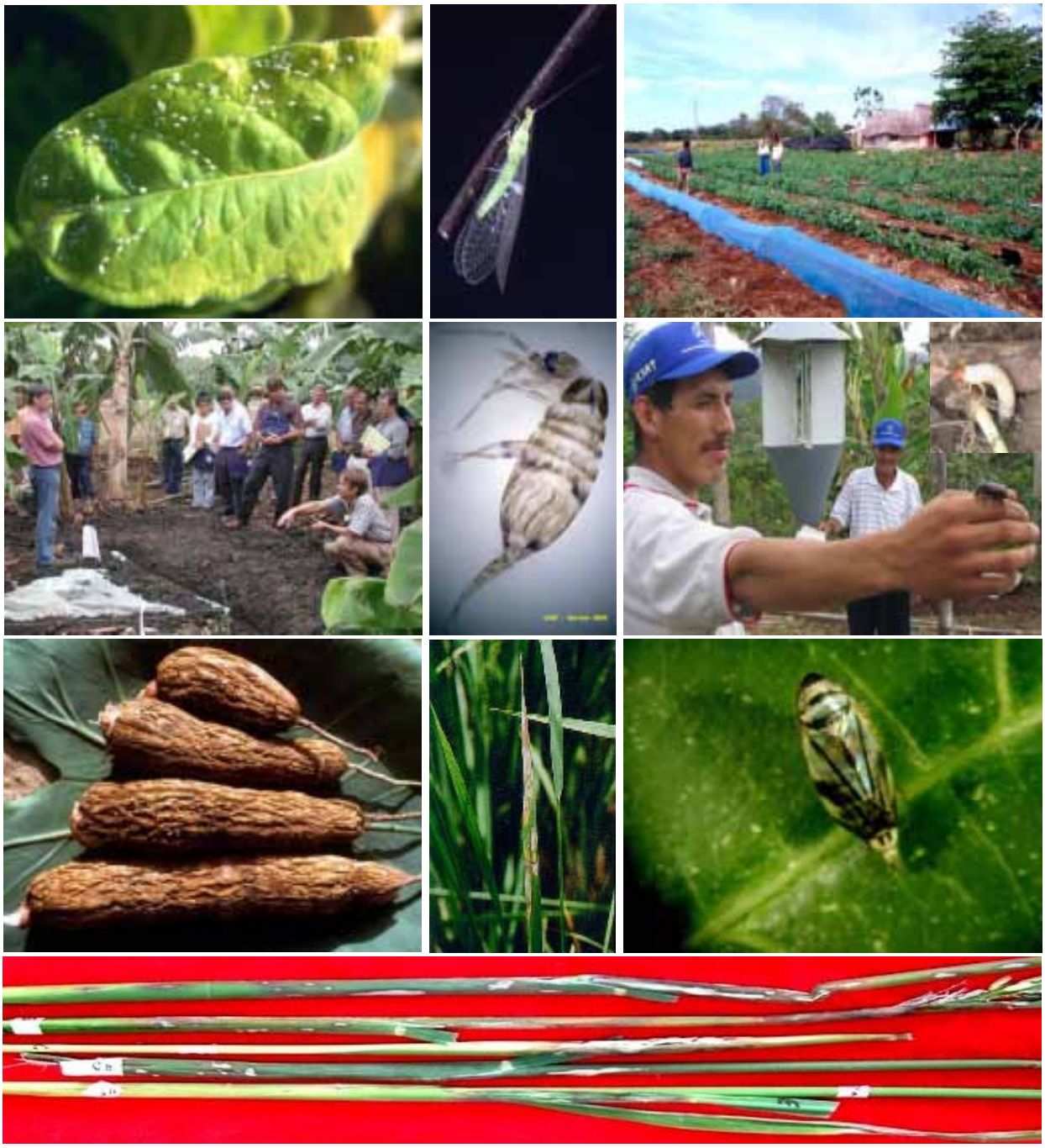
CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Integrated Pest and Disease Management In Major Agroecosystems



PROJECT-PE1 - Annual Report 2004

System – wide Programme on Integrated Pest Management

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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.

References

Gillott, C. 1980. Entomology. Plenum Press, New York. 729 pp.

Ramos, M.; Cristina, G.; Cabreram R,I. 2001. Presencia de *Steneotarsonemus spinki* (Acari: Tarsonemidae) en cuatro variedades de arroz en la República Dominicana. Rev. Protección Veg. Vol. 16(1):6-9.

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

Activity 2. Chrysopidae species associ ated 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).

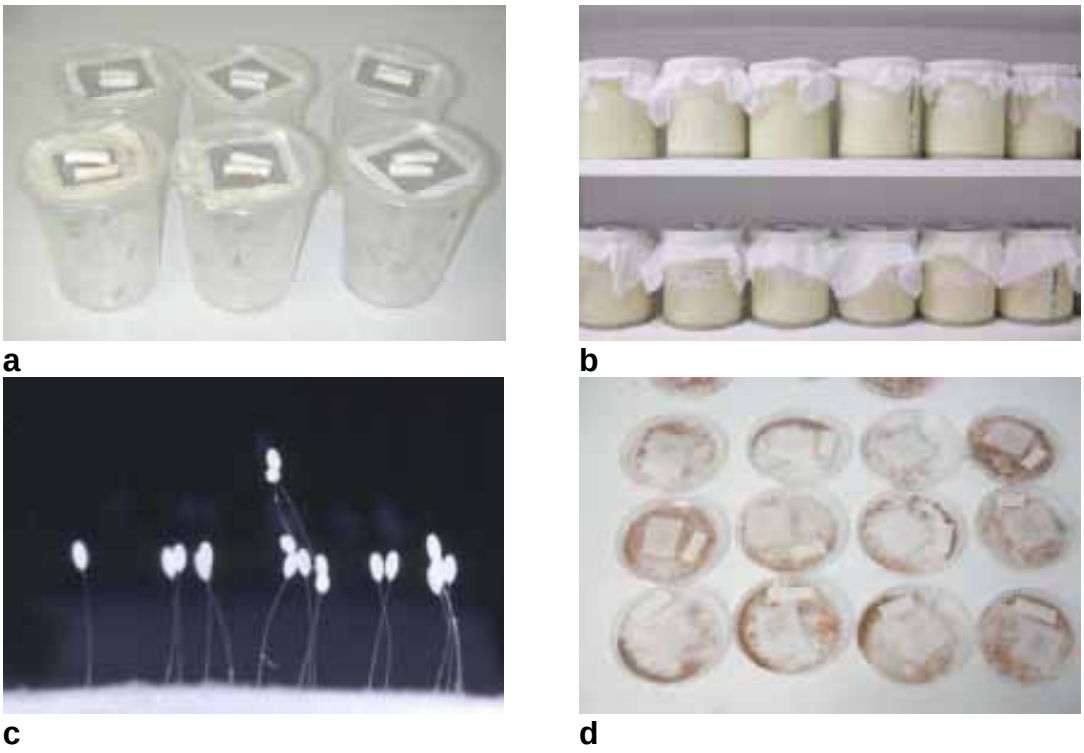


Figure 1. (a) Recipients for transporting Chrysopidae specimens collected in the field. (b) PVC rearing units for individual females. (c) Chrysopa eggs removed from the rearing units. (d) Chrysopid larvae feeding on *Sitotroga cerealella* eggs.

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.

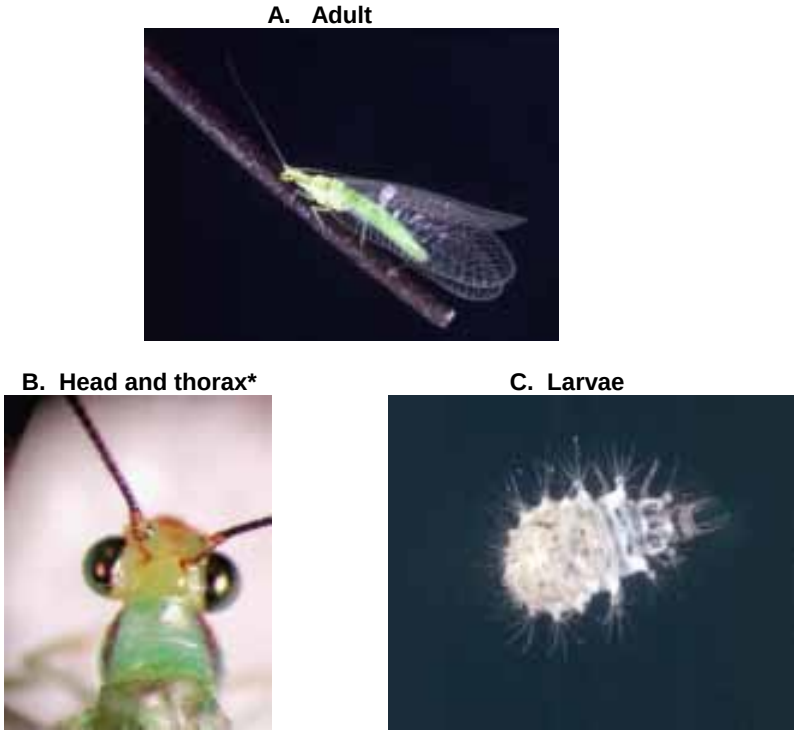


Figure 2. ***Ceraeochrysa claveri*** **(Navás). Characteristics: First–third of antenna darkened, antenna longer than wing extension, reddish-brown lateral spots on the pronotum, lengthening of spots on the shaft, usually on the vertex, palpi clear.**

A. Adult

B. Head and thorax*

C. Larvae

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

A. Adult

B. Head and thorax*

C. Larvae

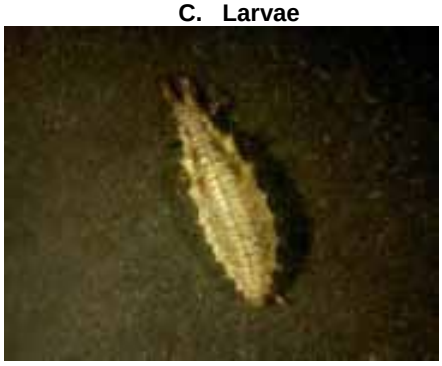


Figure 4. ***Chrysoperla externa*** **(Hagen). C haracteristics: Antenna shorter than w ing extension, yellow bands on the dorsal part of the thorax and abdomen, dark genal spots, pronotum with clear seta originating from dark base, red spotting near the ocular cavity.**

A. Adult

B. Head and thorax*

C. Larvae

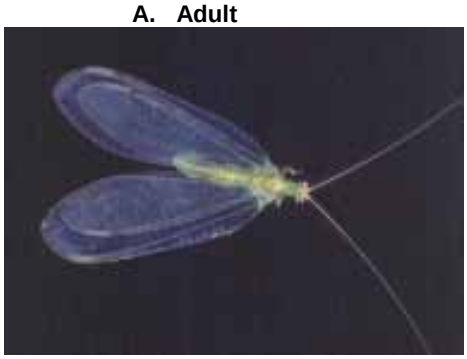
Figure 5. ***Chrysopherla carnea (Stephens).*** **Characteristics: Antenna shorter than the wing extension, yellow bands on the dorsal part of the thorax and abdomen, dark brown genal spots, pronotum with thick seta, blackened at the base.**

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

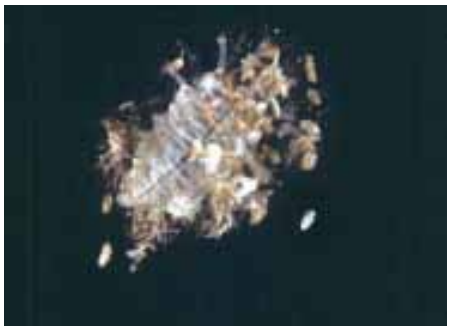


Figure 7. ***Leucochrysa sp.*** **#2. Characteristics: Antenna e qual to or longer than w ing extension, dark spots on the distal third of the w ing, four sp ots on the pronotum that may coalesce to form two lengthening lines.**

Photos by Cristian Olaya.

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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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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**.

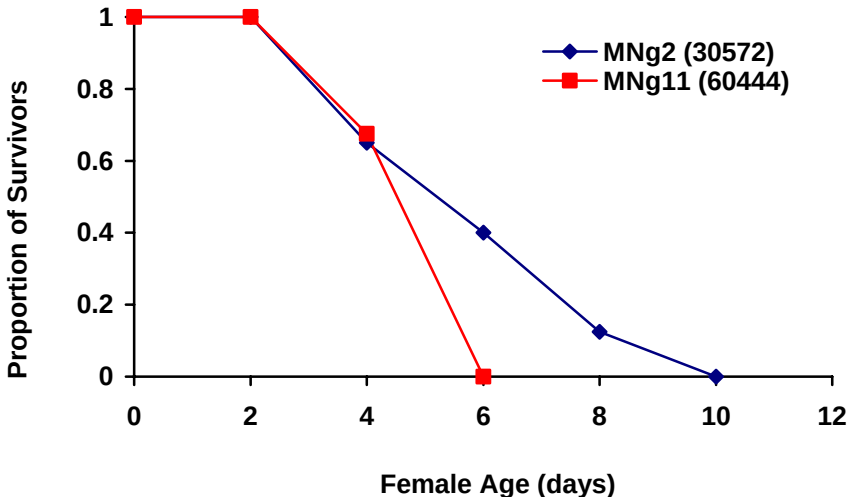

Figure 1. Female survivors of *B. tabaci*, B Biotype, feeding on the African Cassava genotypes MNg 2 (TMS 30572) and MNg 11 (TMS 60444) in the grow th chamber (CIAT, 2004).

Table 1. Average longevity (days), average f ecundity (eggs/female) and oviposition rate (eggs/female/2 days) of *B. tabaci,* B biotype, feeding on the Africa n 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)**.

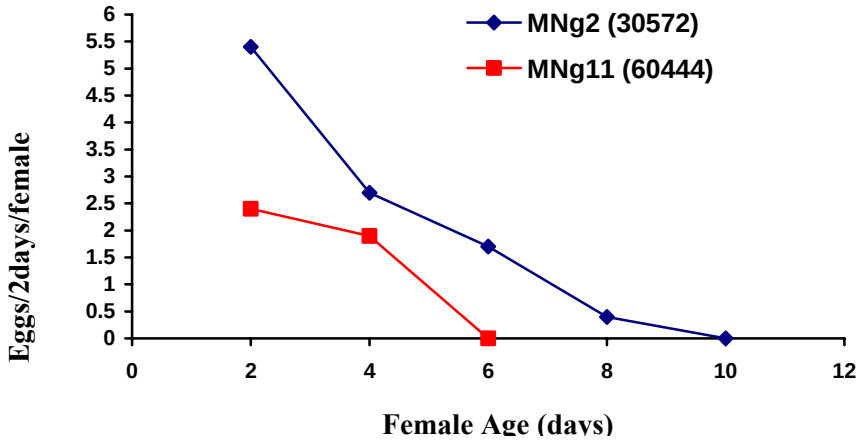

Figure 2. ***B. tabaci*** **reproduction curves when feeding on African cassava genotypes MNg 2 and MNg 11 in the growth chamber (CIAT, 2004).**

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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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 (1_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).

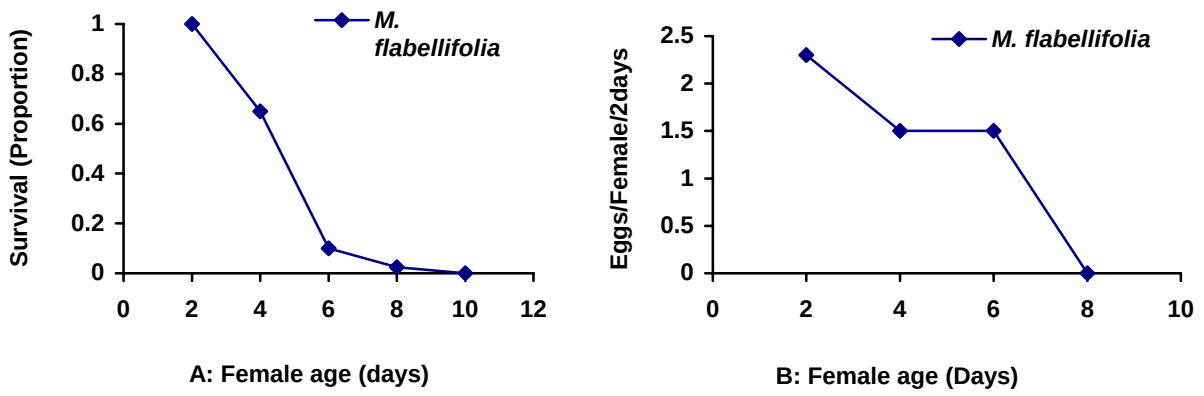

Figure 1. ***Bemisia tabaci*** **(biotyp e B) reproduction (A) and survi val (B) curves when feeding on** ***Manihot flabellifolia*** **in the growth chamber.**

Table 1. Average longevity, average fecundity and rate of oviposition (eggs/female/2 days) of biotype "B" of ***Bemisia tabaci*** **feed ing 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, su rvival and pr oportion 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 w hitefly (*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)**.

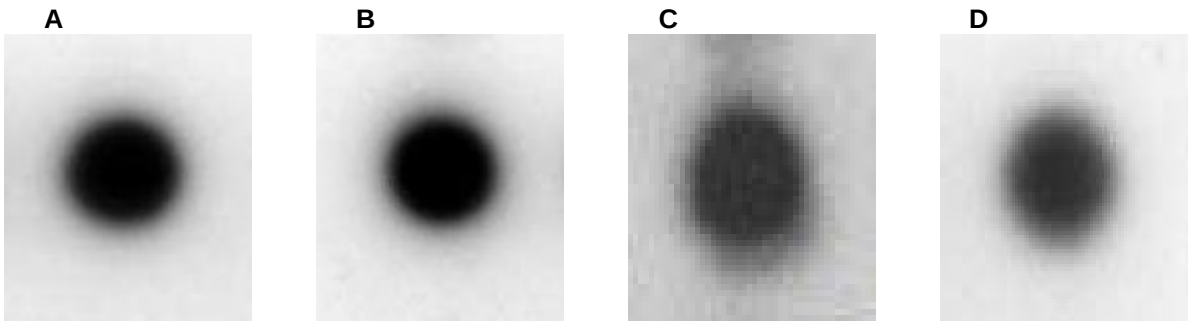

Figure 1. Test for antibody recognition usin g the Dot Blot technique. A: antibodies against MEcu 72, B: antibodies against MEcu 64, C: antibodies against MPer 334, D: antibodies against CMC 40.

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*.

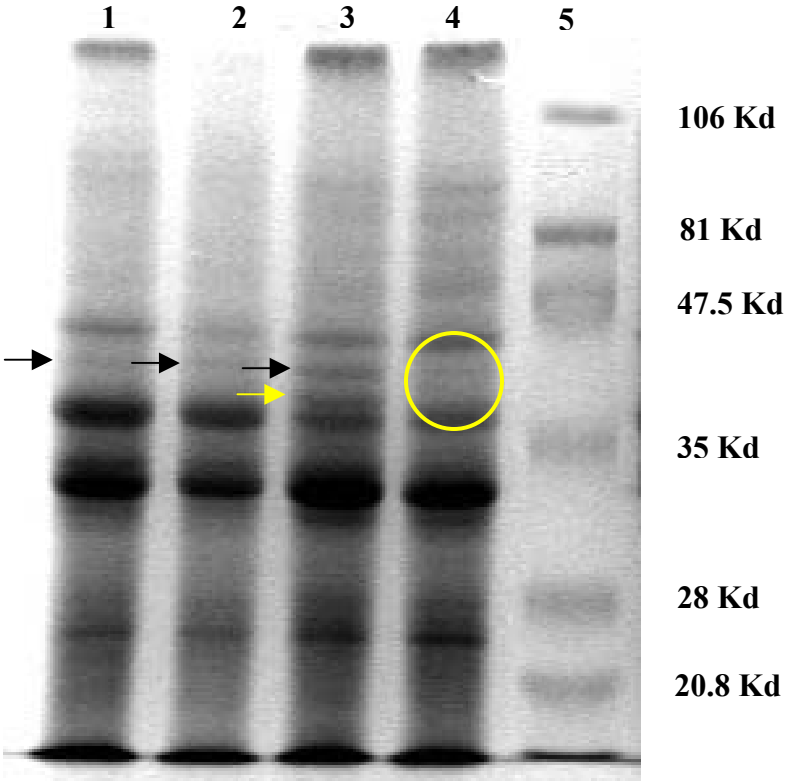

Figure 2. SDS-Page. Phase resolving concentr ation of 14% , Sample concentration of 2 mg/ml. 1: MEcu 72, 2: MEcu 64, 3: MPer 334, 4: CMC 40; 5: Molecular weight marker (Kd). The black arrow indicates the polymorphic band commonly present in the resis tant genotypes and absent in the susceptible, CMC 40, indicated by the yellow circle. The yellow arrow s 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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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 Cicade llidae colle cted fr om cassava fields at several locations in Colombia.

Department	Municip ality	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 grow th 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* (Convelin us) *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 ny mphal stages of *Scaphytopius marginelineatus* under controlled conditions (CIAT, 2004).

Stage Dur	ation (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 an d 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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Contributors: María del Pilar Hernández, Anthony C. Bellotti.

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 w ith the nested -PCR and primers R16F2/R16R2 and R16(III)F2/R16(III)R1.

Samples		Genotype[a]	State Neste	d-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.

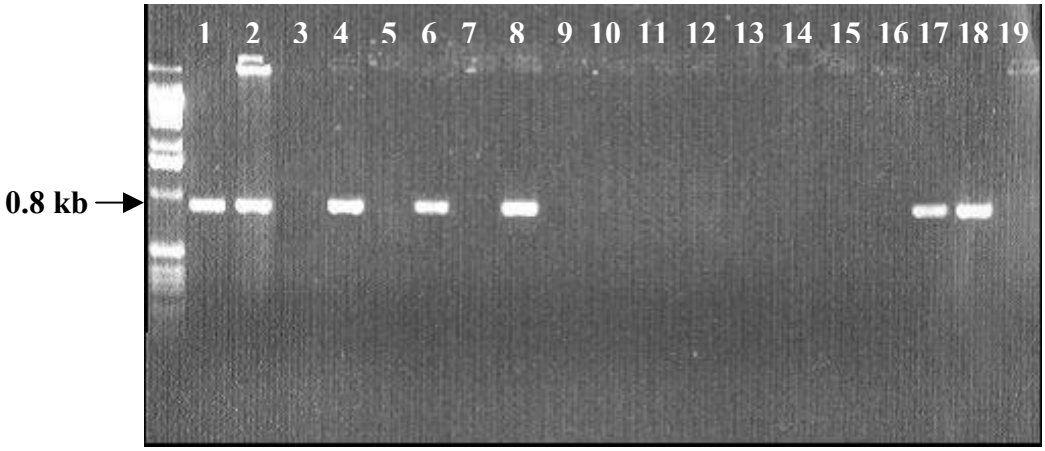

Figure 1. Presence of typical phytoplasm group 16SrIII for *S. marginelineatus* feeding on CFSD infected plants, lanes 1, 2, 4, 6, 8 and 17; lane 18 is th e positive control and lane 19, the negative control; 1kb: Molecular weight.

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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 pestic ide treated and non-treated cassa va (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.

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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.

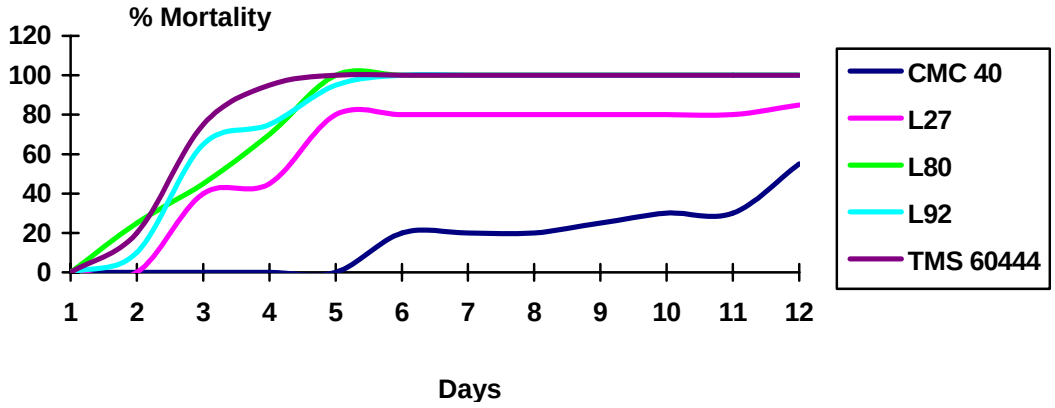

Figure 1. Percent mortality of cassava hornworm (*Erinnyis ello*) larvae consuming leaves from Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) cassava genotypes (CIAT, 2004).

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 ho rnworm (*Erinnyis ello*) la rval mortality occurs on tr ansformed (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)**.

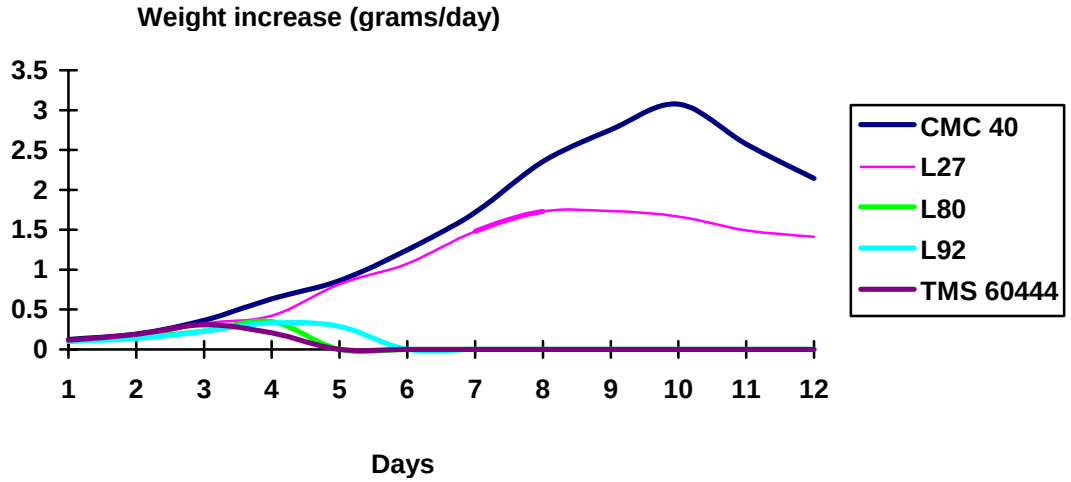

Figure 2. Daily w eight incr ease of cassava hornw orm (*Erinnyis ello*) larvae feeding on leaves from Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) on cassava genotypes (CIAT, 2004).

Table 2. The area below the grow th curve as a function of the w eight and mortality of cassava hornworm (*Erinnyis ello*) larval feeding on Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) cassava genotyp es (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.

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Continued

Nataima-31, A cassava (*Manihot esculenta*) Variety Resistant to the Whitefly, *Aleurotrachelus socialis*

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INTRODUCTION

In recent years, the whitefly *Aleurotrachelus socialis* has been a major pest of cassava, causing over 30% yield losses in different regions of Colombia. Due to its short life cycle (30-35 days), *A. socialis* populations increase rapidly and its ability to develop resistance to pesticides makes chemical control uneconomical. Host plant resistance is sustainable alternative for managing this pest.

Fig. 1. Population and Damage of *A. socialis*

The moderate to high levels of whitefly resistance found in cassava is somewhat unique in cultivated food crops. The CIAT research program to identify whitefly resistance was initiated about 20 years ago and resistance has now been identified in numerous cassava genotypes. Nataima-31 is a resistant commercial hybrid developed in a joint effort between CIAT and CORPOICA/MADR in Colombia.

ORIGIN OF Nataima-31

Evaluation of the CIAT cassava germplasm bank for whitefly (*A. socialis*) resistance was initiated in 1978. A 1 to 6 whitefly damage and population scale was employed, where 1 indicates the absence of whitefly damage and population and 6 indicates the severest damage and highest population (Table 1 and 2).

Table 1. Population scale for evaluating cassava germplasm for resistance to whiteflies

1 = no whitefly stages present
2 = 1-200 individuals per cassava leaf
3 = 201-500 per leaf
4 = 501-2000 per leaf
5 = 2001-4000 per leaf
6 = > 4000 per leaf

More than 6000 cassava genotypes have been evaluated using these scales and different sources of resistance have been detected in several genotypes. Clone MEcu 72 has consistently express high levels of resistance. The variety Nataima-31 is the progeny of a cross between MEcu 72 and MBra 12. MBra 12 was selected as the male parent because of its high yield and desirable agronomic and culinary qualities.

The original cross resulted in 128 progeny and these were evaluated for whitefly resistance, yield and cooking quality at the CORPOICA Research Station in Espinal, Tolima, Colombia. Of the 128 progeny, four (CG 489-34, CG 489-31, CG 489-23 and CG 489-4) were selected for low whitefly populations and no damage as well as the agronomic qualities described above. Nataima-31 is the 31st progeny of the 128 that were evaluated (Bellotti, 2003).

Table 2. Damage scale for evaluating cassava germplasm for resistance to whiteflies

1 = no leaf damage
2 = young leaves still green but slightly flaccid
3 = some twisting of young leaves, slight leaf curling
4 = apical leaves curled and twisted; yellow-green mottled appearance
5 = same as 4, but with sooty mold and yellowing of lobes
6 = considerable leaf necrosis and defoliation, sooty mold on mid and lower leaves and young stems

Fig. 2 Nataima-31 plant type

Fig. 3 Root yield, Nataima-31

VARIETAL REACTION TO WHITEFLY ATTACK

Field evaluations on Nataima-31 reveal that whitefly (*A. socialis*) populations are absent or very low. Whitefly populations and damage were considerably higher on the regional farmers variety, Aroma, and the susceptible control, CMC-40 (Fig. 4 and 5).

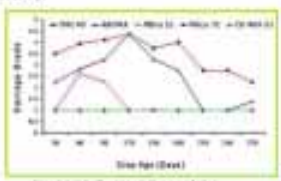

Fig. 4. Whitefly *A. socialis* populations on five cassava genotypes

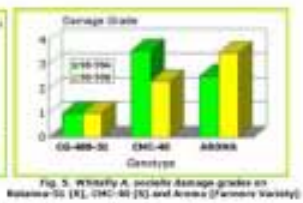

Fig. 5. Whitefly *A. socialis* damage grades on Nataima-31 (N), CMC-40 (S) and Aroma (Farmers Variety)

Additional studies have shown that Nataima-31, as well as its resistant female parent, MEcu 72, have antibiotic effects on *A. socialis* development. The development cycle is longer and there is a higher nymphal mortality ranging from 42.5 to 75.2% (Arias, 1995). Nataima-31 also displays antixenosis with lower ovipositional levels (Table 3).

Table 3. Average per leaf oviposition of *A. socialis* on several cassava genotypes

Genotype	No. Leaves	Eggs/Leaf* Average		C.V
MBra 12	92	82.1	A	291
CMC 40	57	75.2	A	104
CG489-23	54	65.7	AB	119
MCol 1505	70	59.3	B	140
MEcu 72	79	40.4	B	141
CG 489-34	102	20.5	C	154
CG 489-31	77	19.0	C	149

* Values followed by the same letter are not significantly different (Duncan alfa = 0.005).

It is therefore estimated that Nataima-31 will not require chemical pesticide applications for effective whitefly control in area such as Tolima, where *A. socialis* is endemic and, farmers are presently applying up to 6 insecticide applications per crop cycle. These pesticide applications increase production costs and adversely effect the environment. The ideal range of adaptation for Nataima-31 is between 400 to 1000 m.a.s.l., and yields more than the regional variety Aroma (23.0 T/ha vs. 16.2 T/ha).

In addition, roots contain low HCN levels and posses good cooking quality. Roots have a dark brown outer bark and a pink colored inner peel surface. Dry matter is above 30% and adapted to both the fresh and industrial market for starches and animal feed.

THE RELEASE OF Nataima-31

On March 28, 2003, Nataima-31 was officially released by the CORPOICA Research Station in Espinal, Tolima, with ICA (Instituto Colombiano Agropecuario) register number 008, July 10, 2002.

Several presentations were made describing the research process for developing Nataima-31 and explaining its agronomic characteristics and recommended crop management practices.

Newspaper Coverage of the Release of Nataima-31

During the field day, participants visited several field plantings of Nataima-31 where plants were harvested and root quality evaluated. Nataima-31 planting material (stem cuttings) was distributed to field day participants, the initial distribution of this variety.

ECONOMIC, PRODUCTION AND SOCIAL IMPORTANCE OF Nataima-31

It is estimated that the Colombian avicultore industry will require 290,000 tons of cassava for poultry feed. It is planned that the departments of Tolima, Huila and Cundinamarca will plant 14,500 hectares of cassava toward this goal and by 2007; approximately 3,000 hectares may be planted to Nataima-31 in the high, warm Rio Magdalena valley. This could have the following effects on the region:

- An increase of 3,000 hectares with Nataima-31 above the already 8,900 hectares presently being grown using regional varieties.
- A generation of about 177,000 new jobs in the production phase, 48,000 jobs in the post harvest phase and 24,000 indirect jobs.
- A 25% yield increase from the present average of 10 T/ha to 20 T/ha with Nataima-31 for a regional average production of 12.5 T/ha.
- The 3,000 ha sown to Nataima-31 will produce 60,000 tons, and overall production will increase from 89,000 tons annually to 149,000 tons in 2007, an increase of 67.4%.
- A production cost reduction of 6.7% per hectare due to the reduced pesticide applications (a minimum of 3) presently being applied for whitefly control.
- At present between 3,600 to 7,200 kg of active ingredient of pesticides is being applied. This represents an expenditure of 324 to 628 million pesos. Planting Nataima-31 will reduce this cost.
- Nataima 31 maintains the high dry matter and quality of the regional cultivars and is superior in being less susceptible to physiological deterioration; this is an advantage in time of transport to markets.
- Nataima-31 will bring direct benefits to approximately 1,500 rural families and an indirect benefic to 4,500 families in the Rio Magdalena Valley region.

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RECENT ADVANCES IN HOST PLANT RESISTANCE TO WHITEFLIES IN CASSAVA

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INTRODUCTION

Whiteflies are considered one of the world's major agricultural pest groups, attacking a wide range of plant hosts and causing considerable crop loss. There are nearly 1200 whitefly species with a wide host range. As direct feeding pests and virus vectors, whiteflies cause major damage in agroecosystems based on cassava in the Americas, Africa and to a lesser extent, Asia. The most damaging species on cassava in northern South America is *Aleurotrachelus socialis*. Typical damage symptoms include curling of apical leaves, yellowing and necrosis of basal leaves and plant retardation. The "honeydew" excreted is a substrate for a sooty-mold fungus that interferes with photosynthesis (Fig. 1). The rate reduces root yield by 4 to 79% depending on the duration of attack (Bellotti, 2002). More than 5,000 cassava genotypes have been evaluated at CIAT and CORPOICA for whitefly resistance. At present, the major source of host resistance in cassava is the genotype MEcu-72 (Bellotti and Arias, 2001) (Fig. 1). When feeding on MEcu-72 *A. socialis* had less oviposition, longer development periods, reduced size and higher mortality than when feeding on the susceptible genotype, (Fig. 2). Due to the importance of whiteflies as a pest and virus vector, it is important to understand the nature of genes that confer resistance in the genotype MEcu-72. To study the genetics of this resistance, a cross was made between MEcu-72 (resistant genotype) x MCol-2246 (susceptible genotype), to evaluate F1 segregation, using molecular markers. This will accelerate the selection of whitefly resistant germplasm and isolate resistant genes.

Fig.1. A: Nymphal Stages of *A. socialis*, on a cassava leaf. B: Leaf curling on a cassava plant with high populations of *A. socialis*. C: Presence of sooty mold fungus on a cassava leaves attacked by *A. socialis*. D: Resistant genotype MEcu-72 and a susceptible genotype.

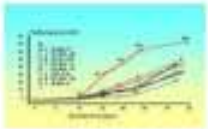


Fig. 2. Whitefly (*A. socialis*) nymphal mortality on resistant (R), tolerant (T) and susceptible (S) cassava clones.

MATERIALS AND METHODS

PLANT MATERIAL

For the present work we have used the cross MEcu-72 (as the resistant parent) x MCol-2246 (as the susceptible parent). A total F1 offspring of 286 genotypes (family CM8996) was produced from this cross. These materials were sowed and evaluated in the field during 2002 and 2003 at two different locations in Colombia: Espinal-Tolima, (CORPOICA-NATAIMA) at 350 m.a.s.l. and Santander de Quilichao, Cauca, at 990 m.a.s.l. With this evaluation we will identify gene segregation in the offspring and we will be able to select the resistant and susceptible materials. The evaluation was performed in the field using population and damage scales.

MOLECULAR ANALYSIS

We are using Simple Sequences Repeat (SSR) and AFLPs to find markers associated with resistance for mapping the resistant gene(s). We are using RGAs sequences (isolated from cassava previously).

RESULTS AND DISCUSSION

FIELD EVALUATION

Field evaluations carried out at Natarma (Tolima) demonstrate that there was considerable whitefly pressure as plant damage and pest populations were high (from 4 to 6 on the damage and population scales). However, some genotypes, in spite of the high pressure, had low damage levels. It can therefore be concluded that these genotypes have resistance levels similar to those of the resistant parent.

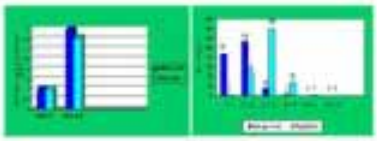


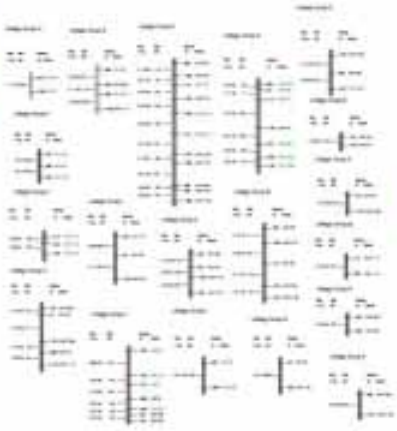
Fig. 3. Cassava damage and whitefly population ratings due to *A. socialis* feeding on parental genotypes MEcu-72, MCol-2246 and clones from the family CM 8996 at CORPOICA, Natalma (Tolima, Colombia).

MOLECULAR ANALYSIS

Both parents, MEcu-72 and MCol-2246, were evaluated with 343 cassava SSR markers (Mba et al, 2001), including 156 cDNA SSRs developed by Mba et al (submitted).

Approximately 155 of the SSRs were polymorphic in the parentals and were evaluated in the F1 (286 individuals). For the construction of the linkage map, 103 SSRs were analyzed, of which 71 were anchored and segregating from the heterozygous female parent (MEcu-72) of an interspecific cross. The map consists of 19 linkage groups, which represent the haploid genome of cassava (Fig. 4). These linkage groups span 550,2 cM and an average marker density of 1 per 7,9 cM. The position of the 71 SSRs markers is shown in figure 5 of the cassava molecular genetic map (LOD = 25 and tetha (θ) = 25). Map distances are shown in Kosambi map units. So far, 26 SSRs markers (shown in green, Fig. 4) have been previously placed on the cassava framework map (Fregene et al, 1997), the other 45 SSRs are new. Thirty one of the 71 SSRs were cDNA sequences (Mba, in preparation) and the others were genomic DNA.

Fig. 4: Preliminary Cassava framework Map of MEcu-72 for Resistance to White Fly, consisting of SSRs and a RGA (Contig39) (Lod = 25 and theta = 25)



AFLPs Analysis

An analysis was done of 128 combinations of primers with both parentals, MEcu-72 and MCol-2246, and both bulks of 10 whitefly resistant and 10 susceptible DNA. We obtained 53 polymorphic combinations, in which we found 425 polymorphic bands between the resistant and the susceptible (Fig. 5).

Fig. 5. Silver stained polyacrylamide gel showing: combination ACA-CTT of AFLP of both parents (R resistant, S susceptible) and Bulks resistants and susceptibles, show the polymorphic band # 10 unique in the resistants.

ASSOCIATION BETWEEN MOLECULAR MARKERS AND RESISTANCE

The molecular data are being analyzed using QTL packages (QTL cartographer Qgene) to determine linkages between the markers and the phenotypic characterization. As preliminary analysis X^2 at the 5% level was done using SAS. Putative associations were found between 43 SSRs markers and the resistance.

CONCLUSIONS

- Field evaluations in the family CM 8996 and their parentals confirm resistance of the genotype MEcu-72 and susceptibility of the parental MCol-2246, this allows us to do preliminary selection of F1 genotypes.
- Using SSR markers, putative association with the parental lines were found.
- A linkage map is being constructed using the SSR data, a RGA and the field phenotypic characterization.

ON GOING WORK

- Saturation of Linkage map of Ecu-72, using AFLPs.
- Isolation, cloning, sequencing and mapping of AFLPs polymorphic bands between resistants and susceptibles genotypes and design of SCARs for marker assisted selection.
- QTLs analysis for resistance to whitefly.
- The whitefly resistance will be the target for map-based cloning using the BAC libraries as tools.
- Isolation of expressed sequences during the defense response of MEcu-72 to white fly attack.
- In order to identify differentially expressed sequences, a new technology known as DNA chips or microarray is available to scan a significant number of clones. Microarray expression profiling detailed experiments will be used to identify putative early-response regulatory and/or signaling genes and to test the function of selected candidate genes using reverse genetics.

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ADAPTATION OF BIOTYPE "B" OF *Bemisia tabaci* TO CASSAVA

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INTRODUCTION

Bemisia tabaci (Fig. 1A) is considered one of the most important pests in tropical and subtropical agriculture, as well as in protective systems in greenhouses (Perring, 1996). Since the 1980s, it has caused considerable economic losses in the southern United States, Mexico, Venezuela, the Eastern Caribbean Basin, and Central and South America due to its proven efficiency as a virus vector, together with damage caused by direct feeding and excretion of honeydew (Oliveira et al., 2001).

To date, a total of 24 biotypes have been identified in different regions of the world, which suggests that *B. tabaci* may be a complex of species and biotypes undergoing continuous evolutionary changes (Perring, 2001). Biotype B of *B. tabaci* is a recognized pest in cassava crops in Asia and Africa, where it transmits the African cassava mosaic virus (ACMV) (Fig. 1B).

Fig. 1. A: Biotype "B" *B. tabaci*; B: Cassava leaves affected by ACMV

Although in the Americas it has been postulated that the absence of ACMV is related to the inability of *B. tabaci* to colonize properly this crop. Hence, the potential adaptation is considered a threat for cassava production in these areas. This work reported from the hypothesis that *B. tabaci* could become gradually adapted to cassava (*Manihot esculenta*).

MATERIALS AND METHODS

The process of adaptation was initiated from a highly susceptible host (*Phaseolus vulgaris*), phylogenetically distant from *M. esculenta*, passing through two intermediate hosts, *Euphorbia pulcherrima* and *Jatropha gossypiifolia*, both Euphorbiaceae, susceptible to *B. tabaci* but phylogenetically close to *Manihot* (Fig. 2). To reach cassava cultivar MCol 2063 "Secundina," well known by its susceptibility to the whiteflies *Aleurotrachelus socialis* and *B. tuberculata* was selected as the final host.

Fig. 2. Host species sequence for the gradual adaptation of *B. tabaci* from *P. vulgaris* to *M. esculenta*

Parameters of the life history of biotype B on *M. esculenta* with individuals previously established on *P. vulgaris*, *E. pulcherrima* and *J. gossypiifolia*

In order to determine the relative importance of the hosts up to the time of their adaptation on *M. esculenta*, population parameters were estimated and evaluated for each specimen of biotype B on *M. esculenta*, reared previously on (a) *P. vulgaris* and passed sequentially to (b) *E. pulcherrima* and (c) *J. gossypiifolia*. In the first case, plants of *E. pulcherrima* were placed in cages and infested with recently emerged adults of Biotype B of *B. tabaci*, coming from the strain established on *P. vulgaris* for five generations. Similarly, after five generations on *E. pulcherrima*, individuals of *B. tabaci* were used to infest plants of *J. gossypiifolia* and lastly, fifth generation individuals coming from *J. gossypiifolia*, were used to infest plants of MCol 2063.

Longevity and fecundity, forty pairs of recently emerged *B. tabaci* coming from each of the three sequences of hosts, were individualized in clip cages, and placed on the underside of the leaves of plants (MCol 2063). Every 48 h the adults were removed to a new area of the leaf. Fecundity was estimated by counting the number of eggs oviposited by the female every 48 h, while longevity was the maximum time (days) that a female lived.

Development time, rate of survival and proportion of females, fifty adults of biotype B, coming from *P. vulgaris*, *E. pulcherrima* and *J. gossypiifolia* on the underside of MCol 2063 leaves. After 6 h the adults were removed, and 200 eggs were selected at random for rearing to adulthood. In each case, the development time from egg to adult, the survival rate of the immature stages, and the proportion of females emerged were recorded.

Demographic parameters, the data on the development time of the immature individuals were combined with experimental data from the reproduction to produce life tables and, used to calculate the demographic parameters defined by Price (1975): 1) Net reproduction rate (Ro), 2) generational time (T), and 3) intrinsic rate of population growth (r_m).

RESULTS AND DISCUSSION

Biology and demographic parameters of biotype B of *B. tabaci* on *M. esculenta* (MCol 2063), coming from three hosts *P. vulgaris*, *E. pulcherrima* and *J. gossypiifolia*

The average longevity of the females of biotype B was significantly higher in females coming from *E. pulcherrima* (9.6 days). The highest oviposition rate (2.64 eggs/female/2 days) was found in females coming from *J. gossypiifolia*, being significantly higher than that of females coming from the other two hosts (Table 1).

Table 1. Longevity (days), fecundity (eggs) and oviposition rate (eggs/female/2 days) of biotype B of *B. tabaci* on *M. esculenta* (MCol 2063) with populations coming from three hosts (n=40).

Average Parameter	Host of Origin		
	J. gossypiifolia	*E. pulcherrima*	*P. vulgaris*
Longevity	3.25 b	5.8 a	3.1 b
Fecundity	8.6 a	7.65 a	1.83 b
Oviposition rate	2.64 a	1.36 b	0.58 c

Averages followed by different letters in the columns differ significantly (Kruskal-Wallis P= 0.0001, followed by Student-Newman-Keuls P= 0.05).

Individuals of biotype B coming from *J. gossypiifolia* took 44.4 days to develop on *M. esculenta*, a significantly shorter time, by about 6 days, as compared with *E. pulcherrima* and *P. vulgaris* (Table 2). On the other hand, it was shown that the highest survival rate (27.5) was reached by individuals grown on *J. gossypiifolia* compared with 3.0 and 2.0 % when come from *E. pulcherrima* and *P. vulgaris*, respectively.

Table 2. Development time, survival and proportion of females of individuals of biotype B of *B. tabaci* on *M. esculenta* coming from *J. gossypiifolia*, *E. pulcherrima* and *P. vulgaris* (n=200).

Parameter	Host of Origin		
	J. gossypiifolia	*E. pulcherrima*	*P. vulgaris*
Development time (days)*	44.4 b	50.8 a	49.5 a
Survival rate (%)	27.5 a	3.0 b	2.0 b
Proportion of females (%)	50.9	50	50

Averages followed by different letters in the columns differ significantly.* Kruskal-Wallis P= 0.0001, followed by Student-Newman-Keuls P= 0.05 ; χ^2 2 df, P= 0.0002, followed by Student-Newman-Keuls P= 0.05

Based on the net reproduction rate (Ro), it was possible to determine that after one generation, on average, the populations of Biotype B can multiply 11.6 times (individuals/individual) on cassava when they come from *E. pulcherrima* (Table 3). A generation time of Biotype B of *B. tabaci* on *M. esculenta* is 44.76 days when the populations come from *J. gossypiifolia*.

Intrinsic growth rates (r_m) revealed a higher population growth on *M. esculenta* when coming from *J. gossypiifolia*, exceeding those from *E. pulcherrima* by 8.3% and up to 58.3% for those from *P. vulgaris*.

Table 3. Demographic parameters for individuals of biotype B of *B. tabaci* on *M. esculenta* coming from *J. gossypiifolia*, *E. pulcherrima* and *P. vulgaris* (n=200).

Parameter	Host of Origin		
	J. gossypiifolia	*E. pulcherrima*	*P. vulgaris*
Ro	8.63	11.6	1.82
T	44.76	50.93	51.3
r_m	0.048	0.044	0.02

Population parameters suggested an increase in the capacity for adapting to the cultivars of *M. esculenta*, favored by the influence of phylogenetically related hosts such as *J. gossypiifolia*. This might act as gradual points in which insect populations undergo an increase in their biotic potential, thereby facilitating their adaptation to *M. esculenta* (Fig. 3). Indeed, this fact constitutes one of the principal findings of this study, as it makes possible determine the adaptive capacity of biotype B on *M. esculenta*, which, according to Costa and Russell (1975), represents a "dead host", in the Americas.

Fig. 3. Populations of biotype B on jatropha and cassava

CONCLUSIONS

Based on the previous findings it is possible to state that in Colombia, *M. esculenta* is a potential host for biotype B of *B. tabaci*.

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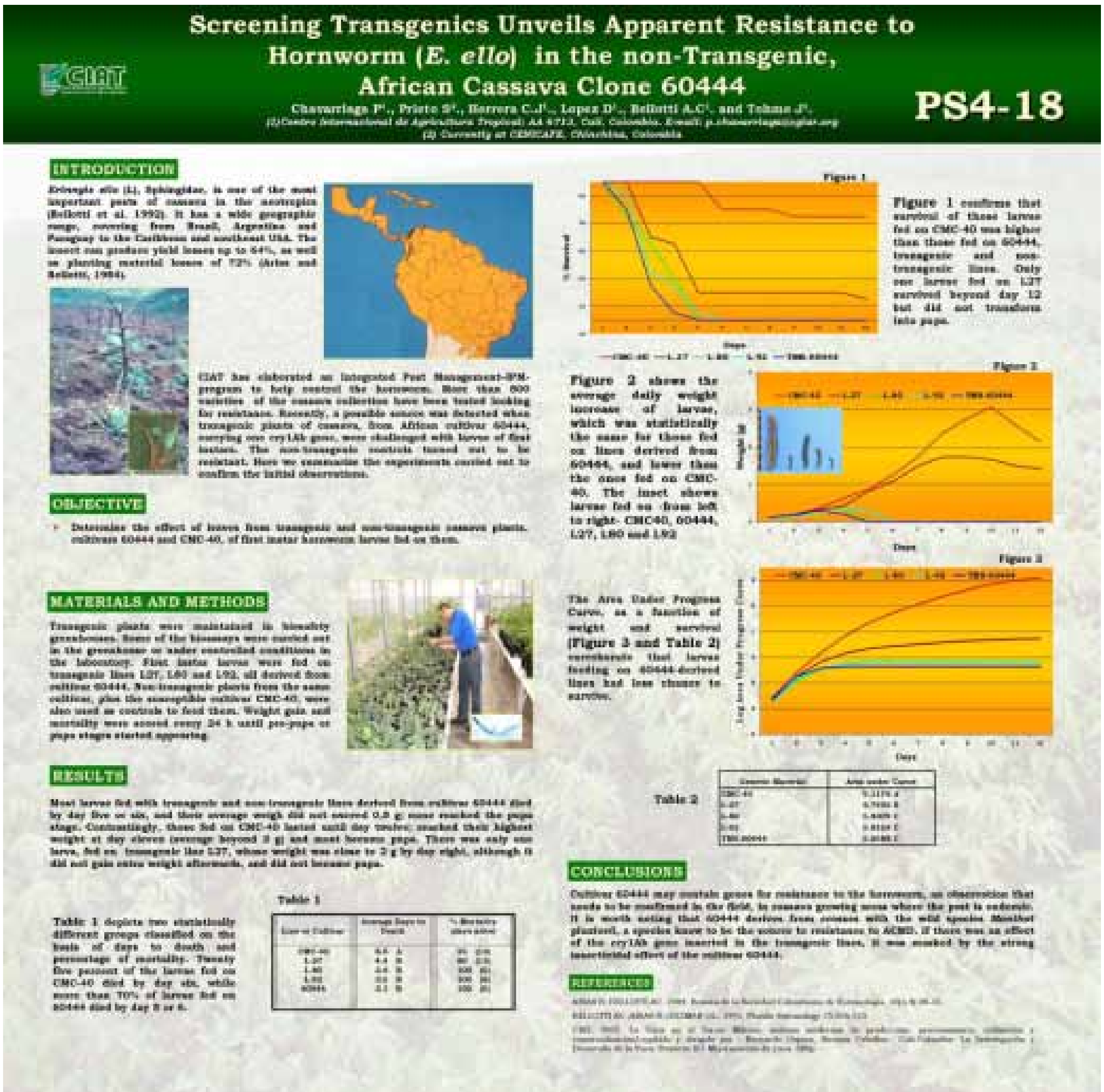
Screening Transgenics Unveils Apparent Resistance to Hornworm (E. ello) in the non-Transgenic, African Cassava Clone 60444
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COMPARACIÓN DE LA ADAPTABILIDAD DEL BIOTIPO "B" DE *Bemisia tabaci* A YUCA COMERCIAL Y SILVESTRE

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INTRODUCCIÓN

Yuca (*Manihot esculenta*) es la principal fuente nutritiva y de calorías para muchos habitantes de África y Asia (Hillocks et al., 2000). En varios países de este último continente su cultivo está limitado por la enfermedad del mosaico Africano de la yuca (ACMO) (Fig. 1A), causada por geminivirus (CMGs), los cuales son transmitidos por el vector *Bemisia tabaci* (Homoptera: Aleyrodidae) (Fig. 1B).

B. tabaci en asocio con el mosaico ocasiona pérdidas económicas estimadas entre 12-25% del total de cultivos de África (Thresh et al., 1997). Aunque se ha especulado que la ausencia del virus de ACMO en las Américas está relacionada con la intabilidad de *B. tabaci* de colonizar yuca (Costa & Russell 1975) o principios de 1990, *B. tabaci* fue encontrado alimentándose de este cultivo en República Dominicana (Brown, datos no publ.) y Cuba (Vázquez et al. 1995). Dada la marcada polifagia de *B. tabaci* sumada a su filial adaptabilidad a nuevos hospederos, existe la posibilidad de que se adapte a especies silvestres y, posteriormente a variedades comerciales de *M. esculenta*.

Fig. 1A: Hoja de yuca afectada con ACMV

Fig. 1B: Adulto y ninfas de *B. tabaci*

Todo lo anterior plantea potenciales para serias amenazas ante la posibilidad de la introducción de ACMO en las Américas, en especial si se tiene en cuenta que las más tradicionales cultivares de yuca son altamente susceptibles a la enfermedad (Bellotti & Arias, 2001).

Con el objetivo de verificar esta posibilidad se realizaron bioensayos, midiendo y comparando el desarrollo de las poblaciones del biotipo B de *B. tabaci* encontrado en Colombia sobre una especie silvestre *Manihot carthaginensis* y una variedad de yuca comercial, *M. esculenta* (MCol 2063).

MATERIALES Y MÉTODOS

Fig. 2.A y B. Biotipo "B"

B. tabaci: La cepa *B. tabaci* Biotipo 'B' (Fig. 2A) fue obtenida a partir de individuos de una colonia establecida en CIAT (Cali, Colombia). Esta fue criada por cinco generaciones sobre *J. gossypiifolia* (Euphorbiaceae), en jaulas de tul y madera (1x1x1m); bajo condiciones controladas de 25±2°C, 70±5% HR y 12 horas de fotoperiodo.

La pureza de la cepa del biotipo B fue verificada periódicamente sobre especímenes adultos utilizando RAPD-PCR (CIAT, 1998).

Yucas silvestres: Cuatro plantas de *Manihot triola* (Mueller) *Manihot peruviana* (Mueller) y *Manihot carthaginensis* (Jacq.) Müll. Arg. de 60 días de edad fueron introducidas en jaulas de tul y madera de (1mx1mx1m) e infestadas con pupas de quinta generación de *B. tabaci* próximas a emerger, provenientes de *J. gossypiifolia* (Fig. 2B). Los insectos permanecieron durante una generación en cada especie silvestre. En este período se realizaron observaciones del desarrollo de los estados inmaduros para determinar sobre cuál de las tres especies silvestres *B. tabaci* presentaba una mayor tasa de supervivencia.

Biología y parámetros demográficos:

Basados en los resultados del establecimiento de *B. tabaci* en las tres especies silvestres del Manihot, se eligió la mejor adaptada (*M. carthaginensis*), para evaluar los cambios que experimentan las poblaciones de *B. tabaci* en longevidad, fecundidad, tiempo de desarrollo, tasa de supervivencia y parámetros demográficos sobre esta especie y comparelos con los obtenidos sobre la variedad comercial *M. esculenta* (MCol 2063).

Longevidad y fecundidad. Cuarenta parejas de *B. tabaci*, recién emergidas provenientes de *J. gossypiifolia* fueron individualizadas en potes pinza y colocadas en el envés de las hojas de *M. carthaginensis* y *M. esculenta* (MCol 2063). Cada 48 horas los adultos fueron removidos a una nueva área de la hoja, hasta la muerte natural de las hembras. La fecundidad fue estimada de acuerdo al número del número de huevos colocados por hembra cada 48 horas, y la longevidad como el máximo tiempo (días) que una hembra vivió.

Tiempo de desarrollo, tasa de supervivencia y proporción de hembras. Cincuenta adultos de *B. tabaci*, de dos días, fueron tomados de plantas de *J. gossypiifolia* y posteriormente colocados en jaulas pinza sobre el envés de las hojas de *M. carthaginensis* y *M. esculenta* (MCol 2063). Después de seis horas los adultos fueron retirados y se seleccionaron al azar 200 huevos. Se registró el tiempo de desarrollo de huevo-adulto, la tasa de supervivencia de los estados inmaduros y la proporción de hembras emergidas.

Parámetros demográficos. Los datos del tiempo de desarrollo fueron combinados con datos experimentales de la reproducción ($l_x m_x$) para generar tablas de vida, usadas para calcular los parámetros demográficos definidos por Price (1975): 1) Tasa de reproducción neta (R_o). 2) Tiempo generacional (T). 3) tasa intrínseca de crecimiento de la población (r_m), estimada mediante la ecuación de Carey (1993): $\sum [exp(-r_m x)] l_x m_x = 1$

RESULTADOS Y DISCUSIÓN

Biología y parámetros demográficos de *B. tabaci* sobre *M. carthaginensis* y *M. esculenta* (MCol 2063)

La longevidad más alta de las hembras de *B. tabaci* se presentó en *M. carthaginensis*, excediendo en aproximadamente dos días a *M. esculenta*. Al día seis, se alcanzó el 55 y 92.5% de mortalidad en hembras provenientes de *M. carthaginensis* y *M. esculenta*. La tasa de oviposición sobre *M. esculenta* fue 2.5 veces mayor comparada con *M. carthaginensis* (Tabla 1).

Tabla 1. Longevidad media (días), fecundidad media (huevos/hembra), y tasa de oviposición (huevos/hembra/2días) de *B. tabaci* sobre *M. carthaginensis* y *M. esculenta* (n=40).

Parámetro	*M. carthaginensis*	*M. esculenta*
Longevidad	**5.1 a**	**3.3 b**
Fecundidad	**6.4 a**	**6.8 a**
Tasa oviposición	**1.1 a**	**2.8 b**

Promedios seguidos por diferentes letras a través de las columnas difieren significativamente (Kruskal-Wallis P< 0.0001, seguido por Student-Newman-Keuls P< 0.05).

En total el ciclo de vida fue 11 días más largo sobre *M. esculenta* comparado con *M. carthaginensis*. Estos resultados podrían interpretarse como una mayor adaptación biológica de los estados inmaduros del insecto a desarrollarse sobre *M. carthaginensis*.

Los resultados de las tasas de supervivencia mostraron que de 200 huevos, el 50% llegaron hasta adulto cuando se desarrollaron sobre *M. carthaginensis* comparado con el 25% que llegaron a adultos sobre *M. esculenta*. Este parámetro es un buen indicador de la capacidad potencial que puede tener *B. tabaci* para desarrollar poblaciones sobre una variedad comercial de *M. esculenta* (MCol 2063) y una silvestre *M. carthaginensis* (Tabla 2).

Tabla 2. Tiempo de desarrollo (días), tasa de supervivencia (%) y proporción de hembras (%) de *B. tabaci* sobre *M. carthaginensis* y *M. esculenta* MCol 2063 (n=200).

Parámetro	*M. carthaginensis*	*M. esculenta*
Tiempo desarrollo (d)*	**33.3 a**	**44.4 b**
Tasa supervivencia (%)§	**50 a**	**25 b**
Proporción hembras (%)	**60**	**62**

Promedios seguidos por diferentes letras a través de las columnas difieren significativamente
* Kruskal-Wallis P< 0.0001, seguido por Student-Newman-Keuls P< 0.05; § Chi-cuadrado= 24.8, 1 d.f., P< 0.0001

Los resultados de la tasa reproductiva neta (R_o) permitieron estimar que, en promedio, el caso de una generación, las poblaciones de *B. tabaci* podrían multiplicarse 8.6 veces sobre *M. esculenta*, siendo 1.6 veces mayor con respecto a *M. carthaginensis*. Estos resultados permiten predecir que el biotipo "B" puede alcanzar diez generaciones por año sobre *M. carthaginensis*, dos generaciones más que en *M. esculenta*.

La población de *B. tabaci* presentó un crecimiento (r_m) igual en *M. carthaginensis* y *M. esculenta* indicando que no existen diferencias en el potencial biótico del insecto al ser criado en cualquiera de estos hospederos y que por consiguiente su potencial adaptativo es igual tanto en la especie cultivada como en la silvestre (Tabla 3).

Tabla 3. Parámetros demográficos de *B. tabaci* sobre *M. carthaginensis* y *M. esculenta* MCol 2063 (n=200).

Parámetro	*M. carthaginensis*	*M. esculenta*
R_o	**5.4**	**8.6**
r_m	**0.040**	**0.040**
T	**35.6**	**44.0**

En síntesis, los resultados de este experimento muestran, en forma global, gran favorabilidad de *M. carthaginensis* para el biotipo "B" de *B. tabaci*. Un corto tiempo de desarrollo, unido a una alta tasa de supervivencia (Fig. 3) son buenos indicadores de la conveniencia de *M. carthaginensis* para el desarrollo del insecto. Este hecho plantea que las especies silvestres de *Manihot* pueden convertirse en puente intermedio, donde la mosca blanca incrementa su potencial biótico fortaleciendo, a posteriori, su completa adaptación sobre *M. esculenta*.

Fig. 3. Supervivencia y desarrollo de estados inmaduros de *B. tabaci* "B" sobre A; *M. carthaginensis* y B: *M. esculenta* MCol 2063

CONCLUSIONES

Basados en los resultados encontrados es posible plantear que las especies silvestres de yuca constituyen hospedería con gran potencial para el desarrollo del biotipo "B" de *B. tabaci* encontrado en Colombia.

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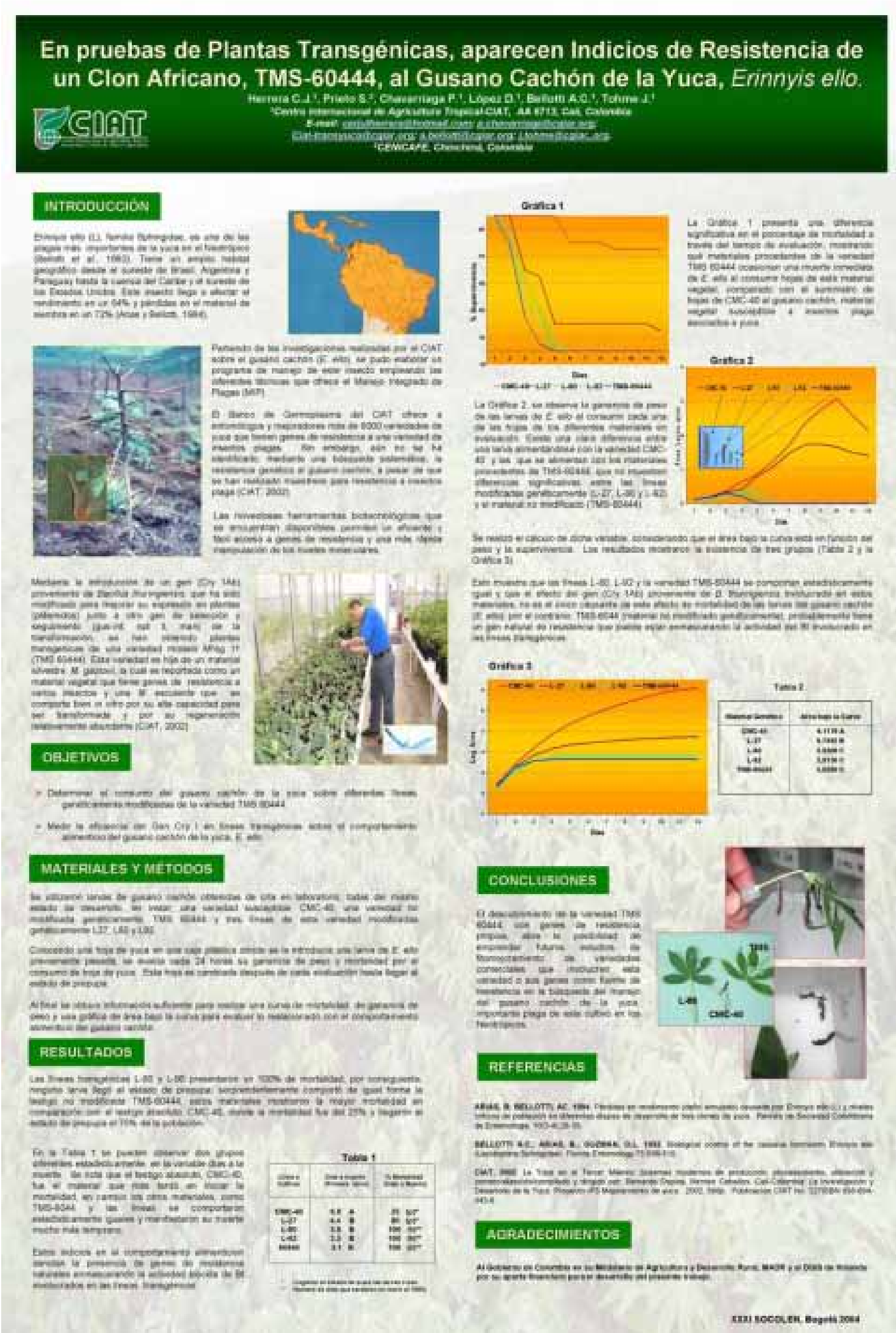
En pruebas de Plantas Transgénicas, aparecen Indicios de Resistencia de un Clon Africano, TMS-60444, al Gusano Cachón de la Yuca, Erinnyis ello.
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SUSCEPTIBILIDAD DEL ADULTO DE *Cyrtomenus bergi* A TRES ESPECIES DE NEMATODOS ENTOMOPATÓGENOS EN INVERNADERO

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INTRODUCCIÓN

Cyrtomenus bergi Froeschner (Fig. 1A), es un hemíptero de la familia Cydnidae, polífago y de hábito subterráneo que se alimenta directamente del producto comercial de diversos cultivos de importancia económica como: yuca, maní, cebolla, espárragos no sólo en Colombia, sino también en países del neotrópico como Panamá, Costa Rica, Venezuela y Brasil. Su control hasta el momento ha sido muy difícil de implementar por su hábito polífago y su adaptación al ambiente del suelo (Bellotti, 2002).

Figura 1A. Adultos de *C. bergi*.

Figura 1B. Juvenil infectivo de *Steinernema sp.*

La evaluación del potencial de biocontrol con nematodos entomopatógenos de las géneros *Steinernema* (Fig. 1B) y *Heterorhabditis* se ha realizado en bioensayos de laboratorio. Encontrándose sólo dos estados, el cuarto y el adulto susceptibles al parasitismo de las diferentes especies evaluadas pero con porcentajes de mortalidad muy bajos 3-40%. Se propone su evaluación en invernadero con el fin de determinar diferencias en su comportamiento en un ambiente más real antes de ser recomendados como una alternativa para su manejo integrado (CIAT, 2003).

MATERIALES Y MÉTODOS

Nematodos y estados de *C. bergi*
Los nematodos seleccionados para la realización del presente trabajo (Tabla 1) fueron producidos en larvas de último instar de *Galleria mellonella* a 23°C de acuerdo a la metodología descrita por Kaya & Stock (1997). Los juveniles infectivos fueron almacenados en agua con formaldehído al 0.01% a 10°C durante 5-7 días y un día antes de su inoculación fueron aclimatados a 23°C. Los adultos de *C. bergi* fueron seleccionados de la colonia del laboratorio de Entomología de Yuca del CIAT.

Tabla 1. Especies de nematodos y su origen.

Especies	Origen
Steinernema riobrave **(Sr)**	**Estados Unidos**
S. Carpocapsae **(S carp.)**	**Estados Unidos**
Steinernema sp. **SNI-0100 (SNI)**	**Colombia**
Heterorhabditis sp. **HNI-0108 (HNI)**	**Colombia**
Heterorhabditis sp. **CIAT 2003 (HCIAT)**	**Colombia**

Ensayos:
El adulto de *C. bergi* fue inoculado con una sola dosis de nematodos, 1.000 nematodos/ml de tres especies diferentes (S carpo., SNI y HNI) en vasos plásticos con 300 g de arena sin esterilizar (ARAYP) y un grano de maíz pregerminado (CIAT, 2003). Los tratamientos fueron organizados en bloques completos al azar con 30 insectos por tratamiento. El control fue inoculado con un mililitro de agua destilada. La evaluación se realizó 10 días después, el parasitismo y la mortandad fueron registrados.

Un segundo ensayo fue realizado con dos especies de nematodos (S carp. y HNI) y 25.000 nematodos/ml). El ensayo fue repetido tres veces veces en bloques completos al azar con 12 insectos por tratamiento. El tratamiento control, la evaluación y parámetros fueron los mismos del ensayo anterior.

El último ensayo constó de tres especies de nematodos (SNI, HCIAT y Sr) a razón de 100.000 nematodos/ml. El ensayo fue repetido tres veces veces en bloques completos al azar con 12 insectos por tratamiento. El tratamiento control, la evaluación y parámetros fueron los mismos de los ensayos anteriores.

Condiciones ambientales
T ° Mín. 23 °C, T Máx. 34 °C y HR Mín. 60% ±5 y Máx. 92% ±5.

Análisis estadístico
Los datos fueron analizados por Chi cuadrado y análisis de varianza (ANOVA).

RESULTADOS Y DISCUSIÓN

Los resultados del primer ensayo mostraron que las tres especies de nematodos evaluados fueron capaces de localizar y parasitar el adulto de *C. bergi* pero con porcentajes de parasitismo muy bajos, 10, 19 y 21% para HNI, SNI y *S. carp.* respectivamente (Fig. 2). Además no fue posible determinar la mayor especie y no se observó mortalidad.

A pesar de la baja respuesta de las especies se confirma la tendencia observada en los ensayos de laboratorio, en los cuales HNI fue la especie con el más bajo parasitismo sobre el estado adulto y las especies SNI y *S. carp.* con las mayores porcentajes de respuesta tanto en laboratorio como en invernadero.

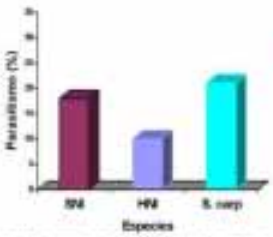

Figura 2. Parasitismo de tres especies de nematodos sobre el adulto de *C. bergi* con 1000 nematodos/ml.

En el segundo ensayo se observó un incremento en la respuesta de parasitismo y mortalidad con el aumento de la concentración de nematodos. La mayor respuesta se obtuvo con *S. carp* con un 84% de parasitismo y 29% de mortalidad y HNI con 54% de parasitismo y 9% de mortalidad (Fig. 3). Pero a pesar del aumento en la respuesta se considera que la interacción entre las especies de nematodos y *C. bergi* es muy baja, pues sólo con el aumento en 25 veces de la concentración de nematodos fue posible duplicar la respuesta del parasitismo.

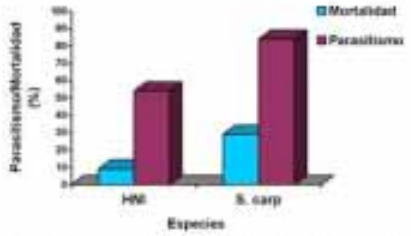

Figura 3. Parasitismo/ Mortalidad del adulto de *C. bergi* con dos especies de nematodos y 25.000 nematodos/ml.

Con el aumento en 25 veces de la concentración de dos de las especies que causaron el mayor porcentaje de parasitismo en los bioensayos de laboratorio, SNI y Sr y de una de las especies con el bajo parasitismo, HCIAT, se observó una disminución en el parasitismo de 100% a 63% para la especie SNI y de 71% a 66% para Sr y de 67% a 56% para HCIAT. En contraste con la mortalidad, la cual mostró un ligero aumento de tres veces a lo obtenido en laboratorio para las especies SNI y Sr y de cinco veces para la especie HCIAT (Fig. 4).

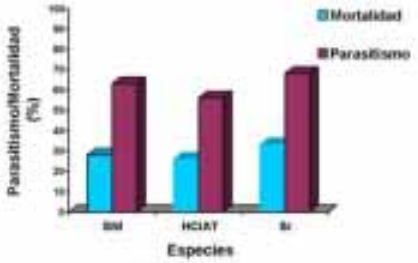

Figura 4. Parasitismo y Mortalidad de tres especies de nematodos sobre el adulto de *C. bergi* y 100.000 nematodos/ml.

Los resultados obtenidos en invernadero confirman los obtenidos en laboratorio con las diferentes especies de nematodos evaluados sobre el adulto de *C. bergi*. Desdeciéndose la relación positiva entre el incremento de la concentración de nematodos, el parasitismo y la mortalidad.

A pesar de la altas concentraciones de nematodos evaluadas, la mortalidad continua siendo muy baja, lo cual permite especular que el número de efectivos que logran penetrar el chinche no son suficientes para causar mortalidad como lo menciona Simoes et al. (1993), un bajo número de nematodos causan una tasa reproductiva baja en el hospedero y un incremento en el riesgo de mortalidad de los nematodos por parte de la respuesta inmune del insecto, y (para Steinernematids) la probabilidad de apareamiento es mucho mas baja debido a la baja población de individuos que logran penetrar el insecto.

Se consideró prioritario iniciar estudios básicos para el conocimiento de los factores físicos y químicos que *C. bergi* está generando cuando entra en contacto con los nematodos entomopatógenos. El conocimiento de este interacción permitirá avanzar en el proceso de selección de las mejores especies de nematodos para ser implementadas en programas de manejo integrado.

CONCLUSIONES

Todas las especies de nematodos entomopatógenos evaluadas fueron capaces de localizar, parasitar y matar el adulto de *C. bergi*.

Aunque se obtuvo un incremento de la mortalidad del adulto de *C. bergi* con el incremento de la concentración de nematodos, es necesario iniciar estudios básicos de los posibles mecanismos de defensa tanto físicos como químicos que *C. bergi* está generando con las diferentes especies de nematodos evaluadas y poder optimizar el proceso de selección de las mejores especies para el control de *C. bergi*.

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Julio 28-30, 2004 – Session XXX

UNA NUEVA ESPECIE DE NEMATODO ASOCIADO AL CHINCHE SUBTERRÁNEO DE LA VIRUELA *Cyrtomenus bergi* Froeschner (Hemiptera : Cydnidae) EN COLOMBIA

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INTRODUCCIÓN

Cyrtomenus bergi Froeschner (Fig. 1A) es considerado una de las principales plagas del suelo de numerosos cultivos en Colombia y varios países tropicales. El aislamiento de enemigos naturales con potencial para su control ha sido una de las metas durante los últimos años como una alternativa para reducir el uso excesivo de plaguicidas químicos (Bellotti, 2002).

C. bergi se alimenta de raíces, tubérculos y frutas subterráneas (E), manil de las plantas hospedantes. Sólo en yuca el daño ha sido caracterizado, tanto adultos como las ninfas insertan su estilete en la epidermis y corteza de la raíz de la yuca dejando lesiones en el parénquima que facilita la entrada de patógenos del suelo tales como *Fusarium*, *Aspergillus*, *Geniculans* y *Pythium* (Bellotti, 2002).

Fig. 1A: Adultos y ninfas de *C. bergi*

Fig. 1B: Raíces de yuca con daño

MATERIALES Y METODOS

Aislamiento y reproducción de nematodos
Durante el año de 1992 se realizó el primer reconocimiento de nematodos nativos asociados a *C. bergi* en ocho localidades de Colombia (Caicedo y Bellotti, 1996). En el año 2000, se realizó un relanzamiento de nematodos de las muestras de suelo de Santander de Quilichao, (Cauca, Colombia) usando Galleria mellonella L. como insecto trampa (Kaya & Stock, 1997). Los nematodos recuperados fueron reproducidos in vivo y en medio sólido y evaluados sobre todos los estados de *C. bergi* bajo condiciones de laboratorio (Barberena y Bellotti, 1998).

Caracterización morfológica
Se realizaron observaciones de especímenes de cada estado, adultos y juveniles infectivos, **IJ**, vivos y muertos con un microscopio de contraste de fases. Las medidas fueron tomadas usando Sicon software de imágenes (Frederick, Maryland, USA) calibrado con un micrómetro.

Caracterización molecular y análisis filogenético
El análisis molecular se realizó mediante la secuenciación de pequeñas unidades de ADN ribosomal (18S rDNA). Muestras de 10-50 especímenes fueron usados para la extracción de ADN, el cual fue cuantificado por espectrofotometría y 100-200 ng fueron usados para PCR. Secuencias de otros tres Rhabditidae: *R. myriophyla* Poinar, *R. blumi* and *C. elegans* fueron recuperados de GenBank usando la opción BLAST.

RESULTADOS Y DISCUSIÓN

Caracterización morfológica

Una nueva especie de nematodo fue aislada e identificada de muestras de suelo y de especímenes adultos y ninfas de *C. bergi* de Santander de Quilichao, Cauca. Este nematodo, perteneciente al género Rhabditis, grupo insectívora, presenta una asociación necromenica con este insecto. La misma especie había sido identificada anteriormente como una raza de la especie *Heterorhabditis bacteriophora* Poinar.

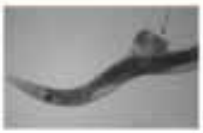

Fig 1. Hembra, manifestando [illegible] Rhabditis necromena (Hembra)

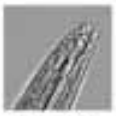

Fig 2. Extremo anterior de la hembra manifestando lóbulos cefálicos Rhabditiformes

Fig 3. Vista de la hembra manifestando anfidios (Hembra)

Descripción

Adultos: Los adultos presentan cutícula lisa, con un grosor de 1um, con puntos finos que forman estrías longitudinales y transversales. Seis labios conectados por una semilla terminal. Región labial 7-9 µm. Anfidios conspicuos con abertura elíptica (Fig.1). Estoma largo y estrecho, 5-6 veces más largo que ancho (Fig.2). Corpus cilíndrico, 48-55% de la faringe. Bulbo medio no bien definido, istmus a 20-24% de la faringe. Bulbo basal piriforme, con válvula bien desarrollada, a 15-17% de la faringe. Poro excretor localizado posterior al anillo nervioso y al nivel del bulbo basal. Anillo nervioso localizado en la mitad del istmus. Faasmidos conspicuos (Fig. 3).

