Guanábana	*Tetranychus sp.*
2651	Colombia	Valle	Palmira	CIAT	Custard-apple	*Oligonychus yothersi*
2652	Colombia	Valle	Palmira	CIAT	Guasimo	*Allonychus reisi*
2653	Colombia	Meta	Villavicencio	Santa Rosa	Rice	*Tarsonemus sp*
2654	Colombia	Valle	Palmira	CIAT	Rice	*Tarsonemus sp*

* Mites with presence of *Neozygites* pathogen.

Other Collections

Ecuador: Twenty-eight arthropod samples collected from *Citrus sinensis* in Ecuador were sent to CIAT for identification. Two samples contained the whitefly *Bemisia tabaci*. Samples were collected and sent by Oswaldo Valarezo (Departamento Nacional de Protección Vegetal, INIA).

Colombia: The parasitoid *Leptomastix dactylopii* Howard (Encyrtidae) was identified parasitizing the guanabana (custard-apple) mealybug by the Natural History Museum, London. The parasitoid was collected in the municipality of Toro, Valle del Cauca. The identification was done for AGRONILO.

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

Activity 2. Chrysopidae species associated with arthropod pests of cassava (*Manihot esculenta* Crantz).

Predators in the Chrysopidae (Neuroptera) family have been successfully produced commercially to control numerous arthropod pests in diverse cropping systems. They have been observed in abundant numbers in cassava agroecosystems, but their role and impact in control of cassava pests, such as the whitefly, *Aleurotrachelus socialis*, is not well studied nor documented. It was therefore decided to survey cassava fields in different regions of Colombia to determine the species of *Crysopa* present. Methodologies for the laboratory rearing of the most frequently collected species were also investigated.

Objective: Determine the Chrysopidae species present in the cassava cropping systems in different regions of Colombia and develop methodologies for rearing key species.

Methodology: Explorations of cassava fields for chrysopid species were accomplished in the Colombia departments of Tolima, Cauca, Valle del Cauca, Risaralda and Quindío. Sampling was done by systematically sweeping cassava fields with an entomological sweep-net, capturing chrysopids present. Captured individuals were removed from the net and placed in plastic containers with a cap containing nylon mesh (for aeration). Orthodonical cotton plugs were impregnated with a diet consisting of water, honey, sugar and pollen, and attached to the nylon mesh on the cap **(Figure 1)**.

Subsequently, individual females were removed to the laboratory and placed in rearing units consisting of a cylindrical PVC tube interiorly lined with a black smooth pasteboard for oviposition. The upper and lower ends of the tube are covered with a nylon mesh where water and the aforementioned diet are made available on a daily basis **(Figure 1b)**. It was thereby made certain that larvae obtained from the same female were sent for identification.

Every second day, eggs were collected from each female by removing the pasteboard from the PVC tube **(Figure 1c)**. Eggs were then placed in lid-aerated petri dishes **(Figure 1d)**. Crysopa larvae were supplied daily with *Sitrotroga cerealella* eggs.

When the larvae arrived at the forth instar, four to five individuals were submerged in hot water for one minute, then bathed in a 10% KOH solution for 20 minutes, and finally transferred to 75% alcohol. These larvae, along with their respective adult were labeled and sent to Catherine Tauber and Maurice Tauber at Cornell University in Ithaca, NY, USA, for identification.

Results: Ten species of Chrysopidae were collected from cassava in the departments sampled **(Table 1)**. Five species have been identified to genus: *Ceraeochrysa* sp. #1, *Ceraeochrysa* sp. #2, *Leucochrysa* sp., *Leucochrysa* (Nodita) sp. #2 and *Leucochrysa* (Nodita) sp. #4. Two species were not previously reported from Colombia, *Ceraeochrysa valida* (Banks) and *Chrysopodes* prob. *Lineafrons* Adams and Penny. The three most frequently collected species were *Ceraeochrysa cubana* (Hagan), *Ceraeochrysa claveri* (Navás) and *Chrysoperla externa* (Hagan).

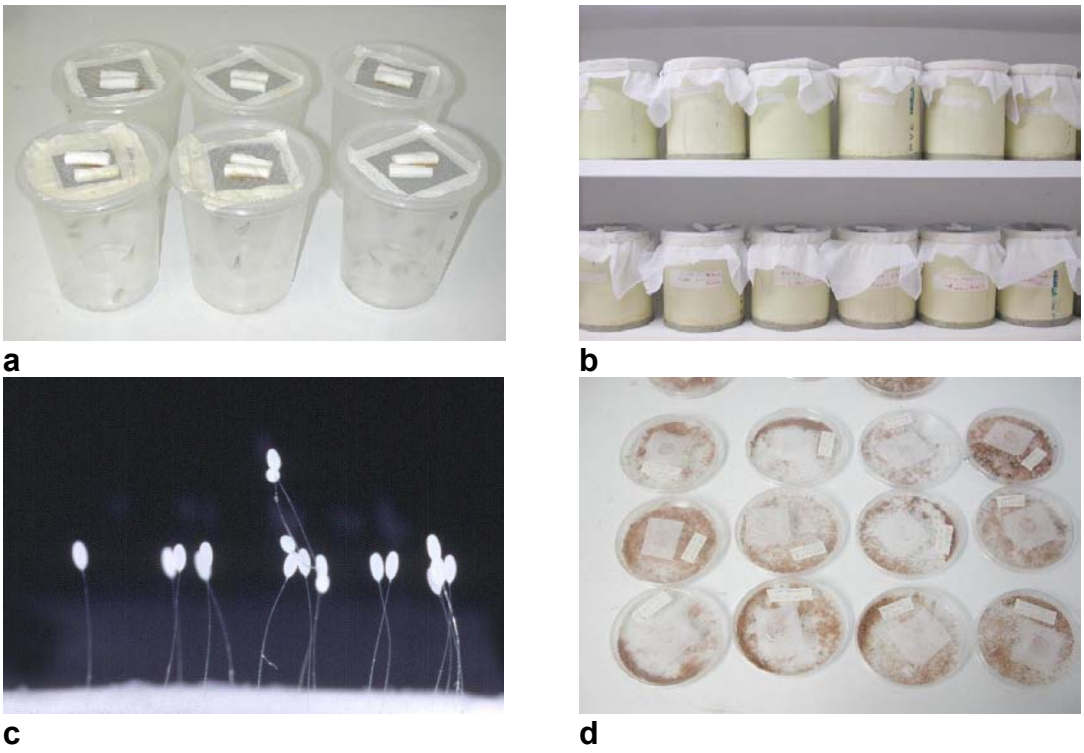


Figure 1. (a) Recipients for transporting Chrysopidae specimens collected in the field. (b) PVC rearing units for individual females. (c) Chrysopa eggs removed from the rearing units. (d) Chrysopid larvae feeding on *Sitotroga cerealella* eggs.

Table 1. Chrysopidae species collected from cassava fields in Colombia.

Department Sampled	Chrysopidae Species Collected
Cauca	*Ceraeochrysa cubana* (Hagen)
	Ceraeochrysa claveri (Navás)
	Chrysoperla externa (Hagen)
Valle del Cauca	*Ceraeochrysa cubana* (Hagen)
	Ceraeochrysa sp. #1
	Ceraeochrysa claveri (Navás)
	Leucochrysa (Nodita) sp. #4
	Chrysoperla externa (Hagen)
	Leucochrysa sp.
Tolima	*Ceraeochrysa cubana* (Hagen)
	Ceraeochrysa claveri (Navás)
	Ceraeochrysa valida (Banks)
	Ceraeochrysa sp. #2
	Chrysoperla externa (Hagen)
	Ceraeochrysa sp. #1
Risaralda	*Ceraeochrysa claveri* (Navás)
	Ceraeochrysa cubana (Hagen)
	Leucochrysa (Nodita) sp. #2
	Chrysoperla externa (Hagen)
Quindío	*Ceraeochrysa claveri* (Navás)
	Chrysoperla externa (Hagen)
	Ceraeochrysa cubana (Hagen)
	Chrysopodes prob. *lineafrons* Adams & Penny
	Leucochrysa (Nodita) sp. #4
	Ceraeochrysa valida (Banks)

Chrysopidae species are taxonomically differentiated using multiple morphological characteristics such as: antenna length in relation to wing extension, wing markings or spotting, banding on the dorsal part of the thorax and abdomen, genal spotting, seta and markings on the pronotum, color and spots present on the vertex, spots on the shaft, among others **(Figures 2 to 7)** (López-Arroyo et al, 1999); Núñez, 1998; Tauber et al, 2000.

At present colonies of the most frequently collected species are being established in the laboratory. These species are being collected from fields at the CIAT station in Santander de Quilichao and Palmira and from the coffee zone (Risaralda and Quindío). The same methodology is being carried out with the species *Chrysoperla carnea*, produced in commercial laboratories. Methodologies are being standardized.

Conclusions: The species *Ceraeochrysa* sp. #1, *Ceraeochrysa* sp. #2, *Leucochrysa* sp., *Leucochrysa* (Nodita) sp. #2 and *Leucochrysa* (Nodita) sp. #4, are presently being described. The species *Chrysopodes* prob. Lineafrons and *Ceraeochrysa valida* were not previously reported from Colombia.

C. cubana, C. claveri and *C. externa* were most frequently found in field collections and therefore a laboratory colony of each has been established for future bioassays.

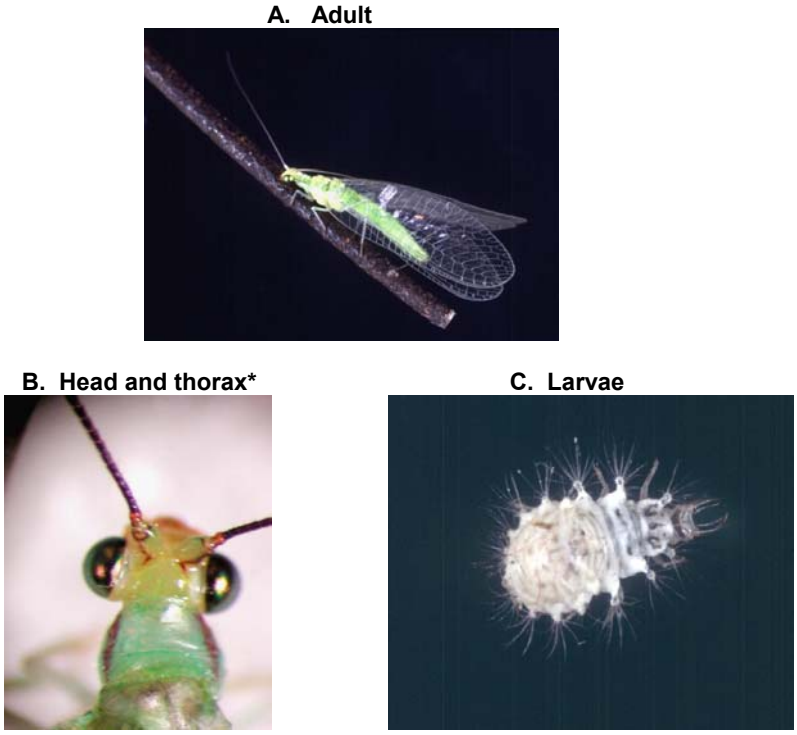


Figure 2. ***Ceraeochrysa claveri*** **(Navás). Characteristics: First–third of antenna darkened, antenna longer than wing extension, reddish-brown lateral spots on the pronotum, lengthening of spots on the shaft, usually on the vertex, palpi clear.**

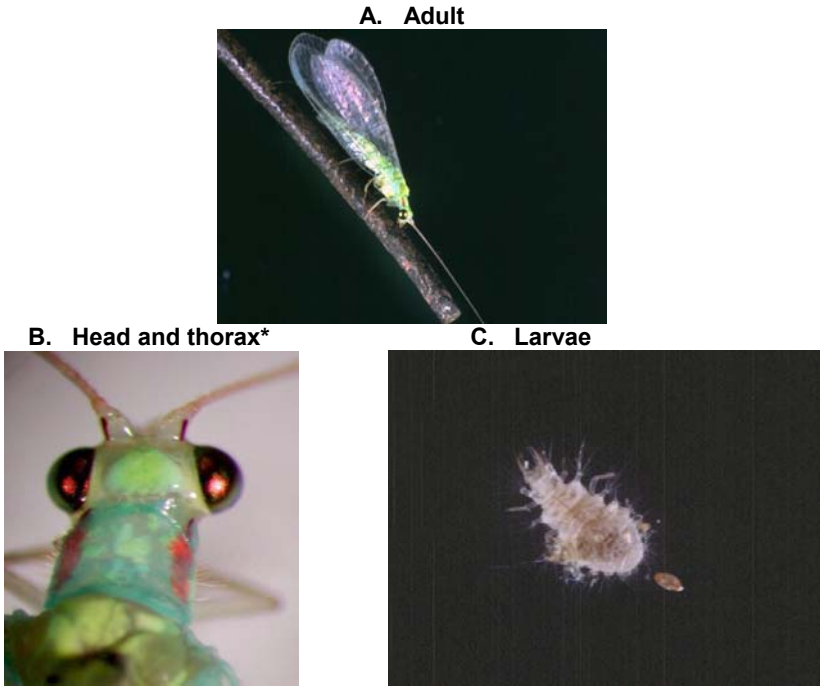


Figure 3. ***Ceraeochrysa cubana*** **(Hagen). Characteristics: Antenna clear and as long as the wing extension thin spots on the shaft, lateral spots with spreading edges, wings with transversal light brown veins, palpi clear.**