Fig 4. Hembra no evolucionadas

Fig 5. Agrupación de juveniles infectivos (IJ)

Hembras hermafroditas: Vulva con apertura transversa, en las hermafroditas jóvenes ligeramente protuberante y labios asimétricos (Fig. 1). Número de huevos presentes por útero menor que en otras especies de *Rhbaditis* (Fig. 4). Longitud del rectum, cerca de 1.5 veces del ancho del cuerpo anal. Los labios anales no protuberantes. Cola cónica y terminando asimétricamente en una punta fina (Fig. 3).

Juveniles infectivos (Fig. 5): Tercer estado con la cutícula del estado anterior, no fuertemente adherida. Cuerpo delgado, gradualmente estrecho de la faringe a la parte final y del istus hasta la punta. Región labial lisa, boca cerrada, estoma 5 veces más largo que ancho, istmus largo y estrecho. Bulbo basal con válvas. Anillo nervioso en el mismo nivel del istmus. Poro excretor cerca de la mitad de la faringe. Cola conoide con punta terminal.

Machos: Con gónadas monórquicas, situadas a la izquierda del intestino. Bursa abierta del tipo peloderan con una parte pequeña de la cola que sobresale de la bursa, el velo en la parte terminal en forma de V (Figs. 6-7). Nueve pares de papas bursales, tres pares precloacales, tres adcloacales y tres post cloacales. Espículas delgadas con puntas en forma de aguja de crochet, con la punta proximal curvada hacia afuera (Fig. 8).

Fig 6. Cola del macho. Vista lateral

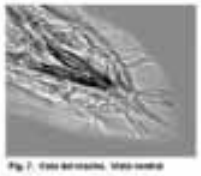

Fig 7. Cola del macho. Vista ventral

Fig 8. Espículas del macho. Vista lateral

Diagnosis diferencial

Rhabditis sp. n. sp. comparte varias características morfológicas con otras especies del grupo insectívora tales como la carencia de un bulbo medio en la faringe bien desarrollado, rectum extremadamente largo, bursa con nueve pares de papilas bursales, espículas con puntas pares 5 y 6 orientadas hacia afuera y las puntas con forma de aguja de crochet. La característica más distintiva de *Rhabditis* sp. n. sp. es la longitud del estoma con respecto a *R. myriophyla*, *R. necromena* y *R. cauleryi*.

Adultos de la nueva especie son más pequeños y delgados que *R. cauleryi*. El número de huevos presentes por útero es menor que en *R. cauleryi*. Con respecto a la especie *R. myriophyla* difiere en la forma y tamaño de las espículas, siendo mucho más grandes las de la nueva especie (42-48 vs. 39-46µm). El poro excretor en la nueva especie está localizado más anteriormente que en *R. myriophyla* y la cola de los juveniles de la nueva especie es también mas larga. Con la especie *R. necromena*, la nueva especie difiere en que en ésta las espículas de los machos son más grandes al igual que el tamaño de los machos y las hermafroditas, pero los juveniles son más pequeños y anchos que los de *R. necromena*.

Caracterización molecular y análisis filogenético

El análisis de parsimonia máximo de las secuencias de las unidades más pequeñas (SSU) produjo 326 caracteres parsimoniosos informativos y produjo un sólo árbol parsimonioso con una longitud de árbol de 400 pasos (CI=0.97). En este análisis *Rhabditis* sp. n. sp. es considerada una hermana de *Rhbaditis myriophila*, una especie asociada con milpiés en California (Fig. 9). Relaciones evolucionarias entre estas dos especies, está soportado por un muestreo completo del 100%. La distancia matriz entre *Rhbaditis* sp n. sp y *R. myriophila* difieren en 22 caracteres.

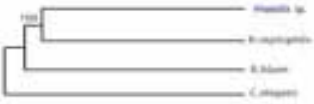

Fig 9. Relaciones filogenéticas de la nueva especie de *Rhabditis* con otras especies *Rhabditidae*

Ecología: Varias especies pertenecientes al grupo *Rhabditis*–insectívora han sido encontradas en asociación con invertebrados muertos del suelo (Poinar, 1996; Sudhaus & Shutte, 1998). En esta asociación, el infectivo penetra los hospederos por sus aperturas naturales, pero nunca se desarrollan los adultos hasta que el hospedero muere y el cadáver es invadido con bacterias (Sudhaus & Shutte, 1998). Los nematodos se alimentan, se aparean y reproducen dependiendo de la multiplicación bacterial en el cadáver del hospedero.

Sin embargo, *Rhabditis* sp. n. sp. fue aislada tanto de chinches como de muestras de suelo usando larvas de *G. mellonella*, multiplicada tanto in vivo como in vitro y evaluada sobre todos los estados de *C. bergi*. Los resultados mostraron al quinto instar como el estado más susceptible con un 90% de mortalidad después de 10 días de inoculados (Barberena & Bellotti, 1998). Además una bacteria del género *Bacillus* fue aislada de los infectivos de esta especie (CIAT, 2002), pero hasta el momento no se ha confirmado si la presencia de esta bacteria es la responsable de la muerte de los hospederos.

CONCLUSIONES

Rhabditis sp. n. sp. es la especie de nematodo que se encontró asociada a especímenes de *C. bergi* y de muestras de suelo de Santander de Quilichao, Cauca y no la especie identificada anteriormente como *Heterorhabditis bacteriophora* Poinar.

Es importante continuar con los estudios básicos sobre esta nueva especie y comprobar su relación con *C. bergi* tanto en laboratorio como en campo.

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Julio, 28-30, 2004 – Socolen XXXI

POTENCIAL DE BIOCONTROL DE SEIS ESPECIES DE NEMATODOS ENTOMOPATÓGENOS SOBRE *Cyrtomenus bergi* EN LABORATORIO

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INTRODUCCION

Nematodos entomopatógenos de los géneros *Steinernema* y *Heterorhabditis* con sus bacterias asociadas *Xenorhabdus* y *Photorhabdus* respectivamente (Fig. 1A) representan un sistema único para el control biológico de insectos-plaga. Ensayos en laboratorio y campo han mostrado que cerca de 17 órdenes y 133 familias de insectos son susceptibles a los nematodos entomopatógenos en algún grado (Akhurst & Smith, 2002).

Figura 1B. Adulto de *C. bergi* con nematodos

Figura 1A. Juvenil infectivo de Heterorhabditis sp

Cyrtomenus bergi Froeschner (Hemiptera: Cydnidae), es un insecto polífago que ha sido encontrado causando daño en diversos cultivos de importancia económica. Desde su descripción como plaga de la yuca en Colombia en 1980, se ha convertido en una plaga importante en todo el neotrópico. Diez años después se iniciaron las primeras evaluaciones en laboratorio del potencial de los nematodos entomopatógenos para su control con la especie exótica, *Steinernema carpocapsae* Weiser (Fig. 1 B) (Caicedo & Bellotti, 1994). Aún no ha sido posible encontrar la mejor especie de nematodo para su control.

MATERIALES Y METODOS

Nematodos y estados de *C. bergi*

Los nematodos seleccionados para la realización del presente trabajo (Tabla 1) fueron producidos en larvas de último instar de *Galleria mellonella* a 23°C de acuerdo a la metodología descrita por Kaya & Stock (1997). Los juveniles infectivos fueron almacenados en agua con formaldehído al 0.01% a 10°C durante 5-7 días y un día antes de su inoculación fueron aclimatados a 23°C.

Los estados de *C. bergi*, quinto y adulto fueron seleccionados de la colonia del laboratorio de Entomología de Yuca.

Table 1. Especies de nematodos y su origen.

Especies	Origen
Steinernema riobrave **(Sr)**	**Estados Unidos**
Heterorhabditis bacteriophora **(Hb)**	**Reino Unido**
Steinernema sp **-SNI-0100 (SNI)**	**Colombia**
Heterorhabditis sp **-HNI-0198 (HNI)**	**Colombia**
Steinernema feltiae cepa Villapinzón **(Sf)**	**Colombia**
Heterorhabditis sp **- CIAT 2003(HCIAT)**	**Colombia**

Ensayos

Se inocularon dos estados de *C. bergi* (quinto y adulto) con una sola dosis de nematodos, 5000 nematodos/ml de cada especie, en vasos plásticos, con 10 g de arena estéril (4%R/P) y un grano de maíz pregerminado (Caicedo & Bellotti, 1994). Cada tratamiento fue repetido cinco veces con 12 insectos por tratamiento en un diseño de bloques completos al azar. El control fue inoculado con un mililitro de agua destilada. La evaluación se realizó 10 días después y el parasitismo y la mortalidad fueron registrados.

Un segundo ensayo fue realizado con tres especies de nematodos (SNI, Sr y HCIAT) y cinco concentraciones (2000, 4000, 6000, 8000 y 10000 nematodos por ml). El ensayo fue repetido cuatro veces en bloques completos al azar con 12 insectos por tratamiento. La evaluación y parámetros fueron los mismos que en el ensayo anterior.

Análisis estadístico

Los datos fueron sometidos a un análisis de varianza (ANOVA) con separación de medias con el test de DUNCAN y análisis Probit respectivamente.

RESULTADOS Y DISCUSION

Los dos estados de *C. bergi* fueron parasitados por todas las especies de nematodos evaluados, pero *Steinernema* sp-SNI fue significativamente la especie más eficiente en parasitar el quinto instar y el adulto de *C. bergi* con 77 y 100% parasitismo respectivamente y la especie menos eficiente fue *Heterorahbditis* sp HNI con 26 y 46% de parasitismo respectivamente (Fig. 2) después de 10 días de inoculados.

El mayor porcentaje de mortalidad se presentó en el quinto instar con la especie *Steinernema* sp-SNI, pero fue de sólo 22% de mortalidad comparado con el 77% de parasitismo. La especie con el menor porcentaje de mortalidad fue *Heterorhabditis* sp HCIAT con sólo 4% de mortalidad (Fig. 3).

Según Koppenhofer, et al. (2000), la eficacia de varias especies de nematodos o cepas difieren significativamente en el control de una misma especie-plaga. Lo cual está influenciado por la tasa de penetración de los infectivos en el insecto, el tiempo de liberación de la bacteria simbiótica y el grado de virulencia de la misma para causar la muerte al insecto.

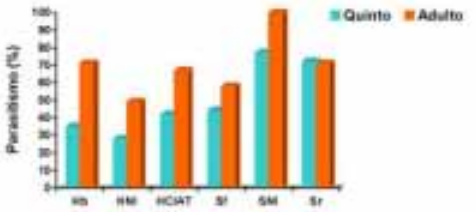

Figura 2. Parasitismo de dos estados de *C. bergi* con seis especies de nematodos.

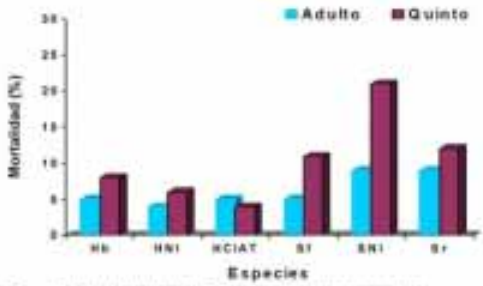

Figura 3. Mortalidad de dos estados de *C. bergi* con seis especies de nematodos.

Cuando el estado adulto de *C. bergi* fue expuesto a cinco dosis diferentes de nematodos no fue posible establecer una relación directa entre la dosis y el parasitismo/mortalidad entre las diferentes especies de nematodos. Tampoco se observó ninguna diferencia significativa entre las tres especies de nematodos evaluados, observándose un rango de parasitismo entre el 65-100% y al igual que en el ensayo anterior la mortalidad del estado adulto fue muy baja, entre el 3-40%.

Los resultados obtenidos confirman lo encontrado por Cespedes & Bellotti (1994) en evaluaciones con la especie *Steinernema carpocapsae* sobre todos los estados del chinche y los encontrados por Barbarena y Bellotti (1998).

En este punto sólo se puede especular sobre los posibles factores que están interactuando entre las diferentes especies de nematodos y los estados de *C. bergi*. Uno podría ser una coevolución de *C. bergi* con los nematodos entomopatógenos y otros patógenos del suelo. Otro sería que *C. bergi* está generando mecanismos de defensa tanto físicos como químicos cuando entra en contacto con los nematodos entomopatógenos, los cuales son desconocidos hasta el momento.

Sólo se puede mencionar la observación de nematodos muertos, con coloración amarilla y sin reproducir dentro de los dos estados de *C. bergi*, lo cual podría relacionarse directamente con los mecanismos de respuesta del sistema inmune de los insectos, tanto a nivel celular como humoral, como son encapsulamiento, melanización y no crecimiento de la bacteria en la hemolinfa del insecto, impidiendo el crecimiento y desarrollo de los nematodos y la muerte del insecto por septicemia.

Para comprobar estas observaciones se planeó la realización de un ensayo preeliminar para determinar si *C. bergi* estaba generando respuesta humoral con los nematodos entomopatógenos evaluados en términos de actividad de phenoloxidasa y la identificación de las células de la hemolinfa responsables de la respuesta a nivel celular (CIAT, 2003).

CONCLUSIONES

Todas las especies de nematodos parasitaron al quinto instar y el estado adulto de *C. bergi*, siendo la especie *Steinernema* sp.SNI la que causó el 77 y 100% de parasitismo respectivamente, pero el alto parasitismo de esta especie no estuvo correlacionado con una alta mortalidad, sólo 9 y 21% de mortalidad respectivamente.

En el momento es una prioridad iniciar los estudios básicos para conocer la respuesta inmune innata de *C. bergi* y determinar la correlación que existe entre el insecto y las diferentes especies de nematodos tanto a nivel celular como humoral y desarrollar una nueva herramienta para la selección de las mejores especies/razas de nematodos entomopatógenos para su control.

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Continued

POTENTIAL OF BIOCONTROL OF SIX ENTOMOPATHOGENIC NEMATODES SPECIES AGAINST *Cyrtomenus bergi* IN THE LABORATORY

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INTRODUCTION

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* together with their symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* respectively (Fig. 1A), represent a unique insect biological control "system". Laboratory and field studies have shown that insects from over 17 orders and 135 families are susceptible to some degree to them (Akhurst & Smith, 2002).

Figure 1 B. *C. bergi* adult infected with nematodes

Figure 1 A. *Heterorhabditis* sp infective juvenile

C. bergi Froeschner (Hem. Cydnidae) is a polyphagous pest and has been reported on cassava, maize, peanuts, potatoes, sorghum, onions, African oil palm, coffee, sugarcane, beans, peas, coriander, asparagus, pasture and weeds. Since its first description as a pest on cassava in Colombia in 1980, it has been considered a serious problem throughout the neotropics. The potential of entomopathogenic nematodes to control pests has been evaluated under laboratory and greenhouse conditions with commercially available and native nematodes (Belotti, 2002).

MATERIALS AND METHODS

Nematodes and *C. bergi* insects

The nematodes used in the present work are listed in Table 1. All nematodes were cultured in the final instar larvae of the greater wax moth, *Galleria mellonella* L. at 23°C according Kaya & Stock's (1997) methodology. Infective juveniles were stored in 0.01% formaldehyde solution at 10°C for 5-7 days and one day before use they were acclimatised to room temperature for at least 24 h before inoculation.

C. bergi fifth and adult stages were selected from the colony that has been established in the entomology laboratory of the cassava program at CIAT, Cali-Colombia.

Table 1. Entomopathogenic nematodes species and origin

Species	Origin
***Steinernema riobrave* (Sr)**	**United States**
***Heterorhabditis bacteriophora* (Hb)**	**United Kingdom**
Steinernema sp Sfnl-0100 (Sfnl)	**Colombia**
***Heterorhabditis* sp Hfnl-0190 (Hfnl)**	**Colombia**
***Steinernema feltiae* strain Villapinzon (Sf)**	**Colombia**
***Heterorhabditis* sp- CIAT 2003(HCIAT)**	**Colombia**

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

In a second test, three species of nematodes were applied in lots of 2000, 4000, 6000, 8000 and 10,000 nematodes per millilitre against the adult stage of *C. bergi*. The experiment was replicated four times in randomised complete blocks. The evaluation period and method were the same as described previously.

Statistical analysis

The data were statistically analysed by ANOVA (GLM) for mean separation by the Duncan test and Probit analyses respectively.

RESULTS AND DISCUSION

The results obtained in this evaluation showed that both *C. bergi* stages were parasitised by all entomopathogenic nematode species. *Steinernema* sp-Sfnl 0100 was the species that showed the highest parasitism in the fifth and adult stage of *C. bergi* with 77 and 100% parasitism respectively and the lowest percentage was showed by *Heterorhabditis* sp Hfnl with 28 and 49% parasitism in the fifth and adult stages respectively (Figure 2) after 10 days of inoculation.

Although higher percentage parasitism was shown in the fifth stage, no correlation was observed with mortality, only 22% mortality compared with 77% parasitism. The lowest mortality was observed with *Heterorhabditis* sp Hfnl with only 4% (Figure 3).

This low mortality observed in both *C. bergi* stages with all the entomopathogenic nematodes, could be because *C. bergi* shows a very strong immune response to all the entomopathogenic nematodes species evaluated.

Koppenhöfer et al. (2003) mentioned that different species/strains of nematode differ in their efficacy significantly in controlling the same insect pest. This can be influenced by the penetration rate of the infective juveniles into the host, the release time of the bacteria, and the virulence degree of killing the insect.

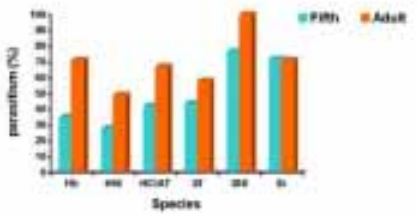

Figure 2. Parasitism of two *C. bergi* stages with six nematodes species

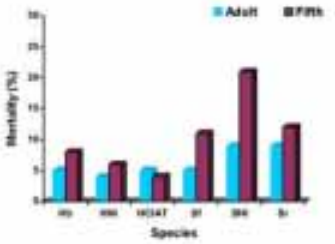

Figure 3. Mortality of two *C. bergi* stages with six nematodes species

When *C. bergi* adults were exposed to different dosages of three nematode species, no significant differences were observed between the lowest and the highest dosages. The results obtained were similar to the above-mentioned, all three nematode species causing parasitism of 85-100% in the adult stage but only able to cause low mortality of 3-40%.

This confirms the results obtained by Caicedo & Bellotti (1994) with *Steinernema carpocapsae* in all the *C. bergi* stages, the adult was the most susceptible to being parasitised with 60%, but very low mortality, 3% after 10 days of inoculation and the youngest instars were least susceptible with 3-17% parasitism. These results are also comparable with those obtained by Barbernna & Bellotti (1998).

At this point we can only speculate about the factors responsible for the interactions among nematode species and *C. bergi* stages. It could be that *C. bergi* has coevolved with entomopathogenic nematodes and other pathogens in the soil but at this time we do not know whether the defence mechanisms reported in other species, such as white grubs which present infrequent CO_2 output, sieve plates covering the spiracles, frequent defecation, other defensive and evasive behaviours and strong immune a response, operate here.

For this reason we consider that is very important to understand the innate immune response of *C. bergi* and to determine if a correlation exists in the insect species between levels of cellular and humoral response of the different species/strains used as a challenge for the ultimate choice of effective species/strains for its control (CIAT, 2003).

CONCLUSIONS

All the entomopathogenic species parasitised both *C. bergi* stages, fifth and adult.

Steinernema sp Sfnl 0100 showed the highest parasitism in both stages, fifth and adult, 77 and 100% respectively, but this high parasitism was not correlated with the mortality, only 9 and 21% mortality respectively was observed.

It is a priority to initiate basic studies into understanding the innate immune response of *C. bergi* and to determine if a correlation exists in the insect species between levels of cellular and humoral response of the different species/strains used as a challenge for the ultimate choice of effective species/strains for its control.

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RECONOCIMIENTO DE NEMATODOS ENTOMOPATÓGENOS ASOCIADOS A *Cyrtomenus bergi* EN TRES LOCALIDADES DE COLOMBIA

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INTRODUCCIÓN

Yuca, *Manihot esculenta Crantz*, ocupa el cuarto lugar a nivel mundial como una de las principales fuentes de carbohidratos. Este importante cultivo es atacado por un gran complejo de insectos-plaga incluyendo a *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) como uno de los más destructivos (Fig.1). *C. bergi* se alimenta directamente del producto comercial, deteriorando su calidad (Fig. 2). Además de la yuca, ataca diversos cultivos de importancia económica como cebolla, maní, espárragos, caña de azúcar, maíz y pastos entre otros, en diferentes países del trópico (Bellotti, 2002).

Figura 1. Adultos de *C. bergi* alimentándose de semillas de maní

El control microbial es una alternativa al uso indiscriminado de plaguicidas para su control. Las estrategias para el uso de patógenos en control biológico de insectos-plaga está determinado principalmente por las interacciones entre patógenos, insecto-plaga, medio ambiente y planta hospedera. Por lo tanto, el primer paso para desarrollar un programa de control microbial es el conocimiento de la ocurrencia natural de los patógenos para ser utilizados como un componente en programas de manejo integrado de plagas (Hominick et. al. 1996).

Figura 2. Raíces de Yuca con daño de *C. bergi*

MATERIALES Y MÉTODOS

Toma de muestras: Se tomaron muestras de suelo de Santander de Quilichao (Cauca), Santágueda (Caldas) y La Florida (Risaralda) durante el período comprendido entre septiembre y diciembre del 2002, de acuerdo a la metodología de Bedding & Akhurst (1975) y Kaya & Stock (1997). De cada sitio se tomaron en promedio tres submuestras a dos profundidades 1-10 cm y 10-25 cm en un área de 10 m². Cada muestra se tomó con un barreno cilíndrico de 10 cm de diámetro. Las muestras fueron colocadas en bolsas plásticas debidamente rotuladas y transportadas al laboratorio en una termonevera. De cada sitio se registró la altitud, temperatura y el tipo de vegetación.

Recuperación de nematodos: Se realizó utilizando larvas de *Galleria mellonella* L. como insecto trampa (Bedding & Akhurst, 1975). Las muestras fueron procesadas dentro de los tres días siguientes de su colección. Un Kg de suelo por cada sitio fue completamente mezclado y submuestras de 250 cc fueron puestas en recipientes plásticos de 300 cc con 10 larvas de *G. mellonella*. Los recipientes fueron guardados en bolsas plásticas en un cuarto con temperatura 21-23°C durante un período de 5-7 días. Este proceso se repitió tres veces en el tiempo. Las larvas muertas fueron puestas en cámara húmeda durante una semana y después transferidas a trampa White para la colección de los infectivos juveniles (Kaya & Stock, 1997).

Los infectivos juveniles recuperados de cada muestra fueron usados para la infección de larvas sanas de *G. mellonella* para verificar su patogenicidad y obtener progenie para su identificación. Las muestras de suelo positivas para nematodos fueron sometidas a análisis físico, MO y pH en el laboratorio de suelos del CIAT.

RESULTADOS Y DISCUSIÓN

De las 193 muestras de suelo colectadas y procesadas de las 11 localidades en los departamentos de Cauca, Risaralda y Caldas, sólo 10 muestras de dos sitios diferentes presentaron 45 y 33% de larvas de *G. mellonella* infectadas con nematodos entomopatógenos (Tabla 1.)

Tabla 1. Características de las localidades muestreadas, en los departamentos de Cauca (SQ), Risaralda (R) y Caldas (C).

Código	Sitio	Fecha	Vegetación	Altitud msnm	% infección
SQ 1	Lagos Brasilia	12-09-02	Yuca	990	0%
SQ 2	Finca Brasilia	12-09-02	Yuca	990	0%
SQ 3	La Agustina	12-09-02	Yuca	990	0%
SQ 4	La Chapa	12-09-02	Yuca	990	0%
SQ 5	La Agustina	1-12-02	Yuca	1340	0%
SQ 6	El Pilal	1-12-02	Yuca	1530	0%
SQ 7	La Independencia	1-12-02	Yuca	1700	0%
SQ 8	Cachimbal	1-12-02	Yuca	1370	0%
SQ 9	Calotefio	1-12-02	Yuca	1530	0%
R 10	La Colonia	3-10-02	Cebolla Medicinales Mora	1900	45%
R 11	La Florida	3-10-02	Cebolla Cilantro Maiz	1740	0% 33% 0%
C 12	Granja Motelindo	1-10-02	Naranjo Bravo Platano	1050	0% 0% 0%

Sólo un género de nematodos entomopatógenos fue recuperado. Heterorhabditis sp **CIAT-2003** de las localidades de La Colonia y La Florida. La identificación hasta nivel de especie se encuentra en proceso por parte del taxónomo P. Stock de la Universidad deTucson, Arizona USA.

Las características generales de sintomatología de infección y morfología de la especie Heterorhabditis sp-CIAT2003 son:

- Coloración rojiza en larvas de *G. mellonella* infectadas (Fig. 3A).
- Ciclo biológico sobre larvas de *G. mellonella* de 13-14 días a 23 °C y 70 % HR.
- Recuperación de juveniles infectivos en trampa white (Fig. 3B)

Figura 3A. Larvas de *G. mellonella* sanas e infectadas

Figura 3B. Recuperación de infectivos en Trampa White

- Primera generación de adultos hermafroditas con el estoma corto característico del género (Fig. 4A); vulva bien desarrollada y desarrollo ovoviviparo (Fig. 4B) y cola terminando en punta (Fig. 4C).
- Adultos machos en la segunda generación también con el estoma corto (Fig. 5A)
- Cola con la bursa del tipo peloderan (Fig. 5B).
- Juveniles infectivos conservando la cutícula del estadio anterior y estoma cerrado

Figura 4A. Estoma hembra primera generación

Figura 4B. Vulva hembra primera generación

Figura 4C. Cola hembra primera generación

Figura 5A. Estoma macho segunda generación

Figura 5B. Cola macho segunda generación

El hallazgo y la identificación de especies de Heterorhabditis es considerada una tarea difícil comparada con especies de Steinernematids y esto se refleja en la cantidad de especies identificadas hasta el momento.

Las especies de este género se consideran como especies morfológicamente conservativas las cuales requieren mucha experiencia y trabajo para la obtención de una adecuada identificación.

Además, la gran confusión que ha generado el hallazgo de razas que difieren biológicamente hace que este proceso sea mucho mas dispendioso. Por lo tanto, aún están sin resolver preguntas como si se tienen diferentes especies o razas y cuál es la relación con su origen.

CONCLUSIONES

El hallazgo de Heterorhabditis sp-**CIAT 2003** en el mismo habitat de *C. bergi* es muy importante al considerarse este género menos diverso que los nematodos del género Steinernematids.

Además el porcentaje de recuperación de 33 y 45% en cada sitio es muy alto comparado con lo reportado en la literatura, el cual no supera el 10-15% de recuperación en diferentes partes del mundo.

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ESPECIES DE LA FAMILIA CHRYSOPIDAE ASOCIADAS AL CULTIVO DE YUCA *Manihot esculenta* Crantz

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INTRODUCCIÓN

Dentro del control biológico, los predadores son los menos estudiados por la dificultad en establecer con precisión su impacto en la población de una plaga dada. Sin embargo, las especies de la familia Chrysopidae han sido estudiadas como controladoras de áfidos y lepidópteros a nivel mundial con óptimos resultados, siendo en la actualidad comercializados para el control de plagas de diversos cultivos. En yuca, específicamente sobre especies de mosca blanca, se carece de investigaciones sobre este grupo de predadores.

Por esta razón, los objetivos de este trabajo son:

a) Determinar las especies de la familia Chrysopidae presentes en cultivos de yuca de diferentes zonas de Colombia con y sin presencia de mosca blanca.

b) Establecer colonias en laboratorio de las especies de Chrysopa más frecuentes, para futuros bioensayos de predación con mosca blanca.

METODOLOGÍA

Se realizaron exploraciones de crysópidos en cultivos de yuca con y sin presencia de mosca blanca, en los departamentos del Tolima, Cauca, Valle del Cauca y parte de la zona cafetera colombiana (Risaralda y Quindío).

Los muestreos y las colectas se realizaron en lotes comerciales de yuca entre 3 y 8 meses de edad.

Los especímenes (larvas y adultos) se enviaron al Departamento de Entomología de la Universidad de Cornell (USA) para identificación.

Establecimiento de las colonias

Veinte adultos de las especies Ceraeochrysa cubana, Ceraeochrysa claveri y Chrysoperla externa, procedentes de la zona cafetera, Valle del Cauca y Cauca, fueron introducidos en unidades de pvc **(Fig. 1a)** revestidas con cartulina negra para obtener posturas **(Fig. 1b)**. Dos veces por semana los huevos fueron extraídos y se trasladaron a cajas petri **(Fig. 1c)**.

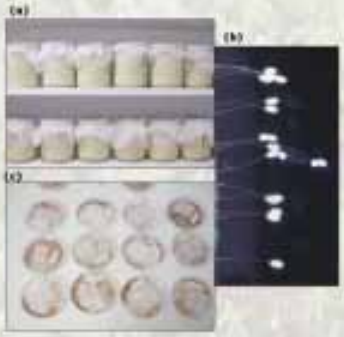

Figura 1. (a) Unidades de cría de adultos de crysopa (b) Posturas de crysopa extraídas de las unidades de pvc (c) Cajas petri con posturas de *Sitotroga cerealella* para alimentación de larvas de crysopa.

Inicialmente los adultos se alimentaban con una dieta tradicional de levadura, germen de trigo, miel, azúcar y agua.

Las larvas emergidas se alimentaban con huevos de *Sitotroga cerealella* hasta el estado de pupa. Al emerger los adultos se introducían nuevamente en las unidades de cría.

Con estas mismas condiciones se estableció la cría de *Chrysoperla carnea* (Stephens) obtenida de un laboratorio comercial.

Figura 2. *Ceraeochrysa claveri* (Hagen). (a) larva (b) adulto (c) características: Mancha laterales pardas/grises en el pronoto, mancha en el escapo prolongada casi siempre sobre el vertex, palpos claros.

Figura 3. *Ceraeochrysa* sp. #2 (a) larva (b) adulto, características: Manchas laterales pardas/grises en el pronoto, dorso del escapo sin manchas, 10 primeros segmentos antenales más oscuros.

Figura 4. *Ceraeochrysa cubana* (Hagen) (a) larva (b) adulto (c) características: Antenas claras, manchas del escapo ausentes, manchas laterales en el pronoto con bordes difusos, palpos claros.

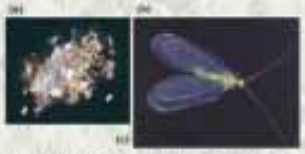

Figura 5. *Leucochrysa* sp. #2 *cubana* (j)adulto, características: Mancha oscura en el ápice distal de las alas, cuatro manchas sobre el pronoto que pueden unirse para formar dos líneas alargadas.

Figura 6. *Chrysoperla externa* (Hagen) (a) larva (b)adulto (c) características: Banda amarilla en el dorso del tórax y el abdomen, mancha general oscura, pronoto con setas claras saliendo de una base oscura, manchas cerca a los cuatro ocelares.

Figura 7. *Chrysoperla carnea* (Stephens) (a) larva (b) adulto (c) características: Banda amarilla en el dorso del tórax y el abdomen, mancha general marrón oscuro, setas del pronoto gruesas y negras en su base.

RESULTADOS

Se encontraron diez especies de crysopa en cultivos de yuca. De las cuales cinco aún no han sido determinadas y tres son las más frecuentemente encontradas en todas las zonas del país muestreadas **(Table 1)**.

Tabla 1. Especies de Crysopa Encontradas en Cultivos de Yuca de Colombia.

Zona de Muestreo	Especie de Crysopa
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. #3
	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. Limaflava Adams & Penny
	Leucochrysa (Nodita) sp. #4
	Ceraeochrysa valida (Banks)

Múltiples características diferencian las especies de Crisópidos, algunas generales son: tamaño de los antenas respecto a la extensión ala, manchas en las alas, tonalidades en el dorso del tórax y abdómen, mancha genal, setas y manchas en el pronoto, color y presencia de manchas en el vertex, manchas en el escapo, entre otras **(Fig. 2 a 7)**.

Establecimiento de colonias

Con la metodología utilizada se logró establecer la cría de las diferentes especies de Chrysopidae. Respecto a la alimentación, con la dieta inicial la oviposicion fue baja o nula para las especies *C. cubana*, *C. claveri* y *C.carnea*. Por esta razón se modificó la dieta tradicional reemplazando la levadura por proteína hidrolizada de levadura. Con esta nueva dieta se logró establecer la colonia de las especies nativas eficientemente. Para *C. carnea* ambas dietas fueron efectivas en la cría de la especie, siendo mejor la tradicional que la modificada.

CONCLUSIONES

- Las especies *Ceraeochrysa* sp. #1, *Ceraeochrysa* sp. #2, *Leucochrysa* sp., *Leucochrysa* (*Nodita*) sp. #2 y *Leucochrysa* (*Nodita*) sp. #4, se encuentran en proceso de descripción.
- La metodología de cría permitió multiplicar las especies fácilmente a nivel de laboratorio.
- La dieta de levadura, germen de trigo, miel, azúcar y agua, permitió multiplicar la especie *C. carnea* fácilmente en laboratorio.
- La dieta modificada con proteína hidrolizada de levadura permitió establecer fácilmente las colonias de las especies nativas *C. cubana*, *C. claveri* y *C. externa* a nivel de laboratorio.

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Búsqueda de poblaciones nativas de nematodos entomopatógenos en regiones de Colombia y Panamá

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Introducción

Los nemátodos entomopatógenos (NEP's) Heterorhabditidos y Steinernematidos son usados como agentes potenciales de control de numerosas plagas de suelo. A través del mundo se han llevado muchas investigaciones partiendo de capturas de poblaciones nativas de estos organismos y estudiando su distribución, encontrándoselos en variedad de habitats como: pastos, forestales, hortalizas, frutales, flores, etc., los cuales presentan diferentes características de suelos (Rueda *et al*., 1993).

Una de las formas de captura de estos nematodos es por medio del insecto trampa *Galleria mellonella* (Lepidoptera: Pyralidae), la cual presenta mas susceptibilidad ante estos patógenos que los insectos hospederos comunes. Este insecto provee una excelente detección de nematodos *in situ*, o para muestras procesadas en laboratorio (Bedding y Akhurst, 1975).

Objetivo

Identificar las especies nativas presentes en determinadas regiones de Colombia y Panamá, asociadas a cultivos atacados por insectos rizófagos, como aporte al conocimiento de estos enemigos naturales en el manejo biológico de plagas de suelo.

Material y Métodos

Se toman muestras de diferentes sitios de Colombia y Panamá (Figura 1), eligiendo suelos agrícolas con cultivos afectados con rizófagos. En el país se muestrean los departamentos Quindío, Risaralda, Caldas y Cauca; y, en Panamá las provincias Coclé, Veraguas, Chiriquí.

La toma de muestras de suelo se realiza al azar en el campo (Figura 2), con un barreno (4,5 cm de diámetro), a una profundidad entre 15 a 20 cm, extractando 1000 cm^3 por submuestra, las que se conservan en termonevera con hielo (8 a 15 °C). Se identifica el sitio muestreado y se toman datos de localización, humedad relativa, temperatura, cultivos y fitófagos asociados; y, rotaciones efectuadas. Estas muestras se mantienen en laboratorio a 15 °C, en incubadora y en oscuridad.

Figura 1. Sitios muestreados en Colombia (qje: Caldas y Cauca) y Panamá (Coclé, Veraguas y Chiriquí).

Figura 2. Esquemas para la toma de muestras en campo.

Adicionalmente se toman muestras de suelo (500 g), para su análisis fisicoquímico.

El proceso para la búsqueda, reactivación y almacenamiento de NEP's en laboratorio se explica en la Figura 3.

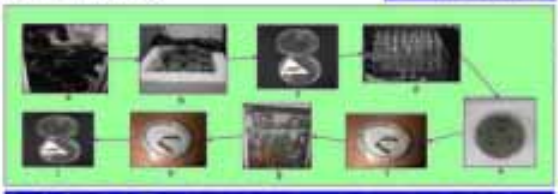

Figura 3. Extracción de NEP's en laboratorio (tres ventas: a, suelos almacenados en recipientes; b, varias placas con muestras; c, trampa White modificada; e, fusión con muestras de remuestreo; d, cajas petri con agua con NEP's; f, larvas extraídas de la trampa con síntomas; g, muestras almacenadas en recipientes; h, aplicación de plaguicidas de tipo (verificación); i, recuperación de NEP's).

Resultados y Discusión

Los sitios se seleccionaron por encontrarse cultivos (cebolla, pastos, yuca y otros menos frecuentes como: Maracuyá, Mango, Árbol del pan) afectados por plagas rizófagas, en Colombia y Panamá.

En las muestras de Panamá, se identifican nematodos que por sus características morfológicas resultan ser saprófitos, estos se murieron en la solución de almacenamiento de formaldehído, imposibilitando su multiplicación por otros métodos. Las muestras de Colombia se procesan con la metodología propuesta, manteniéndose unas en solución fijadora TAF (Formol + Trietanolamina + agua destilada) y otras vivas en solución de formaldehído al 0.05 %. Todo este material es enviado a Alemania para su identificación por un especialista en técnicas moleculares.

Durante la evaluación se encontraron en las larvas de *G. mellonella* organismos que al parecer se estaban alimentando de ellas, identificando entre éstos a ácaros pertenecientes a las familias Acaridae e Histionidae, que son conocidos por ser saprófagos y fungívoros.

También se observaron crecimientos de hongos, los cuales fueron identificados como *Fusarium* sp. y *Metarhizium* sp.

En casi todos los casos se encontraron nematodos sobre las larvas de *G. mellonella*. La separación entre saprófagos y patógenos se realizó aplicando los postulados de Koch, registrando síntomas en larvas así como comportamiento típicos. De las 320 muestras (300 g c/una) en 15 cultivos, se obtienen positivas para hongos (24), ácaros (16) y nematodos patógenos y saprófagos (21) (Tabla 1).

Tabla 1. Sitios de muestreo de NEP's en Colombia y Panamá y los organismos asociados.

País	Departamento o Provincia	Municipio	Cultivo	No. de muestras	Organismo presente: Ácaros	Hongos	Nematodos
Panamá	Coclé	El Valle de Antón	Cebolla	4	2	2	1
			Maíz	4	0	2	1
	Veraguas	Ocú	Yuca	10	0	2	1
	Chiriquí	Dolega	Yuca	8	0	2	0
		Cerro Punta	Cebolla	3	1	2	1
Colombia	Quindío	Montenegro	Yuca	21	1	2	1
			Plátano	20	1	2	1
			Maíz	20	0	1	1
	Risaralda	Santa Rosa	Cebolla	4	0	1	0
		Pereira	Pasto	30	1	1	1
			Yuca	33	0	1	1
		La Florida	Cebolla	34	1	0	1
			Pasto	31	1	0	1
	Caldas	Manizales	Quimbo	2	0	1	1*
			Árbol del Pan	3	0	0	1
			Maíz	3	0	0	1
			Fique	4	1	0	1
			Yuca	4	1	0	1
			Aguacate	2	0	0	0
			Plátano	3	0	0	0
			Mango	3	0	0	0
			Manzanita	3	1	1	0
			Limón	3	1	0	0
			Maracuyá	3	0	1	0
			Café	4	0	1	1
			Cebolla	6	0	1	1
	Cauca	Santander de Quilichao	Pasto	20	1	0	1
			Yuca	21	1	1	1*
			Maíz	2	1	0	1
TOTAL				320	16	24	21 (6.6%)

*Muestras analizadas para entomopatógenos, con la especie *Steinernema kraussei* (Steiner, 1923).

De esta búsqueda (20 muestras aparentemente patógenas), se reporta por primera vez en Colombia, en Cauca (Yuca) y Caldas (Guamo), la especie *Steinernema kraussei*, descrita por Steiner (1923). La identificación la realiza Susurluk (2004) con la técnica molecular de PCR (Figura 4).

Figura 4. PCR de *Steinernema kraussei* de procedencia de la región ITS digerido con seis enzimas de restricción.

Según el análisis de suelo de estas regiones, donde se identificó la especie, presentan un pH entre 6,4 (Cauca) y 5,2 (Caldas), con una textura entre franco-arcillosa y franco-arcillosa-limosa, respectivamente.

Conclusiones y Recomendaciones

Se colecta e identifica por primera vez en Colombia *Steinernema kraussei*, especie reportada en otros países como controladora de chisas.

Según algunos autores los suelos de textura franco-arcillosas presentan menos ocurrencia de NEP's (Parada, 2001), sin embargo, en este caso en uno de los sitios evaluados con esta textura se hallaron NEP's, mostrando esta especie un amplio rango. Los steinernemátidos se hallaron dentro del rango de pH de 4 a 8 que reportan Koppenhofer *et al*. (1996).

Del total de las muestras evaluadas (320) solo el 6,6% presentan nematodos, lo que puede deberse a que el muestreo se realizó en suelos de cultivos bajo manejo químico (cultivados), lo que podría influenciar la presencia de estos organismos, por lo que es recomendable muestrear paralelamente sitios sin intervención química.

De las 20 muestras de nematodos enviadas a Alemania para su identificación, dos fueron positivas como entomopatógenos (*S. kraussei*), continuándose la identificación de las restantes.

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Awards

The "Hernán Alcarez Viecco" an Honorable Mention to CIAT's Cassava Entomology team (Arturo Carabalí, Anthony C. Bellotti, James Montoya, and María Helena Cuéllar). Second place for their paper "Adaptación del biotipo B *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) a yuca *Manihot esculenta* (Crantz)". XXX Congress of the Colombian Entomological Society, SOCOLEN. July 28-30, Bogotá, Colombia.

Training Received

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Course "Taxonomia de Coleopteros: Sacarabaeidae de Importancia Agricola (Coleoptera: Scarabaeidae "Pleurosticti)." Twenty Technicians, Students and Professionals of Integrated Pest and Disease Management Unit. 6-10 Sep. 2004. (Professor: Jhon César Neita Moreno, Universidad Nacional, Bogotá).

Training and Consultancy Services offered during 2004.

Organizer	Place	Date	Participants	Received by	Service
CIAT-MADR	CIAT	12 Oct. 2004	28	Farmers, administrators	Management of Pests
CIAT/Biotechnología	Vereda Alegrías Santander de Q.	25 Aug. 2004	70	Professionals, extensionists and farmers from Cauca-AMUC (Asoc. Municipal de Usuarios Campesinos del Cauca)	Conference and field day. MIP in whitefly and Cassava Frogskin (CFS)
Universidad Nacional (Medellin)	CIAT	13 May 2004	13	Agronomy Students	Cassava Pests and biological control
CLAYUCA	CIAT	31 May-12 Jun. 2004	40	Scientists, farmers, extensionists, administrators	Conference and field day (MIP on cassava whitefly) in an International Course about Modern Systems of Production, Processing and Utilization of Cassava

Organizer Place		Date	Participants	Received by	Service
Universidad Nacional Abierta y a Distancia-Pereira	CIAT	30 April 2004	18	Students	Integrated Pest Management in Cassava
Colegio Tomás Cripriano Mosquera	CIAT	27 April 2004	32	Students	Workshop on Integrated Management of Soil Pests, with Emphasis on Microbiological Control
Universidad de Antioquia	CIAT	16 April 2004	13	Students of the University's Biology Institute	Workshop on Integrated Management of Soil Pests, with Emphasis on Microbiological Control
CIAT-MADR	Pereira	26 Feb. 2004	28	Farmers, Producers, Administrators, and Cassava Processors	Integrated Pest Management in Cassava in the Coffee-growing Region
CLAYUCA	CIAT	20 Feb. 2004	2	Ernesto Espinosa and José Ventura from INIVIT-Cuba	Integrated Management of the Whitefly *Aleurotrachelus socialis* with Emphasis on Varietal Resistance
CIAT-Subterranean Pests Project	Quimbaya	17 Feb. 2004	32	Farmers, Producers, Students, Administrators, and Cassava Processors	Workshop on IPM in Cassava in the Coffee-growing Region, with Emphasis on Soil Pests
Universidad de Medellín	CIAT	17 Feb. 2004	20	Students of Biological Control Course	Biological Control of Cassava Pests, Nematodes and Taxonomy of Parasitoids
CENICAÑA	CIAT	16-20 Feb. 2004	4	Luis A. Gómez, Germán Vargas (Cenicaña); María Marín (Fedepanela); Luis A. Hincapié (IcA, Risaralda)	Workshop on Bioecology and IPM of the Spittlebug
Inst. Nal. de Investigaciones Forestales y Agropecuarias-Yucatán, Mexico	CIAT	1-2 Dec. 2003	1	Raúl Díaz	Training in whitefly identification and mounting of specimens
Universidad Nacional, Palmira	CIAT	1-30 Nov. 2003	1	Sergio Prieto, Visiting Researcher	Evaluation of the level of mortality and body weight gain of the cassava hornworm (*E. ello*)
INIAP-Experiment Station in Portoviejo, Ecuador	CIAT	10-14 Nov. 2003	3	Oswaldo Valarezo, Ernesto Cañarte, Bernardo Navarrete	Mite taxonomy, preparation and mounting of mite specimens
PROINPA, Bolivia	CIAT	24 Oct. 2003	1	Oscar Barea, Coordinator of Integrated Crop Management	Whitefly taxonomy
ESPE (Escuela Superior Politécnica del Ejército) in Quito, Ecuador; CIAT	CIAT-IPDM Unit	17-19 Sep. 2003	3	ESPE (Escuela Superior Politécnica del Ejército) in Quito, Ecuador	Integrated management of the whitefly, the cassava hornworm, and entomopathogens and mites

Organizer	Place	Date	Participants	Received by	Service
CIAT	CIAT	27-28 Aug. 2003	1	Edwin Iquize, Visitor from Bolivia	Visit to the CIAT experiment station in Santander de Quilichao / Training in sampling techniques to analyze the incidence and population fluctuations of the spittlebug and soil arthropods

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MFAT – New Zealand Ministry of Foreign Affairs
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Linkages with Other CIAT Projects and with CIAT's Partner Institutions
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Instituto de Investigaciones de Viandas Tropicales – INIVIT, Cuba
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EMBRAPA, Cruz das Almas, Brazil
Escuela Politécnica del Ejército (ESPE), Ecuador
CLAYUCA
Conservation and Use of Tropical Genetic Resources SB-2
Improved Cassava for the Developing World IP-3
Tropical Fruits IP-6
GIS

Evaluating the Impact of Biotechnology on Biodiversity: Effect of Transgenic Maize on Non-Target Soil Organisms

Activity 1. Response of Non-Target Soil Arthropods to Chlorpyrifos in Colombian Maize.

Introduction

Quantitatively and qualitatively, arthropods constitute the most important group of soil macro-organisms, whether in terms of number of individuals, biomass, trophic function, or species diversity (Paris 1979, Jaramillo 1997). The majority of these arthropods are detritivores, playing an important role in the transformation and mineralization of organic material (Marasas *et al*. 2001), as well as regulation of microbial populations, decomposition of organic material, and nutrient cycling within the soil (Doles *et al*. 2001).

Mites and springtails constitute nearly half of all soil arthropods (ECA 2001). Springtails can occur in very high abundance, up to 40,000 individuals /m^2; mite populations can approach 200,000/m^2 and species diversity up to 200/m^2 (Jordan 1996). In some habitats, diplopods and other arthropods such as fly larvae are important, and can represent the principal detritivores at the soil surface when earthworms are absent (Jordan 1996). Overall, arthropods are expected to have a higher diversity and abundance in less perturbed ecosystems such as forests and permanent prairies (Raw 1971).

There are a diversity of beneficial insects that occur in the soil and function in biological control, lowering populations of pest arthropods and being an important component of integrated pest management (Kirsten *et al*. 1998). In agricultural systems, diversity can be viewed as an indicator of agroecosystem balance, where the application of chemical controls to reduce the effect of pest insects in the crop generates a disequilibrium in the populations of beneficial fauna, creating conditions favorable for the increase, resurgence and/or appearance of potential pests (Kirsten *et al*. 1998). In one study that compared the soil surface entomofauna in maize/bean systems (Zanin *et al.* 1995), it was established that insecticide application reduced the population of almost all arthropods in the individual crops, especially when the product was applied to the whole plant versus the soil.

In Colombia, maize was planted on 574,117 ha in 2001, with technified and traditional maize accounting for 26.0 and 74.0% of that area, respectively. National production was 1,239,346 tons, 44.5 and 55.5% corresponding to technified and traditional, respectively. Mean yield was 2.2 tons/ha (Ministerio de Agricultura 2001). The most important pests to maize during the germination and early plant stage are associated with the soil and include the cutworms *Spodoptera frugiperda* (J.E. Smith), *S. eridiana* (Cramer) and *Agrotis ipsilon* (Hufnagel), *Solenopsis* sp. ants, the scarab *Euetheola bidentata* (Burmeister) and the chinch bug *Blissus* sp. (Corpoica 2001). In general terms, the attacks are localized and when damage is greater than 10% of the seedlings, some type of control should be initiated (Corpoica 2001).

Spodoptera frugiperda (Lepidoptera: Noctuidae) is considered the most important pest of maize in Colombia and often achieves very high populations (García Roa 1996). Although known as the fall armyworm, *S. frugiperda* acts as a soil-borne cutworm, but also attacks the shoot and fruit (García Roa 1996). Chlorpyrifos (Lorsban) is the most common of the chemical control products used to combat this insect, incorporated into the soil before planting to reduce the impact of *S. frugiperda* as a cutworm (Ospina 1999).

As part of the project "Assessing the Impact of Biotechnology on Biodiversity: Effect of Transgenic Maize on Non-Target Soil Organisms" we conducted a study at CIAT to determine the effect of chlorpyrifos on soil arthropods in Colombian maize over two consecutive growing cycles (2002-2003). We expect that the results of this study will establish the usefulness of pitfall traps as a technique to monitor soil arthropod populations under tropical conditions and will generate data on the fauna associated with maize in the Cauca Valley of Colombia.

Objectives

General Objective: Determine the impact of soil insecticides on non-target soil arthropods in maize.

Specific Objectives

- Evaluate the effect of chlorpyrifos application to non-target soil arthropods in field plots.
- Generate information on the species richness of soil arthropods associated with maize.
- Quantify and compare the biodiversity of soil arthropods in maize with and without the use of soil insecticides.

Establishment and execution of w ork plan. Research was conducted at the International Center for Tropical Agriculture (CIAT), located at 3°31' N, 76°21' W, 956 m elevation, mean annual rainfall 1000 mm, mean temperature 24° C, and Holdridge life zone classification Dry Tropical Forest.

The experimental area consisted of eight experimental plots each with an area of 1849 m^2 (43 x 43 m) and evaluated over two consecutive cycles of maize (second semester 2002 and first semester 2003). In the semester previous to the start of the experiment, the plots were planted to *Crotalaria juncea* that was incorporated as a green manure. Planting date was 30 September 2002 and the plant material was the commercial hybrid "Master" from Syngenta. Plants were spaced 0.2 m apart in rows 0.75 m apart for a density of 12,326 plants/plot. At planting the graminicide "Dual" was applied at 1.5 l/ha.

Two treatments with four replicates were evaluated: maize with and without soil insecticides. Once treatments were assigned to field plots, chlorpyrifos (Lorsban 2.5%, 25 g AI/kg, product of Dow AgroSciences) was applied to the corresponding plots. No other pesticides were used and any weed control was done by hand.

Permanent pitfall traps were put out once germination reached 50%. Eight traps were used for each plot, one placed randomly along rows 5, 10, 15, 20, 25, 30, 35. Pitfalls were evaluated every week from germination to harvest except when rainfall interrupted sampling. The pitfall

traps had three components. The fixed part of the trap was a disposable 12 oz plastic cup with mouth diameter 7.5 cm; this was placed in each of the corresponding rows, dug into the soil so the top rim was even with the soil surface **(Figure 1A)**. The removable part of the trap was a 4 oz disposable plastic cup with mouth diameter 6.5 cm; this part of the trap was put in for 24 hours and then lidded and brought back to the lab for evaluation **(Figure 1B)**. When the traps were not being used for collecting samples they were covered with the lid of a petri dish to prevent arthropods from falling in **(Figure 1C)**.

Field samples were brought to the laboratory for their processing on the same day. Larger arthropods were picked out by hand. To recover the microarthropods, the samples were processed in a small funnel lined with a very fine mesh. The field sample was washed into the funnel with water. By capping the end of the funnel, the sample was floated, and the supernatant removed after discarding the larger debris. Then the remaining precipitate was floated again, this time in 35% salt solution and the supernatant removed. Both supernatant samples were then combined and stored in 70% ethyl alcohol until analysis and identification **(Figure 2)**.

Figure 1. (A) Fixed component and removable component and (B) lid of the pitfall traps in the field.

The samples were counted and identified under a dissecting scope and with appropriate taxonomic keys. Specimens that could not be identified to family or order were labeled and stored for shipping to Cornell for identification by specialists.

Figure 2. Cleaning and storage of samples in the laboratory.

Analysis of information: The experiment was set up as a completely randomized design. Differences in the abundance of organisms between treatments were tested with an ANOVA.

For the more abundant groups, the area under the abundance curve (accumulated insect-days) was calculated to determine differences between treatments in insect load. To compare arthropod diversity between treatments, we used taxonomic data on the level of order to calculate three indices of diversity (Shannon, Margalef and Simpson), a dominance index (Simpson), and an equitability index.

Results

Arthropod Taxonomic Composition: Over both cycles of evaluation (2002B –2003A), 11,850 specimens were captured representing 16 orders and 5 classes of arthropods **(Tables 1, 2)**. Of these, 98.9% were identified to order and 64.8% to family. Class Collembola was the most represented, with 46.0% of all individuals evaluated. Class Chilopoda was the least abundant, with 0.3% **(Table 1)**. Of total individuals captured, 58.7% corresponded to the insecticide treatment and 41.3% to the control. The majority of specimens (71.4%) were captured during the first cycle (2002).

Table 1. Number of individuals and compositio n of arthropod classes caught in pitfall traps in maize, 2002B and 2003A, with and without insecticide.

Class 2	002B	%	2003A	%	Total
Arachnida	2,000	23.6	1,214	35.8	3,214
Chilopoda	17	0.2	13	0.4	30
Collembola	4,737	56.0	707	20.9	5,444
Diplopoda	32	0.4	164	4.8	196
Insecta	1,677	19.8	1,289	38.1	2,966
Sum	8,463	100	3,387	100	11,850

Table 2. Number of individuals and compositio n of arthropod orders caught in pitfall traps in maize, 2002B and 2003A, with and without insecticides.

Order	With insecticide		Without insecticide		Sum with and without	
	Total %		Total %	To	tal %	
Acarina	1,411	20.3	922	18.9	2,333	19.7
Araneae	567	8.1	314	6.4	881	7.4
Blattaria	3	0.0	1	0.0	4	0.0
Chilopoda[1]	12	0.2	18	0.4	30	0.3
Coleoptera	446	6.4	307	6.3	753	6.4
Dermaptera	12	0.2	5	0.1	17	0.1
Diplopoda[1]	102	1.5	94	1.9	196	1.7
Diptera	113	1.6	105	2.1	218	1.8
Entomobryomorpha	394	5.7	404	8.3	798	6.7
Hemiptera	121	1.7	108	2.2	229	1.9
Homoptera	39	0.6	53	1.1	92	0.8
Hymenoptera	536	7.7	733	15.0	1,269	10.7
Lepidoptera	64	0.9	78	1.6	142	1.2
Neuroptera	2	0.0	2	0.0	4	0.0
Others	93	1.3	75	1.5	168	1.4
Orthoptera	17	0.2	23	0.5	40	0.3
Poduromorpha	2,934	42.1	1,548	31.7	4,482	37.8
Symphypleona	89	1.3	75	1.5	164	1.4
Thysanoptera	6	0.1	24	0.5	30	0.3
Total	6,961	100	4,889	100	11,850	100

[1] Taxonomic Class (Including for analysis).

The orders of greatest abundance were Poduromorpha and Acarina with 37.8 and 19.7% of all individuals captured **(Table 2)** . Only the orders Acarina and Thysanoptera exhibited a significant difference in abundance between treatments **(Table 3)**, while only orders Araneae, Diptera, Diplopoda, Hemiptera and Thysanoptera exhibited a significant difference in abundance between cycles. The orders Acarina and Collembola had a significant difference between treatments in area under the curve during both cycles **(Figures 3, 4)**. The greatest abundance of Collembola was in the insecticide treatment with 59.2% more individuals than the control. Of all Collembola collected, 82.3% of individuals were from the family Poduromorpha **(Table 5)**.

Of the 753 Coleoptera captured, 59.2% were from the insecticide treatment and 40.8% from the control. Analysis of the area under the curve showed statistically higher accumulated area for the control treatment during first growing cycle **(Figure 5)**. The Carabidae and Cicindellidae were the most represented families, comprising 46.6 and 6.6% of all beetles, respectively **(Table 4)**; 47.8% of Carabidae and 6.8% Cicindellidae were captured in the insecticide treatment and control, respectively. The most represented genera of the Carabidae were *Calosoma* (especially *C. granulatum*) with 85.8% of individuals and tribe Galeritini with 7.1%. For the family Cicindellidae all individuals corresponded to the genus *Megacephala* (*Tetracha).*

Of the 229 individuals captured from the order Hemiptera, 52.8% were captured in the insecticide treatment **(Table 6)**. The family Pyrrhocoridae was the most represented with 79.5% of total individuals captured, all belonging to the genus *Dysdercus*.

Table 3. Abundance of arthropods (mean ± S.E. number of individuals caught per evaluation date) associated with maize, 2002B and 2003A, with and without insecticide.

Order	Between Treatments		Between Semesters	
	With Insecticide	Without Insecticide 2	002B	2003A
Acarina	11.38±15.23 a	7.44±8.59 b	10.65±14.79 a	8.08±9.35 a
Araneae	4.57±24.61 a	2.53±8.66 a	4.98±25.59 a	2.03±1.48 b
Blattaria	0.02±0.15 a	0.01±0.09a	0	0.03±0.18
Chilopoda[1]	0.10±0.39 a	0.15±0.55 a	0.13±0.54 a	0.11±0.41 a
Coleoptera	3.60±6.46 a	2.49±4.21 a	3.38±6.02 a	2.67±4.82 a
Dermaptera	0.10±0.45 a	0.04±0.24 a	0.09±0.44 a	0.05±0.25 a
Diplopoda[1]	0.82±1.79 a	0.76±1.64 a	0.25±0.69 b	1.37±2.23 a
Diptera	0.91±2.07 a	0.85±1.85 a	0.43±0.97 b	1.36±2.55 a
Entomobryomorpha	3.18±3.62 a	3.26±4.83 a	1.22±1.81 b	5.35±5.03 a
Hemiptera	0.98±2.43 a	0.87±1.79 a	1.43±2.79 a	0.38±0.75 b
Homoptera	0.31±0.63 a	0.43±0.88 a	0.36±0.87 a	0.38±0.64 a
Hymenoptera	4.32±8.37 a	5.91±15.08 a	5.71±15.89 a	4.48±6.21 a
Lepidoptera	0.52±1.06 a	0.63±1.42 a	0.56±1.42 a	0.58±1.06 a
Neuroptera	0.02±0.13 a	0.02±0.13 a	0.01±0.09 a	0.03±0.16 a
Orthoptera	0.14±0.41 a	0.19±0.50 a	0.13±0.42 a	0.20±0.50 a
Poduromorpha	23.66±112.35 a	12.48±84.12 a	34.77±136.23 a	0.26±1.05 b
Symphypleona	0.72±1.86 a	0.60±1.41 a	1.02±2.13 a	0.28±0.71 b
Thysanoptera	0.05±0.22 b	0.19±0.62 a	0.20±0.60 a	0.04±0.20 b
Unidentified	0.75±1.46 a	0.60±0.97 a	0.81±1.54 a	0.53±0.79 a

[1] Taxonomic Class (Including for analysis).

For each row, means followed by different letters are statistically different at $P<0.05$ (Tukey-Kramer test for multiple comparisons).

Table 4. Number of individuals and composition of Coleoptera families caught in pitfall traps in maize, 2002B and 2003A, with and without insecticides.

Family	With Insecticide		Without Insecticide		Sum With and Without	
	Total	%	Total	%	Total	%
Bruchidae	1	0.2	--	--	1	0.1
Carabidae	213	47.8	138	45.0	351	46.6
Cicindellidae	29	6.5	21	6.8	50	6.6
Chrysomelidae	6	1.3	3	1.0	9	1.2
Cucujidae	1	0.2	0	0.0	1	0.1
Curculionidae	2	0.4	1	0.3	3	0.4
Elateridae	1	0.2	0	0	1	0.1
Geotrupidae	2	0.4	0	0	2	0.3
Immature	138	30.9	86	28.0	224	29.7
Lycidae	1	0.2	1	0.3	2	0.3
Melolonthidae	0	0	1	0.3	1	0.1
Myxophaga	0	0	2	0.7	2	0.3
Nitidulidae	11	2.5	12	3.9	23	3.1
Scarabaeidae	24	5.4	23	7.5	47	6.2
Scolytidae	0	0.0	1	0.3	1	0.1
Staphylinidae	8	1.8	8	2.6	16	2.1
Unidentified	9	2.0	10	3.3	19	2.5
Sum	446	100.0	307	100.0	753	100.0

Table 5. Number of individuals and compositio n of Collembola orders caught in pitfall traps in maize, 2002B and 2003B, with and without insecticides.

Order	With Insecticide	%	Without Insecticide	%	Total	%
Entomobryomorpha	394	11.5	404	19.9	798	14.7
Poduromorpha	2,934	85.9	1,548	76.4	4,482	82.3
Eysymphypleona	89	2.6	75	3.7	164	3.0
Sum	3,417	100	2,027	100	5,444	100

Table 6. Number of individuals and composition of Hemiptera families caught in pitfall traps in maize, 2002B and 2003A, with and without insecticides.

Family	With Insecticide	%	Without Insecticide	%	Total	%
Gelastocoridae	1	0.8	1	0.9	2	0.9
Immature	97	80.2	85	78.7	182	79.5
Lygaeidae	5	4.1	2	1.9	7	3.1
Pentatomidae	4	3.3	4	3.7	8	3.5
Pyrrhocoridae	4	3.3	1	0.9	5	2.2
Reduviidae	0	0	1	0.9	1	0.4
Tingidae	9	7.4	9	8.3	18	7.9
Unidentified	1	0.8	5	4.6	6	2.6
Sum	121	100.0	108	100.0	229	100.0

The order Hymenoptera represented 10.7% of total individuals captured, with 91.8% representing the family Formicidae where 58.4% were captured in the control treatment. Analysis of the area under the curve showed significant differences in accumulated area in favor of the control during first cycle. During the second cycle the area under the curve showed significant differences in favor of the treatment with insecticide.

Arthropod Taxonomic Diversity : Taxonomic richness, measured at the level of order, was highest in the control maize without insecticide **(Table 7)**. The index of species richness was not significantly different between treatments or between semesters. Some of the other measures, however, did exhibit differences between semesters. In terms of species similarity, the Jaccard Index for differences between treatments was 0.93 for 2002 and 1.00 for 2003. Comparing semesters, this index was 0.88 for the insecticide treatment and 0.94 for the control treatment.

Conclusions

- The protocols used to determine differences in abundance of soil active and surface active arthropods were adequate to provide the information needed for statistical analyses.
- Differences in abundance between semesters were greater than the differences between the two treatments.
- Contrary to expected results, the insecticide treatment had a higher overall arthropod abundance than the control.
- Analysis of the area under the population curve showed statistically significant differences between treatments. Only the orders Coleoptera and Hymenoptera exhibited differences between semesters.
- Indices of species diversity and dominance exhibited differences between semesters but not between treatments.
- There was 100% overlap of arthropod orders between treatments for semester 2003A

Table 7. Indices of arthropod taxonomic (ordinal level) diversity, dominance and equity in maize, 2002B and 2003A, with and without insecticides.

Index	Between Treatments		Between Semesters	
	With Insecticide	Without Insecticide 2	002B	2003A
Wealth (S)	13.00 a	13.25 a	12.88 a	13.38 a
Shannon diversity index	1.61 a	1.79 a	1.48 b	1.92 a
Margalef diversity index	1.85 a	1.98 a	1.76 a	2.06 a
Simpson diversity index	0.70 a	0.76 a	0.66 b	0.801 a
Simpson dominance index	0.30 a	0.23 a	0.34 a	0.20 b
Equity index	0.63 a	0.69 a	058 b	0.74 a

For each row, means followed by different letters are statistically different at $P<0.05$ (Tukey-Kramer test for multiple comparisons).

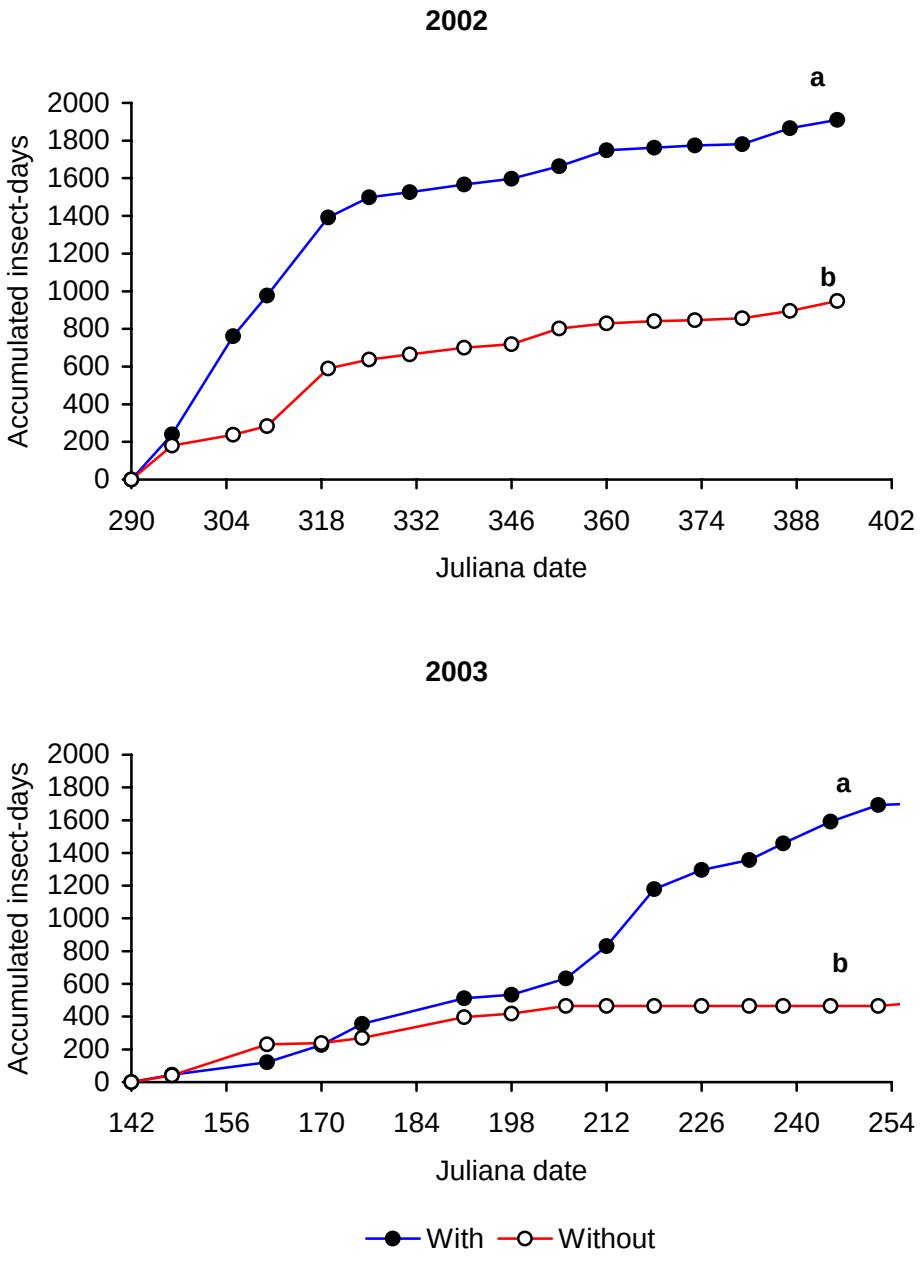

Figure 3. Area under the abundance curve for Acarina in maiz e, 2002 and 2003, w ith and without insecticides.

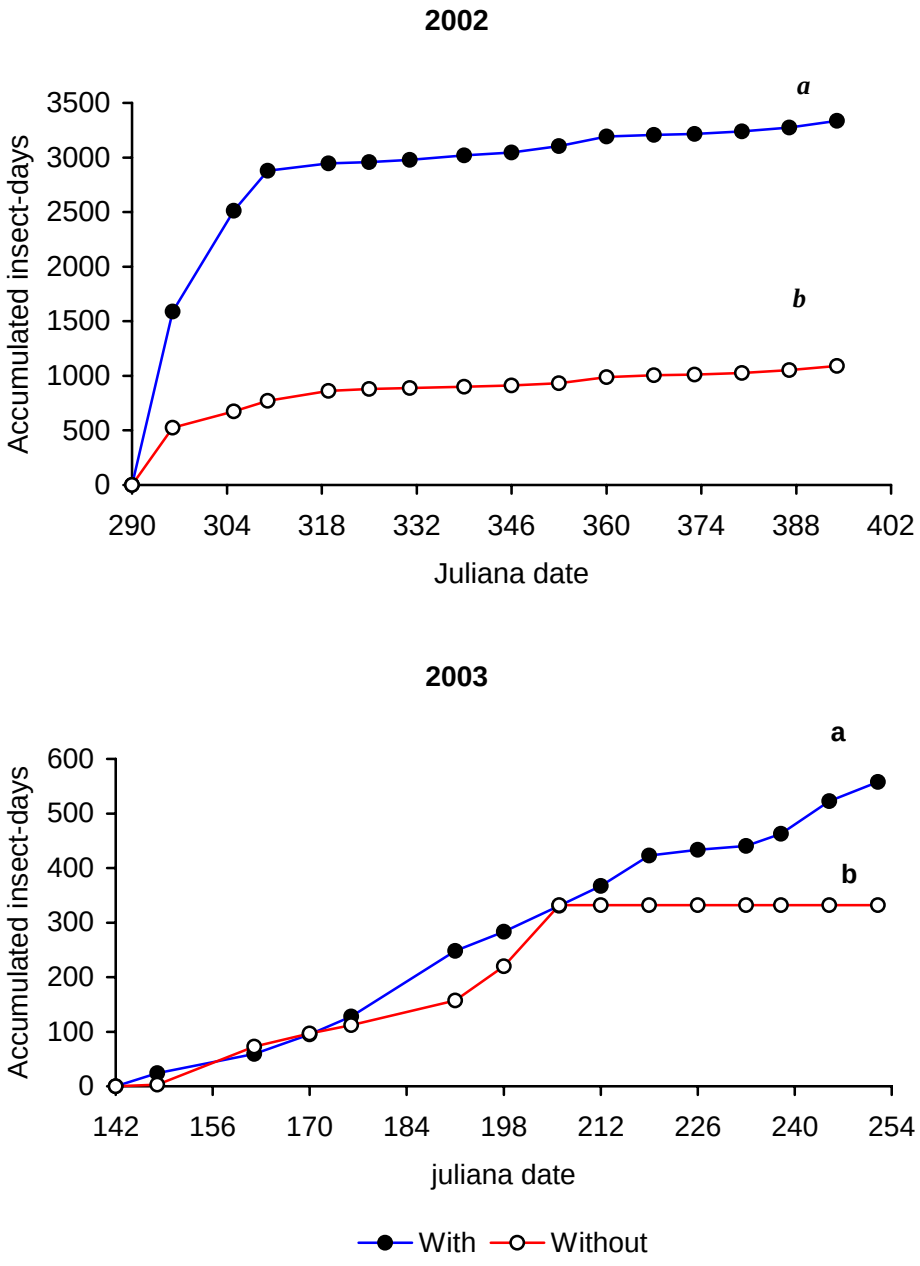

Figure 4. **Area under the abundance curve fo r Collembola in maize, 2002B and 2003A, with and without insecticides.**

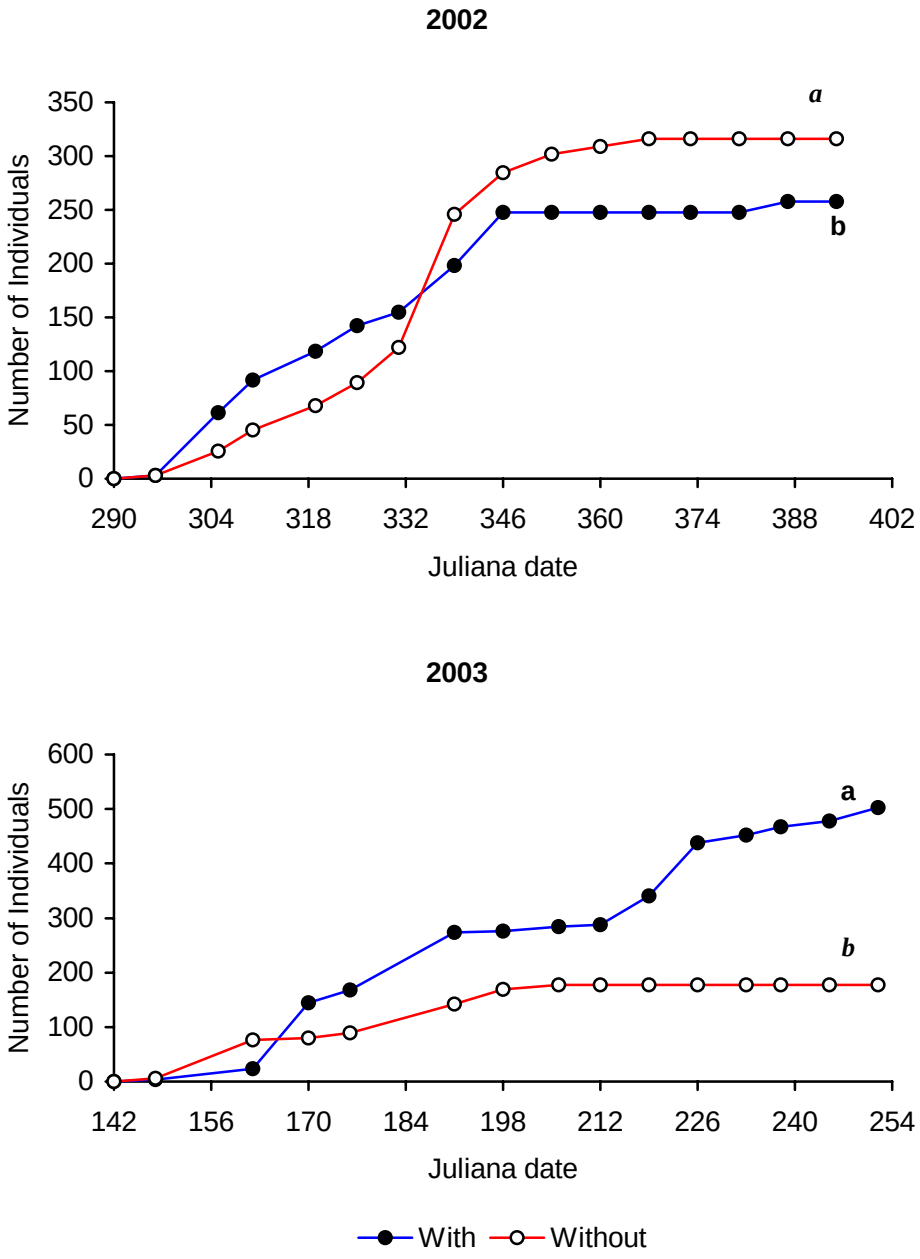

Figure 5. **Area under the abundance curve fo r Coleoptera in maize, 2002B a nd 2003A, with and without insecticides.**

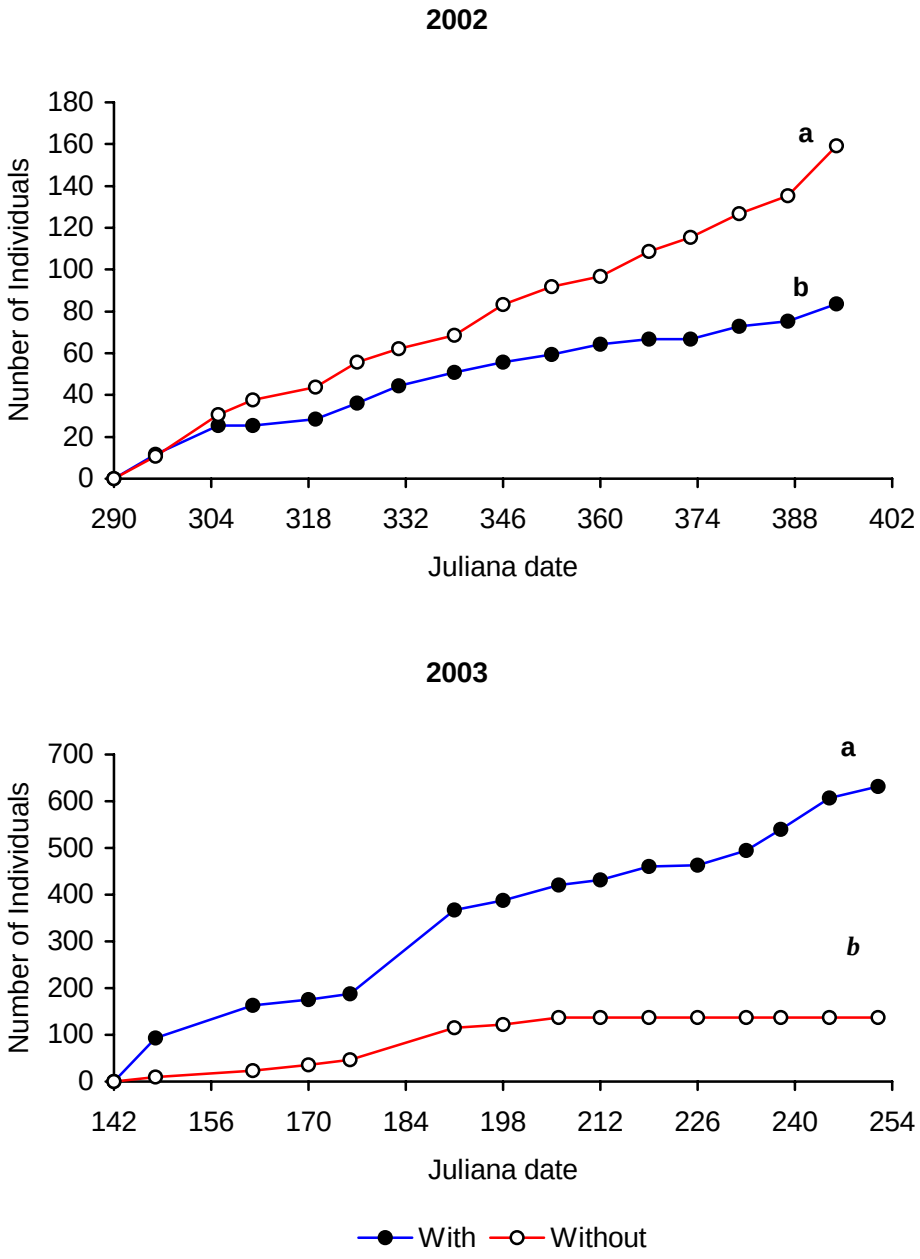

Figure 6. Area under the abundance curve for Hymenoptera in maiz e, 2002B and 2003A, with and without insecticides.

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Activity 2. Effect of transgenic cotton [Bollgard® Bt Cry1A(c)] on Non-Target Soil Arthropods in the Cauca Valley of Colombia.