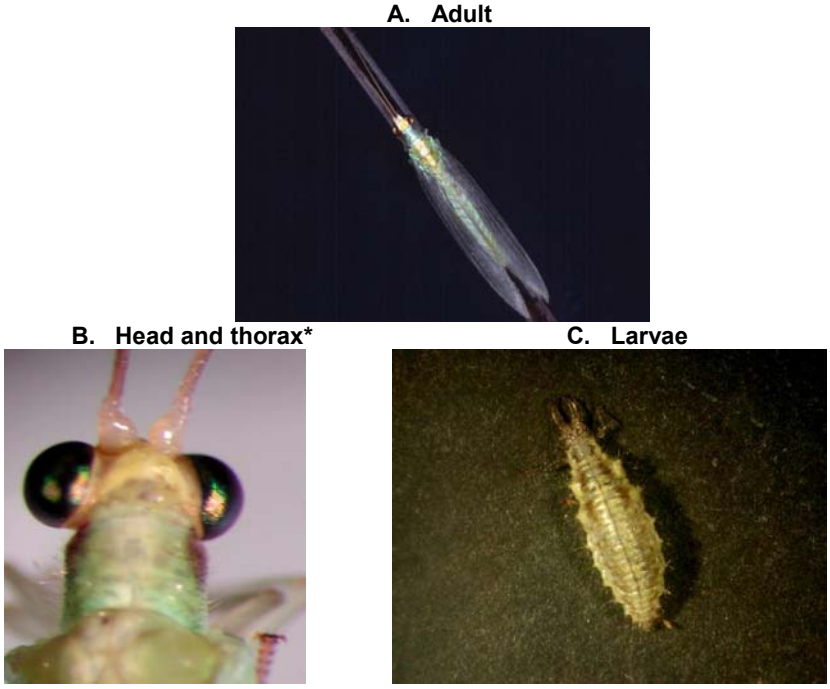


Figure 4. ***Chrysoperla externa*** **(Hagen). Characteristics: Antenna shorter than wing extension, yellow bands on the dorsal part of the thorax and abdomen, dark genal spots, pronotum with clear seta originating from dark base, red spotting near the ocular cavity.**

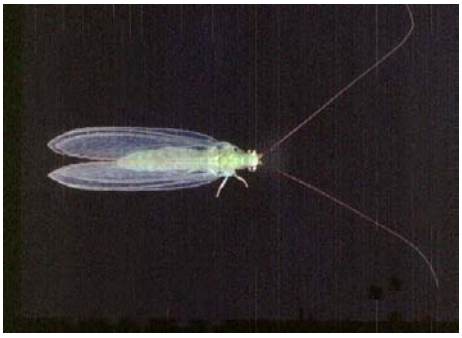
A. Adult

B. Head and thorax*

C. Larvae

Figure 5. ***Chrysopherla carnea (Stephens).*** **Characteristics: Antenna shorter than the wing extension, yellow bands on the dorsal part of the thorax and abdomen, dark brown genal spots, pronotum with thick seta, blackened at the base.**

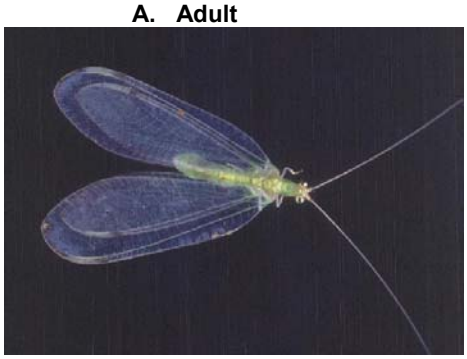
A. Adult



B. Larvae

Figure 6. ***Ceraeochrysa sp.*** **#2. Characteristics: Antenna clear with the first 10 segments darker and as long as the wing extension reddish-brown lateral spots on the pronotum, dorsal end of the shaft without spots.**

A. Adult



B. Larvae

Figure 7. ***Leucochrysa sp.*** **#2. Characteristics: Antenna equal to or longer than wing extension, dark spots on the distal third of the wing, four spots on the pronotum that may coalesce to form two lengthening lines.**

Photos by Cristian Olaya.

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Contributors: Claudia M. Holguín, Carmen Elisa Mendoza, Adriano Muñoz, Anthony C. Bellotti.

Activity 3. Laboratory studies on the biology of *Ceraeochrysa claveri* (Neuroptera: Chrysopidae) feeding on two prey hosts.

During explorations to determine the Chrysopidae species associated with cassava arthropod pests in different regions of Colombia, *Ceraeochrysa claveri* was one of the species most frequently collected. Owing to the limited information available on the biology, ecology and behavior of this predator, research was initiated to determine its developmental biology while feeding on the cassava whitefly *Aleurotrachelus socialis* and *Sitotroga cerealella* (the Angoumois grain moth)**.** *S. cerealella* eggs are commercially available and a common host of *C. claveri.*

Objective: Determine the duration of the developmental stages of *C. claveri* on two hosts, *A. socialis* and *S. cerealella* in the laboratory.

Methodology: Studies were carried out in the cassava entomology laboratories at CIAT, Palmira, Colombia. Experimental units consisted of $2.5^{(D)}$ x $1.5^{(H)}$ cm plastic vials, containing a 2% nutrient agar. A cassava leaf disc with 150-200 first instar nymphs of *A. socialis* was placed on the agar. In separate units, *S. cerealella* eggs were placed in the experimental arena. Subsequently, one *C. claveri* egg, obtained from the laboratory colony (25°C, 65% RH and 12:12 photo period), was placed in each vial containing host eggs. Each vial was covered with a plastic wrap (Seran-wrap) with small aeration holes, to prevent larval escape. Evaluations of instar changes were made on a daily basis by detecting the presence of exuviate; caste skins were immediately removed to avoid confusion with later instars. Observations were continued until *C. claveri* reached adult stage. The cassava leaf discs, first instar *A. socialis* nymphs and *S. cerealella* eggs were changed every second day.

Results obtained were analyzed using the SAS statistical package, employing the Turkey (HSD) at P<ó=0.05.

Results and Discussion

Eggs: *C. claveri* eggs have an entirely smooth opaque corion; recently oviposited eggs are green, gradually changing to yellow at the extremes and when close to hatching they become white. Eggs are deposited individually on the apex of a rigid pedicle made of a hardened secretion. The average duration of the egg stage is 4.3 days when placed with 1st instar *A. socialis* nymphs and 4.5 days when placed with *A. cerealella* eggs (no significant differences) **(Table 1)**.

Table 1. Duration (days) of the developmental stages of *C. claveri* feeding on *A. socialis* 1st instar nymphs and *S. cerealella* eggs (CIAT, 2004).

Development Stage	Prey Species	
	A. socialis	***S. cerealella***
Egg	***4.3 a****	***4.5 a***
Larvae I	***6.0 a***	***4.3 a***
Larvae II	***6.0 a***	***2.2 a***
Larvae III	***4.5 a***	***2.5 a***
Prepupae	***2.0 a***	***4.2 a***
Pupae	-	14.0 a

* Averages followed by the same letter are no significantly different at P< 0.05.

Larvae: Larvae are flat deiform with a flattened prognathous (heavy-jawed) head and actively passing through three instars. Primary setas are well defined on body segments and covered with prey residuals (*A. socialis* 1st instar nymphs and *S. cerealella* eggs). Regardless of prey consumption (*A. socialis* nymphs or *S. cerealella* eggs), *C. claveri* larvae were very similar in external aspects; both, in the developmental stages displayed translucid legs and white abdominal sides. The most visible difference was in the coloration of the central abdomen; it was a light brown when larvae fed on *S. cerealella* eggs, and white when larvae fed on 1st instar *A. socialis* nymphs. There was a marked increase in size for each instar; instar I was 1.61 mm long x 0.43 mm wide and instar II was 2.52 mm long x 0.68 mm wide when feeding on 1st instars of *A. socialis*.

The average duration of 1st instar *C. claveri* larvae was 6.0 days when feeding on *A. socialis* and 4.3 days when feeding on *S. cerealella* eggs (not significantly different) **(Table 1)**.

Second instar larvae of *C. claveri* had a duration of 6.0 days when feeding on *A. socialis* and 2.2 days when feeding on *S. cerealella* eggs. The duration of third instar larva was 4.5 days when feeding on *A. socialis* and 2.5 days when feeding on *S. cerealella*.

Prepupa: Upon reaching their maximum growth potential, *C. claveri* suspend feeding and initiate constructing a cocoon, terminating activity. The duration of the prepupal stage was 2.0 days when larvae feed on *A. socialis* and 4.2 days when they feed on *S. cerealella* eggs **(Table 1)**.

Pupae: Pupae are white in color, spherical and cottony in appearance, attaching themselves to the side of the vials. Those individuals that were limited to feeding on *A. socialis* nymphs did not reach pupal stage, while the pupal stage of those feeding on *S. cerealella* eggs pupated for 14 days before reaching the adult stage.

The duration of the egg to pupa stages was 22.8 days for *C. claveri* feeding on *A. socialis*, while the complete life cycle of *C. claveri* feeding on *S. cerealella* eggs (from egg to adult emergence) was 31.7 days.

These studies have shown that *C. claveri* has similar developmental and morphological characteristics as other Chrysopidae species. The larvae pass through three instars distinguishable by an increase in size (width and length). Secondly, feeding ceases during the pre-pupal stage and pupal coloration and shape is similar to other species (López-Arroyo et al, 1999).

Those *C. claveri* individuals feeding on *A. socialis* nymphs did not pupate, possible owing to the change in diet, as the progenitors of these individuals had been reared on *S. cerealella* eggs. Future research should take this into consideration and the diet should be varied between generations. These results also indicate that *C. claveri* may need an alternate or additional host in *A. socialis* infested cassava fields to complete its life cycle.

This research is a preliminary study of the possible role of Chrysopidae predators in the biological control of cassava whiteflies. Studies need to be undertaken with additional species to

determine prey preference and consumption rates, and eventually their effectiveness under field conditions.

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Contributors: Luz Paola Velásquez, Claudia M. Holguín, Anthony C. Bellotti.

Activity 4. Intrinsic rate of increase of Biotype "B" *Bemisia tabaci* on two African cassava genotypes MNg 2 and MNg 11.

Bemisia tabaci (Homoptera: Aleyrodidae) as the vector of Africa Cassava Mosaic Disease (CMD), caused by a geminivirus (CMGs) (Legg et al, 2002), causes yield losses reported as ranging from 12-25% of the cassava crop in Africa (Thresh et al, 1997). It has been speculated that the absence of CMD in the Americas is related to the inability of *B. tabaci* to colonize cassava in the Neotropics (Costa and Russell, 1975). However, in the early 1990's a new biotype "B" of *B. tabaci* was collected feeding on cassava in the Americas. Biotype "B" is considered by some authors and taxonomists to be a separate species, *Bemisia argentifolii* (Bellows and Perring) (Bellows et al, 1994). *B. tabaci* "B" is now viewed as a possible threat to vector CMD (or other geminiviruses) in the Americas if the disease were inadvertently introduced; traditional landrace cassava varieties cultivated in the Americas are considered highly susceptible to CMD (Bellotti and Arias, 2001). In addition, cassava damage evaluation caused by the increase in a *B. tabaci* population in East and Central Africa indicate yield losses above 50% due to direct feeding by whiteflies, even on varieties known to be resistant to CMD (CIAT, 2004). Those reports thereby indicate that cassava varieties that contain resistance only to CMD may not be adequate to resist yield losses due to the direct feeding damage caused by *B. tabaci.*

The search for resistance (HPR) to the whitefly, *B. tabaci*, in cassava genotypes offers an alternative and additional low cost and stable option for maintaining lower populations of the whitefly and reducing crop losses. Research experiments were designed to measure and compare the development of "B" biotype of *B. tabaci* populations found in Colombia, on two African cassava genotypes, TMS 30572 (MNg 2) and TMS 60444 (MNg 11). These genotypes were developed during the 1950 as part of a project to identify germplasm resistant to CMD (CIAT, 2003).

Objective: Determine the intrinsic rate of increase of populations of Biotype "B" of *B. tabaci* on two African cassava genotypes, MNg 2 and MNg 11.

Methodology

1. **Genotypes of Manihot esculenta:** In vitro plantlets (20) of the *M. esculenta* genotypes MNg 2 (TMS 30572) and MNg 11 (TMS 60444) were obtained from the CIAT Biotechnology Project (Agrobiodiversity and Biotechnology SB-2). Plantlets were subsequently planted in plastic bags and pots. Eight, 40 day old plants of each genotype were placed in nylon mesh, wooden framed cages (1m x 1m x 1m).
2. ***Bemisia tabaci:*** The source of *B. tabaci* was obtained from a CIAT colony established on *Jatropha gossypiifolia* (Euphorbiacea). The colony had been established for 15 generations on *J. gossypiifolia* in the previously described cages under growth chamber conditions (25±2°C, 70±5% RH and 12:12 photoperiod). The colony is periodically checked for species purity by RAPD-OCR of adult specimens (CIAT, 1999).
3. **Biological and demographic parameters of *B. tabaci* on MNg 2 and MNg 11.**

Longevity and fecundity: Forty pairs (40 males: 40 females) of recently emerged *B. tabaci* adults were collected from *J. gossypiifolia* using a technique described by Eichelkraut and Cardona (1989). One pair was placed in clip-cages (2.5 mm diameter x 2.0 mm depth) and attached to cassava leaves of MNg 2 and MNg 11 so that whiteflies fed on the leaf undersurface. Every 48 hours, the whiteflies were moved to a different area of the leaf. This procedure was repeated throughout the study until the natural death of the females; males were replaced whenever they perished before their mate. Fecundity was estimated by recording the number of eggs oviposited by each female during the 48 hour periods, while longevity was calculated as the time (days) that the female survived.