Despite the controversy over the use of genetically-modified organisms (GMOs), the number of countries with GM commercial crops has grown from one in 1992 to 13 in 1999 (Shelton *et al.* 2002). From 1996 to 2000, the global area under GMOs increased 42.5 million ha (James 2004). The countries that reported the greatest increases were USA, Canada and Argentina, with 98% of the total area (Shelton *et al.* 2002). During 2003/04 67.7 million ha of GMOs were planted worldwide, of which soybean, maize, cotton and colza occupied 99% of the total area. Soybean plantings made up 61%, followed by maize and cotton with 23 and 11%, respectively (ICAC Recorder 2004).

For 2003/04, the area planted to GM cotton was 6.8 million ha, representing 21% of the total area planted to cotton worldwide. The countries that currently plant GM cotton are Argentina, Australia, China, Colombia, India, Indonesia, Mexico, South Africa and USA. The proportion of cotton that was GMO in 2003/04 was 77% for USA and 58% for China with an increase of 7% with respect to the previous harvest (ICAC Recorder 2004).

The Ministry of Agriculture and Rural Development, through the Colombian Agricultural Institute (ICA), designed a scheme to determine the viability of incorporating GMOs into the agricultural production system. ICA therefore passed Resolution 03492 in 1998 to establish and regulate the process of introduction, production, liberation and commercialization of GMOs. In two other provisions (Agreements 013/98 and 0002/02) ICA created the National Technical Council of Agricultural Biosecurity (CTN) to function in the assessment and support of GMO technology.

Since the establishment of those regulations, applications have been submitted for *Brachiaria*, carnations, cassava, coffee, cotton (resistance to lepidopterans), maize, rice, *Stylosanthes* and sugar cane. Of these, only four have been approved to date: (i) carnations for cut-flower production, (ii) cotton for commercial production, (iii) rice for small scale field trials, and (iv) maize for biosecurity tests (Díaz 2003).

For the period 1991-2002, Colombia experienced a reduction of 83% in the area planted to cotton. The 2001/02 harvest only included 39,000 ha in the two cotton-growing regions of Tolima-Valle and Costa-Meta (ICA 2001). One aspect that has greatly influenced the loss of area planted to cotton in Colombia is the high incidence of pests. The greatest losses are caused by the boll weevil (*Anthonomus grandis*, Coleoptera: Curculionidae) that affects 89% of the growing area in the departments of Córdoba, Cesar and Tolima, causing 15% loss of flower heads. *Heliothis virescens* (Lepidoptera: Noctuidae) affects 100% of the cotton planting area of Colombia, causing damage to 15-20% of the flower heads and bolls. Some 10% of the cultivated area is additionally affected by the foliovore "gusano rosado" (*Sacadodes pyralis*, Lepidoptera: Noctuidae) and whiteflies (Homoptera: Aleyrodidae).

Control of these pests is largely based on extensive use of agrochemicals and these represent 23% of the direct costs of the crop to the Colombian producer. In the Atlantic Coast, there was an average of 26 applications of pesticides per crop cycle, with 69.2% of those directed toward the control of lepidopterans. In the Cauca Valley, the number of applications has been reduced

73% to an average of 7 applications per crop cycle, with 57.1% directed towards the control of lepidopterans.

Given this scenario, ICA and the CTN implemented the first studies to determine the effect of the Bollgard® technology (Monsanto) on populations of arthropods and annelids in the cotton zone of Córdoba department in the Caribbean Region. The Bollgard® technology, generated by Monsanto, has the Cry1Ac insert whose target pests include the following lepidopterans: *Alabama argillacea* (Noctuidae, cotton leafworm), *Heliothis virescens* (Noctuidae, tobacco budworm), *Helicoverpa zea* (Noctuidae, corn earworm), *Pectinophora gossypiella* (Gelechiidae, pink bollworm), *Sacadodes pyralis* (Noctuidae, "el gusano rosado colombiano"), *Spodoptera frugiperda* (Noctuidae, fall armyworm), *Trichoplusia* sp. (Noctuidae, looper) and *Bucullatrix* sp. (Lyonetiidae, cotton leaf perforator)

Based on results obtained during the 2001-2002 growing cycle, ICA authorized the first commercial plantings of cotton with resistance to lepidopterans. The department of Córdoba was the first to commercially plant GM cotton with 6,187 ha planted in the second semester of 2003. During the first semester of 2004, 4,495 ha were planted in Tolima-Huila and 696 ha in the Cauca Valley.

Objectives

General Objective: Determine the impact of Bt transgenic cotton on non-target soil arthropods.

Specific Objective

- Evaluate the short-term effect of Bollgard® technology on non-target soil arthropods in field plots.
- Generate information on the species richness of soil arthropods associated with transgenic and non-transgenic cotton in the Cauca Valley.
- Evaluate the long-term effect of Bollgard® technology on non-target soil arthropods in field plots.
- Compare and contrast the non-target effects of traditional chemical and Bt-transgenic plant protection technologies.

Establishment and ex ecution o f the w ork plan: In collaboration with ICA's division of Agricultural Regulation and Protection, we initiated field studies for the first cycle of cotton at the ICA research station in Palmira, located at 03°31'N, 76°19'W, 975 m elevation, annual precipitation 1295 mm, mean temperature 24^{o}C, relative humidity 76%, and corresponding to the Holdridge life zone of Dry Tropical Forest.

The evaluations were conducted within the methodology implemented by ICA to evaluate the effect of Bollgard® technology on arthropod populations in the cotton crop in the departments of Tolima, Huila and Valle del Cauca.

The experimental units were plots measuring 225 m^2 (15 m x 15 m) in a completely randomized block design. Each block had 6 plots for a total of 24 plots under evaluation. Plant material was

(1) Bollgard® technology represented by the variety NuCont 33B that contains the Cry1A(c), and (2) the conventional technology represented by variety DP 5415.

Sampling: Information was gathered from two types of samples: pitfall traps and berlese funnels. Pitfall traps were located between plants within the rows; eight were put out in each experimental plot **(Figure 1)**. A total of 192 pitfall traps were deployed, and these were opened to sampling for a 24-hour period each week.

Field samples were brought to the laboratory for their processing on the same day. Larger arthropods were picked out by hand. To recover the microarthropods, the samples were processed in a small funnel lined with a very fine mesh. The field sample was washed into the funnel with water. By capping the end of the funnel, the sample was floated, and the supernatant removed after discarding the larger debris. Then the remaining precipitate was floated again, this time in 35% salt solution and the supernatant removed. Both supernatant samples were then combined and stored in 70% ethyl alcohol until analysis and identification **(Figure 2)**.

In addition to the pitfall traps, a cup cutter was used to take soil samples every 2 weeks. The cup cutter had a diameter of 10 cm and the sample was taken to a depth of 10 cm in the row between plants **(Figure 2)**. Four samples were taken per plot for a total of 96 samples per evaluation. Samples were placed in berlese funnels for 24 hours after which the samples of separated arthropods were stored in 70% ethyl alcohol until analysis. Because only 48 funnels were available, blocks 1 and 2 were done the first period, followed by blocks 3 and 4 which were maintained at 11^{o}C during the interim 24 hours. Arthropod samples were separated, sorted and processed as in the pitfall trap samples.

Figure 1. (A) Fixed component and removable component and (B) lid of the pitfall traps in the field.

Analysis of information : The statistical model used for the analysis of the data was a completely randomized block design. With this design an ANOVA will be used to determine differences in abundance among treatments and determine the effect of their interactions. In addition, for the most abundant groups we will conduct an analysis of the area under the population curve (accumulated insect-days) to determine differences among treatments during the trial. We will also compare the diversity and abundance among treatments using various indices of taxonomic diversity, dominance and equity.

Figure 2. Field collection of samples for berl ese extraction of arthropods using a "Lever Action Hole Cutter."

Results

Arthropod Taxonomic Composition

Pitfall traps: During the first cycle (2003), 438,934 specimens were captured, belonging to 20 different taxonomic orders in 8 taxonomic classes. Sixty-five different species were identified, with a difference of only three species between those reported in NuCotn 33B and DP 5415 **(Table 2)**. The most abundant class was Collembola with 52.3% of total individuals captured **(Table 1)**.

Table 1. Number of individuals and compositio n of arthropod classes caught in pitfall traps and berlese funnels in cotton, du ring 2003 in the Cauca Valley, Colombia.

Class	Pitfall trap			Berlese funnels		
	NuCotn 33B	DP 5415*	Total	NuCotn 33B	DP 5415*	Total
Aracnida	37,321	38,677	75,998	24,127	28,218	52,345
Chilopoda	6	2	8	36	44	80
Collembola	138,061	91,364	229,425	4,709	4,638	9,347
Diplopoda	17	29	46	14	42	56
Diplura	0	0	0	48	70	118
Insecta	62,666	70,443	133,109	7,679	8,717	16,396
Malocostraca	112	48	160	46	63	109
Nematoda	25	108	133	12	20	32
Oligochaeta	0	1	1	155	215	370
Protura	0	0	0	3	4	7
Symphyla	27	27	54	729	952	1,681
Sum	238,235	200,699	438,934	37,558	42,983	80,541

* 44% of samples evaluated.

Of all individuals captured, 54.3% of those were associated with transgenic cotton and 45.7% with conventional cotton. Of the 20 identified orders, the most abundant were Poduromorpha, Hymenoptera and Acari with 50.4, 29.7 and 17.2%, respectively **(Table 3)**. Abundance, in terms of individuals per order, was 1.2 times greater in NuCotn 33B where only Acarina and Hymenoptera were more abundant in DP 5415 **(Table 4)**. Only Collembola and Isopoda exhibited differences between the treatments, both being more abundant in NuCotn 33B.

Table 2. Number of individuals and compositio n of arthropod orders caught in pitfall traps and berlese funnels in cotton, du ring 2003 in the Cauca Valley, Colombia.

Order	NuCotn 33B		DP 5415	
	Pitfall Traps	Berlese Funnels	Pitfall Traps	Berlese Funnels
Acarina	37,139	24,114	38,497	28,198
araneae	182	13	180	20
Blattaria	27	3	37	9
Chilopoda[1]	6	36	2	44
Coleoptera	257	743	251	768
Dermaptera	3	0	--	1
Diplopoda[1]	17	14	29	42
Diptera	91	224	159	370
Diplura	0	48	0	70
Entomobryomorpha	4,171	829	3,360	928
Hemiptera	61	5	61	7
Homoptera	782	62	878	68
Hymenoptera	61,385	6,311	68,977	7,228
Isopoda	112	46	48	63
Lepidoptera	23	91	31	93
Neuroptera	0	0	2	0
Orthoptera	26	2	38	3
Poduromorpha	133,382	3,880	87,930	3,706
Protura	--	3		4
Symphyla[1]	27	729	27	952
Symphypleona	508	0	74	4
Thysanoptera	9	6	5	2
Unidentified	2	232	4	168
Sum 23	**8,210**	**37,391**	**200,590**	**42,748**

[1] Taxonomic Class (Including for analysis).

***** 44% of samples evaluated.

Given their overall abundance, the class Collembola was examined in more taxonomic detail. Seven families were identified belonging to three suborders **(Table 5)**. Of the springtail orders identified to date, Poduromorpha, Entomobryomorpha and Simphypleona were more abundant in NuCotn 33B.

In the analysis of the area under the population development curve, NuCotn 33B accumulated significantly more area than DP 5415 **(Figure 3)**. Among the Hymenoptera, the most abundant family was Formicidae, with 99.9% of total individuals captured. Overall 52.9% of those specimens were captured from DP 5415; DP 5414 also had significantly more accumulated area than NuCotn 33B **(Figure 4)**. Although Acarina had 1.36 times more individuals captured in NuCotn 33B, no difference was detected in the area under the curve **(Figure 5)**.

Berlese funnels. To date, 44% of the samples collected in the first cycle of cotton (2003) have been evaluated. These number 80,541 specimens representing 11 classes and 21 orders **(Table 1)**. The most abundant classes were Arachnida and Insecta with 65.0 and 20.4% of total specimens, respectively. The most abundant order was Acarina, with 65% of total captures and 1.2 times more abundant in DP 5415.

Table 3. Abundance of arthropods (mean ± S.E. number of individuals caught per evaluation date) associated w ith cotton, during 2003 in the Cauca Valley, Colombia.

Group Nu	Cotn 33B	DP5415
Acarina	22.76±54.60 a	23.59±50.05 a
Araneae	0.11±0.33 a	0.11±0.34 a
Blattaria	0.02±0.14 a	0.02±0.16 a
Chilopoda[1]	0.00±0.06 a	0.00±0.03 a
Coleoptera	0.16±0.48 a	0.15±0.47 a
Dermaptera	0.00±0.04 a	0.00±0.00 a
Diplopoda[1]	0.01±0.10 a	0.02±0.16 a
Diptera	0.06±0.33 a	0.10±1.24 a
Entomobryomorpha	2.56±10.58 a	2.06±8.17 a
Hemiptera	0.04±0.24 a	0.04±0.22 a
Homoptera	0.48±1.53 a	0.54±4.48 a
Hymenoptera	37.61±79.69 a	42.27±82.57 a
Isopoda	0.07±0.50 a	0.03±0.19 b
Lepidoptera	0.01±0.18 a	0.02±0.15 a
Neuroptera	0	0.00±0.05 a
Orthoptera	0.02±0.13 a	0.02±017 a
Poduromorpha	81.73±436.77 a	53.88±333.46 b
Symphyla	0.02±0.15 a	0.02±0.15 a
Symphypleona	0.31±3.80 a	0.05±0.30 b
Unidentified	0.00±0.03 a	0.00±0.03 a

[1] Taxonomic Class (Including for analysis).

For each row, means followed by different letters are statistically different at P<0.05 (Tukey-Kramer test for multiple comparisons).

Arthropod Taxonomic Diversity: The species richness and Shannon indices were not significantly different between the treatments NuCotn 33B and DP 5415. The Simpson index showed dominance for one species, presenting values of 0.6 and 0.7 for NuCotn 33B and DP 5415, respectively. The Margalef index was 4.8 for NuCotn 33B and 4.6 for DP 5415. In comparing the similarity (Jaccard, Sorenson and Morisita) between NuCotn 33B and DP 5414 in terms of the identified species, values ranged from 0.8 for Jaccard to 0.97 for Morisita.

Table 4. Collembola families collected fr om pitfall traps during 2003 in the Cauca Valley, Colombia.

Order Family		Genus
Poduromorpha	Hypogasturidae	*Ceratophysella*
	Brachystomellidae	*Brachystomella**
	Neanuridae	*Arlesia*
	Cyphoderidae	*Cyphoderus***
Entomobryomorpha	Entomobryidae	*Seira, Lepidocyrtus*
	Isotomidae	*Isotoma, Proisotoma, Folsomides*
	Paronellidae	*Paronella, Salina*
Symphypleona	Dicyrtomidae	*Calvatomina*

* Only on the soil surface (pitfall traps).

** Only in soil (soil cores).

Table 5. Indices of arthropod taxonomic (o rdinal level), diversity, domin ance and equity in cotton, during 2003 in the Cauca Valley, Colombia.

Index	NuCotn 33B	DP 5415
Species richness (S)	14.1 a	14.2 a
Shannon diversity index	1.0 a	1.0 a
Margalef diversity index	1.3 a	1.4 a
Simpson diversity index	0.6 a	0.6 a
Simpson dominance index	0.4 a	0.4 a
Equity index	0.4 a	0.4 a

For each row, means followed by different letters are statistically different at $P<0.05$ (Tukey-Kramer test for multiple comparisons)

Conclusions

- These studies have identified a high abundance and diversity of soil-active and surface-active fauna associated with the cotton crop under the conditions of the Cauca Valley, Colombia.
- Pitfall traps are an appropriate method for measuring the abundance of surface-active arthropods and comparing their activity and diversity across treatments.
- Extracting soil cores with berlese funnels is an adequate method for measuring the abundance of soil-active arthropods and comparing their activity and diversity across treatments.
- The various indices of taxonomic diversity, richness, dominance and equity are useful tools for comparing ecological communities and will allow us to make long-term comparison of the effects of different plant protection technologies under the conditions of the Cauca Valley, Colombia.
- The abundance differences observed between treatments in the first cycle of cotton should be studied in more detail to define how GMOs affect those differences. The protocols established in the first cycle will therefore be implemented in two additional cycles to better describe abundance effects over time, and to gather information to compare differences in species composition of key groups such as the springtails.
- Although abundance and diversity differences may exist in response to GMO technology, it is important to determine whether the magnitude of those differences is ecologically relevant, i.e. have an effect on ecological function or overall soil health.

Recommendation

It is a recommendation of this research group that this information be used in the best possible way to benefit the general community. We highlight that this information is preliminary and should be interpreted as such, keeping in mind that this information.

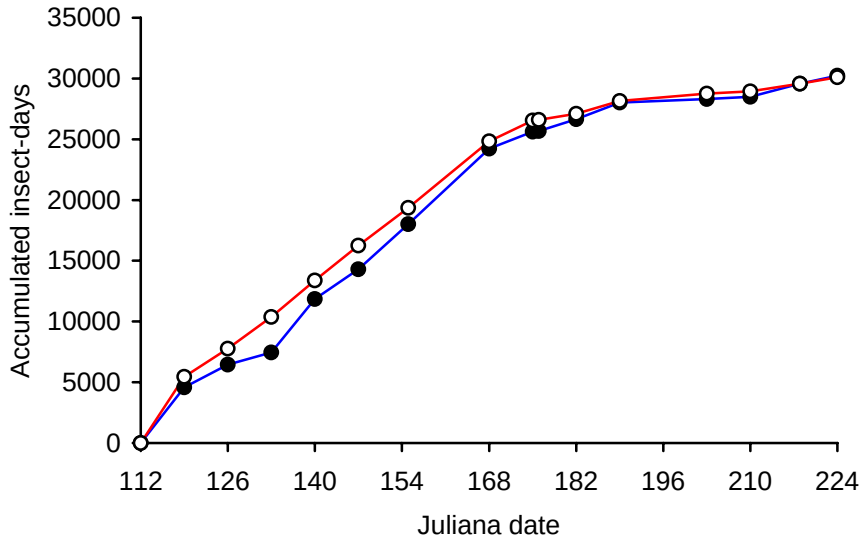

Figure 3. Area under the abundance curve fo r Acarina in Cotton, 2003 in the Cauca Valley, Colombia.

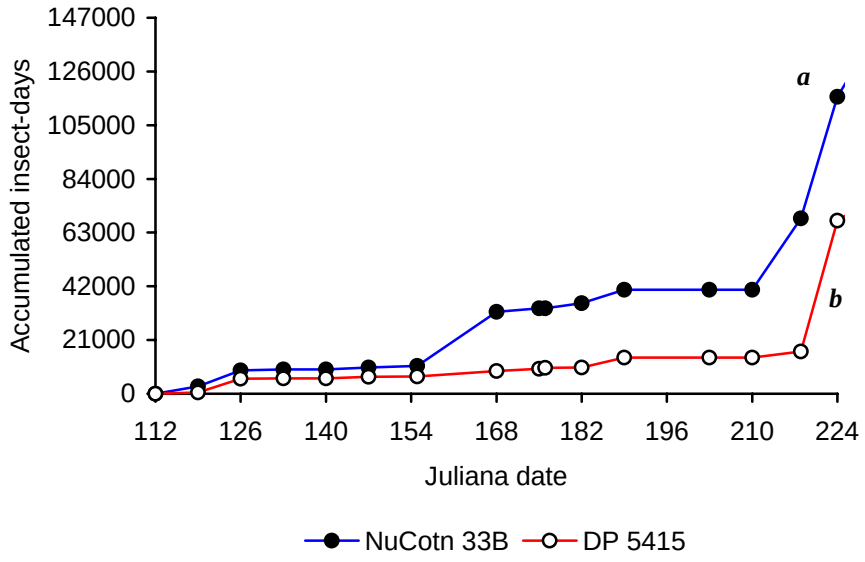

Figure 4. Area under the abundance curve fo r Collembola in cotton, 2003 in the Cauca Valley, Colombia.

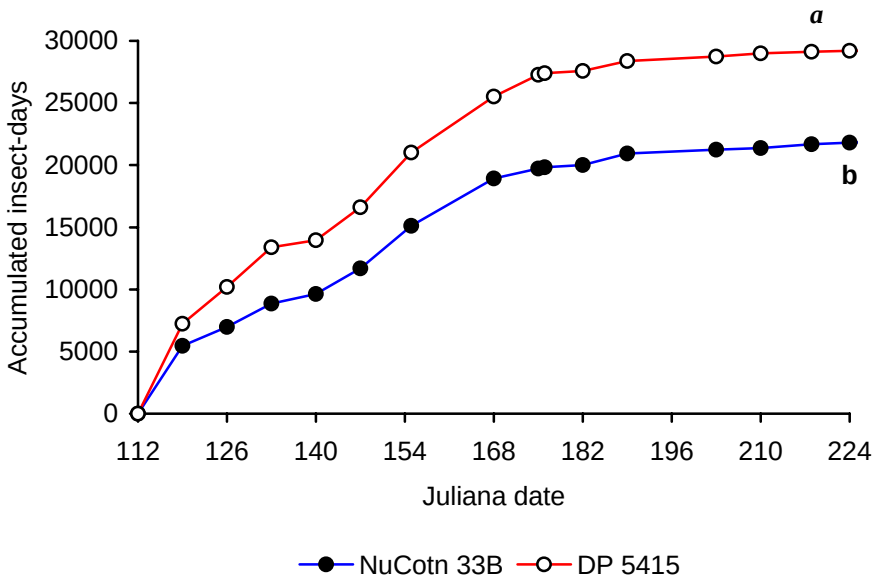

Figure 5. Area under the abundance curve for Hymenoptera in cotton, 2003 and in the Cauca Valley, Colombia.

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Continued

Activity 3. Taxonomy of the Springtails (Collembola) Associated with Cotton and Maize of the Cauca Valley, Colombia.

Introduction

The entomofauna associated with crops plays an important role in soil quality and consequently in crop yield. Agricultural practices can affect the diversity and abundance of groups of non-target organisms, among which we highlight the class Collembola, or springtails. These are small (250 microns to 10 mm in length) wingless arthropods that are similar to insects. Springtails are entognathous, have a postantenal organ, a maximum of 8 ocelli on each side of the head (8+8) or completely lack visual organs, and possess 4 antennal segments sometimes subdivided. The thorax has three segments, the first reduced in some groups; the legs have basically four segments. The abdomen has three segments on which specialized appendages are located on segment 1 (collophore), 3 (retinaculum) and 4 (furcula); the genital opening is located on segment 5. Developmental instars have gradual metamorphosis (ametabolous) (Greenslade 1991).

Springtails are ecologically important because of their influence in improving soil structure and accelerating the decomposition of animal and plant material. Most soils contain millions of springtail feces that can be beneficial in slowing the liberation of nutrients essential for plant roots. They also serve as substrate for a large number of microorganisms, stimulating the activity of fungi and bacteria, accelerating the processes of decomposition, and indirectly improving the structure, absorption capacity and fixation of interchangeable bases of the soil (Villalobos 1990; Cutz Pool 2002). On the other hand, springtails are the prey of many arthropods, particularly ants, beetles and predaceous mites, and thereby form a fundamental element of trophic interactions (Palacios-Vargas 2000).

Given the high abundance and diversity of organisms associated with the soil of agroecosystems, it is important to begin to understand the patterns of diversity and ecological function of groups like the Collembola. In Colombia, a study was conducted by the Agronomy Department of the National University of Colombia in Bogotá (Entomological Museum, UNAB) on the families of springtails associated with forage crops in the Department of Antioquia. This study identified 14 springtail families, seven of which constituted first reports of those families in Colombia (Ospina *et al.* 2003). This work was conducted as part of a broader project ("Collembola (springtails) of Colombia") initiated to make a preliminary inventory of the springtail fauna present in Colombia.

This technical report summarizes activities related to the separation, mounting and identification of springtails collected as part of a series of studies conducted in the Cauca Valley as part of the project "Evaluating the Impact of Biotechnology on Biodiversity." The first subproject focused on maize, and was designed to gauge the effect of chlorpyrifos on non-target soil fauna over two consecutive growing cycles (2002B and 2003A). The second subproject focused on cotton, designed to compare the non-target effects of conventional and Bt-transgenic plant protection strategies. Springtails were a dominant fauna collected in the course of these studies. In order to shed higher resolution of the response of this group to the experimental treatments, the specimens collected are being identified to better understand the diversity and function of this fauna in cotton and maize crops of the Cauca Valley.

Objectives

General Objective: Describe the springtail fauna associated with the soil of maize and cotton crops in the Cauca Valley.

Specific Objectives

- Identify the taxonomic families of springtails associated with maize and cotton crops.
- Compare the composition of the springtail fauna present in conventional cotton with that of Bt-transgenic cotton.
- Compare the composition of the springtail fauna captured in pitfall traps (surface-active) and soil cores extracted with Berlese funnels (soil-active).

Establishment and execution of the work plan: This work was based on specimens collected from two localities: (1) the CIAT experimental station, Palmira, located at 3º31'N, 76º21'W with an elevation of 965 m, mean annual temperature of 24ºC and (2) the ICA experimental station, Palmira, located at 3º31'N, 76º19'W with an elevation of 1295 m, mean annual precipitation of 24ºC and relative humidity 76%. Both localities correspond to the Holdrige classification of Dry Tropical Forest. At CIAT, specimens were collected as part of the activities of the project "Response of Non-Target Soil Arthropods to Chlorpyrifos in Colombian Maize", where the two treatments of maize with and without insecticide were evaluated over two consecutive cropping cycles (Subproject 1). At ICA, specimens were collected as part of the activities of the project "Effect of Transgenic Cotton [Bollgard® Bt Cry1A©] on Non-Target Soil Arthropods in the Cauca Valley of Colombia" where two treatments of conventional cotton (DP 5415) and Bt-transgenic Bollgard® cotton (NuCotn 33B) were evaluated (Subproject 2).

Springtails were separated from the original samples of arthropods that had been collected from maize and cotton and stored in 70% ethyl alcohol. While the springtails had already been separated from the other arthropods in the maize samples, they had to first be separated from the mixed arthropod samples collected from the cotton plots. This was performed by examining the samples under a stereoscope, separating all springtails, and returning the remaining arthropods to their original specimen vials.

Preparation and processing of springtail specimens: Springtails were initially sorted into morphospecies, taking into account the form of the body and the presence or absence of scales. These morphospecies were separated into different vials. In order to prepare the specimens for identification, they were fixed by clearing for 5 min in 10% KOH in individual glass wells, followed by submersion in lactophenol. The time in lactophenol varied depending on the size and pigmentation of the specimen; heat was used to accelerate the process as needed. In order to avoid the formation of crystals in the final fixing phase, the specimens were rinsed with Hoyer's solution to totally remove any reactives adhering to the body.

The fixed mounting was done under a stereoscope where dissecting pins were used to arrange the specimen in a drop of Hoyer's solution. The cover slip was placed on top with care to avoid forming bubbles. Finally, the slide was labeled, placed on a warming plate at 45-50ºC for a period of 4 days, after which the slide was examined to confirm that the Hoyer's solution had hardened. Once drying was finished, the excess Hoyer's solution was scraped off and the edges

of the cover slip were sealed with varnish. Finished mounts were stored in slide cases until identification of the specimen.

Identification of springtail specimens: Once the specimens were mounted, they were identified to family using the taxonomic keys of Palacios-Vargas (1990, 1991), Greenslade (1991), Jaensen (2001) and Ospina *et al.* (2003). Identification of genera was done in collaboration with Dr. José G. Palacios-Vargas, springtail specialist in Mexico, using the keys of Jaensen (2002) and Cristiensen and Bellinger (1980a, 1980b, 1980c, 1981). Finally, we (C. Ospina) developed a taxonomic key to the families of springtails associated with maize and cotton crops of the Cauca Valley based on the specimens mounted as part of this study (Appendix 1).

The reference collection produced from this work is housed at CIAT, in the office of the research group "Evaluating the impact of biotechnology on biodiversity." Voucher specimens will also be housed in the collaborating institutions of the Colombian National University in Bogotá (entomological museum UNAB) and Cornell University (CU Insect Collection).

Analysis of information: The information obtained in these studies will be analyzed with the indices of similarity of Jaccard and Sorensen appropriate for qualitative data. The degree of similarity will be determined and compared between crop type (maize vs. cotton) and sampling method (pitfall vs. soil cores extracted with Berlese funnels) at the level of family and genus.

Results

Springtails in maiz e: Over the two semesters of evaluation, a total of 5,444 specimens were captured from the class Collembola. Of those, 62.5% were captured in the insecticide treatment and 87.0% were captured during the first growing cycle (2002B).

The identified springtails belonged to three orders and six families, each of which has been previously documented in Colombia **(Table 1)**. The most abundant order was Poduromorpha with 27.3 and 5.6 times more individuals than the orders Symphypleona and Entomobryomorpha, respectively. In terms of abundance, no differences were detected between treatments at the level of order. There were statistically significant differences between semesters for each order. While Entomobryomorpha was more abundant in the second semester (2003A), the other two orders were more abundant in 2002B (Subproject 1)

Springtails in cotton: During the cropping cycle of cotton (2003), 229,425 specimens from the class Collembola were captured in pitfall traps, and 9,347 in soil samples (only partially analyzed to date). Of total captures, 59.8% were captured in Bt-transgenic cotton (Pitfall and Berlese), with 60.0% belonging to Poduroporpha. Both Poduromorpha and Symphypleona exhibited differences between treatments, being significantly more abundant in the Bt-transgenic plots (Subproject 2).

Eight families were identified to date. Of those, seven were detected in pitfall traps, and of those six were common to both treatments. The family Neanuridae was only detected in the conventional plots, and also only in pitfall traps **(Table 2)**. Six families were detected in soil samples and of those five were common to both treatments. The family Dicyrtomidae was only

detected in the conventional plots. In addition the family Cyphoderidae was only detected in soil samples (in both treatments).

Table 1. Families a nd genera of springtails asso ciated w ith maiz e at t he CIAT experimental station, Palmira, Colombia.

Family	Genus
Hypogastruridae	*Ceratophysella*
Isotomidae	*Isotoma*
Entomobryidae	*Seira*
	Entomobrya
Sminthurididae	*Sphaeridia*
	Denisiella
Bourletiellidae	*Deutosminthurus*

Of the 13 genera identified, eight were common to both treatments and collection techniques. The genera *Brachystomella* and *Arlesia* were only detected in pitfall traps (in both treatments), while the genus *Chypoderus* was only detected in soil samples (in both treatments). The genus *Salina* was only detected in the conventional treatment (in both berlese and soil samples)

Table 2. Families and genera of surface active (pitfall traps) and soil active (soil samples processed in Berlese funnels) springtail s associated w ith cotton at the ICA experimental station, Palmira, Colombia.

		Pitfall		Berlese	
Family	**Genus**	**NuCotn 33B**	**DP 5415**	**NuCotn 33B**	**DP 5415**
Hypogastruridae	*Ceratophysella*	1	1	1	1
Brachystomellidae	*Brachystomella*	1	1	0	0
Neanuridae	*Arlesia*	0	1	0	0
Isotomidae	*Isotoma*	1	1	1	1
	Proisotoma	1	1	1	1
	Folsomides msp 1	1	1	1	1
	Folsomides msp 2	1	1	1	1
Entomobryidae	*Seira*	1	1	1	1
	Lepidocyrtus	1	1	1	1
Paronellidae	*Paronella*	1	1	1	1
	Salina	1	1	0	1
Cyphoderidae	*Cyphoderus*	0	0	1	1
Dicyrtomidae	*Calvatomina*	1	1	0	1

(1) Present; (0) Absent.

Arthropod Taxonomic Diversity: In terms of similarity in families between maize and cotton, for pitfall traps the Jaccard and Sorensen indices were 0.33 and 0.50, respectively, in other words 41.5% of the families captured in pitfall traps were common to both systems. In comparing collection techniques within the cotton crop, the similarity indices were 0.75 and 0.85 for Jaccard and Sorensen, respectively, i.e. 80% of families were sampled by both pitfalls and soil cores.

In terms of similarity in genera between maize and cotton, for pitfall traps the Jaccard and Sorensen indices were 0.19 and 0.32, respectively, or 25.5% overlap in genera between systems for pitfall traps. In comparing collection techniques within the cotton crop, the similarity indices were 0.85 and 0.92 for Jaccard and Sorensen, respectively, i.e. 88.5% of families were sampled by both pitfalls and soil cores **(Table 2)**.

A general view of the identified specimens is shown in **Figures 1-10**. These photos are of specimens in the reference collection mounted by C. Ospina and photographed by C. Olaya (CIAT).

Conclusions

- This report on the taxonomic diversity of springtails associated with maize and cotton crops is the first of its kind for any agricultural production system (other than forage crops) in Colombia.
- Of the genera identified to date, only the detection of *Brachystomella* and *Cyphoderus* depended on collection method; captures of these genera were limited to pitfall and soil cores, respectively.
- The similarity in family and genus composition of springtail fauna was low in comparing crop (maize and cotton), but high in comparing collection method (pitfall and soil core in cotton).
- For the first time, a key to the families of springtails associated with maize and cotton crops in the Cauca Valley of Colombia has been made developed, complemented with photos of select morphospecies.

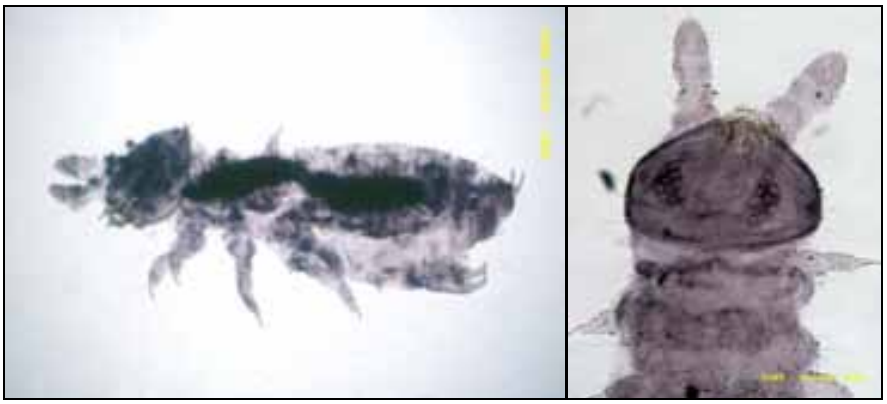

Figure 1. General habitus of *Ceratophysella* (Hypogastruridae), detail of the head, showing the 8 ocelli on each side of the postantenal organ (OPA), as well as the mouthparts formed from the mandibles and maxillae. Species present in maize and cotton.

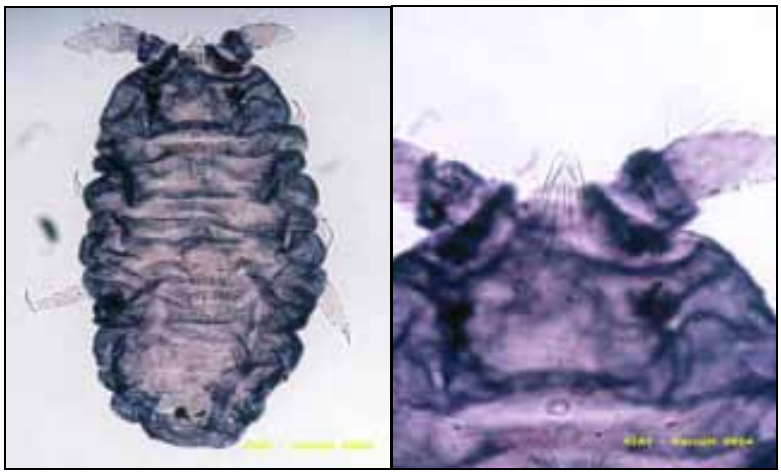

Figure 2. **General habitus of *Arlesia* (Neanuridae), detail of the stiletiform mouthparts. Species present in cotton.**

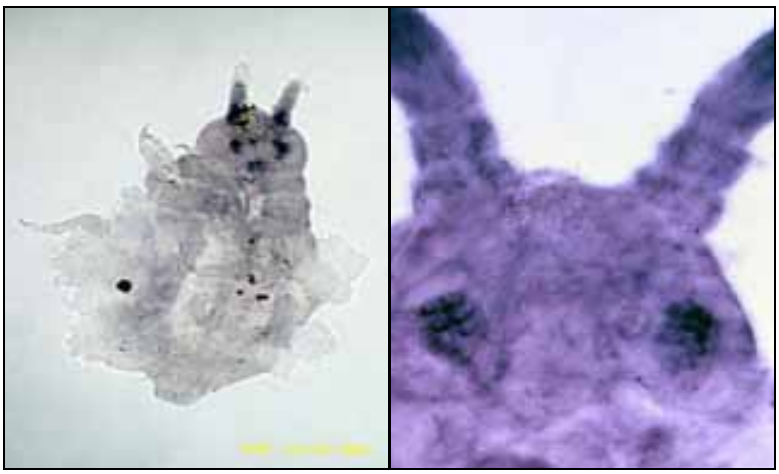

Figure 3. **General habitus of *Brachystomella* (Brachystomellidae), detail of the quadrangular mouthparts. Specimens collected in cotton.**

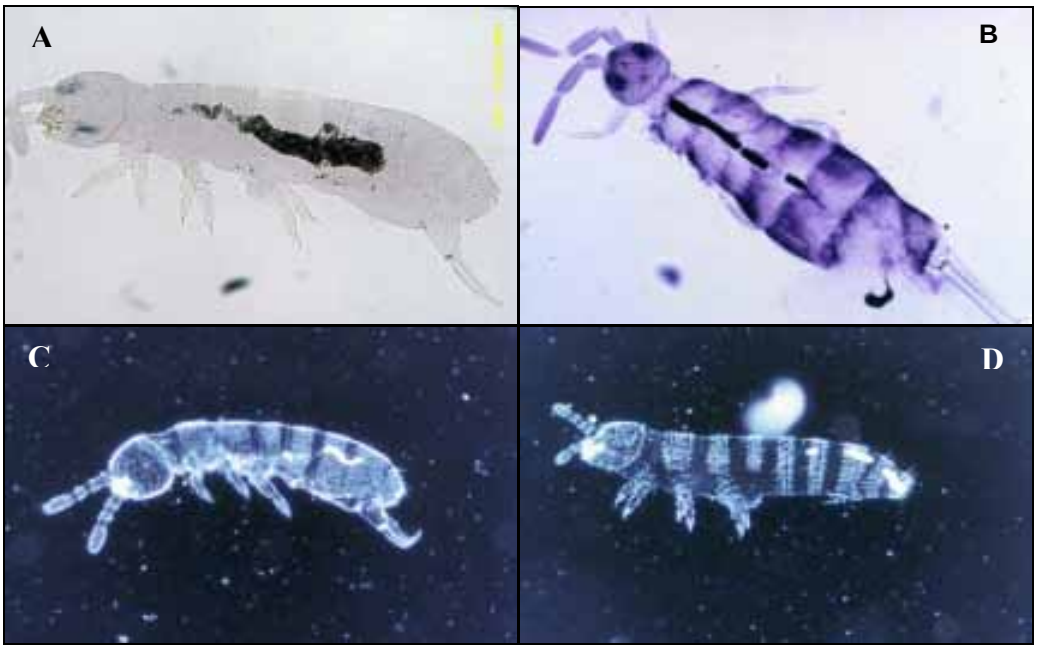

Figure 4. **General view of the genera of Isotomidae: (A) *Isotoma* (specimen from maize), (B) *Proisotoma,*(C) *Folsomides* sp. 1 and (D) *Folsomides* sp. 2 (specimens from cotton).**

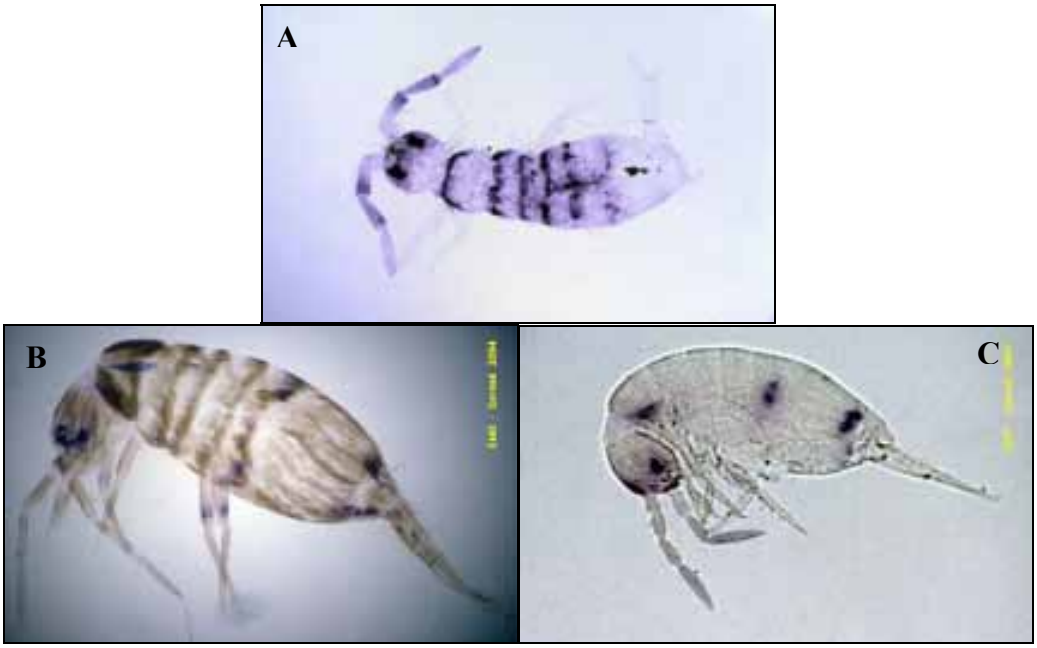

Figure 5. **Habitus of the gene ra of Entomobryidae: (A) *Entomobrya* (speci men fro m maize), (B) *Seira* and (C) *Lepidocyrtus* (specimens from cotton).**

Figure 6. **Habitus of the genera of Paronellidae: (A) *Paronella* and (B) *Salina*, collected from cotton.**

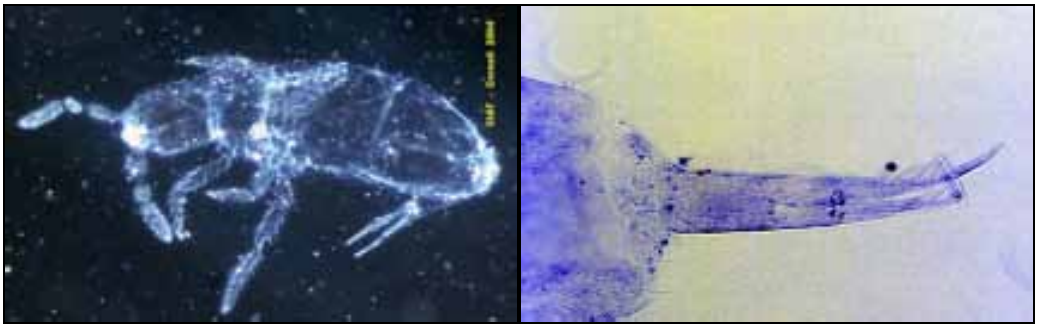

Figure 7. **General habitus of *Cyphoderus* (Cyphoderidae) with detail of the furcula, found in a sample of soil from cotton.**

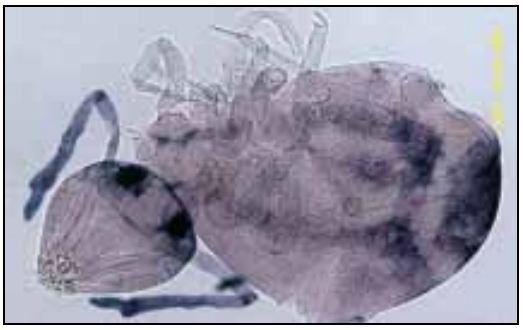

Figure 8. **General habitus of *Calvatomina* (Dicyrtomidae), specimen from maize.**

Figure 9. **General habitus of *Sphaeridia* (S minthurididae), (A) male and (B) female, specimens collected in maize.**

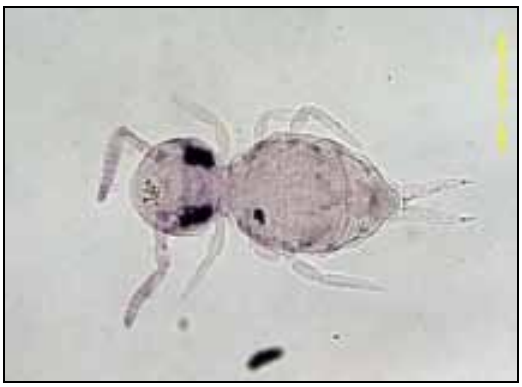

Figure 10. General view of Deutosmin thurus (Bourletiellidae), s pecimen c ollected in maize.

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Contributors: Claudia M. Ospina**,** Jairo Rodríguez Chalarca, Mariluz Mojocoa, and Daniel C. Peck.

APPENDIX 1

KEY TO IDENTIFICATION OF THE FAMI LIES OF SPIRNG TAILS AS SOCIATED WITH THE MAIZE AND COTTON CROPS IN THE CAUCA VALLE, COLOMBIA.

1. Body elongate (Figure 1) never globular; thorax and first four abdominal segments not fused; furcula present, well developed…… .....….**2**

1’ Body globular (Figure 2); at least the first four abdominal segments fused; furcula always well developed…….....…. **8**

2. Prothorax well developed (Figure 1), with dorsal setae; furcula not well developed……..... .....**3**

2’. Prothorax reduced (Figure 3), without dorsal setae; furcula frequently well developed..... .....**5**

3. With chewing mouthparts; mandibles with molar surface (Figure 4) ..... **HYPOGASTRURIDAE**

3’ With modified mouthparts.....……..... .....**4**

4. Mandibles and maxillae present; maxillae stylifrom (Figure 5).....**NEUANURIDAE***

4’. Mandibles absent; maxillae square and usually with teeth (Figure 6) ....**BRACHYSTOMELLIDAE***

5. Body segments similar length (Figure 7); post-antennal organ (PAO) simple (Figure 8) ....**ISOTOMIDAE**

5’ IV Abdominal segment elongated (alargado) (Figure 3), PAO absent.....**6**

6. Dens spined or toothed; mucro square, much shorter than the dens (Figure 9) ....**PARONELLIDAE***

6’. Dens spineless and toothless.....….....**7**

7. Dens crenulate; mucro short, hook like with 1 or 2 teeth (Figure 10)..... .....**ENTOMOBRYIDAE**

7’. Dens smooth; mucro elongate with variable number of teeth .....**CYPHODERIDAE***

8. Antennae elbowed between segments II y III, segment IV much shorter than III (Figure 12).....…… .....….....**DICYRTOMIDAE***

8’. Antennae elbowed between segments III y IV, (Figure 13), segment IV longer that III.....….....**9**

9. Abdominal segments V and VI fused , males with prehensile antennae (Figure 14), female lacking anal appendages..…… ..... ….....**SMINTHURIDIDAE****

9’ Abdominal segments V and VI separate, males with simple antennae; female with anal appendages (Figure 15); mucro spatulate (Figure 16) ..... .**BOURLETIELLIDAE****

* Only family in cotton.

** Only family in maize.

FIGURES

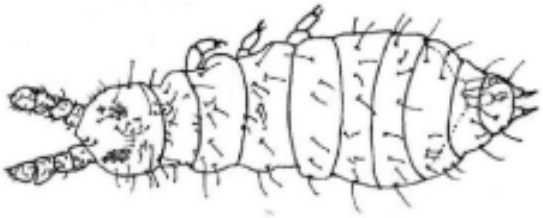

Figure 1. Habitus of Hypogastruridae.

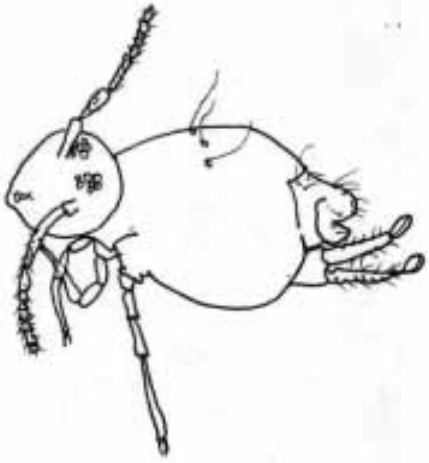

Figure 2. Habitus of Bourletiellidae.

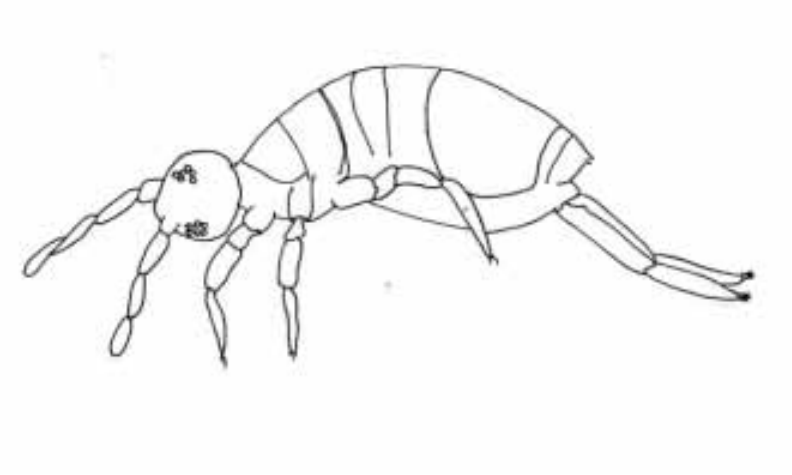

Figure 3. Habitus de Paronellidae.

Figure 4. Mandible, *Ceratophysella.*

Figure 5. Mandibles and maxillae modified, Neanuridae.

Figure 6. Maxillae of *Brachystomella.*

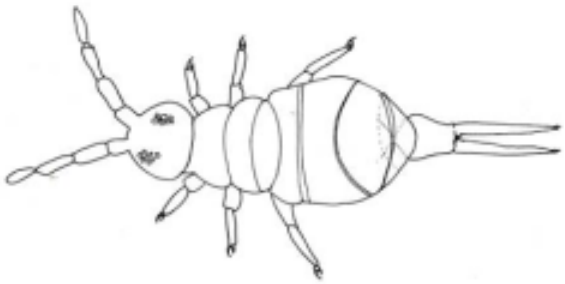

Figure 7. Habitus of *Isotoma.*

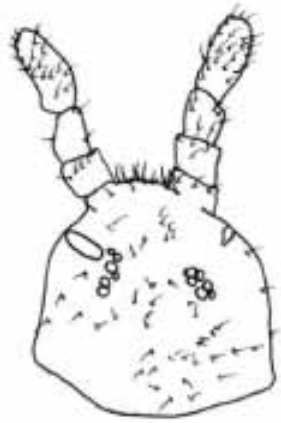

Figure 8. OPA of *Folsomides.*

Figure 9. Furcula of *Paronella.*

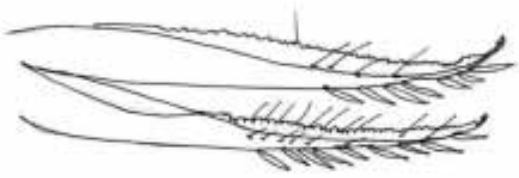

Figure 10. Furcula of *Lepidocyrtus.*

Figure 11. Furcula of *Cyphoderus.*

Figure 12. Head of *Dicytoma.*

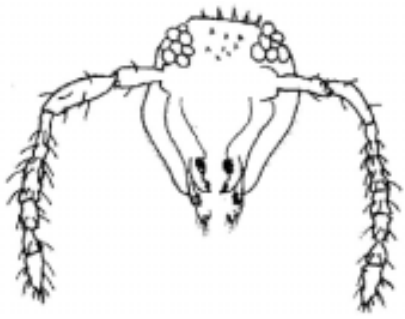

Figure 13. Head of Sminthuridae Ospina *et al* 2003).

Figure 14. Antennae of *Sphaeridia* (Macho) (Ospina *et al* 2003).

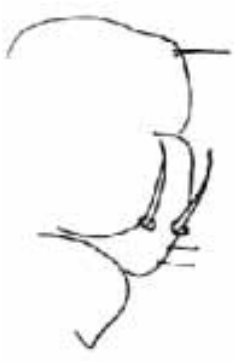

Figure 15. Anal appendages of *Deutosminthurus.*

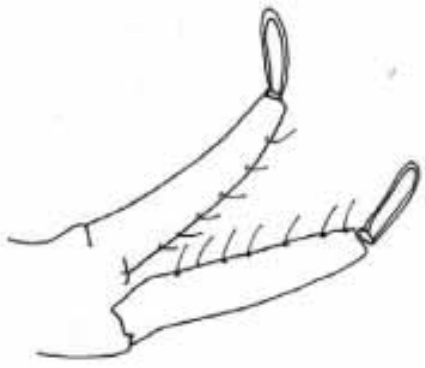

Figure 16. Mucro of Bourletiellidae.

Activity 4. Publications, posters, conferences, training and consultancies.

Publications

Rodríguez, Ch.J.; Castro, U.; Morales, A.; Peck, D.C. 2003. Biología del salivazo *Prosapia simulans* (Walker) (Homoptera: Cercopidae), nueva plaga de gramineas cultivadas en Colombia, Revista Colombiana de Entomología, 29(2):149-155.

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Posters

Rodríguez, Ch.J.; Peck, D.C. 2004. Gauging the effect of transgenic maize and cotton on non-target soil arthropods in Colombia. 8th International Symposium on the Biosafety of Genetically Modified Organisms. September 26-30, Montpellier, France.

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Rodríguez, Ch.J.; Peck, D.C. 2004. Gauging the effect of transgenic maize and cotton on non-target soil arthropods in Colombia. 8th International Symposium on the Biosafety of Genetically Modified Organisms (September 26-30, Montpellier, France). p. 102-104.

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Gauging the effect of transgenic maize and cotton on non-target soil arthropods in Colombia

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INTRODUCTION

[illegible]

OBJECTIVE

[illegible]

MATERIALS AND METHODS

[illegible]

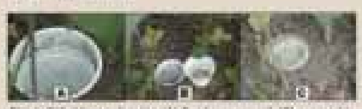

Fig. 1. Pitfall traps showing (A) fixed component, (B) removable component and (C) lid.

[illegible]

Fig. 2. Field collection of samples for berlese extraction of arthropods (A) cup cutter, (B) soil sample, (C) berlese funnels.

[illegible]

RESULTS

[illegible]

Table 1. Number of individuals and composition of invertebrate classes caught in pitfall traps in maize (2002-2003) and cotton (2003).

[illegible]

Table 2. Abundance of invertebrate orders (mean number of individuals captured per evaluation date) in maize (2002-2003) and cotton (2003).

[illegible]

[illegible]

Table 3. Number of individuals and composition of invertebrate classes extracted from soil cores in cotton (2003).

Taxonomic class	NuCotn 33B	DP 5415	Total
Arachnida	24,127	28,218	52,345
Chilopoda	36	44	80
Collembola	4,709	4,638	9,347
Diplopoda	14	42	56
Diplura	48	70	118
Insecta	7,679	8,717	16,396
Malacostraca	46	63	109
Nematoda	12	20	32
Oligochaeta	155	215	370
Protura	3	4	7
Symphyla	729	952	1,681
Total	37,598	42,983	80,541

CONCLUSIONS

[illegible]

REFERENCES

[illegible]

ACKNOWLEDGMENTS

[illegible]

8th International Symposium on the Biosafety of Genetically Modified Organisms (September 26-30, 2004, Montpellier, France)

Training Received

Visiting Scholar in the Department of Entomology. Jairo Rodríguez, IPM Project. NYSAES, Cornell University, 15 Sep.-15 Dec., 2003.

Workshop on Gene Flow Analysis and Environmental Briosafety. Jairo Rodríguez, IPM Project. CIAT, Cali, Colombia, 7-9 Oct. 2004.

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SOIL PESTS – CASSAVA AND OTHER CROPS

Introduction

Whitegrubs of the family Melolonthidae and the burrower bug (*Cyrtomenus bergi*) (BB) belong to the most important soil pests in South America. Until about 20 years they were not considered as pests, however, in the recent past they have caused considerable yield losses on many crops that show the necessity of developing efficient and environmentally sound strategies of an integrated control of these insects.

This project is divided in two phases: the diagnostic and strategic research. The first phase is almost coming to its end and has generated important results: we have identified the key pest species in the Savanna of Bogotá, East and North Antioquia, Northern Cauca, Risaralda, and Quindío and we understand their seasonality in Northern Cauca, Risaralda, and Cundinamarca. We also have identified many natural enemies of the most noxious whitegrub species and the BB. Moreover, we developed a communication network on Internet base to facilitate the information exchange of researchers on specialists of the soil pest community (CIAT 2003). In the project's second phase or strategic research these findings will be verified and applied in on farm experiments.

The objectives of the present study were

I. Diagnostic of pest species and understand the life cycle of key pest species in Antioquia and Quindío
II. To identify biological control agents of white grubs and burrower bug
III. To develop biological control tactics against white grubs
IV. To develop biological control tactics against *C. bergi*

References

CIAT. 2003. *Annual Report*. Soil Pests – Cassava and other crops. Pp 53-70.

Activity 1. Identification of key pest species in three regions of Antioquia (Colombia).

Introduction

Key pest species have been collected and identified in the survey's areas of Cauca, Quindío, Risaralda, and Cundinamarca (CIAT, 2003), and presently field experiments are being carried out in such areas. The data of these surveys will be presented by the end of this year.

In Antioquia, the quantity of collected individuals is enormous (CIAT, 2003). Hence, we continued with systematic surveys including the seasonality of the beetles and larvae. The activities in Antioquia are conducted with the collaboration of Corpoica Rionegro ("La Selva"); ICA's program "Epidemiology in Agriculture" in Medellin; the National University Medellin (unit of Postgraduate Studies in Entomology), and the Umata of the municipalities Rionegro, San Vicente, El Carmen de Viboral, and La Union.

Methodology: In Antioquia, we surveyed three zones: The two cold savannas in the North and the East (both 2600-2800 m. a. l. s.), and the moderate cold zone in the East (2100-2400 m.a.l.s). Beetles were collected in a weekly basis from December 2002 to December 2003) and monthly in the case of whitegrubs (in the North where *Ancognatha* dominates we collected all over the year and in the other zones where *Phyllophaga* spp. dominate from August to November) and their natural enemies. We focused our surveys on pasture and potato fields. We installed six light traps in Rionegro (2110 m. a. s. l.), El Carmen de Viboral (2258 m. a. l. s.), San Vicente (2300 m. a. s. l., La Union (2460 m. a. s. l.), Santa Rosa (2486 m. a. s. l.), and Entrerrios (2437 m. a. s. l.). Extensionists of the Umata and in some cases farmers collected and prepared the insects for storage. The collected material was transferred to the laboratory of Corpoica for identification (CIAT, 2003). We have revised approximately 190.000 beetles until December 2003.

Results and Discussion: The collections of adults in the three zones revealed some major peaks during the months of March and June. In the North of Antioquia we captured the greatest number of individuals. Here, the subfamily Dynastinae dominated with 92,589 specimens, followed by Melolonthinae with 669 and a minimal presence of Rutelinae. The collected genera were (order from highest to lowest abundance): *Ancognatha, Cyclocephala, Heterogomphus* (all Dynastinae), *Astaena* (Melolonthidae), *Golofa* (Dynastinae), *Megaceras*, *Anomala* (both Rutelinae) and *Plectris* (Melolonthinae). We collected 61,103 beetles of *Ancognatha* in Santa Rosa and 12,725 in Entrerrios, followed by *Cyclocephala* with 7,731 and 10,473 individuals, respectively. Among the phytophagous genera *Astaena* dominated with 276 and 417 captures specimens, respectively.

In La Union, municipality in the cold zone of Eastern Antioquia, we captured during February and November 27,366 beetles of Dynastinae, followed by Melolonthinae (49) and Rutelinae (2). The captured genera were *Ancognatha, Astaena, Golofa, Cyclocephala, Heterogomphus, Plectris, Anomala,* and *Isonychus*. The dominance of *Ancognatha scarabaeoides* (27,187 specimens) was again overwhelming, followed by *Ancognatha vulgaris* (139) and *Astaena* (45).

In the moderate cold zone of Eastern Antioquia (Rionegro, San Vicente, and El Carmen de Viboral) 65% of the captured adults were Dynastinae, 33% Melolonthinae, and 2% Rutelinae. The captured genera were *Ancognatha, Cyclocephala, Phyllophaga, Isonychus, Astaena,*

Plectris, *Anomala*, *Golofa*, *Heterogomphus*, and *Macrodactylus*. The presence of the phytophagous genera was surprisingly high in this zone.

It was striking that a small difference of about 150 meters of altitude rigidly changed the species complex. In the lower zone of Rionegro (2100 m. a. s. l.) Melolonthidae (*Phyllophaga*, *Plectris*, *Astaena*) was the dominant subfamily, whereas El Carmen de Viboral (2258 m. a. s. l.) Dynastinae (*Ancognatha* and *Cyclocephala*) was the most present group.

Comparing the peaks of seasonality of the beetles with precipitation it was surprising that the population of *A. scarabaeoides* was high during the whole year without pronounced peaks. In contrast, *Cyclocephala sexpunctata* and *Astaena* performed their only peak in April.

In the cold zone of East Antioquia *A. scarabaeoides* performed a similar seasonality as in North Antioquia. In opposite, *A. vulgaris* showed two peaks in April and November, however, population was extremely low compared to *A. scarabaeoides*. *Astaena* has as in the north a pronounced peak in April.

Peaks of flight activity were recorded for *Phyllophaga obsoleta*, *Plectris* sp., and *Cyclocephala sexpunctata* in April in the moderate cold zone, whereas *Astaena* sp. presented a peak in May and *A. scarabaeoides* in June. We captured the greatest number of adults of *Isonycus* sp. in March, a second peak followed in September.

The studies on the taxonomy of larvae are based on three big surveys where they have been individualized for rearing and characterization of their morphology. Additionally, we have successfully recollected some adults in order to obtain a progeny for later description and drawings.

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Contributors: Martha Londoño, Diana Acevedo, Luis Carlos Pardo, Andreas Gaigl.

Activity 2. Development time studies on key pest species under controlled conditions.

Introduction

Although the whitegrubs *Phyllophaga* sp., *P. menetriesi* (subfamily Melolontinae), and *Anomala inconstans* (subfamily Rutelinae) belong to the economically most important pest species (particularly *Phyllophaga menetriesi*) little is known about their biology and behavior. In most of the cases farmers only react when damage has already occurred. The control of the larvae is difficult and expensive. It is necessary to understand the biology of these whitegrubs to be able to develop a successful integrated control.

Methodology: We selected three species of whitegrubs (Melolonthidae) for these studies: *Phyllophaga* sp., *P. menetriesi*, and *Anomala inconstans*. All of them are considered as important soil pests (Morón 1994). Adults were captured by black light traps installed in Pescador (1500 m. a. l. s. Cauca) and Calucé (1637 m. a. s. l., Valle del Cauca). We filled the containers of the traps with sawdust in order to maintain captured adults alive. The captured specimens were transferred to CIAT. The specimens were stored at a temperature of 21 °C and a relative humidity of 70%. Seven pairs of adults were confined in plastic buckets equipped with a transparent plastic cylinder of 40 cm height to allow the beetles to fly. The bucket was filled with a mixture of sterile soil and sand (3:1 soil:sand). Larvae were fed with carrot slices. Eggs were removed once a week. After hatching larvae were individualized. We recorded the day of moulting, and measured the size of the cephalic capsule, diameter and length of larvae during their development. In order to define the duration of each instar we measured the width of the cephalic capsule. A significant increase of the width was the indicator that the larvae have moulted to the next instar. According to Pardo[1] and Quintero[2] (2004) (personal communications) larvae have moulted to the next instar when the cephalic capsule has doubled its size. However, we observed a maximal growing rate of 37.6% from the first to the second instar, and 36% from the second to the third. Hence, when these growing rates were reached we considered moulting as realized.

Results and Discussion : The life table data of the three species evaluated during this study are presented in **Table 1**.

Table 1. Development time of *Phyllophaga* sp., *P. menetriesi,* and *Anomala inconstans.*

Species	N	Mean Development of L1	Max L1	Min L1	Mean Development of L2	Max L2	Min L2	Mean Development of L3	Max L3	Min L3	Pupae	Duration Total
Phyllophaga menetriesi	12	19	19	19	28	15	32	175	201	82	37	259
Phyllophaga sp.	59	24	30	16	29	36	21	171	194	138	30	254
Anomala inconstans	53	27	30	24	29	38	21	163	173	140	30	249

* Development time in days; L1, L2, L3 indicate the instar of the larvae.

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Development time: The development time from egg to adult was similar for all three species (250 – 259 days); however, both *Phyllophaga* species passed a considerably longer period as third instar than *A. inconstans*.

Our results indicate a shorter development time of *P. menetriesi* than previous studies report. This might be due to the fact that they did not carry the experiments under controlled temperature and humidity (Vallejo 1996) or they used elevated temperatures (Hidalgo 1993; Aragon & Pérez 1999). Posterior field samples corroborated these explanation: we found several specimen of *P. menetriesi* in more advanced stages (prepupae or pupae) than expected according to the season while the specimen in the lab still continued as larvae without showing any signs to soon instar change.

Body size: Both *Phyllophaga* species had a wider cephalic capsule than *Anomala inconstans* **(Table 2)**. *P. menetriesi* presented the greatest width and length of the three species. Completing the first instar this species reached a length of 1.51 cm, similar to that of *Phyllophaga* sp. *A. inconstans* was considerably smaller (1.12 cm). The larvae of *P. menetriesi* were also the longest of the three species. Completing the second instar the larvae of *P. menetriesi* reached a width of 0.39 cm, whereas *Phyllophaga* sp. and *A. inconstans* did not pass 0.27 and 0.30 cm, respectively. The body width was the only of the three variables where we could observe significant differences between the species.

Table 2. Body sizes of *P. menetriesi, Phyllophaga* sp., and *A. inconstans* measured at the beginning of the first and the end of the second instar.

Species	Cephalic capsule		Body width		Body length	
	1st initial	2nd end	1st initial	2nd end	1st initial	2nd end
Phyllophaga menetriesi	0.22	0.39	0.22	0.39	1,45	2.24
Phyllophaga sp.	0.18	0.27	0.18	0.27	1.32	1.82
Anomala inconstans	0.18	0.30	0.18	0.30	1.10	1.77

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Contributors: Germán Andrés Calberto, Luis Carlos Pardo, Oscar Yela, Andreas Gaigl.

Activity 3. Search for natural enemies of in Northern and Eastern Antioquia.

Methodology: The physiographical zones where we carried out the surveys and the collaborators were the same as in Activity 1.

Results

- In the moderate cold North (2600 m. a. s. l.) we realized 13 surveys in the municipalities Santa Rosa de Osos, Entrerrios and San Pedro, where we collected 1,150 specimens.
- In the cold North (2600 m. a. s. l.) we realized 10 surveys in the municipality of La Union and collected 862 specimens.
- In the East (2100 m. a. s. l.) we collected 718 individuals in 12 surveys. The municipalities were Rionegro, El Carmen de Viboral, Guarne, and San Vicente.

Most of the collected specimens were larvae, some adults and pupae. These samplings corroborated the surveys described in Activity 1 and are not mentioned here. **Table 1** lists the collected larvae:

Table 1. Genera of collected whitegrub la rvae in thr ee ecolog ical z ones in Antioquia (Colombia) during 2003.

Region	Municip ality	Subfamily	Genera
Savanna North (2600 – 2800)		Dynastinae	*Ancognatha* *Cyclocephala* *Heterogomphus*
		Rutelinae	*Anomala*
		Not determined	sp.
		Melolonthinae	*Phyllophaga*
Cold East (2600 – 2800)	La Union	Dynastinae	*Ancognatha* *Cyclocephala* *Heterogomphus*
		Rutelinae	*Anomala*
		Melolonthinae	*Astaena* *Phyllophaga* *Plectris*
		Not determined	sp.
Savanna East (2100 m. a. s. l.)	El Carmen de Viboral	Dynastinae	*Ancognatha* *Cyclocephala*
		Melolonthinae	*Plectris* *Phyllophaga*
	Rionegro	Melolonthinae	*Phyllophaga* *Plectris*
		Not identified	*Astaena* sp.
		Dynastinae	*Cyclocephala* *Ancognatha* *Heterogomphus*
		Rutelinae	*Anomala*
	San Vicente	Rutelinae	*Anomala*
		Dynastinae	*Ancognatha*

Region	Municipality	Subfamily	Genera
	Guarne	Not identified	sp.
		Melolonthinae	*Plectris*
		Rutelinae	*Anomala*
		Melolonthinae	*Astaena* *Phyllophaga*
		Dynastinae	*Ancognatha* *Cyclocephala*
		Not identified	sp.

Nearly all of 2730 collected larvae died. 56% of them showed signs and symptoms of infections by entomopathogens. These parasitized larvae were transferred to the laboratory in order to isolate and identify the pathogens. **Table 2** shows the mortality of the collected larvae.

Table 2. Mortality of collected whitegrubs in three zones in Antioquia.

Zone		No. of Living Grubs	No. of Dead Grubs	%
North	2600-2800 m. a. s. l.	5	1145	99
East	2600-2800 m. a. s. l.	0	862	100
East	2100-2400 m. a. s. l.	0	718	100

The reasons of the extremely high mortality were mainly bacteria and fungi **(Table 3**).

Table 3. List of isolated entomopathogens from whitegrubs collected in all surveys in Antioquia (Colombia).

	Genus	Mortality reason	%	Principal Pathogens
Savanna North (2600 – 2800 m. a. s. l.)	*Ancognatha*	Bacteria	56	*B. popilliae* *Clostridium sp.* *B. sphaericus*
		Others	34	
		Fungi	6	Ma – 1 Ma – 2 Ma – 3
		Nematodes	1	Small Mermitidae
		Parasitoids	1	Ectoparasites
East (2600 – 2800 m. a. s. l.)				
La Union	*Ancognatha*	Fungi	56	Ma – 1 Ma – 2 *Paecilomyces sp.*
		Bacteria	30	*B. popilliae* *Clostridium sp.* *B. cereus*
		Others	14	
Savanna East (2100 – 2400 m. a. s. l.)				
El Carmen de Viboral	*Ancognatha*	Fungi	42	Ma – 1 Ma – 2
		Others	25	
		Bacteria	17	*B. popilliae* *B. larvae* *B. shaericus*
		Nematodes	14	Small Mermitidae
		Parasitoids	2	Endoparasites

	Genus	Mortality reason	%	Principal Pathogens
Savanna North (2600 – 2800 m. a. s. l.)	*Ancognatha*	Bacteria	56	*B. popilliae* *Clostridium sp.* *B. sphaericus*
		Others	34	
		Fungi	6	Ma – 1 Ma – 2 Ma – 3
		Nematodes	1	Small Mermitidae
		Parasitoids	1	Ectoparasites
Rionegro	*Phyllophaga*	Others	52	
		Bacteria	17	*B. popilliae* *B. larvae* *B. sphaericus*
		Fungi	8	Ma – 1 Ma – 2 Ma – 3
		Parasitoids	3	Ectoparasites
		Protozoa	3	
San Vicente	*Anomala*	Bacteria	39	*B. popilliae* *Clostridium sp.* *B. cereus*
		Others	40	
		Fungi	7	Ma – 3
		Nematodes	7	Mermitidae
		Parasitoids	7	Ectoparasites Endoparasites
Guame	*Anomala and Astaena*	Others	42	
		Bacteria	36	*B. popilliae* *Clostridium sp.* *B. cereus*
		Nematodes	11	small Mermitidae
		Fungi	6	Ma – 3
		Parasitoids	5	Ectoparasites Endoparasites

Since August 2004 we conducted weekly surveys on potato farms in Northern Antioquia in order to identify the pest species among the whitegrub complex.

Conclusions

- The dominating whitegrub species differ in every region.
- The well-defined peaks of flight activity facilitate the development of control strategies.
- The surveyed areas harbor a great diversity of microorganisms that can be a promising tool for the biological control of whitegrubs when following parameters are defined: prevailing whitegrub genus, climate and soil type.

Contributors: Martha Londoño, Elizabeth Meneses (Corpoica Rionegro), and Andreas Gaigl.

Activity 4. Search for natural enemies of Whitegrubs in the Colombian departments o f Cauca, Quindío, Risaralda, and Cundinamarca.

Entomopathogenic fungi

CIAT maintains since four years a collection of entomopathogenic fungi and bacteria stored in a ceparium. At this moment our ceparium harbors 411 strains of entomopathogenic fungi (EPF) isolated from spittlebug, whitefly, burrower bug and whitegrubs. In the first two years of this project we have isolated 213 strains of entomopathogenic fungi (EPF) only from whitegrubs and burrower bugs. Of those we have identified 118 strains, belonging to nine genera and 12 species.

On occasion we have isolated two entomopathogenic species from one insect species, without knowing, which one caused mortality and which one is saprophytic. Most EPF are saprophytes and act as facultative entomopathogens.

The ceparium includes of 4 *Aspergillus* sp., 5 *B. bassiana*, 2 *Beauveria* sp., 45 *Fusarium* sp., 13 *Gliocladium* spp., 18 *M. anisopliae*, 6 *Metarhizium* sp., 1 *Mucor* sp., 2 *Paecilomyces fumosoreus*, 13 *Paecilomyces* sp., 5 *Penicillium* sp., and 2 *Trichoderma* sp.

We isolated 126 EPF strains from six (identified) whitegrub species: *P. menetriesi* (55 isolations), *Phyllophaga* sp. (22), *Galleria mellonella* (10), *Cyrtomenus bergi* (35), *Anomala* sp. (1), *A. inconstans* and unidentified whitegrubs (82).

The 213 isolates are from 11 different origins **(Table 1**). The most frequent isolated fungus was *Fusarium* sp., followed by *Metarhizium* spp. Cauca, Risaralda and Quindío dominate the frequency because these departments were place of our surveys.

Table 1. Origin of CIAT ceparium strains.

Genera	Department							TOTAL
	Caldas	Ca uca	C/marca[1]	Quindío	Risaralda	Valle	Others[2]	
Aspergillus	0	0	0	0	3	1	0	4
Beauveria	0	2	0	0	5	0	0	7
Fusarium	0	29	0	7	9	0	0	45
Gliocladium	0	9	0	0	4	0	0	13
Metarhizium	1	10	1	1	12	1	1	27
Mucor	0	0	0	0	1	0	0	1
Paecilomyces	0	10	0	2	1	0	0	13
Penicillium	0	3	0	1	1	0	0	5
Trichoderma	0	2	0	0	0	0	0	2
Unidentified[3]	0	51	0	11	27	3	4	96
TOTAL	1	116	1	22	63	5	5	213

[1]Cundinamarca, [2]One strain from *C. bergi* colony at Hanover University, [3]unidentified.

Entomopathogenic bacteria

We isolated 89 strains of entomopathogenic bacteria (EPB) from whitegrubs collected in the three departments Cauca, Risaralda, and Cundinamarca **(Table 2**). *Bacillus popilliae* and *B.*

lentimorbus represent the major part of this collection. Other species are *B. larvae, B. sphericus* and two strains of *Serratia* sp.

Table 2. Host and origin of entomopath ogenic bacteria isolated from whitegrubs collected in the Colombian depa rtments of Valle, Risaralda, and Cundinamarca.

Genera	Department			Total
	Valle Risa	ralda	Cundinamarca	
Clavipalpus spp.	0	0	8	8
Ancognatha spp.	0	0	7	7
H. dilaticollis	0	0	2	2
Anomala	4	1	0	50
P. menetriesi	60	7	0	67
Total	64	8	17	89

Contributors: Sonia Ximena Restrepo, Anuar Morales, Rosalba Tobón, Oscar Yela, Andreas Gaigl.

Activity 5. Search for entomopathogenic nematodes in Colombia and Panama: Firs t description of *Steinernema kraussei* as native entomopathogenic nematode in Colombia.

Introduction

The entomopathogenic nematodes (EPN) of the family Heterorhabditidae and Steinernematidae are used as control agents of a wide range of soil pests. The search of native organisms is realized all over the world, where they have been found in a wide range of soil and crops (Rueda *et al*. 1993). The objective of this study was to identify native EPN species associated with pasture, cassava, onion, groundnut, and other crops in the survey zones.

Methodology: We surveyed different regions in Colombia (Quindío, Risaralda, Caldas, and Cauca) and Panama **(Table 1**. Ten random samples per hectare were taken in a profundity of 15 to 20 cm.) From each hole we took one liter of soil to the lab for further processing. The shovels were disinfected with alcohol before digging a new hole. Once in the lab the samples were stored at 15 °C until processing. We sent 500 g of each sample to the CIAT soil analysis unit to determine pH, humidity, soil organic matter and texture.

300 g of the sample was placed in a plastic cup where 10 larvae of *Galleria mellonella* were confined as baits. This procedure was repeated three times. After five days the infested moth larvae were transferred to 'White traps' (Kaya & Stock 1997) where the EPN abandoned the dead insect and migrated over filter paper to water. In order to test the virulence of the isolated EPN these were transferred into Petri dishes filled with sand harboring *Galleria* larvae (Koch's postulates). The newly extracted EPN were stored in sterile and distilled water. We fixed about 5% of the samples in TAF (Formaldehyde + Triethanoamine + sterile distilled water), the rest were stored alive in sterile distilled water.

The isolated EPN were sent alive for identification to the Department of Biotechnology (DBT), Kiel University, Germany. Molecular techniques were used which were based on Restricted Fragment Length Polymorphism (RFLP) of the Internal Transcribed Spacer Region (ITS). This region was amplified by PCR. PCR was digested by eight restricted enzymes and made visible by electrophoresis.

We evaluated the following parameters:

a. Coloring of dead *G. mellonella* larvae
b. Morphological and behavioral characteristics
c. The response to the test of Koch's postulates
d. Identification of the EPN
e. Soil physical characteristics

Table 1. Survey sites in Colombia and Panama for entomopathogenic nematodes.

Country	Department	Municipality	Date of Sampling	Crop
Panama	***El Valle de Antón***	*Coclé*	Oct-02	Onion-Groundnut
	Ocú	Veraguas	Oct-02	Cassava
	Sioguí	Chiriquí	Oct-02	Cassava
	Cerro Punta	Estación IDIAP	Oct-02	Onion
Colombia	Quindío	Quimbaya	Mar-03	Cassava-Banana-Maize
	Risaralda	Santa Rosa	Mar-03	Onion
		Pereira	Mar-03	Pasture-Cassava-Onion
			May-03	Onion
			Feb-03	Pasture, Onion, Cassava, Pea
		Dosquebradas	Mar-03	
		La Florida	Jun-03	**Pasture**
	Caldas	Manizales	Jun-03	Onion
				Guamo, Breadfruit Tree, Maize, Beans
	Cauca	S/Quilichao	Mar-03	Cassava, Avocado, Banana, Mango, Mandarin, Lemon, Passionfruit, Café
				Groundnut, Pasture, Cassava

Results and Discussio n: In total we analyzed 320 soil samples taken from soils of 15 crops **(Table 2)**. 24 of these samples harbored fungi (*Fusarium* sp. and *Metarhizium* sp.), 16 mites (Acardiae and Histiomidae) and about 300 of them nematodes. We classified the majority of the isolated nematodes as saprophagous due to their size, movement and survival. Most of them died during conservation. We used the surviving nematodes for re-infection of *Galleria mellonella* larvae (Koch's postulates). The 21 nematodes with corroborated entomopathogenic characters are listed in **Table 2**. We sent 20 of them to Kiel University for identification where two samples were identified as *Steinernema kraussei* **(Figure 1)**. It is the first time that this species was reported in Colombia.