Development time, rate of survival and proportion of females: Fifty two day old *B. tabaci* adults (male and females) were removed from *J. gossypiifolia* plants with the aid of a buccal aspirator (constructed with a Pasteur pipette). Adults are then placed in small clip cages (2.5 cm diameter x 3.0 cm depth) and attached to the undersides of MNg 2 and MNg 11 leaves. Adults are allowed to oviposit for six hours before being removed and 300 eggs are selected at random. The development time from egg to adult is obtained and survival rate of the immature stages and proportion of females is determined.

Demographic parameters: Data on development time is combined with experimental data on reproduction '1_x-m_x,' generating life tables which are used to calculate the demographic parameters as defined by Price (1975): 1) Net reproduction rate (R_o), the average number of females descendents produced by one female per generation; 2) generational time (T), equivalent to the time contained between parental birth and progeny birth and 3) the intrinsic rate of population increase (r_m) estimated using the equation (Carey, 1993),

$$\sum \exp(-r_m x)\ 1_x m_x = 1$$

Where x is the female age (days); l_x is specific survival age and m_x, the proportion of females from a female progeny at age x. To calculate the values of r_m, the corrected age X+0.5 and the equation $1n\ 2/r_m$ were used to estimate the days required to double the population (Carey, 1993).

Statistical analysis: Statistical analysis was carried out by utilizing the program Stat View, version 5.0.1 (SAS Institute, 1999). The values for longevity, fecundity, oviposition rate and development time were analyzed using Mann-Whitney test; this permits comparing the means of two distributions without needing to determine the supposition that the error is normally distributed. Rate of survival values were compared using chi-square (χ^2).

Results and Discussion

1. Biology and demographic parameters of *B. tabaci* feeding on MNg 2 (TMS 30572) and MNg 11 (TMS 60444).

Longevity and fecundity: the most extensive longevity range, 2 to 10 days, was achieved by *B. tabaci* females feeding on MNg 2, exceeding by approximately 4 days those females feeding on MNg 11. After six days, mortality reached 60% and 100% on MNg 11 and MNg4 respectively

(Figure 1) and their respective longevities were significantly different (Mann-Whitney P < 0.05) **Table 1**.

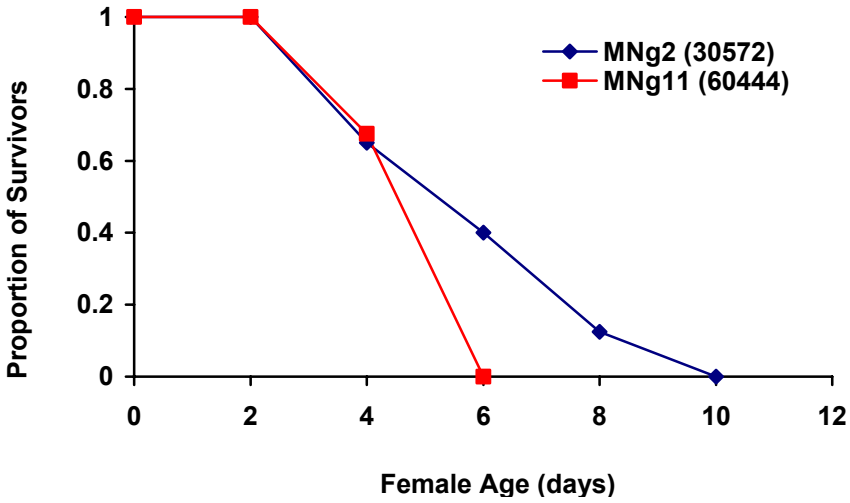

Figure 1. Female survivors of *B. tabaci*, B Biotype, feeding on the African Cassava genotypes MNg 2 (TMS 30572) and MNg 11 (TMS 60444) in the growth chamber (CIAT, 2004).

Table 1. Average longevity (days), average fecundity (eggs/female) and oviposition rate (eggs/female/2 days) of *B. tabaci*, B biotype, feeding on the African cassava genotypes MNg 2 and MNg 11 (CIAT, 2004).

Parameter	MNg 2	MNg 11
Average longevity	4.5 a	3.3 b
Range	2-8	2-4
Average fecundity	8.1 a	3.7 b
Range	1-25	2-16
Average oviposition rate	1.8 a	1.1 b
Range	0.5-11.5	0.5-4

Averages followed by different letters across the columns are significantly different (Mann-Whitney P< 0.05).

Initial oviposition on both genotypes was similar in that *B. tabaci* females oviposited 66% of their total oviposition within the first 48 hours. The difference in average ovipositional rate for each genotype permits predicting that, in a limited way, either of the two hosts would the adequate for development of nymphal stages. The highest ovipositional rate (1.8 eggs/2 days/female) was achieved on MNg 2 with a significantly higher value than achieved on MNg 11 (Mann-Whitney P < 0.05). Maximum oviposition on both genotypes occurred during the first two days. These differences reveal a certain preference for *B. tabaci* to oviposit on MNg 2.

The average fecundity was significantly higher on MNg 2 compared with that on MNg 11 (Mann-Whitney P < 0.05) **(Figure 2, Table 1)**.

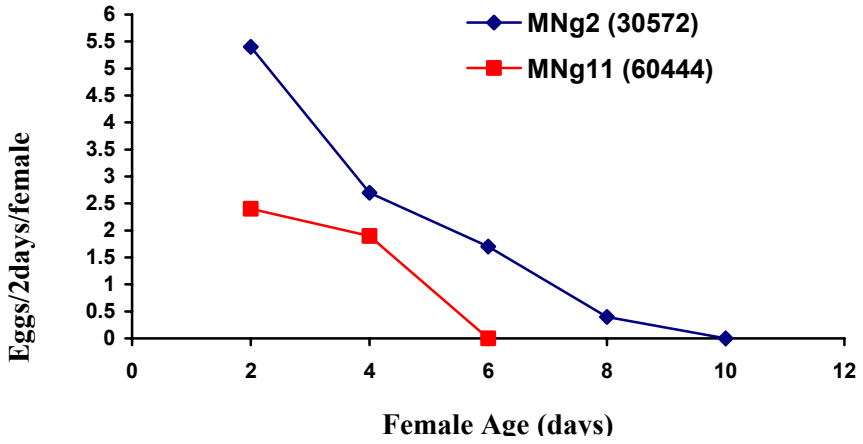

Figure 2. ***B. tabaci*** **reproduction curves when feeding on African cassava genotypes MNg 2 and MNg 11 in the growth chamber (CIAT, 2004).**

2. Development time, rate of survival of immature stages and proportion of females.

The development time of *B. tabaci* feeding on MNg 2 was significantly shorter by 30 days than those feeding on MNg 11 **(Table 2)**. Development time for *B. tabaci* feeding on MNg 2 was 37.9 days, and 68.0 days while feeding on MNg 11. The highest levels of nymphal mortality occurred in the first instar on both genotypes. Mortality also occurred during the second and third instars feeding on MNg 2, but only occurred during the second instar for those feeding on MNg 11. In each case, nymphs entered into a latent state without reaching the adult stage. These results suggest *B. tabaci* biologically adapts more readily on the genotype MNg 2. *B. tabaci* survival rates for immature stages were significantly different on the two genotypes (Chi-Square = 44.58, 1d.f., $P < 0.0001$) **(Table 2)**. Results show that of the 200 *B. tabaci* eggs, 45 individuals survived to adult stage when feeding on MNg 2; compared to only 2 adults surviving on MNg 11. This parameter is a good indication of the potential capacity of *B. tabaci* to develop higher populations on MNg 2, compared to that on MNg 11. In general, the proportion of females and males was not affected by genotype.

Table 2. **Development time, survival and proportions of female** ***B. tabaci*** **feeding on two African genotypes, MNg 2 and MNg 11 (CIAT, 2004).**

Parameter	MNg 2	MNg 11
Development time (days)*	37.9 b	68 a
No. Insects	45	2
Survival rate (%)♣	22.5 a	1 b
No. Insects	200	200
Proportion of females (%)	60	50
No. Insects	45	2

* Averages followed by different letters across columns are significantly different Mann-Whitney $P<0.05$.
♣ Chi-Square = 44.58, 1d.f., $P<0.0001$ (CIAT, 2004).

2. Demographic parameters.

The net rate of reproduction (R_o) allows us to estimate that, on average, at the end of a generation, *B. tabaci* populations could multiply 8.1 times (individual/individual) on MNg 2 **(Table 3)**, this being 1.9 times greater than on MNg 11. One generation of *B. tabaci* would be completed in 39.6 and 68.8 days on MNg 2 and MNg 11 respectively **(Table 3)**. These results allow us to predict that *B. tabaci* would complete nine generations per year on MNg 2, while only five generations on MNg 11.

The results are equally consistent when comparing the intrinsic rate of increase (r_m). This analysis shows a greater population build up on MNg 2, 62% greater than on MNg 11. Likewise, the value of r_m reflects the time of population doubling. On MNg 2, *B. tabaci* requires 21 days less to duplicate its population compared to MNg 11 **(Table 3)**.

Table 3. Demographic parameters of biotype B of *Bemisia tabaci* feeding on MNg 2 (TMS 30572) and MNg 11 (TMS 60444) in the growth chamber (CIAT, 2004).

Parameter	MNg 2	MNg 11
Net reproduction rate (R_o) $\Sigma l_x m_x$	8.1	4.2
Generation time (T)	39.6	68.8
Intrinsic rate of increase (r_m)	0.053	0.02
Days to duplicate population (TD) $\ln 2/r_m$	13	34.5

Results on longevity, fecundity, development time, survival rate and demographic parameters, suggest that the genotype MNg 11 (TMS 60444) is not a suitable host for biotype B of *B. tabaci* in Colombia. These results, however, do differ than those reported by Costa and Russell (1975), where none of the *M. esculenta* genotypes tested permitted survival or reproduction of *B. tabaci*. Bird (1957) also reported that he was not able to rear *B. tabaci* on *M. esculenta*, with whiteflies previously reared on *J. gossypiifolia*. In addition, the results from this study suggest that the African genotypes of *M. esculenta* are potential hosts of the B biotype of *B. tabaci* found in Colombia.

In recent experiments with the genotype TMS 60444 (MNg 11), resistance to the cassava hornworm, *Erinnyis ello*, was observed on this genotype (Chavarriaga et al, unpublished data) (Activity 12). *E. ello* is an important cassava pest in the Neotropics (Bellotti, 1981). The TMS 60444 genotype was developed in Nigeria in the 1950's by using the third backcross derived from an interspecific cross between *M. esculenta* and *M. glaziovii*, as a source of resistance to CMD (CIAT, 2003). The other progeny TMS 30572, also derived from the backcross with *M. glaziovii* was used to construct the genetic map of cassava (Fregene et al, 1997) and shows genomic regions that are probably inherited from *M. glaziovii.* One of their regions is found in ligament D, which shows QTLs for resistance associated with CMD and CBB (CIAT, 2003). These findings, together with the results of this study permit speculating about a possible resistance in TMS 60444 (MNg 11) to biotype B of *B. tabaci* found in Colombia. This could be related to that region on the genome for the QTL's previously mentioned.

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Contributors: Arturo Carabalí, Adriano Muñoz, Anthony C. Bellotti.

Activity 5. Studies on the biology and behavior of biotype "B" of *Bemisia tabaci* on a wild *Manihot* sp, *M. flabellifolia*.

Whiteflies are a major agricultural pest group, attacking a wide range of crop species. As direct feeding pests or virus vectors, whiteflies cause yield losses in cassava based agroecosystems in the Americas, Africa and Asia. The origin of cassava (*Manihot esculenta*) is in the neotropics and two whitefly species cause considerable crop damage in the region; *Aleurotrachelus socialis* predominates in northern South America (Colombia, Venezuela and Ecuador), while *Aleurotrixus aepim* is the major species in Brazil. *Bemisia tabaci* is a pantropical species that is the vector of Africa Cassava Mosaic Disease (CMD) in Africa and parts of Asia. Biotype "B" of *Bemisia tabaci* has been collected feeding on cassava in the Americas but has not been reported, nor observed, transmitting virus diseases on cassava in the neotropics. Host plant resistance in cassava to whiteflies is seen as a practical, low cost, long-term solution for reducing whitefly populations and damage.

The wild species within the genus *Manihot* are seen as potential source of genes for resistance in the control of major cassava pests (see 2003 Annual Report; Project IP-3). There is a precedence for this in that resistance to CMD resulted from an interspecific cross between *M. esculenta* and *M. glaziovii*. However, apart from this one successful case, wild Manihot species have not been exploited as a source of resistance to cassava pests and diseases (also see Activity 12).

The development of pest and diseases resistant varieties resulting from interspecific crosses involving wild *Manihot* species is difficult and time consuming and no continued effort has been attempted to take advantage of this potential source of resistance genes. However recent advances in the development of the molecular genetic map of cassava facilitates gene transfer and transformation. It is presently considered that with the modern tools of genetic engineering now available, access to resistance genes in the wild species will be more efficient, providing quicker manipulation at the molecular level.

Objective: The objective of this present study is to evaluate biological, populational and demographic aspects of Biotype "B" of *B. tabaci* found in Colombia, on *Manihot flabellifolia*.

Methodology:
a) Source of *M. flabellifolia* and *B. tabaci.*

Plantlets of *M. flabellifolia* were obtained from the CIAT Biotechnology Unit (Agrobiodiversity and Biotechnology Project, SB-2) where they were propagated in-vitro. These were transplanted to plastic bags or pots. Eight 40-day old plants were selected and placed in nylon mesh wooden frame cages (1m x 1m x 1m).

The source of *B. tabaci* whiteflies was a CIAT established colony being reared on *Jatropha gossypiifolia* (Euphorbiacea). These had been reared for 15 generations on *J. gossypiifolia* in nylon meshed wooden cages (1m x 1m x 1m) in the growth chamber (25±2ºC, 70±5% RH, 12:12 photoperiod). The species quality (uncontaminated) of the *B. tabaci* colony is periodically verified through RAPD-PCR testing of adults (CIAT, 1999).

b) Biology of *B. tabaci* on *M. flabellifolia.*

Longevity and fecundity were evaluated by placing 40 recently emerged adult pairs (40 males + 40 females) of *B. tabaci* from the *J. gossypiifolia* colony, in small clip cages (1.5 cm diameter + 2.0 cm depth) (one pair per cage), placed on the underside of *M. flabellifolia* leaves. Adults were removed every 48 hours to a different site on the leaf; this procedure was repeated until the natural death of the females. Fecundity was estimated by counting the number of eggs oviposited every 48 hours by each female, while longevity was estimated based on the number of days that females survived.

Development time, survival and female/male ratio was estimated by placing 50 two day old adults (males and females) removed from the *J. gossypiifolia* colony, in round clip cages (2.5 x 2.0 cm) on the underside of *M. flabellifolia* leaves. After six hours, adults were removed and 200 eggs were randomly selected. Egg to adult development time, survival of immature stages and proportion of females was observed and recorded.

Demographic parameters were calculated by combining data on development time and reproduction (l_x-m_x), generating life tables (Price, 1975): 1) net reproduction rate (R_o), the average number of females that one female produces in one generation; 2) generational time (T), equal to that period between birth of the parents and of the progeny and 3) intrinsic rate of increase of the population (r_m), estimated using Carey's formula (1993),

$$\sum \exp(-r_m x)\, l_x m_x = 1$$

where x is the age of the female in days, l_x, the age of species survival, and m_x, the proportion of female progeny of one female at age x.

Results

Longevity and Fecundity: Results show a range of *B. tabaci* female survival of 2 to 8 days when feeding on *M. flabellifolia*, with an average of 3.5 days **(Figure 1A and Table 1)**. An average of 3.3 eggs (range 1-16 eggs) were oviposited per female. Ninety percent of female initiated oviposition during the first 48 hours and by the 4th day, 87% of oviposition had occurred **(Figure 1B).**

Development Time, Survival, Proportion of Females: Development time of *B. tabaci* (biotype B) individuals feeding on *M. flabellifolia* was 47.2 days **(Table 2)**. The proportion of females was 50% and survival 8%.

The net reproduction rate (Ro) estimates that *B. tabaci* population will increase three fold during one generation **(Table 3)**. *B. tabaci* will complete one generation in 48 days feeding on *M. flabellifolia*, resulting in seven generations in one year. In addition the r_m value indicates a 77% population decrease when compared to the reproductive rate of *B. tabaci* on its original host, *J. gossypiifolia*. Feeding on *M. flabellifolia*, *B. tabaci* requires 31 days to duplicate its population, compared to only 25 days on *J. gossypiifolia* (Carabalí, 2004).

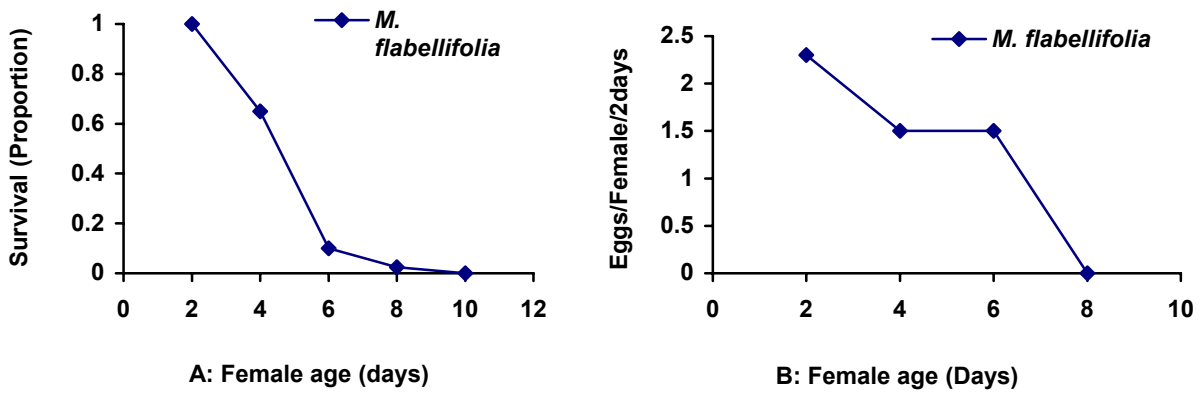

Figure 1. ***Bemisia tabaci*** **(biotype B) reproduction (A) and survival (B) curves when feeding on** ***Manihot flabellifolia*** **in the growth chamber.**

Table 1. Average longevity, average fecundity and rate of oviposition (eggs/female/2 days) of biotype "B" of ***Bemisia tabaci*** **feeding on** ***Manihot flabellifolia*** **in the growth chamber.**

Parameter	*M. flabellifolia*
Average longevity	3.5
Range	2-8
No. insects	40
Average fecundity	3.3
Range	1-16
Average Oviposition rate	0.98
Range	0.25-4

Table 2. Development time, survival and proportion of females of Biotype "B" of ***Bemisia tabaci*** **feeding on** ***M. Flabellifolia*** **(n=200) in the growth chamber.**

Parameter	Values
Development time (days)	47.2
Rate of survival (%)	8
Proportion of females (%)	56

Table 3. Demographic parameters of individuals of biotype "B" of ***Bemisia tabaci*** **feeding on** ***Manihot flabellifolia*** **(n=200) in the greenhouse.**

Parameter	Values
Net reproduction rate (Ro) ∑lxmx	3.0
Generation time (T)	48.3
Intrinsic rate of increase (r_m)	0.0222
Days to duplicate population Ln2/rm	31.2

In recent studies, *M. flabellifolia* was evaluated for resistance to the cassava mealybug (*Phenacoccus herreni*), the cassava green mite (*Mononychellus tanajoa*), and the whitefly (*Aleurotrachelus socialis*). *M. flabellifolia* showed moderate levels of resistance to the mealybug and mite and high levels to the Whitefly (Burbano, 2003). Present results further indicate that the wild *Manihot* species are a potential source of whitefly resistance genes and in particular a resistance source to biotype B of *B. tabaci*.

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Contributors: Arturo Carabalí, Adriano Muñoz, Anthony C. Bellotti.

Activity 6. Determining the plant metabolites involved in whitefly (*Aleurotrachelus socialis*) resistant cassava varieties, MEcu 64, MEcu 72 and MPer 334.

The whitefly, *Aleurotrachelus socialis*, is a major pest of cassava, reducing root yield and the formation of cassava planting material (cuttings or stakes). Field evaluations during a 1, 6- and 11-month attack resulted in yield losses of 5, 42 and 79% respectively (Bellotti and Vargas, 1986). Whiteflies cause direct damage to cassava by feeding on the phloem of leaves, inducing leaf chlorosis and abscission, which results in reduction in root yield if feeding is prolonged (Bellotti, 2002). Additional yield reduction can be caused by the growth of a "sooty-mold" that grows on whitefly exudates deposited on cassava leaves and deters photosynthesis (Bellotti and Vargas, 1986).

The CIAT cassava germplasm bank contains nearly 6000 accessions, of which 93% are landraces (locally selected cultivars), collected from tropical and subtropical regions of the world, but mainly from the Neotropics. This germplasm collection has been extensively screened in the field for whitefly (*A. socialis*) resistance, more than 5400 landrace cultivars have been evaluated. Sources of resistance to *A. socialis* have now been identified. The clone "MEcu 72" has consistently expressed high level of resistance. Several additional cultivars, including "MEcu 64; MPer 334, MPer 415, MPer 317, MPer216, MPer 221, MPer 266 and MPer 365, have expressed moderate to high levels of resistance. These results also indicate that *A. socialis* resistance may be concentrated in Peruvian and Ecuadorian germplasm. In greenhouse and field studies show that *A. socialis* feeding on resistant clones had less oviposition, longer development period reduced size and higher mortality than those feeding on susceptible one (Arias, 1995). *A. socialis* nymphal instars feeding on MEcu 72 suffered a 72.5% mortality, mostly in the early instars (Arias, 1995, Bellotti and Arias, 2001).

Recent studies under controlled conditions in the growth chamber, *A. socialis* had a longer development cycle when feeding on MEcu 64, MEcu 72 and MPer 344 when compared to the susceptible control, CMC 40. Nymphal mortality was highest on MPer 334 (77.5%), followed by MEcu 64 and MEcu 72 with 68.5% and 68.0% respectively.

In addition genomic sequences possibly involved in *A. socialis* resistance have been detected in MEcu 72 using AFLP and microsatelite markers (Bellotti, et al, 2003).

Plant strategies for resisting insect attack often involved biochemical factors or activities. Studies were therefore initiated to determine what plant metabolites might be involved in the development of *A. socialis* resistance found in the resistant genotypes. MEcu 64, MEcu 72 and MPer 334.

Materials and Methods: Electrophoresis, employing polyacrylamide gels (PAGE) has proven to be a very useful technique for the analysis and characterization of complex protein mixtures. Nevertheless, since access into the interior of protein matrixes is limited, information generated about the individual components is usually restricted to molecular weight and isoelectric dots. The transfer of proteins by PAGE to an unfixed membrane, permits the utilization of diverse tests for an improved characterization. One of the more precise applications for the transfer of proteins to membranes, is through inmunodetection which consists of the identification and

characterization of a fixed antigen by means of antibody tests (Timmons and Dunbar, 1990); Garfin, 1990; Anderson, 1988; Hames and Richwood, 1988; Dunbar, 1987).

Inmune-detection permits estimating by semiquantitative means, the mass or abundance of a specific protein in a determinate tissue. This technique is regularly employed in experimental studies in which the objective is to detect a specified protein or to observe its variation under diverse conditions.

It was decided that the first stage of this study would be carried out to determine if a relationship exists between leaf proteins in the resistant genotypes, MEcu 64, MEcu 72 and MPer 334, and the resistant characteristics they display to *A. socialis*; the susceptible genotype CMC 40 was used as the control. The plan includes obtaining polyclonal antibodies from the immunization of rabbits against protein extracts for each of the materials, and later to determine by means of immunodetection, and the combination of Western Blot and 2D SDS-PAGE techniques, the differences between each of the protein extracts. This process will be carried out using healthy plants (non-infested), and plants infested with *A. socialis*, for each of the genotypes, to see if a proteic response occurs in infested plants. In addition, *A. socialis* feeding on resistant plants will be examined for the presence of a plant protein.

Total Protein Extraction: To extract the total protein, cassava leaves (without petioles) were macerated in liquid nitrogen, obtaining a very fine powder that was subsequently homogenized for five hours at 4ºC with the buffer Tris HCL, pH 8.0, and containing 1mM of EDTA (metalloprotease inhibitor), 5 mM of DTT (reduction agent), 1% PVP (antiphenolic), and 5 mM of PMSF (serine protease inhibitor) at a proportion of 1g macerated leaf to 3ml of buffer. The following step consisted of filtering this mixture and centrifuging it at 15000 rpm for 30 minutes at 4ºC, to clarify the extract and eliminate vegetative tissue. The supernadant is dialyzed with a dialysis membrane of W.M. Co. 3.5 Kd and finally lyophilized to obtain an extract in powder form, in order to manipulate the concentration by weight units.

Immunization and Production of Polyclonal Antibodies against Cassava Proteins

Polyclonal antibodies were used as they contain different sub-classes of antibodies, including IgG, IGM, IGE, IgA and IgD. Each antibody represents the product of only one stimulated lymphocyte and its clonal progeny. An antigen complex such as a protein can contain several distinct or epitopes or determinant antigens, each of which is specifically recognized by antibodies from only one clonal lymphocyte (Dunbar and Schwoebel, 1990).

To produce polyclonal antibodies the following steps were developed:

- Two milligrams of each protein was dissolved in 1 ml of the buffer Tris-Glicina pH 6.8 and later emulsified with one ml of Freund's complete adjuvant.
- Four New Zealand breed rabbits were employed. Each of them was subcutaneously injected four times with 0.5 ml of each of the prepared proteins. The injections were applied to the animal's loin.
- After three weeks, the four applications were repeated on each rabbit, but at this time the proteins were emulsified with 1ml of Freund's incomplete adjuvant. Two of the injections were intermuscular.