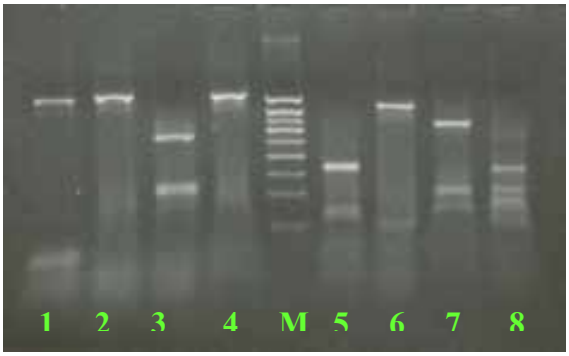

Figure 1. PCR amplified products from the ITS region of *Steinernema kraussei* digested with 8 restric tion en zymes. Lanes 1-8 indicate follow ing enzymes: 1. *Desulfovibrio desulfuricans*, strain Nor way, 2. *Haemophilus aegytius,* 3. *H. haemolyticus,* 4. *H. influenzae*, M. Molecular w eight markers (band siz es 1000, 800, 700, 600, 500, 400, 300, 200, 100 base pairs), 5. *H. influenzae* Rf, 6. *H. aphrophilus,* 7. *Acidiphilium facilis,* 8. *Staphylococcus aureus* 3A.

Table 2. Organisms isolated from soil samples taken in various parts in Colombia and Panama.

Country	Department	Municipality	Crop	No. of Samples (300 g)	Isolated organisms		
					Mites	Fungi	Supposed EPN
Panamá	El Valle de Antón	Coclé	Onion	5	2	2	1
			Groundnut	5	0	2	1
	Ocú	Veraguas	Cassava	10	0	2	1
	Sioguí	Chiriquí	Cassava	6	0	2	0
	Cerro Punta	Estac. IDIAP	Onion	5	1	2	1
Colombia	Quindío	Quimbaya	Cassava	21	1	2	1
			Banana	20	1	2	1
			Maize	20	0	1	1
	Risaralda	Santa Rosa	Onion	5	0	1	0
		Pereira	Pasture	38	1	1	1
			Cassava	33	0	1	1
		La Florida	Onion	24	1	0	1
			Pasture	35	1	0	1
	Caldas	Manizales	Guamo	3	0	1	1
			Breadfruit tree	3	0	0	1
			Maize	3	0	0	1
			Bean	3	1	0	1
			Cassava	4	1	0	1
			Avocado	2	0	0	0
			Banana	2	0	0	0
			Mango	2	1	0	0
			Mandarin	2	1	1	0
			Lemon	3	1	0	0
			Passionfruit	3	0	1	0
			Café	4	0	1	1
			Onion	6	0	1	1
	Cauca	Santander de Quilichao	Pasture	20	1	0	1
			Cassava	31	1	1	1
			Groundnut	2	1	0	1
Total				320	16	24	21 (6,6%)

 = Samples with *Steinernema kraussei*

Abiotic factors play an important role in the successful performance of EPN, affecting the searching behavior, finding and invading the host (Parada 2002). EPN can tolerate a wide range of pH: 4 to 8 for *Steinernema* and 4 to 6 for *Heterorhabditis*. Humidity is a key factor for survival, movement, persistence and infectivity of the EPN (Koppenhöfer & Kaya 1996). Sandy and loamy soils harbor more species than clayish (Parada 2001). Our soil analyses showed that we carried out the samplings in zones with a pH range from 6.4 (Cauca) to 5.8 or 5.2 (Caldas). The soil texture varied from loamy (Cauca) to loamy-sandy (Caldas) **(Table 3**).

Table 3. Soil analyses of the surveyed sites.

Site	Crop	pH	SOM *	Texture
Risaralda	Onion	6,2	90,0	Loamy
	Pasture	5,3	77,7	Loamy
	Cassava	5,7	135,3	Loamy
	Pasture	5,9	81,9	Loamy
	Onion	6,0	96,1	Loamy
Quindío	Cassava	5,4	52,5	Loamy
Cauca	Groundnut	4,6	51,5	Clay
	Pasture	5,3	53,4	Clay
	Cassava	6,4	44,9	Loamy
	Fallow land	5,0	51,1	Clay
Caldas	Guamo	5,8	56,3	Loamy-sandy

 = Samples with *Steinernema kraussei;* SOM = Soil organic matter.

The fact that our survey sites were in the range of these favorable characteristics we hypothesize that are reasons are responsible for the absence of EPN, for example contamination by pesticides.

Conclusions and recommendations

- *Steinernema kraussei* was described in Colombia for the first time.
- However, *S. kraussei* showed a wide range of soil characteristics like pH (4-8) and texture.
- EPN were present only in 6.6% of all evaluated soil samples, suggesting that much of the area the major part where the survey was realized the use of synthetic insecticides is common. We suggest continuing with these surveys in zones free of insecticide use.

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Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Andreas Gaigl, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 6. Efficiency of entomopathogenic nematodes for w hitegrubs control under laboratory conditions.

Introduction

Globally, whitegrubs belong to the most important soil pests of many crops. During the last 20 years their pest status has dramatically increased, especially in the tropics, resulting in considerable yield reductions and environmental degradation: The whitegrub complex is characterized by considerable species richness. Many of these scarab species are important for the decomposition of organic material in the soil. However, the ever increasing reduction of plant biodiversity in many farming systems in the tropics, accompanied by losses of organic soil matter diminishes scarab diversity and leads to a selection of the most resistant and aggressive species. Most often the only control tactics for farmers is the excessive use of synthetic pesticides, with all the inherent health hazards to both farmers and rural and urban consumers. Moreover, synthetic pesticides pollute the environment and through the selection of resistant strains of whitegrubs and burrower bug can aggravate pest status by selecting for resistance.

Entomopathogenic nematodes (EPN) are considered as a promising strategy for the biological control of soil pests (Kaya & Gaugler, 1993). EPNs can be used as microbiological insecticides, suitable for small farmers. Industrial and semi-industrial mass rearing techniques for EPNs have been successfully developed (e.g. Capinera & Epsky, 1992; Ehlers, 1998), and EPNs can be applied using conventional pesticide equipment (Georgis, 1990).

Searching for feasible alternatives for the whitegrub control we tested the effect of ten EPN strains as antagonist of three important pest species: *Phyllophaga menetriesi*, *Phyllophaga* sp., and *Anomala cincta*.

Methodology: The experiment was conducted in the CIAT laboratory (23 ± 2 C, 70 ± 5% RH). We used whitegrubs collected in the fields in Caldono in Cauca (1570 m. a. s. l.) and Calucé in Valle (1637 m. a. s. l.) as target insect. We used larvae in the third instar of *Phyllophaga menetriesi*, *Phyllophaga* sp. (Cauca) and *Anomala cincta* (Calucé). After field collection we quarantined the larvae for four weeks. The larvae were maintained at 19 °C in plastic cups of 100 cm^3 and in a mixture of sand : sterile soil (1:3) and fed with carrots **(Figure 1**).

We tested 10 strains of native and introduced nematodes **(Table 1**). They were multiplied before every experiment in larvae of *G. mellonella*. We evaluated following variables: 1) Infection and mortality rate of whitegrubs and larvae of *G. mellonella* as control. 2) Impact of age of larvae on performance of EPN.

Figure 1. Whitegrub rearing cabins (a. *Phyllophaga menetriesi*, b. *Phyllophaga* sp., and c. *Anomala cincta*) in the CIAT laboratory of E ntomology (23 ± 2 C, 70 ± 5 % RH, 12 hours photoperiod)

Table 1. Evaluated EPN strains as control agents of three w hitegrub species (*Phyllophaga* sp., *P. menetriesi*, *Anomala cincta*) under lab conditions.

Species	Place of Origin			Date of Collection / Arrival	Collector
	CIAT-Code	Country	Institution		
Steinernema riobravis	1	USA	Certis, USA	Jan - 2003	Certis
Steinernema feltiae	2	Colombia	Univ. Nacional Bogotá	Mar - 2003	Parada
Heterorhabditis bacteriophora	3	Italia	CABI/Bioscience	2002	López
Steinernema carpocapsae	4	USA	CABI/Bioscience	2002	López
Steinernema sp.	5	Colombia	Cenicafé	2002	López
Heterorhabditis sp.	6	Colombia	Cenicafé	2002	López
Steinernema arenarium	8	Russia	Kiel University	Jun - 2003	Ehlers
Steinernema feltiae	10	Germany	E-nema	Jun - 2003	E-nema
Heterorhabditis bacteriophora	11	Germany	E-nema	Jun - 2003	E-nema
Steinernema scarabaei	14	USA	Rutgers University	May-2004	Koppenhöfer

The experimental design was completely randomnized, evaluating only one factor (EPN) with a control without any application and another control with *G. mellonella* in order to evaluate the mortality after 48 hours. Every experimental unit consisted of 12 larvae and was repeated four times. We applied 1000 EPN / ml. The experimental unit consisted of plastic cups (56 ml) filled with soil and sterilized sand (3:1). We adjusted the field capacity by aggregating 3 ml water. We applied the EPN one day after the whitegrubs were introduced into the experimental units. The cups were stored in plastic bags in order to avoid the loss of humidity and were maintained in cabins under controlled conditions. We realized these experiments between January and July 2004 with repititions over the time in order to study the impact of the larval development on the eficiency of the EPN. These repititions had to be adjusted according to the quantity of available specimen of whitegrubs.

We used the statistical software Infostat for performing ANOVA and the treatment means were compared by Tukey ($P<0.05$). The non-homogenous data were homogenized by the formula $\sqrt{x+1}$ before analysis.

Results and Discussion: On *Phyllophaga* sp. all *Heterorhabditis* strains were the most efficient EPN. Only the native strain *S. feltiae* from Bogotá showed a similar performance as the imported

H. bacteriophora from E-nema **(Figure 2**). However, mortality and infection rate of EPN were significantly lower when larval age oncreased **(Figure 3**).

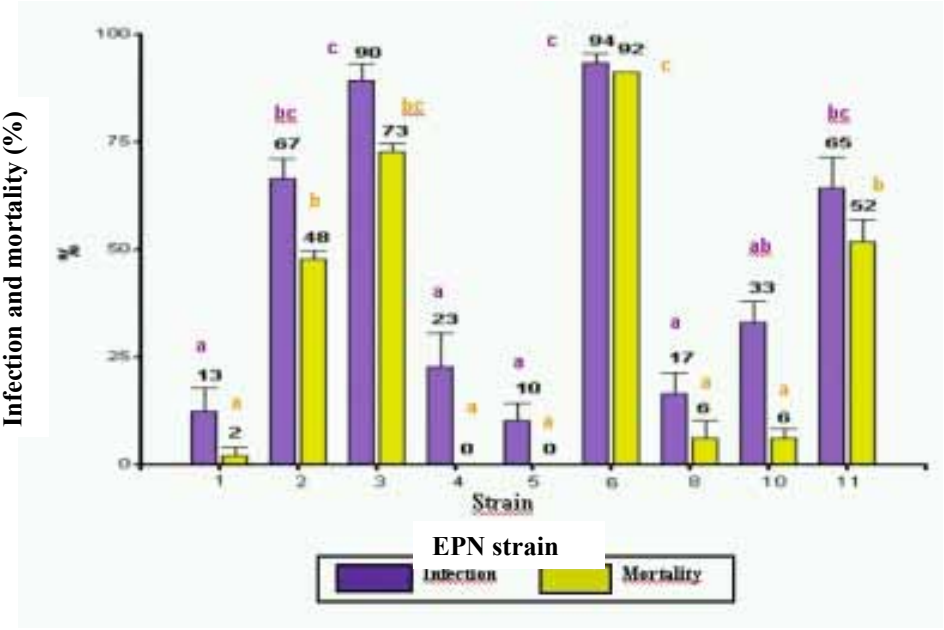

Figure 2. **Infection and mortality rate of nine EPN str ains vs. *Phyllophaga* sp. (in %). Strains: 1. *S. riobravis,* 2. *S. feltiae* (UN Bogotá), 3. *H. bacteriophora* (Italia, Cenicafé), 4. *S. carpocapsae,* 5. *Steinernema sp.,* 6. *Heterorhabditis sp.* (Cenicafé), 8. *S. arenarium,* 10. *S. feltiae* (E-nema), 11. *H. bacteriophora* (E-nema).**

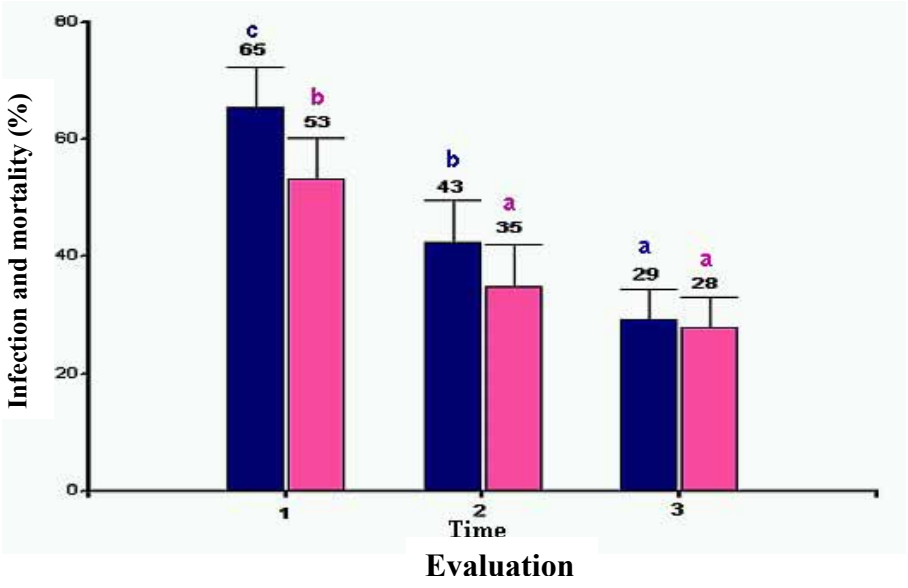

Figure 3. **Means of infection and morta lity ra te of sev en EPN strains vs. the increa sing age of the third instar of *Phyllophaga* sp. Evaluations took place in a period of four w eeks. Evaluations w ere subsequent ly carried out in intervals of four weeks.**

We tested seven EPN strains vs. *P. menetriesi* where EPN were less efficient than vs. *Phyllophaga* sp. Interestingly, *S. carpocapsae* caused the highest whitegrub mortality (29%), *Heterorhabditis* sp. from Cenicafé showed the highest infection rate (40%, but mortality was very low (8%) **(Figure 4**)). After four weeks *H. bacteriophora* (Italia, Cenicafé) and *Heterorhabditis* sp. (Cenicafé) obtained a mortality of 12 and 10%, respectively (**Figure 5).**

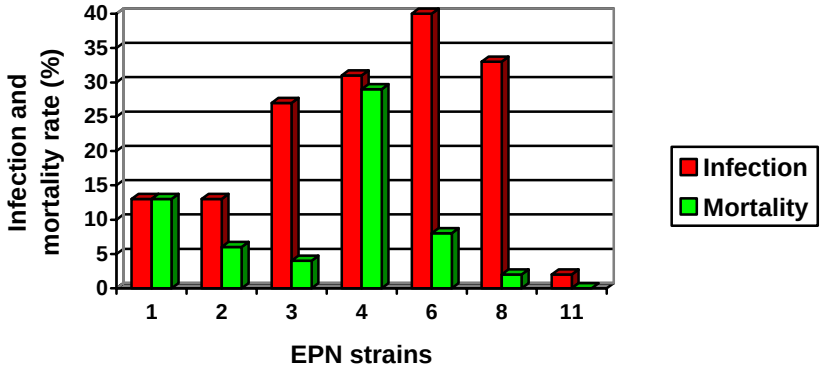

Figure 4. **First evalu ation of infection a nd mortality rate of seven EPN strains vs. *P. menetriesi*. Strains: 1. *S. riobravis,* 2. *S. feltiae* (UN Bogotá), 3. *H. bacteriophora* (Italia, Cen icafé), 4. *S. carpocapsae,* 6. *Heterorhabditis sp.* (Cenicafe), 8. *S. arenarium,* 11. *H. bacteriophora* (E-nema).**

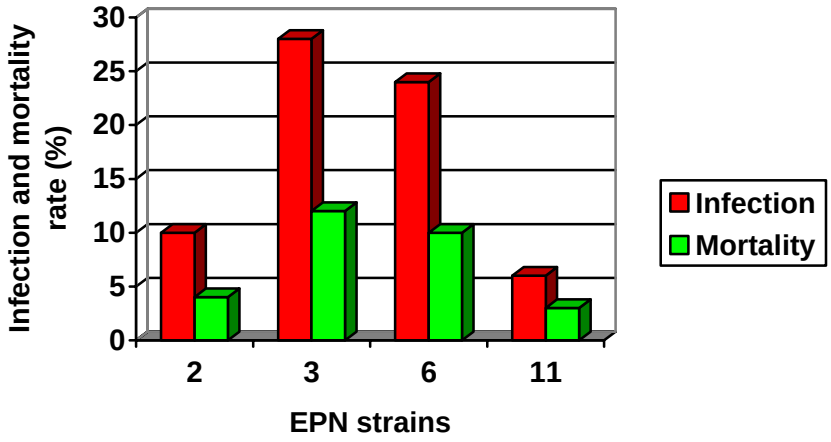

Figure 5. **Second evaluation of four EPN strains vs. *P. menetriesi* (in %). Strains: 2. *S. feltiae* (UN Bogotá), 3. *H. bacteriophora* (Italia, Cenicafé), 6. *Heterorhabditis* sp. (Cenicafé), 11. *H. bacteriophora* (E-nema).**

The EPN performed the best control of *P. menetriesi* during the second evaluation **(Figure 6).**

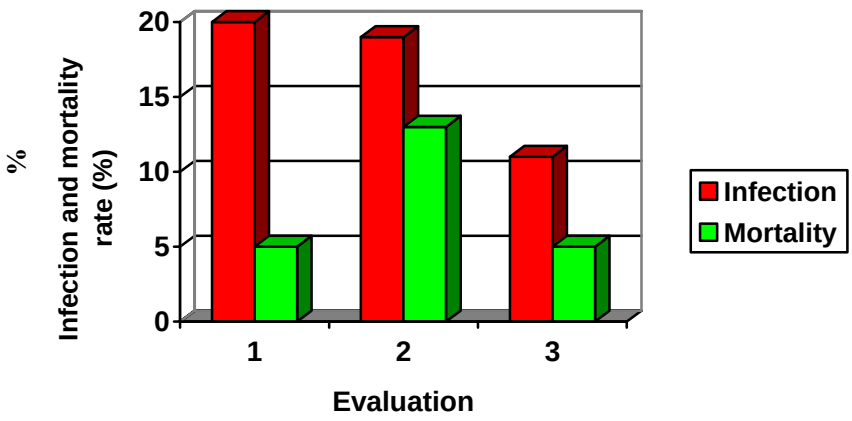

Figure 6. Means of infection and mortality rate of four EPN strains vs. the increasing age of the third instar of *Phyllophaga* sp. Evaluations were subsequently carried out in intervals of four weeks.

The most effective EPN vs. *Anomala cincta* were *H. bacteriophora* (Italia, Cenicafé) and *Heterorhabditis* sp. (Cenicafé) **(Figure 7**). The tested EPN were most efficient vs. *Phyllophaga* sp., followed by *A. cincta*. As mentioned before *P. menetriesi* is the whitegrub with the best defense against EPN: Earlier experiments with EPN (Quintero 2003) and *Metarhizium anisopliae* vs. third instar larvae of *P. menetriesi* (Martha Londoño 2004, personal communication[3]) corroborate this observation.

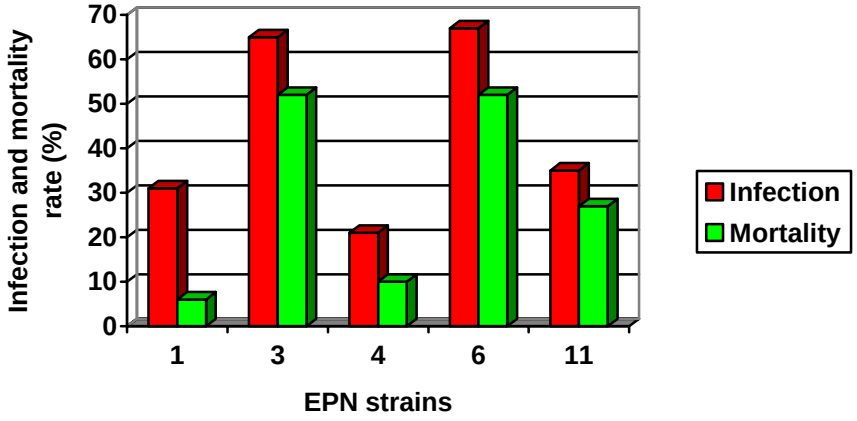

Figure 7. Infection and mortality rate of nine EPN strains vs. *Anomala cincta* (in %). Strains: 1. *S. riobravis* (Certis, USA), 3. *H. bacteriophora* (Italia, Cenicafé), 4. *S. carpocapsae*, 6. *Heterorhabditis* sp. (Cenicafé), 11. *H. bacteriophora* (E-nema).

[3] Martha Londoño is research associate of Corpoica, Rionegro

The evaluations during the first two development phases of *A. cincta* didn't show any significant differences in mortality **(Figure 8**).

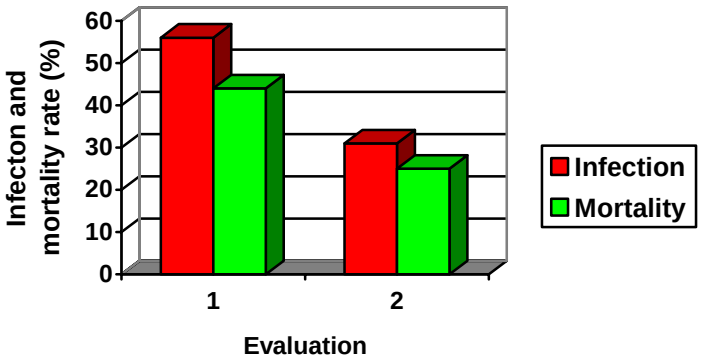

Figure 8. Comparing two consecutive evaluations of infection and mortality rate of four EPN vs. third instar of *Anomala cincta*.

When we compared all tested EPN vs. each whitegrub species we found that the nematodes showed greatest mortality on *Phyllophaga* sp., followed by *Anomala cincta. P. menetriesi* was the most resistant species to EPN infection and mortality **(Figure 9**).

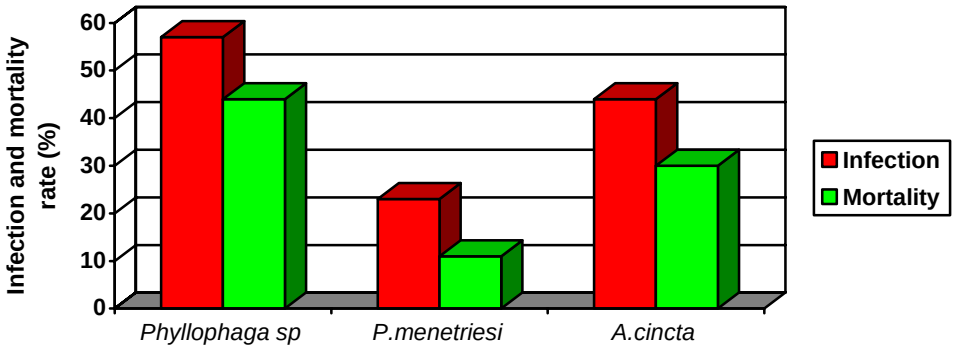

Figure 9. Infection and mortality rates of all EPN strains on three whitegrub species

Conclusions

- *P. menetriesi* was the most resistant whitegrub species to EPN attacks.
- Larval age significantly affects the impact of EPN. The third instar is the most resistant probably due to morphological and functional reasons that still have to be studied and identified.
- *Heterorhabditis* sp. (Cenicafé) was the most promising EPN against *Phyllophaga* sp. and *Anomala cincta*, followed by *H. bacteriophora* from Italia, *H. bacteriophora* (E-nema) and *S. feltiae* (Universidad Nacional).

Recommendations

- Conduct studies with EPN vs. the first two instars of *P. menetriesi.*
- Realize studies to evaluate the optimal concentration of EPN.
- Repeat these experiments under semi-controlled conditions.
- We recommend studying only mortality in order to reduce dissection work; moreover, mortality is the most important variable.

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Contributors: Elsa Melo, Carlos Alberto Ortega, Andreas Gaigl, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 7. Infection and mortality rate of *S. scarabaei* vs. *Phyllophaga* sp.

Introduction

Steinernema scarabaei Stock & Koppenhöfer was recently isolated from *Exomala* (= *Anomala*) *orientalis* Waterhouse and *Popillia japonica* Newman in New Jersey, USA (Stock & Koppenhöfer 2003). This EPN reached mortality rates of almost 100% of the whitegrubs *P. japonica*, *Cyclocephala borealis* and *E. orientalis* under field conditions in New Jersey. *S. scarabei* outperformed clearly other EPN species such as *H. bacteriophora* and the unidentified *Heterorhabditis* sp. (Koppenhöfer & Fuzy 2003). Due to our experience that *P. menetriesi*, the economically most important whitegrub between 1000 and 1500 m. a. s. l., is hard to kill (CIAT 2003, Quintero 2003) we decided to import this EPN species for experiments in the laboratory in spite of the fact, that it has not been possible to mass produce these nematodes in vitro (Ralf-Udo Ehlers 2003, personal communication).

Methodology: We evaluated six concentrations (0, 25, 50, 100, 200, and 400 EPN / ml) of *S. scarabaei* vs. larvae of *Phyllophaga* sp. The further procedure is explained in the previous part A (12 specimen per treatment and four replicates). The nematodes were provided by Albrecht Koppenhöfer, Rutgers University, Brunswick, New Jersey.

Results: *S. scarabaei* obtained greatest infection and mortality rate vs. *Phyllophaga* sp. with the highest concentration. Differences between the concentrations 200 and 400 Dauer Juveniles (DJ) per ml were not significant **(Figure 1**). Interestingly, infection and mortality rate were equal in all treatments. In the control treatment, all *G. mellonella* died after 48 hours.

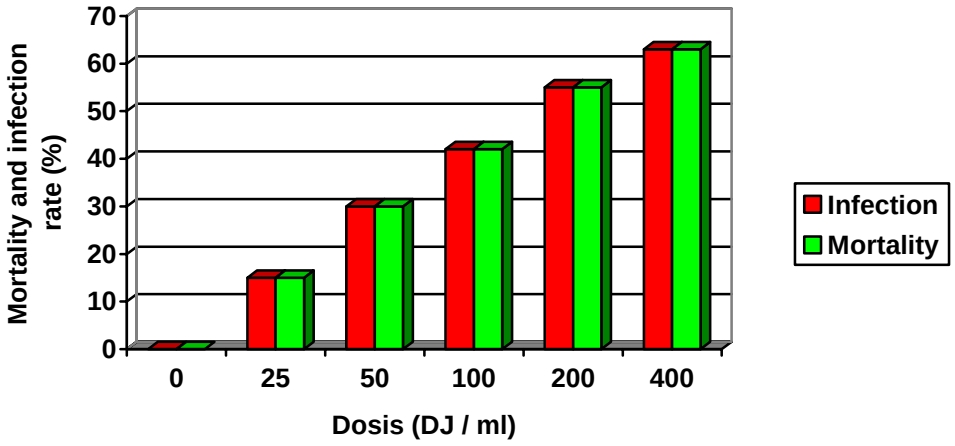

Figure 1. Infection and mortality rate of six concentrations of Dauer juveniles (DJ) of *S. scarabei* vs. *Phyllophaga* sp.

However, in the second experiment the performance of these nematodes was very poor. Infection and mortality was not greater than 5%, the larvae of *G. mellonella* didn't show a high mortality, either. It is possible that the loss of this efficiency was due to the prolonged transport period due to importation problems. Since the mortality in the first experiment wasn't very high and even

100% mortality of *G. mellonella* may not be a reliable indicator of EPN pathogenicity (Albrecht Koppenhöfer 2004, personal communication) this experiment should be repeated with healthier, fresher material.

Conclusions

- Infection and mortality rate of whitegrubs are positively correlated with increasing DJ concentration
- The time of EPN application had an effect on infestation and mortality rate. At the first evaluation using a concentration of 200 and 400 DJ / ml control was greater than 50%

Recommendations

- Reactivate and multiply *S. scarabei* in order to continue these experiments
- Considering the tedious dissection of hosts in order to count the number of pentrated DJ we recommend to include in further trials only the variable “mortality”. Moreover, this variable is much more important

References

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Koppenhöfer, A.M., and E.M. Fuzy. 2003. Steinernema scarabaei for the control of whitegrubs. *Biological Control*, 28: 47-59.

Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Andreas Gaigl.

Activity 8. Preliminary studies on path ogenicity of entomopath ogenic fungi against *Phyllophaga menetriesi.*

Introduction

At this moment, our ceparium harbors 213 strains of entomopathogenic fungi (EPF) that have been isolated from adult beetles or whitegrub larvae. Reactivation of a fungus is the first step of the selection process of the most virulent strains. Here, we describe the process of reactivation of fungi on the third instar of *Phyllophaga menetriesi* generating some important data for selection of the most virulent strains.

Methodology: The larvae of *P. menetriesi* were collected in Pescador (Caldono, Cauca). The larvae were placed in plastic cups of 12 ounces filled with soil and transferred to CIAT. Here, the grubs were confined in sterile soil and observed for at least 40 days in order to detect entomopathogens.

We selected 46 isolates (23 *M. anisopliae*, 9 *B. bassiana*, 4 *Paecilomyces* sp., 3 *Fusarium* sp., 3 *Gliocladium* sp., 2 unidentified strains and 1 *Penicillium* sp.) **(Table 1**). Once they showed a considerable sporulation we applied them to the insects. We confined the grubs in plastic cups filled with soil; three pregerminated maize seeds and one larva of *P. menetriesi* (third instar). Before covering the experimental unit with a perforated lid we added 2 ml of a suspension with spores. The cups were placed in plastic bag filled with wax sheets in order to maintain humidity 70% and stored at 27 °C and 100% darkness.

We counted living and dead larvae once a week during a two months period. The dead insects were transferred to a chamber with a RH of 80% in order to enhance sporulation. Once the growing of the fungus was visible we inoculated it on Agar. We realized the studies on reactivation on larvae using four groups: 7 strains and one control as pilot treatment where we tested the viability of the methodology; the second inoculation with 9 strains; the third inoculation with 20 and the fourth with 26 strains.

Results: Some strains caused mortality of the grubs at 16 days after application. After five weeks the following strains of *M. anisopliae* caused a mortality of 100%: CIAT 323, CIAT 328, CIAT 393, and CIAT 405. In a second experiment the following strains of *M. anisopliae* caused a mortality of 100%: 1p, CIAT 300, CIAT 348, CIAT 418, CIAT 513, and 515.

Recommendations: For the following tests we will use the strains CIAT515 (*M. anisopliae*) and CIAT418 (*M. anisopliae*) due to their high pathogenicity. We also strongly suggest including CIAT 338 and CIAT 405, because those represent the species *Gliocladium* sp. and *Beauveria bassiana*, respectively, and both caused 100% mortality. We plan to test these strains vs. the second instar of *P. menetriesi*. The most pathogenic strain will be tested vs. the third instar of the larvae of the same species.

Table 1. Applied EPF strains vs. larvae of the third instar of *Phyllophaga menetriesi* for reactivation.

	Strain		Host		Origin		
	Code	Genera	Species	Genus	Species	Department	Municipality
1	1p	*Metarhizium*	*Anisopliae*				
2	CIAT014	*Metarhizium*	*anisopliae*	*Aeneolamia*	*varia*	Valle	Palmira
3	CIAT044	*Beauveria*	*bassiana*	*Zulia*	*carbonaria*	Cauca	Sder Quilichao
4	CIAT224	*Metarhizium*	*anisopliae*	*Cyrtomenus*	*bergi*	Valle	Pradera
5	CIAT245	*Metarhizium*	*anisopliae*	*Hypotenemus*	*hampei*	Caldas	Chinchiná
6	CIAT300	*Metarhizium*	*anisopliae*	*Galleria*	*mellonella*	Cauca	Sder Quilichao
7	CIAT302	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
8	CIAT308	*Paecilomyces*	spp.	*Galleria*	*mellonella*	Cauca	Sder Quilichao
9	CIAT309	*unidentified*	*unidentified*	Chisa		Risaralda	Pereira
10	CIAT314	*Gliocladium*	sp	Chisa		Cauca	Caldono
11	CIAT316	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
12	CIAT317	*Beauveria*	*bassiana*	Chisa		Risaralda	Pereira
13	CIAT320	*Fusarium*	spp.	Chisa		Risaralda	Pereira
14	CIAT323	*Metarhizium*	*anisopliae*	Chisa		Cauca	Caldono
15	CIAT325	*Fusarium*	spp.	Chisa		Cauca	Sder Quilichao
16	CIAT328	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
17	CIAT329	*Penicillium*	sp	Chisa		Cauca	Caldono
18	CIAT338	*Gliocladium*	sp				
19	CIAT344	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
20	CIAT345	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
21	CIAT347	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
22	CIAT348	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
23	CIAT349	*Metarhizium*	*anisopliae*	*Galleria*	*mellonella*	Risaralda	Pereira
24	CIAT351	*Gliocladium*	sp	Chisa		Risaralda	Pereira
25	CIAT354	*Metarhizium*	*anisopliae*	Chisa		Caldas	Manizales
26	CIAT359	*Beauveria*	*bassiana*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
27	CIAT360	*Beauveria*	*bassiana*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
28	CIAT363	*Beauveria*	*bassiana*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
29	CIAT388	*Metarhizium*	*anisopliae*	*Cyrtomenus*	*bergi*	Valle	Florida
30	CIAT389	*Metarhizium*	*anisopliae*	Chisa		Valle	Florida
31	CIAT390	*Metarhizium*	*anisopliae*	*Phyllophaga*	sp	Risaralda	Pereira
32	CIAT393	*Metarhizium*	*anisopliae*	Chisa		Cauca	Sder Quilichao
33	CIAT395	*Paecilomyces*	spp.	*Phyllophaga*	sp	Risaralda	Pereira
34	CIAT401	*Beauveria*	*bassiana*	Chisa		Cauca	Caldono
35	CIAT405	*Beauveria*	*bassiana*	*Trialeurodes*	*vaporariorum*	Valle	Roldanillo
36	CIAT412	*Metarhizium*	*anisopliae*	*Phyllophaga*	sp	Cauca	Sder Quilichao
37	CIAT418	*Metarhizium*	*anisopliae*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
38	CIAT422	*Paecilomyces*	sp	*Galleria*	*mellonella*	Cauca	Caldono
39	CIAT513	*Metarhizium*	*anisopliae*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
40	CIAT515	*Metarhizium*	*anisopliae*	*Phyllophaga*	*sp*	Cauca	Sder Quilichao
41	Cuc. CIAT	*Beauveria*	*bassiana*	*Anomala*	Sp	Cauca	Caldono
42	CUN 087A	*Metarhizium*	*anisopliae*				
43	CUN 087B	*Beauveria*	*bassiana*				
44	CUN PER2	*Fusarium*	sp				
45	CUN059	*Metarhizium*	*anisopliae*	*Aeneolamia*	*varia*	Valle	Palmira
46	P.crus.	*Paecilomyces*	*crustaceus*				

Activity 9. Preliminary studies on pathogenicity of *Bacillus popilliae* against *Phyllophaga menetriesi.*

Introduction

Larvae of *Phyllophaga menetriesi* are the economically most important whitegrub species in Colombia in zones from 1200 to 1600 m. a. s. l. (CIAT 2003). The bacteria *Bacillus popilliae* (Bp) has been reported as one of the most important natural antagonists of about 70 species of whitegrubs causing the milky disease (Tanada & Kaya 1993) and is also one of the most frequent pathogen of larvae we collected during this project (CIAT 2003; see also Activity 4 of this report). The objective of this recently initiated undergraduate thesis is to describe pathogenicity and virulence of *B. popilliae* in larvae of *P. menetriesi*. Here we present the results of isolations of native strains of this bacteria and its multiplication.

Methodology: In the first experiment we tested four strains of Bp (LF24, 381, B386, LG) on three species (*P. menetriesi*, *Phyllophaga* sp. and *Anomala cincta*) using 25 insects for each strain and species. Then, we could include two more strains (Bp4 and Bp1) that had been isolated from field-collected whitegrubs. Due to the lack of specimens of *P. menetriesi* we applied these six strains on *Phyllophaga* sp. (100 individuals / strain). For the following experiment sufficient larvae of *P. menetriesi* were available for testing the six Bp strains (75 individuals / strain). We injected in each grub a concentration of 3×10^8 spores. The conditions in the laboratory were: 21 °C ± 2, 70% RH ± 5, 965 m. a. s. l., 24 hours darkness.

Results: Figure 1 shows the different mortalities caused by four Bp strains vs. three whitegrub species. LG caused only a mortality of 4% on *A. cincta*, whereas the mortality of the genus *Phyllophaga* varied from 88 to 100%. In contrast, LF24 killed all grubs by 100%. 381 caused a relatively low mortality of *A. cincta* and B386 of *P. menetriesi*. *Phyllophaga* sp. was the species where all bacteria strains caused almost a 100% control. Latter result was repeated when we included the two new strains.

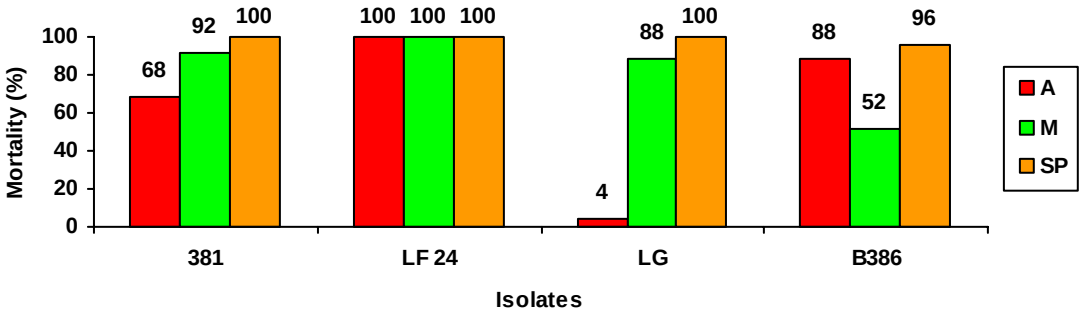

Figure 1. Mortality of three w hitegrub s pecies (A= *Anomala cincta*, M= *Phyllophaga menetriesi*, SP= *Phyllophaga* sp.) caused by four stra ins of *B. popilliae* in the laboratory

The strains LF24 and LG gave the best reproduction in *Phyllophaga* sp. followed by Bp4, Bp1, 381, and B386 **(Figure 2**).

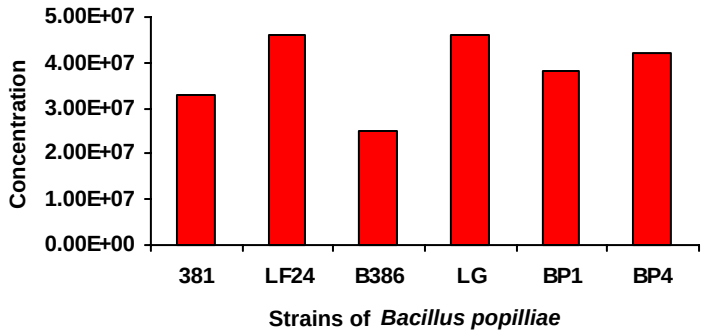

Figure 2. Reproduction of six *Bacillus popilliae* strains in *Phyllophaga* sp. in the laboratory

The control of the *P. menetriesi* by the six *B. popilliae* strains oscillated between 97 and 100% where the strains 381 and Bp4 showed the lowest results (97%) **(Figure 3**). These data suggest that all six strains can be selected for further tests of pathogenicity and virulence.

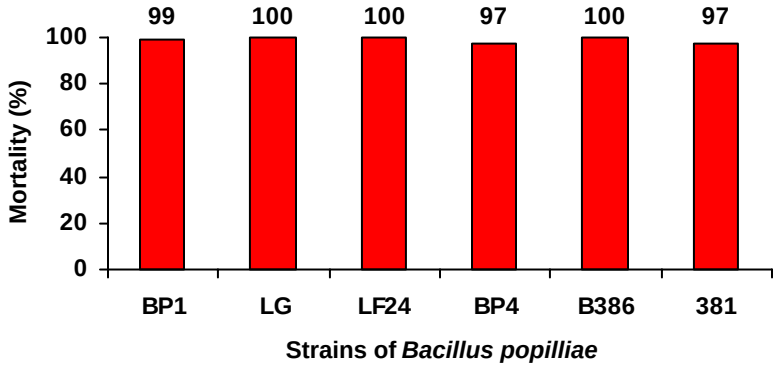

Figure 3 Mortality of *Phyllophaga menetriesi* caused by six strains of *Bacillus popilliae*.

Discussion. Injection of bacteria spores showed good results in the sense of control; however, results indicated that it is not the appropriate method for bacteria multiplication in the insect. This can be explained by the fact that injection is an unnatural way of inoculation. Normally, the larva takes in the bacteria by feeding. For this reason we suggest to use forced alimentation for future studies. All six strains caused a high mortality of all three insect species and are promising candidates as biological control agents of *P. menetriesi* and other whitegrub species.

Recommendations

- We recommend using all six strains for further studies.
- Study pathogenicity of the selected Bp on the *P. menetriesi* using the concentrations of $1x10^5$ and $1x10^8$.
- Morphological, molecular and biochemical characterization of these six strains

References

CIAT. Annual Report 2003. Integrated pest and disease management in major agroecosystems. Soil pests – Cassava and other crops. Pp 53-70.

Tanada, Y. & H. Kaya. 1993.Insect Pathology. California, Academic Press Inc. USA.

Contributors: Carolina Buitrago, Anuar Morales, Rosalba Tobón, Sonia Ximena Restrepo, Andreas Gaigl (CIAT), James Montoya (Universidad del Valle).

Activity 10. Control of *Cyrtomenus bergi* using entomopathogenic nematodes.

Introduction

During the last 13 years researchers in Colombia tested native and introduced strains for the biological control of the burrower bug *Cyrtomenus bergi* (BB): *Heterorhabditis bacteriophora* as the endemic species and *Steinernema carpocapsae* as introduced strain from the US (Caicedo & Bellotti 1994; Barbarena & Bellotti 1998).

The mentioned research revealed that adults and nymphs at fifth instar are the most susceptible stages to EPN. 60% of the adults and 10 to 50% of the fifth instar nymphs were penetrated by exotic EPN. 65 to 100% of the Dauer Juvenile (DJ) of native strains parasitized the fifth instar, 85 to 100% adults (ibid). Recent greenhouse studies showed that 50% of 25,000 DJ / ml penetrated into the host (CIAT 2003).

The objectives of this study were
1. To determine the efficiency of various strains of entomopathogenic nematodes as antagonists of *Cyrtomenus bergi* under laboratory conditions.
2. To evaluate of the lethal dose of two commercial EPN strains vs. adult burrower bugs in the laboratory.

Methodology: We selected a commercial *H. bacteriophora* strain (E-nema) and two *S. feltiae* strains for these studies **(Table 1**). We obtained some control of *C. bergi* with the commercial strain in greenhouse experiments (see following section). Now, we used this strain as contrast to the native *Steinernema feltiae* (Villapinzón, Cundinamarca). The latter nematode is not only of interest due to its native origin, but also for combining ambusher and cruiser characters (Parada 2003, personal communication). The experiments were conducted with adult *C. bergi.*

Table 1. EPN strains applied for the control of *C. bergi* in the laboratory.

Species	Origin	Source	Date of reception
Steinernema feltiae	Colombia	J.C. Parada*	March / 2003
Steinernema feltiae	Germany	E-nema**	June / 2003
Heterorhabditis bacteriophora	Germany	E-nema**	June / 2003

Supplied by * Julio Cesar Parada, National University Bogotá; ** E-nema, Raisdorf, Germany.

EPN Rearing: We reared EPN "in vivo", using larvae of *Galleria mellonella* as hosts. This method is divided in three parts: infection, harvest, and storage. We maintained the nematodes in the incubator at 10 to 15 °C in complete darkness. The steinernematids are cleaned and reactivated every six months, the heterorhabditids every four months.

Experimental Unit: We filled transparent plastic cups (2 ounces) with 20 g sterile and hydrated (3%) sand. We added one pregerminated maize seed and one BB to each cup and sealed it. We applied a suspension of 1 ml with EPN and 1 ml of distilled and sterile water (DSW). For the control treatments we used only DSW. These units were stored in black plastic bags at complete darkness in order to maintain humidity. Food was renewed once a week. Evaluations were carried out every 14 days according to the periods of moulting, also in the case of the fifth instar

that lasts about 30 days. In the second experiment the evaluation interval for adults was three weeks.

Evaluated Variables

i. Rate of infectivity of EPN. We evaluated dead and live bugs, dissecting them under stereoscope.
ii. Rate of mortality of the BB. We dissected every bug to check the presence of EPN.
iii. Rate of melanization. Melanized EPN in the dissected bugs were counted.
iv. Rate of mortality of larvae of *Galleria mellonella.* In order to have a control for the described experiments we evaluated the mortality of moth larvae after 72 hours.
v. Rate of infectivity and mortality according to EPN strain and EPN concentration. We took the commercial concentration of EPN as base for these experiments and two higher and two lower concentrations, following a geometric progression; the control without EPN was the sixth dose **(Table 2**).

Table 2. Dose of commercial EPN strains (E-nema) for the control of *C. bergi.*

Concentration	Steinernema feltiae	Heterorhabditis bacteriophora
C1	100,000	50,000
C2	10,000	5,000
C3	1,000	500
C4	100	50
C5	10	5
C6	0	0

C3 = commercial dose.

Experimental Design: The experiments were completely randomized, where the factors were the two EPN strains and the six stages of the bug with a check without EPN and another one observing the infectivity of Galleria. In the second experiment we used two EPN strains at six concentrations. We used 10 bugs per treatment with four replicates. As check with *G. mellonella* we used 20 larvae per strain.

Statistical Analysis: An ANOVA and a posterior Duncan Test were realized to establish and evaluate differences between variances. It was necessary to transform the value with the equation $\sqrt{x+1}$. In the figures we used the original data in percentage. Finally, we made a regression analysis to measure the impact of the treatments on the experiments.

Results

Objective 1: Efficiency of various strains of entomopathogenic nematodes as antagonists of *Cyrtomenus bergi* under laboratory conditions

Infectivity of tw o EPN strains vs. six stages of *C. bergi*: We did not observe any significant differences between the strains but between the stages of the BB. The fourth instar was the most invaded (42%), followed by the adult bug and the fifth instar (28.1% and 25%, respectively) **(Figure 1**).

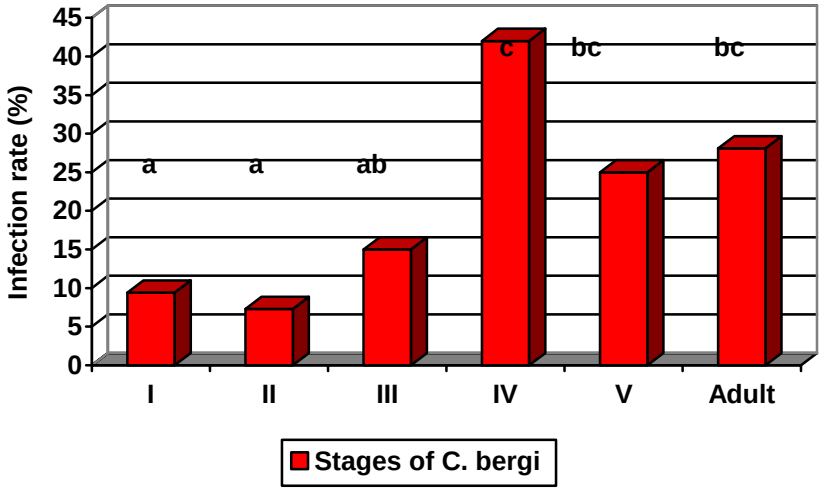

Figure 1. Infection rate of two EPN strains on six stages of the burrower bug (bars with the same letter are not different; p < 0.05)

Mortality of two EPN strains vs. six stages of *C. bergi*: Similar to the rate of infectivity of the two EPN strains **(Figure 1)** didn`t differ in mortality of their hosts. For this reason we present here the means of both strains. The fourth and fifth instar were the most suceptible and differed significantly from the the first two instars **(Figure 2)**. All larvae of *G. mellonella* were invaded by the EPN after 48 hours, indicating that the EPN were in good conditions.

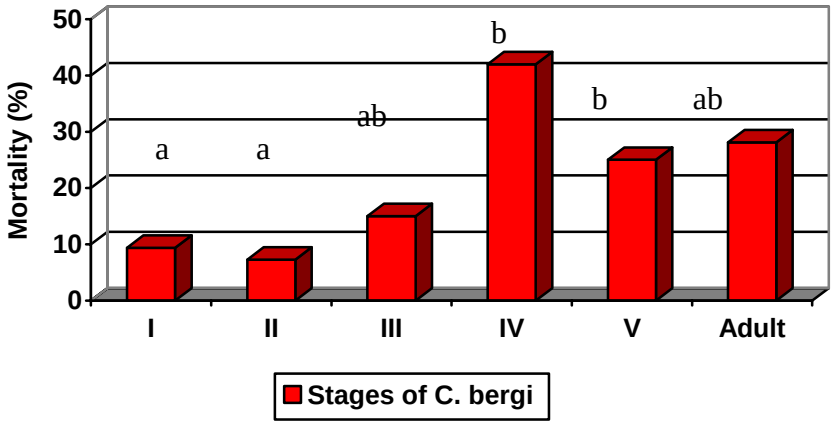

Figure 2. Mortality of six stages of the burrower bug vs. two EPN strains (bars with the same letter are not different; p < 0.05)

Objective 2: Evaluation of the lethal dose of two commercial EPN strains vs. adult burrower bugs in the laboratory.

Infectivity rate of two EPN strains applied in six concentrations vs. *C. bergi:* Much more *S. feltiae* penetrated the host than *H. bacteriophora* **(Figure 3)**. The highest penetration was in

correlation with the highest concentration; however, it is difficult to explain, why concentration C2 obtained a penetration of almost 60%.

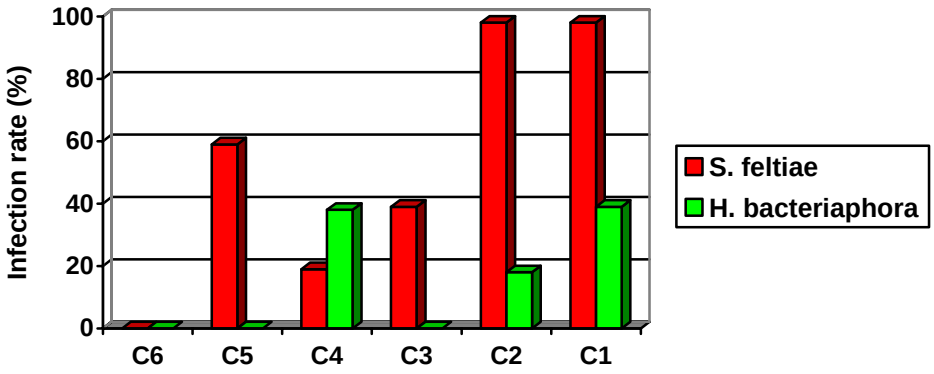

Figure 3. Rate of infection on EPN in six concentrations vs. adults of *C. bergi*

Mortality rate of tw o EPN strains applied in six concentrations vs. *C. bergi:* Surprisingly, only *S. feltiae* caused mortality of the BB **(Figure 4**). Mortality was correlated with EPN concentration (r = 0.8293, p<0.05).

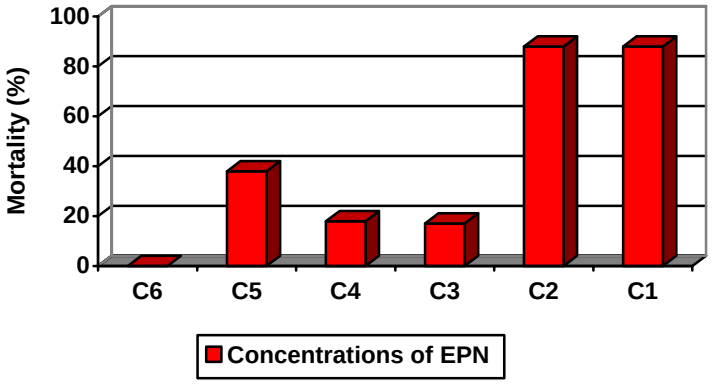

Figure 4. Mortality rate of adult burro wer bugs exposed to different doses of *S. feltiae* (in percent) (concentrations see Table 3-Activity 5).

Melanization of EPN: *S. feltiae* showed a higher susceptibility to melanization than *H. bacteriophora* **(Figure 5**). Melanization rate and EPN concentration were correlated (r = 0.8068).

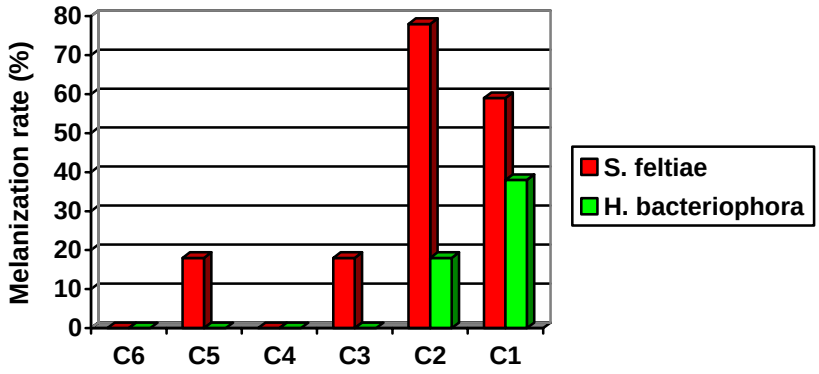

Figure 5. Rate of melaniz ation of EPN as defense mechanism of C. bergi (in percent) (concentrations see Table 3-Activity 5)

Control Treatment: We observed that a native and an introduced strain killed all *G. mellonella* larvae within a period of 48 hours.

Conclusions

- Infectivity and mortality caused by EPN did not differ in any stage of *C. bergi* between introduced *H. heterorhabditis* (E-nema) and native S. feltiae. However, we observed significant differences between host stages. The most susceptible stages were fifth and fourth instar nymphs. This finding should be considered for further attempts of biological control vs. *C. bergi*.
- The two commercial strains of *S. feltiae* and *H. bacteriophora* (E-nema) differed considerably. *H. bacteriophora* didn't cause mortality in any of the six stages. As expected we also observed differences between EPN concentrations.
- It is curious that *S. feltiae* suffered the highest melanization rate but still remained as the more efficient strain.
- We obtained a high mortality of BB when we applied 100,000 EPN / ml. This is an extremely high dose rate and not feasible for a biological control under field conditions. Recognizing the relatively low mortality of even the most efficient strains (S. feltiae)of about 20% and the efficient defense mechanisms of *C. bergi* question if EPN are the appropriate tool for successful biological control. However, the defense mechanism must be understood to be able to make a final conclusion. Moreover, EPN can be combined with sublethal doses of insecticides as we successfully performed with entomopathogenic fungi (see later section) vs. BB or authors vs. whitegrubs (*e.g.* Koppenhöfer *et al*. 2000).
- In opposite to BB, young whitegrubs in the first, second, or even early third instar seem to be susceptible for EPN suggesting to study the set new experiments with these young whitegrub larval stages.

Recommendations: Some values were far out from normal and were are difficult to be explained suggesting to repeat these experiments.

References

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Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 11. Evaluation of two strains of entomopathogenic nematodes as natural enemies of *Cyrtomenus bergi* under greenhouse conditions.

Introduction

During recent years there has been an increasing interest in the biological control of pests by means of entomopathogenic nematodes (EPN). As a consequence, it became necessary to describe native species and the interest in exchanging material between institutions has increased. The classical biological control has generated various examples for the successful introduction of exotic EPN as control agents of important pests (Bedding & Akhurst 1975; Parkman *et al*. 1993, Kaya, H.K. 1993). Janssen (1993) mentioned some limiting factors for a successful introduction of EPN: Target host and habitat, nematode suitability and quality, seasonality, application strategy, soil characteristics, compatibility with pesticides, and biotic factors in the soil. The introduction of these organisms may include some risks: range expansion, host range and impact on non-target organisms, effect on community dynamics and displacement, permanence of introduction and vulnerability of target habitat, and the relationship with symbiotic bacteria (ibid.)

The deliberate release of exotic EPN has taken place for more than a century around the globe. The releases have often been ineffective but have very rarely been troublesome. Unlike classical biological control agents, EPN are not normally expected to establish following release. Wild-type EPN have very complex survival repertoires that may be sacrificed in favor of better production and performance of the DJ and in favor of ecological safety in inundative application (Downes 1996).

The objective of this research was to determine the efficiency of *Steinernema feltiae* and *Heterorhabditis bacteriophora* vs. the burrower bug (BB) *Cyrtomenus bergi*. Both are commercial strains and were provided by E-nema, Germany.

Methodology: As continuance of the previously described laboratory experiment we realized this work in the greenhouse at CIAT experimental station. The temperatures oscillated between 18 and 28 °C and RH between 55 and 85%. The BB were taken from the lab colony. We used adults and the fifth instar, which are the most vulnerable stages to EPN according to earlier experiments presented in an earlier section of this report.

The experiment was designed as a completely randomized block with the factors 2x2+1 and three replicates. We evaluated two factors: the two ages of the BB and the two EPN strains. As the first control treatments we applied distilled water to the bugs. As second control we infested larvae of *G. mellonella* with both EPN strains. We evaluated EPN infection and mortality rates of the insects after 15 and 30 days after the application of nematodes (daa).

We used groundnuts (*Arachis hypogaea*) as host plant which were sowed 15 days before we released 20 bugs /pot. Each unit had six pots (15 x 15 x 10 cm), which were combined with transparent acetates as cages **(Figure 1**).

Figure 1. Experimental units w ith acetate c ages w here host plants and insects (adults and fifth instar) were confined

We applied the EPN according to the provider's instructions: Nemaplus (*S. feltiae*) with 1000 DJ / ml water and 7.7 ml / pot and Nematop (*H. bacteriophora*) with 417 DJ / ml water and 18.47ml / pot. After the application we watered each pot with 40 ml water in order to facilitate the propagation of the EPN.

The data were subjected to ANOVA and the treatment means were compared by Tukey-Test ($P<0.05$). The data were homogenized by $\sqrt{x+1}$ before analysis. We used the original percentage values for the figures.

The evaluated variables were:

1. Infectivity of EPN (Infection). The bugs were dissected and checked for daa. Only the infected bugs were considered counted independently of the quantity of penetrated EPN.
2. Mortality rate.
3. Melanization rate.
4. We verified the virulence of EPN in a control treatment using larvae of *G. mellonella* and "White Traps". We applied simultaneously in a multiple well dish BB and moth larvae and applied a solution of 300 µl with EPN in each well that contained 3 g sand. Moreover, we checked the external characteristics of larvae of *G. mellonella* five daa of EPN as additional control of nematode virulence.

Results: Both EPN strains did not show any significant differences in rate of infection. There was almost no penetration of EPN into fifth instar nymphs; however, adults were infected by 90% and 75%. Infestations between days after application were not significant ($p<0.01$) **(Figure 2**).

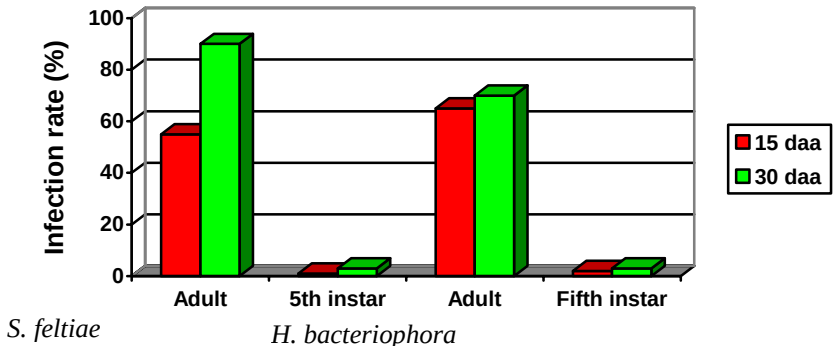

Figure 2. Infection rate of two commercial EPN strains on two stages of *C. bergi* and after 15 and 30 days of application (daa)

Both EPN strains achieved a higher mortality on adults than on fifth instar (p<0.01). We also observed a higher mortality caused by *H. bacteriophora* at 15 daa **(Figure 3**). After 30 daa differences were not significant.

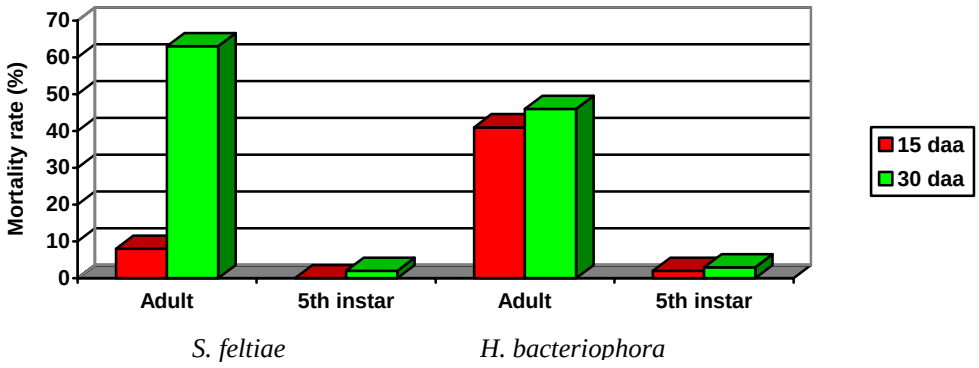

Figure 3. Mortality rates of two commercial EPN strains on two stages of *C. bergi* and after 15 and 30 days of application (daa)

Only EPN in adult showed symptoms of melanization. *H. bacteriophora* caused a higher rate of this defense mechanism after 30 days (37.5%) than *S. feltiae* (13.2%). Of all 180 evaluated adult bugs 120 specimens were invaded by EPN after 15 days and 160 after 30 days **(Figure 3**). 45 individuals (28%) of these bugs harbored melanized EPN after 30 days (dead, rigid, and dark brown coloring) **(Figure 3**). The period of harboring (15 and 30 daa) EPN did not show significant differences. The low number of BB with melanized EPN and the low mortality indicate that this insect possesses more defense mechanism than only melanization. This conclusion is strengthened by the observation that the utilized EPN killed all *G. mellonella* larvae. More research is needed to understand the defense mechanisms of the BB.

All inoculated EPN maintained their viable character confirmed by the 100% mortality of *Galleria* larvae. However, we didn't find any penetration of EPN in the fifth instar. Moreover, *S. feltiae* didn't cause any mortality in adults, whereas *H. bacteriophora* obtained a mortality of 49%. This

result is contradictory to the experiment in the lab (see **Figure 4**) where *S. feltiae* obtained the highest mortality.

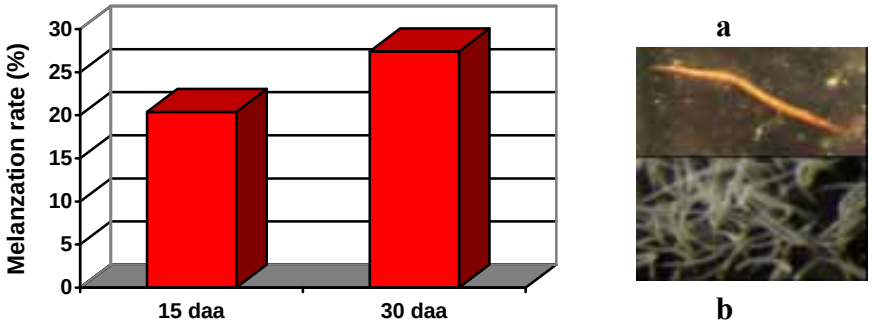

Figure 4. Percentage of BB harboring melani zed EPN; a. mela nized EPN, b. healthy EPN after 15 and 30 days of application (daa)

Conclusions

- The adult BB is more susceptible to EPN than the fifth instar
- Infectivity can be evaluated 15 daa; however, mortality should be determined after 30 dda
- *S. feltiae* was the most affected strain by melanization
- The applied commercial products remained viable during the entire experiment

Recommendations

- These experiments should be repeated in order to verify the obtained results and include other EPN strains.
- Experiments should be realized in order to understand the defense mechanisms of BB

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Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 12. Control of *Cyrtomenus bergi* using entomopathogenic fungi.

M.Sc. Thesis. Faculty of Horticulture. University of Hannover, Germany.

In this study we investigated the pathogenicity of four strains of entomopathogenic fungi (EF) against the subterranean burrower bug *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) under laboratory and greenhouse conditions. In order to select the most promising EF species/strain for *C. bergi* control, in the first part of the study the pathogenicity of three native Colombian strains of *Metarhizium anisopliae* (CIAT 224, CIAT 230, CIAT 245) and one of *Paecilomyces* sp. (CIAT 308) were tested against fourth instar nymphs of *C. bergi* in sand bioassays under laboratory conditions. In the initial screening as well as in the re-infection host passage experiments, comparatively low levels of corrected mortality were obtained never exceeding 50% (twenty days after treatment). *M. anisopliae* strains CIAT 224 and CIAT 245 caused the highest corrected mortality (34.7 and 49.3%, respectively) at the end of the initial screening. Based on these results the efficacy of combined applications of *M. anisopliae* strain CIAT 224 and a sub-lethal dose of imidacloprid (Confidor® SC 350) was evaluated under laboratory and greenhouse conditions. Initially, the *in vitro* fungitoxic effects of three different concentrations of imidacloprid on the germination and vegetative growth of *M. anisopliae* colonies were studied. After 24 hours germination ranged from 97-98.6% and was not significantly affected by any dose rate of imidacloprid present in the Potato Dextrose Agar (PDA) culture medium. On the other hand, the vegetative growth of the fungus was affected by the presence of imidacloprid in the medium. Significantly greater *M. anisopliae* colony size was found when the fungus was cultured in PDA medium amended with 300 ppm of imidacloprid. Subsequently, *C. bergi* nymphal mortality was assessed in a sand bioassay. Significantly higher *C. bergi* nymphal mortality was always recorded when *M. anisopliae* was applied in combination with imidacloprid compared to sole applications of the fungus. A *M. anisopliae* treatment (1E+07 conidia/gram of sand) in combination with 30 ppm of imidacloprid resulted in 87.2% nymphal mortality compared to 34.2% when the fungus was applied alone at the same dose rate 20 days after application. Sub-lethal doses of imidacloprid not only enhanced the efficacy of the fungus but also lead to a reduced quantity of inoculum needed to cause high levels of nymphal mortality. For instance 80.3% mortality was recorded 25 days after application of 1E+06 conidia/gram of sand of *M. anisopliae* strain CIAT 224 and a sub-lethal dose of imidacloprid; this level of mortality did not differ significantly from a 10-fold decreased concentration (i.e., 1E+05 conidia/gram of sand) and the same imidacloprid dose. Combined applications of *M. anisopliae* and a sub-lethal dose of imidacloprid also resulted in high *C. bergi* mortality when applied to a native Colombian soil under greenhouse conditions. Mortality levels in nymphs in the combined treatment 30 days after application were 87.1 and 82.6% in sterile and non-sterile soil, respectively, compared to 66.5 and 32.4% in the same soil types following a sole application of *M. anisopliae*. In conclusion, the results of this thesis indicate that the use of combinations of *M. anisopliae* and a sub-lethal dose of imidacloprid might become an important component within an IPM strategy against *C. bergi* and possibly also other subterranean pests in Colombia, thereby enabling farmers to reduce the use of highly toxic synthetic insecticides like chlorpyrifos and carbofuran, currently the most common control strategy for *C. bergi*. However, in a next step combination of *M. anisopliae* and a sub-lethal dose of imidacloprid need to be tested under field conditions.

Contributors: Juliana Jaramillo-Salazar, Christian Borgemeister, Lemma Ebssa (IPP, University Hanover), Gisbert Zimmermann (BBA, Darmstadt), Rosalba Tobón, Sonia Ximena Restrepo, Rodrigo Zúñiga, Carmen Elisa Mendoza, Andreas Gaigl (CIAT), and Esther Cecilia Montoya (Cenicafé).

Activity 12. Behavior of *Cyrtomenus bergi* as res ponse to the presence of entomopathogenic fungi by means of radiography.

Introduction

Soil insects like whitegrubs and crickets are able to avoid the sites where their natural enemies are present. using a methodology that includes the creation of a microcosm and the use of radiography it was possible to corroborate this behavior for larvae of the Japanese Beetle (*Popillia japonica)*, European Chafer (*Rhizotrogus majalis*), Oriental Beetle (*Anomala orientalis*) and nymphs or adults of crickets. Determining the soil insect's behavior is important for the selection of a natural enemy. Moreover, biochemical studies should identify responsible metabolites emitted by the entomopathogenic fungi (EPF) that have this repellent effect.

Methodology

Experiment 1: We used white PVC tubes of ¾ and ½ inches diameter and filled 60g soil type Cornell with 10.5% humidity from both ends with 100 in order to have an equal density. Then, we introduced one 5th instar of the BB and a pregerminated maize grain via an aperture of 0.6 cm diameter in the middle of the tubes **(Figure 1)**. We took radiographies of different potential (45, 50, 55, 60, 65, and 70 Hz) and exposition periods (5, 10, 15, and 20 sec). We realized eight replicates of each treatment. The treatments are presented in **Figure 2**.

The units were placed in an incubator (25 °C, 70% RH, 100% darkness). We took radiographies after 0, 1, 2, 3, 4, 5, 6, 12, 24, 36, 48, 60, 72, and 96 hours after introducing the insect into the experimental unit.

Figure 1. Experimental units and assembling of experiment. The left photo show s the tubes for taking radiography; the right the introduction of bugs of the 5th instar.

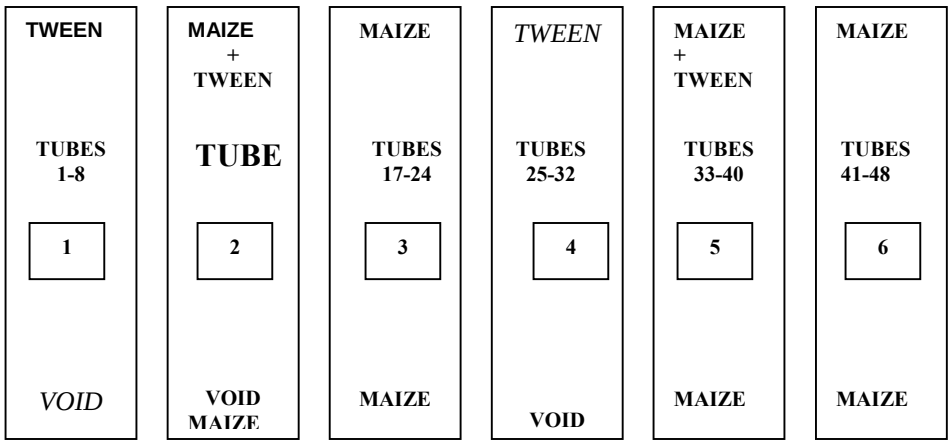

Figure 2. Treatments of Experime nt 1: movement detection of *C. bergi* by means of radiography.

Experiment 2: The experimental setup was the same as in Experiment 1 with some modifications: we used only 60 Hz and exposed the film only 15 sec. The treatments are displayed in **Figure 3**.

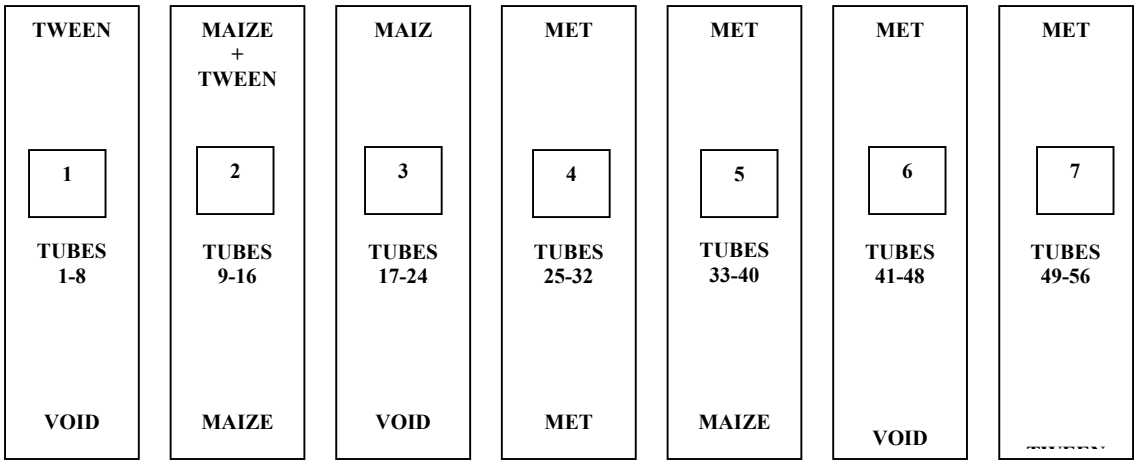

Figure 3. Treatments of Experime nt 2: movement detection of *C. bergi* by means of radiography. MET= *Metarhizium anisopliae* (USDA collection, this strains was isolated from the Japanese Beetle).

Results

Experiment 1: It was not possible to detect the insect in the tubes of ¾ inches because the photography became blurred. Resolution was much better with the tubes of ½ inches. Hence, we could define that the potential of 60 Hz and an exposition of 15 seconds were the optimal

adjustments of the camera. Moreover, we decided to replace the maize by peanuts due to the fast growing roots of the maize that probably allowed the insect not to move.

Experiment 2: The fungus didn't cause any reaction of avoidance by the bug; however, we could observe the insect moving within the tube. The insect moved less when the fungus was present at both ends of the tube compared to the tubes where this pathogen was absent.

Discussion: Following conclusions can be drawn from this preliminary experiment: The deployed methodology is useful to detect movements of the BB; however the lack of a clear tendency of the bug behavior needs to be reflected. One reason may be that the fungus wasn't directly isolated from the BB and it might be possible, that it is not even pathogenic to the insect. Another explanation might be that the spore concentration was too low compared to former experiments realized with whitegrubs and crickets. Hence, we recommend continuing these interesting studies deploying higher concentrations and volumes of spores and use strains with known pathology to *C. bergi*.

Contributors: Anuar Morales (Cornell University), Daniel Peck, Andreas Gaigl.

Activity 13. Production of *Galleria mellonella* within 44 days for studies with entomopathogens.

The larvae of *Galleria mellonella* are commonly used in entomological studies on biological control, such as tests of infection and mortality by entomopathogenic organisms such as nematodes, fungi or bacteria, capturing entomopathogens from the soil, and mass production of these organisms. For this reason we need a high and rapid production of healthy larvae of the last instar. Here we present the 7 steps of our new technology.

Step 1
Sterilize all recipients with hypochlorite and UV-radiation.

Step 2
Introduce glass paper with paraffin bended like an accordion into recipient, then, adults (10 males and 20 females) are confined in these pots and kept during five days in darkness.

Step 3

100 g artificial diet per recipient.
Normal: 500g wheat bran, 145g yeast, 72g bee wax, 150ml glycerin, 270ml bee honey, 1% formaldehyde of all liquids.

Alternative: 400g wheat bran, 100g yeast, 70g bee wax, 400g glycerin, 300g honey, 200g milk powder, 200g maize mill, 100g wheat germs.

The paraffin paper is introduced into the trays. Once the larvae have hatched, the food has been consumed and the larvae have moulted into the third instar the trays have to be moved to bigger trays and added more food. Moreover, the paraffin paper has to be removed in order to prevent eggs from hatching and to obtain a greater homogeneity of size and age of the larvae.

Step 4
The larvae should be permanently revised for infection by fungi or bacteria. Is this the case then the tray has to be removed immediately. If the infection is limited to only some individuals it is sufficient to replace them by healthy ones.

Step 5
Diet should be provided twice a week. The alternative diet should be given twice a month. Larvae have to be divided when the tray is overcrowded. Larvae climbing the walls of the trays in spite of sufficient food is available is an indicator that the tray is overcrowded. Then, a part has to be transferred to a new tray.

Step 6
When the first larvae start to web the cocoon and reduce food consumption it is time to select larvae for experiments. The not considered larvae can be left in their trays and stored in an incubator in order to delay moulting to pupae and adults.

Step 7
It is recommended to carry the whole time a big tray with paper towels in order to transfer potential pupae into this tray. The future adults that are necessary for the colony will be obtained in these trays.

Contributors: Catalina Ramirez, Elsa Liliana Melo, and Andreas Gaigl.

Activity 14. Publications, Conferences, Workshops, Training, Students.

Publications

Accepted

Struck, E., L. Ebssa, R.-U. Ehlers, H.-M. Poehling, A. Gaigl and C. Borgemeister. 2004. Interactions between host plants, the subterranean burrowing bug, *Cyrtomenus bergi*, and the entomopathogenic nematode *Heterorhabditis megidis.* Nematology (July 2, 2004).

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Awards

Zuluaga, C.A., M.S. Serrano, L.C. Pardo and A. Gaigl. 2004. Fluctuacion poblacional, identificacion de larvas y enemigos naturales de chisas en pasto Kikuyo en Cundinamarca. Presented at XXX Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 17-19, Cali, Colombia. Third place of Premio Francisco Luis Gallego, July 30, 2004.

Training

- Training in taxonomy of scarabs Melolonthidae for the assistants of CIAT Cassava Entomology. September 6–10, 2004. The course was held by Jhon Cesar Neita, taxonomist of the Universidad Nacional, Bogotá.
- Training of farmers and extensionists in Pescador (Cauca), Quimbaya and Armenia (Quindío) in biology and basic taxonomy of whitegrubs. In both sites we realized the capacitating twice. The first course was to evaluate farmer's knowledge of whitegrubs, in the second course farmers learned to identify the most important whitegrub species and they got familiar with the project objectives and activities (February 17 and June 2, 2004, in Quimbaya and Armenia, respectively) and April 6 in Pescador (Cauca).
- Field day organized by Erupcion, Manizales, an association of asparagus producers, where the latest results of research on the control of the burrower bug were presented. (June 15, 2004).

Thesis Students

Carlos Alberto Ortega (M.Sc., Escuela Politécnica del Ejército, Quito). Evaluation of whitegrub damage (Coleoptera: Melolonthidae) after natural infestation on cassava *Manihot esculenta* in Pescador (Cauca).

Carolina Buitraga Aya (Universidad del Valle). Evaluación de la patogenicidad y virulencia de *Bacillus popilliae* Dutky sobre larvas de tercer instar de *Phyllophaga menetriesi* Blanchard (Coleoptera: Melolonthinae).

Thesis Completed

German Andreas Calberto (Universidad Autonoma de Occidente, Cali). Estudio del ciclo de vida de *Phyllophaga menetriesi* B. (Col: Melolonthidae) en condiciones de cría artificial.

Catalina Ramirez (Universidad Autonoma de Occidente, Cali). Obtencion de *Galleria mellonella* (Lepidoptera: Pyralidae) en un periodo de 44 días para estudios con entomopathogenos.

Project Staff List

Elsa Liliana Melo
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BEAN ENTOMOLOGY

Activity 1. Developing germplasm with resistance to pests: Bruchids, pod weevil, leafhopper, and *Thrips palmi*

Screening for sources of resistance to major insect pests

Rationale: Identification of sources of resistance to major insect pests of beans is a continuous activity. Additional work is conducted trying to identify and characterize the mechanisms of resistance to specific major pests.