- Ten days after the last injections, the animals were bled, obtaining 15-20 ml of blood from each.
- The collected blood was left at room temperature for 24 hours, then centrifuged and the serum was stored coagulated in aliquots for later analysis.

Test for Antibody Recognition using the Dot Blot Technique

A test for antibody recognition using the Dot Blot technique was carried out to verify that the antibodies produced were in good condition. The following steps were developed:

- One milligram of each of the proteins was dissolved with 200 µl of Tris Glycine (pH 6.8) buffer. On each nitrocellulose membrane 5 µl of the stock solution was applied to each of the proteins.
- Blockage of the nitrocellulose membrane with the sample in TBS containing 1% gelatine.
- Exposure of the membrane to 30 µl of the first antibody dissolved in 30 ml of blockage solution.
- Four washings of the membrane of 15-minutes each. The first three with TTBS (TBS containing 1% tween 20) and the last with TBS.
- Exposure of the membrane in 30 µl of the second antibody (Bound to PER) dissolved in 30 ml of the blockage solution.
- Four washings of the membrane of 15-minutes each. The first three with TTBS (TBS containing 1% tween 20) and the last with TBS.
- Addition of 5 ml of revealed solution (40 ml of TBS, 3 µl of hydrogen peroxide and 30 mg of 4 Chloro-1-Naphtol dissolved in 10 ml of methanol). This solution is preheated at 35°C.

SDS-PAGE Electrophoresis

Using electrophoresis trials with polyacrilamide gels in disnatured conditions (SDS-PAGE) it was determined:

- Protein sample concentrations (mg/ml) carried on gel pools for a visualization of the bands. To do this, concentrations of 200 mg/ml, 100 mg/ml, 75 mg/ml, 50 mg/ml, 25mg/ml, 10 mg/ml and 2mg/ml were tested.
- Adequate concentrations of the resolving phase of the gel were achieved for a good view of the protein bands. To do this, concentrations of 10%, 14%, and 17% were tested. It should be noted that the phase stacking concentration was 4% at all times.
- Polymorphism by molecular weight for each of the proteins for each genotype evaluated. To do this a marker of the Prestained SDS-PAGE from Biorad Laboratories (with a arrange of 106 to 20.8 Kd) molecular weight was utilized.

These tests were carried out in a Biorad Mini Protean electrophoresis chamber and followed the protocol established by the manufacturer for both the electrophoresis as well as the staining of the gels.

Results: Tests for antibody recognition using Dot Blot. By sing the afore-described methodology a clear recognition of the antibodies for each of the genotype extracts was achieved and evaluated. In addition a good staining (concentration) of the polyclonal antibodies

originating from each genotype was observed, owing to the high intensity of each marker **(Figure 1)**.

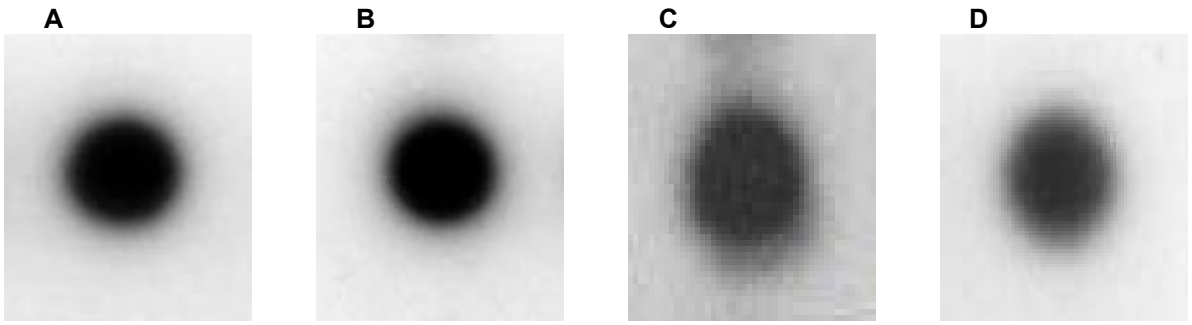

Figure 1. Test for antibody recognition using the Dot Blot technique. A: antibodies against MEcu 72, B: antibodies against MEcu 64, C: antibodies against MPer 334, D: antibodies against CMC 40.

These results indicate that the process for immunization and production of the antibodies using the described procedures was successful; therefore it is possible to continue with the cross-tests for immunodetection of proteins for both the varieties being evaluated, as well as for *A. socialis*.

SDS-PAGE Electrophoresis

It was determined that the protein sample concentration that best provides a good visualization of the bands is 2mg/ml. This concentration provided for well defined bands without vertical streaking of protein, as occurred with the other concentration evaluated **(Figure 2).**

The protein concentration that gave adequate results for the resolving phase by providing good visualization of the protein bands was 14% **(Figure 2)**. With the other concentrations the distribution of the bands along the gel were not uniform and very congested on the lower part of the get at the 10% concentration, while they were congested at the top of the gel at the 17% concentration.

In **Figure 2**, polymorphic bands can be observed between the resistant and susceptible genotypes, with molecular weights between 47.5 and 35 Kd. A common polymorphic band is clearly noted in the resistant genotypes (black arrow), although it is less intense for MEcu 64. The genotype MPer 334 shows a high polymorphism as well as an additional band that is absent in the other genotypes (yellow arrow). The yellow circle on **Figure 2**, indicates the absence of these aforementioned protein bands on the susceptible genotype, CMC 40. These results are a good indication that these protein immunodetection tests should be continued on these genotypes; the differences shown between the resistant and susceptible genotypes is a good indication that a relationship exists between these proteins and the presence of resistance to *A. socialis*.

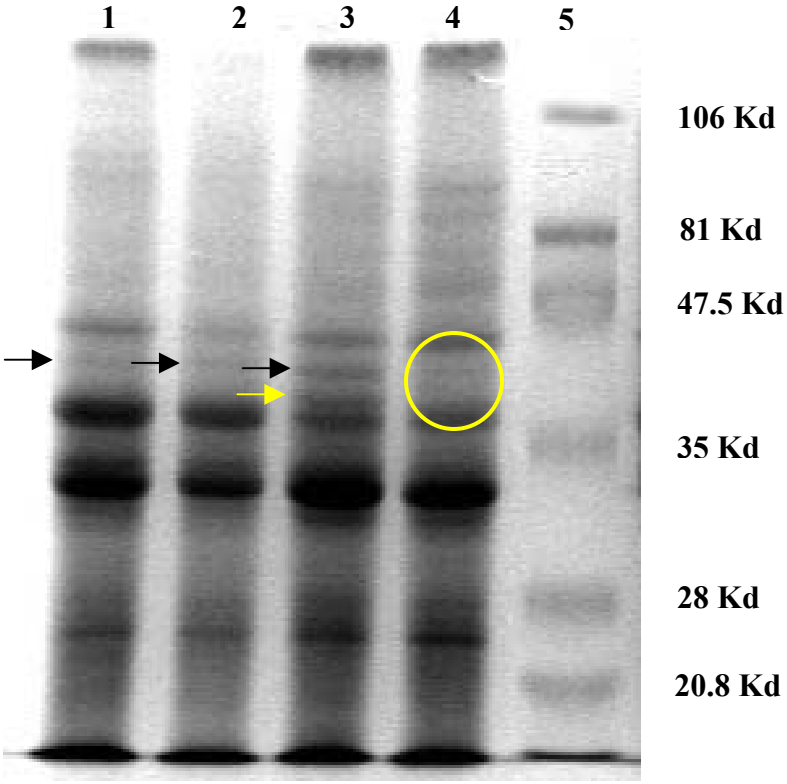

Figure 2. SDS-Page. Phase resolving concentration of 14%, Sample concentration of 2 mg/ml. 1: MEcu 72, 2: MEcu 64, 3: MPer 334, 4: CMC 40; 5: Molecular weight marker (Kd). The black arrow indicates the polymorphic band commonly present in the resistant genotypes and absent in the susceptible, CMC 40, indicated by the yellow circle. The yellow arrows show an additional polymorphic band that is only evident in the resistant genotype MPer 334.

Projections

With the polycloned antibodies tested and the standardization of conditions for the SDS-PAGE achieved, we can proceed to develop cross-immunodetection tests of the genotypes and *A. socialis* utilizing the Western Blot and 2D SDS-PAGE techniques.

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Collaborators: Diego Fernando Múnera S., Anthony C. Bellotti, Arnubio Valencia. Universidad de Caldas, Manizales, Colombia, Stephen L. Lapointe. United States Department of Agriculture (USDA), Paul-André Calatayud, IRD, France.

Activity 7. The identification and evaluation of homopteran species as possible vectors of cassava Frogskin Disease (CFSD).

Cassava Frogskin Disease (CFSD) can cause severe yield losses on cassava in several regions of Colombia. It disrupts the movement of germplasm within and between countries and hinders our ability to carry out cassava field research in infested areas. CFSD probably originated in the Amazon Region of South America and is now reported from several countries in South America. Dissemination by infected planting material is well documented (Calvert and Thresh, 2002). However neither its transmission within cassava fields, nor its epidemiology is well understood.

Recent research at CIAT (Annual Report PE-1, 2003) with infested plants has indicated the presence of a phytoplasm and a virus of the family Reoviridae. These results may also demonstrate a possible association between these two potential causal organisms.

Leafhoppers (Homoptera) are known to be important vectors of plant pathogens (viruses, phytoplasm, spiroplasm and bacteria) (Nielson, 1968; Maramorosch and Harris, 1979). During 2003 and up to August 2004, surveys and explorations were carried out in cassava fields of several CFSD infested regions of Colombia (see Annual Report PE-1, 2003 for additional information). These explorations collected numerous homopteran species from cassava agroecosystems, including weeds. Collections were made from fields with and without the presence of CFSD.

A list of homopteran captured was presented in previous Annual Reports (Project PER-1, 2002, 2003). In this report, the family Cicadellidae was the most frequently collected, followed by other plant hoppers from the families Cixiidae and Delphacidae. Identification to species level of the latter two families is difficult to obtain due to a lack of taxonomic expertise in tropical species. Therefore, identification to species level is limited to the Cicadellidae.

Objective: Determine the Cicadellidae species associated with cassava in CFSD infected and non-infested fields.

Methodology: Cassava fields; with and without the presence of CFSD were sampled at 16 different localities in nine Colombian departments. Nymph and adult cicadellids were captured by using a sweep-net or direct removal and preserved in 70% alcohol. Specimens were grouped by morpho species for identification.

Results: Collected leafhoppers were identified by Dr. Paul Freytag, taxonomist at the University of Kentucky, USA **(Table 1)**.

Two species of the genus *Scaphytopius* were identified associated with cassava, *S. fuliginosus* (Osborn) and *S. marginelineatus* (Stal). The former was registered in the department of Tolima (Chicoral and Gualanday municipalities) while the latter was amply distributed throughout the departments of Cauca, Valle del Cauca and Quindío. The leafhopper species *Empoasca bispinata* was collected from a majority of the sites sampled and at a relatively higher population than that observed for the *Scaphytopius* species.

Table 1. Leafhoppers from the family Cicadellidae collected from cassava fields at several locations in Colombia.

Department	Municipality	Site	Species	Observations*
Valle del Cauca	Palmira	CIAT	***S. marginelineatus*** *(Stal)* ***Empoasca bispinata*** *Davidson & DeLong*	6-month cassava field plot (with weeds and some cassava plants with CFSD
Cauca	Santander de Quilichao	Hacienda Bariloche	***S. marginelineatus***	Two-month cassava field plot
		Granja CIAT	***S. marginelineatus*** ***E. bispinata*** ***Tylozygus fasciatus*** (Walker) ***Hortensia similis*** (Walker) ***Stirellus bicolor***	Some cassava plants with CFSD and presence of weeds around the crop
Quindío	La Tebaida		***Planicephalus flavicosta*** (Stal) ***Agallia nielsoni*** Freytag n. sp.	Five-month cassava field plot with weeds.
	Armenia	La Primavera	***S. marginelineatus*** ***E. bispinata*** ***Stirellus bicolor*** ***Agallia nielsoni***	Four-month cassava field plot
	Quimbaya	Vereda Querman	***S. marginelineatus*** ***Hortensia similis*** ***Stirellus bicolor*** ***Planicephalus flavicosta***	4-5 month cassava field plot
Risaralda	Morelia	Santa Rita	***Agallia*** n. sp. ***Stirellus bicolor***	4-month cassava field plot
Tolima	Chicoral	Granja Nataima	***Scaphytopius fuliginosus*** (Osborn) ***Empoasca bispinata***	Some cassava plants with CFSD
	Gualanday		***S. fuliginosus***	7-month cassava field plot
	Ambalema	Via Ambalema	***Empoasca bispinata***	6-month cassava field plot
	Espinal	San Francisco	***Empoasca bispinata***	6-7 month cassava field plot
Meta	Villavicencio	Corpoica	***Stirellus bicolor***	
Atlántico	Pitalito		***Empoasca bispinata*** ***S. marginelineatus***	7-8 month cassava field plot
	Barahona Caracoli	Palapa	***Hortensia similes***	
Córdoba	Ciénaga de Oro		***Empoasca bispinata***	7-8 month cassava field plot
Sucre	Corozal	Las Penas	***Empoasca bispinata*** ***S. marginelineatus***	7-8 month field plot (CFSD present)

* CFSD = Cassava Frogskin Disease.