Materials and Methods: Bruchid nurseries are tested in the laboratory simulating normal storage conditions (20° C, 80% R.H., and 14 % seed humidity). Genotypes are tested using 3-5 replications of 50 seeds per genotype. Evaluation units (replicates) are infested with 7 pairs of *Z. subfasciatus* per 50 seeds or two eggs per seed in the case of *A. obtectus*. *T. palmi*, leafhopper and pod weevil nurseries are planted in the field under high levels of natural infestation, usually with 3-4 replicates per genotype in randomized complete block designs. Evaluations for resistance include damage and bean production ratings, insect counts, damage counts, and, in some cases, yield components and yields.

Results and Discussion

Bruchids

Acanthoscelides obtectus: Using a novel Double Congruity Backcross technique developed at CIAT, the Biotechnology Unit has been able to develop fertile insterspecific *Phaseolus vulgaris* x *P. acutifolius* (common x tepary) bean hybrids using the tepary genotype NI576 (a genotype competent to *Agrobacterium*-mediated genetic transformation). Some of these crosses involve the tepary accession G 40199 an excellent source of resistance to the bean weevil, *Acanthoscelides obtectus*. In 2002 and 2003 we identified several progenies containing both *P. vulgaris* and *P. acutifolius* cytoplasm with very high levels of antibiosis resistance to *A. obtectus*. In 2004, emphasis was placed upon the reconfirmation of resistance in previously selected progenies. As shown in **Table 1** , one hybrid containing *P. vulgaris* cytoplasm and seven containing *P. acutifolius* cytoplasm showed high levels of resistance to the insect (< 20% adult emergence). Resistance in some cases was as high as that of G 40199, the resistant check.

Table 1. Resistance to *Acanthoscelides obtectus* in selected $F_{3,5}$ hybrid progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses.

Code and Generation	Cross	Percentage Adult Emergence	Days to Adult Emergence	Percentage Seeds Damaged
Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. vulgaris* cytoplasm				
T7K – 2F F_3	V-DCBC5 x V-DCBC4	67.5	38.7	66.6
T7K – 2E F_3	V-DCBC5 x V-DCBC4	7.1	71.0	10.0
T7K – 2 8B F_4	V-DCBC5 x V-DCBC4	100.0	39.2	100.0
T7K – 2 8A F_4	V-DCBC4 x GNV	99.2	39.6	100.0
T7K – 2 - 6 F_5	V-DCBC5 x V-DCBC4	100.0	38.6	100.0

Code and Generation	Cross	Percentage Adult Emergence	Days to Adult Emergence	Percentage Seeds Damaged
Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm				
GKA – 12R F_3	A-DCBC7-2 x A6	54.8	53.9	86.1
GKA – 12R F_3	A-DCBC7-2 x A6	77.4	44.5	96.0
GKX – 6B F_3	A-DCBC8-2	12.7	48.0	33.3
GNVAV – 200A F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	12.2	44.4	20.7
GNVAV – 200B F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	88.2	42.9	100.0
GNVAV – 200D F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	2.7	41.0	8.7
GNVAV – 200G F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	1.1	67.0	3.3
GNVAV – 200H F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	0.0	N.E	0.0
GVV – 101 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	55.9	47.1	76.7
GVV – 102 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	71.1	47.1	90.0
GVV – 104 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	55.6	49.4	79.3
GVV – 107 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	57.1	45.5	100.0
GVV - 108 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	11.1	56.6	36.1
GVV - 108 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	62.2	44.2	75.0
GVV - 110 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	36.3	48.6	57.8
Checks				
G 12882 Arc 1	Susceptible wild *P. vulgaris* accession	78.3	35.8	100.0
G 12952 Arc 4	Susceptible wild *P. vulgaris* accession	75.0	46.3	100.0
G 40168	Susceptible *P. acutifolius* accession	88.3	41.8	100.0
G 25410	Susceptible *P. lunatus* accession	93.3	42.7	100.0
RAZ 44	Susceptible *P. vulgaris* line	98.3	36.9	100.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	96.6	31.7	100.0
G 40199	Resistant *P. acutifolius* accession	3.3	85.7	12.2
G 25042	Resistant *P. lunatus* accession	1.6	80.0	5.3

After multiplication of resistant seeds in the greenhouse, some of these hybrids again showed high resistance to *A. obtectus* (< 20% adult emergence) in replicated tests **(Table 2)**.

Table 2. Resistance to *Acanthoscelides obtectus* in selected $F_{5,6}$ hybrid progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses.

Cross Code	Hybrid Number	Cross	Percentage Adult Emergence	Days to Adult Emergence	Percentage Seeds Damaged
		Hybrids			
GNVAV	200A9 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	18.7	54.0	32.6
GNVAV	200D21 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	47.9	51.2	52.6
GNVAV	200D22 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	31.9	52.7	63.8
GNVAV	200G16 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	14.4	58.1	31.3
GNVAV	200G17 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	30.6	52.0	45.2
GNVAV	200G18 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	0.6	71.0	1.8
GNVAV	200G19 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	13.0	56.0	25.5
GNVAV	200H5 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	0.8	71.0	2.8
GVV	110G F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	5.2	58.0	18.8
GVV	110 I F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	21.1	48.9	36.6
GVV	108 N F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	14.6	55.7	37.0

Cross Code	Hybrid Number	Cross	Percentage Adult Emergence	Days to Adult Emergence	Percentage Seeds Damaged
		Checks			
G 12882 Arc1		Susceptible wild *P. vulgaris* accession	66.4	36.0	100.0
G 12952 Arc4		Susceptible wild *P. vulgaris* accession	60.0	47.6	100.0
G 40168		Susceptible *P. acutifolius* accession	65.2	43.4	100.0
G 25410		Susceptible *P. lunatus* accession	90.4	42.4	100.0
ICA Pijao		Susceptible *P. vulgaris* cultivar	93.9	30.6	100.0
G 40199		Resistant *P. acutifolius* accession	16.3	58.5	39.0
G 25042		Resistant *P. lunatus* accession	1.5	62.0	7.1

We also tested three interspecific hybrids with *P. vulgaris* cytoplasm (all susceptible) and eight with *P. acutifolius* cytoplasm, three of which (BWG – 5N F_3, BWG – 6Y F_3, and BWG – 1F F_3) showed resistance **(Table 3)**. After multiplication of selected seeds, replicated reconfirmation tests revealed intermediate resistance (20-50% adult emergence) in some of these hybrids **(Table 4)**.

Table 3. Resistance to *Acanthoscelides obtectus* in selected segregating F_3 hybrid progenies derived from *Phaseolus vulgaris* x P. *acutifolius* crosses.

Code and Generation	Type of Material	Percentage Adult Emergence	Days to Adult Emergence	Percentage Seeds Damaged
	Hybrids			
TZT – 4A3 F_4	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with P. vulgaris cytoplasm	82.2	35.9	73.3
TZT – 4A1 F_4	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with *P. vulgaris* cytoplasm	73.3	42.1	80.0
TZT – 1E F_4	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with *P. vulgaris* cytoplasm	96.0	40.0	100.0
BWG – 5N F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with *P. acutifolius* cytoplasm	11.3	58.5	31.1
BWG – 5M F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with *P. vulgaris* cytoplasm	52.6	45.9	78.1
BWG – 5S F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with *P. acutifolius* cytoplasm	80.0	50.2	96.7
BWG – 6Y F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm	48.8	52.8	85.0
BWG – 6W F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm	94.5	41.9	100.0
BWG – 1F F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm	39.8	49.3	67.0
BWG – 1A F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm	64.4	46.3	76.7
BWG – 1G F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm	95.6	39.2	93.3
	Checks			
G 12882 arc 1	Susceptible wild *P. vulgaris* accession	78.3	35.8	100.0
G 12952 arc 4	Susceptible wild *P. vulgaris* accession	75.0	46.3	100.0

Code and Generation	Type of Material	Percentage Adult Emergence	Days to Adult Emergence	Percentage Seeds Damaged
G 40168	Susceptible *P. acutifolius* accession	88.3	41.8	100.0
G 25410	Susceptible *P. lunatus* accession	93.3	42.7	100.0
RAZ 44	Susceptible *P. vulgaris* line	98.3	36.9	100.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	96.6	31.7	100.0
G 40199	Resistant *P. acutifolius* accession	3.3	85.7	12.2
G 25042	Resistant *P. lunatus* accession	1.6	80.0	5.3

Table 4. Resistance to *Acanthoscelides obtectus* in selected segregating F_4 hybrid progenies derived from *Phaseolus vulgaris* x P. *acutifolius* crosses.

Cross Code	Hybrid Number	Percentage Adult Emergence	Days to Adult Emergence	Percentage Seeds Damaged
	Hybrids			
BWG	1F7 F_4	43.2	43.4	70.1
BWG	1F13 F_4	45.4	46.4	90.5
BWG	1F14 F_4	44.4	43.4	73.0
BWG	1F18 F_4	25.6	43.7	53.8
BWG	5N1 F_4	33.8	49.9	64.1
BWG	5N4 F_4	24.4	55.3	55.1
BWG	6Y6 F_4	29.5	50.7	64.8
BWG	6Y15 F_4	17.5	51.4	35.1
	Checks			
G 12882 Arc 1	Susceptible wild *P. vulgaris* accession	66.4	36.0	100.0
G 12952 Arc 4	Susceptible wild *P. vulgaris* accession	60.0	47.6	100.0
G 40168	Susceptible *P. acutifolius* accession	65.2	43.4	100.0
G 25410	Susceptible *P. lunatus* accession	90.4	42.4	100.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	93.9	30.6	100.0
G 40199	Resistant *P. acutifolius* accession	16.3	58.5	39.0
G 25042	Resistant *P. lunatus* accession	1.5	62.0	7.1

The tedious but important process of testing individual seeds to detect segregation in interspecific hybrids continued in 2004 **(Table 5)**. Those selected for resistance were multiplied but the seed did not germinate. One intraspecific *P. lunatus* hybrid that did germinate (coded V5) showed a very high level of resistance comparable to that of the resistant accession G 25042. Two double congruent hybrids with *P. acutifolius* cytoplasm (GKVGAG 1B 4D F_5 and GKVGAG 1E 2C $F_{5)}$ were selected for further testing **(Table 6)**.

Table 5. Reconfirmation of resistance to *Acanthoscelides obtec*tus in pre-selected segregating hybrid progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses and intraspecific *Phaseolus lunatus* crosses

Code and Generation Type	of Material	Number of Seeds Evaluated	Number of Resistant Seeds	Days to Adult Emergence
	Hybrids			
GNVAV-21 F_3	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with *P. acutifolius* cytoplasm	4	3	N.E[a]
GKA 11 F_2	Interspecific *P. vulgaris* x *P. acutifolius* hybrid with *P. acutifolius* cytoplasm	15	3	N.E
Z99ZX6 F_2	Double congruent hybrid with *P. acutifolius* cytoplasm	6	1	N.E
Z99ZX–1A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	20	8	N.E[1]
Z99ZX–11A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	7	1	N.E
ZX99-15 F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	7	3	N.E
ZXTG31-4-10 F_4	Double congruent hybrid with *P. acutifolius* cytoplasm	12	5	N.E
GKVGAG-1A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	31	31	N.E
A6 F_2	Intraspecific *P. lunatus* hybrid	9	7	N.E
VS42-14 F_2	Intraspecific *P. lunatus* hybrid	5	4	N.E
V5 F_2	Intraspecific *P. lunatus* hybrid	31	31	N.E.
VS42-7 F_2	Intraspecific *P. lunatus* hybrid	6	6	N.E
	Checks			
G 40168	Susceptible *P. acutifolius* accession	15	0	42.6
G 25410	Susceptible *P. lunatus* accession	15	0	44.4
RAZ 44	Susceptible *P. vulgaris* line	15	0	38.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	15	0	31.9
G 40199	Resistant *P. acutifolius* accession	15	9	N.E
G 25042	Resistant *P. lunatus* accession	15	9	N.E
G 25713	Resistant *P. lunatus* accession	26	24	N.E

[a] N.E., no adult emergence from resistant seeds.

Table 6. Reconfirmation of resistance to *Acanthoscelides obtec*tus in pre-selected segregating F_5 hybrid progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses.

Code and Generation	Type of Material	Number of Seeds Evaluated	Number of Resistant Seeds	Days to Adult Emergence
	Hybrids			
GKVGAG 1B 4D F_5	Double congruent hybrid with *P. acutifolius* cytoplasm	25	22	N.E[a]
GKVGAG 1E 2C F_5	Double congruent hybrid with *P. acutifolius* cytoplasm	59	58	N.E
	Checks			
G 12882 ARC1	Susceptible wild *P. vulgaris* accession	20	0	37.5
G 12952 ARC 4	Susceptible wild *P. vulgaris* accession	20	0	53.3
G 40168	Susceptible *P. acutifolius* accession	20	0	45.2
G 25410	Susceptible *P. lunatus* accession	19	0	43.7
RAZ 44	Susceptible *P. vulgaris* line	20	0	37.3
ICA Pijao	Susceptible *P. vulgaris* cultivar	20	0	30.5
G 40199	Resistant *P. acutifolius* accession	19	19	N.E
G 25042	Resistant *P. lunatus* accession	20	20	N.E

[a] N.E., no adult emergence from resistant seeds.

Contributors: C. Cardona, J. F. Valor, A. Mejía, S. Beebe, and J. Tohme.

Pod weevil (*Apion godmani*)

Rationale: The pod weevil is one of the most important pests of beans in Mexico and Central America. As indicated in previous reports, we are attempting to develop a molecular marker for *Apion* resistance. This work has been conducted in close collaboration with Dr. Ramón Garza from INIFAP. In order to support the molecular work, new phenological data were obtained by testing for resistance in the field a set of 54 recombinant inbred lines (RILs) developed in 2002. The lines are derived from a cross between Jamapa (a susceptible cultivar) and J-117 (a highly resistant Mexican landrace). The materials were tested at two locations (Santa Lucía de Prías in Mexico State and Atotonilco in Hidalgo State) in replicated nurseries using three replications per material in a randomized complete block design. The infestation in Santa Lucía was low and unreliable for proper resistance evaluation. That in Atotonilco was high and reliable to discriminate between susceptible and resistance genotypes.

The population of RILs was normally distributed for *Apion* resistance **(Figure 1)**, suggesting that the inheritance of resistance to the pod weevil may be governed by more than a single major resistance gene. Even though overall levels of infestation in 2003 were higher than in 2002, there was a significant correlation (r = 0.423; $P < 0.01$) between damage scores obtained in 2002 and in 2003 **(Figure 2)**. The phenological data obtained in 2003 is being used in the development of a molecular marker for pod weevil resistance (for details see SB-2 Report).

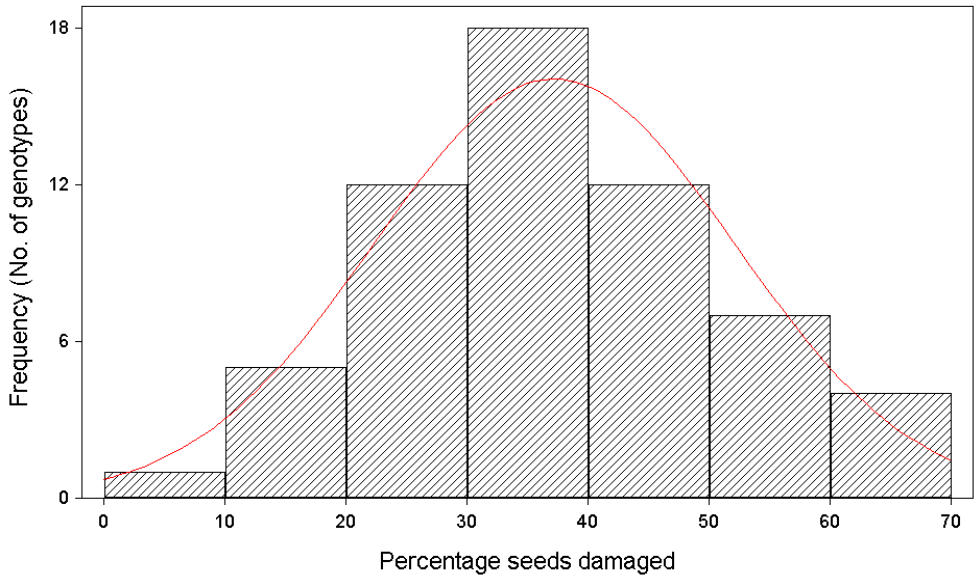

Figure 1. Frequency histogram of percentage seeds damaged by the pod weevil (*Apion godmani*) in a population of 54 recombinant inbred lines derived from a cross between Jamapa (a highly susceptible cultivar) and J-117 (a highly resistant Mexican landrace). The lines were screened under field conditions and high insect populations in a replicated nursery. Atotonilco, Hidalgo State, Mexico, 2003B.

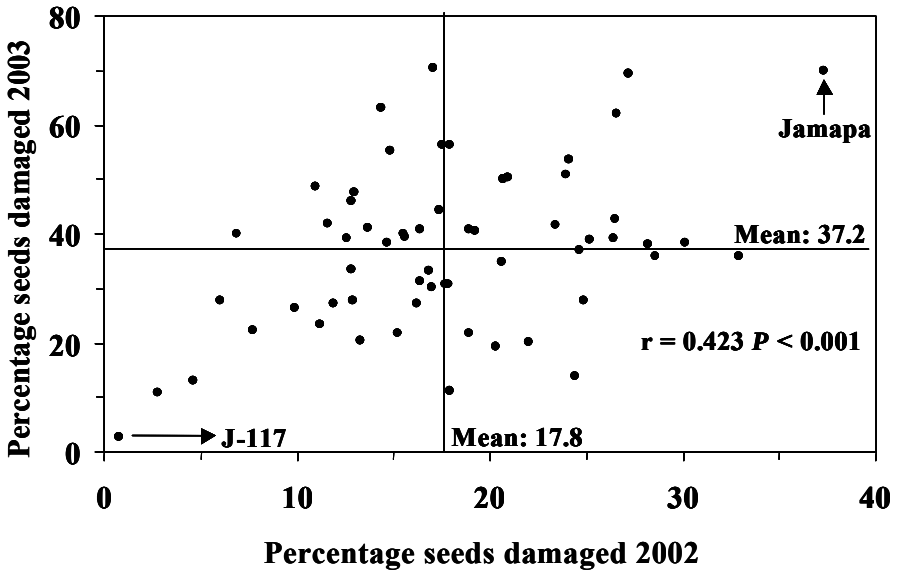

Figure 2. Percentage seed damage in 54 recombinant inbred lines (RILs) tested for resistance to the pod weevil (*Apion godmani*) in two consecutive trials. RILs derived from a cross between Jamapa (a susceptible cultivar) and the resistance source J-117 (a Mexican landrace). Tests conducted in Atotonilco, Estado de Hidalgo, Mexico.

Contributors: R. Garza (INIFAP), C. Cardona, M. Blair.

Leafhopper (*Empoasca kraemeri*)

In 2004 we screened a total of 549 bean germplasm accessions for resistance to the leafhopper. Those selected in 2003 (33) were reconfirmed in replicated nurseries. Of these, 21 were selected for further testing in 2004. We also gave support to the mainstream breeding activities of the Bean Project by screening a series of nurseries. These included 29 selections made in 2003 individual plant selections in Andean crosses performed with selected EMP lines as parents. Thirteen were yield-tested in 2004. Other yield tests included 13 lines derived from crosses with EMP 250 and lines from crosses with Saladin and 16 Andean lines.

We will highlight the work on evaluation of interspecific *P. vulgaris* x *P. acutifolius* hybrids. Similar to the work with bruchids these progenies were obtained by means of the Double Congruity Backcross technique developed at CIAT. We tested 189 progenies (F_2 and F_3) of crosses made with the tepary sources of resistance to leafhopper G 40019 and G 40036. Selected progenies and their reaction to leafhopper are shown in **Tables 7-9**. In general, the best lines show an intermediate level of resistance comparable to that found in the tolerant check, ICA Pijao. It can also be said that resistance to leafhopper in interspecific hybrids is not as good as the resistance found in *P. acutifolius* accessions G 40036, G40019, and G 40019.

Table 7. Resistance to *Empoasca kraemeri* in selected F_{2-5} progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses.

Code Pedigree	[a]	Damage Scores[b]	Reproductive Adaptation Scores[c]	Overall Rating
	Hybrids			
KKQ-11 F_5	V-DCBC x V-DCBC	6.8	3.8	Intermediate
A99Y-15 F_4	V-DCBC x (G40199 X A-DCBC)	6.0	5.3	Resistant
A19Y-103 F_5	V-DCBC x (G40019 X A-DCBC)	6.3	4.0	Intermediate
A19Y-117 F_4	V-DCBC x (G40019 X A-DCBC)	6.4	4.8	Intermediate
A36Y-42 F_5	V-DCBC x (G40036 X A-DCBC)	6.1	4.3	Intermediate
A99Y-86 F_4	V-DCBC x (G40199 X A-DCBC)	6.4	4.8	Intermediate
A99Y-90 F_4	V-DCBC x (G40199 X A-DCBC)	5.9	5.0	Resistant
A99Y-91 F_4	V-DCBC x (G40199 X A-DCBC)	6.3	4.3	Intermediate
ANIY-101 F_4	V-DCBC x A-DCBC	6.9	4.0	Intermediate
A36Y-42 F_4	T-6FB x G36NGP-3FL	6.5	-	Intermediate
EMPZ-2 F_3	A99Y-90 x ZXTGS21-9	6.2	-	Intermediate
EMPZ-5 F_3	A36Y-42 x ZXTGS-21-11	6.7	-	Intermediate
EMPZ-8 F_3	A99Y-103 x ZXTGS49-8	7.0	-	Intermediate
EMPZ-9 F_3	A99Y-103 x ZXTGS49-8	7.0	-	Intermediate
TZTE - 9F F_2	TZT-12FL x EMPZ-3FB	7.0	-	Intermediate
TZTE – 11F_2	TZT-3FL x EMPZ-2FB	7.0	-	Intermediate
TZTE - 20B F_2	TZT-3FL x EMPZ-3FB	6.5	-	Intermediate
TZTE - 71B F_2	TZT-4FL x EMPZ-3FB	5.7	-	Intermediate
	Checks			
BAT 41	Susceptible *P. vulgaris* line	8.8	3.5	Susceptible
EMP 250	Tolerant *P. vulgaris* line	6.4	6.0	Intermediate
EMP 508	Tolerant *P. vulgaris* line	6.2	6.3	Intermediate
EMP 512	Tolerant *P. vulgaris* line	6.0	5.8	Resistant
G40016	Susceptible *P. acutifolius* accession	8.6	3.0	Susceptible
G40019	Resistant *P. acutifolius* accession	5.6	5.5	Resistant
G40036	Resistant *P. acutifolius* accession	5.3	6.0	Resistant
G40056	Susceptible *P. acutifolius* accession	8.8	2.5	Susceptible
G40065	Susceptible *P. acutifolius* accession	8.1	4.8	Susceptible
G40119	Resistant *P. acutifolius* accession	5.1	6.0	Resistant
ICA Pijao	Tolerant *P. vulgaris* cultivar	7.3	6.0	Intermediate
NI576	Susceptible *P. acutifolius* line	8.8	2.0	Susceptible

[a] V-DCBC = Double congruent hybrid with *P. vulgaris* cytoplasm; A-DCBC = Double congruent hybrid with *P. acutifolius* cytoplasm.

[b] On a 1-9 visual scale (1, no damage; 9, severe damage).

[c] On a 1-9 visual scale (1, no yield, no pod formation; 9, excellent pod formation and filling, excellent yield).

Table 8. Resistance to *Empoasca kraemeri* in selected F_{3-6} progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses.

Code Pedigree	[a]	Damage Scores[b]	Reproductive Adaptation Scores[c]	Overall Rating
	Hybrids			
A99Y-15 F_5	V-DCBC x (G40199 X A-DCBC)	6.8	4.5	Intermediate
A19Y-103 F_6	V-DCBC x (G40019 X A-DCBC)	6.8	4.0	Intermediate
A19Y-117 F_5	V-DCBC x (G40019 X A-DCBC)	7.2	4.0	Intermediate
A99Y-86 F_5	V-DCBC x (G40199 X A-DCBC)	6.8	4.5	Intermediate
A99Y-90 F_5	V-DCBC x (G40199 X A-DCBC)	6.6	4.5	Intermediate
A99Y-91 F_5	V-DCBC x (G40199 X A-DCBC)	6.8	4.3	Intermediate
ANIY-101 F_5	V-DCBC x A-DCBC	7.2	3.5	Intermediate
A36Y-42 F_5	T-6FB x G36NGP-3FL	7.0	4.5	Intermediate
EMPZ-8 F_4	A99Y-103 x ZXTGS49-8	7.2	3.5	Intermediate
EMPZ-9 F_4	A99Y-103 x ZXTGS49-8	7.0	3.8	Intermediate
TZTE - 20B F_3	TZT-3FL x EMPZ-3FB	7.4	3.8	Intermediate
EMPZ-2 F_4	A99Y-90 x ZXTGS21-9	6.4	5.0	Resistant
EMPZ-5 F_4	A36Y-42 x ZXTGS-21-11	6.8	4.5	Intermediate
TZTE - 71B F_3	TZT-4FL x EMPZ-3FB	6.5	4.0	Intermediate
	Checks			
G40019	Resistant *P. acutifolius* accession	5.6	5.5	Resistant
G40036	Resistant *P. acutifolius* accession	5.6	5.8	Resistant
NI576	Susceptible *P. acutifolius* line	7.8	2.8	Susceptible
G40033	Susceptible *P. acutifolius* accession	8.8	2.3	Susceptible
G40119	Resistant *P. acutifolius* accession	5.7	5.0	Resistant
EMP 512	Tolerant *P. vulgaris* line	6.1	5.3	Resistant
EMP 508	Tolerant *P. vulgaris* line	6.5	4.3	Intermediate
EMP 250	Tolerant *P. vulgaris* line	6.8	4.3	Intermediate
BAT 41	Susceptible *P. vulgaris* line	9.0	2.0	Susceptible
ICA Pijao	Tolerant *P. vulgaris* cultivar	6.8	4.3	Intermediate

[a] V-DCBC = Double congruent hybrid with *P. vulgaris* cytoplasm; A-DCBC = Double congruent hybrid with *P. acutifolius* cytoplasm.

[b] On a 1-9 visual scale (1, no damage; 9, severe damage).

[c] On a 1-9 visual scale (1, no yield, no pod formation; 9, excellent pod formation and filling, excellent yield).

Table 9. Resistance to *Empoasca kraemeri* in selected F_{3-6} progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses.

Code[a]	Pedigree	Damage Scores[b]	Reproductive Adaptation Scores[c]	Overall Rating
	Hybrids			
TSC	TZTA-1A2L FB x Row 3 FB (A36Y 42)	6.3	4.0	Intermediate
TSC	TZTA-1A2L FB x Row 3 FB (A36Y 42)	6.0	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2L FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTTZ-85R FB x Row 10 FB (EMPZ2)	6.3	4.3	Intermediate
TSC	TZTTZ78M FB x Row 30 FB (TZTE-71)	6.3	4.3	Intermediate
ZXTGS	ZXTG6FB x Row 49 Entry 70FL	6.3	4.3	Intermediate
ZXTGS	ZXTG6FB x Row 49 Entry 70FL	6.3	4.3	Intermediate
ZXTGS	ZXTG6FB x Row 21 FLG36NGP-3F_2	5.7	4.3	Resistant
SCO	ZXTG6FB x Row 49 Entry 70FL	6.3	4.7	Intermediate
SCO	ZXTG6FB x Row 21 FLG36NGP-3F_2	5.7	4.7	Resistant
TSC	TZTA-1A2L FB	6.3	5.5	Resistant
TSC	TZTTZ-98B FL	6.0	5.0	Resistant
	Checks			
G40019	Resistant *P. acutifolius* accession	4.5	6.0	Resistant
G40036	Resistant *P. acutifolius* accession	4.3	6.1	Resistant
NI576	Susceptible *P. acutifolius* line	8.0	2.8	Susceptible
G40033	Susceptible *P. acutifolius* accession	8.8	2.3	Susceptible
G40119	Resistant *P. acutifolius* accession	4.6	5.6	Resistant
EMP 512	Tolerant *P. vulgaris* line	5.9	6.0	Resistant
EMP 508	Tolerant *P. vulgaris* line	6.5	4.3	Intermediate
EMP 250	Tolerant *P. vulgaris* line	6.2	6.0	Intermediate
BAT 41	Susceptible *P. vulgaris* line	8.3	2.6	Susceptible
ICA Pijao	Tolerant *P. vulgaris* cultivar	6.7	5.3	Intermediate

[a] TSC = Double congruent hybrid with *P. vulgaris* cytoplasm; A-DCBC = Double congruent hybrid with *P. acutifolius* cytoplasm; both ZXTGS and SCO possess *P. acutifolius* cytoplasm.

[b] On a 1-9 visual scale (1, no damage; 9, severe damage).

[c] On a 1-9 visual scale (1, no yield, no pod formation; 9, excellent pod formation and filling, excellent yield).

Contributors: J. M. Bueno, C. Cardona. A. Mejía, J. Tohme.

Developing germplasm resistant to insects

For details of breeding activities, please refer to section 2.2.1. As in 2003, studies were aimed at developing Andean type bean with improved tolerance to the leafhopper, *Empoasca kraemeri.* We will highlight results of the work trying to develop Andean type beans (crosses with PVA 773 and CAL 143) with improved tolerance to the leafhopper, *Empoasca kraemeri.* Lines selected for lower damage scores and higher reproductive adaptation scores in previous years performed relatively well under moderate levels of leafhopper infestation (4.3 nymphs per leaf, seasonal average) **(Figure 3)**. Given that susceptibility to leafhopper is usually very high in large-seeded Andean beans, these results indicate that substantial progress has been made in incorporating resistance to leafhopper in these types of beans. Another set of lines derived from crosses between Saladin and selected EMP lines did not perform so well **(Figure 4)**, possibly due to the inherent susceptibility of Pompadour-type beans. Nevertheless, eight lines that showed moderate tolerance were selected for further testing

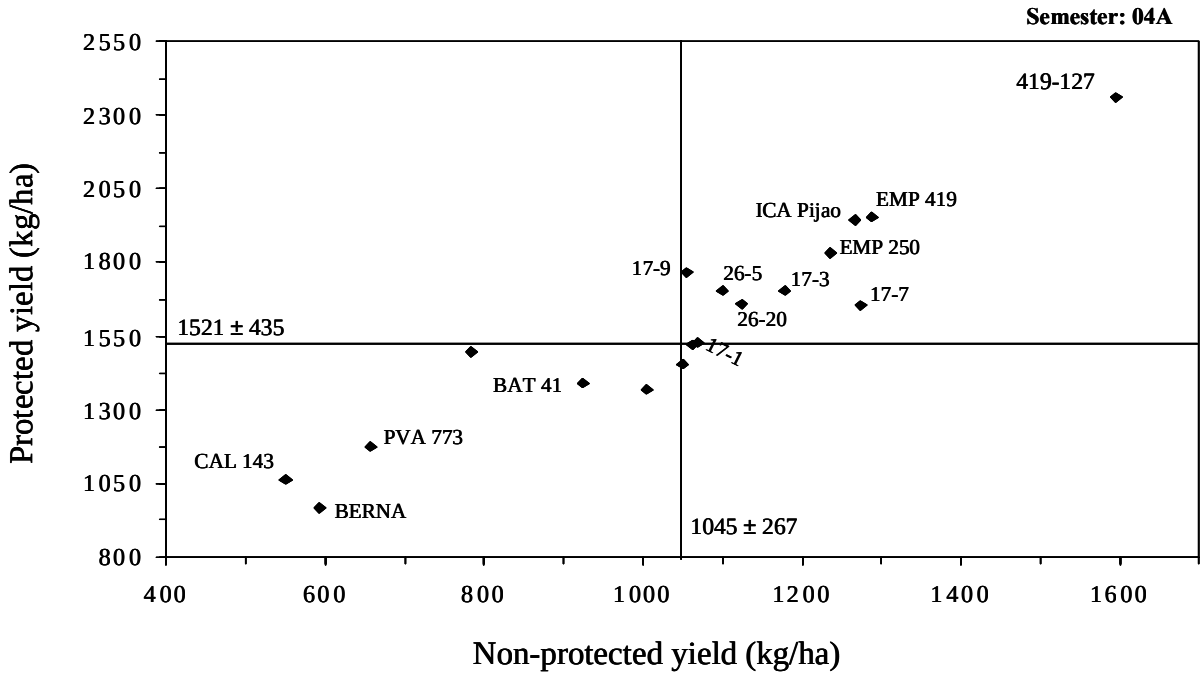

Figure 3. The relationship between protected and non-protected yield in selected Andean bean lines bred for tolerance to *Empoasca kraemeri*. PVA 773 and CAL 143 are susceptible parents. EMP 250 is the tol erant parent. BAT 41 and Pijao are susceptible and tolerant checks, respectively.

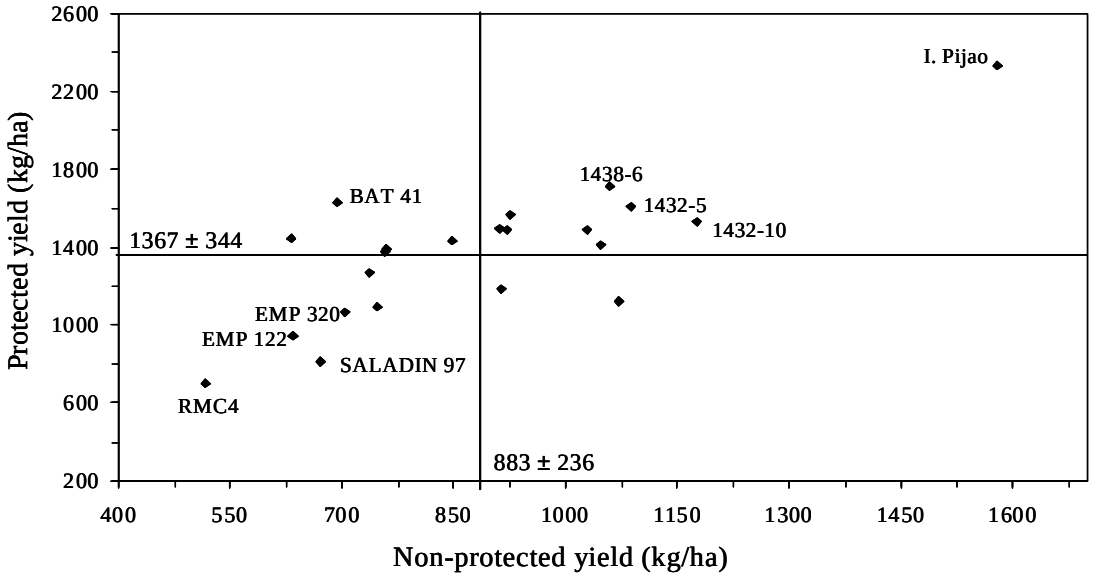

Figure 4. The relationship between protected and non-protected yield in selected Andean bean lines for tolerance to *Empoasca kraemeri*. Saladin 97 is a commercial variety in Dominican Republic.

Contributors: J. M. Bueno, C. Cardona, M. Blair.

Tolerance to leafhopper studies

In 2004 we finished our studies on progress in incorporating tolerance to leafhopper. We performed the combined analysis of variance for the five consecutive trials aimed at measuring the response of EMP lines (bred for leafhopper resistance) and checks to two levels of infestation (3 and 6 nymphs per leaf). These were obtained by exercising chemical control at pre-established action levels. There was not a significant interaction between trials and treatments. At all levels of infestation, EMP 250, EMP 542, EMP 544, and EMP 588 yielded significantly better than the susceptible check BAT 41 and EMP 124. None performed better than ICA Pijao, the tolerant check **(Figure 5).** However, in terms of percentage yield losses, new lines (the EMP 500 series) performed better at all levels of infestation than the improved checks EMP 124 and EMP 250, and, in some cases, better than the standard tolerant check, ICA Pijao. At very high levels of infestation (6 nymphs per leaf) average yield losses in EMP lines was above the 30% level, meaning that even tolerant materials would benefit from integration with chemical control exercised at pre-established action levels.

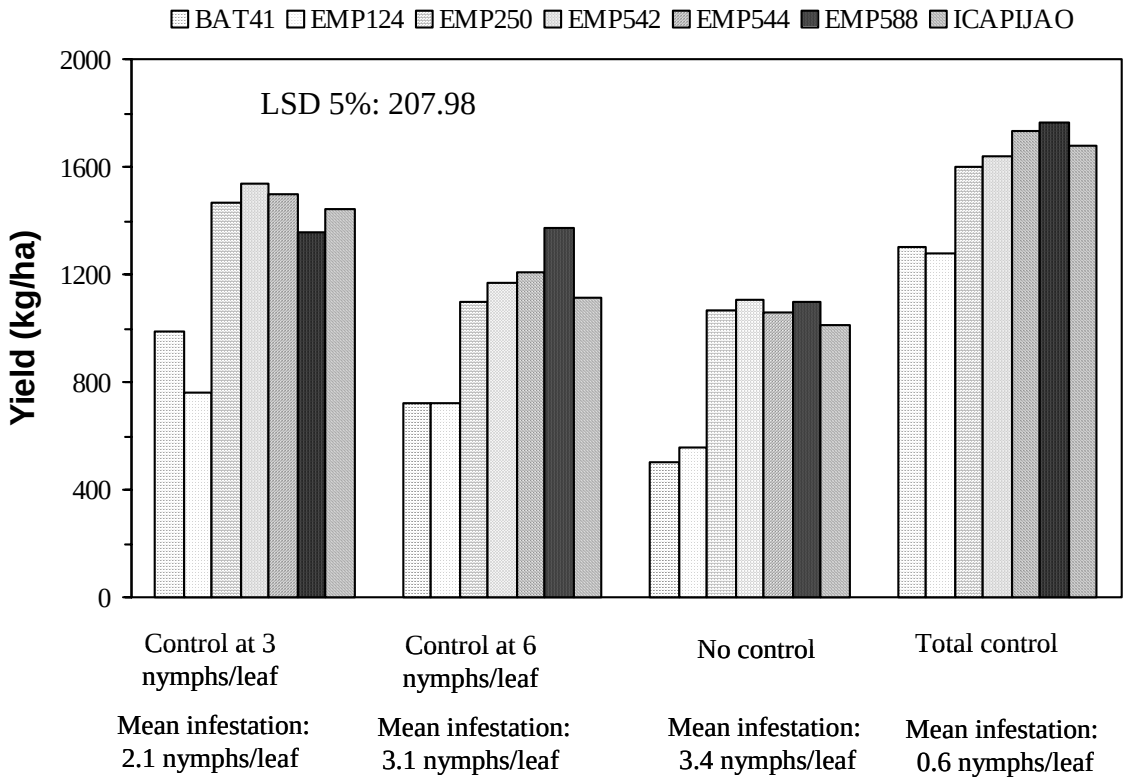

Figure 5. Yields of selected EMP lines and checks (BAT 41, ICA Pijao) at different levels of infestation with the leafhopper *Empoasca kraemeri*. Means of five trials.

Contributors: J.M. Bueno, C. Cardona.

Progress toward achieving output milestones

- Identification of sources of resistance, understanding of mechanisms of resistance to insects, and development of insect resistant bean lines contribute to the mainstream breeding objectives of the Bean Project.
- Insect resistant beans may be basic components for management of insect pests in beans.
- The development of molecular markers for pod weevil, thrips, and bruchids should facilitate breeding for resistance.

Activity 2. Publications, book chapters, workshops, conferences and training.

Journals

Frei, A., H. Gu, J. M. Bueno, C. Cardona, S. Dorn. 2003. Antixenosis and antibiosis of common beans to *Thrips palmi* Karny (Thysanoptera: Thripidae). J. Econ. Entomol. 96: 1577-1584.

Manzano, M. R., J. van Lenteren, C. Cardona. 2003. Comportamiento de búsqueda de *Amitus fuscipennis* (Hymenoptera: Platygasteridae): Tiempo de permanencia en la planta hospedera y actividad de búsqueda. Rev. Colombiana Entomol. 29(2): 221-226.

Frei, A., J. M. Bueno, J. Díaz-Montaño, H. Gu, C. Cardona, S. Dorn. 2004. Tolerance as a mechanism of resistance to *Thrips palmi* in beans. Entomol. Experim. et Appl. 112: 73-80.

Murray, J. D., T. E. Michaels, K. P. Pauls, C. Cardona, A. W. Schaafsma. 2004. Yield and insect injury in leafhopper (*Empoasca fabae* Harris and *Empoasca kraemeri* Ross & Moore) infested dry beans in Ontario and Colombia. Canadian J. of Plant Science 84: 891-900.

Rodríguez, I., H. Morales, J. M. Bueno, C. Cardona. 2004. El biotipo B de *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) adquiere mayor importancia en el Valle del Cauca. Rev. Colombiana de Entomología Vol 30 (In press, accepted for publication March 10, 2004)

Accepted for Publication

Frei, A., M. W. Blair, C. Cardona, S. E. Beebe, H. Gu, S. Dorn. 2004. QTL mapping of resistance to *Thrips palmi* in common beans. Crop Science.

Book Chapter

Cardona, C. 2004. Common beans – Latin America. *In*: Hodges R.J. and G. Farrell, (eds). Crop Post-harvest: Science and Technology Volume 2: Durables. Blackwell Science Ltd. London. ISBN 0632057238.

Workshops and Conferences

Cardona, C. 2004. Tendencias actuales y futuras en el manejo de insectos plaga de importancia agrícola. pp. 38-44 *In*: Memorias I Seminario Internacional y II Nacional de Control Biológico de Plagas y Enfermedades de los Cultivos. Abril, 2004. Escuela Politécnica del Ejército, Quito, Ecuador.

Bueno, J. M., C. Cardona, P. Chacón. 2004. Fenología, desarrollo de métodos de muestreo y distribución espacial de Trialeurodes vaporariorum (Homoptera: Aleyrodidae) en habichuela y fríjol. p. 29 *In*: Resúmenes XXXI Congreso Sociedad Colombiana de Entomología, Bogotá, Julio 28-30, 2004.

Students

Name	Degree	Status	University	Title
Frei, Andrea	Ph. D.	Completed	ETH (Switzerland)	Resistance to Thrips palmi in beans
Bueno, Juan Miguel	M. Sc.	Completed	U. del Valle	Sampling methods for whiteflies on beans and snap beans
Prieto, Sergio	B. Sc.	Completed	U. Nacional, Palmira	Molecular markers for arcelin
Montenegro, María Fernanda	B. Sc.	Completed	U. Nacional, Palmira	Effect of insecticides on natural enemies of whiteflies
Valencia, Sandra Jimena	B. Sc.	Continuing	U. Nacional, Palmira	Sub-lethal effects of antibiosis on the demography of *Zabrotes subfasciatus* and *Acanthoscelides obtectus*, storage pests of beans

Trips

Date	Destination	Event or purpose
November, 2003	Texcoco, Mexico	Evaluate Apion nurseries
March, 2004	Chota, Ecuador	Visit whitefly management trials
May, 2004	Beijing, China	Attend International Plant Protection Congress

Special Projects

Title	Donor	Funding period	Total amount
Integrated management of whiteflies in the tropics	DFID	2001 - 2004	US$ 65,000
Biotechnological tools to improve beans	ABOS	2001 – 2004	US$ 15,000
Post-harvest losses in beans	ZIL	2002 – 2004	US$ 36,000

Courses and Workshops

Date	Title	Duration (days)	Total No. Participants	No. of Women Participants	No. of CIAT Instructors
10/17/03	The B biotype in the Cauca Valley	1	25	3	3
12/16/03	The B biotype in the Cauca Valley	1	25	?	3
01/29/04	The B biotype in the Cauca Valley	1	110	?	1
04/10/04	The B biotype in the Cauca Valley	1	35?	?	1
05/05/04	Whiteflies and their control (field day)	1	76	15?	3
06/04/04	Pests of beans and their control	1	60	?	1
07/27/04	Sampling methods for whiteflies	3	275	?	2
08/05/04	The B biotype B in the Cauca Valley	1	197	?	1

AFRICA: BEAN ENTOMOLOGY

Activity 1. Bean IPM Promotion in eastern, central and southern Africa.

Rationale

The promotion of bean IPM strategies among bean farming communities in eastern and southern Africa had in the past three seasons focused mainly on the management of bean insect pests using both traditional and improved technologies. During the reporting period however, the dimension of the promotional activities was expanded through additional funding support to include the promotion and dissemination of products/outputs from other bean research projects. These include disease tolerant germplasm, improved high yielding pest tolerant varieties and soil fertility management technologies that have been generated from activities supported by different NARS programmes, ECABREN, CIAT, NGOs and other active partners.

Methodology: The participatory approach involving innovative farmers, farmer groups and locally active partners from the local government administration (policy makers, extension personnel, etc.), NGOs, community based organisations-CBOs (civil and religious), local schools and the private sector (market traders and input suppliers) continued to be adopted. Participating farmers, collaborators and partners at activity sites continued to play the major role in planning, implementation and evaluation of project activities with backstopping from the other stakeholders. Traditional and improved pest management technologies were promoted in pilot and satellite sites. Farmers were reached through the standard farmer field school (FFS) approach in the case of south western Uganda and parts of western Kenya, and the modified farmer field school approach (MFFS), i.e. farmer research group (FRG) approach as were the cases in parts of Kisii site in western Kenya, northern and southern Tanzania and central Malawi. Linkages with existing partners was maintained and strengthened. New farmers, farmer groups and partners joined in to support and participate in project activities. The MEDIEA Company Ltd produced a radio programme (Pilika Pilika) on agricultural production, i.e. crops (with focus on beans) and livestock in Kiswahili. The programme has been on air in 4 national radio stations (3 private, 1 public) in Tanzania from March 2004. Pilot studies in to document community behaviour in IPDM uptake have been initiated with a Masters degree student in Hai district site in northern Tanzania. More and new promotional materials were prepared and distributed to target village information centres and partners.

Findings/Observations

Project activities and IPDM awareness creation have spread to wider and new areas during the reporting period and more farmers have received the message **(Table 1)**. The participatory farmer research group approach, farmer meetings, field demonstrations combined with field days and exchange visits), promotional materials including farmer activity reports, village information centres, small seed packets, local farmer seed displays and exchanges, visits to farmer groups (by local administrators and policy makers, donor representatives, CIAT DG and other staff), radio, etc. are proving to be very effective tools in getting the message to the bean farming communities. Observations show that these tools work differently at different sites depending on the community culture and behaviour. No one tool seem to be self propelling at any of the active

sites. Participating and non participating farmers are happy with the approach of involvement in management of their own resources. Partners are willing to contribute to costs involved in farmer exchange visits when such activities are linked to areas of priority for their development goals in those particular communities.

The government policy makers in each of the participating countries (Malawi, Tanzania, Kenya and Uganda) have declared a "YES" to the community group approach. Tanzania has gone ahead to declare the community group approach for its district focus new national planning policy in rural development and community empowerment for food security, poverty eradication and in addressing the HIV/AIDS pandemic. In the uptake studies, 39 farmer groups (out of 77) in 27 villages (out of 54) in Hai district, northern Tanzania have been surveyed. Data processing is in progress. Project promotional materials have been on high demand by participating and non- participating partners. Postage on the CIAT website has led to demands from outside the continent, e.g. a recent request on the leaflet on "Cultivation of climbing beans" from Chile.

Table 1. Spread of bean IPDM project message in eastern, central and southern Africa as per June 2004.

Pilot Site	Satellite Sites	Number of Farmers Reached with at least 1 Technology	Estimated Number of Farmers aware of Bean IPDM Message
Malawi (Dedza)	Kasungu	500	> 1000
Tanzania - Southern (Mbeya and Mbozi)	Mbeya, Mbozi, Iringa, Njombe, Chunya,	7000	>10000
Tanzania - Northern (Hai, Lushoto, Arumeru)	Babati, Rombo, Moshi,	8800	>31000
Kenya (Kisii, Kabondo)	Homabay, Gucha, Marani, Rachuonyo, Vihiga, Hamisi, Kakamega	2500	>3000
Uganda	Kabale, Bushenyi, Kisoro, Iganga		
DR Congo	Katana, Kavumu, Mudaka		
Rwanda	Runyinya		

Discussion: The FFS and FRG members and participating partners were instrumental in training new farmers and helping in the formation of groups. For example, in south-western Uganda, the Kabamare FFS members trained 4 new groups including a polytechnic school community. The FFS group leader has trained several neighboring farmers, helped in setting up demonstrations for the five groups at his site and trained groups collaborating with other partners including NGOs. The whole concept is to use trained farmer groups to be trainers community members at their locations. These innovators were also the key players in spreading the word by mouth to neighbouring farmers and relatives and to the various visitors. Farmers were very happy learning together, sharing information, experiences and resources (e.g. seed, etc.). For example, Rombo district farmers invited by Shari village IPDM groups in Hai (~ 150 km away) for field day with a bean seed sharing event in March 2004, brought local bean seed for 6 different cultivars and in exchange they selected both improved (from bean programme) and local bean cultivar seed from Hai to experiment with in Rombo. In the same field day, visiting Babati

farmers collaborating with and sponsored by Farm Africa, also selected some of the bean seed for experimentation in their fields.

The tools used in disseminating bean and other crop and livestock production products among bean farming communities have helped the project reach farmers beyond expectation. The radio programme in Tanzania, has played a key role in sending the message across communities in the past six months because every bean growing community that we have interacted with have farmers asking questions pertaining to the programme captions. Some of the farmers have participated in the radio question time and won prizes that were contributed by the national bean research programme (improved bean seed packs) and the IPDM project (leaflets).

More farmers in Malawi, Tanzania, Kenya and Uganda have accessed the improved high yielding and pest tolerant bean variety seeds (from the national programmes) and high yielding pest tolerant germplasm (from NARS, ECABREN and CIAT). Dissemination of improved pest tolerant bean varieties particularly focused on products generated in previous bean research projects in the southern highlands of Tanzania, Malawi and Uganda. Despite the unreliable weather conditions that prevailed in most areas in the region during the past bean production period, a number of farmers received the seed and some were able to harvest the grain.

Contributors: E.Minja, E. Ulicky, P. Mviha, H. Mlenga, C.Madata, D. Kabungo, J. Ogecha, F. Makini, F. Opio, M.Ugen, R. Buruchara, K.Ampofo, P. Kanaura, IPDM Project Farmers.

Collaborators: M. Pyndji, R.Chirwa, ECABREN and SABRN partners, AHI, World Vision, ADRA, Farm Africa (Babati), Concern Universal, PLAN International - Malawi, CARE, KADFA, NARS research and extension services.

FORAGE ENTOMOLOGY

Activity 1. Screening *Brachiaria* genotypes for spittlebug resistance.

Continuous mass rearing of spittlebug species in Palmira and Macagual

A permanent supply of insects is essential in the process of evaluating genotypes for resistance to spittlebug. At present, the progress made in mass rearing of nymphs and in obtaining eggs from adults collected in the field allows us to conduct simultaneous screening of large number of *Brachiaria* genotypes for resistance to all major spittlebug species present in Colombia. Insects produced in our mass rearing facilities are used for greenhouse evaluations in Palmira and field evaluations in Caquetá.

Contributors: G. Sotelo, C. Cardona.

Identify *Brachiaria* genotypes resistant to spittlebug

Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species

Rationale: Assessment of resistance to spittlebugs is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. In 2004, intensive screening of selected hybrids was conducted under greenhouse and field conditions.

Materials and Methods: Screenings for resistance were conducted with *Aeneolamia varia, A. reducta, Zulia carbonaria,* and *Prosapia simulans*. Test materials were usually compared with five checks fully characterized for resistance or susceptibility to *A. varia*. Plants were infested with six eggs per plant of the respective spittlebug species and the infestation was allowed to proceed without interference until all nymphs were mature (fifth instar stage) or adult emergence occurred. Plants (usually 5-10 per genotype) were scored for symptoms using a damage score scale (1, no visible damage; 5, plant dead) developed in previous years. Percentage nymph survival was calculated. Materials were selected on the basis of low damage scores (<2.0 in a 1-5 scale) and reduced percentage nymph survival (<30%). All those rated as resistant or intermediate were reconfirmed. All susceptible hybrids were discarded.

Results and Discussion: A set of 731 pre-selected sexual (SX03) hybrids were simultaneously screened for resistance to *A. varia*, *A. reducta*, and *Z. carbonaria*. We used one rep per hybrid per insect species. For comparison, we used 16 well-known checks replicated 10 times per insect species. In terms of damage scores, 78.3%, 84.3%, and 74.9% of the hybrids were rated as resistant to *A. varia*, *A. reducta*, and *Z. carbonaria*, respectively **(Table 1)**. After percentage survival was recorded, 120 hybrids combining low damage levels and high levels of antibiosis resistance were selected for reconfirmation tests. These were conducted using five replications per genotype per insect species. Results **(Table 2)** clearly indicated that a very significant progress has been made in incorporating antibiosis resistance to all of the three test species in a relatively short period of time. The rapid progress made in incorporating resistance to spittlebug

is also illustrated in **Figure 1**. There has been a steady increase in the frequency of resistant genotypes as a result of recurrent selection through cycles.

Table 1. Frequency distribution (percentages) of resistance reactions in a set of 731 sexual *Brachiaria* hybrids screened for resistance to three spittlebug species.

Category	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*	All Three Species
Resistant	64.2	75.2	59.1	39.5
Intermediate	14.1	9.1	15.8	33.9
Susceptible	21.7	15.7	25.1	26.6

Table 2. Levels of resistance to three spittlebug species in selected sexual *Brachiaria* hybrids.

Genotype	Damage Scores			Percentage Nymph Survival		
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*
Elite hybrids						
SX03/2483	1.0	1.0	2.4	8.0	0.0	26.7
SX03/2226	1.0	1.0	1.7	3.3	6.7	16.7
SX03/2061	1.3	1.0	1.3	16.7	0.0	16.7
SX03/4043	1.3	1.0	1.2	10.0	10.0	6.7
SX03/3744	1.0	1.4	1.6	13.3	6.7	3.3
SX03/4351	1.1	1.4	1.4	20.0	13.3	10.0
SX03/3882	1.0	1.3	1.2	13.3	20.0	10.0
SX03/2053	1.0	1.0	2.4	20.0	20.0	33.3
SX03/1100	1.0	1.5	2.7	25.0	21.7	13.3
SX03/4224	1.4	1.2	1.2	20.0	23.2	4.2
SX03/0282	1.0	1.0	1.5	30.0	6.7	6.7
SX03/0770	1.3	1.3	1.2	30.0	10.0	3.3
SX03/1090	1.3	1.7	1.7	30.0	17.3	13.3
SX03/1408	1.0	1.2	1.2	26.7	23.3	13.3
SX03/2784	1.5	1.1	2.0	26.7	16.7	0.0
Checks						
CIAT 36062[a]	1.0	1.4	2.2	25.0	21.7	60.0
SX01NO/0102 [a]	1.6	1.0	2.2	26.7	10.0	20.0
CIAT 0606 [b]	4.6	3.8	4.0	91.7	75.0	53.3
BRX-44-02 [b]	4.8	4.6	3.8	83.3	80.0	68.3

[a] Resistant check.
[b] Susceptible check.

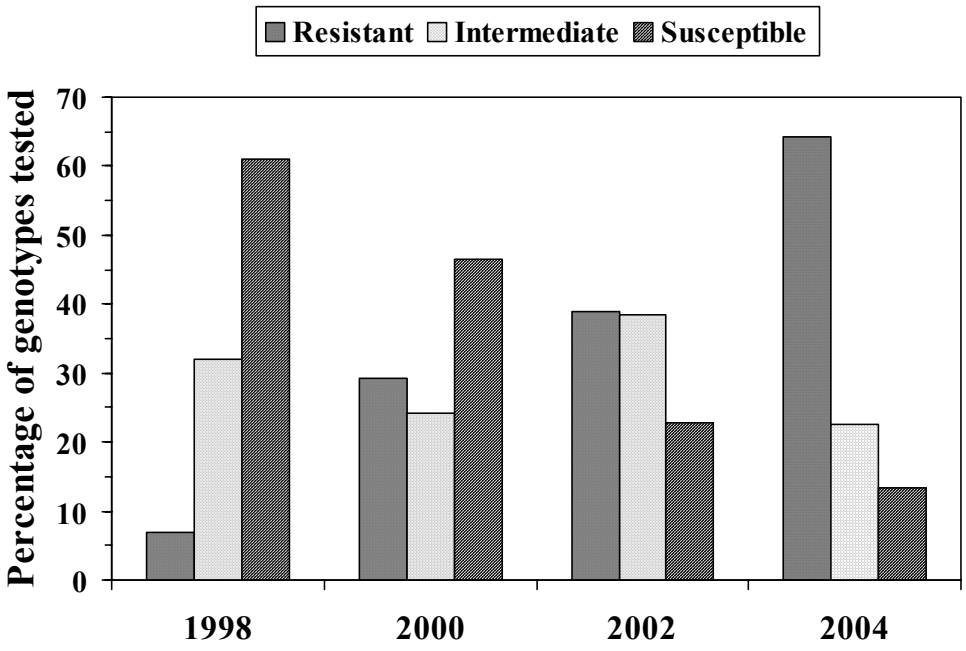

Figure 1. Progress in the incorporation of resistance to *Aeneolamia varia* in *Brachiaria*; note the steady increase in the frequency distribution of resistance genotypes and the decline in the frequency of susceptible genotypes as a result of continuous cycles of selection.

As part of on-going studies on mechanisms of resistance to spittlebug species of economic importance in Mexico, we screened 34 hybrids for resistance to *Prosapia simulans*. These hybrids had been pre-selected in Mexico for good adaptation and desirable agronomic characteristics. Using a level of infestation of six nymphs per plant and 10 replications, the hybrids were compared with four accessions, and two susceptible and two resistant checks. Results **(Table 3)** showed that 11 hybrids have antibiosis resistance to *P. simulans*. This information will be crossed with that obtained in Mexico with the species *Aeneolamia albofasciata* and *A. postica* (part of Ulises Castro's M. Sc. thesis on mechanisms of resistance to Mexican species).

In progress is the evaluation for resistance to *A. varia*, *A. reducta* and *Z. carbonaria* of 422 apomictic hybrids derived from crosses between the highly resistant sexual hybrid SX01NO/0102 and *B. decumbens* 'Basilisk' and other susceptible genotypes. The main purpose of this study is to identify patterns of segregation of resistance for each of the spittlebug species involved. Results will be reported in 2005.

Table 3. Levels of resistance to *Prosapia simulans* in *Brachiaria* hybrids pre-selected for Mexican conditions.

Genotype Dam	age Scores	Percentage Nymph Survival Ra	ting
Hybrids			
MX 1905	1.1	3.3	Resistant
MX 1561	1.3	5.6	Resistant
MX 3056	1.6	1.7	Resistant
MX 1423	1.8	1.7	Resistant
MX 1880	1.8	29.6	Intermediate
MX 3641	2.0	25.0	Intermediate
MX 2295	2.2	10.0	Resistant
MX 1809	2.2	16.7	Resistant
MX 1388	2.2	16.7	Resistant
MX 3567	2.2	26.7	Intermediate
MX 2552	2.2	33.3	Intermediate
MX 1788	2.3	31.5	Susceptible
MX 3426	2.3	26.7	Intermediate
MX 1263	2.4	42.6	Susceptible
MX 3731	2.4	18.5	Resistant
MX 2135	2.5	48.3	Susceptible
MX 2531	2.6	50.0	Susceptible
MX 1942	2.6	41.7	Susceptible
MX 3850	2.7	50.0	Susceptible
MX 2783	2.7	60.0	Susceptible
MX 3213	2.8	9.2	Resistant
MX 1660	2.9	47.9	Susceptible
MX 1769	3.0	3.3	Resistant
MX 1548	3.1	50.0	Susceptible
MX 1565	3.1	31.7	Susceptible
MX 2775	3.1	40.7	Susceptible
MX 1638	3.2	56.7	Susceptible
MX 3861	3.2	66.7	Susceptible
MX 2273	3.2	6.2	Resistant
MX 2090	3.2	23.3	Susceptible
MX 1614	3.3	46.7	Susceptible
MX 3626	3.4	71.7	Susceptible
MX 3582	3.7	75.9	Susceptible
Checks			
CIAT 16827	1.2	5.0	Resistant
CIAT 26110	1.8	21.7	Resistant
CIAT 36087	1.8	1.7	Resistant
CIAT 36061	3.1	27.1	Intermediate
CIAT 36062	1.8	0.0	Resistant check
CIAT 06294	1.6	6.7	Resistant check
CIAT 0606	3.6	50.0	Susceptible check
BRX-44-02	4.0	68.3	Susceptible check
LSD 5%	1.97	12.5	

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Activity 2. Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species.

Rationale: Assessment of spittlebug resistance under natural levels of infestation in the field is very difficult due to the focal, unpredictable occurrence of the insect. This problem has been overcome since 1998 when we developed an artificial infestation technique that allows us to properly identify resistance under field conditions. The purpose of field evaluations is to reconfirm levels of resistance identified under greenhouse conditions.

Materials and Methods: Using the experimental unit described in our 1998 Annual Report, the genotypes (usually 10 replicates) are initially infested in the greenhouse with an average of 10 eggs per stem. Once the infestation is well established, with all nymphs feeding on the roots, the units are transferred to the field and transplanted 10-15 days after infestation. The infestation is then allowed to proceed without interference until all nymphs have developed and adults emerge some 30-35 days thereafter. The plants are then scored for damage by means of the 1-5 visual scale utilized in greenhouse screenings. The number of stems per clump is counted before and after infestation and a tiller ratio (tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process) is then calculated. Using this methodology, 12 major screening trials (four with *A. varia*, four with *Zulia carbonaria*, two with *Z. pubescens*, and one with *Mahanarva trifissa*) were conducted in Caquetá in 2004. The main purpose of these trials was to reconfirm resistance in 22 sexual hybrids (SX01) previously selected in Palmira under greenhouse conditions.

Results an d Discussio n: As shown in **Table 1** , virtually all of the sexual hybrids showed adequate levels of field resistance to all four species tested. Consistently, average damage scores were significantly lower than those obtained with the susceptible checks, CIAT 0606 and BRX-44-02. Tiller ratios for the sexual hybrids were significantly higher than those of susceptible checks, suggesting that antibiosis resistance present in the hybrids protects the plants from intense insect damage, allowing the plant to grow and produce new tillers. On the contrary, susceptible plants lose tillers As in previous occasions there were significant ($P < 0.01$) negative correlations between damage scores and tiller ratios (r = -0.844 for *A. varia*, -0.887 for *Z. carbonaria*, -0.785 for *Z. pubescens*, and -0.697 for *M. trifissa*). This means that damage scores are useful in predicting tiller losses resulting from intense insect damage. One of the commercial checks (CIAT 36087, 'Mulato 2') was resistant. Surprisingly, the commercial check CIAT 36061 ('Mulato'), which is not antibiotic to any spittlebug species, showed a very interesting level of field tolerance both in terms of damage scores and tiller ratios **(Figure 1)**.

Table 1. Damage scores and tiller ratios obtained with 22 selected sexual *Brachiaria* hybrids and checks tested for resistance to *Aeneolamia varia* (Av), *Zulia carbonaria* (Zc), *Z. pubescens* (Zp), and *Mahanarva trifissa* (Mt) under field conditions.

Genotype	Damage Scores				Tiller Ratiosa			
	Av	Zc	Zp	Mt	Av	Zc	Zp	Mt
SX01/NO/0067	1.8	2.1	1.6	1.3	1.09	1.21	1.29	1.62
SX01/NO/0102	2.0	1.7	1.6	1.4	1.29	1.76	1.92	1.60
SX01/NO/0159	1.6	1.8	1.5	1.3	1.37	1.66	1.53	1.82
SX01/NO/0233	2.7	1.9	1.8	1.9	0.94	1.29	1.43	1.33
SX01NO/0263	2.0	1.9	1.6	1.2	1.34	1.48	1.55	1.76
SX01/NO/0446	1.8	1.8	1.6	1.1	1.09	1.26	1.38	1.47
SX01/NO/0878	1.9	1.8	1.8	1.7	1.38	1.44	1.65	1.71
SX01/NO/1090	1.9	1.9	1.8	1.2	1.06	1.61	1.26	1.24
SX01/NO/1175	1.8	2.0	1.8	1.3	1.12	1.26	1.35	1.43
SX01NO/1186	2.1	2.1	1.9	1.4	1.22	1.46	1.34	1.18
SX01NO/1710	1.7	2.0	1.6	1.5	1.33	1.52	1.46	2.72
SX01/NO/2017	1.9	2.0	1.6	1.3	1.48	1.39	1.72	1.83
SX01/NO/2420	1.9	1.8	1.6	1.7	1.35	1.57	1.46	1.18
SX01/NO/2619	1.7	1.7	1.7	1.2	1.11	1.46	1.69	1.49
SX01/NO/3168	1.8	1.8	1.8	1.4	1.10	1.33	1.52	1.38
SX01/NO/3178	1.9	1.8	1.8	1.3	1.12	1.41	1.34	1.47
SX01/NO/3390	2.1	2.3	1.8	1.9	0.92	1.14	1.30	1.14
SX01/NO/3439	1.9	1.9	1.6	1.0	1.25	1.61	1.58	2.22
SX01/NO/3615	1.7	1.8	1.7	1.4	1.22	1.54	1.32	1.47
SX01/NO/4506	2.1	2.1	1.7	1.6	0.92	1.17	1.29	1.44
SX01/NO/4785	1.9	2.0	1.6	1.1	1.22	1.27	1.57	1.83
SX01/NO/4861	1.7	1.7	1.7	1.6	1.28	1.64	1.62	1.74
Mean 22 hybrids	1.9b	1.9b	1.7b	1.4b	1.19b	1.43b	1.48a	1.59a
CIAT 36087	2.0	1.6	1.6	1.4	1.31	1.60	1.50	1.50
CIAT 36061	1.7	1.5	1.3	1.3	1.44	2.00	1.59	1.71
Mean commercial checks	1.8b	1.6bc	1.4c	1.3b	1.37a	1.80a	1.54	1.60a
CIAT 36062	1.6	1.3	1.4	1.1	1.64	1.92	1.69a	1.89
CIAT 6294	1.1	1.4	1.2	1.1	1.22	1.58	1.33	1.46
Mean resistant checks	1.3c	1.3c	1.3c	1.1b	1.43a	1.75a	1.51a	1.67a
CIAT 0606	4.0	3.1	2.9	3.7	0.37	0.62	0.59	0.46
BRX-44-02	4.5	3.5	3.4	4.0	0.30	0.64	0.70	0.54
Mean susceptible checks	4.2a	3.3a	3.1a	3.8a	0.33c	0.63c	0.64b	0.50b

a Tillers per plant at the end of the infestation process/Tillers per plant at the beginning of the infestation process. Means of 10 reps per genotype per species, 4 trials in the case of A. varia and Z. carbonaria, two trials with Z. pubescens and one trail with M. trifissa. Means within a column followed by the same letter are not significantly different at the 5% level according to Scheffe's multiple range test for arbitrary comparisons. Each species analyzed separately.

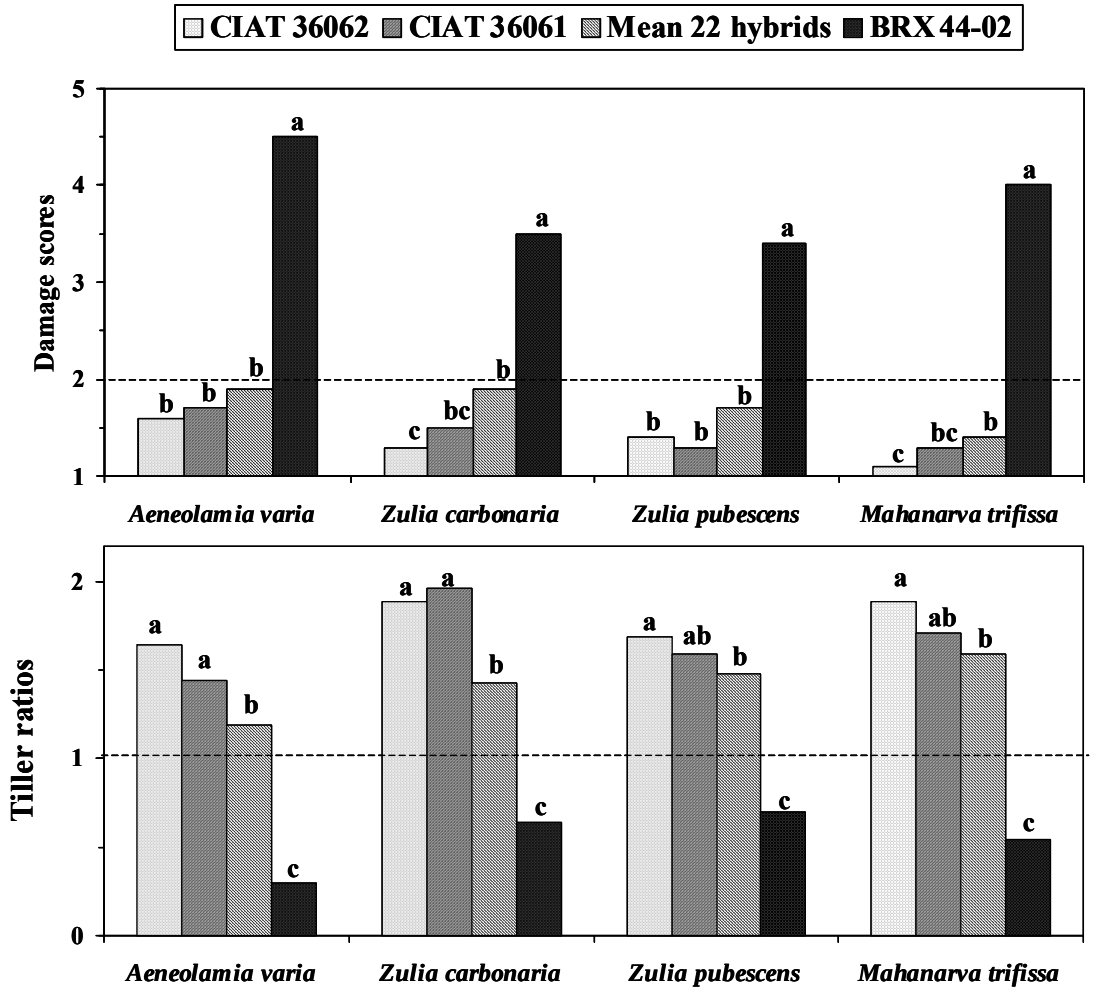

Figure 1. Resistance to four spittlebug species in selected *Brachiaria* genotypes tested under field conditions. Dotted lines represent cut-off points for resistance rating and selection. Within a given spittlebug species, bars with the same letter are not significantly different at the 5% by LSD. Each species analyzed separately.

Damage scores obtained with the 22 sexual hybrids and assorted *Brachiaria* accessions in the greenhouse correlated very well (r = 0.76; $P < 0.01$) with damage scores recorded in the field **(Figure 2)**. This is further proof that the technique we are using to screen for resistance in the field is a reliable one to reconfirm resistance detected under greenhouse conditions.

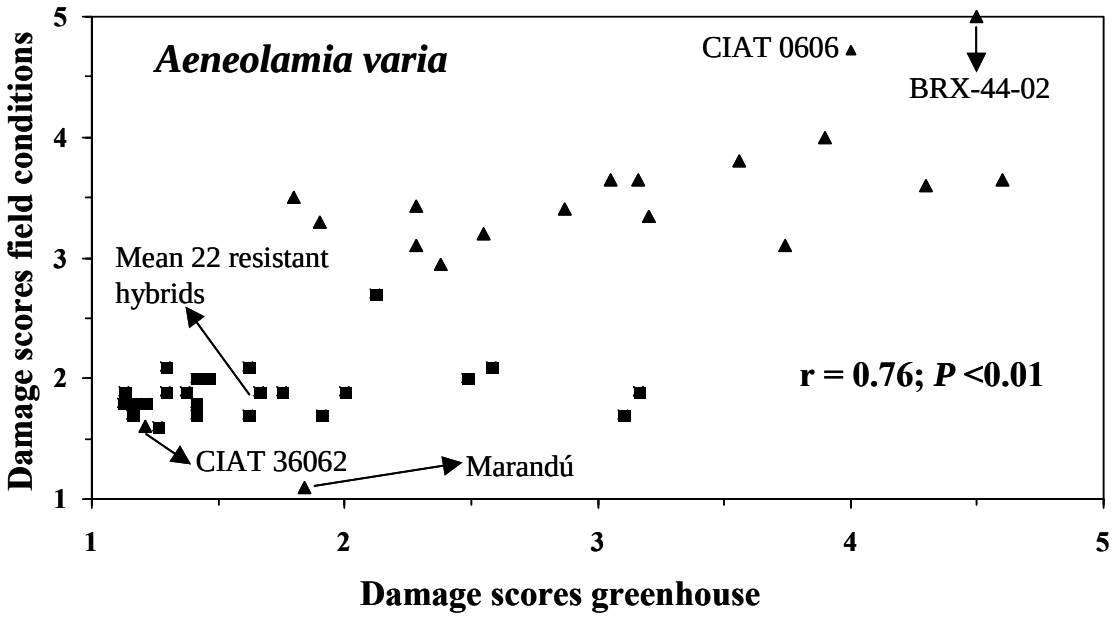

Figure 2. Damage scores obta ined w ith selected sexual *Brachiaria* hybrids (■) and accessions (▲) tested for resistance to *Aeneolamia varia* under greenhouse and field conditions.

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Activity 3. Identify host mechanisms for spittlebug resistance in *Brachiaria.*

Effect of host plant resistance on the demography of *Aeneolamia varia*

Rationale: Varying levels of antibiosis resistance to nymphs of several spittlebug species have been well characterized in a number of resistant *Brachiaria* genotypes. The effects of antibiosis on the biology of nymphs have also been studied. Not much was known about possible direct effects of antibiotic genotypes on the biology of adults. Even less was known about sub-lethal effects (i. e., reduced oviposition rates, reduced longevity, prolonged generation times, reduced rates of growth, etc.) on adults resulting from nymphs feeding on antibiotic genotypes. We initiated a series of studies aimed at measuring how antibiotic genotypes may directly or indirectly (through sub-lethal effects) affect the biology of adults of *A. varia*. We used the life-table technique, which is widely recognized as one of the most effective means of teasing apart the subtle, interrelated aspects of changes in population density. Longevity, age-specific fecundity, sex ratio and generation time can be examined and compared among treatments as they relate to the most important demographic parameter, the intrinsic rate of natural increase.

Materials and Methods: A comprehensive series of experiments aimed at determining whether antibiosis to nymphs has an adverse effect on the demography of *A. varia* were conducted. For this, 18 life tables (nine fecundity, nine complete) were constructed. Treatment combinations are shown in **Table 1**. For each of these treatments we established cohorts of 105 pairs of spittlebug and the fate and reproductive rate of individuals were recorded until death occurred. From these data the following life-table statistics were derived: net reproductive rate (R_o) [net contribution per female to the next generation]; mean generation time (T) [mean time span between the birth of individuals of a generation and that of the next generation]; doubling time (D) [time span necessary to double the initial population]; finite rate of population increase (λ) [multiplication factor of the original population at each time period]; and intrinsic rate of natural increase (r_m) [innate capacity of the population to increase in numbers]. Life-table statistics were analyzed using the SAS program based on jackknife estimates of demographic parameters. Other variables recorded were sex ratios, percentage egg fertility and adult dry weights. These data were submitted to analysis of variance and when the *F* test was significant, we performed mean separation by LSD.

Table 1. Treatment combinations to study po ssible sub-lethal effects of in termediate and high levels of nymphal antibiosis on adults of *Aeneolamia varia*.

Nymphs Reared on:	Resulting Adults Feeding on:	Null hypothesis
BRX 44-02[a]	BRX 44-02	Absolute check
BRX 44-02	CIAT 06294	A genotype that is moderately antibiotic to nymphs does not affect adults
BRX 44-02	CIAT 36062	A genotype that is highly antibiotic to nymphs does not affect adults
CIAT 06294	BRX 44-02	Intermediate antibiosis to nymphs does not affect resulting adults
CIAT 06294	CIAT 06294	Intermediate antibiosis to nymphs does not affect resulting adults even when these are feeding on a moderately antibiotic genotype
CIAT 06294	CIAT 36062	Intermediate antibiosis to nymphs does not affect resulting adults even when these are feeding on a highly antibiotic genotype

Nymphs Reared on:	Resulting Adults Feeding on:	Null hypothesis
CIAT 36062	BRX 44-02	High antibiosis to nymphs does not affect resulting adults
CIAT 36062	CIAT 06294	High antibiosis to nymphs does not affect resulting adults even when these are feeding on a moderately antibiotic genotype
CIAT 36062	CIAT 36062	High antibiosis to nymphs does not affect resulting adults even when these are feeding on a highly antibiotic genotype

[a] BRX44-02 is a highly susceptible accession; CIAT 6294 (an accession) and CIAT 36062 (a resistant hybrid) possess intermediate and high levels of antibiosis resistance to nymphs of A. varia, respectively.

Results and Discussion:

A. Sub-lethal effects of re sistance on adults of *Aeneolamia varia*: The impact of antibiosis to nymphs on the reproductive biology of resulting adults

Both resistant genotypes caused significant effects on the demography of *A. varia*. For simplicity, we will limit the discussion to the results obtained with the most resistant genotype, CIAT 36062. In general, rearing of nymphs of *A. varia* on the resistant genotype had a deleterious effect on the weight of resulting males and on the number and fertility of eggs laid per female **(Table 2)** . Females feeding on the susceptible genotype BRX-44-02 weighted significantly more than those feeding on the resistant genotype.

Table 2. Life history parameters of *Aeneolamia varia* as affected by all possib le combinations of rearing immatu re stage s and feeding resulting adults on susceptible (BRX 44-02) or resistant (CIAT 36062) *Brachiaria* genotypes.

Treatment[a]		Adult Dry Weight (g x 10^{-3})			
Nymphs Reared on:	Resulting Adults Feeding on:	Females	Ma les	Eggs Per Female	Percentage Egg Fertility
BRX 44-02 (S)	BRX 44-02 (S)	5.73a	3.79a	130.4ab	93.0a
BRX 44-02 (S)	CIAT 36062 (R)	4.90b	3.66ab	147.8a	92.6a
CIAT 36062 (R)	BRX 44-02 (S)	5.46ab	3.28b	108.0bc	80.6b
CIAT 36062 (R)	CIAT 36062 (R)	4.37c	3.10c	86.1c	80.4b

[a] S, susceptible; R, resistant.
Within a column, means followed by the same letter are not significantly different at the 5% level by LSD.

Age-specific survival and age-specific fecundity curves for *A. varia* adults are presented in **Figure 1**. Mean survival times for the four treatment combinations did not differ at the 5% level, meaning that there was not a major impact of nymphal antibiosis on the survival of resulting males or females. On the contrary, rearing of the insect on the resistant genotype CIAT 36062 did have a pronounced effect on the ability of resulting females to lay eggs. Independently of the food substrate used to feed the adults, females obtained from rearing the nymphs on the resistant genotype laid less eggs for a slightly shorter period of time, than those obtained from rearing the insect on the susceptible genotype. This can be interpreted as a sub-lethal effect of nymphal antibiosis on the reproductive capacity of the insect.