Additional species collected, although in reduced numbers, but equally important for our studies were *Hortensia similis, Stirellus bicolor* and *Tylozygus fasciatus*. These were collected from the cassava crop, as well as from weeds bordering cassava fields.

Cicadellidae species collected from CFSD free fields in the coffee growing regions (Quindío and Risaralda departments) include *Planicephalus flavicosta, Agallia nielsoni* and *Agallia* sp. n sp. Based on these observations and collections, the leafhoppers *S. marginelineatus* and *E. bispinata* were selected for initiating studies as possible vectors of CFSD, since they were the species most frequently observed in surveyed sites.

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

Activity 8. Methodologies developed for laboratory rearing of *Scaphytopious marginelineatus (Stal)* and *Empoasca bispinata* Davidson & Delong on cassava.

From 2002 to 2004 a complex of leafhopper (Cicadellidae) species have been collected from cassava and adjacent weeds at several localities in Colombia (Annual Reports PE-1, 2002 to 2004). Two species that are frequently recovered from field surveys are *Scaphytopious marginelineatus* and *Empoasca bispinata*. Based on these results these two species were selected for further studies as possible vectors of Cassava Froskin Disease (CFSD). In order to carry out effective CFSD transmission studies a pathogen free leafhopper colony is required. Therefore, all field-collected individuals of the above mentioned species were "quarantined" by utilizing an acceptable alternate host, such as beans (*Phaseolus vulgaris*).

Objective: Develop and maintain laboratory colonies of *S. marginelineatus* and *E. bispinata* (Homoptera: Cicadellidae) for CFSD transmission studies.

Methodology: Several field collections of *S. marginelineatus* and *E. bispinata* were made from cassava at the CIAT experiment station in Santander de Quilichao (Cauca), during the months of August and September, 2003 and 2004, when population of these species are highest in this regions. Approximately 50 to 60 individuals of each species were collected and placed in plastic boxes containing cassava leaves; leaf petioles were placed in sealed tubes containing water, to maintain freshness during transport.

Initially these insects were housed in nylon-mesh cages containing 20-day-old cassava plants. Later, when these plants reached two months, they were replaced with bean plants(var. Ica-pijao), a highly acceptable host of these species. By transferring the individuals from cassava to beans it is calculated that this will free the specimens of any CFSD pathogens that they might have acquired feeding on cassava in the field. When bean plants began to produce pods, they were replaced with younger plants. Leafhopper infested leaves of the older plants were placed on the younger plants, allowing for recuperation of eggs and nymphs. The initial colony was housed in a growth room under controlled conditions (30-30.5ºC, 60-94% RH and 12:12 photoperiod). Once the leafhopper colonies were established and "quarantined" on beans, a slow transfer was initiated from beans back to cassava **(Photos 1 and 2)**. The cassava varieties CM 6740-7, CMC 40 (MCol 1468) and Secundina were used.

Results and Discussion: *S. marginelineatus* was successfully reared on cassava using the above-described methodology; adequate populations were maintained both on beans and cassava with no apparent effect on colony adaptation on either host.

S. marginelineatus was able to feed on, and become established on, all the aforementioned cassava varieties. These results question the previously held observation that this species is only a sporadic visitor to the cassava crop. It was determined that the approximate development period on cassava is 40.5 days under the aforementioned conditions. With an initial population of 15 females and 15 males, a nymphal population of approximately 345 individuals was achieved per cage.

The colony of *E. bispinata* is in the process of becoming established and several adjustments in relation to humidity and temperature are being made to achieve more favorable rearing conditions. Nevertheless, the first generation of *E. bispinata* has been reared on cassava in the growth room **(Photo 3)**.

Photo 1

Photo 2

Photo 1 and 2. Growth room colonies of *Scaphytopius marginelineatus* feeding on bean (*Phaseolus vulgaris*) and cassava (*Manihot esculenta*).

Photo 3. Second instar nymph of *Empoasca bispinata* reared on cassava in the growth room.

Contributors: María del Pilar Hernández, Claudia María Holguín, Carmen Elisa Mendoza, Adriano Muñoz, Anthony C. Bellotti.

Activity 9. The biology and morphology of *Scaphytopius* (Convelinus) *marginelineatus* feeding on cassava leaves.

There are approximately 21,000 described leafhopper species and 151 have been reported as plant disease vectors; of these 117 are found within 47 genera of the subfamily Deltocephalinae (Knight, 1993). The genus *Scaphytopius* Ball, contains 6 virus vector species; *S. acutus cimus, S. acutus acutus, S. acutus delongi, S. irroratus, S. magdalensi* and *S. nitidus* (Maramorosch, 1979).

Objective: Determine certain aspects of the biology and life cycle of *S. marginelineatus* on cassava in the growth room.

Methodology: Individuals were removed from the colonies maintained in the growth room and placed in small clip cages attached to cassava leaves (Var. CMC 40). Daily observations were made on the developmental stages.

Results:

Taxonomic Position

Order: Homoptera
Family: Cicadellidae
Subfamily: Deltocephalinae
Genus: *Scaphytopius*
Species: *Scaphytopius (Convelinus) marginelineatus* (Stal).

Distribution: This species has been reported from Brazil, Guyana and Colombia (departments of Cauca, Quindío, Córdoba, Atlántico, Magdalena and Valle del Cauca). *S. fuliginosus* (Osborn) has been reported from Costa Rica, Puerto Rico, México and Colombia (department of Tolima).

Hospederos: The genus *Scaphytopius* in Colombia is associated with cassava, soybean, common bean and weed species.

Morphological Description

Eggs: Individual eggs are approximately 0.94 mm long and 0.32mm wide. They are elongated and slightly curved**;** recently oviposited eggs are translucid but gradually become whitish in color and are thereby easily detectable on the leaf surface. Eggs are inserted by the adult ovipositor below the epidermal layer of the leaf and usually dispersed along and/or between the leaf veins and along the leaf edge.

Eggs are generally oviposited individually but 2 to 5 closely aligned eggs have been observed. As the incubation period advances, red ocular spots begin to appear and are characteristic of the embryo **(Photo 1 and 2)**. Egg hatch occurs 10 to 12 days after oviposition; 75.3% of the eggs hatched.

Photo 1

Photo 2

Photo 1 and 2. Eggs of *Scaphytopius marginelineatus* oviposited on cassava leaves.

Nymphs: There are five nymphal instars. Recently emerged nymphs are cream colored, later some spotting occurs (generally 5 pairs) symmetrically spaced on the body as such: 2 pair on the pronotum, one pair on the sides of the scutellum, and the other 2 pair between the 4th and 5th abdominal segment. As nymphal development advances, spots begin to fuse until a band is formed on both sides of the body. Occasionally 5th instar nymphs are reddish in color **(Photo 3)**. Males can be easily distinguished by the pair of dark spots located on the thorax **(Photo 4)**.

Photo 3

Photo 4

Photo 3 and 4. Nymph and adult of *Scaphytopius marginelineatus* feeding on cassava leaves (female and male respectively).

Nymphs are mobile but can remain on the same leaf for parts of their cycle, and occasionally nymphal instar exudates can be observed. The five nymphal stages last about 31.5 days **(Table 1)**.

Adults: Adult females are approximately 4.5 mm long and males about 4.0 mm. Adults are coffee colored (dark brown); both nymphs and adults have a pointed head that is narrower than

the pronotum; the clypeus is a characteristic pale yellow color. The pronotum and wings have numerous dark brown reticulations, interspaced with gray spots **(Photo 5)**.

Table 1. Development period of eggs and nymphal stages of *Scaphytopius marginelineatus* under controlled conditions (CIAT, 2004).

Stage	Duration (Days)*
Preoviposition period	8-10
Egg	7-9
Nymph I	5-8
Nymph II	4-7
Nymph III	4-5
Nymph IV	4-7
Nymph V	7-9

* Duration is calculated based on 105 individuals per stage.

Photo 5. Adult of *Scaphytopius marginelineatus* feeding on cassava (CIAT, 2004).

Biology and Behavior: Preliminary observations on the egg to adult durational period under laboratory conditions indicate that several factors influence *S. marginelineatus* development and determine its life cycle duration. Generally, adults initiate copulation about three days after emerging and begin to oviposit 8 to 10 days later. Copulation is observed as most active in the afternoon. Female/male ratio is 1:1.

Egg to adult duration is about 40.5 days and adults can survive for 60 days. The population is polyvoltine. Adults are very mobile and fly rapidly when the foliage is disturbed; peak activity is during the morning hours.

Damage: *S. marginelineatus* is a piercing-sucking insect and damage is mechanical and physiological in that most Cicadellidae are phloem feeders and may inject a toxin during feeding. Under greenhouse conditions and with high populations, a gradual leaf yellowing occurs that increases in size until the whole leaf lobe is effected; after three to four days damage results in leaf fall **(Photo 6 and 7)**.

Photo 6 and 7. Cassava leaves with typical *Scaphytopius marginelineatus* damage.

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Activity 10. Transmission of cassava frogskin disease; evaluation of homopteran species as vectors.

Cassava frogskin disease (CFSD) is a limiting factor in cassava productions in several regions of the neotropics. In many cassava genotypes, leaves of infected plants may be symptom-less, making detection of the disease in the field difficult. Root symptoms can be severe, surface ridges develop on the root resulting in a knarled appearance. Severely infected roots do not accumulate starch, remaining thin and knarled. CFSD is known to be transmitted through infected stem cuttings. An insect vector is suspected but results are inconclusive at present. Indications are that whiteflies, especially *Bemisia tuberculata* may be involved in transmission of the causal agent but efficiency of transmission is low. Field surveys in CFSD endemic regions of Colombia have identified numerous homopteran species associated with the cassava crop (see Activity 7, this report; also Annual Reports 2002, 2003, Project PE-1).

Objective: Determine if the Ciccadellidae, *Scaphytopius marginelineatus* is the vector of cassava frogskin disease.

Methodology: *S. marginelineatus* has been collected feeding on cassava from several sites in Colombia. It is the non-whitefly homopteran species most frequently collected from the cassava agroecosystems.

Transmission studies were carried out in the entomology growth room (Temp. ≃ 26.5°C, 65% RH and 12:12 photoperiod). Ten to fifteen adult *S. marginelineatus* were removed from the established colony (Activity 8) with the aid of a bucal aspirator. These adults were released into a nylon meshed cage containing CFSD infested cassava plants (Var. MCol 2063, "Secundina") and allowed to feed for 7 days. "Secundina" is an CFSD indicator variety in that leaves readily express disease symptoms. After 7 days, surviving *S. marginelineatus* adults were removed and placed in cages with healthy Secundina plants and allowed to feed for 30 days. Plants were observed on a daily basis for CFSD leaf symptoms.

At the same time, *S. marginelineatus* adults were collected from a colony being reared on healthy Secundina plants, and were released into nylon meshed cages containing CFSD infected plants of the variety MBra 383. The adults remained on these plants until nymphs were obtained.

Adults that completed the 30 days feeding period on Secundina did not cause CFSD leaf symptoms on the healthy plants. It was therefore decided to do DNA extractions from the adults that fed on the CFSD infected Secundina as well as the adults and nymphs that fed on the infected MBra 383. Adults from the healthy (CFSD free) colonies being reared on Secundina and beans were also evaluated. DNA extractions were carried out using the method described by Girbertson et al (1983) for PCR analysis.

Nested PCR Analysis. Fifty ng of genomic DNA, from Nested PCR, were amplified using universal primers R16F2/R16R2 and R16(III)F2/R16(III)R1 (specific primers from the 16Sr III X-Disease) groups. This cocktail is prepared with 2/mM of dNTP's Buffer of Tag 1X, 2.5 mM of M of $MgCL_2$, 1 U of Tag polimerase and 10 μM of each primer. Initial denaturing was for 2

minutes at 94°C and 35 cycles, at 94°C for one minute, 50°C for 2 minutes and 72°C for three minutes followed by a final extension of 72°C during 10 minutes. The PCR products were analyzed by electrophoresis on a 1.5% agar gel.

DNA Sequencing. PCR amplifications were cleaned with a Qiagen purification kit and later sequenced by an automated dideoxy sequencing (AB1 Prism 377-96 DNA Sequencer), using a DNA-sequencing kit from Applied Biosystems. The sequences obtained were *homologized* with sequences reported in the gene bank for identifying the organisms detected in the samples evaluated.

Results

DNA Extractions. Seventeen samples from the different developmental stages of *S. marginelineatus* were extracted and 1 to 2 individuals per sample were processed **(Table 1).**

Table 1. Phytoplasm identified in the homopteran *Scaphytopius marginelineatus*, evaluated with the nested-PCR and primers R16F2/R16R2 and R16(III)F2/R16(III)R1.