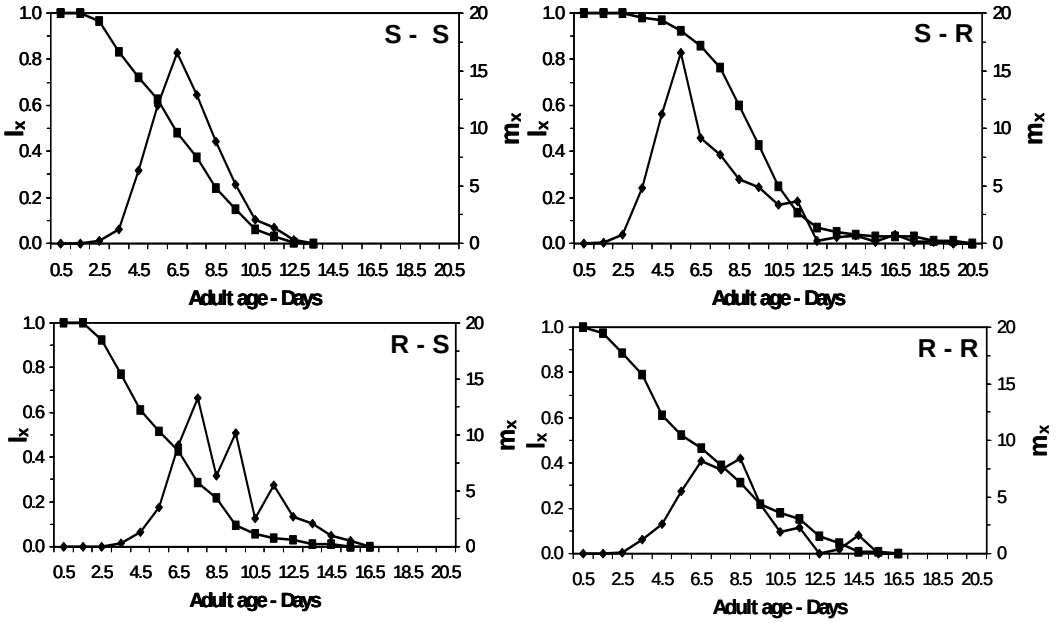

Figure 1. Age-specific survival (l_x) (■) and age-specific fecundity (m_x) (♦) curves for adults of *Aeneolamia varia* as affected by all possible combinations of food substrate for adults and nymphs. First initial letter combinations indicates the food substrate for nymphs followed by the initial for the food substrate for resulting adults. S, susceptible genotype (BRX 44-02); R, resistant genotype (CIAT 36062).

All demographic parameters of *A. varia* adults were significantly affected by the antibiotic effect of CIAT 36062 on the nymphs **(Table 3)**. Females originating from nymphs reared on the resistant genotype had lower net reproductive rates, lower intrinsic rates of natural increase, lower finite rates of increase and longer generation times than those reared on the susceptible genotype. We conclude that antibiosis to nymphs in the resistant *Brachiaria* hybrid CIAT 36062 causes significant sub-lethal effects on the reproductive biology of resulting adults.

Table 3. Fecundity life-table statistics for *Aeneolamia varia* adults as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (BRX 44-02) or resistant (CIAT 36062) *Brachiaria* genotypes.

Treatment[a]		Demographic Parameters			
Nymphs Reared on:	Resulting Adults Feeding on:	Net Reproductive Rate (R_o)	Intrinsic Rate of Natural Increase (r_m)	Mean Generation Time (T)	Finite Rate of Increase (λ)
BRX 44-02 (S)	BRX 44-02 (S)	65.8a	0.724a	5.8b	2.06a
BRX 44-02 (S)	CIAT 36062 (R)	69.5a	0.747a	5.7b	2.11a
CIAT 36062 (R)	BRX 44-02 (S)	52.5b	0.576b	6.9a	1.77b
CIAT 36062 (R)	CIAT 36062 (R)	42.2b	0.574b	6.3a	1.80b

[a] S, susceptible; R, resistant.
Within a column, means followed by the same letter are not significantly different at the 5% level by LSD Jackknife estimates of the intrinsic rate of increase (per capita rate of population growth).

B. Total effects of resistance on the demography of *Aeneolamia varia*

To measure the total impact of antibiosis resistance on the demography of *A. varia*, we took into account the rates of immature mortality caused by both the resistant and the susceptible genotypes. Age-specific survival curves for nymphs and adults, as well as age-specific fecundity curves for *A. varia* adults are presented in **Figure 2**. The antibiosis to nymphs present in the resistant genotype CIAT 36062 had a significant deleterious effect on the biology of the insect, which reflected in very high levels of immature mortality. As a result, survival curves were very low as compared to those obtained with the susceptible genotype. Rearing of the insect on the resistant genotype caused a delay of about 15 days in the emergence of adults. Antibiosis also had a significant effect on the ability of resulting females to lay eggs. Independently of the food substrate used to feed the adults, females obtained from rearing the nymphs on the resistant genotype laid less eggs than those obtained from rearing the insect on the susceptible genotype.

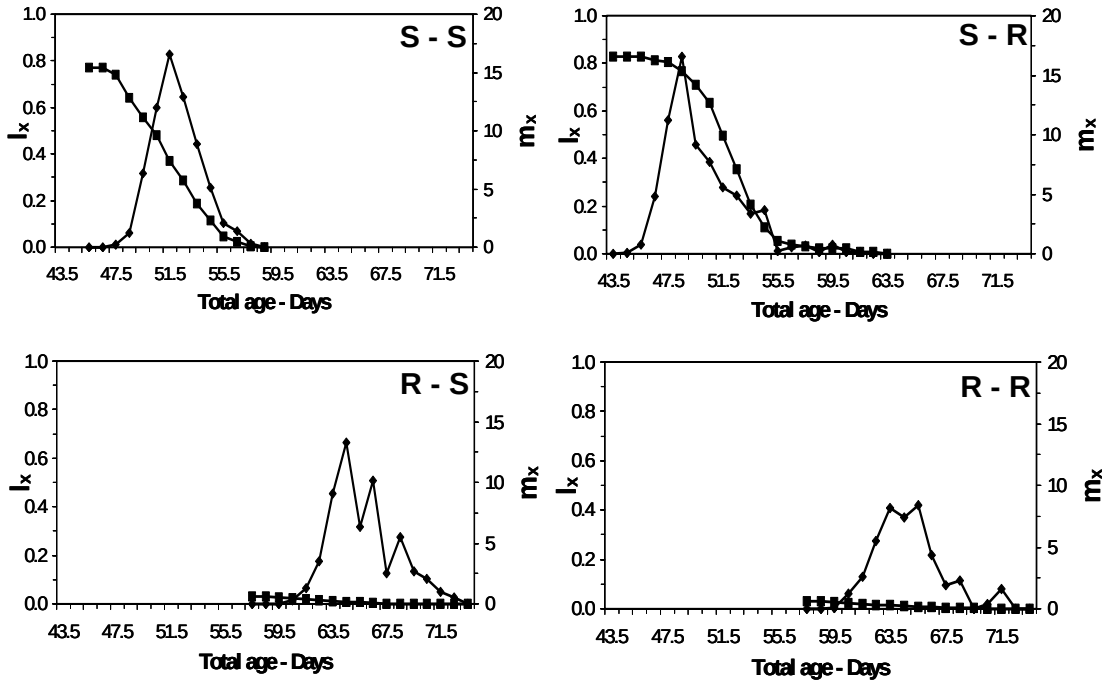

Figure 2. Age-specific survival (l_x) (■) and age-specific fecundity (m_x) (♦) curves for *Aeneolamia varia* as affected by all possible combinations of food substrate for adults and nymphs. First initial in letter combinations indicates the food substrate for nymphs followed by the initial for the food substrate for resulting adults. S, susceptible genotype (BRX 44-02); R, resistant genotype (CIAT 36062).

As a result of high immature mortality and sub-lethal effects on resulting adults, all demographic statistics of the *A. varia* population tested were significantly affected by the antibiosis present in CIAT 36062 **(Table 4)**. Populations derived from the resistant genotype had lower net

reproductive rates, lower intrinsic rates of natural increase, lower finite rates of increase and longer generation times than those obtained from rearing the insect on the susceptible genotype.

The finite rate of increase is a parameter that describes deleterious effects on a given population. It is defined as a multiplication factor of the original population at each time period. The decimal part of the finite rate of increase corresponds to the daily rate of increase expressed as a percentage. This means that populations reared on the susceptible genotype would grow by 9.5 to 10.3% whereas those on the resistant genotype would grow by 0.4-0.8% **(Table 4)**. We conclude that high immature mortality and sub-lethal effects of antibiosis on resulting adults caused by the resistant *Brachiaria* hybrid CIAT 36062 have a major impact on the demography of *A. varia*.

Table 4. Life-table statistics for *Aeneolamia varia* as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (BRX 44-02) or resistant (CIAT 36062) *Brachiaria* genotypes.

Treatment[a]		Demographic parameters			
Nymphs Reared on:	Resulting Adults Feeding on:	Net Reproductive Rate (R_o)	Intrinsic Rate of Natural Increase (r_m)	Mean Generation time (T)	Finite Rate of Increase (λ)
BRX 44-02 (S)	BRX 44-02 (S)	50.7a	0.090b	43.3b	1.095b
BRX 44-02 (S)	CIAT 36062 (R)	57.7a	0.098a	41.5c	1.103a
CIAT 36062 (R)	BRX 44-02 (S)	1.6b	0.008c	54.4a	1.008c
CIAT 36062 (R)	CIAT 36062 (R)	1.3b	0.004c	53.8a	1.004c

[a] S, susceptible; R, resistant.
Within a column, means followed by the same letter are not significantly different at the 5% level by LSD Jackknife estimates of the intrinsic rate of increase (per capita rate of population growth).

Contributors: P. Sotelo, G. Sotelo and C. Cardona.

Activity 4. Publications, Workshop and Conferences, Awards.

Journal papers

Cardona, C., P. Fory, G. Sotelo, A. Pabon, G. Díaz, and J. W. Miles. 2003. Antibiosis and tolerance to five species of spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: Implications for breeding for resistance. J. Econ. Entomol. 97(2): 635-645.

Kelemu, S., C. Cardona, and G. Segura. 2004. Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea* (L.), a tropical forage legume. Plant Physiology and Biochemistry (accepted for publication August, 2004; in press).

Sotelo, G., C. Cardona y J. Miles. 2003. Desarrollo de híbridos de *Brachiaria* resistentes a cuatro especies de salivazo (Homoptera: Cercopidae). Rev. Colombiana de Entomología 29(2): 157-163.

Workshop and Conference Papers

Cardona, C., J. Miles, and G. Sotelo. 2004. Allopatric resistance to several species of spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: Sources, mechanisms, and progress in plant breeding. p.756 *In*: Proceedings, XV International Congress of Plant Protection, 10-15 May, 2004. Beijing, China.

Kelemu, S., C. Cardona, and G. Segura. 2004. Antimicrobial and insecticidal properties of a protein isolated from seeds of the tropical forage legume *Clitoria ternatea* (L). (Abstract) Phytopathology 94: S50. .

Sotelo, P., C. Cardona, G. Sotelo y J. Montoya. 2004. Resistencia de *Brachiaria* spp. al salivazo*:* Posibles efectos subletales de cultivares resistentes sobre los adultos de *Aeneolamia varia* (F.) (Homoptera: Cercopidae). p. 64 *In*: Resúmenes XXXI Congreso Sociedad Colombiana de Entomología, Socolen, Bogotá, Julio 28-30, 2004.

Pabón , A., G. Sotelo y C. Cardona. Resistência de dois genótipos híbridos de *Brachiaria* spp. ao ataque combinado de quarto espécies de cigarrinha das pastagens (Homoptera: Cercopidae). XX Congreso Brasileiro de Entomología, Gramado, Brasil, 5-10 Setembro, 2004. (Poster).

Awards

"Francisco Luis Gallego Award" to the best paper presented by an undergraduate student. XXX Congress of the Colombian Entomological Society. Awarded to: A. Pabón, G. Sotelo, and C. Cardona

Personnel

César Cardona, Guillermo Sotelo, Gilberto Córdoba, Reinaldo Pareja, William Mera.

Students

Alejandro Pabón, M. Sc. candidate, Universidad de Viçosa, Brazil. Thesis title: Mechanisms of resistance to *Deois incompleta* and *Notozulia entreriana* en *Brachiaria* spp.

Ulises Castro, M. Sc. candidate. Colegio de Postgraduados de Chapingo, Chapingo, Mexico. Thesis title: Mechanisms of resistance to *Aeneolamia albofasciata* and *Prosapia simulans* en *Brachiaria* spp.

Paola Sotelo. B. Sc. Thesis (finalized): Resistencia de *Brachiaria* spp. al salivazo*:* Efectos subletales de cultivares resistentes sobre los adultos de *Aeneolamia varia* (F.) (Homoptera: Cercopidae).

María Fernanda Miller. B. Sc. Thesis: Resistencia de *Brachiaria* spp. al salivazo*:* Efectos subletales de cultivares resistentes sobre los adultos de *Zulia carbonaria* (Lallemand) (Homoptera: Cercopidae).

Collaborators: J. W. Miles, Personnel in the Breeding Section, CORPOICA – Macagual.

VIROLOGY

Activity 1. Resistance for cassava frogskin disease is widespread in cassava germplasm.

Introduction

In the Amazon regions of Brazil and Colombia, it was observed that there where apparent difference in the reaction of varieties to cassava frogskin disease (CFSD). Some varieties developed typical root symptoms, while other varieties that were planted in same fields did not develop symptoms. This led to the idea that some cassava landraces are resistant to CFSD. In 1995, it was decided to test the 640 accessions of the CIAT cassava core collection for resistance to cassava frogskin disease (CFSD). The results have shown that tolerance to CFSD is widespread in cassava germplasm. More than 100 tolerant lines have been identified and are potential sources of resistant to CFSD. In the last year, 42 lines were evaluated for their agronomic characteristics and resistance to other pests. All these lines, that are rated as tolerant, have remained infested with CFSD at least eight growing cycles.

Evaluation of cassava for resistance to CFSD

The plants tested were from the core collection of 640 cassava lines that are representative of the CIAT cassava collection that consists of over 6000 lines. All plants in this trial were graft inoculated using stem cuttings of the cassava line CT5460-10 infested with CFSD. Five plants from each line were inoculated by grafting with the CFSD affected stem cuttings of line 5460-10. In the last four years, these lines were grown in randomize block design of four repetitions with 10 plants per repetition and evaluated visually for root symptoms. Representative plants in these lines were assayed for CFSD by grafting stem cuttings (rootstock) to Secundina (scion), and the new leaves were examined for mosaic symptoms. All of the plants tested were positive for CFSD. The roots of each plant were rated using the following scale: 1 for no symptoms, 2 for very mild symptoms, 3 for moderate symptoms, and 4 for severe symptoms.

The ratings of 28 best lines and their yields during the last four years are summarized in **Table 1**. These are the best cassava lines in the CIAT cassava core collection that yield well in the conditions at the CIAT experiment station at Santander de Quilichao, Cauca, Colombia and are tolerant to CFSD. This year the yields are much lower than in the previous years. This was due to the lack of rain during the last year. Even in the harsh conditions of this last year, several of the lines yielded more than 10 t/ha. There was also an increase in the severity of CFSD with eight of the best lines had modest levels of disease pressure. An analysis of the temperatures, rainfall and symptoms during the last four years needs to be done. There are still twenty lines that never have developed significant symptoms over the course of this experiment.

There is ample resistance in the cassava germplasm for CFSD. It is a form of tolerance because the plants remain infected and the disease is transmitted thought the infected stem cuttings. Under the condition of mid-altitude tropics, these lines have remained tolerant year after year. After nine years of field trials, we have a solid base to state that the resistance is stable and holds up under the range of climatic variation that occurs at the screening site. From just the core collection of CIAT, landraces or varieties have been identified for most of the countries where CFSD is endemic and an important production constraint.

Although the yields were disappointing, the commercial varieties HCM-1 and CMC40 (Mcol 1468) were in the low end for yield and are moderately tolerant to CFSD as compared with the most resistant varieties. There are about 15 lines that have been identified from the CIAT core collection that are tolerant to CFSD and have yields potential that are relatively high for varieties that are directly consumed. There is also data on 100 other lines with tolerance. This means that there is a wide range of germplasm options for cassava growing areas where CFSD is a problem.

The core collection represents about 10% of the total number of cassava accession at CIAT. The process of identification of tolerant varieties is time consuming, and the climatic conditions affect the level of disease expression. If a molecular marker or set of markers were developed that could identify CFSD tolerance in cassava, the remaining germplasm could be screened rapidly. This along with the agronomic information available on the cassava germplasm collection and commercial varieties could quickly lead to the identification of germplasm for farmers in areas where CFSD is endemic. Breeding programs in Colombia, Brazil, and Costa Rica should try to incorporate CFSD resistance into their new varieties.

Table 1. The best lines in the CIAT core collection for resistance to CFSD.

20	00-2001		2001-2002		2002-2003		2003-2004		4 Years
Variety	Symptom Rating	Yield	Symptom Rating	Yield	Symptom Rating	Yield	Symptom Rating	Yield	Symptom Rating
M Per 183	1.00	3.95	1.02	5.50	1.00	3.07	1.00	0.98	1.005
M Per 438	1.00	3.95	1.00	2.69	1.00	17.9	1.02	1.28	1.005
M Chn 2	1.00	3.32	1.00	2.16	1.00	1.69	1.00	0.71	1.000
M Mex 95	1.03	2.79	1.04	2.35	1.00	2.00	1.00	0.72	1.018
M Per 213	1.00	2.70	1.00	2.16	1.00	2.11	1.00	0.61	1.000
M Bra 886	1.08	2.32	1.50	2.56	1.08	1.71	1.38	0.61	1.260
MEcu 68	1.00	1.18	1.00	1.91	1.00	3.15	1.00	0.58	1.000
MCol 634	1.00	2.54	1.19	2.04	1.29	1.56	1.07	1.22	1.140
MMal 50	1.00	3.13	1.00	1.58	1.08	1.38	1.00	0.36	1.020
MPer 431	1.00	2.12	1.00	1.86	1.00	1.98	1.00	0.91	1.000
MGua 78	1.00	1.97	1.20	1.63	1.04	2.21	1.00	0.56	1.060
MCol1468	1.03	2.21	1.30	1.83	1.33	1.52	1.13	0.10	1.200
HMC 1	1.00	1.72	1.23	1.62	1.25	1.69	1.50	0.30	1.245
MBra 325	1.00	2.22	1.00	1.68	1.25	1.12	1.33	0.20	1.145
MPer 209	1.00	1.99	1.12	1.91	1.00	1.08	1.00	1.13	1.030
MCr 59	1.13	2.00	1.06	1.59	1.20	1.43	1.24	1.17	1.160
MPer 243	1.00	1.29	1.00	1.49	1.06	1.98	1.08	0.27	1.035
MMal 24	1.00	1.91	1.04	1.66	1.08	1.65	1.70	0.64	1.220
MGua 41	1.05	1.67	1.00	1.39	1.06	1.56	1.00	0.44	1.030
MMex 80	1.00	1.40	1.07	1.82	1.06	1.04	1.28	0.57	1.100
MMal 13	1.00	1.16	1.02	2.16	1.00	1.77	1.00	0.33	1.005
MCr 79	1.23	1.71	1.23	1.22	1.21	0.50	1.31	0.55	1.245
MMal 38	1.03	0.95	1.07	1.38	1.14	1.06	1.11	0.33	1.090
MCol2157	1.00	1.13	1.00	1.14	1.04	0.77	1.65	0.53	1.200
MPer 377	1.03	1.07	1.00	0.96	1.00	0.73	1.00	0.43	1.010
MPar 163	1.03	1.47	1.09	0.48	1.23	0.58	1.00	0.26	1.090
MBol 1	1.00	0.89	1.00	0.77	1.03	0.63	1.00	0.28	1.010
MMex102	1.00	0.73	1.00	0.43	1.00	0.74	1.00	0.40	1.000

Contributors: Calvert, L.A. and Cuervo, M.

Activity 2. The association a reolike virus in *Manihot esculenta* affected with cassava frogskin disease.

Introduction

The evidence for a reolike virus in cassava includes multiple double stranded RNA species, virus-like particles and cDNA clones that have homology with rice ragged stunt virus. The consistent association of the virus with the disease has been more difficult because of low virus titers and reoviruses tend to be extremely liable. Therefore the detection of cassava frogskin virus (CFSV) is not as consistent as it needs to be. This study used nine different isolates of cassava frogskin disease (CFSD) and used two techniques for the detection of the virus. CFSV was detected in all nine of the isolates but was not found in the healthy controls. This is further evidence of the association of CFSV with CFSD.

Materials and Methods

Source of host plants and isolates. The CFSD isolates were collected both in the Andean and Amazonian regions of Colombia and maintained in greenhouses by vegetative propagation. The isolates of CFSD were Secundina 5, Secundina 80, Valluna 29, CM-5460-10, SM 909-25, Regional Tolima, CMC40, Amazonas 16, and Catumare Jamundi. The first part of the name designates the name of the cassava landrace or breeding line affected by CFSD.

The healthy control plants were obtained from materials that were subjected to heat therapy and cultured *in vitro*. The *in vitro* plants were hardened and subsequently maintained in a greenhouse free of CFSD. When Secundina is affected with CFSD, it has mosaic leaf symptoms. All the test plants were grafted to Secundina to determine if the were healthy or affected with CFSD.

Extraction of dsRNA. Three grams of tissue were collected and the dsRNA was extracted (Morris and Dodds, 1979). The ds-RNAs were treated with Dnase (10µg/ml) for 40 minutes at 40°C. The samples were then subjected to an ethanol precipitation and run on agarose or polyacrylamide gels.

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

The first strand synthesis was done in a final volume of 40µl containing 50Mm Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM each dATP, dCTP, dGTP, dTTP, 40 U of Rnasin (Promega) y 400 U of Superscript II RT (Gibco, BRL). The mixture was incubated for 60 min a 37° C. Then an additional 200 U of SuperScript II RT was added to the mixture and the reaction was allowed to continue for another 30 minutes. The reactions were then subjected to 70° C for 1 minute and placed in ice water for 2 minutes. To the 40µl of the first strand reaction 25 mM Tris-HCl (pH 7.5), 100 mM KCl, 5 mM $MgCl_2$, 10 mM$(NH_4)_2SO_4$, 0.15 mM β-NAD^+, 0.25 mM each dATP, dCTP, dGTP, dTTP, 1.2 mM DTT, 25 U *E. coli* Ligase, 40 U *E. coli* Polymerase, 4 U *E. coli* Rnase H were added and the final volume was 150µl. After the mixture

was incubated for 3 h at 16° C, 30 U of T4 DNA Polymerase was added and the reaction was continued at 16° C for 10 minutes. The reaction was stopped by the addition of 10 µl of 0.5 M EDTA, pH 8.0 and treated with phenol:chloroform:isoamyl alcohol (25:24:1). The cDNAs were precipitated with 1/10 volume of 7 M ammonium acetate and 2.5 volumes of 95% ethanol and resuspended in sterile DEPC treated water.

PCR amplificat ion. The PCR reaction (25 µL) consisted of 0.2 µM of each primer S5 up(5'GTT AGC ATT ACC ATT CTC ACA T 3'), 2.5 mM MgCl2, 20 ng DNA, buffer 1x (100 mM Tris-HCl, 500 mM KCl, 1% Triton X-100), 0.25 mM dNTPs and 1 U Taq polymerase (Perkin Elmer). The reaction was carried out in a thermocycler using the following program: initial denaturation at 94ºC for 5 min; 39 cycles at 94ºC for 1min, 50ºC 2 min, 72ºC for 2.30 min; a final extension at 72ºC for 10 min. The PCR products were separated in agarose and capillary blotted into nylon membranes and hybridized with S5 probe labeled using DIG high prime DNA labeling kit (Roche Applied Science).

Results and Discussion

The detection of a Gen omic Segments of Cassava Frogskin Virus. Nine CFSD isolates were tested using reverse transcriptase PCR for the presence of CFSV. The primers were specific for the CFSV segment S5. Multiple PCR products were amplified including a product in the controls. The products were transferred to a membrane and a CFSV S5 probe was used to detect specific PCR products. The products in the healthy controls were not specific while multiple bands in CFSD affected plants reacted with the cDNA CFSV S5 probe **(Figure 1)**. This method is highly specific but requires many steps including the purification of dsRNA, reverse transcriptase, PCR, and hybridization.

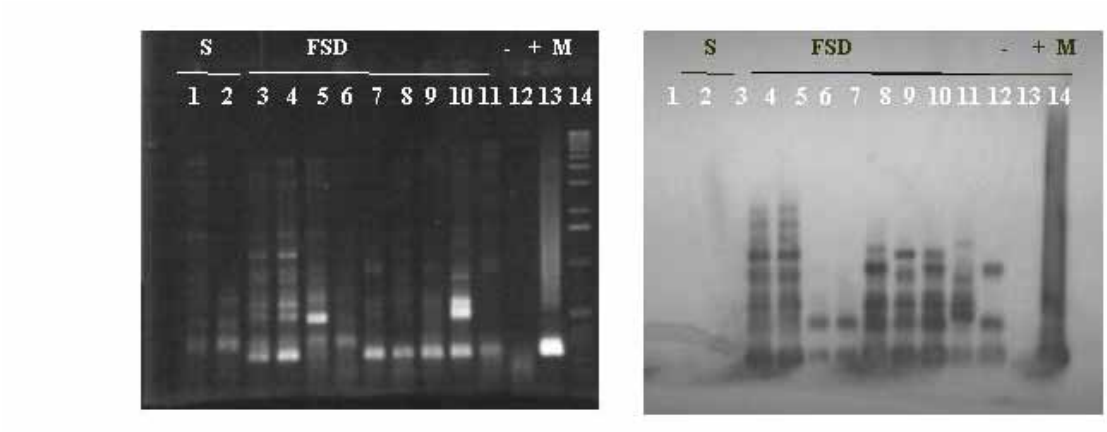

Figure 1. Detection of the S5 of cassava frogsk in virus using rt-PC R. On the left is a gel showing the PCR products stained w ith ethidium bromide, and on the right a hybridazation using a probe specific fo r CFSV segment 5. The isolates are health 1: Secundina , 2: CMC40; CFSD affected 3: Secundina 5, 4: Secundina 80, 5: CF SD 29, 6: CM-5460-10, 7: SM 909-25, 8: Regi onal Tolima, 9: CMC40, 10: Amaz onas 16, 11: Catuma re Jamundi, 12: negative control, 13: positive control, 14: 1 kb molecular weight marker.

The same cassava plants affected with CFSD were tested for the presence of CFSV dsRNA genomic segment S5. The CFSV genomic segment S5 was consistent detected in the nine isolates affected with CFSD, but not in the negative controls **(Figure 2)**. This genomic segment is estimated to by 2800-3000 nucleotides in length and has homology with the rice ragged stunt virus segment S5.

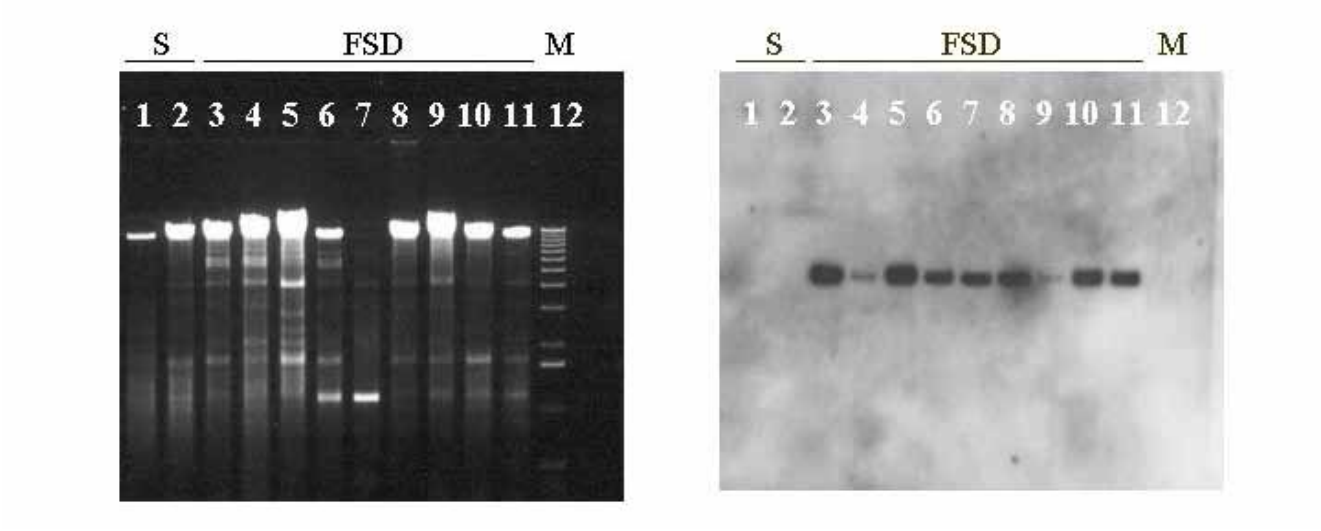

Figure 2. Detection of the dsRNA genomic se gment S5 of cassava frogskin virus. On the left is a gel showing t he dsRNAs staine d with ethidium bromide, and on the right a hybridaz ation using a probe speci fic for CFSV segment 5. The isolates are healthy 1: Secundina , 2: CMC40. CFSD affected : 3: Secundina 5, 4: Secundina 80, 5: CDSD 29, 6: CM- 5460-10, 7: SM 909-25, 8: Regional Tolima, 9: CMC40, 10: Amaz onas 16, 11: Catumare Jamundi, 12: 1 kb molecular weight marker (M).

The detection of the genomic segment is not as consistent as the rt-PCR. The virus titer tends to be very low and the virus is very prone to degradation. PCR methods are more sensitive than direct methods that do not involve amplification. Nevertheless, after optimization of the conditions, we were able to detect the genomic segment S5 of CFSD.

Further characteriz ation of CFSV. In addition to the CFSV S5 cDNA clone, there is the cDNA clone CFSV that represents a part of segment 1 (S1). Both these cDNA clones have homology at the amino acid level with rice ragged stunt virus (RRSV). A unique cDNA product was the identified by AFLP. This fragment was cloned and represents part of the genomic segment of CFSV S1. Using the same methods for CFSV S5, the full-length genomic segment S1 was detected in dsRNA gels by the clone cDNA S1. This area was purified, and cDNA clones were generated using a variety of strategies. These cDNA clones are in the process of being analyzed. The best characterized is around 1000 nucleotides and has homology at the amino acid level with segment 4 of RRSV. The genomic segments RRSV S1, S2, and S4 are 3849, 3810 and 3823 nucleotides respectively. Therefore the purification of dsRNA in the area of the CFSV S1 also should yield the S2 and S4 genomic segments. The successful cloning of the S4 segment confirms the strategy and demonstrates how previous information is helping in the

characterization of the virus. The S1 clone is 235 nucleotides and the S5 clone is 327 nucleotides. The CFSV S4 cDNA clone is the largest fragment cloned to date, and represents a significant step in the further characterization of CFSV.

Contributors: Lee A. Calvert, Maritza Cuervo, Ivan Lozano, and Natalia Villareal.

CASSAVA AND TROPICAL FRUIT PATHOLOGY

Activity 1. DNA sequence analysis of specific regions of phytoplasma, *Glomerella*, *Sphaceloma*, *Ralstonia*, *Phytophthora*, *Pythium*, and cassava.

Objective

Report in GenBank the sequences of fungi, bacteria, and phytoplasmas that affect important crops in Colombia (*Manihot esculenta* Crantz, *Elaeis guineensis*, *Solanum quitoense*, *Coffea arabica*, *Anona muricata* and *Musa* AAB*)* and several resistance genes.

Methodology

DNA fragments of phytoplasma from *Elaeis guineensis*, *Manihot esculenta* Crantz, *Solanum quitoense* and *Coffea arabica* were obtained using the polymerase chain reaction (PCR). Fungi and bacteria affecting different crops—for example *Phytophthora* sp. (*Manihot esculenta* Crantz), *Sphaceloma* sp. (*Manihot esculenta* Crantz), *Glomerella* sp. (*Anona muricata*), and *Ralstonia solanacearum* (*Musa* AAB)—and resistance gene analogs of cassava (obtained from varieties resistant to *Phytophthora* sp. and *Xanthomonas axonopodis* pv *manihotis*) were purified and then ligated in pGEM-T Easy vector, which was introduced into the *Escherichia coli* strain DH5-a by electroporation at 2.4 kV/cm^2. Transformants were selected on blue/white color screening by plating on LB/ampicillin/IPTG/X-gal media. Positive inserts were observed by plasmid restriction with *Eco*RI and electrophoresis in 1.5% agarose gel. Different-sized fragments were selected for sequencing by automated dideoxy sequencing (ABI Prism 377-96 DNA Sequencer), using a DNA-sequencing kit from Applied Biosystems. Using the DNAMAN software with the Assambly option, different fragments of each microorganism or gene were aligned to obtain complete sequences. To report the sequences in the GenBank (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov), the read bases and their taxonomic classification at both morphologic and molecular levels were analyzed for each species **(Table 1)**.

Table 1. Sequences submitted to the GenBank database.

Accession GenBank Nam	e	Size (bp)	Org anism	Isolate/ Clone	Host/ Source Genotype	Location
AY525125	*Coffee crispiness phytoplasma* 16S rRNA gene	941	*Phytoplasma X-Disease group*		*Coffea arabica*	Caldas, Colombia
AY737646	*Cassava frogskin disease phytoplasm* (FSD)	1260	*Phytoplasma X-Disease group*	FSDY17	*Manihot esculenta*	Valle del Cauca, Colombia
AY737647	*Cassava frogskin disease phytoplasm* (FSD)	1298	*Phytoplasma X-Disease group*	FSDY29	*Manihot esculenta*	Valle del Cauca, Colombia
AY731819	*Solanum quitoense machorreo phytoplasma* 16S rRNA gene	1567	*Phytoplasma X-Disease group*		*Solanum quitoense*	Valle del Cauca, Colombia
AY739023	*Lethal decline oil palm phytoplasma* strain PO8.90OilCol 16S rRNA gene	1235	*Phytoplasma*	PO8.90 OilCol	*Elaeis guineensis*	Villanueva, Colombia
AY739024	*Lethal decline oil palm phytoplasma* strain PC2.1014R 16S rRNA gene	1424	*Phytoplasma Aster Yellows*	PC2.1014R	*Elaeis guineensis*	Villanueva, Colombia
AY737648	*Colletotrichum acutatum* isolate CA15 5.8S rRNA gene ITS1 ITS2	438	*Glomerella acutata*	CA 15	*Annona muricata*	Valle del Cauca, Colombia
AY739025	*Colletotrichum gloesporioides* isolate CG5 18S rRNA gene ITS1 ITS2	524	*Glomerella cingulata*	CG 5	*Annona muricata*	Valle del Cauca, Colombia
AY739018	*Sphaceloma manihoticola* 18S rRNA gene ITS1 ITS2	644	*Sphaceloma manihoticola*	S2	*Manihot esculenta*	Brazil
AY739019	*Sphaceloma manihoticola* 18S rRNA gene ITS1 ITS2	625	*Sphaceloma manihoticola*	S47	*Manihot esculenta*	Colombia
AY739020	*Sphaceloma krugii* 18S rRNA gene ITS1 ITS2	629	*Sphaceloma krugii*	S1	*Euphorbia heterophylla*	Brazil
AY737489	*Ralstonia solanacearum* isolate G175 16S rRNA gene fragment	255	*Ralstonia solanacearum*	G175	*Solanum melongena*	Kenya
AY745758	*Ralstonia solanacearum* isolate CIAT 1017 16S rRNA gene fragment	225	*Ralstonia solanacearum*	CIAT 1017	*Canna indica* (Indian shot)	La Dorada, Colombia
AY745759	*Ralstonia solanacearum* isolate G 218 16S rRNA gene fragment	223	*Ralstonia solanacearum*	G 218	*Capsicum sp.*	Philippines
AY745760	*Ralstonia solanacearum* isolate G 217 16S rRNA gene fragment	216	*Ralstonia solanacearum*	G 217	*Heliconia sp.*	Costa Rica
AY745757	*Ralstonia solanacearum* isolate CIAT 1016 16S rRNA gene fragment	235	*Ralstonia solanacearum*	CIAT 1016	*Solanum tuberosum*	Popayán, Colombia
AY745755	*Ralstonia solanacearum* isolate Urabá 6 16S rRNA gene fragment	203	*Ralstonia solanacearum*	Urabá 6	*Musa* sp.	Urabá, Colombia

Accession GenBank Nam	e	Size (bp)	Org anism	Isolate/ Clone	Host/ Source Genotype	Location
AY745761	*Ralstonia solanacearum* isolate Quindío 1 16S rRNA gene fragment	223	*Ralstonia solanacearum*	Quindío 1	*Musa* AAB	Montenegro, Colombia
AY737486	*Ralstonia solanacearum* isolate Jamundí soil 16S rRNA gene fragment	235	*Ralstonia solanacearum*	Jamundí a	Soil – plantain	Jamundí, Colombia
AY737487	*Ralstonia solanacearum* isolate 16a soil 16S rRNA gene fragment	244	*Ralstonia solanacearum*	16a	Soil – plantain	Montenegro, Colombia
AY737488	*Ralstonia solanacearum* isolate CIAT 1043 16S rRNA gene fragment	220	*Ralstonia solanacearum*	CIAT 1043	*Nicotiana tabacum*	Socorro, Colombia
AY745756	*Ralstonia solanacearum* isolate CIAT 1077 16S rRNA gene fragment	225	*Ralstonia solanacearum*	CIAT 1077	*Lycopersicon Esculentum*	North Carolina USA
AY730038	*Manihot esculenta* resistance gene analog clone N37 NBS-LRR	325	*Manihot esculenta*	N37	M Bra 1045	Palmira, Colombia
AY730040	*Manihot esculenta* resistance gene analog clone N38 NBS-LRR	474	*Manihot esculenta*	N38	M Bra 532	Palmira, Colombia
AY730041	*Manihot esculenta* resistance gene analog clone K1 NBS-LRR	496	*Manihot esculenta*	K1	M Bra 532	Palmira, Colombia
AY737490	*Manihot esculenta* resistance gene analog clone N33 NBS-LRR	342	*Manihot esculenta*	N33	M Bra 1045	Palmira, Colombia
AY745762	*Manihot esculenta* resistance gene analog clone N31 NBS-LRR	210	*Manihot esculenta*	N31	CM6438-14	Palmira, Colombia
AY745763	*Manihot esculenta* resistance gene analog clone P32 kinase	449	*Manihot esculenta*	P32	CM 3311-4	Palmira, Colombia
AY745764	*Manihot esculenta* resistance gene analog clone W5 kinase	487	*Manihot esculenta*	W5	CM 7772-13	Palmira, Colombia
AY745765	*Manihot esculenta* resistance gene analog clone X1 kinase	441	*Manihot esculenta*	X1	CM 3311-4	Palmira, Colombia
AY745766	*Manihot esculenta* resistance gene analog clone X5 kinase	535	*Manihot esculenta*	X5	CM 7772-13	Palmira, Colombia
AY745767	*Manihot esculenta* resistance gene analog clone X9 kinase	336	*Manihot esculenta*	X9	CM 6438-14	Palmira, Colombia
AY745768	*Manihot esculenta* resistance gene analog clone W6 kinase	117	*Manihot esculenta*	W6	CM 6438-14	Palmira, Colombia
AY745769	*Manihot esculenta* resistance gene analog clone W10 kinase	442	*Manihot esculenta*	W10	CBB resistant bulk	Villavicencio, Colombia
AY745770	*Manihot esculenta* resistance gene analog clone P36 kinase	336	*Manihot esculenta*	P36	CM 3311-4	Palmira, Colombia
AY745771	*Manihot esculenta* resistance gene analog clone P41 kinase	599	*Manihot esculenta*	P41	M Nga 19	Palmira, Colombia

Accession GenBank Nam	e	Size (bp)	Org anism	Isolate/ Clone	Host/ Source Genotype	Location
AY745752	*Phytophthora cinnamomi* 5.8S and 28S rRNA gene ITS1-ITS4	883	*Phytophthora cinnamomi*	CLT	*Calathea* sp.	The Netherlands
AY745749	*Phytophthora cryptogea* 5.8S and 28S rRNA gene ITS1-ITS4	860	*Phytophthora cryptogea*	HMA	*Heliconia* sp.	Palmira, Colombia
AY739022	*Phytophthora tropicalis* 18S rRNA gene ITS1 ITS2	747	*Phytophthora tropicalis*	P71	*Manihot esculenta*	Quindío, Colombia
AY739021	*Phytophthora melonis* 18S rRNA gene ITS1 ITS2	920	*Phytophthora melonis*	P12	*Manihot esculenta*	Brazil
AY745754	*Phytophthora nicotianae* 18S, 5.8S and 28S rRNA gene ITS4-ITS5	839	*Phytophthora nicotianae*	STD3	*Manihot esculenta*	Santander de Quilichao, Colombia
AY745753	*Phytophthora melonis* 18S, 5.8S and 28S rRNA gene ITS4-ITS5	869	*Phytophthora melonis*	B10	*Manihot esculenta*	Brazil
AY745750	*Phytophthora palmivora* 5.8S and 28S rRNA gene ITS1-ITS4	657	*Phytophthora palmivora*	PP7	*Theobroma cacao*	Caldas, Colombia
AY745751	*Phytophthora palmivora* 5.8S and 28S rRNA gene ITS1-ITS4	791	*Phytophthora palmivora*	FLMA1	*Theobroma cacao*	Caldas, Colombia
AY745748	*Pythium chamaehyphon* 5.8S and 28S rRNA gene ITS1-ITS4	773	*Pythium chamaehyphon*	MTR4	*Manihot esculenta*	Mitú, Colombia

Activity 2. Sample collection and isolation of the bacterium *Ralstonia solanacearum* obtained from plantain, its conservat ion, identifica tion by PCR, DNA sequencing, and determination of races, biovars, and pathogenicity.

Objectives

1. To isolate *R. solanacearum* from soil and diseased plants of plantain and banana by culturing in semi-selective medium, and identifying through the PCR technique
2. To evaluate the pathogenicity of *R. solanacearum* isolates
3. To biochemically characterize the isolates to determine biovars
4. To identify the isolates, using DNA sequencing

Methodology

Obtaining R. solanacearum isolates

We processed samples collected from 14 farms suffering from problems of moko, a bacterial wilt that attacks various crops of economic importance. Samples were obtained from plants of plantain (31) and banana (4) infected with the wilt, soil (193), weeds (2), and water (1) in the production regions of the Colombian Departments of Quindío, Antioquia, Valle del Cauca, Caquetá, and Meta.

From these samples, we obtained and selected, according to their growth in the SMSA semi-selective culture medium (which reduces growth of saprophytic bacteria) and to their positive reaction to the oxidase and KOH tests, bacterial isolates that seemed to be *R. solanacearum*. These were later identified as such by the PCR technique, using specific primers to amplify a sole fragment of *R. solanacearum* DNA.

DNA extraction and PCR analysis

Genomic DNA was extracted from bacterial cells, using pure colonies of isolates that had had 36 h of growth in nutritive agar (Seal et al. 1992). A colony was suspended in 100 µL of sterilized distilled water, heated to 96°C for 5 min, and centrifuged at 12,000 rpm for 2 min. We took 2.5 µL of the supernatant as DNA mold for the PCR reaction. The volume of the reaction was 12.48 µL and, moreover, contained 1X *Taq* polymerase buffer, 0.16 mM of each dNTP, 1.5 mM $MgCl_2$, 0.25 U *Taq* polymerase, and 0.16 µM of each of the specific primers Oli1 (5'-GGGGGTAGCTTGCTACCTGCC-3') and Y2 ((5'-CCCACTGCTGCCTCCCGTAGGAGT-3'), previously reported by Martins (2000).

DNA amplification was carried out in a thermocycler (MSJ-Research PTC-100), using the following program: 2 min at 96°C, 50 cycles of denaturation for 20 s at 94°C, annealing for 20 s at 62°C, extension for 30 s at 72°C, and another final extension for 5 min at 72°C. The PCR products were separated on 1.5% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light.

Determining biovars

Isolates belonging to *R. solanacearum* could be classified into different biovars by their acid production, using three disaccharides (cellobiose, lactose, and maltose), and their oxidation of

three hexose alcohols or polyols (sorbitol, dulcitol, and mannitol) in a base medium for determining biovars as according to Hayward (1964).

The disaccharides and polyols were sterilized through filtration, and the sterilized base medium then added. To inoculate with bacteria, we used a colony from a culture grown in nutritive agar over 24 h. We then observed the reactions over 1, 3, and 7 days of incubation at 28°C. A change of color from olive green to yellow, from top to bottom, indicated the production of acids from the disaccharides, whereas a color change from purple to yellow indicated oxidation of the polyols.

The reaction to 3% KOH was assessed by placing 1 drop of reagent on a glass slide and then dissolving in it a colony from a pure and metabolically active culture with a 24-h growth. The reaction was regarded as positive if it gave rise to a viscous strand 15 to 30 s afterwards.

For the oxidase test, 2 drops of 1% aqueous solution of tetramethyl-*p*-phenylenediamine dihydrochloride (Kovács' oxidase reagent) were placed on a piece of filter paper and a colony rubbed in the drops. A positive reaction was determined by appearing purple color 10 sec later.

Testing for pathogenicity on plantain

The pathogenicity test was carried out by inoculating different isolates identified by PCR as *R. solanacearum* on plants of plantain (*Musa* sp.) derived from in vitro meristem culture. Before inoculation, these plants had been transplanted to plastic bags containing sterilized soil and left for 15 days in a humid chamber to ensure the plants' development.

Four plantain plants per *R. solanacearum* isolate were inoculated with an injection of 1 mL of bacterial suspension over two sites on the pseudostem. The suspension was prepared with cultures of each bacterium identified as *R. solanacearum* grown over 24 h in nutritive agar at an absorbance of 0.6 to 600 nm wavelength, corresponding to an approximate concentration of 1×10^8 cfu/mL. As positive check, inoculations were also carried out with a strain of *R. solanacearum* (CIAT 1008) collected from Ibagué, Tolima, and now part of the strain bank at CIAT. The negative check was inoculation with sterilized water.

The inoculated plants were incubated in a humid chamber at temperatures between 24°C and 29°C, relative humidity between 80% and 91%, and about 13 h of light. At day 4, the plants were evaluated for symptoms such as leaf flaccidity; between days 8 and 10, signs of yellowing appeared and growth declined; and at day 15 onwards, lodging and wilting occurred.

Testing for hypersensitivity

The isolates grown in nutritive agar over 24 h were inoculated onto tobacco plants, infiltrating a bacterial suspension of 1×10^8 cfu/mL through the intercellular spaces of two leaves per plant, and three plants per isolate. Tissue collapse as reaction was observed at 24 h.

DNA sequencing

Fragments measuring 288 bp, amplified by PCR, were sequenced at the DNA sequencing laboratory of the Iowa State University. The sequences were then cleaned and homologized with reported sequences in the GenBank database. A phylogenetic tree that included sequences

reported in the database was constructed with Phylogenetic Analysis Using Parsimony (PAUP) and bootstrapping of 1000 replications.

Results

Obtaining R. solanacearum isolates

The samples yielded 52 isolates of bacteria that were tentatively considered as *R. solanacearum* for their growth in the SMSA semi-selective medium. At 48 h after incubating at 28°C, their growth was similar to that of check strain CIAT 1008.

PCR analysis

In a 1.5% agarose gel, a band, indicating a fragment located at gene 16S rRNA and with a molecular weight of 288 base pairs, was detected in 21 of the 52 isolates, identifying the isolates as *R. solanacearum*. **Figure 1** shows 6 of the isolates eventually identified.

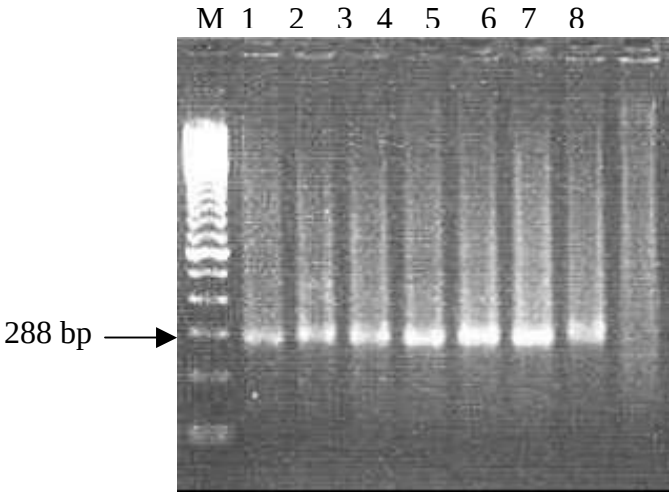

Figure 1. A 288-bp-long product amplified in the 16S rRNA region of DNA from the bacterium *Ralstonia solanacearum*. Six of the 22 isolates eventually identified are shown here: M = 100-bp marker; lanes 1–3 = isolates from Quindío, Colombia; lanes 4–6 = isolates from Urabá, Colombia; lane 7 = *R. solanacearum* strain CIAT 1008; lane 8 = negative control.

Determining biovars

Table 1 lists the reaction of each of the 21 isolates to the sugars and alcohols evaluated, indicating that all the isolates characterized belonged to biovar I. That is, none of the isolates used the three sugars, nor oxidized the three alcohols, in the biochemical tests made. Moreover, the 21 isolates reacted positively to the oxidase and KOH tests.

Table 1. Characteristics of 22 isolates of the bacterium *Ralstonia solanacearum* from Colombia as determined by biochemical tests, PCR amplification, and pathogenicity.

Isolate	Origin	Source/Treatment	SMSA[a]	Oxidase[b]	3% KOH[c]	PCR	HR tobacco[d]	Pathogenicity	Biovar[e]
1	Quindío	Plantain/Flower	(+)	(+)	(+)	(+)	(+)	(+)	1
2	Quindío	Plantain/Petiole	(+)	(+)	(+)	(+)	(+)	(+)	1
3	Quindío	Plantain/Petiole	(+)	(+)	(+)	(+)	(+)	(+)	1
4	Antioquia	Banana/Pseudostem	(+)	(+)	(+)	(+)	(-)	(+)	1
5	Antioquia	Plantain/Strain	(+)	(+)	(+)	(+)	(-)	(+)	1
6	Antioquia	Plantain/Fruit	(+)	(+)	(+)	(+)	(-)	(+)	1
7	Antioquia	Plantain/Fruit	(+)	(+)	(+)	(+)	(-)	(+)	1
15	Quindío	Soil/Farmer	(+)	(+)	(+)	(+)	(+)	(+)	1
16a	Quindío	Soil/Mucuna mulch	(+)	(+)	(+)	(+)	(+/-)	(+)	1
16b	Quindío	Soil/Mucuna mulch	(+)	(+)	(+)	(+)	(+/-)	(+)	1
17	Valle	Soil	(+)	(+)	(+)	(+)	(+)	(+)	1
18	Valle	Plantain/Sucker	(+)	(+)	(+)	(+)	(+)	(+)	1
32	Caquetá	Plantain/Pseudostem	(+)	(+)	(+)	(+)	(+)	(+)	1
33	Caquetá	Plantain/Pseudostem	(+)	(+)	(+)	(+)	(+)	(+)	1
34	Caquetá	Plantain/Raceme rachis	(+)	(+)	(+)	(+)	(+)	(+)	1
38	Quindío	Soil/Agroplus® + coffee pulp	(+)	(+)	(+)	(+)	(+)	(+)	1
39	Quindío	Soil/Agroplus® + coffee pulp	(+)	(+)	(+)	(+)	(+)	(+)	1
40	Quindío	Soil/Center of disease pressure	(+)	(+)	(+)	(+)	(+)	(+)	1
41	Quindío	Soil/Center of disease pressure	(+)	(+)	(+)	(+)	(+)	(+)	1
42	Meta	Plantain/Pseudostem	(+)	(+)	(+)	(+)	(+)	(+)	1
43	Meta	Plantain/Pseudostem	(+)	(+)	(+)	(+)	(+)	(+)	1
48	Quindío	Plantain/Pseudostem	(+)	(+)	(+)	(+)	(+)	(+)	n.d.
CIAT 1008[f]	Tolima	Plantain	(+)	(+)	(+)	(+)	(+)	(+)	1

a. Spizizen's minimal salts, a semi-selective medium.
b. Oxidase test, using Kovács oxidase reagent.
c. Reaction to 3% KOH.
d. Test for hypersensitivity to *R. solanacearum* in tobacco.
e. n.d.= Not determined.
f. Check strain from CIAT collection.

Testing for pathogenicity, and confirming "Race 2"

The *R. solanacearum* isolates obtained from infected plantain tissue were pathogenic on inoculating plantain plants, confirming that they belonged to Race 2. This was further confirmed by the typical hypersensitivity reaction obtained with tobacco leaves to 15 isolates **(Figure 2)**. The soil isolates, coded 16a and 16b, caused leaf yellowing on tobacco, which is possibly related to differences in pathogenicity.

Likewise, isolates 4, 5, 6, and 7 from banana samples, reacted negatively to the hypersensitivity test **(Table 1)**. These last four isolates were pathogenic to banana and plantain.

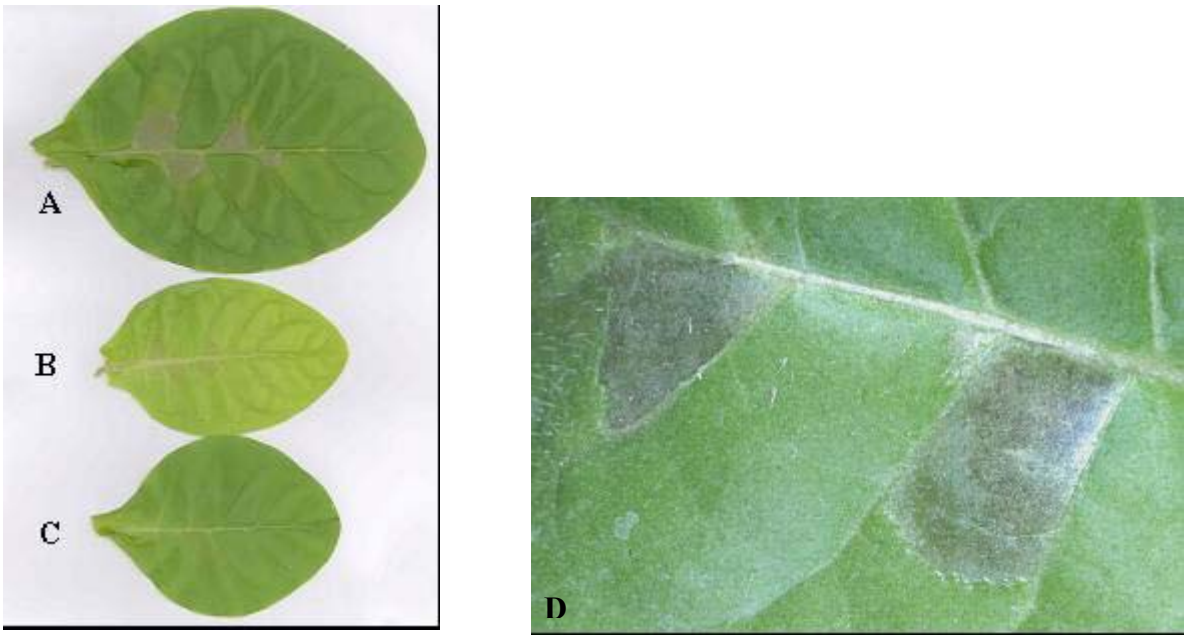

Figure 2. Reaction of hypersensitivity in tobacco to isolates of the bacterium *Ralstonia solanacearum* Race 2. Tobacco leaves were inoculated by infiltration. (A) and (D) Typical reactions of hypersensitivity; (B) an atypical reaction, induced by isolates from soil and banana; and (C) control infiltrated with sterilized distilled water.

DNA sequencing

Figure 3 shows a certain degree of similarity between isolates collected from different production regions. Isolate Caquetá 32, obtained from plantain, separated into a cluster next to isolate Urabá 6, obtained from banana. The latter isolate, in its turn, showed phylogenetic differences with isolate Urabá 4, also from banana. Isolates from the soil at Quindío also separated among themselves, except for 38 and 16a, which formed a cluster. Meanwhile, isolates from Meta, Quindío soils, and Tolima (1008) showed greater similarities with isolates from potato and tobacco than with either the three sequences reported in GenBank (AY 216796, obtained from soil; PS01716SR, and AY642432) or with the sequences of different bacterial species (*R. mannitolilytica*, *R. picketii*, *R. thomasii*, and *Burkholderia solanacearum*).

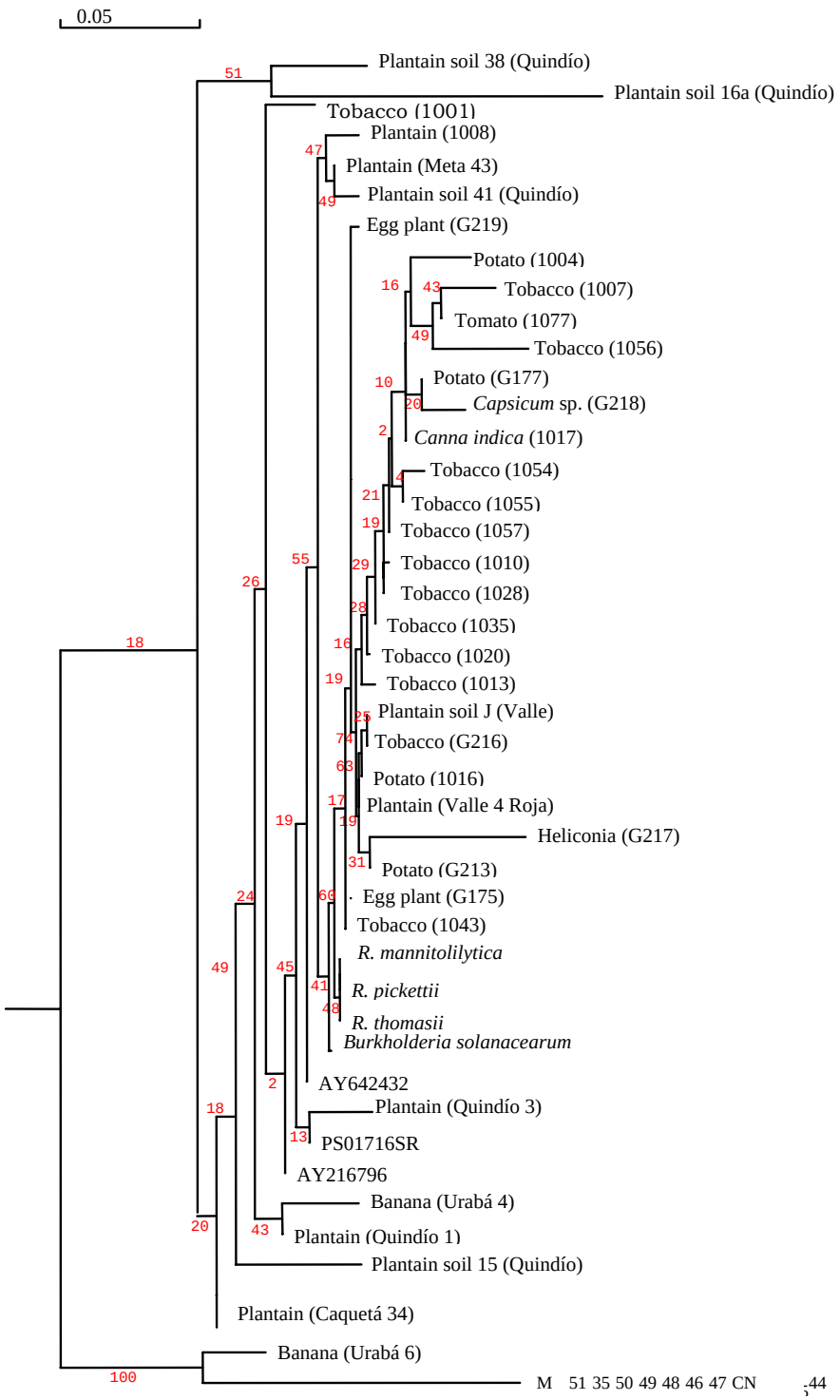

Figure 3. Phylogenetic tree constructed with PAUP and bootstrapping of 1000 replications from 44 isolates of the bacterium *Ralstonia solanacearum* from plantain in Tolima (1008), Valle del Cauca, Quindío, Meta, Caquetá; banana from Urabá; and soil from Quindío and Valle del Cauca, Colombia. The tree compares the isolates with those from several crops and with accessions in GenBank (AY 216796, AY642432, and PS01716SR of *R. solanacearum*, *R. picketii*, *R. thomasii*, *R. mannitolilytica*, and *Burkholderia solanacearum*).

Activity 3. DNA sequence analysis of *Ralstonia solanacearum* obtained from banana, *Heliconia* sp., eggplant, potato, tomato, tobacco, and Indian shot (*Canna indica* L.)

Specific objectives

1. To confirm the identity of strains of *R. solanacearum* isolated from tomato, tobacco, potato, plantain, *Heliconia* sp., Indian shot (*C. indica*), banana, and eggplant through PCR with the specific primers Oli1/Y2 and sequencing
2. To identify, by sequencing, 18 bacterial strains isolated from plantain and now part of the bacterial collection held at CIAT's Cassava Pathology
3. To characterize, through biochemical tests, strains of *R. solanacearum*

Methodology

DNA extraction and PCR analysis

We wanted to confirm the identity of 41 isolates of *R. solanacearum* belonging to the bacterial collection at Cassava Pathology, CIAT, obtained from different crops **(Table 1**, codes 1 to 41), and to discover the identity of 18 strains more, collected from plantain suckers from a farm located in Jamundí (Valle del Cauca), both before and after thermotherapy **(Table 1**, codes 42 to 59).

Table 1. Characteristics of isolates of the bacterium *Ralstonia solanacearum* from Colombia as determined by PCR amplification[a].

Code	Number in Collection[b]	Race	Source	Origin	Host[c]	PCR
1	1001		CIAT	Floridablanca, Colombia	Tobacco	+
2	1003	1	CIAT	Trinidad	Tomato	-
3	1004	1	CIAT	Nambour, Australia	Potato	+
4	1005	3	CIAT	Atherton, Australia	Potato	-
5	1006	1	CIAT	Worthi Co., Georgia, USA	Tomato	-
6	1007	1	CIAT	Quency, Florida, USA	Tobacco	+
7	1008	2	CIAT	Ibagué, Tolima	Plantain	+
8	1010		CIAT	Floridablanca, Colombia	Tobacco	+
9	1011	3	CIAT	Toowoomba, Australia	Potato	-
10	1012	3	CIAT	Las Palmas, Colombia	Potato	-
11	1013	1	CIAT	North Carolina, USA	Tobacco	+
12	1014	3	CIAT	Africa	Potato	-
13	1015		CIAT			-
14	1016	3	CIAT	Popayán, Colombia	Potato	+
15	1017	2	CIAT	Dorada, Colombia	Indian shot	+
16	1018	3	CIAT	Popayán, Colombia	Potato	-
17	1020		CIAT	Girón, Colombia	Tobacco, Col. var. 37	+
18	1028		CIAT	Girón, Colombia	Tobacco, Col. var. 37	+
19	1035		CIAT	Socorro, Colombia	Tobacco, Col. var. 37	+
20	1043		CIAT	Socorro, Colombia	Tobacco, Col. var. 37	+
21	1051		CIAT	Villanueva, Colombia	T, woolly var.	-
22	1052		CIAT	Girón, ICA, Colombia	Tobacco, Col. var. 37	-
23	1053		CIAT	Girón, ICA, Colombia	Tobacco, Col. var. 37	-
24	1054		CIAT	San Gil, La Flora, Colombia	Tobacco	+
25	1055		CIAT	San Gil, El Comunismo, Colombia	Tobacco	+

Code	Number in Collection[b]	Race	Source	Origin	Host[c]	PCR
26	1056		CIAT	Floridablanca, Colombia	Tobacco	+
27	1057		CIAT	Floridablanca, Colombia	Tobacco	+
28	1077	1	CIAT	Wake Co., North Carolina	Tomato	+
29	G212	3	Dinamarca		Potato	-
30	G213	3	Dinamarca		Potato	+
31	G175		Dinamarca	Kenya	Eggplant	+
32	G176	2	Dinamarca	Peru	Banana	-
33	G177	3	Dinamarca	Australia	Potato	+
34	G215	3	Dinamarca	French Réunion	Potato	-
35	G216	1	Dinamarca	Japan	Tobacco	+
36	G217	2	Dinamarca	Costa Rica	*Heliconia* sp.	+
37	G218	1	Dinamarca	Philippines	*Capsicum* sp.	+
38	G219	1	Dinamarca	Sri Lanka	Eggplant	+
39	R3		CIAT	Quindío	Soil	-
40	R18		CIAT	Quindío	Plantain	-
41	R22		CIAT	Quindío	Plantain	-
42	1		CIAT	Jamundí, Colombia, no. 1	Pl w. th.	+
43	2		CIAT	Jamundí, Col., no. 3	Pl w. th.	+
44	3		CIAT	Jamundí, Col., no. 4	Pl w. th.	+
45	4		CIAT	Jamundí, Col., no. 9	Pl w. th.	-
46	5		CIAT	Jamundí, Col., no. 10	Pl w. th.	+
47	6		CIAT	Jamundí, Col., no. 11	Pl w. th.	+
48	7		CIAT	Jamundí, Col., no. 1, red	Pl bef. th.	+
49	8		CIAT	Jamundí, Col., no. 1, pink	Pl bef. th.	+
50	9		CIAT	Jamundí, Col., no. 4, red	Pl bef. th.	+
51	10		CIAT	Jamundí, Col., no. 4, clear	Pl bef. th.	+
52	11		CIAT	Jamundí, Col., no. 11a	Pl bef. th.	+
53	12		CIAT	Jamundí, Col., no. 11b	Pl bef. th.	-
54	13		CIAT	Jamundí, Col., Colony a	Soil	+
55	14		CIAT	Jamundí, Col., Colony b	Soil	+
56	Termoterapia 1 roja		CIAT	Jamundí, Col., no. 1, red	Pl bef. th.	+
57	Termoterapia 1 clara		CIAT	Jamundí, Col., no. 1, pink	Pl bef. th.	+
58	Termoterapia 4		CIAT	Jamundí, Col., no. 4	Pl bef. th.	+
59	Termoterapia 11		CIAT	Jamundí, Col., no. 11	Pl bef. th.	+

[a] The first 41 are from CIAT collection. Codes 42 to 59 corresponds to plantain suckers from Jamundí (Valle del Cauca).

[b] Th. = thermotherapy.

[c] Pl bef. th = plantain before thermotherapy; Pl w. th. = plantain with thermotherapy; T, woolly var. = tobacco, woolly variety; Tobacco, Col. var. 37 = tobacco, Colombian variety 37.

We evaluated a band of 288 base pairs generated by amplification with the specific primers Oli1 (5'-GGGGGTAGCTTGCTACCTG CC-3') and Y2 (5'-CCCACTGCTGCCTCCCGTAG GAGT-3'), previously reported by Martins (2000). To do this, we conducted a direct PCR, using 24-h-old cultures grown in nutritive agar (Seal et al. 1992). We resuspended a colony in 100 µL sterilized distilled water and heated it to 95°C for 5 min. It was then centrifuged at 2800 rpm for 5 min, and 5 µL of the supernatant was taken as mold for the PCR reaction.

Each PCR reaction was carried out in a final volume of 25 µL, taking into account the following final concentrations: 0.1 mM of each of dATP, dCTP, dGTP, and dTTP; 1X *Taq* polymerase buffer; 1.5 mM $MgCl_2$; 0.5 U *Taq* polymerase; and 0.16 µM of each primer. Amplification was carried out in an MSJ-Research PTC-100 thermocycler with the following program: 2 min at 96°C, 50 cycles of denaturation for 20 s at 94°C, annealing for 20 s at 68°C, extension for 30 s at 72°C, and another final extension for 10 min at 72°C. The amplified product was visualized in 1.5% agarose gels, stained with ethidium bromide.

DNA sequencing

The products that had been amplified with primers Oli1 and Y2 (fragments of 288 bp) were sequenced. The generated sequences were then homologized with sequences of *R. solanacearum* reported in the NCBI's GenBank database (www.ncbi.nlm.nih.gov), using the application BLAST®n.

Using the program DNAMAN 4.13 and the sequences homologized with *R. solanacearum* and some sequences previously reported in the GenBank, we generated a phylogenetic tree with PAUP and bootstrap statistical analysis with 1000 replications. We took the strains presenting high homology, while taking into account that they may represent the same isolate if they came from the same host and place of origin.

Biochemical testing

When the presence of the 288-bp band was confirmed in the strains of *R. solanacearum* held at Cassava Pathology, We then determined their macroscopic morphology and biochemically characterized each strain to determine its biovar, using eight different tests on 24-h-old pure cultures.

We evaluated reaction to 3% KOH by placing 1 drop of reagent on a glass slide and then dissolving in it a colony from a pure and metabolically active culture with a 24-h growth. A reaction was considered positive if it gave rise to a viscous strand 15 to 30 s later.

The oxidase test was carried out by placing 2 drops of 1% aqueous solution of tetramethyl-*p*-phenylenediamine dihydrochloride (Kovács' oxidase reagent) on a piece of filter paper and rubbing a colony in the drops. A positive reaction was determined by appearing purple color 10 sec later.

Tests were also conducted with the disaccharides cellobiose, lactose, and maltose; and with the oxidation of hexose alcohols (or polyols) dulcitol, mannitol, and sorbitol. The methodology described by Hayward (1964) and Schaad (1988) was followed. The disaccharides and alcohols were sterilized by filtering. Three milliliters of culture medium were placed in test tubes and inoculated with a colony from a 24-h-old culture. They were then incubated at 30°C and reactions were observed at days 3, 7, and 14 after inoculation. The change of color from olive green to yellow, from top to bottom, indicated the production of acids from the disaccharides. When the color changed from purple to yellow, it indicated oxidation of the polyols. Biovars were determined according to the classification proposed by García et al. (1999) and Gunawan et al. (2002).

Results

PCR analysis

The presence of a 288-bp band generated by amplifying with primers Oli1 and Y2 enabled us to confirm the identity of 24 of the 41 strains from the Cassava Pathology collection **(Table 1**, codes 1 to 41). We could also identify 16 of the 18 isolates from plantain as *R. solanacearum* **(Table 1**, codes 42 to 59), 14 of which are shown in **Figure 1**.

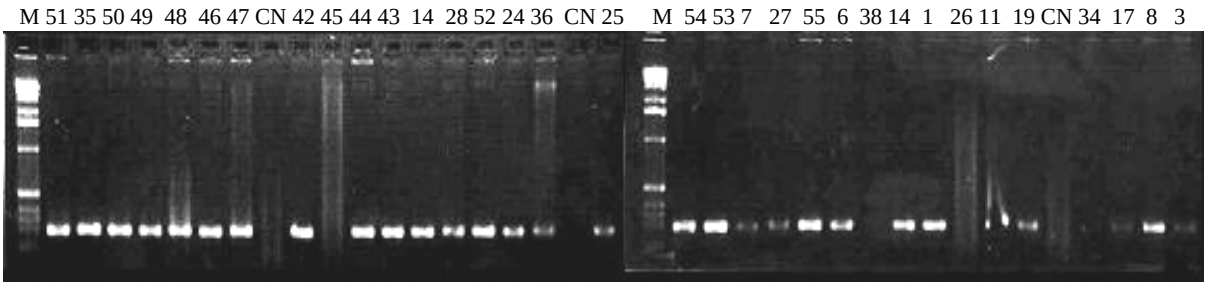

Figure 1. Product amplified from DNA of the bacte rium *Ralstonia solanacearum* wi th primers Oli1/Y2. M = 1-kb-long marker; NC = negative control. The numbe r above each lane corresponds to the strain code number described in Table 2.

DNA sequencing

The DNA sequences of the 288-bp amplified fragment presented high homology with *R. solanacearum*, with a statistical significance (e-value) between 4^{-88} and 1^{-128}. We also saw the presence of identical sequences when bacteria were isolated from different samples of a common origin. For strains isolated from plantain from Jamundí (Valle del Cauca), strain 59 presented a homology of 99% and 100% with strains 50 and 49, respectively. Strains 1, 27, and 8, isolated from tobacco in Floridablanca (Santander), behaved in a similar fashion.

Likewise, identical sequences were isolated from the same host, but of different geographical origins. The sequences of strains 31 and 38, isolated from eggplant, presented a homology of 100%, even though they had come from Kenya and Sri Lanka, respectively.

The results of the sequencing indicated that primers Oli1 and Y2 enabled us to detect a region of the genome of *R. solanacearum* that was being conserved among different races and biovars isolated from different crop species and even among different bacteria species.

The phylogenetic tree **(Figure 1)** was constructed with 44 sequences, including those of strains isolated from plantain, potato, tobacco, tomato, eggplant, Indian shot, and *Heliconia* sp, and selected from GenBank (7, i.e., *R. mannitolilytica*, *R. picketii*, *R. thomasii*, *Burkholderia solanacearum*; and AY 216796, PS01716SR, and AY642432 of *R. solanacearum*). The tree showed that some of the sequenced isolates formed a homogeneous cluster together with isolates from *R. solanacearum*, *R. mannitolilytica*, *R. picketii*, *R. thomasii*, and *Burkholderia solanacearum* from GenBank. However, they presented minor differences with isolate CIAT 1001 from tobacco and G219 from eggplant, which tended to separate from the rest.

On generating the phylogenetic tree with the sequenced strains, we could not separate them on an evolutionary basis. Nor could we find clusters according to geographical origin or type of host, which indicates that the sequenced region is highly conserved, even among strains of different species and isolated from different hosts.

Biochemical testing

The biochemical tests enabled us to classify some of the strains from the Cassava Pathology collection into different biovars. All the strains tested as Gram negative, and reacted positively to both the oxidase and 3% KOH tests. The results of other tests are described in **Table 2**.

Table 2. Results of biochemical tests carried out with isolates of the bacterium *Ralstonia solanacearum* that had amplified in the specific PCR.

Number in Collection	Crop	Maltose	Lactose	Cellobiose	Mannitol	Sorbitol	Dulcitol	Biovar[a]
1001	Tobacco	-	-	-	-	-	-	1
1004	Potato	-	-	-	+	+	+	4
1007	Tobacco	-	-	-	-	-	-	1
1008	Plantain	-	-	-	+	+	-	D
1010	Tobacco	-	-	-	-	-	-	1
1013	Tobacco	-	-	-	-	-	-	1
1016	Potato	-	-	+	-	-	-	2
1017	Indian shot	-	-	-	-	-	-	1
1020	Tobacco	-	-	-	-	-	-	1
1035	Tobacco	-	-	-	-	-	-	1
1043	Tobacco	-	-	-	+	-	-	D
1054	Tobacco	-	-	-	-	-	-	1
1055	Tobacco	-	-	-	-	-	-	1
1056	Tobacco	+	+	-	+	+	-	3
1057	Tobacco	-	-	-	-	-	-	1
1077	Tomato	-	-	-	-	-	-	1
G213	Potato	-	-	-	-	-	-	1
G175	Egg plant	-	-	-	+	+	+	4
G177	Potato	-	-	-	+	-	-	D
G216	Tobacco	-	-	-	+	+	+	4
G217	*Heliconia* sp.	-	-	-	-	-	-	1
G218	*Capsicum* sp.	-	-	-	-	-	+	D
G219	Egg plant	-	-	-	+	+	+	4

a. D = strain that had biochemical reactions that were outside the expected for the five classes of biovar. No biovar 5 was detected.

Most of the strains isolated from tobacco, together with strains from potato, Indian shot, tomato, and *Heliconia* sp., were classified as Biovar 1. One strain from potato was classified as Biovar 2, another strain from tobacco as Biovar 3, and strains from eggplant, potato, and tobacco as Biovar 4. No strains were classified as Biovar 5, and 4 strains had biochemical reactions that were outside the five classes (marked as "D" in **Table 2**).

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Activity 4. Isolation of *Fusarium oxysporum* f. sp. *cubense*, causal agent of Panama disease of banana, and evaluating its pathogenicity.

Specific objective

To isolate *Fusarium oxysporum* f. sp. *cubense*, causal agent of Panama disease of banana, and evaluate its pathogenicity

Methodology

From processed samples of wilted banana that came from the Department of Quindío, three fungi were isolated and identified by microscope as *Fusarium* spp. They were also selected for their growth in PDA and a selective medium with 0.1% pentachloronitrobenzene (PCNB), developed by Nash and Snyder (1962) to isolate *Fusarium* species from plant tissue. Microscopic observation (40X) showed morphological characteristics of the fungus grown in the above-mentioned selective medium and in PDA.

Isolates identified as *Fusarium* and previously grown for 6 days in PDA were evaluated for their pathogenicity in plants of the banana known as 'Cocos' and plantain under the controlled conditions of the greenhouse. Inoculation was carried out by infiltration in the pseudostem with 3 mL of a suspension with 1×10^6 conidia per milliliter, as calculated in the Neubauer chamber. For an absolute check, pseudostems were also inoculated with sterilized water.

Results

Microscopic examination showed abundant proliferation of microconidia, usually loose and oval, the presence of chlamydospores formed in the hyphae, branched and unbranched monophialids, and scarce presence of macroconidia. In the PDA medium, the colony showed white aerial mycelium and the presence of red pigment, whereas in the Nash and Snyder culture, the mycelium was white and grew slowly.

The isolates were not pathogenic to two genotypes of banana from the FHIA collection, whereas they did infect the regional variety Cocos, cultivated in Quindío.

Reference

Nash SM; Snyder WC. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567–572.

Activity 5. DNA sequence analysis of the 16S rRNA region of phytoplasmas obtained from oil palm, insect vectors, and weeds in Casanare, Colombia.

Specific objectives

1. To identify the causal agent of lethal wilt in oil palm by using PCR and sequencing.
2. To determine possible insect vectors of the causal agent of lethal wilt in oil palm.

Methodology

Sample collection

We collected 268 samples of tissue from oil palm showing early, intermediate, and advanced symptoms (as evaluated by each plantation) of the disease known as "lethal wilt". The causal agent had been found to be a phytoplasma (CIAT 2002). The collection included 42 samples from healthy plants and 7 from weeds collected at affected lots. The samples originated from three plantations **(Table 1)**, located in the Department of Casanare.

Table 1. Detecting phytoplasmas, using nested PCR and primers Fu5/Ru3 and R16F2N/R16R2, in samples of oil palm affected by lethal wilt. The samples were taken from three plantations at Casanare, Colombia.

Sample Code Number	Plot	Tree Line	Palm Tree Number	Plantation	Plant Part	Dev't Dtage of Lethal Wilt	PCR (+)	
							Fu5/ Ru3	R16F2N/ R16R2
P7	9H	162	14	1	Inflorescence	Intermediate		+
P8	9H	162	14	1	Leaf spear	Intermediate		+
P9	9H	162	14	1	Base of leaf spear	Intermediate		+
P10	9H	162	14	1	Meristem	Intermediate		+
P24	9H	17	6	2	Flower primordia	Intermediate		+
P73	8E	16	28	3	Base of leaf spear	Advanced		+
P108	10H	239	2	2	Flower primordia	Advanced		+
P109	10H	88	4	2	Meristem	Advanced		
P110	23H	126	7	2	Flower primordia	Intermediate		+
P114	10H	88	4	2	Forming inflorescence	Advanced		+
P115	23H	126	7	2	Forming inflorescence	Intermediate		+
P120	23H	126	7	2	Base of leaf spear	Intermediate		+
P125	8G	79	11	2	Base of leaf spear	Advanced		+
P130	8G	79	11	2	Base of inflorescence	Advanced		+
P131	G16 (88)	62	P6	1	Inflorescences	Advanced		+
P138	G16 (88)	80	P5	1	Inflorescences	Advanced		+
P143	G17 (88)	65	P1	1	Leaf spears	Advanced		+
P144	G17 (88)	65	P1	1	Meristem	Advanced		+
P 147	10H	99	2	2	Forming inflorescence	Intermediate	+	+
P 148	10H	99	2	2	Leaf spears	Intermediate	+	
P153	10H	99	2	2	Peduncle of inflorescence	Intermediate	+	
P155	9H	3	3	2	Forming inflorescence	Advanced	+	+
P156	9H	3	3	2	Leaf spears	Advanced	+	
P157	9H	3	3	2	Base of leaf spears	Advanced	+	
P158	9H	3	3	2	Isolated meristem	Advanced	+	
P159	9H	3	3	2	Base of meristem	Advanced	+	
P161	9H	3	3	2	Peduncle of inflorescence	Advanced	+	
P174	G18	39	3	1	Base of meristem	Advanced	+	+
P175	G18	39	3	1	Forming inflorescence	Advanced	+	+
P 178	G18	39	3	1	Isolated meristem	Advanced		+
P 185	G17	21	16	1	Lower stipe	Intermediate		+
P 201	10H	96	3	2	Cylinder of stipe	Intermediate		+
P 202	6H	21	9	2	Cylinder of stipe	Intermediate		+
P 203	6H	48	1	2	Cylinder of stipe	Intermediate		+
P 207	6H	20	6	2	Cylinder of stipe	Intermediate		+
P 209	10H	96	2	2	Cylinder of stipe	Intermediate		+
P 213	6H			2	Grass	Infected plot		+
P 214	10H			2	Grass	Infected plot		+

Sample Code Number	Plot	Tree Line	Palm Tree Number	Plantation P	lant Part	Dev't Dtage of Lethal Wilt	PCR (+)	
							Fu5/ Ru3	R16F2N/ R16R2
P 220	MP1C	4	28	1	Base of leaf spear	Affected by Bud Rot		
P 224	9A	10	3	3	Base of meristem	Intermediate		+
P 225	9A	10	3	3	Isolated meristem	Intermediate		+
P 226	9A	10	3	3	Upper meristem	Intermediate		+
P 227	9A	10	3	3	Base of leaf spear	Intermediate		+
P 228	9A	10	3	3	Forming inflorescence	Intermediate		+
P 229	9A	10	3	3	Flower primordia	Intermediate		+
P 230	9A	10	3	3	Fruits	Intermediate		+
P 231	9A	10	3	3	Roots	Intermediate		+
P 233	G16	62	10	1	Roots	Advanced		+
P 234	10H	67	7	2	Leaf spears	Advanced		+
P 235	10H	67	7	2	Base of leaf spear	Advanced		+
P 236	10H	67	7	2	Flower primordia	Advanced		+
P 237	10H	67	7	2	Base of inflorescence	Advanced		+
P 238	10H	67	7	2	Forming inflorescence	Advanced		+
P 239	10H	67	7	2	Base of meristem	Advanced		+
P 240	10H	67	7	2	Meristem	Advanced		+
P 241	10H	67	7	2	Roots	Advanced		+
P 243	9G	35	11	2	Adventitious roots	LW in Bud Rot plot[a]		+
P 245	10H	190	3	2	Adventitious roots	Early		+
P 246	10H	190	3	2	Roots	Early		+
P 247	10H	190	3	2	Forming male inflorescence	Early		+
P 248	10H	190	3	2	Base of meristem	Early		+
P 249	10H	190	3	2	Leaf spears	Early		+
P 250	10H	190	3	2	Base of leaf spears	Early		+
P 251	10H	190	3	2	Flower primordia	Early		+
P 252	10H	190	3	2	Base of inflorescence	Early		+
P 253	10H	190	3	2	Meristem	Early		+
P 254	10H	237	8	2	Roots	Intermediate		+
P 255	10H	237	8	2	Meristem	Intermediate		+
P 256	10H	237	8	2	Base of meristem	Intermediate		+
P 257	10H	237	8	2	Leaf spears	Intermediate		+
P 258	10H	237	8	2	Base of leaf spear	Intermediate		+
P 259	10H	237	8	2	Base of inflorescence	Intermediate		+
P 260	10H	237	8	2	Flower primordia	Intermediate		+
P 261	10H	237	8	2	Forming inflorescence	Intermediate		+
P 262	10H	237	8	2	Fruit	Intermediate		+
P 266	1OH	190	3	2	Leaves	Early		+
P 267	9A	10	3	3	Base of meristem with yellowing	Intermediate		+
P 268	10H	67	7	2	Base of meristem with yellowing	Advanced		+

a. LW = lethal wilt in a site affected by Bud Rot.

DNA extraction

The DNA from palm tissues was extracted as described by Gilbertson et al. (1993) from samples of leaf spear, base of leaf spear, inflorescence, meristem, base of meristem, flower primordium, raceme peduncle, inflorescence peduncle, fruit, stipe, and roots. As positive controls, we used DNA from lulo (*Solanum quitoense*) and periwinkle (*Catharantus roseus*) plants, and samples of palm tissue in which the phytoplasma was originally detected in extractions made in July 2002. The DNA was diluted in sterilized distilled water at a final concentration of 20 µg/µL.

Six collections of insects were made in three plantations at Casanare (Colombia) from November to December 2002, and September 2003 to March 2004. A total of 170 samples were processed, covering different homopterous species of the families Cicadellidae, Nogodinidae, and Membracidae **(Figure 1)**. The insects were collected from the lower parts of infected palms located in plots under the highest disease pressure. The samples were sent either in 70% alcohol or NaCl solution, or dehydrated and wrapped in cotton wool. A total of 24 species of Homoptera were received. From some species, we extracted the following organs: the ovariole or eggs, parts

of the intestine, and glands (female), and intestine and glands (male). To prevent the organs dehydrating, they were extracted with the insects submerged in serum (NaCl at 8.5 g/L).

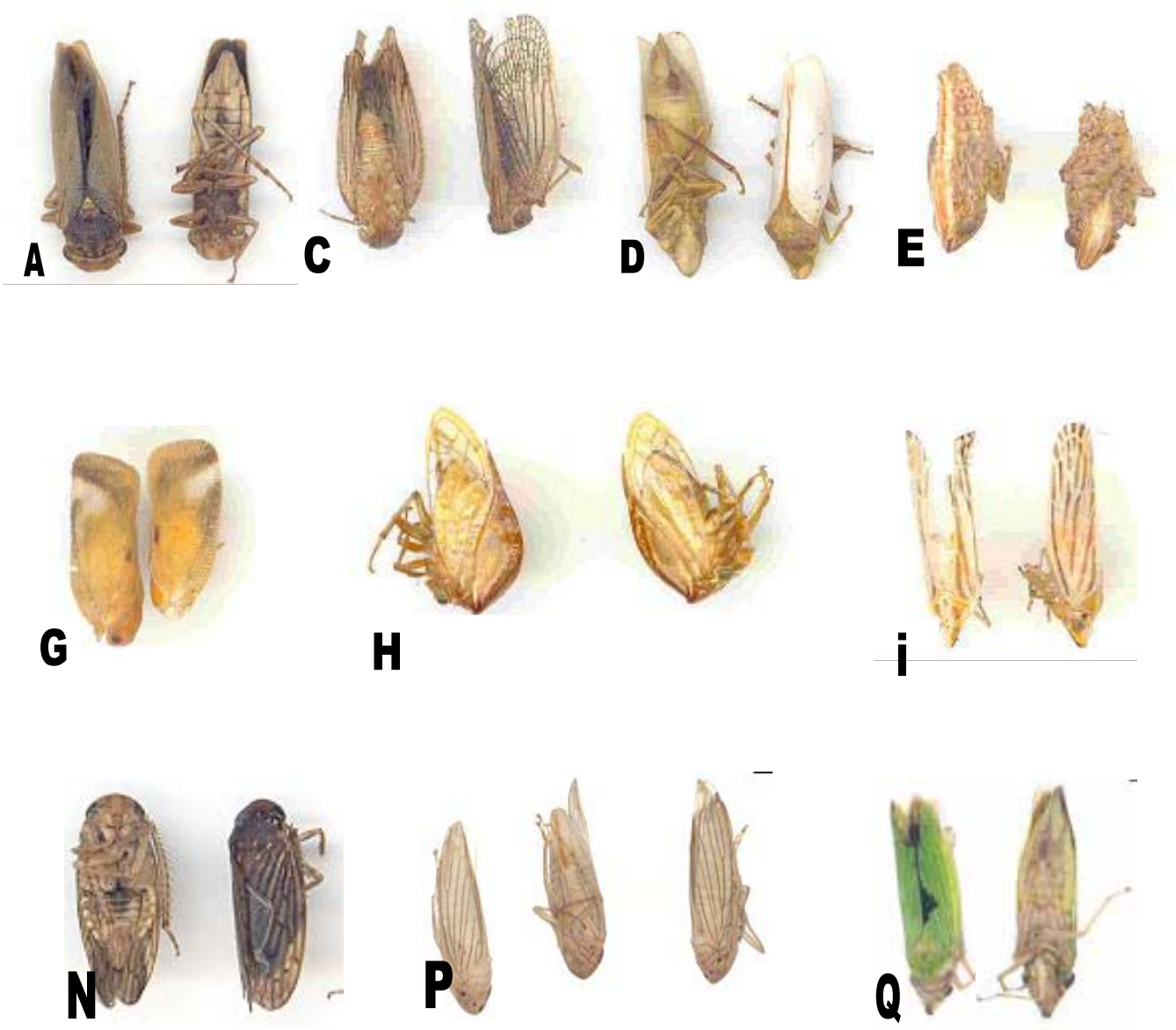

Figure 1. Insect species collected from three oil-palm plantations at Casanare, Colombia, where phytoplasmas were detected. A, E, G, I, N, P, Q = Cicadellidae family ; C = Nogodinidae family; H = Membracidae family.

Detection by PCR in plant or insect tissues

Samples of DNA from healthy and infected palms, and from weeds from infected plots were amplified, using direct and nested PCR. For the first amplification we used the primer pairs P1/P7 or R16mF2/R16mR1 under the following conditions: 100 ng DNA, 1X buffer, 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.1 µM of each primer, and 1 U *Taq* polymerase (CIAT). For the primers P1/P7, 35 cycles were conducted in a thermocycler (PTC-100), with the following

conditions: 30 s (90 s for the first cycle) of denaturation at 94°C, annealing for 50 s at 55°C, extension for 80 s at 72°C, and another final extension for 10 min at 72°C. For the primers R16mF2/R16mR1, 28 cycles were conducted under the same conditions as for the previous primers. The PCR products were diluted to 1:50 with sterilized distilled water for use as 1-µL DNA molds in nested PCR. This PCR was amplified with primer pairs R16F2N/R16R2 and Fu5/Ru3, with an annealing temperature of 50°C and 53°C, respectively. The PCR products were analyzed by electrophoresis in 1.5% agarose gel to visualize the bands.

PCR-RFLP

To classify them into phytoplasma groups, the amplified fragments were digested with restriction enzymes *Msp*I, *Alu*I, and *Taq*I. We took 5.4 µL of PCR product and added 2 µL of 10X buffer enzyme and 0.6 µL of restriction enzyme (500 units/µL), and completed the final volume with water to 20 µL. The suspension was incubated for 16 h at 37°C (the enzyme *Taq*I at 65°C). Then 3 µL of loading buffer (0.25% bromphenol blue and glycerol in water at 30%) was added and the whole was run under electrophoresis in Synergel for 6 h at 100 V, 24 mA, in 1X TBE buffer, staining the gel with ethidium bromide at 10 mg/mL.

Electron microscopy

From each sample of plant tissue, 1–3 mm fragments were cut out and pre-fixed in 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2). Post-fixing was done in 1% OsO_4 buffer. The fragments were then dehydrated by immersing for 20 min in pure isopropanol alcohol for each of the following concentrations: 25%, 50%, 70%, 80%, 95%, and 100%. They were then washed three times in pure acetone. The samples were embedded, using 100% and different mixtures of epoxy resin (Spurr's medium) and acetone, all polymerized at 60°C for 8 h.

The embedded samples were cut with a microtome, using a diamond knife, to obtain ultra-thin sections 60 to 90 nm thick. The dispersion of atoms and staining were done with uranyl acetate and Reynold's lead citrate. Observations were then made with a transmission electron microscope. The fixed fragments from a total of 37 tissue samples were evaluated by Dr. Tracey Pepper, specialist in electron microscopy, at the Iowa State University.

Results

DNA extraction

With the Gilbertson extraction method, high quality and high concentration DNA (between 50 and 600 ng/µL) was obtained both from plant tissues and insect organs. However, smaller quantities of DNA (10 to 30 ng/µL) were normally obtained from roots, tending to increase with young, unlignified roots.

Detection by PCR in plant or insect tissues

We obtained amplifications from 40% of processed samples with intermediate or advanced symptoms, 17.5% with early symptoms, and none from healthy samples. Meristem bases presented the highest percentage of amplifications (78%), followed by flower primordia (75%), leaf spears (60%), and stipes (33%). Amplifications were also obtained from all four samples from inflorescence bases. The positive checks from lulo, periwinkle, and palm indicated that the

band obtained from the samples with lethal wilt of palm corresponded to a phytoplasma. **Figures 2 and 3** show the results obtained for the amplifications. For sequencing, we selected samples on the basis of different tissues.

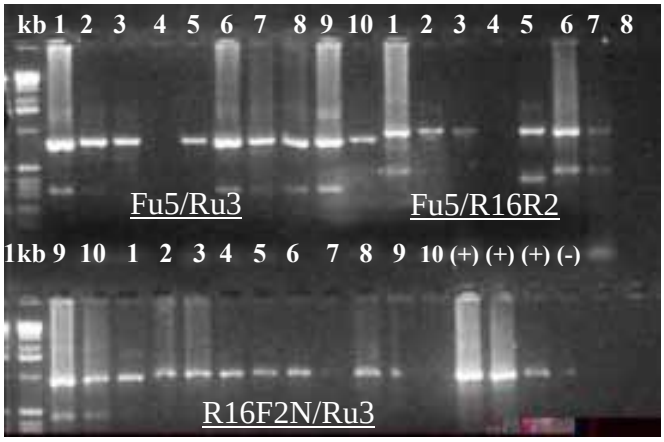

Figure 2. Amplifications w ith universal pr imers fo r phytoplasmas (P1/P7 nested w ith R16F2N/R16R2–Fu5/Ru3) from oil palms w ith early sy mptoms of lethal w ilt. M = marker. Samples are lane 1 = p229; 2 = p233; 3 = p 236; 4 = p256; 5 = p257; 6 = p258; 7 = p259; 8 = P8; 9 = lulo ; 10 = periwinkle. Positive controls for phytoplasmas are lulo and periwinkle; negative control is water.

From crop weeds sampled in infected plots, we obtained PCR amplifications from a grass known as *pasto negro* (black grass), which harbored a high population of insects of the Nogodinidae family. A phytoplasma was also detected by PCR in this insect's tissues.

Phytoplasmas appear exclusively in phloem vessels, have a normally heterogeneous distribution in the plant, and are found in low concentrations. These characteristics make their detection and identification difficult (Seemüller et al. 1998). Hence, we had to design specific primers from the sequences obtained in our study to increase sensitivity for detecting the pathogen in plants with very low levels of inoculum (i.e., early symptoms).

Of the 23 species of insects collected at points of highest disease pressure, 11 presented the highest percentages of amplification, between 45% and 100%, regardless of the organ DNA amplified, indicating the samples as positive (**Figure 3**). Detection by PCR in Nogodinidae, Cicadellidae, and Membracidae was 40%, 57.1%, and 60%, respectively, on preparing a mixture of DNA of different organs extracted from the insects.

According to insect organ, detection for glands was 51.2%; intestines, 68.3%; and eggs, 37.5%, with no significant differences between them. This indicates that, in future evaluations of new species, fragments of the abdomen and entire thoraxes can be taken, without having to dissect each insect, thus making detection more efficient. According to the species of insect, 100% amplifications were obtained from individuals coded as A, E, I, N, and Q; and 50% from individuals coded as G and P, both groups from the Cicadellidae family. Moreover,

amplifications were observed for insects coded as C (Nogodinidae family; 40%) and H (Membracidae family, 60%) **(Figure 1**).

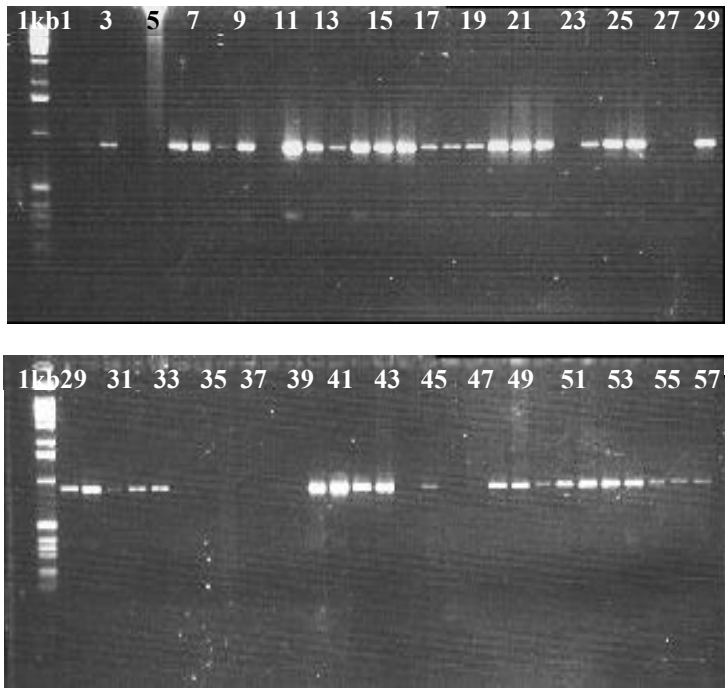

Figure 3. DNA from insect tissu es amplified with primers P1/P7 nested with Fu5/Ru3. Lanes 1–24 = Membracidae family; lane s 25–37 = Nogodinidae family; lanes 38–57 = Cicadellidae family.

PCR-RFLP

Taking into account the polymorphism indicated in the patterns of bands (**Figure 4**), we observed that at least two groups of phytoplasmas existed. One corresponded to samples from plantation number 1 (lane 7) and the other to samples from plantation number 2 (lane 8). These results were similar to those obtained with samples from both plantations sequenced at the Iowa State University in 2001 by Elizabeth Álvarez and Thomas Harrington. Lanes 6 and 9 correspond to *Acholeplasma.*

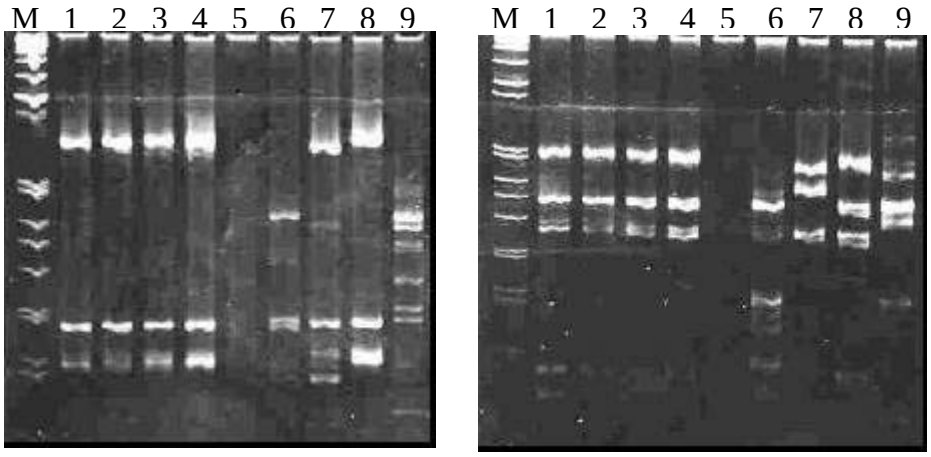

Figure 4. Restriction patterns of DNA amplified and later digested with enzymes *Alu*I and *Rsa*I. M = 1-kb marker; lanes 1–5 = cassava controls; lane 6 = *Acholeplasma* from leaf-base tissue from the oil-palm plantation number 1; lane 7 = phytoplasma from leaf-base tissue from plantation number 1; lane 8 = phytoplasma from inflorescence tissue from the oil-palm plantation number 2; lane 9 = *Acholeplasma* from leaf-base tissue from plantation number 2, Colombia.

Sequencing analysis

The sequence of the DNA band, measuring 1200 nucleotides, was determined, using primers R16F2N/R16R2 and Fu5/Ru3. It was then compared with sequences of phytoplasmas reported in GenBank, using the tool BLAST®n. Some of the amplified fragments were cloned with *Escherichia coli* and sequenced using primers T7 and Sp6.

The phytoplasma sequences analyzed in this study showed homology with sequences of the 16Sr I group (aster yellows; 92%), the 16Sr IV group (coconut lethal yellowing; 93%), and of *Acholeplasma palmae* (94%). All three groups belong to the Acholeplasmataceae family. The computer-assisted aligning of sequences revealed that the sequence of nucleotides of the 16S rRNA region of the some oil-palm phytoplasma was similar to the GenBank accessions AY180932.1, AY249248, and AF 487779, corresponding to Aster Yellows group. Other phytoplasma isolates were similar to the GenBank accessions AF 105316 and AF361019, corresponding to Pigeonpea Witches' Broom group (**Figure 5**).

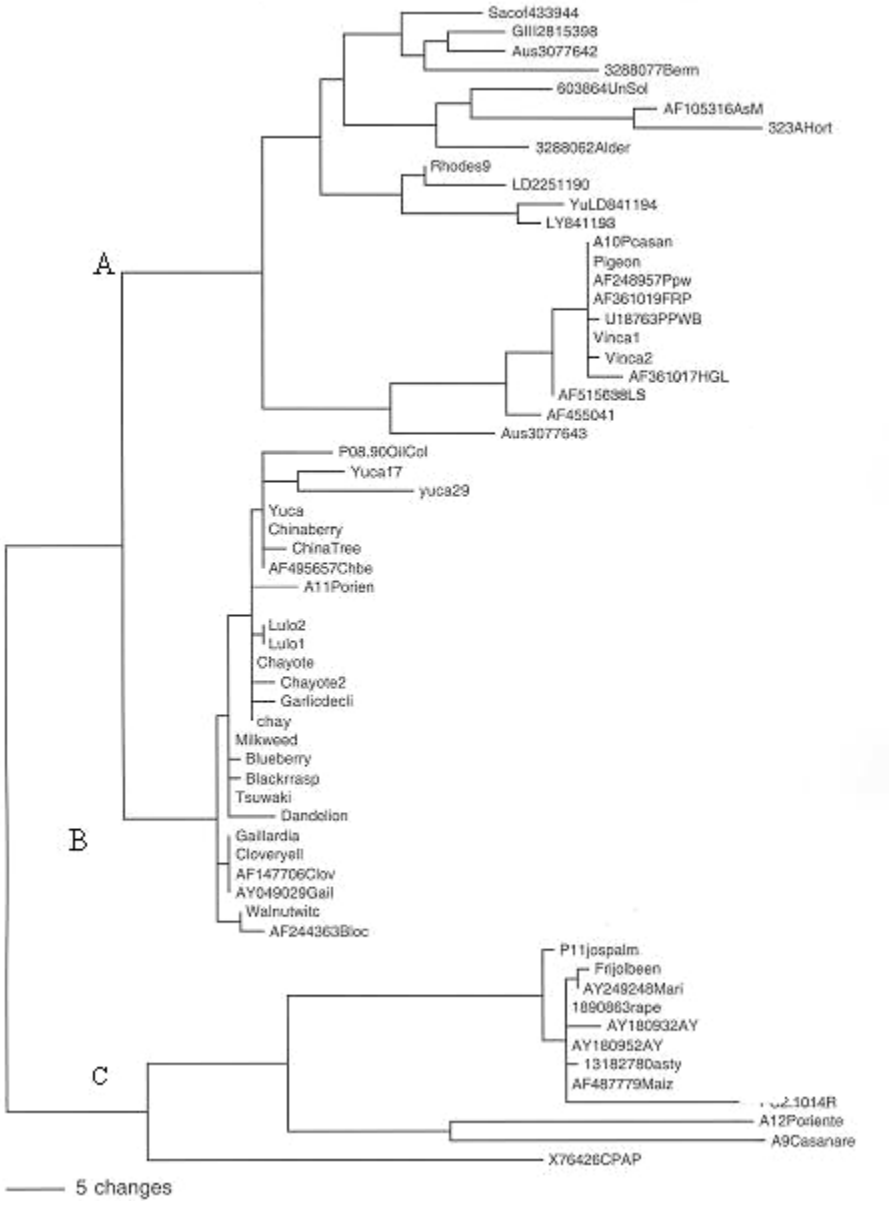

Figure 5. Phylogenetic tree constructed for oil-palm samples, using PAUP and bootstrapping of 1000 replications. Cluster A = 16Sr IX (pigeonpea witches' broom); cluster B = 16Sr III (x-disease); cluster C = 16Sr I (aster yellows). A10Pcasan = plantation number 1 (base of leaf spear); P08.90OilCol = plantation number 2 (inflorescence); A11Porien = plantation number 2 (inflorescence); P11jospalm = plantation number 1 (base of leaf spear); A12Poriente = plantation number 2 (leaf spear); A9Casanare = plantation number 1 (base of leaf spear).

The homology tree (**Figure 6**) presented two genetic groups that separated at 75%. One relatively uniform group was made up of phytoplasmas detected in palm or insect tissues from the three plantations. The other group comprised phytoplasmas from various groups registered at the GenBank database.

The differences between the homologies resulted when, in the first case, comparisons were made between two sequences—one from a palm and the other from GenBank—indicating their similarity; whereas, in the second case, many sequences were compared, thus accentuating their differences, even though these are small. Moreover, the primers used by each researcher reporting sequences to GenBank are different, such that different subregions of the 16S rRNA region are amplified, thus influencing the generation of the homology tree. The GenBank group of phytoplasmas, however, included two sequences of oil-palm phytoplasma corresponding to this study (A9 and FP 2001).

The sequence of phytoplasma DNA obtained from insects and the grass *pasto negro* showed high homology with oil-palm phytoplasmas. Diversity was also found among phytoplasmas detected in palms, insects, and the grass, thus confirming the polymorphism observed with the PCR-RFLP. However, transmission tests must be made to verify that the insect species involved is capable of transmitting the phytoplasma, as an insect may carry the phytoplasma without necessarily being able to transmit it.

The homology analysis of the sequence from the 16S rRNA region and the tRNA gene confirmed the presence of a phytoplasma in association with lethal wilt of oil palm. To produce a more specific diagnosis when detecting the phytoplasma, DNA fragments obtained in this study could be cloned for use as specific probes for pathogenic phytoplasmas in oil palm.

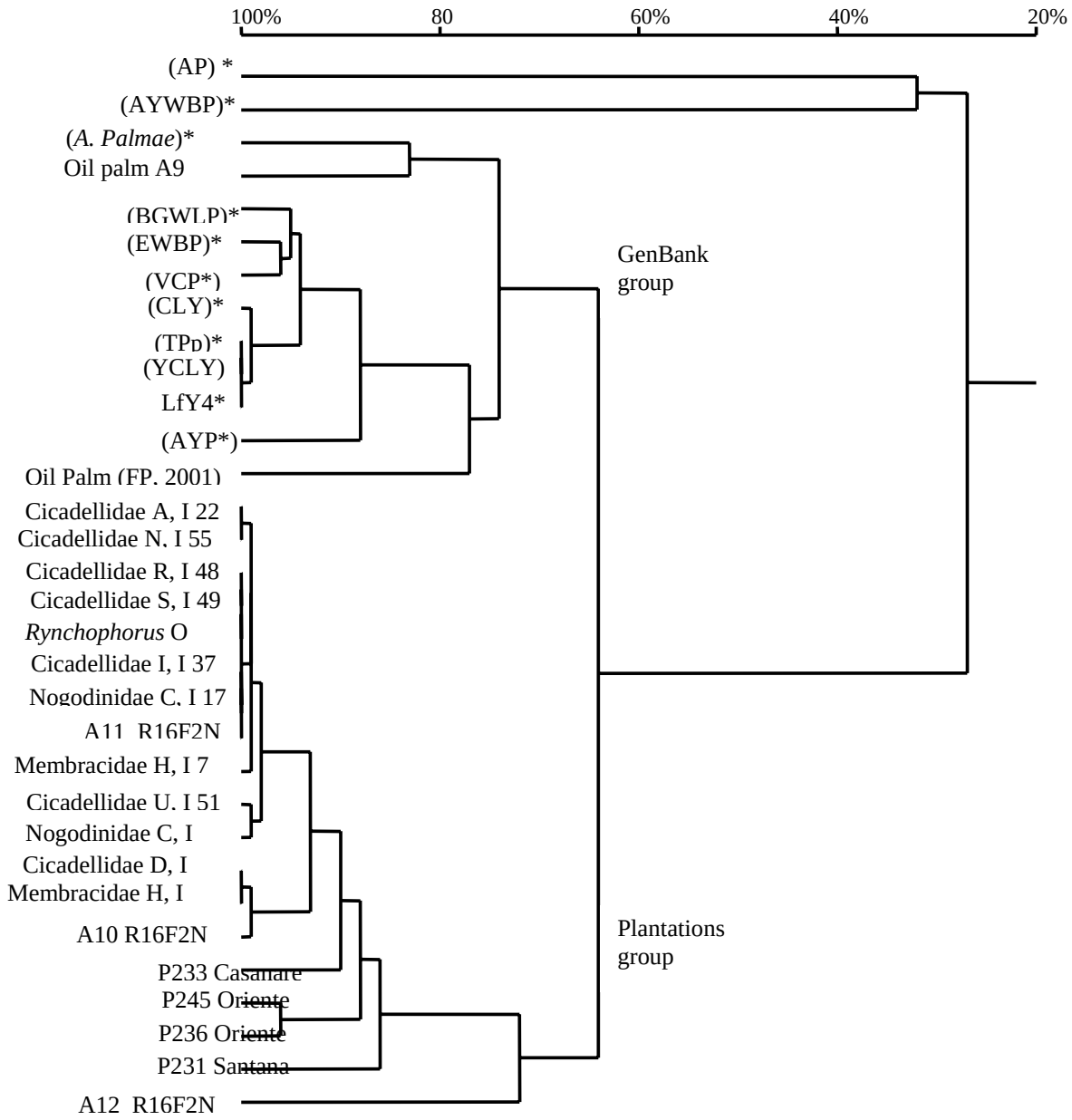

Figure 6. Homology tree of sequences from the 16S rRNA region of phytoplasmas detected in oil palm and insects. * = GenBank accessions: Apple proliferation (AP), Aster yellows witches'-broom phytoplasma (AYWBP), *Acholeplasma palmae* (A. palmae), Bermuda-grass white-leaf phytoplasma (BGWLP), Erigeron witches'-broom phytoplasma (EWBP), Virginia creeper phytoplasma (VCP), Coconut lethal-yellowing phytoplasma (CLY), Texas Phoenix palm phytoplasma (TPP), Yucatan coconut lethal-decline phytoplasma (YCLY), Phytoplasma sp. LfY4 (MA5)-Oaxaca (LfY4), Aster yellows phytoplasma (AYP), and Phytoplasma palma (FP). The codes for the insect ranks correspond to those in Figure 1.

Electron microscopy

In Dr Tracey Pepper's laboratory at the Iowa State University, phytoplasmas were observed in sieve tubes in samples from plantation number 1 and 2, compared with *Catharanthus roseus,* using as a control (**Figure 7**).

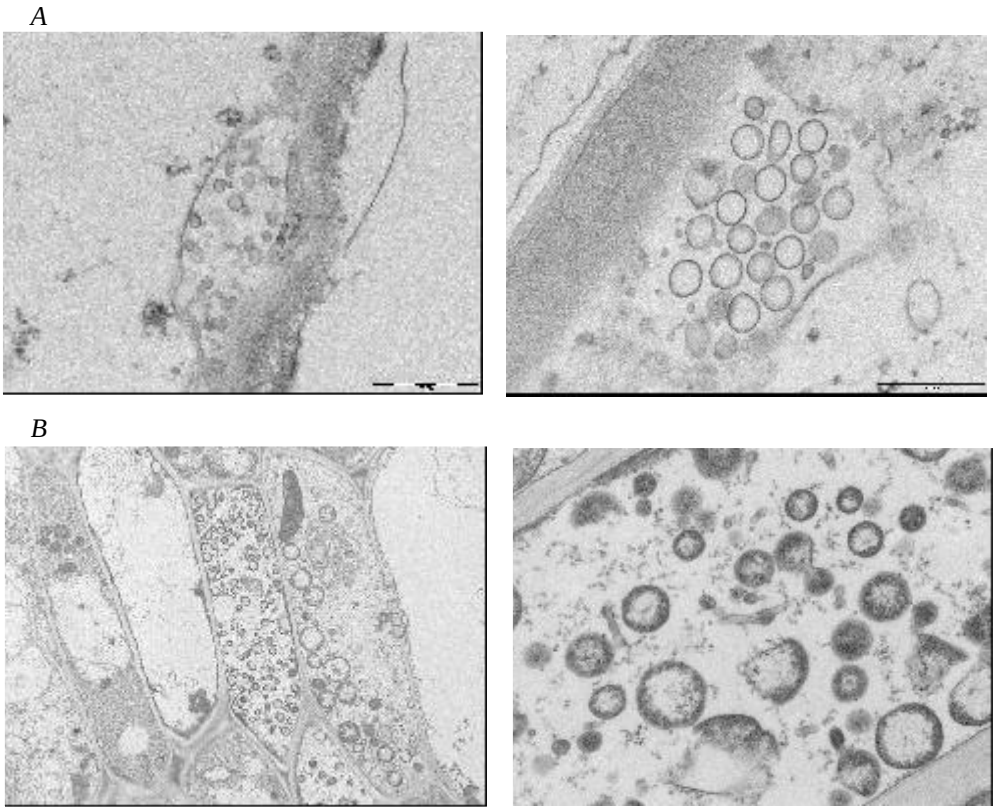

Figure 7. Phytoplasmas found in samples of (A) oil palm infected by lethal wilt, and (B) periwinkle (*Catharanthus roseus*), using transmission electron microscopy.

Conclusions

From our study, we arrive at the following conclusions:

1. We developed a method for detecting, through nested PCR and using primers P1/P7 and re-amplifying with primers R16F2N/R16R2 and Fu5/Ru3, phytoplasmas in (a) 40% of samples of palm tissue showing intermediate and advanced stages of lethal wilt, and (b) in 17.5% of samples of palm tissue showing early symptoms.
2. Phytoplasmas were detected in infected palms, principally in tissues from meristem bases, flower primordia, and leaf spears.
3. Phytoplasmas were detected in insects of different species of the following families: Cicadellidae, Membracidae, and Nogodinidae.

4. Phytoplasmas detected in insects were found principally in glands and intestines.
5. By DNA sequencing, we identified different phytoplasmas in plant and insect tissues.
6. The homology found between sequences of phytoplasmas from plant and insect tissues was almost 100%.
7. A phytoplasma amplified from a grass that grew in an infected plot at plantation number 2 showed high homology with phytoplasmas from palms and insects.

References

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Gilbertson, L. Dellaporta, S. L 1983. Molecular extraction DNA protocols. In Molecular Biology of Plants (pp. 395-397). Cold Spring Harbor, N.Y. Cold Spring Harbor Laboratory.

NCBI (National Center for Biotechnology Information). http://www.ncbi.nlm.nih.gov.

Seemuller, E.C., U. Marcone, A. Lauer, A. Ragozzino, and M. Goschl. 1998. Current status of molecular classification of the phytoplasma. J. Plant Pathol. 80:3-26.

Activity 6. Collecting oomycetes and bacteria from oil palm, and evaluating their pathogenicity.

Objectives

1. To isolate and conserve bacteria and Pythiaceae microorganisms obtained from oil palm infected with lethal wilt
2. To evaluate their pathogenicity and establish their association with the disease

Introduction

Lethal wilt is considered as an economically significant disease of oil palm. It spreads very quickly, although only over short distances, and causes plant death within 4 to 6 months after the first symptoms appear. To date, the disease cannot be controlled. Activity 5 presented the results of research on the association between phytoplasmas and lethal wilt. In this Activity, we present results of evaluations of not only their pathogenicity but also of bacteria found in association

Methodology

Isolating and conserving bacteria. We took 42 samples from palms infected with lethal wilt and growing in two plantations at Villa Nueva, Department of Casanare, Colombia. The sampled tissues were from roots, leaf spear and base, cylinder of stipe, meristems and base, forming inflorescence, peduncle of inflorescence, and flower primordium. The samples were washed with deionized water for 15 min. We then cut 3-mm fragments from each sample and placed them on nutritive agar medium. The petri dishes were incubated at 30°C for 24 to 48 h. The isolates were then purified, using the same culture medium. The isolates obtained were conserved in 60% glycerol in cryopreservation tubes at -80°C.

Pathogenicity of bacteria. Bacterial colonies isolated from tissues of infected oil palm were planted in nutritive agar and incubated at 30°C for 48 h. A bacterial suspension was then prepared to a concentration of about 1×10^9 cfu/mL. Inoculation with the suspension was done in two ways: the first was to inject 5 mL per point located diagonally on the base of shoots. The second way was to spread 50 mL of the suspension on the soil surface around each palm and cutting the palm's root apices.

The inoculated palms were 2–3 years old, of the genotype AR Malasia, and considered as susceptible to lethal wilt. They had been planted in 60-lb bags containing a mixture of 2 parts sand to 1 part soil and pasteurized by steaming for 5 h at about 80°C. Two palms per isolate were inoculated and incubated for 3 days at 20°C to 30°C in a humidity chamber. Relative humidity was at first 98%, then 60%, and raised again to 90%. Negative controls were palms or bags inoculated with sterilized distilled water.

Obtaining Pythiaceae isolates. Roots were washed with deionized water, and 3-mm fragments were cut out. These were disinfected first in 1% sodium hypochlorite and then in 50% ethanol for 1 min. They were then dried on sterilized paper toweling and five fragments were planted in

each of three selective media: oat agar (OA), potato dextrose agar (PDA), and V8-agar (V8A). The media contained benomyl at 15 ppm, rifampicin (10), penicillin (200), hymexazol (50), and PCNB (200). Tissue fragments were also planted in OA, PDA, and V8A without antibiotics or fungicides. The petri dishes were incubated at 20°C in the dark for 10–15 days. The cultures were checked every day for the presence of oomycetes. Tips of hyphae were also planted to ensure purity of the cultures.

Conserving Pythiaceae isolates. The isolates were conserved in 10 mL of nutritive broth and incubated at 26°C for 48 h and then at 15°C. Fragments of mycelia from PDA were also conserved in water at 15°C.

Extracting total DNA. We took about 0.1 g of fresh mycelia from pure isolates planted in OA and PDA and placed them in 1.5-mL Eppendorf tubes. We added a small quantity of beach sand, 50 mg polyvinylpolypyrrolidone (PVPP), and 750 µL extraction buffer (200 mM Tris HCl, pH 7.5; 250 mM NaCl; 25 mM EDTA; and 0.5% SDS). The mixture was homogenized with a plastic Eppendorf micro-pestle, centrifuged at 13,000 rpm for 5 min, and the supernatant removed to a fresh tube. We then added 500 µL of phenol:chloroform:isoamyl alcohol at 25:24:1 to the supernatant, and gently inverted and re-inverted the whole several times. It was then centrifuged at 13,000 rpm for 5 min, and the aqueous phase removed. To the remaining volume, we added cold isopropanol and vigorously inverted and re-inverted the whole several times. It was then centrifuged at 13,000 rpm for 10 min, after which the entire supernatant was discarded. The remaining pellet was washed with 1 mL 70% ethanol, mixed vigorously, and centrifuged for 2 min to later discard excess ethanol. The pellet was dried and resuspended in 30 µL sterilized bi-distilled water. We added 1 µL RNase at 10 mg/mL, and the whole was conserved at -20°C (http://www.phytid.org/methods.htm).

PCR-RFLP and sequencing. Total DNA was used at a concentration of 5 ng/µL to amplify the internal transcribed spacer regions (ITS 1 and ITS 2) and subunit 5.8S of rDNA with the universal primers ITS 4 and ITS 6. Conditions were as follows: 5 ng DNA, 1X buffer, 1 mg/mL bovine serum albumin (BSA), 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 µM of each primer, and 1 U *Taq* polymerase. We conducted 35 cycles in a PTC-100 thermocycler, using the following conditions: 30 s (3 min for the first cycle) of denaturation at 94°C, annealing for 30 s at 55°C, extension of the primer for 60 s at 72°C, and a final extension for 10 min at 72°C. The range of molecular weight expected in the bands was 860 to 950 bp for Pythiaceae. As positive control, we used an isolate of *P. tropicalis*.

To determine the species and classify the isolates, the amplified fragments were digested with restriction enzymes *Msp*I, *Alu*I, and *Taq*I. We took 5.4 µL of the PCR product and added 2 µL 10X enzyme buffer and 0.6 µL restriction enzyme (500 U/µL), and completed the final volume of 20 µL with water. This suspension was incubated for 16 h at 37°C (that with the enzyme *Taq*I was incubated at 65°C). Then, 3 µL loading buffer (0.25% bromphenol blue and glycerol in water at 30%) were added. Electrophoresis was run in Synergel™ for 6 h at 100 V, 24 mA, in TBE 1X buffer and stained with 10 mg/mL ethidium bromide.

The DNA fragments, amplified in the ITS region, were purified by adding a volume of a 20% solution of polyethyleneglycol (PEG) with 2.5 M NaCl for later sequencing with BigDye

Terminator Kit (Applied Biosystems) in an ABI Prism® 377 sequencer. Sequencing analysis was conducted with the programs Sequencher 4.1 and DNAMAN 4.13. Homology was sought in GenBank (www.ncbi.nlm.nih.gov), using the tool Blast®n. Homology was determined on the basis of the region ITS 1, 5.8S and ITS 2.

Pythiaceae pathogenicity. The six Pythiaceae isolates obtained from planting in PDA culture medium were incubated for 8 to 12 days at 20°C, and then inoculated into 6 to 8-month-old palms of the variety AR Malasia. The inoculation method was to inject the base of shoots of 5-month-old plants, standing 125 to 150 cm high, through perforations 3 to 5 cm deep into the plant and with 4-mm diameters. The inoculum was 10 mL of a suspension of mycelia from PDA or OA had been colonized by the microorganism. Once the perforation was injected, the orifice was covered with cement. For controls, we had plants inoculated with sterilized water.

The inoculated plants were incubated at a relative humidity of 90%–100% and 22°C and 30°C for 3 days. The plants were then incubated at an RH between 50% and 80% and 19°C and 30°C.

Results

Isolating bacteria and evaluating their pathogenicity. From the processed plant samples, we obtained 102 bacterial isolates. Colonies of different colors and growth types were isolated. **Table 1** presents the origins of the isolates that are now being evaluated for their pathogenicity. The bacterial colonies were grouped according to morphological characteristics, and 66 isolates were selected for inoculating the palms. Four months after inoculation, the bacteria's pathogenicity was evaluated.

Table 1. Origins of the bacteri al isolates obtained from tissue s amples of oil pa lms infected with lethal wilt, Department of Casanare, Colombia.

Tissue	No. of Samples	No. of Isolates	
		Isolated	Inocul ated
Base of leaf spear	5	10	6
Base of meristem	4	11	10
Cylinder of stipe	5	5	5
Leaf spear	2	5	5
Forming inflorescence	3	7	6
Meristem, including base	1	1	1
Meristem, not including base	2	6	5
Peduncle of inflorescence	3	10	5
Flower primordium	3	4	3
Root	14	43	20
Total 42		**102**	**66**

Isolating and identifying Pythiaceae, and evaluating their pathogenicity. From processed root samples we obtained four isolates belonging to the Pythiaceae family. **Table 2** indicates their origins.

Table 2. Origins of Pythiaceae isolat es obtained from root tissues of oil palms infected with lethal wilt (LW), Department of Casanare, Colombia.

Isolate No.	Sample No.	Plot	Tree Line	Palm No.	Plantation	Severity of LW
P216	P216	G16	57	4	1	Healthy in disease pressure point
P217	P217	G16	62	10	1	Intermediate
P231	P231	9A	10	3	2	Intermediate
P241	P241	10H	67	7	3	Advanced

For the four isolates evaluated, on amplifying the primers ITS 4 and ITS 6, we obtained a band of about 950 bp, larger than that of the positive control, *P. tropicalis*, which was 900 bp. These fragments were then digested with restriction enzymes. Although enzymes *Alu*I and *Taq*I had different band patterns, they remained consistent for the four isolates. Enzyme *Msp*I digested only the positive control and isolate P241. The band patterns obtained with the three enzymes were compared with patterns reported for some *Phytophthora* species (Cooke and Duncan 1997). No similarities were found, indicating that either an unreported species or a different Pythiaceae such as *Pythium* sp. was found.

To confirm these results, we sequenced the region ITS 1, 5.8S, and ITS 2 (between 600 and 750 bp) of the four isolates and sought for homology in GenBank. Isolates P216, P217, P231, and P241 showed a homology of 97% with *Pythium chamaehyphon* and 96% with *Pythium vexans* **(Table 3)**.

Table 3. Homology found between sequences of DNA obtained from oomycetes by PCR and sequences of Pythiaceae reported in GenBank.

Pairing with GenBank[a] GenBank	Code	Probability of Greatest Homology[b]	Homologized Bases[c]	Homology (%)
Pythium chamaehyphon	PCH233440	0	457/469	97
ITS 1, 5.8S rRNA gene, and		1e-78	204/220	92
ITS 2, strain MS6-10-8V				
(792)				
Pythium vexans ITS 1, 5.8S	AY269998.1	1e-139	290/301	96
rRNA gene, and ITS 2,		1e-132	241/241	100
complete sequence (789)				

a. Italicized value in parentheses indicates total number of bases reported in GenBank.
b. The value 0 is expected for the highest percentages of homology.
c. Values indicate number of bases homologized in different regions of the sequence reported in GenBank.

The alignment of the sequences revealed that the sequence of the nucleotides of region ITS 1, 5.8S, and ITS 2 of GenBank the isolates was similar to that for the same region in the GenBank accessions AY269998.1 and PCH233440 belonging to the *Pythium* genus, thus confirming the results obtained for the restriction patterns.

Although no symptoms have appeared so far in the palms inoculated with the bacterial and oomycete isolates, we are still evaluating the trials established at CIAT.

References

Cooke D. E. L. & Duncan J. M. (1997) Phylogenetic analysis of *Phytophthora* species based on the ITS1 and ITS2 sequences of ribosomal DNA. *Myc. Res.* 101, 667-677.

NCBI (National Center for Biotechnology Information). GenBank overview. http://www.ncbi.nlm.nih.gov/Genbank/ (June, 2004).

PhytID - Identification of Plant Pathogenic *Phytophthora* Species by ITS Fingerprinting, http://www.phytid.org/methods.htm (September, 2003).

Activity 7. DNA sequence analysis of the ITS region of oomycete species obtained from oil palm.

From processed samples of roots we obtained four isolates (**Figure 1** shows two) belonging to the Pythiaceae family. The origins of the isolates are described in **Table 1**.

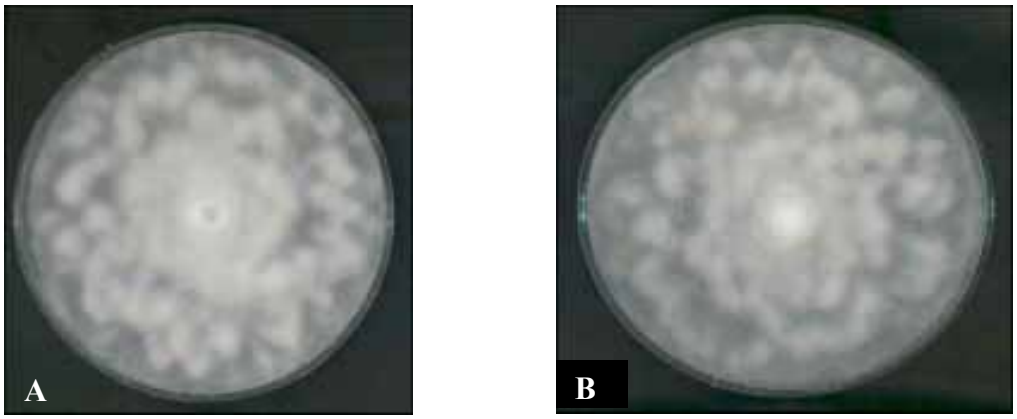


Figure 1. Isolates of oomycete species obtained from oil-palm roots in plot G16 of plantation number 1, Villanueva, Department of Casanare, Colombia. (A) Isolate P216 obtained from an oil palm infected with lethal wilt; (B) isolate P217 from a palm from the same plot but not showing lethal wilt.

Table 1. Origins of Pythiaceae isolates obtained from roots of oil palms infected with lethal wilt (LW) in the Department of Casanare, Colombia.

Isolate Code Number	Sample Code Number	Plot	Tree Line	Palm Tree Number	Plantation T	issue	Dev't Stage of LW
P216	P216	G16	57	4	1	Roots	Healthy in site of highest disease pressure
P217	P217	G16	62	10	1	Roots	Intermediate
P231	P231	9A	10	3	2	Roots	Intermediate
P241	P241	10H	67	7	3	Roots	Advanced

Although no symptoms, bacteria, or oomycetes have yet been reproduced, we are continuing with the evaluations of the trials established at CIAT.

PCR-RFLP and sequencing

On amplifying with primers ITS 4 and ITS 6, we obtained, for the four isolates, a band size of about 950 bp, contrasting with the 900 bp of the positive control of *Phytophthora tropicalis* **(Figure 2A)**. When these fragments were digested with restriction enzymes, we saw that enzymes *Alu*I and *Taq*I had band patterns that differed among themselves but were exactly the same for the four isolates **(Figures 2B** and **2C)**. Enzyme *Msp*I only digested the positive control and isolate P241 **(Figure 2D)**. The band patterns obtained with the three enzymes were compared with patterns reported for some *Phytophthora* species (Cooke and Duncan 1997). No

similarities were found among them, indicating either an unreported species or another Pythiaceae such as *Pythium* sp.

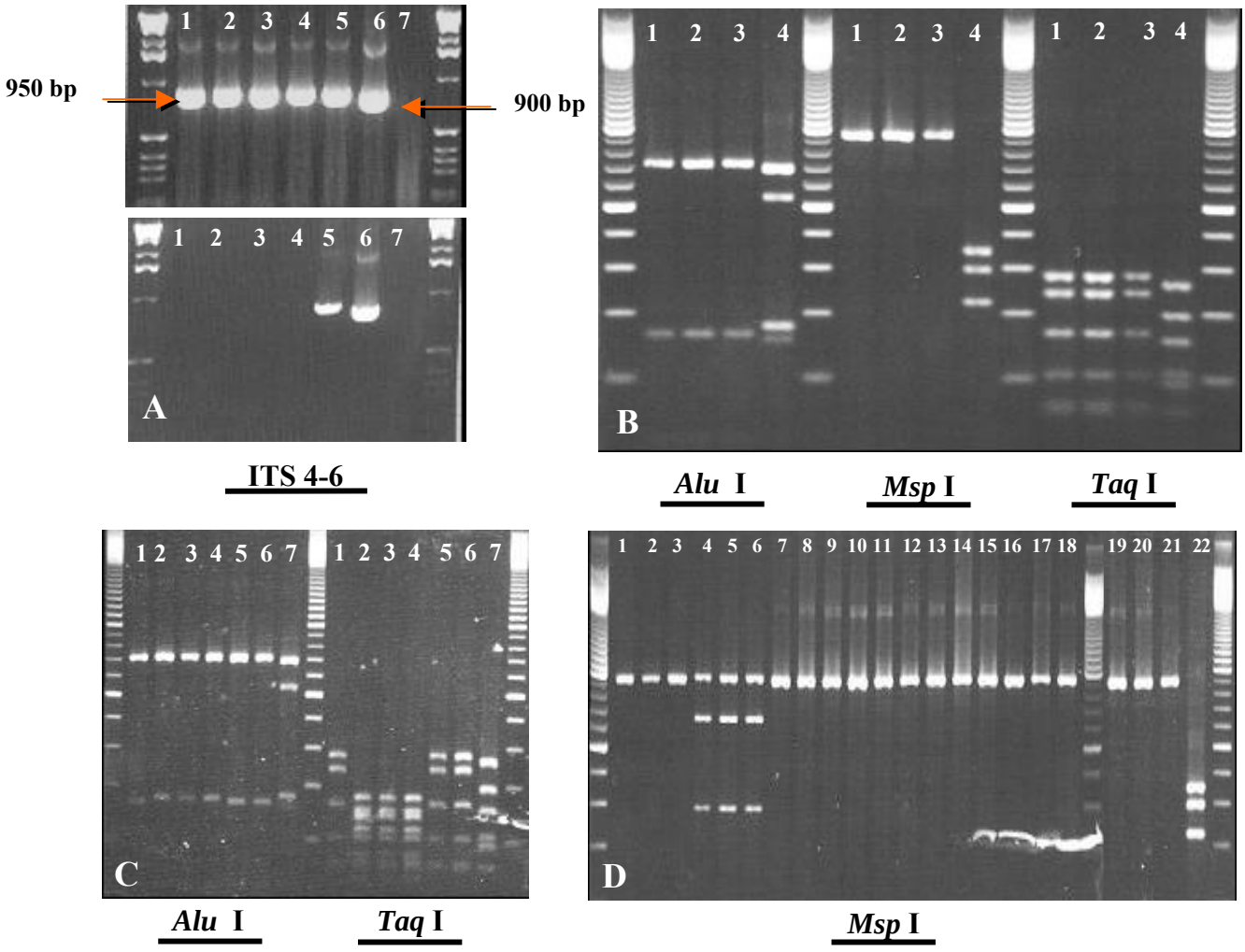


Figure 2. **(A) Amplification of an oomycete species with primers ITS 4 and ITS 6: lanes 1 and 2 = P216; lane 3 = P217; lane 4 = P231; lane 5 = P241; lane 6 = *Phytophthora tropicalis*; lane 7 = negative control; end lanes = 1-kb marker. (B) Cuts of the amplified product by restriction enzymes *Alu*I, *Msp*I, and *Taq*I: lane 1 = P216; lane 2 = P217; lane 3 = P231; lane 4 = *P. tropicalis.* (C) Cuts of the amplified product by restriction enzymes *Alu*I and *Taq*I: lane 1 = P231; lanes 2–4 = P241; lane 5 = P217; lane 6 = P216; lane 7 = *P. tropicalis.* (D) Cuts of the amplified product by restriction enzyme *Msp*I: lanes 1–3 = P231; lanes 4–6 = P241; lanes 7–12 = P217; lanes 13–18 = P216; lanes 19–21 = P231; lane 22 = *P. tropicalis.***

To confirm these results, the four isolates were sequenced and homologized according the whole region ITS 1, 5.8S, and ITS 2 at GenBank. Between 600 and 750 bases could be sequenced for each isolate. The isolates P216, P217, P231, and P241 showed a homology of 97% with *Pythium*

chamaehyphon and 96% with *Pythium vexans* **(Table 2)**. Alignment of the sequences showed that the sequence of the nucleotides in the region ITS 1, 5.8S, and ITS 2 in GenBank was similar to that same region of the GenBank accessions AY269998.1 and PCH233440, belonging to the *Pythium* genus, thus confirming the results obtained with the restriction patterns.

Table 2. Homology found betw een sequences of DNA from oomycete species obtained by PCR a nd sequences of Pythiaceae reported in the GenBank database. Homology report refers to two-fragment sequence.

Pairing with GenBank[a]	GenBank Code Number	Probability of high Homology[b]	Homologized Bases[c]	Homology (%)
Pythium chamaehyphon ITS 1, 5.8S rRNA gene and ITS 2, strain MS6-10-8V (792)	PCH233440	0	457/469	97
		1e-78	204/220	92
Pythium vexans ITS 1, 5.8S rRNA and ITS 2, complete sequence (789)	AY269998.1	e-139	290/301	96
		e-132	241/241	100

a. Italicized value in parentheses indicates total number of bases reported in GenBank; ITS = internal transcribed spacer; rRNA = ribosomal RNA.
b. The value 0 is expected for the highest percentages of homology.
c. Values indicate number of bases showing homology in different regions of the sequence reported in GenBank.

Reference

Cooke D. E. L. and Duncan J. M. 1997. Phylogenetic analysis of *Phytophthora* species based on the ITS1 and ITS2 sequences of ribosomal DNA. Mycological. 101, 667-677.

Activity 8. Detecting phytoplasmas in cassava affected by frogskin disease (FSD), using nested PCR.

Specific objective

To detect phytoplasmas in cassava plants affected by frogskin disease (FSD)

Methodology

Plant tissues. Asexual planting materials (stakes) from 40 plants of the commercial cassava varieties Catumare and Manzana were obtained. Twenty of the plants came from Rozo and Palmira, Department of Valle del Cauca, Colombia, and were either moderately infected (Catumare) or severely affected (Manzana) by FSD. The other 20 plants were disease-free and came from Montenegro, Department of Quindío, Colombia.

The stakes, 20 cm long, were planted in plastic bags containing pasteurized soil that was free of FSD. The bags were placed on plates to prevent contamination during watering.

All the plants were fertilized periodically and left in anti-aphid cages. The plants and cages were fumigated periodically, rotating the following products: Vertimec® 1.8% CE (abamectin at 0.5 cc/L of the commercial product), Malathion® (malathion at 1 cc/L), and Sistemin® (dimethoate at 3 cc/L).

As control we used healthy 'Secundina' from in vitro plants, placing them in the cages with the varieties being evaluated. They also functioned as monitors for the presence of insect vectors.

The trials were established in a greenhouse and screen house under different conditions of relative humidity and temperature. The greenhouse had an RH of 31% to 98%, and temperatures varied from 19°C to 28°C. Four replications of 10 plants were used per variety in each of the greenhouse and screen house, and placed in the same cages of their respective varieties.

Healthy plants from Armenia, Quindío, were also established under equal conditions in the same greenhouse and screen house but in separate cages.

Insects. In a separate experiment, Homopterans (*Scaphytopius marginelineatus*) were collected from cassava crops infected with FSD, and breeding was established in cages containing diseased plants. After a couple of generations, adult insects were transferred to healthy plants to test for transmission of disease (CIAT Cassava Entomology Section, personal communication, 2004).

DNA extraction. Total DNA was extracted as described by Gilbertson et al. (1983).

Nested-PCR analysis. We amplified 50 ng of genomic DNA, using nested PCR with the universal primers R16F2/R16R2 and the primers specific to the 16SrIII group (X-disease), R16(III)F2/R16(III)R1. The cocktail was prepared with 2 mM dNTPs, 1X Taq buffer, 2.5 mM $MgCl_2$, 1 U Taq polymerase, and 10 µM of each primer. The conditions for amplification were

94°C for the initial denaturation for 2 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, extension at 72°C for 3 min, and a final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis on 1.5% agarose gel.

RFLP analyses. All amplified PCR products were digested with the restriction endonucleases *Alu*I, *Rsa*I, and *Taq*I to confirm the presence of a single group of phytoplasmas associated with the disease. The restriction products were analyzed by electrophoresis on 5% polyacrylamide gel. These enzymes had been used previously to classify FSD phytoplasmas.

DNA sequencing. The amplified PCR products were cleaned, using a purification kit (QIAGEN) and then sequenced by automated dideoxy sequencing (ABI PRISM® 377-96 DNA Sequencer, Applied Biosystems). The sequences obtained were homologized with the sequences reported in GenBank to identify the organism detected in the evaluated samples.

Results

DNA extraction. A total of 320 DNA samples were obtained from infected plant tissues —160 from roots and 160 from leaf midribs and petioles—and another 80 from healthy tissues. All samples were from the varieties Catumare and Manzana **(Table 1)**. In addition, 17 samples were extracted from tissues at different developmental stages of the insect *S. marginelineatus*, processing 1 to 2 individuals per sample **(Table 2)**.

Table 1. Tissues evaluated, using nested PCR and sequencing, to determine the incidence of phytoplasmas in cassava plants infected with frogskin disease.

		Samples processed[a] PCR				[b] sequencing	
Tissue	Variety	Roots P	and MR	No. of Positive Samples	% Nested PCR	Roots P	and MR
Infected[c]	Catumare[d]	80	80	124	77	8	10
Infected	Manzana[e]	80	80	138	86	10	12
Healthy[f]	Catumare	20	20	0	0	-	-
Healthy	Manzana	20	20	0	0	-	-

a. Total number of samples processed in the greenhouse and screen house; P = leaf petioles; MR = leaf midribs.
b. The same number of samples was taken for both greenhouse and screen house. P = leaf petioles; MR = leaf midribs.
c. Seed came from plots infected with frogskin disease in Rozo and Palmira, Valle del Cauca.
d. Moderately infected.
e. Severely infected.
f. Seed came from plots free of frogskin disease in Montenegro, Quindío.

Nested-PCR analysis. Of the 320 infected-plant-tissue samples evaluated, 262 were detected as having a phytoplasma (82%); of the 17 samples from insects fed on diseased plants, 50% showed amplification; and of the 80 healthy plant tissues, no amplifications were obtained.

The presence of a phytoplasma was shown by visualization in agarose gels. Bands of about 800 bp—typical of the 16SrIII group—appeared when the primer pair R16(III)F2/R16(III)R1 was used. The rates of detecting the presence of phytoplasmas in plants (82%) **(Figure 1A)** and insects (50%) **(Figure 1B)** are high, considering that a rate of no detection of phytoplasmas is possible in plants presenting symptoms typically associated with them. Lack of detection could

be attributed to substances in plant-tissue extracts inhibiting amplification, irregular distribution of phytoplasmas in the plant, or low concentrations of the microorganism in either plant or insect tissues (Chen and Liao 1975; Lee et al. 1994; Bianchini 2001).

Table 2. Identifying phytoplasmas in Ho mopterans (*Scaphytopius marginelineatus*) as evaluated by nested PCR w ith pr imers R16F2/R16R2 and R16(III)F2/ R16(III)R1.

	Sample Genotype	[a]	Stage Neste	d PCR[b]
1	1[a]	M Col 2063 [(I)]	Adult	+[(S)]
2	1B	M Col 2063 [(I)]	Nymph	+
3	1C	M Col 2063 [(I)]	Nymph	-
4	2[a]	M Col 2063 [(I)]	Adult	+
5	2B	M Col 2063 [(I)]	Nymph	-
6	2C	M Col 2063 [(I)]	Nymph	+
7	3B	M Col 2063 [(I)]	Nymph	-
8	3C	M Col 2063 [(I)]	Nymph	+
9	4[a]	M Col 2063 [(H?)]	Adult	-
10	4B	M Col 2063 [(H?)]	Nymph	-
11	4C	M Col 2063 [(H?)]	Nymph	-
12	SE1	M Col 2063 [(I)]	Adult	-
13	Ss1	M Col 2063 [(H?)]	Adult	-
14	F1	Bean[(H)]	Adult	-
15	383 (1)	M Bra 383[(I)]	Male nymph	-
16	383 (2)	M Bra 383[(I)]	Female nymph	-
17	383 (3)	M Bra 383[(I)]	Adult	+[(S)]

a. Cassava germplasm materials facilitated and qualified as healthy or diseased by the CIAT Virology Unit. [(I)] = infected; [(H)] = healthy.

b. [(S)] Samples sequencing.

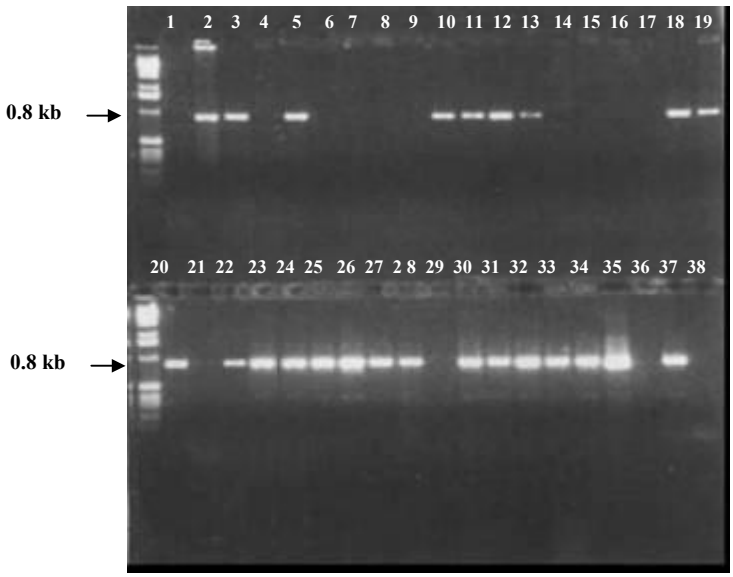


Figure 1A. DNA from infected tissues amplified with primers R16F2/R16R2 nested with R16(III)F2/R16(III)R1. Lanes 1–19 = Screenhouse tissues; lanes 20–38 = Glasshouse tissues.

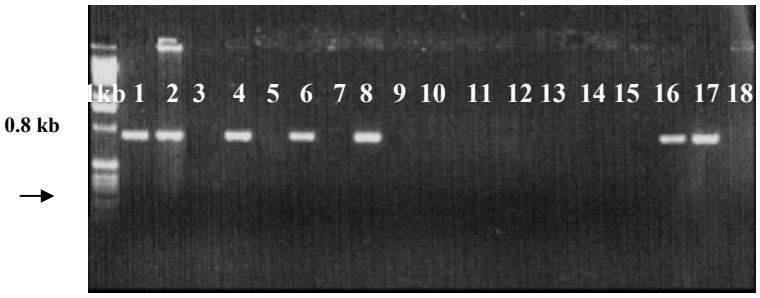
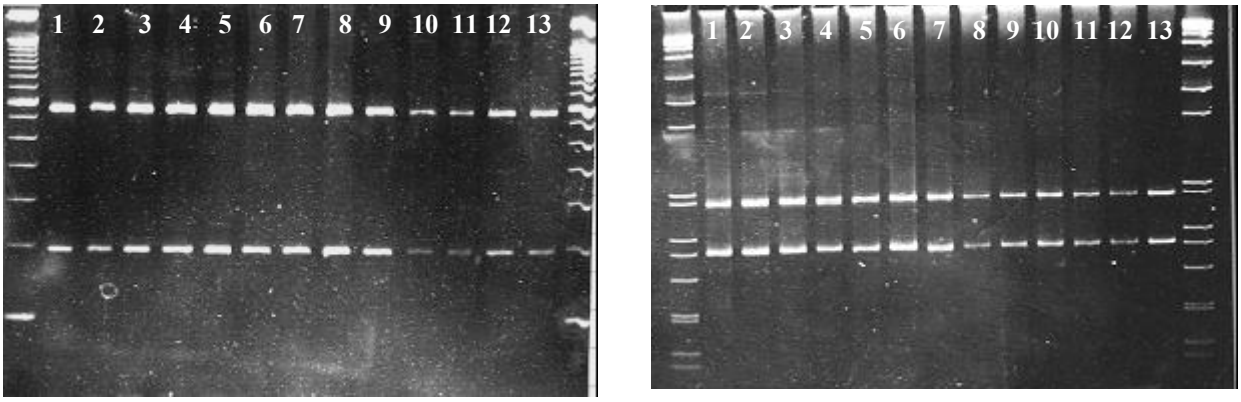


Figure 1B. Presence of phytoplasm typical o f the group 16Sr III for *S. marginelineatus*, fed on infected plants, line 1,2,4,6,8 and 17; line 18 positive control and line 19 negative control, 1kb: Marker of molecular weight.

RFLP analyses. The band pattern obtained for the 262 samples amplified with the three evaluated enzymes enabled us to confirm that the products belonged to the 16SrIII group (X-disease) **(Figure 2)**.



***Alu* I** ***Taq* I**

Figure 2. Cuts of the amplified product w ith primers R16(III)F2/R16(III)R1 by restriction enz ymes *Alu*I and *Taq*I. Lines 1-6 (Glasshouse) and 7-13 (Screenhouse).

DNA sequencing. For the DNA amplifications, we took representative tissue samples from the roots and leaves (midrib and petiole) of infected cassava varieties in the field and in the greenhouse and screen house where the trials took place. The 40 fragments of plant DNA and 2 of insect DNA **(Table 1 and 2)** were then directly sequenced, purifying the PCR products.

The sequence analysis of the 42 fragments revealed that the cassava phytoplasma was similar to Cirsium white-leaf phytoplasma (GenBank accession no. AF373106, 16SrIII or X-disease group), with a sequence homology of 100% in fragments measuring 800 bp. These findings thus confirmed that the amplified products belong to a phytoplasma associated with FSD in cassava (CIAT 2003).

A homology of 90% was found among the sequenced fragments from insect tissue and from

tissues of the varieties Manzana and Catumare. Given these homology results, being based on the nested-PCR technique, new transmission trials are being evaluated by Cassava Entomology Section at CIAT. The Section will first evaluate plants regarded as healthy or diseased and then evaluate plants on which those homopterans insects identified as possible vectors have fed.

This study shows evidence of an association between FSD and phytoplasmas. By applying molecular tools, a phytoplasma was successfully detected in FSD-infected cassava roots and leaf midribs.

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Acknowledgments

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Activity 9. Identifying phytoplasmas by sequencing PCR products.

Specific objective

To identify, through DNA sequencing, the phytoplasma associated with frogskin disease (FSD) of cassava.

Methodology

Plant tissues. Roots, petioles, and leaf midribs of both FSD-infected and healthy cassava plants, grown in the field and greenhouse, were processed. We evaluated 41 samples from cassava genotypes and varieties of three areas of Colombia—Atlantic Coast, Valle del Cauca, and Cauca—where FSD has high incidence. The goal was to confirm the presence of phytoplasmas in plants showing symptoms of FSD **(Table 1)**.

Table 1. List of DNA fragments obtained from samples of tissues of 41 cassava varieties infected w ith frogs kin disease. The samples were amplified by nested and direct PCR, using universal primers and primers specific for phytoplasmas.

	Variety	Tissue	Site[a]	PCR	Primers[b]
1	CM 6740-7	Leaf midrib	Agrovélez	+	A
	CM 6740-7	Root	Agrovélez	+	A
	CM 6740-7	Leaf midrib[c]	CIAT-greenhouse	+	A
	CM 6740-7	Root	CIAT-greenhouse	+	A
	CM 6740-7	Leaf midrib[c]	Santa Elena-field	+	C
	CM 6740-7	Root	Santa Elena-field	-	C
2	Parrita	Shoot	Agrovélez	+	B
	Parrita	Leaf midrib	Agrovélez	+	B
	Parrita	Stem	Agrovélez	+	B
	Parrita	Petiole	Agrovélez	+	B
	Parrita	Root	Agrovélez	+	B
	Parrita	Leaf midrib	CIAT-greenhouse	-	B
	Parrita	Petiole	CIAT-greenhouse	-	B
	Parrita	Stem	CIAT-greenhouse	-	B
	Parrita	Rootlet	CIAT-greenhouse	-	B
3	Catumare	Leaf midrib	Montenegro	-	B
	Catumare	Root	Montenegro	-	B
	Catumare	Leaf midrib[c]	Rozo-field	+	B
	Catumare	Root	Rozo-field	+	B
	Catumare	Leaf midrib	CIAT-screen house	+	C
4	Manzana	Leaf midrib	Montenegro	-	B
	Manzana	Root	Montenegro	-	B
	Manzana	Leaf midrib	Rozo	+	B
	Manzana	Root	Rozo	+	B
5	M Bra 383	Root	Quilichao	+	B
	M Bra 383	Root	Quilichao	+	B
	M Bra 383	Root	CIAT-field	+	B
	M Bra 383	Root	CIAT-field	+	B
6	CM 849-1	Leaf midrib	Agrovélez	+	B
	CM 849-1	Petiole	Agrovélez	+	B
	CM 849-1	Stem	Agrovélez	+	B
	CM 849-1	Rootlet	Agrovélez	+	B
7	SM 1219-9	Leaf midrib	CIAT-field	+	B

	Variety	Tissue	Site[a]	PCR	Primers[b]
	SM 1219-9	Root	CIAT-field	+	B
8	CM 2177-2	Leaf midrib	CIAT-field	+	B
	CM 2177-2	Root	CIAT-field	+	B
9	CM 4919-1	Leaf midrib	CIAT-field	+	B
	CM 4919-1	Root	CIAT-field	+	B
10	M Col 2063	Leaf midrib[c]	CIAT-greenhouse	+	B
	M Col 2063	Root	CIAT-greenhouse	+	B
	M Col 2063	Leaf midrib	CIAT-screen house	+	B
	M Col 2063	Root	CIAT-screen house	+	B
11	M Bra 383	Leaf midrib[c]	CIAT-greenhouse	+	B
	M Bra 383	Rootlet	CIAT-greenhouse	+	B
12	Venezolana	Root	Sincelejo-field	+	A-B
	Venezolana	Root	Sincelejo-field	+	A-B
13	CM 3306-9	Leaf midrib[c]	CIAT-greenhouse	+	B
	CM 3306-9	Petiole	CIAT-greenhouse	+	B
14	CM 3306-19	Leaf midrib[c]	CIAT-greenhouse	+	B
	CM 3306-19	Petiole	CIAT-greenhouse	+	B
15	M Bra 856-54	Leaf midrib[c]	CIAT-greenhouse	+	B
	M Bra 856-54	Petiole	CIAT-greenhouse	+	B
16	M Col 634	Leaf midrib	Quilichao-field	+	C
	M Col 634	Root	Quilichao-field	+	C
17	M Bra 829	Leaf midrib	Quilichao-field	+	C
	M Bra 829	Root	Quilichao-field	-	C
18	M Per 16	Leaf midrib	Quilichao-field	+	C
	M Per 16	Root	Quilichao-field	+	C
19	M Bra 856	Leaf midrib	Quilichao-field	+	C
	M Bra 856	Root	Quilichao-field	+	C
20	M Bra 856	Leaf midrib	Quilichao-field	+	C
	M Bra 856	Root	Quilichao-field	+	C
21	M Chn 2	Leaf midrib	Quilichao-field	-	C
	M Chn 2	Root	Quilichao-field	-	C
22	HMC 1	Leaf midrib	Quilichao-field	+	C
	HMC 1	Root	Quilichao-field	+	C
23	M Arg 2	Leaf midrib	Quilichao-field	-	C
	M Arg 2	Root	Quilichao-field	-	C
24	M Bra 325	Leaf midrib	Quilichao-field	+	C
	M Bra 325	Root	Quilichao-field	+	C
25	M Bra 839	Leaf midrib	Quilichao-field	+	C
	M Bra 839	Root	Quilichao-field	+	C
26	M Col 1178	Leaf midrib	Quilichao-field	+	C
	M Col 1178	Root	Quilichao-field	+	C
27	M Col 1468	Leaf midrib	Quilichao-field	+	C
	M Col 1468	Root	Quilichao-field	+	C
28	M Cub 74	Leaf midrib	Quilichao-field	-	C
	M Cub 74	Root	Quilichao-field	+	C
29	M Bra 886	Leaf midrib[c]	Quilichao-field	+	C
	M Bra 886	Root	Quilichao-field	+	C
30	M Bra 882	Leaf midrib	Quilichao-field	-	C
	M Bra 882	Root	Quilichao-field	-	C
31	CM 5460-10	Leaf midrib[c]	CIAT-screen house	+	C
	CM 5460-10	Petiole	CIAT-screen house	+	C
32	SM 909-25	Leaf midrib[c]	CIAT-screen house	+	C
	SM 909-25	Petiole	CIAT-screen house	+	C
33	CG 6119-5	Leaf midrib[c]	Santa Elena-field	+	C
	CG 6119-5	Root	Santa Elena-field	+	C

	Variety Tissue		Site[a]	PCR Primers	[b]
34	M Per 335	Root	Santa Elena-field	+	C
35	ICA Nataima	Leaf midrib	Santa Elena-field	-	C
	ICA Nataima	Root	Santa Elena-field	+	C
36	SM 1201-5	Leaf midrib	Santa Elena-field	-	C
37	GM 228-14	Leaf midrib	Santa Elena-field	-	C
38	CM 9582-64	Leaf midrib	Rozo-field	+	A-B-C
	CM 9582-64	Root	Rozo-field	+	A-B-C
39	CM 9582-65	Leaf midrib	Rozo-field	+	A-B-C
	CM 9582-65	Root	Rozo-field	+	A-B-C
40	CM 9582-24	Leaf midrib	Rozo-field	+	A-B-C
	CM 9582-24	Root	Rozo-field	+	A-B-C
41	M CR 81	Leaf midrib	Rozo-field	+	A-B-C
	M CR 81	Root	Rozo-field	+	A-B-C

a. Agrovélez S.A., CIAT, Rozo, and Santa Elena are found in the Department of Valle del Cauca; Quilichao in Cauca; Sincelejo in Atlántico; and Montenegro in Quindío.

b. Primers used for amplification were **(A)** P1/P7–R16F2N/R16R2, **(B)** R16mF2/R16mR1–R16F2N/R16R2, and **(C)** R16F2/R16R2–R16(III)F2/R16(III)R1.

c. Also showing foliar symptoms of mosaic and deformation of leaf blade.

Sequencing the 16S rRNA region. DNA obtained from plants with symptoms of FSD was used to amplify fragments of the 16S region of ribosomal DNA, using polymerase chain reaction (PCR) and two pairs of universal primers P1/P7 and R16mF2/R16mR1. The products were re-amplified, using nested PCR and primers R16F2N/R16FR2, to detect and confirm that the phytoplasma is associated with the disease. The products of the nested PCR (1.2 kb) were digested with the enzymes *Alu*I, *Mse*I, *Rsa*I, and *Taq*I. The band patterns obtained with the restriction fragment length polymorphism (RFLP) technique made it possible to locate the group to which the phytoplasma belongs.

These results were confirmed by re-amplifying the products R16F2/R16R2 with primers R16(III)F2/R16(III)R1 (0.8 kb) specific to the 16SrIII group. The fragments of 1.2 kb and 0.8 kb were cloned and sequenced. Purified PCR products were ligated in pGEM®-T Easy Vector, which was introduced into the *Escherichia coli* strain DH5-α by electroporation at 2.4 kV/cm^2.

Transformants were selected on a blue-white screen by plating on an LB/ampicillin/IPTG/Xgal medium. Positive inserts were observed with plasmid restriction with *Eco*RI and electrophoresis in 1.5% agarose gel. Different-sized fragments were selected and sequenced by automated dideoxy sequencing (ABI PRISM® 377-96 DNA Sequencer, Applied Biosystems).

Results

A phytoplasma was successfully detected, using nested PCR, in all FSD-infected tissues. Of the methods used in this study, PCR was the most sensitive for detecting, identifying, and classifying phytoplasmas.

A sequence from a cloned fragment, obtained from an infected cassava plant, showed a 99% homology with the Chinaberry yellows phytoplasma and 100% with that of Cirsium white leaf.

These results allow us to infer that a phytoplasma plays a role in this disease.

As criteria, we took the number of correctly read bases of the amplified fragment, amplifications of the characteristic symptoms of the disease, and differences of genotype, and obtained two complete sequences, measuring 1260 and 1298 bp of 16Sr DNA gene region of two different cassava varieties, M Col 2063 (Y17) (leaf midrib and petiole) and SM 1219-9 (Y29) (external phloem from roots), which were classified and reported in GenBank with the accession numbers AY737646 (1260 bp) and AY737647 (1298 bp).

Acknowledgments

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Continued

Activity 10. Designing specific primers for hi gh-specificity detec tion of a phytoplasma associated with frogskin disease (FSD) of cassava.

Specific objective

To obtain high specificity in the technique and improve it for detecting phytoplasmas in cassava plants with symptoms of FSD, weeds, and potential insect vectors

Methodology

Sequencing and analyzing phytoplasma rDNA. We previously described obtaining complete sequences of DNA fragments through PCR from samples of two cassava varieties. They were reported to GenBank, which gave them accession numbers AY737646 and AY737647 (CIAT 2004). We conducted analyses of homology with these sequences against 24 sequences of the 16SrIII group and accessions of phytoplasmas representing at least 14 primary phytoplasma groups, using multiple alignments among the sequences (DNAMAN, version 5.2.2, Lynnon BioSoft). Specific differences in nucleotides were sought, seeking a series of bases that would be specific to the cassava phytoplasma. The homology of the sequences was calculated (in %) by taking the identical number of bases over the difference of aligned sequences and total size of gaps (in %). "Gap (%)" is the number of gaps of all sequences over the size of aligned sequences.