	Samples	Genotype[a]	State	Nested-PCR[b]
1	1A	M Col 2063 (I)	Adults	+(S)
2	1B	M Col 2063 (I)	Male-nymphs	+
3	1C	M Col 2063 (I)	Female-nymps	-
4	2A	M Col 2063 (I)	Adults	+
5	2B	M Col 2063 (I)	Male-nymphs	-
6	2C	M Col 2063 (I)	Female-nymps	+
7	3B	M Col 2063 (I)	Male-nymps	-
8	3C	M Col 2063 (I)	Female-nymps	+
9	4A	M Col 2063 (H)	Adults	-
10	4B	M Col 2063 (H)	Nymphs	-
11	4C	M Col 2063 (H)	Nymphs	-
12	SE1	M Col 2063 (I)	Adults	-
13	Ss1	M Col 2063 (H)	Adults	-
14	F1	Bean(H)	Adults	-
15	383 (1)	M Bra 383(I)	Male-nymphs	-
16	383 (2)	M Bra 383(I)	Female-nymphs	-
17	383 (3)	M Bra 383(I)	Adults	+ (S)

[a] Clean/healthy plant material provide dby the CIAT Virology Unit (I) Infected, (H) Healthy.
[b] (S) Evaluated by sequence.

Nested PCR Analysis. Of the 17 samples evaluated, a 50% amplification of the insects feeding on infected plants was obtained; the majority of these pertain to adults of *S. marginelineatus.* The presence of phytoplasm was visually evident in the agar gels; bands consisting of approximately 800 pb, typical bands of the 16SrIII group when the pair of primers R16(III) F2/R16(III)R1 are used **(Figure 1)**.

DNA Sequencing. Two of the bands were obtained from direct sequencing, purifying the PCR products **(Table 1)**. The sequences analyzed from the fragments revealed that the phytoplasm from the insects was similar to Cirsium while leaf phytoplasm (GenBank acc. No. AF373106, 16SrIII X-disease group) with a 100% homologue in both fragments with a total of 800 pb sequenced. In addition a strong homologue was found between the sequenced fragments from

the insects and sequences reported in GenBank for phytoplasm associated with FSD in the genotypes MCol 2063 (acc. No. AY737646) and SM 1219-9 (acc. No. AY73647) (procedures carried out by the Cassava Pathology section). This confirms that the amplified insect products are related to the phytoplasm associated with CFSD in cassava (CIAT, 2003). Based on these homologous results and on the Nested-PCR technique, new transmission studies are being evaluated, taking into consideration the previous evaluations done in plants considered healthy or diseased and later evaluating plants where the feeding homopterous insects were identified as possible vectors.

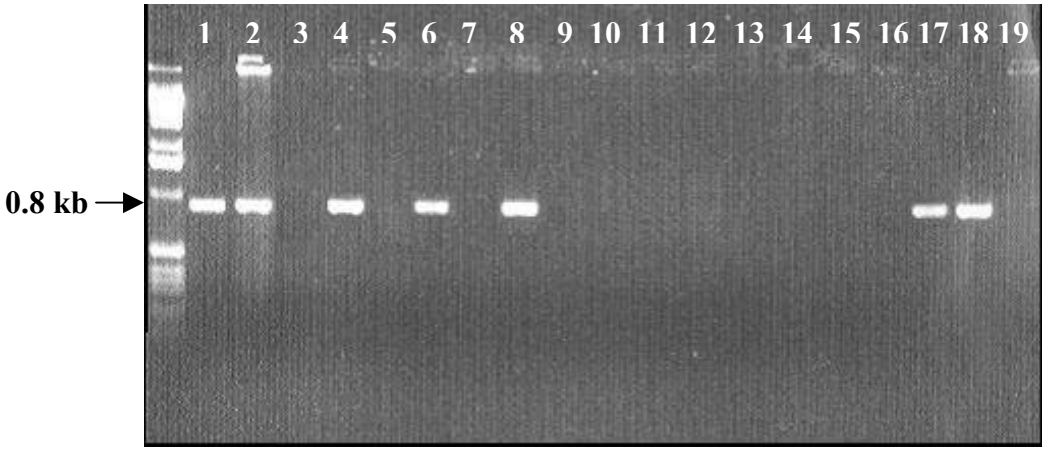

Figure 1. Presence of typical phytoplasm group 16SrIII for *S. marginelineatus* feeding on CFSD infected plants, lanes 1, 2, 4, 6, 8 and 17; lane 18 is the positive control and lane 19, the negative control; 1kb: Molecular weight.

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Collaborators: Claudia M. Holguín, Anthony C. Bellotti, Elizabeth Alvarez, Juan Fernando Mejía, Adriano Muñoz.

Activity 11. Field evaluation and identification of homopteran species as possible vectors of Cassava Frogskin Disease.

Cassava frogskin disease (CFSD) is a virus like disease that can cause considerable crop damage resulting in root yield losses as high as 90%. The epidemiology of CFSD is not completely understood; while dissemination by infected planting material is well documented, transmission by insect vectors is still being investigated. Transmission by the whitefly *Bemisia tuberculata* is suspected but not sufficiently confirmed. Therefore studies are in progress to determine other possible vectors of CFSD.

Objectives: The objective of the present research is to determine possible insect vectors associated with the transmission of the causal agent of CFSD: emphasis is being given to collecting and evaluating several homopteran species, especially those of the Cicadillidae and Delphacidae families.

Methodology: An experiment was designed to identify the arthropod species complex in a cassava field during its growth cycle and correlate this to the presence/absence of CFSD. The experimental field was located on the CIAT research station in Santander de Quilichao, Cauca.

A completely random experimental design with two treatments and 12 replications per treatment was employed. The experimental arena consisted of 49m^2 plots with one meter between rows and one meter between plants. The cassava variety sown was MCol 1505 (Manihoica P-12) that originated from an in-vitro source, free of CFSD. The experimental field had usually been planted in legumes and not cassava.

The treatments considered of:

T1: All arthropods present (no pesticide application)

T2: Absence or minimal presence of arthropods. Weekly application of imidacloprid (1 ml/l) + tamaron (2.5 ml/l) for the first six months of plant growth.

Evaluations of arthropod presence was carried out weekly beginning at 15 days after planting up to six months, on six plant in each plot. All arthropods present at the time of evaluation were recorded (including biological control agents). A visual scale was utilized to determine whitefly populations (eggs, nymphs and adults) **Table 1**.

Table 1. Population scale for development stages of the whitefly *Aleurotrachelus socialis* Bondar 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

When above ground evaluations were completed, root sampling for CFSD symptoms was initiated. Five plants from each plot were harvested monthly up to total plot harvest (about one year).

Results: Sampling plots with and without pesticide application resulted in 17 insect species identified. Four of these were whitefly natural enemies; the predator, crysopa, and parasites such as *Amitus* sp., *Encarsia* sp., and *Eretmocerus* sp. **(Table 2)**.

Table 2. Arthropods collected from pesticide treated and non-treated cassava (MCol 1505 at CIAT Farm, Santander de Quilichao, Cauca, 2004.

Arthropods	Total Arthropods	Treated Plots	Non-treated Plots
Aleurotrachelus socialis	3*	2	4
Bemisia tuberculata	64	1	63
Trialeurodes variabilis	9	-	9
Scaphytopius marginelineatus	5	3	2
Cicadellidae	6	4	2
Empoasca sp.	54	17	37
Fulgoridae	14	4	10
Thrips	910	16	894
Hornworm	254	104	150
Mites	20286	13226	7060
Aphids	1	-	1
Lacebugs	17	4	13
Diabrotica	1	-	1
Predator: Crysopa	458	168	300
Whitefly Parasitoids:	1182	65	1117
Amitus macgowni			
Encarsia sp.			
Eretmocerus sp.			

* Average adult, eggs, and nymphs of whiteflies, based on 1-6 population scale **(Table 1)**.

Arthropod populations of the different species varied. The whitefly species *A. socialis* was recorded present throughout the cassava growth cycle with highest populations, as expected, in the non-treated plots **(Table 2)**. Other whitefly species, *Bemisia tuberculata* and *Trialeurodes variabilis*, were observed in low numbers on non-treated plots and during the last evaluations (6 months).

During the early growth stages (0-3 months), thrips dominated. As thrips populations decreased, mite populations increased and remained high until evaluations terminated (6 months). In general, arthropod populations were higher on the non-treated plots than on the treated; mites were the exception where treated plots had higher populations **(Table 2)**.

Homopteran species such as *Scaphytopius marginelineatus*, *Empoasca* sp., and other Cicadellidae and Fulgoridae, (identification pending) were observed sporadically in the plots. *Empoasca* predominated, and was followed by the Fulgoridae, in non-pesticide plots. *S. marginelineatus* and the Cicadellidae in general were represented by only 11 individuals, primarily in non-pesticide applied plots **(Table 2)**.

Evaluations were terminated when the crop reached 6 months and root sampling was initiated. Five plants in each plot were randomly selected and harvested to detect CFSD root symptoms.

Plots were sampled four times before the final harvest. CFSD symptoms were observed on only three plants in all the experimental plots. One infected plant was detected in the non-applied plot and two in the pesticide applied plots. The arthropods that predominated in plots where CFSD was detected consisted primarily of whitefly (*A. socialis*), thrips, hornworm and mites, as well as the natural enemies (Crysopa and whitefly parasitoids) **(Table 3)**.

Table 3. Arthropods collected from cassava field plots where the presence of CFSD was detected (Santander de Quilichao, Cauca, 2004).

Insects	Treated Plots[1]	Non-treated Plots[2]
Aleurotrachelus socialis	1.72*	2.49
Bemisia tuberculata		1
Thrips	1	46
Empoasca sp.		1
Hornworm	2	4
Mites	1078	761
Crysopa	12	32
Whitefly parasitoids	9	70
Fulgoridae	1	1

1 One plant detected with CFSD symptoms.
2 Two plants detected with CFSD symptoms.
* Average whitefly adult, eggs and nymphs population using 1 to 6 population scale.

The species found that are possible vectors of CFSD were *Empoasca* sp. in the pesticide applied plot, and fulgorids in the non-applied plots. In addition, the whitefly *A. socialis* was found in both plots.

These results are not conclusive to establish any possibility of identifying possible CFSD vectors. However, considering the high populations observed of the whitefly, *A. socialis* and the low incidence of CFSD, it is a strong indication that *A. socialis* is not a vector of CFSD. A second experiment was planted in the same fields to verify these results. Evaluation methodology was changed in favor of using yellow sticky traps. Evaluations of this experiment are presently being carried out.

Contributors: Claudia M. Holguín, Adriano Muñoz, Carmen Elisa Mendoza, Anthony C. Bellotti.

Activity 12. Testing of transgenic cassava (Africa genotype TMS 60444) plants displaying indications of resistance to the cassava hornworm, *Erinnyis ello.*

Erinnyis ello, the cassava hornworm, is one of the most serious cassava pests in the neotropics (Bellotti et al, 1992). It has a broad geographic range, extending from the southern cone (Brazil, Argentina and Paraguay) of South America to the Caribbean Basin and southern USA. Hornworm larval feeding will defoliate cassava plants causing considerable yield reductions, especially if repeated attacks occur. Based on extensive research of this pest by CIAT and NAR's scientists an IPM program for hornworm control has been developed. The basis of this program is centered around biological control, especially the use of a baculovirus that has recently been developed as a commercial biopesticide (CIAT Annual Report, Project PE-1, 2002 and 2003).

The CIAT cassava germplasm bank consists of nearly 6,000 genotypes. Most accessions are traditional land race cultivars collected from farmers' fields and this material offers entomologists and breeders a potential pool of pest resistant genes. A high (60 to 70%) percentage of genotypes in this germplasm bank are consistently being grown in the field and subject to pest attack. Periodic evaluations of these genotypes when hornworm attacks have occurred have indicated that genetic resistance to *E. ello* is not available in cultivated cassava, *Manihot esculenta.*

Several years ago CIAT initiated research based on introducing insect resistant *Bacillus thuringiensis* (Bt) genes (*Cry 1ab*) through *Agrobacterium* mediated transformation into cassava embryonic tissue to develop lepidopteran resistant cultivars. Transgenic plants of the model variety of African origin, TMS 60444 (MNg 11) have been developed. This genotype is the progeny of an interspecific cross of the wild species *Manihot glaziovii* and *M. esculenta*. *M. glaziovii* is also the source of resistance to ACMD (African Cassava Mosaic Disease), and in preliminary evaluations at CIAT has displayed resistance to pest such us whiteflies. TMS 60444 was selected because of its high transformation capacity and relatively rapid regeneration (Bellotti et al, 2002).

Objective

1. Determine the leaf consumption rate of the cassava hornworm, *E. ello*, on different genetically modified lines the variety TMS 60444.
2. Quantify the effect of the *Gen Cry 1Ab* in transgenic lines on the behavior and feeding of the cassava hornworm.

Methodology: Hornworm larvae were obtained from the laboratory/field colony maintained at CIAT. The cassava variety CMC-40, a susceptible genotype was grown out in farmers and CIAT fields. TMS 60444, non-modified genetically and resistant to the hornworm was grown at CIAT. The genetically modified lines L27, L80 and L92, originally from TMS 60444 were produced at CIAT.

The cassava hornworm, *E. ello*, colony is maintained by placing adults (male and females) in large field cages (2m x 2m x 2m) where females can readily oviposit on growing cassava plants. Eggs are removed to the laboratory where larval instars (5) develop in cages while feeding on

cassava leaves. Recently emerged first instar larvae were used in all experiments; the first leaves fed-upon were those of each respective treatment.

The experiment had six treatments and twenty replications per treatment. The experimental arena was a plastic petri dish (15mm x 2.5mm) that contained excised cassava leaves. One first instar *E. ello* larvae was introduced into each petri dish and allowed to feed on the cassava leaf. All larvae were weighed on an analytical balance prior to being placed in the petri dish. It was therefore possible to record any weight gain or loss during the larval feeding period. Larvae were weighed every 24 hours and cassava leaves were replaced on a daily basis, until pupation or larval mortality occurred. Chi square analysis was used to evaluate mortality vs. variety (treatment).

Results: Hornworm (*E. ello*) mortality reached 100% on the transgenic lines L80, L92, and 85% mortality on L27 **(Figure 1)**. On the latter 15% of the larvae reached the prepupal stage. Mortality on the non-modified control variety, CMC-40 was 25%. Mortality on the non-modified variety, TMS 60444, was 100%. The Chi square test showed that the mortality was independent of the genotype.

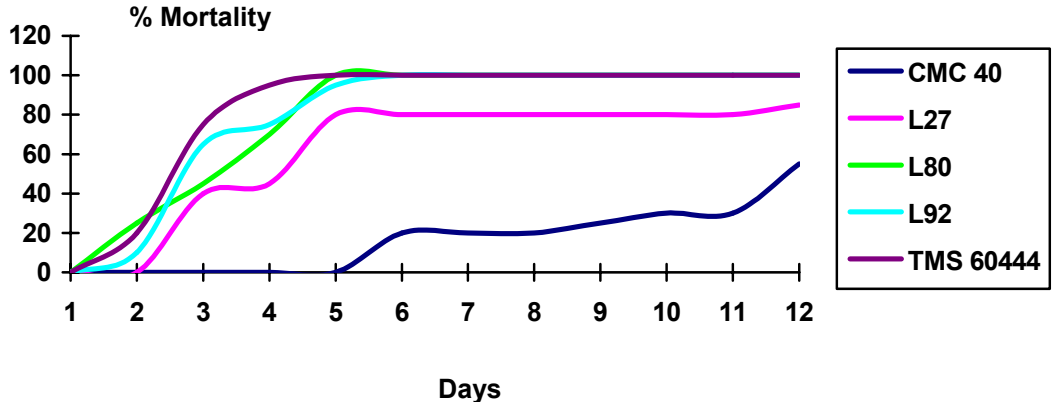

Figure 1. Percent mortality of cassava hornworm (*Erinnyis ello*) larvae consuming leaves from Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) cassava genotypes (CIAT, 2004).

Peak mortality on the transgenic lines and non-modified TMS 60444 occurred during the first 3 to 5 days of larval development. Larval mortality on the susceptible control, CMC-40, first occurred at 6.6 days **(Table 1)**. There were no statistical differences between the transgenic genotypes and TMS 60444, but all four genotypes were statistically different from CMC 40 **(Table 1)**.

These results show that the TMS 60444 genotypes, have a "natural" resistance to *E. ello* and that this resistance masks the effect of the Bt gene inserted into the transgenic lines. The rapid mortality of *E. ello* larvae feeding on the modified or non-modified TMS 60444 genotypes, when compared to the susceptible control (CMC 40) is additional evidence of the effectiveness of the natural resistance in TMS 60444.

Table 1. **Average number of days when initial hornworm (*Erinnyis ello*) larval mortality occurs on transformed (BT, Cry 1Ab) (L27, L92, L80) and non-transformed (CMC 40, TMS 60444) cassava genotypes (CIAT, 2004).**

Genotypes	Days Mortality Initiated (Average)
CMC-40	6.6 A
TMS 40666	3.1 B
L-27	4.4 B
L-92	3.5 B
L-80	3.6 B

E. ello larvae feeding on TMS 60444 and the transgenic lines show a significant reduction in daily weight gain when compared to the susceptible control, CMC 40 **(Figure 2)**. Daily weight gain on TMS 60444, L80 and L92 was significantly lower than on L27, which was significantly lower than on CMC 40 **(Table 2)**.

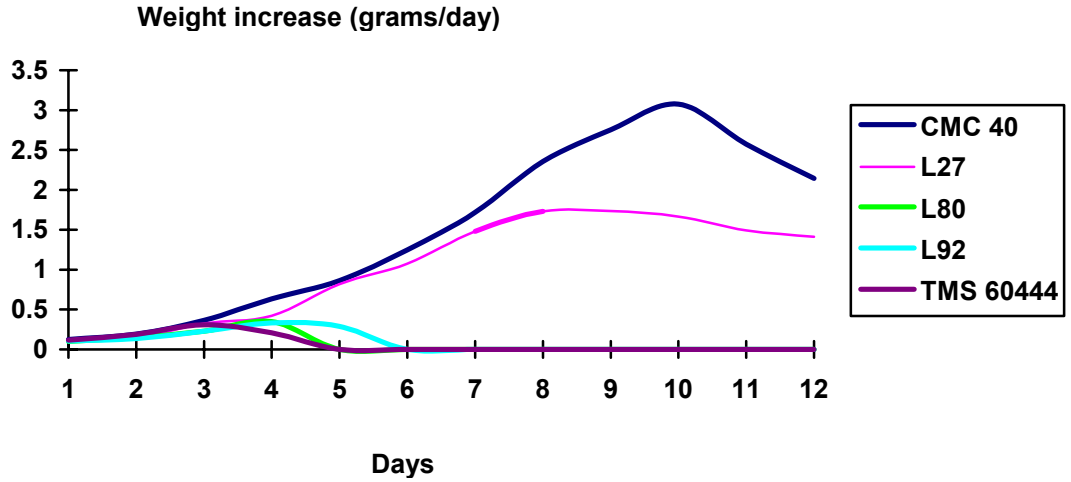

Figure 2. **Daily weight increase of cassava hornworm (*Erinnyis ello*) larvae feeding on leaves from Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) on cassava genotypes (CIAT, 2004).**

Table 2. **The area below the growth curve as a function of the weight and mortality of cassava hornworm (*Erinnyis ello*) larval feeding on Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) cassava genotypes (CIAT, 2004).**

Cassava Genotypes	Area Below Curve
CMC 40	9.1176 A
L27	6.7492 B
L80	5.8309 C
L92	5.8156 C
TMS 60444	5.6588 C

The area below the growth curve is a function of larval weight increase and mortality on the Bt transformed and non-transformed genotypes.

These results show no significant difference between the transgenic lines L80, L92 containing the Cry 1Ab gene from *Bacillus thuringienses* and the non-modified TMS 60444. This indicates that the TMS 60444 genotype has genes independent of Cry 1Ab that expresses resistance to the cassava hornworm, *E. ello*. As stated earlier, numerous years of observation (at least 30) of the CIAT *M. esculenta* germplasm bank did not detect any resistance to *E. ello*. This leads to the speculation that the source of the "natural" resistance found in TMS 60444 originated from the interspecific cross with *M. glaziovii*, a parent in its development in Africa nearly 70 years ago.

A genetic study is needed to identify the gene or gene sequence responsible for the *E. ello* resistance detected in the TMS 60444 lines.

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Collaborators: Carlos Julio Herrera, Anthony C. Bellotti, Paul Chavarriaga, Danilo López.

Activity 13. Toxicity of *Jatropha gossypiifolia* leaf extracts on three Lepidoptera species.

Leaf extract of *Jatropha gossypiifolia* (Euphorbiaceae), known to contain toxics to insects (one Tenebrionid and one Pseudococcid) were tested under artificial conditions on larvae of three Lepidoptera species *Busseola fusca* (Noctuidae), *Ostrinia nubilalis* (Pyralidae) and *Sesamia nonagrioides* (Noctuidae), which are important pests of maize in Africa, Europe and Mediterranean countries respectively.

Using three distinct leaf extract concentrations, the mortality of *B. fusca* neonates is shown in **Table 1**. The results indicate clearly that *J. gossypiifolia* leaf extracts were toxic. Surprisingly, the toxicity was significantly lower at 50 mg/ml as compared to the one obtained at 10 mg/ml, probably due to some phago-deterrent effect of the high dose, on which some larvae did not feed and therefore not dead after 24 hours. Moreover, the corrected LC_{90} after 24 hours was 19 mg/ml. This concentration is close to the lowest concentration tested (10 mg/ml). After 48 hours, calculation of the LC_{90} was not possible because almost 100% of the neonates had already died at the first concentration tested **(Table 1)**.

Table 1. Percent mortality and LC_{90} of *B. fusca* neonates due to exposure to leaf extract of *J. gossypiifolia* at different concentrations in the diet.

	% [1] Mortality (means ± SE [2])	
Extract Concentration in the Diet (mg/ml)	**24 Hours After**	**48 Hours After**
0	0 a	0 a
10	70.0 ± 5.8 c	90.0 ± 10.0 b
50	50.0 ± 10.0 b	100 b
100	100 d	100 b
LC_{90} [confidence interval, p=0.05] (mg/ml)	19 [9 – 29]	not possible to calculate

[1] % Without correction.

[2] Means followed by the same letter are not significantly different at 5% level (Fisher's PLSD test following ANOVA).

The concentration inducing the highest *B. fusca* mortality after 24 hours was 100 mg/ml, consequently the subsequent experiments were performed at this concentration.

J. gossypiifolia leaf extract revealed also to be toxic to *O. nubilalis* neonates **(Table 2)**. In fact, 100 mg/ml of extract in the diet induced 75% of mortality after 24 hours and 100% after 48 hours. In the case of *S. nonagrioides* no such toxicity level was found. It is important to point out that toxicity of leaf extract remained after boiling treatment for both *B. fusca* and *O. nubilalis*. This suggests strongly that the toxicity of the extract could be due to proteins hence in support of the extraction method used, and that such proteins are thermo-stable.

Almost no toxicity was found to neonates of *S. nonagrioides*. Accordingly to the leaf extraction method used, the toxicity to *B. fusca* and *O. nubilalis* neonates could have been due to protein(s). Nevertheless, this toxicity decreased strongly with the larval age, disappearing completely for *O. nubilalis* at the fourth instar larvae.

Table 2. Percent mortality of *B. fusca*, *O. nubilalis* and *S. nonagrioides* neonates due to exposure to leaf extract of *J. gossypiifolia* at 100 mg/ml in the diet before or after boiling.

	% Mortality (Means ± SE [1])	
B. fusca	**24h after**	**48h after**
Control diet	0	0
With extract	100	100
With extract (after boiling)	100	100
O. nubilalis		
Control diet	5.0 ± 5.0 a	10.0 ± 5.8 a
With extract	75.0 ± 9.6 b	100 b
With extract (after boiling)	65.0 ± 9.6 b	100 b
S. nonagrioides		
Control diet	5.0 ± 5.0	10.0 ± 10.0
With extract	25.0 ± 12.6	45.0 ± 9.6
With extract (after boiling)	20.0 ± 8.2	20.0 ± 8.2

[1] Means followed by the same letter are not significantly different at 5% level (Fisher's PLSD test following ANOVA). For *B. fusca*, no letter was given by the impossibility to calculate the ANOVA. For *S. nonagrioides*, no letter was given because $p>0.05$ for ANOVA.

Subsequent assays for feeding activity of the larvae was carried out by using 1% (w/w) of pH indicator powder. In this experiment we consistently observed that all neonates intestinal ducts were colored by the presence of the pH indicator into the diet, demonstrating that the larvae indeed fed on the diet and that their deaths were related to the toxicity of the leaf extract.

The toxicity of *J. gossypiifolia* leaf extract decreased with the age of the larvae. For *B. fusca*, when the same experiment was conducted with the fourth instar larvae: no mortality (0%) was recorded after 24 hours at 100 mg/ml, but 70% of mortality was obtained after 48 hours. For *O. nubilalis* and *S. nonagrioides,* no mortality (0%) was recorded at 100 mg/ml in the diet after 48 hours and even after five days. Because all larvae presented a colored intestinal duct after pH indicator treatment, the low toxicity on old larvae was not related with starvation but more probably to a better and effective detoxification system, developed later during their development.

In conclusion, *J. gossypiifolia* leaf extract was toxic to *B. fusca* and *O. nubilalis*. Neonates revealed to be more sensitive than older larvae. Since neonate stage represents the first stage of host plant colonization, it is therefore reasonable to consider that the compounds and probably protein(s) involved in this toxicity can be studied to develop improved pest-resistant plants by transgenic strategies. In spite of these results, however, the real involvement of a putative protein(s) in this toxicity still remains to be not only demonstrated, but also established in more detail. According to the quasi-absence of toxicity found in *S. nonagrioides* and to the decrease of toxicity with the larvae age, it is possible to suggest the existence of acquired resistance mechanism in these insect species to *J. gossypiifolia* leaf extract.

During evolution, pests have often been in contact with a variety of natural toxins and they have, consequently, developed strategies to deal with their toxic effects. These may include the degradation of the toxic compound by digestive enzymes, which are not susceptible to these

"proteinaceous" toxins. The ability of target pests to overcome the effects of the introduced "proteinaceous" toxin should be considered to ensure their successful implementation as bio-insecticides in transgenic plant production programs.

Contributors: A. Valencia-Jimenez, Departamento de Química. Facultad de Ciencias Exactas y Naturales. Universidad de Caldas, Manizales, Colombia; B. Frérot, H. Guénégo, Institut National de la Recherche Agronomique (INRA), Unité de Phytopharmacie et Médiateurs Chimiques, Versailles, France; D.F. Múnera, A.C. Bellotti, International Centre for Tropical Agriculture (CIAT), Cassava Entomology, CIAT, Cali, Colombia; P.-A. Calatayud, Institut de Recherche pour le Développement (IRD) c/o International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.

Activity 14. Publications, book chapters, posters, conferences, training and consultancies.

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