We used the option "Quick Alignment" to perform pairwise alignment with all sequences, using the method developed by Wilbur and Lipman (1983). With this method, DNAMAN aligns each pair of sequences, constructs a homology tree from the results of pairwise alignment, and finally builds up alignment based on the homology tree with the previously established alignment. This tree is set up with the distance matrix, using the UPGMA method (Sneath and Sokal 1973). The matrix can be built up only with Observed Divergence (this method uses directly unmatched residues divided by compared length between two sequences. No correction is applied to distances). After the tree is constructed, dynamic programming is finally used to optimize group alignment (Feng and Doolittle 1987; Thompson et al. 1994).

The phylogenetic analysis was constructed with the distance matrix, using the neighbor-joining method (Saitou and Nei 1987). Bootstrapping statistically shows typical variations (Felsenstein 1985). It involves creating a new data set by sampling randomly with replacements, so that the resulting data set has the same size as the original, but some characters have been left out and others are duplicated. The method assumes that the characters evolve independently. Phylogenetic analysis of the 16Sr RNA sequences was resolved, using the PAUP Software Program, version 3.1.

Results

Designing primers. The results of the phylogenetic and homology analyses show that the FSD phytoplasma clustered closely with other known X-disease (16SrIII) group strains, thus supporting its assignment to this group. We found multiple differences among the sequences of the FSD phytoplasma and the group 16SrIII phytoplasmas **(Figure 1)**, generating sufficient

information to design primers. The primers for the specific amplification of the phytoplasma associated with FSD were designed with the assistance of the program PRIMER 3.0 (www-genome.wi.mit.edu/cgi-bin/primer/primer3-www.cgi [2004]), taking into account certain criteria such as the contents of G + C and A + T, close to 50%, a minimum of nitrogenous bases, absence of extensive palindrome sequences within the primers, and that mating among their pairs was minimum. The primers obtained were synthesized by Integrated DNA Technologies, Inc.

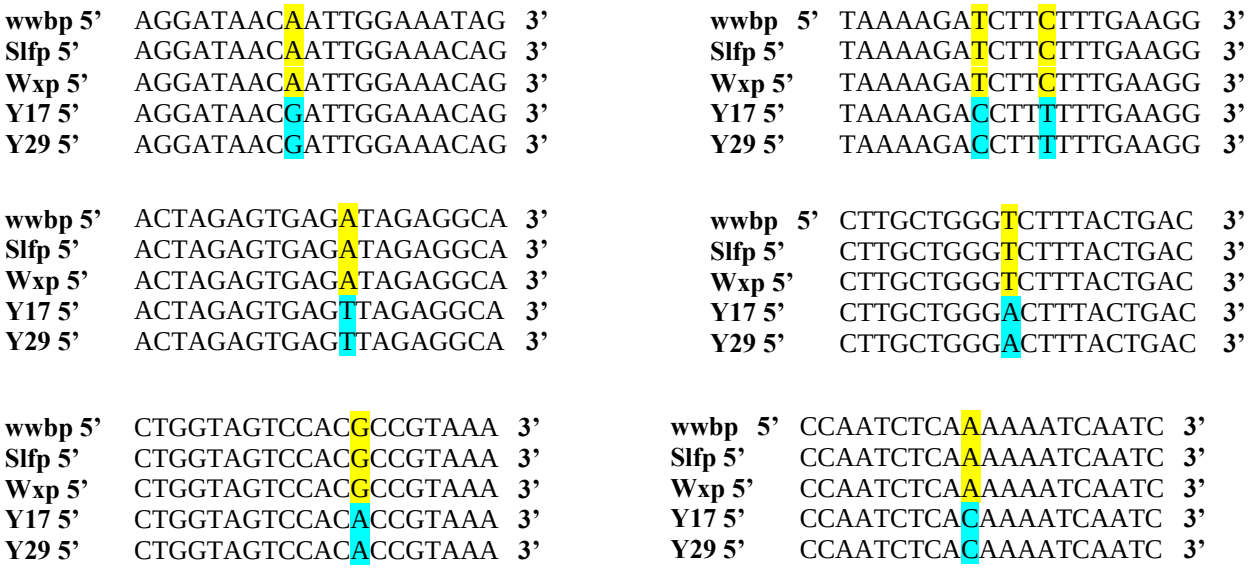

Figure 1. Some differences found in region 16Sr DNA between phytoplasmas of the 16SrIII group and the cassava frogskin disease phytoplasma. Wwbp (Walnut witches'-broom phytoplasma), Slfp (Strawberry leafy fruit phytoplasma), Wxp (Western X phytoplasma), and Y17 indicate the cassava frogskin disease phytoplasma AY737646 (1260 bp). Y29 indicates the cassavafrogskin disease phytoplasma AY737647 (1298 bp).

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Acknowledgments

We thank the following people for their support: Tom Harrington and Joe Steimel of the Plant Pathology Department, and Gary Polking of the DNA Sequencing and Synthesis Facility, Iowa State University.

Activity 11. Detecting phytoplasmas by electron microscopy.

Specific objective

To detect, through electron microscopy, phytoplasma structures in tissues infected with FSD that was positive to nested PCR

Methodology

Tissues. Portions of roots exhibiting typical FSD symptoms were chosen from four different cassava genotypes. Typical symptoms are small longitudinal fissures distributed all over the root. On healing, these fissures develop "lips". The root portions for each variety comprised different cuts directed mainly at the phloem. Healthy plant samples were also processed to act as control **(Table 1)**.

Table 1. Processed samples of insects (*Scaphytopius marginelineatus*) and cassava plant tissues for detecting phytoplasmas by electron microscopy.

Tissue sample	Insect's developmental stage	Cassava genotype	Electron microscopy
Insect SE1	Adult	M Col 2063	In process
Insect Ss1[a]	Adult	M Col 2063	In process
Insect 383 (1)	Male nymph	M Bra 383	In process
Insect 383 (2)	Female nymph	M Bra 383	In process
Insect 383 (3)	Adult	M Bra 383	In process
Roots-petiols	-	CM 9582-64	+
Roots-petiols	-	CM 9582-65	+
Roots-petiols	-	CM 9582-24	+
Roots-petiols	-	M CR 81	+

a. Tissues in this sample were healthy, whereas tissues in the other samples were infected.

We also processed Homopteran insects (*S. marginelineatus*) collected from cassava crops infected with FSD and bred them in cages with different susceptible cassava genotypes that would show severe symptoms of the disease. Three individuals per developmental stage of the insect were taken as samples **(Table 1)** (CIAT Cassava Entomology, personal communication, 2004). The tissue fragments were cut into 1 × 2 mm pieces to be prefixed in 2%–3% glutaraldehyde (0.1 M phosphate buffer, pH 7.3). Complete insects were also fixed in the same buffer.

Electron microscopy. The samples for electron microscopy were prepared by making ultra-thin (60–90 nm) sections with a Reichert Ultracut S ultramicrotome (North Central Instruments, Plymouth, MN). After post-fixation and precontrasting in uranyl acetate, they were dehydrated in an acetone series 50, 70, 90 (15 min each) and 100% (15 min, three times), and were embedded in Spurr's resin. A previous 18-h infiltration with acetone-Spurr (1:1) was done to facilitate the entry of resin into the tissues. The ultra-thin sections were mounted on copper grills, and images taken, using a Megaview III digital camera system with SIS software (Soft Imaging System Corp., Lakewood, CO) on a JEOL 1200EX Woburn, MA scanning/transmission electron microscope (Japan Electron Optics Laboratory, Peabody, MA).

Results

In the previous studies, diverse tissues (stem, leaf midrib, petioles, and roots) were evaluated for numerous cassava plants, but only some could be compared with the results obtained for nested PCR. In this study, guided by the results of the nested PCR, root tissues of four cassava genotypes susceptible to FSD were first examined. These showed severe symptoms of the disease. Cells characteristic of phytoplasmas were detected in root phloem. The phytoplasma structures observed were pleomorphic, comprising round, elongate, dumbbell, and ring-shaped elements, mostly 150 to 250 nm wide and 1000 nm long **(Figure 1)**. The phytoplasma structures were limited only to phloem tubes and were never seen in large quantities (Andersen *et al.* 2001). The insect-tissue samples are still being processed.

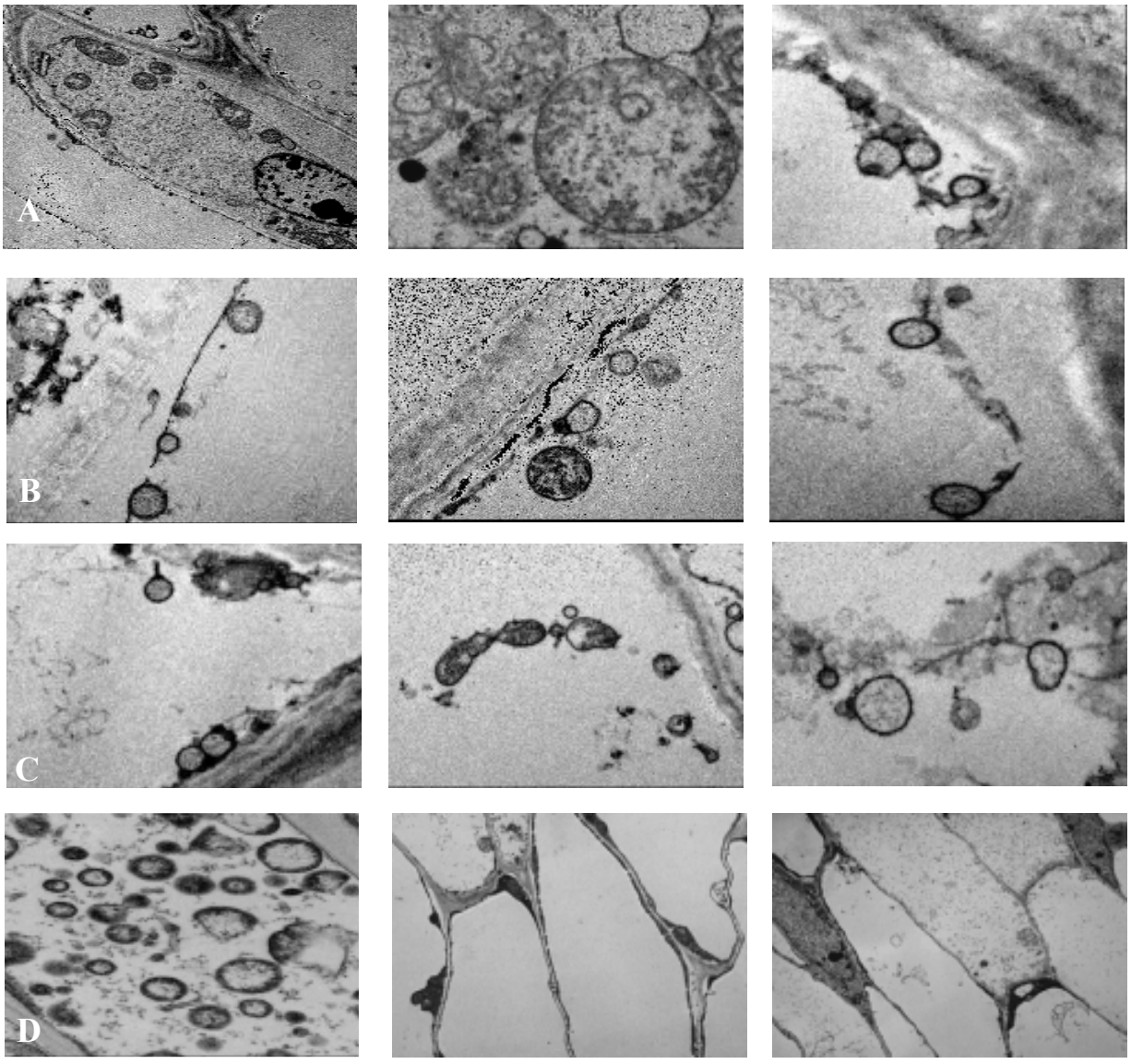

Figure 1. Micrographs, taken by cell transm ission microscopy, of phytoplasmas FSD. (A) and (B) Infected cassava petiols. (C) Infected cassava roots and (D) Positive control (Periwinkle) and healthy cassava petiols. Photos (Alvarez, 2004).

Reference

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Acknowledgments

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Activity 12. The detection and molecular cha racterization of a phytoplasma associated with *Machorreo* of Lulo (*Solanum quitoense*) in Colombia.

Objective

To detect presence of phytoplasmas in lulo attacked by *machorreo*.

Introduction

Phytoplasmas are microorganisms that lack cell walls. They were first observed under the electron microscope in 1967. Currently classified within the class Mollicutes, phytoplasmas are associated with plant diseases, causing more than 600 diseases in several hundreds of plant species, usually concentrating in phloem sieve tubes (Oshima et al. 2001). The affected plants show yellowing or reddening of leaves, reduced leaf size, stunting with shortened internodes, and loss of apical dominance. This leads to poor development of the producing plant, proliferation of shoots and roots, witches' broom, necrosis of phloem tissues, branch death in woody plants, reduced production, plant decline, and, occasionally, death. Several symptoms unique to diseases related to phytoplasmas involve flowers: virescence (green coloring in flowers), phyllody (conversion of petals and sepals into leaves), and flower sterility causing floral abortion (Agrios 1997; Oshima et al. 2001).

Phytoplasmas are commonly transmitted between plants by homopterous insects. The microorganism first multiplies in the intestinal cells of their insect vectors and subsequently in the hemolymph after passing through the salivary glands. They infect internal organs such as the thoracic ganglion and fatty bodies (lipids) (Kawakita et al. 2000).

These Mollicutes are detected through electron-microscopy, using both light and immunofluorescence with 4', 6-diamidino-2-phenylindole (DAPI). These techniques are limited by the concentration of the microorganism used (Ahrens and Seemüller 1992). Recently, the polymerase chain reaction (PCR) method was used to detect phytoplasmas by amplifying, with universal and specific primers located in the 16S rDNA region, the intergenic spacer (IS) and the 23S rDNA of a given phytoplasma's genome (Ahrens and Seemüller 1992). Sequence analysis and restriction fragment length polymorphisms (RFLPs) (Lee et al. 1993) are also used to determine and classify phytoplasmas.

Most authors who use PCR to identify phytoplasmas also use, as positive control, *Catharanthus roseus* (syn. *Vinca rosea*; also periwinkle). This indicator plant is highly susceptible to infection by phytoplasmas from different crops. It enables the conservation of live phytoplasmas isolated from different species of affected plants (Ahrens and Seemuller 1992; Davis and Lee 1982; Deng and Hiruki 1991; Firrao et al. 1993; Prince et al. 1993).

Lulo or naranjilla (*Solanum quitoense*) is a fruit with commercial potential for food processing in Colombia. It is attacked by a disease known as *machorreo*, the causal agent of which has yet to be determined. The disease is a serious constraint to lulo production in the country, with reports of production having been reduced by as much as 70%. The disease characteristically stunts the plant and causes floral abortion, which symptoms suggest the presence of a phytoplasma.

For our study, to ascertain the presence of phytoplasmas in lulo attacked by *machorreo*, we used molecular techniques, particularly those based on PCR, RFLPs, and sequencing of the 16S rDNA region, as being the most likely to detect this type of microorganism in plant crops.

Materials and Methods

Tissue samples

Lulo plants showing stunting, phyllody, and floral abortion were collected from the municipalities of Manizales and Dosquebradas in the Departments of Caldas and Risaralda, respectively **(Table 1)**. In vitro lulo plants were included as negative controls. Positive controls were samples of *C. roseus* of the type 'besito' or 'vinca' that were clearly stunted or showed reddening in their terminal buds—typical symptoms of phytoplasma infection. Also used was DNA from the coffee crispiness phytoplasma of the X-disease group (NCBI's GenBank accession no. AY525125), provided by the Colombian Centro Nacional de Investigaciones de Café (*National Coffee Research Center*; CENICAFE).

Table 1. List of lulo (*Solanum quitoense*) materia ls used to study the possibility of phytoplasmas being the causal agent of the Colombian phylody and virescense disease *machorreo*.

No. of Samples[a]	Variety Departme	nt[b]	Municipality	Village District	Positive samples from PCR per processed sample (%)
3	Lulo hybrid 'La Selva'	Caldas	Manizales	La Trinidad	100
3	Lulo hybrid 'La Selva'	Caldas	Manizales	Alto Bonito	100
3	Lulo hybrid 'La Selva'	Risaralda	Dosquebradas	Chaquiro	100
1	–	Valle del Cauca	El Cerrito	Los Cuchos	100
1	Lulo 'Castilla'	Valle del Cauca	Buga	El Janeiro	100

a. Total number of samples received from each farm.
b. An administrative and political division of Colombia.

Transmission by grafting

We conducted tests for transmission by grafting, using infected lulo plants showing symptoms of phyllody, viresecence, and flower abortion. The plants were collected from the municipalities of Manizales (Caldas), Dosquebradas (Risaralda), and El Cerrito and Buga (Valle del Cauca). We grafted buds and petioles from both diseased and healthy lulo plants onto plants of *C. roseus*. The grafted plants were then kept in the greenhouses at ICA's Palmira Experiment Station until symptoms appeared.

DNA extraction

DNA was extracted according to the protocol of Gilbertson and Dellaporta, 1983. Tissues from leaf veins, stems, and petioles, which had been conserved at -80°C, were macerated with liquid nitrogen, using a porcelain mortar. About 0.4 g of the pulverized tissue were mixed with 0.51 mL of extraction buffer (50 mM EDTA, pH 8.0; 500 mM NaCl; and 10 mM 2-mercaptoethanol) and agitated for 2 min at speed 8 in a blender (Vortex-Genie 2, model G560, Scientific Industries, Bohemia, NY). Then 90 µL of SDS at 10% were added and the whole agitated for 2

min before being incubated at 65°C for 10 min. Subsequently, 150 µL of potassium acetate at 5 M, pH 5.5, were added and the whole agitated for 2 min. The mixture was centrifuged at 14,000 rpm for 10 min and the supernatant (about 600 µL) collected. Then, 0.5 volumes of isopropanol at 100% (300 µL) were added and left to precipitate at -20°C for 30 min. The whole was then centrifuged at 14,000 rpm for 10 min, the supernatant eliminated, and the pellet washed with 500 µL of ethanol at 70%, centrifuging at 10,000 rpm for 5 min. Finally, the supernatant was eliminated and the pellet re-suspended in TE at 30 and 50 µL and 50°C. It was then incubated overnight with 2 µL of RNase A (10 mg/mL) at 4°C.

Detection by PCR

Seven primer pairs were used for amplification of the 16S rRNA gene, 23S rRNA gene, rp genes, and 16S/23S spacer region, to detect phytoplasmas in lulo **(Table 2)**. The locations of these primers are shown in **Figure 1**.

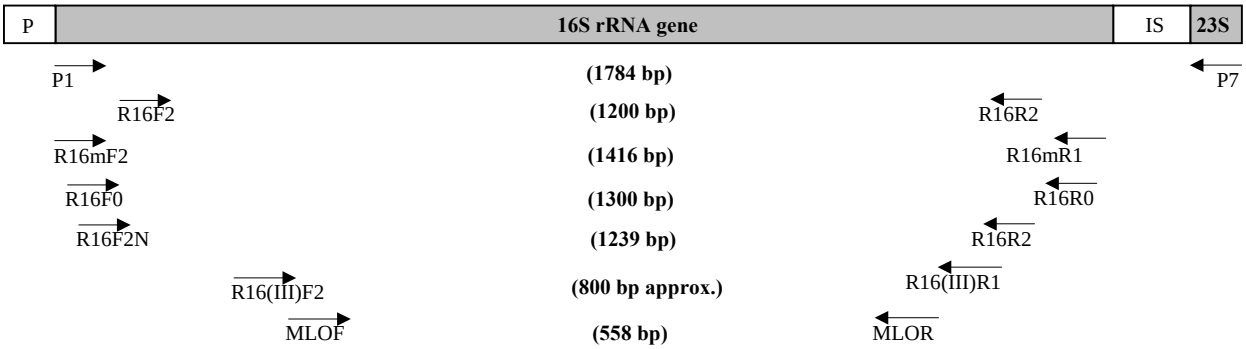

Figure 1. Location of primers in the rRNA operon. P = promoter; IS = intergenic spacer; 23S = 23S rRNA gene (From Guo et al. 2000; Heinrich et al. 2001).

Table 2. Primers us ed for PCR amplif ication and sequencing of genes 16S and 23S rRNA from phytoplasma-infected plants of lulo (*Solanum quitoense*).

Primers[a]	Sequence (5' → 3')	Amplified region[b]
[C]R16F2/[E]R16R2	ACG ACT GCT GCT AAG ACT GG TGA CGG GCG GTG TGT ACA CCC G	***16S (universal)***
[A]R16F0/R16R0	CTGGCTCAGGATTAACGCTGGCGGC GGATACCTTGTTACGACTTAACCCC	16S (universal)
[B]R16mF2/R16mR1	CAT GCA AGT CGA ACG GA CTT AAC CCC AAT CAT CGA C	16S
[D]R16F2N	GAA ACG GCG GTG TGT ACA AAC CCC G	16S
[B]P1/[F]P7	AAG AGT TTG ATC CTG GCT CAG GAT T CGT CCT TCA TCG GCT CTT	16S 23S
[B]LD16-1/23S	CGG AAA ACC TTC GGG TTT TAG TCT TTT CCT GCG GTT ACT TAG AT	16S 23S
[D]P4	GAA GTC TGC AAC TCG ACT TC	16S
[D]R16(III)F2/R16(III)R1	AAGAGTGGAAAAACTCCC TCCGAACTGAGATTGA	16S (X-disease group)
[A]MLOF/MLOR	ACGAAAGCGTGGGGAGCAAA GAAGTCGAGTTGCAGACTTC	16S

a. (A) Primers used in direct PCR; (B) primers used in nested PCR for the first cycle; (C) primer used in direct and nested PCR for the first cycle; (D) primers used in nested PCR for the second cycle; (E) primer used in direct PCR, and nested PCR for the first and second cycles; (F) primer used in nested PCR for the first and second cycles.

b. Location of primer within rRNA operon, that is, within each of the genes 16S rRNA and 23S rRNA.

Samples of DNA from healthy and diseased lulo and the controls *C. roseus* and coffee were evaluated with direct and nested PCR based on dilutions made at 20 ng/µL. For direct and nested amplification in the first cycle, we used primers that had amplified a broad region of the 16S rRNA and 23S rRNA genes **(Table 2)**. Conditions were as follows: 100 ng DNA, 1X buffer, 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.1 µM of each primer, and 1 U *Taq* polymerase.

For the primers R16F0/R16R0 and R16F2/R16R2, we carried out 35 cycles in a MJR PTC-100 thermocycler (MJ Research), using the following conditions: 1 min of denaturation (2 min for the first cycle) at 94°C, annealing for 2 min at 50°C, and primer extension for 3 min (10 min in the final cycle) at 72°C.

For the primers LD16-1/23S, we used 29 cycles, reducing annealing and extension time by 1 min.

For the P1/P7 primers, we carried out 35 cycles: denaturation for 30 s (90 s for the first cycle) at 94°C, annealing for 50 s at 55°C, and primer extension for 80 s (10 min in the final cycle) at 72°C. We used the same conditions for the primers R16mF2/R16mR1 (Gundersen and Lee 1996; Schneider et al. 1995), but carried out 28 cycles.

For the MLOF/MLOR primers, we used 24 cycles: denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and primer extension for 30 s (4 min in the final cycle) at 72°C.

For the nested PCR, the amplified product of the first pair of primers was diluted to 1:50 with sterilized distilled water to use it as DNA mold, in quantities of 1 µL **(Table 3)**. The four pairs of the second-cycle primers were evaluated under the same conditions as for the first-cycle primers, except for R16F2N/R16R2 for which the annealing temperature was lowered to 50°C.

Table 3. Primers used in nested PCR, conducted to detect the presence of phytoplasmas in lulo attacked by the phylody and virescense disease *machorreo*.

First Cycle	Second Cycle	Approx. Fragment Size (Base Pairs)
P1/P7	R16F2N/R16R2	1200
R16mF2/R16mR1	R16F2N/R16R2	1200
LD16-1/23S	P4/P7	550
R16F2/R16R2	R16(III)F2/R16(III)R1	800

Restriction fragment length polymorphisms (RFLPs)

To classify the phytoplasma in terms of the 15 groups so far reported, we amplified—using nested PCR, with the universal primers P1/P7 and R16F2N/R16R2, and the specific primers for the 16Sr III group R16F2/R16R2 and R16(III)F2/R16(III)R1—the sequence of gene 16Sr RNA (1.2 kb and 0.8 kb). The nested PCR product of universal and specific primers was then analyzed by digestion with restriction enzymes *Rsa*I, *Alu*I and *Mse*I (Invitrogen Life Technologies, Carlsbad, CA). The enzyme *Taq* I was evaluated only with the PCR product obtained with specific primers. , as controls, phytoplasmas from the 16Sr I group (aster yellows, as represented by palm phytoplasma), 16Sr III group (X-disease, as represented by coffee phytoplasma), and 16Sr IX group (pigeonpea witches' broom, as represented by phytoplasma from periwinkle), were used.

15 µL of the PCR product and added 2 µL of 10X buffer enzyme and 1 µL of the restriction enzyme (500 units/µL), were taken. This mixture was incubated for 16 h at 37°C (except for enzyme *Taq*I, which was incubated at 65°C). We then added 3 µL loading buffer (bromophenol blue at 0.25%, glycerol in water at 30%) and ran it in acrylamide gel at 5% for 1 h at 100 V, 24 mA, in TBE 1X buffer and stained with ethidium bromide at 10 mg/mL. The PCR product obtained with the specific primers for the X-disease group, was also digested.

Sequence analysis

The product amplified from direct and nested PCR was purified, following the protocol described for the QIAquick PCR Purification Kit, and sequenced. Another analysis was carried out with cloned DNA. The purified PCR products were ligated to the vector (pGEM®-T Easy Vector). Competent cells were transformed by electroporation and planted in selective culture medium (LB agar, Xgal, IPTG, and ampicillin). Colonies presenting white coloring were selected. The plasmid's DNA was purified for the clones that had inserts of the expected size. These clones were sequenced with primers T7 and Sp6, using the BigDye Terminator Kit (Applied Biosystems) in an ABI PRISM® 377 DNA Sequencer. Sequence analysis was done with the programs Sequencher 4.1 and DNAMAN 4.13. Homology was sought in the NCBI's GenBank (www.ncbi.nlm.nih.gov), using the tool BLAST®n.

Results

DNA extraction

The method of extracting nucleic acids showed satisfactory results for detecting phytoplasmas. High quality and concentration (between 300 and 600 ng/µL) were obtained, sufficient to conduct molecular tests. The highest yields were observed when extraction was based on a mixture of leaf-petiole and leaf-stem tissues. On visualization in agar gel at 0.8%, a strong band was observed in all the samples examined, applying 50 V consistently over 1 h.

Transmission by grafting

Results of the tests for transmission by grafting indicated that the disease is contagious. The C. roseus cv. Periwinkle plants, grafted with buds and petioles from diseased lulo plants, showed yellowing of leaves, noticeably reduced leaf size, and diminished plant development. These symptoms are similar to those described for diseases caused by phytoplasmas (Agrios 1977; Oshima et al. 2001). The Periwinkle plants grafted with tissues from healthy lulo plants had no symptoms.

Detection by PCR

To conduct the PCR, we had to carry out nested PCR, as one PCR only was insufficient to detect this phytoplasma, given its specificity (many bands) and low sensitivity **(Table 4)**. Primers that gave the best results were P1/P7–R16F2N/R16R2, R16mF2/R16mR1–R16F2N/R16R2, LD16-1/23S–P4/P7 as universal, and R16F2/R16R2–R16(III)F2/R16(III)R1 as specific **(Table 3)** . These last primers were specific to all the phytoplasmas of the 16Sr III group (X-disease). They detected with greater sensitivity and specificity several positive samples than did the universal pairs. The expected sizes in base pairs were obtained for each primer in the lulo samples and the periwinkle and coffee controls **(Figure 2)**.

Table 4. Identifying phytoplasmas in samples of lulo (*Solanum quitoense*) with symptoms of the phylody and virescense disease *machorreo*, using universal and specific primers.

Primers	Positive Samples per Symptomatic Sample (%)	Reproducibility (%)
Nested PCR (P1/P7 and R16F2N/R16R2)	100	75
Nested PCR (R16mF2/R16mR1 and R16F2N/R16R2)	100	75
Nested PCR (LD16-1/23S and P4/P7)	100	80
Nested PCR (R16F2/R16R2 and R16(III)F2/R16(III)R1)	100	100
MLOF/MLOR	Amplification of healthy samples	
R16F2/R16R2	Non-specific	
R16F0/R16R0	Non-specific	

The primers of the X-disease group amplified only for the phytoplasmas obtained from lulo and the coffee control. The periwinkle controls did not amplify for this region, suggesting an approximation to the group to which the lulo phytoplasma would belong **(Figure 2D)**.

Phytoplasmas are found exclusively in phloem vessels. Normally, they have a heterogeneous distribution in the plant, and are found in low concentrations. These characteristics make their detection and identification difficult (Seemüller et al. 1998). Hence, specific primers are needed to increase sensitivity for detecting the pathogen in plants with very low levels of inoculum (i.e., with initial or intermediate symptoms).

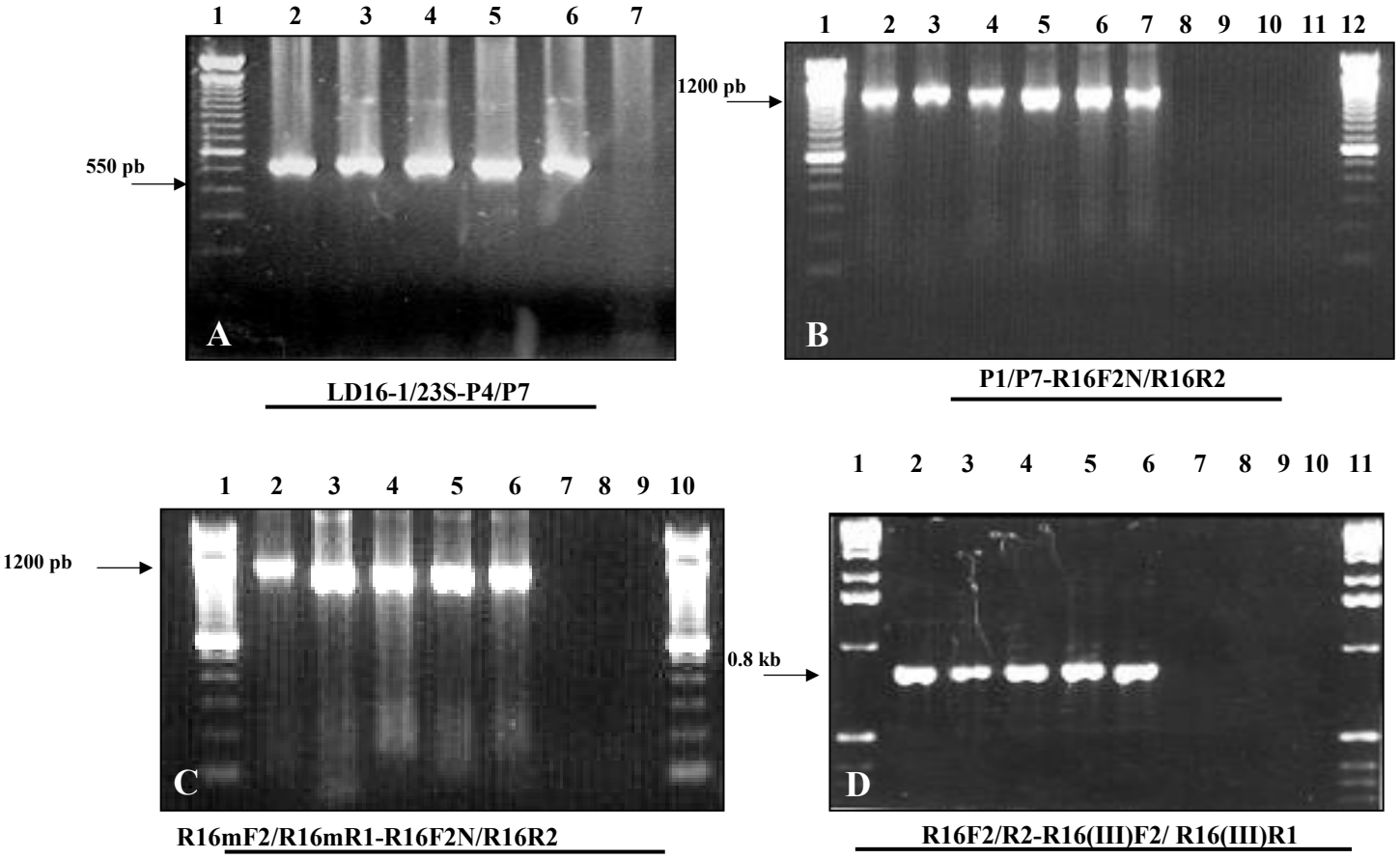

Figure 2. **Amplifications w ith nested PCR, using universal and specif ic p rimers, for detecting phytoplasmas. (A) La ne 1 = 10 0-bp marker; lanes 2–4 = lul o phytoplasma; lane 5 = phytoplasma from p eriwinkle; lane 6 = phytoplasma from c offee; lane 7 = healthy lulo plan t. (B) Lanes 2–5 = lulo ph ytoplasma; lane 6 = phytoplasma from periw inkle; lane 7 = phytoplasma from coffee; lane 8 = health y lulo plan t; lane 9 = negat ive c ontrol. (C) Lanes 2– 4 = lulo phytoplasma; lane 5 = phytoplasma from periw inkle; lane 6 = phytoplasma from coffee; lane 7 = healthy lu lo plant; lane 8 = negative control. (D) Lanes 1 and 11 = 1- kb marker; lanes 2–5 = lulo phytoplasma sampled from different village districts, Colombia: lane 2 = La Trinidad, lane 3 = Alto Bonito, lane 4 = Chaquiro, and lane 5 = Los Cuchos; lane 6 = phytoplasma from co ffee; lane 7 = healthy lulo plant; lane 8 = phytopla sma from periw inkle; lane 9 = negative control.**

Restriction fragment length polymorphisms (RFLPs)

The positive samples were amplified with primers R16F2N/R16R2 and digested with the four enzymes. Their band patterns were compared with phytoplasmas of the groups 16Sr I (aster yellows, as represented by palm phytoplasma), 16Sr III (X-disease, as represented by phytoplasma from coffee), and 16Sr IX (pigeonpea witches' broom, as represented by

phytoplasma from periwinkle) **(Figure 3)**. The lulo phytoplasma was placed in the 16Sr III group (Lee et al. 1993). For each enzyme evaluated, the band patterns were different, clearly differentiating among the phytoplasma groups. The amplified products were digested, using specific primers for the 16Sr III group, and compared with the phytoplasmas from coffee and cassava (*Manihot esculenta* Crantz) (Alvarez et al. 2003) **(Figure 4)**. The same band pattern was observed for all isolates, suggesting the presence of only one phytoplasma in the set of samples evaluated.

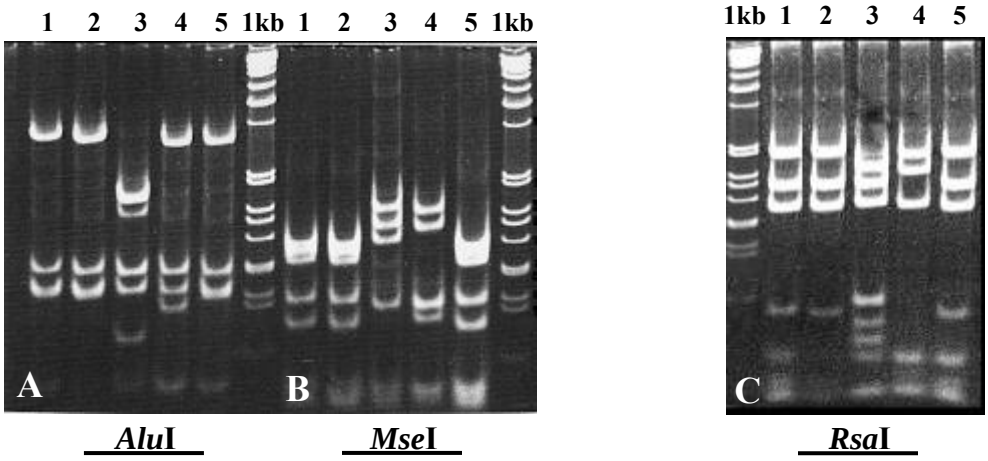

Figure 3. Restrictio n fragment length polymorphisms (RFLPs) compared across thre e restriction enz ymes (*Alu*I, *Mse*I, and *Rsa*I) for the fra gments amplified by nested PCR with primers R16F2N/R16R2. Lanes 1 and 2 = lulo phytoplasma; lane 3 = 16Sr I group (palm phyto plasma); lane 4 = 16Sr IX group (phytoplasma from periw inkle); lane 5 = 16Sr III group (phytoplasma from coffee).

Sequence analysis

The determination and comparison of the phytoplasma sequence in the 16S rRNA region involved about 1050 nucleotides of the sequence of each PCR fragment determined by direct sequencing and cloning, using the primers R16F2N/R16R2. The sequenced samples were from leaf-petiole and leaf-stem tissues. An extensive analysis was performed, based on the results of the sequencing (maximum pairing and higher percentages of homology), using the tool BLAST®n in GenBank.

The phytoplasma analyzed in this study presented high levels (97%) of homology with the sequences of the 16Sr III group (X-disease) **(Table 5)**. The annealing of the computer-assisted sequences indicated the sequence of the nucleotides from the 16S rRNA region of the lulo phytoplasma was very similar to that of 16S rRNA of the GenBank accessions AF147706, AF510724, and AY034090. The lulo phytoplasma differed from the other phytoplasmas of the 16Sr III group by its nitrogen bases varying in the position of 4 cytosines and 1 thymine throughout the sequence of 16Sr RNA.

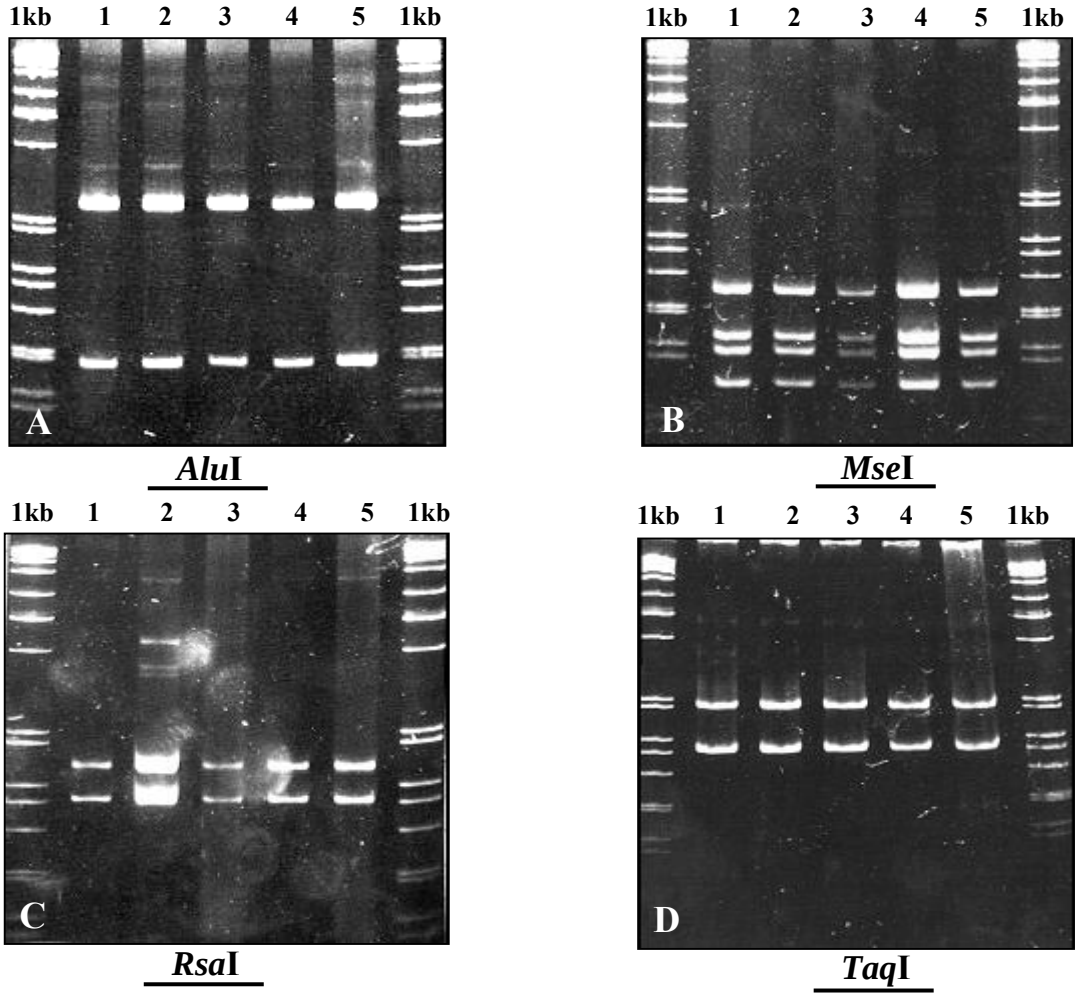

Figure 4. **Restriction fragment length poly morphisms (RFLPs) compared across four restriction enz ymes (*Alu*I, *Mse*I, *Rsa*I, and *Taq*I) for f ragments a mplified by nested PCR w ith primers R16(III)F2 /R16(III)R1. Lanes 1–3 = lulo phytoplasma; lane 4 = cassava phytoplasma; lane 5 = 16Sr III group (phytoplasma from coffee).**

Table 5. The degree of homology found betw een sequences of phytoplasma DNA obtained, through nested PCR, from lea f-petiole and le af-stem tiss ues of lulo (*Solanum quitoense*) attacked by the phylody and virescense disease *machorreo* and sequences of phytoplasmas reported in NCBI's GenBank.

Pairing with GenBank[a]	GenBank Code	Probability of Favorable Homology[b]	Homologized Bases[c]	Homolog y (%)
Chayote witches'-broom phytoplasma (ChWBIII), strain ChWBIII(Ch10), 16S ribosomal RNA gene; 16S-23S ribosomal RNA intergenic (1813)	AF147706	0.0	1004/1031	97
Milkweed yellows phytoplasma 16S ribosomal RNA gene, partial sequence; 16S-23S intergenic spacer region and tRNA-Ile gene, complete sequence; and 23S ribosomal RNA gene, partial sequence (1812)	AF510724	0.0	1003/1031	97
Blueberry proliferation phytoplasma 16S ribosomal RNA gene, partial sequence; tRNA-Ile gene, complete sequence; and 23S ribosomal RNA gene, partial sequence (1770)	AY034090	0.0	1002/1031	97

a. Values in italics refer to the total number of bases reported in GenBank.
b. The value 0 is expected for high percentages of homology.
c. Number of homologized bases in different regions of the sequence reported in GenBank.

When several accessions of the 15 groups of phytoplasmas reported in the GenBank were homologized with the lulo sequences, using the DNAMAN 4.13 program (Homology Tree), the high homology already found with the 16Sr III group **(Figure 5)** was confirmed.

Conclusions

We detected the presence of a phytoplasma in association with *machorreo* in lulo, using phylogenetic analysis and homology with the sequence of the 16S rRNA region and the gene tRNA. Using the following techniques, we identified the phytoplasma as belonging to the 16Sr III group (X-disease; 97% homology): nested PCR to obtain, with universal primers, amplifications of fragments measuring about 1.2 kb and, with specific primers, fragments measuring 800 bp in plants affected by *machorreo*; restriction enzymes and partial sequencing of the phytoplasma's DNA to obtain cloned fragments; amplification of these with primers SP6 and T7, using pGEM®-T Easy Vector and PCR; and direct sequencing with internal primers.

For future studies, we recommend identifying possible vectors through samplings of crop insects, particularly of the order Homoptera. Attempts should be made to reproduce symptoms of the disease in healthy plants of lulo to confirm the insects' association with the disease. Through molecular techniques, crop weeds should also be evaluated for their potential as hosts to the phytoplasma. Finally, as a possible management practice, resistance of promising lulo hybrids to *machorreo* should also be evaluated.

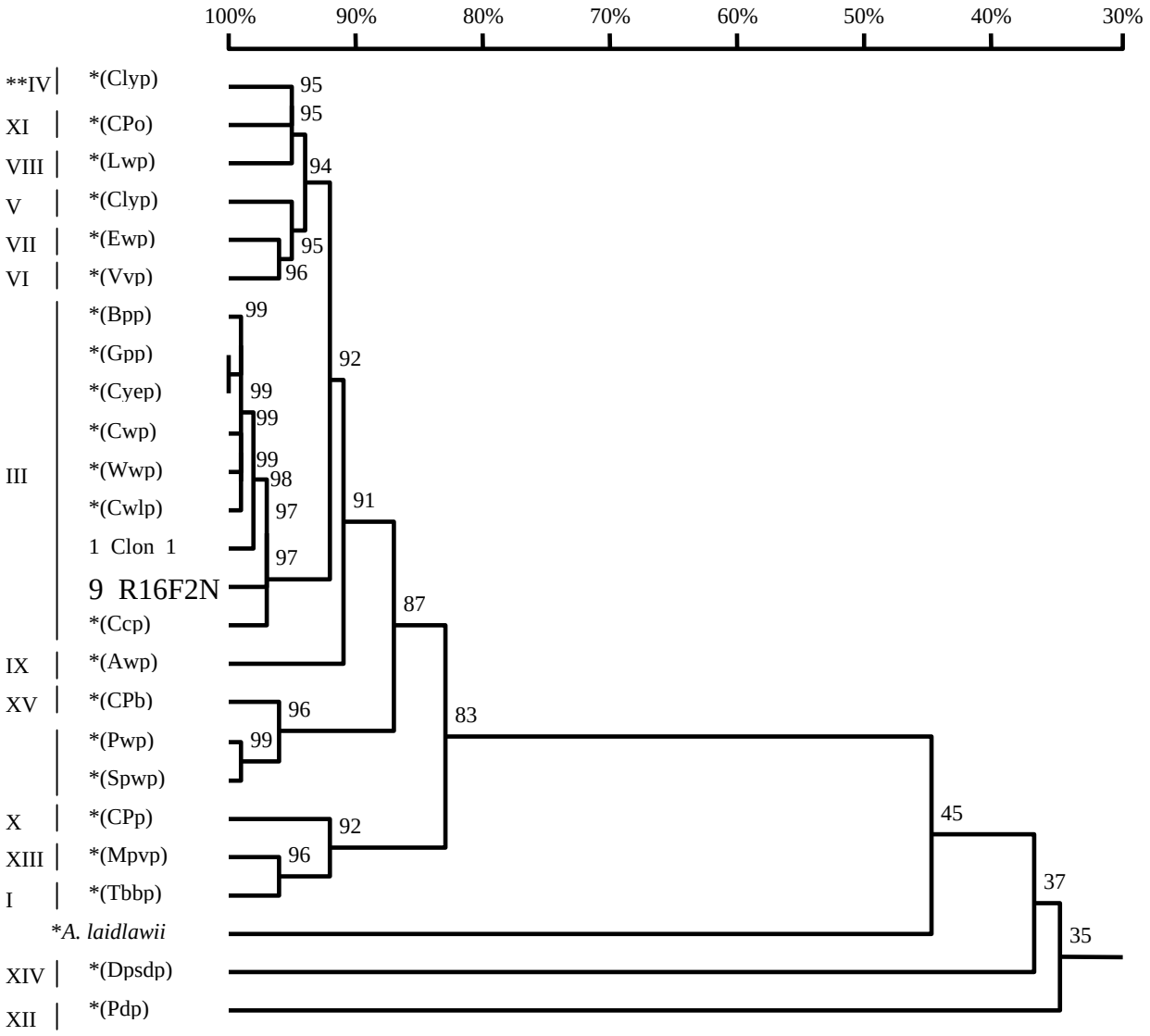

Figure 5. 16S rRNA homology tree of sequences of 22 phytoplasmas and *Acholeplasma laidlawii* (*A. laidlawii*) belonging to the same family of phytoplasmas. It includes the sequences of fragments of direct PCR (9_R16F2N) and one of the cloned fragments (1_Clon_1) obtained from lulo. * = GenBank accessions; ** = phytoplasma group number.

Clyp = coconut lethal yellowing phytoplasma
CPo = *Candidatus* Phytoplasma oryzae
Lwp = loofah witches'-broom phytoplasma
Clyp = cherry lethal yellows phytoplasma
Ewp = Erigeron witches'-broom phytoplasma
Vvp = periwinkle virescence phytoplasma
Bpp = blueberry proliferation phytoplasma
Gpp = Gaillardia phyllody phytoplasma
Cyep = clover yellow-edge phytoplasma
Cwp = chayote witches'-broom phytoplasma
Wwp = walnut witches'-broom phytoplasma
Cwlp = Cirsium white-leaf phytoplasma
[a]Ccp = coffee crispiness phytoplasma
Awp = almond witches'-broom phytoplasma
CPb = *Candidatus* Phytoplasma brasiliense
Pwp = peanut witches'-broom phytoplasma
Spwp = sweetpotato witches'-broom phytoplasma
CPp = *Candidatus* Phytoplasma prunorum
Mpvp = Mexican periwinkle virescence phytoplasma
Tbbp = tomato big-bud phytoplasma
Dpsdp = date-palm slow-decline phytoplasma
Pdp = papaya dieback phytoplasma

a. Check used in this study.

Acknowledgements

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Activity 13. Evaluating the effects of various control practices on the incidence and severity of *Phytophthora* root rots under field conditions in Quindío, Colombia.

Objective

Reduce cassava root rot caused by *Phytophthora* by ecological practices.

Methodology

Different control practices for *Phytophthora* spp. were evaluated for disease incidence and severity, and for yield in a field trial conducted at "La Elena" Farm, Municipality of Montenegro, Department of Quindío. The trial was planted with the local variety Chiroza (M Col 2066). Treatments were as follows:

1. An integration of the following practices:
   a. Selection of high-quality stem cuttings, including for root yield per plant harvested.
   b. Thermotherapy: planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.
   c. Biological control: strain 14 PDA-4 of *Trichoderma* spp. was used to make a suspension of 1×10^4 conidia/mL. Planting stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil in which the stakes were planted, using 100 mL/plant.
2. Traditional farmer's practice, including chemical control. Planting stakes were immersed for 5 min in a mixture of Orthocide® (captan) at 4 g/L and Ridomil® at 3 g/L of water.

All plots were fertilized 45 days after planting, using 500 kg/ha of the fertilizer mix Nitrax®, DAP, and KCl, applied at a rate of 1:2:2. The plots were planted according to a randomized complete block experimental design with six replications and 150 plants per treatment.

Results

The commercial variety Chiroza is highly susceptible to Phytophthora root rot. The disease affected cassava development, as only 82.7% of the plants (all treatments) were harvested **(Table 1)**. This percentage is relatively low for this region. In this trial, seed selection, heat treatment of stakes, and immersion of stakes in a suspension of conidia of the fungus *Trichoderma* and its application to the soil did not affect germination nor plant development, compared with the plants under farmer agronomic management, which involved using fungicides.

Table 1. The effects on yield of two different control strategies to manage root rots in cassava, Montenegro, Department of Quindío, Colombia. The local variety Chiroza was used and evaluated 12 months after planting.

Control strategy	Plants harvested	Roots harvested (t/ha)	Yield of Healthy Roots (t/ha), Type: Commercial	Non-com.	Com. + non-com.	Commercial and non-commercial roots affected by root rots (t/h)	(%)	Proximal	Distal
Integrated mgt based on ecological practices[a]	80%	26.3	15.1	4.3	19.4	6.9	26.2	14.1	44.9
Traditional farmer management[b]	85%	23.1	10.6	4.0	14.6	8.5	36.8	14.6	57.3
Average	82.7%	24.7	12.9	4.1	17.0	7.7	31.2	14.3	51.1

a. Planting stakes were selected for their quality. They were immersed for 49 min in water heated to 49°C over a wood fire. Strain 14 PDA-4 of the fungus *Trichoderma* sp., which attacks root-rot fungi (*Phytophthora* spp.), was used to make a suspension of 1×10^6 conidia/mL. The stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil near the base of each stake planted.

b. Planting stakes were immersed for 5 min in a mixture of Orthocide® (captan) at 4 g/L and Ridomil® at 3 g/L of water.

The package of ecological practices increased yield of commercial roots by 4.5 t/ha (42.5%), compared with conventional farmer practices.

From the plots under farmer agronomic management, 8.5 t/ha of roots with rot was obtained, as against 6.9 t/ha (i.e., 18.8% less) from the plots under ecological management. Of the harvest in each treatment, 36.8% of roots suffered from rot under traditional farmer management, whereas only 26.2% of the crop was attacked under integrated management based on ecological practices.

At harvest, distal parts of roots were observed to be more affected by rot than proximal parts. This observation was confirmed through trials in which roots were inoculated with *Phytophthora tropicalis*.

Conclusions

Ecological practices increased yield in commercial roots by 42.5% and reduced, by a smaller proportion, the number of roots with rot 18.8% compared with the chemical control used by farmers in the region. We therefore conclude that using a combination of different practices is essential for managing the disease, and that these practices must be strengthened by planting cassava varieties that are resistant to rot.

Acknowledgments

We thank the following for their support: Silverio González and Camilo López, Special, La Tebaida, Department of Quindío; and Rosy Santacruz, Universidad de Quindío, Armenia.

Activity 14. Developing and validating sustainable methods of preventing and controlling FSD and SED.

To determine the effect of sustainable methods of preventing and controlling FSD and SED, experiments are recently started or ongoing.

Objective

To examine the effect of heat treatment on stakes of cassava plants affected by superelongation disease (SED), their germination, and yield

Methodology

We used thermotherapy to treat six stakes from each of 168 cassava genotypes taken from a field affected by SED. The stakes were immersed in hot water at 49°C for 49 min and then planted at Santander de Quilichao. The percentage of germination and yield were estimated, and the data analyzed by T test.

Results

The group of genotypes treated with thermotherapy had a germination rate of 90.2%, and yield was 22.2 t/ha, whereas the untreated group had a germination rate of 98.5%, and yield was 22.9 t/ha. The T test for germination gave a value of 1.79 and a probability of 0.077, whereas for yield the value was 0.41 and the probability of 0.66. The results indicated that there were no significant differences between the two treatments.

Acknowledgments

Bernardo Arias, Cassava Entomology, CIAT.

Activity 15. Greenhouse and on-farm evaluations of the effects of amendments, cover crops, organic fertilizer, and green manure on *Ralstonia solanacearum*, with practices based on chemicals included as controls.

Objective

To evaluate the effect of non-contaminating alternatives to formol on a population of *R. solanacearum* Race 2, causal agent of bacterial wilt of plantain, using bacteria inoculated into soil under greenhouse conditions

Methodology

Greenhouse

In an arrangement of randomized complete blocks, with five replications, the following treatments were tested:

- Incorporation of marigold (*Tagetes patula*) at 1 kg/m^2
- Incorporation of calfos, a fertilizer for P-deficient acid soils, at 0.5 kg/m^2
- Liquid fertilizer Fulvan®, an organic fertilizer based on "first froth" from boiled sugarcane juice and enriched with microorganisms, at a dosage of 20 L/m^2
- Lixiviate of plantain compost, produced on an artisanal basis by farmers in Quindío, Colombia, at a dosage of 2.7 L/m^2

These treatments were compared with 20% formol at 9.3 L/m^2. The controls used were inoculated soil without treatment, and soil that was neither inoculated nor treated. The experimental unit consisted of 2 plant pots with 250 cc of soil. The bacterial population was evaluated weekly over 70 days.

Field

Trials were established on six farms located in the Department of Quindío to evaluate the following treatments:

- Fulvan®, an organic fertilizer
- Lixiviate of plantain compost
- A practice recommended by ICA, where glyphosate is injected into the infected plant and its healthy neighbors, followed by weeding and applying formol into holes in the soil and covering with plastic
- Agroplus®, an organic product
- Coffee pulp
- Basamid®, a soil fumigant
- Formol
- Covering the soil with *Mucuna* mulch
- Covering the soil with *Crotalaria* mulch

Results

Greenhouse

The formol reduced the bacterial population by 100%, according to the evaluation made 20 days after application to the soil. Marigold was the next most effective treatment, reducing the bacterial population by 84.8%, according to a count made 70 days after the treatment was applied. Differences between the two treatments were not significant (Tukey's test, $\alpha = 5\%$).

The bacterial populations in the soil were also reduced by lixiviate of plantain compost (55.8%), calfos (39.3%), and Fulvan® (36.7%), whereas the control with no application had a reduction of 50%.

The effect of marigold may be attributed to one or several of the following organic components: linalol, carvona, cineole, ocimene, phenol, eugenol, anethole, dextralinolene, quercetagine, quercetagetine, quercetagitrine, tagetiine, flavonoids, pyretrines, bi-thienylacetylene, heleniene, derivatives of selenophene, fatty acids (myristic, palmitic, stearic, lauric, and oleic), aterthienyl, lutein, campherol, campheritrine, (SIAMAZONIA 2003), and sulfur derivatives such as thiophene.

Figure 1 shows that the formol and marigold affected the bacterial population from application, whereas calfos required about 27 days to act, possibly because it dissolves slowly.

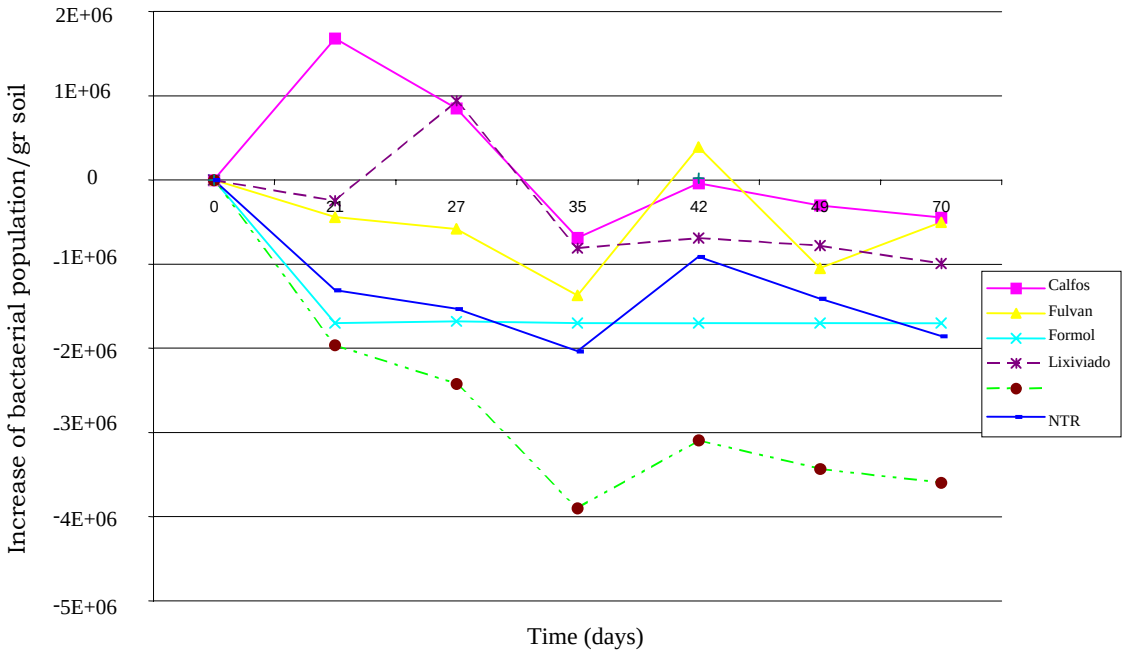

Figure 1. Changes in population sizes of the soil bacterium *Ralstonia solanacearum* after the applica tion of d ifferent ecolo gical a lternatives to formol. Trials w ere carried out under greenhouse conditions. NTR = no treatment.

Field

In the field, the bacterial population in the soil was limited almost exclusively to the site where the infected plant was found in the point of highest disease pressure focuses where control treatments were carried out. The bacterium was isolated only from the Lusitania farms, from the check at 1.6×10^6 cfu/g of soil; La Habana, from the treatment Agroplus® + coffee pulp at 7.2×10^6 cfu/g of soil; and Belén Farm, from the treatments Basamid® and formol at 60,545 cfu/g.

Reference

SIAMAZONIA. 2003. "Rosa sisa". (http://www.siamazonia.org.pe/Publicaciones/2003/Enero Plantas_medicinales/ rosasisa.htm SIAMAZONIA.2003. "Rosa sisa".

Activity 16. Evaluating the resistance of banana FHIA 17 to *Ralstonia solanacearum.*

Objective

To evaluate the resistance of banana FHIA 17 to three isolates of the bacterium *R. solanacearum*

Methodology

A bacterial suspension of *R. solanacearum* was prepared for each of three isolates—CIAT 1008 (from Tolima), Rs2 (Quindío), and Jamundí-a (Valle del Cauca)—with an optical density of a 0.6 to 600 nm wavelength. We then injected 3 mL of the suspension into FHIA 17 banana plants, which are believed to be resistant to the bacterium. The inoculum was distributed across three points on the pseudostem. The inoculated plants were incubated in a chamber at 30°C for 10 days under constant moisture. They were then moistened for 1 h per day until evaluation, 20 days after inoculation.

Results

Twenty days after inoculation, the plants began showing leaf yellowing, and 5 to 8 days later, wilting was observed, which progressed until the plants died. The cuts on the plants' pseudostems showed necrosis of vascular bundles. The symptoms caused by isolate Rs2 were mild, whereas those of the other two isolates caused plant death.

Activity 17. Diagnosing plant diseases and technical assistance.

Bacteriological and fungal diagnoses were performed on different samples obtained from Colombian farmers and institutions (see **Table 1**).

Table 1. Bacteria and fungi isolated from different crops and identified at the CIAT Cassava Pathology Laboratory (Palmira, Colombia).

Location Host plant	Disease	Detection method	Microorganism identified
Palmira, Valle del Cauca — Cassava	Wilting and root and stem root in seedlings	Isolation on selective media	*Fusarium sp.*
Valle del Cauca, Meta, Quindío — *Plantain*	Moko	Isolation, pathogenicity and DNA sequence analysis	*Ralstonia solanacearum*
Valle del Cauca, Putumayo — *Plantain*	Watery rot	Isolation	*Erwinia carotavora*
Antioquia — *Banana*	Moko	Isolation, pathogenicity and DNA sequence analysis	*Ralstonia solanacearum*
Quindío, Cauca — *Banana*	Fusarium wilt	Isolation and pathogenicity	*Fusarium oxysporum f.* sp. *cubense*
Palmira, Valle del Cauca — Dendrobium	Petals with brown-colored necrotic wounds	Isolation and direct observation under light microscope	*Colletotrichum* sp.
Palmira, Valle del Cauca — Dendrobium	Black stem rot	Isolation and direct observation under light microscope	*Fusarium sp*. associated with the stem borer *Xylosandrus mongenus*
La Unión, Valle del Cauca — Melon	Stems presenting internal orange-yellow dry rot	Isolation on selective media	*Fusarium sp.*
Cali, Valle del Cauca — *Tomato*	Powdery mildew	Direct observation under light microscope	*Oidium lycopersicum*

An *in vitro* bioassay to evaluate the effect of fungicides on *Colletotrichum* sp. isolates from *Dendrobium* showed that Mertect was the most effective of the six products tested.

Activity 18. Training researchers from La tin America , the Caribb ean, and Africa on managing cassava diseases and research technology.

2003

- 23 October. Philipp Aerni, Senior Researcher, Center for Comparative and International Studies (CIS) of the Swiss Federal Institute of Technology (ETH) at Zürich. Disease diagnosis, molecular characterization of pathogens, molecular markers associated with resistance to cassava diseases, and management of cassava diseases.

2004

- 10 February. José Ventura and Ernesto Espinoza, INIVIT, Cuba. Disease detection and diagnosis in cassava.
- 15 February. Natali Cortés, Student, Tver State University, Moscow, Russia. Applied biotechnology to detect and control phytopathogenic agents.
- March. Okechukwu Eke-Okoro (Nigeria), Titus Alicai (Uganda), Christopher Omongo (Uganda), William Sserubombwe (Uganda), Mayanne Apok (Uganda), Steven Tumwesigye (Uganda). Disease diagnosis, molecular characterization of pathogens, molecular markers associated with resistance to cassava diseases, management of cassava diseases.
- 20 May. Colombo–Japanese Association (15 participants). Integrated management of diseases for cassava, plantain, palm, and flowers.
- 31 May to 12 June. CIAT (30 participants). International course on modern systems of cassava production, processing, and use. Organized by CLAYUCA.
- 9 September. Reinaldo Tovar. Universidad Nacional Experimental de Guayana. Puerto Ordaz, Venezuela. Thermotherapy for *in vitro* production of cassava plantlets.
- 30 September. Manuel Valdivié. Instituto de Ciencia Animal (ICA), Cuba. Management of cassava diseases.
- 1 October. Gustavo Córdova. Instituto Nicaragüense de Tecnología Agropecuaria. Managua, Nicaragua. Biological control of pathogens.

Training students, farmers, technicians, and researchers through field days and meetings on modern, sustainable, cassava production systems in different regions of Colombia to manage major cassava diseases, emphasizing selection of stem cuttings

2003

- 4 November. Five people from Chemonics International and farmers of Putumayo. Disease diagnosis and management for cassava and plantain.

2004

- February. Lorena Escobar. Universidad Nacional de Colombia. Isolation of *Phytophthora* species from chili pepper.
- March. Liliana Cadavid and Susana Mejía, Biology Students, Universidad del Valle, Cali, Colombia. Isolation, detection, and pathogenicity tests of pathogens.
- 29 February. 18 participants, including farmers and students from Pereira, Department of Risaralda. Disease diagnosis and management for cassava.

- 12 March. 12 participants, including Chemonics International, Fundación Futuro Ambiental, and Fundación Catatumbo, and farmers. Disease diagnosis and management for cassava, rubber, cacao, and vanilla.
- 15–17 March. Ana Claudia Gordillo, CORPOICA "La Libertad", Villavicencio. Diagnosis and identification of *Ralstonia solanacearum* in plantain, banana, soil, weeds, and water.
- 24 March. Meeting with Nicolás Cock Duque in Ecoflora. Integrated management of diseases through plant extracts.
- May. Meeting in Palmar del Oriente, with technicians from Palmar del Oriente, Palmas de Casanare, and Palmeras Santana oil-palm plantations; and with researchers from CENIPALMA. Discussion of progress in diagnosing and identifying the causal agent of lethal wilt of oil palm.
- 16 May. 30 participants, including farmers, functionaries from national bodies, and NGOs in Orito, Department of Putumayo. Diagnosis and management of frogskin and other cassava diseases.
- 18 May. Dr Octavio Vargas, Mitsui & Co., Ltd Natural products in disease management.
- 4 June. 37 Students, University of Caldas. Cassava and plantain diseases.
- 5 June. 350 participants, including farmers, functionaries from national bodies, and private enterprises in Montería, Department of Córdoba, at the release of new cassava varieties. Management of cassava diseases, with a presentation on the diagnosis and management of frogskin disease.
- 1 July. 6 farmers, Pescador, Department of Cauca. Diagnosis and management of cassava diseases.
- 22 July. Visitors from the Colombian Association of Banana Growers (AUGURA) and CENIBANANO, including Luis Fernando Patiño, León Toné Gaviria, and Ramiro Jaramillo Sosa of the Board of Directors of CENIBANANO. Integrated management of diseases for plantain, cassava, and oil palm.
- 26 July to 6 August. Hernán Zapata, Agrobiológicos SAFER (Natural Control). Isolation and conservation of pathogens and biocontrollers, inoculum preparation, and pathogenicity tests.
- 3 September. 3 farmers, Department of Quindío. Progress on the management of bacterial wilt (*moko*) of plantain.
- 9 to 10 August. Workshop. Diagnosis of diseases caused by viruses and phytoplasmas. CIAT, Cali.

Attendance at Meetings in 2004

8–14 March. Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia

31 July to 4 August. Annual Meeting of the American Phytopathological Society (APS), Anaheim, CA

13–15 August. XXV National Congress of Phytopathology, Cali, Colombia (held by ASCOLFI)

Awards

13 August. Premio Nacional de Fitopatología "Rafael Obregón" categoría profesional, for the paper "Detección de marcadores microsatélites asociados con la resistencia al Añublo Bacterial de la yuca (*Manihot esculenta* Crantz) en Colombia". PX Hurtado, E Alvarez, M Fregene and GA Llano. Presented at XXIV Congress of ASCOLFI. June 25 – 27, 2003.

Feria de innovaciones, México 2004. Desarrollo de medidas de manejo del Moko (*Ralstonia solanacearum*) de plátano (*Musa AAB*) en Colombia, mediante investigación participativa con agricultores. Grupo Consultivo para la Investigación Agrícola Internacional (CGIAR).

Publications

Alvarez E; Mejía JF; Huertas C; Varón F. Detección y caracterización molecular de un fitoplasma, asociado con el "machorreo" del lulo (*Solanum quitoense*) en Colombia. Fitopatol Colomb 27(2):71–76.

Hurtado PX; Alvarez E. Búsqueda de genes análogos de resistencia asociados con la resistencia al añublo bacterial de la yuca. Fitopatol Colomb 27(2):59–64.

Loke JB; Pérez JC; Alvarez E; Cuervo M; Mejía JF; Llano G.; Pineda B. Cuero de Sapo: *Una Enfermedad de la Yuca* -Once Preguntas Muy Interesantes de Agricultores-. Poster presented June 5 during the release of new cassava varieties.

Extension Brochures

Alvarez, E., Llano, G. 2004. Añublo Bacterial de la yuca.

Alvarez, E., Mejia, J.M. 2004 Superalargamiento de la yuca.

Submitted

Alvarez E. and Ospina C.A. 2004. Morphological, genetic, and pathogenic characterization of *Colletotrichum gloeosporioides,* causal agent of anthracnose in soursop (*Annona muricata*) in the production areas of Valle del Cauca, Colombia. Plant. Dis.

Loke JB; Alvarez E; Vallejo FA; Marín J; Fregene M; Rivera S; Llano GA. Análisis de QTLs de la resistencia a pudrición de raíz causada por *Phytophthora tropicalis* en una población segregante de yuca (*Manihot esculenta* Crantz). Acta Agron.

Llano GA; Alvarez E; Muñoz JE; Fregene M. Identificación de genes análogos de resistencia a enfermedades en yuca (*Manihot esculenta* Crantz), y su relación con la resistencia a tres especies de *Phytophthora*. Acta Agron 53(1/2).

Calle F; Pérez JC; Gaitán W; Morante N; Ceballos H; Llano GA; Alvarez E. Genetics of relevant traits in cassava (*Manihot esculenta* Crantz) adapted to acid-soil savannas. Euphytica.

Hurtado PX; Alvarez E; Fregene M; Llano GA. Detección de marcadores microsatélites asociados con la resistencia a *Xanthomonas axonopodis* pv. *manihotis* en una familia de yuca (bc1). Rev Fitopatol Colomb.

Concept Notes and Proposal

Identification of insect vectors and alternative hosts of phytoplasmas causing cassava frogskin disease. Presented to USAID. Funds requested: US$ 12.000 for 1 year. Approved.

Desarrollo de estrategias de manejo de cuero de sapo y superalargamiento en yuca, mediante investigación participativa. Presented to Ministerio de Agricultura y Desarrollo de Colombia. Funds requested: US$ 14.546 for 1 year. Approved.

Combating Hidden Hunger in Latin America: Biofortified crops with improved Vitamina A, Essential Minerals and Quality Protein (English). Presented to CIDA. Funds requested: US$ 122.880 for 6 years. Approved.

Manejo integrado de la enfermedad del Moko en plátano. Presented to Ministerio de Agricultura y Desarrollo de Colombia. Funds requested: US$ 37.469. Approved.

Pest and disease resistance, drought tolerance and increased shelf life genes from wild relatives of cassava and the development of low-cost technologies to pyramid them into elite progenitors. Presented to The generation challenge programme. Funds requested: US$ 289.200 per year, for 3 years. Submitted.

Desarrollo de métodos rápidos de detección de *Ralstonia solanacearum*, agente causante de Moko de plátano, en plantas, malezas, agua y suelo. Presented to ASOHOFRUCOL. Funds requested: US$ 47.235. Submitted.

Manejo Integrado de Enfermedades del Cultivo de Yuca. Presented to Ministerio de Agricultura y Desarrollo de Colombia and IICA. Funds requested: US$ 77.700. Submitted.

Desarrollo de prácticas de manejo de Pudrición de Raíz de yuca mediante la detección molecular de *Phytophthora* en zonas semi áridas en Brazil y Colombia. Presented to Cassava Biotechnology Network for Latin America and the Caribean (CBN-LAC). Funds requested: US$ 77.700. Submitted.

Confirmación de fitoplasma, como agente causante de la Marchitez Letal en palma de aceite. Presented to Palmar del Oriente, Palmas de Casanare y Pameras Santana. Funds requested: US$72.288. Submitted.

Presentations at Meetings

25 Febrero. Hurtado PX. Internal seminar at CIAT. Detección de marcadores microsatélites con la resistencia al añublo bacterial de la yuca.

8–14 March. Llano GA; Alvarez E; Fregene M; Muñoz JE. Identification of resistance-gene analogs in cassava (*Manihot esculenta*), and their relationship to three *Phytophthora* species. Poster presented at the Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia. Page 121.

8-14 March. Loke JB; Alvarez E; Corredor JA; Folgueras M; Jaramillo G; Ceballos H. Preliminary evidence between foliar and root resistance to root rot caused by *Phytophthora tropicalis* in cassava. Poster presented at the Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia. Page.79.

8–14 March. Loke JB; Alvarez E; Fregene M; Marín J; Rivera S; Llano GA; Mejía JF. QTL mapping for resistance to root rot caused by *Phytophthora tropicalis* in cassava. Poster presented at the Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia. Page 158.

11–13 August. Alvarez E; Mejía JF; Llano GA; Loke JB. Detección de un fitoplasma asociado a cuero de sapo de yuca (*Manihot esculenta* Crantz) en Colombia. Paper presented at the XXV ASCOLFI Congress, Cali.

11–13 August. Arenas A; López D; Llano GA; Alvarez E; Loke JB. Efecto de prácticas ecológicas sobre la población de *Ralstonia solanacearum* Smith, causante de moko de plátano. Paper presented at the XXV ASCOLFI Congress, Cali.

Thesis

Postgraduate Theses in Cassava

Paula X. Hurtado. Evaluación de marcadores microsatélites y genes análogos, asociados a la resistencia de yuca a *Xanthomonas axonopodis* pv. *manihotis*. Universidad de los Andes—Bogotá. For a Master's in Biology, emphasizing Plant Molecular Biology.

John B. Loke. Análisis genético de la resistencia de yuca (*Manihot esculenta* Crantz) a *Phytophthora tropicalis*, causante de pudrición radical. Universidad Nacional de Colombia—Palmira. For a Master's in Plant Breeding.

Undergraduate Theses in Cassava

Alejandro Corredor. Evaluación de la asociación de Marcadores bioquímicos y morfológicos con la resistencia a pudrición de raíz (Phytophthora tropicalis) y el deterioro fisiológico en yuca (Manihot esculenta Crantz). Universidad de Caldas, Manizales. For a degree in Agronomy.

Eduardo Gómez. Identificación y caracterización de aislamientos de Ralstonia solanacearum, obtenidos de zonas afectadas por moko de plátano y banano en Colombia. Universidad Pontificia Javeriana, Bogotá. For a degree in Microbiology.

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TROPICAL FORAGE PATHOLOGY

Activity 1. Antifungal proteins in tropical forages.

An antifungal protein from *Clitoria* and its direct application in disease control

Rationale

When wounded, or attacked by harmful microorganisms, plants can trigger an array of potent defense mechanisms, one of which is to synthesize proteins, peptides and low-molecular-weight compounds that have antimicrobial effects. Antimicrobial proteins and peptides are widely distributed in nature and are synthesized not only by plants but also by bacteria, insects, fungi and mammals.

Seeds use strategies to germinate and survive in soils that are inhabited by a wide range of microfauna and microflora. Various antifungal and/or antibacterial proteins such as chitinases, β-glucanases, thionins, ribosome-inactivating proteins and permatins have been detected in seeds. Antimicrobial proteins and peptides have been isolated and characterized from seeds of maize (*Zea mays* L.), radish (*Raphanus sativus* L.) and various other plants. They are believed to play a role in plant defense because of their strong antimicrobial activity *in vitro*. This belief is further supported by their ability to confer resistance (to pathogens) in transgenic plants containing genes that encode them. The list of antifungal proteins from various organisms is long, with new ones continuously being discovered.

Other plant-derived proteins have insecticidal properties that can, for example, protect seeds from attack by larvae of various bruchids and inhibit the growth and development of *Helicoverpa punctigera* (Wallengren) larvae. Of particular interest are plant-derived proteins called cyclotides (circular proteins in which the N and C termini are linked via a peptide bond), which have antimicrobial and insecticidal properties. Ocatin, a protein isolated from the Andean tuber crop oca (*Oxalis tuberosa* Mol.), is reported to have antibacterial and antifungal effects.

We reported the isolation, purification and characterization of a protein with an antifungal, antibacterial and insecticidal properties from seeds of *Clitoria ternatea* (L.) [*IP-5 AR 2003*; Kelemu, S., Cardona, C., and Segura, G. 2004. *Plant Physiology and Biochemistry* (in press); Kelemu, S., Cardona, C., and Segura, G. 2004. *Phytopathology* 94:S50). In this study, we examined the direct applications of the crude preparations of the protein in disease control on various plants.

Materials and Methods

Protein extractions: Large quantities of seeds *Clitoria ternatea* CIAT 20692 were produced on field plots at CIAT headquarters in Palmira, Colombia, for protein extractions. Seeds (100 g) of *C. ternatea* CIAT 20692 were surface-sterilized in 3.25% NaOCl solution for 10 min, then in 70% ethanol (3 min), and rinsed 6 times with sterile distilled water. The seeds were left in sterile distilled water overnight to facilitate maceration. The imbibed seeds were then macerated in 1,000 mL of sterile distilled water with a sterilized mortar and pestle. The macerated solution

was filtered through several layers of cheesecloth to get rid of the seed debris. The filtrate was then centrifuged at 4 ^{o}C in tubes (50 mL) at 13 000 × g for an hour. To remove any potential microbe associated with the filtrate, the supernatant was filtered through 0.22-µm-pore-size cellulose acetate membranes. Aliquots (7 mL) of the filtrate were distributed in 15-mL tubes and lyophilized for 7 hours. The lyophilized samples were stored at - 20 ^{o}C for further use.

This lyophilized crude protein extracts were re-suspended in sterile distilled water (10 % of the original volume) to conduct the antifungal activity bioassay on plants.

Inoculum: A highly virulent isolate (PG8 HND) of the pathogen *Phaeoisariopsis griseola*, causal agent of angular leaf spot, was grown on V8 agar at 24^{o}C for 12 days. Conidia were collected and suspended in sterile distilled water at a concentration of 2 x 10^{4} conidia per mL. This inoculum was used on *Phaseolus vulgaris* variety Sprite bean plants. This variety is one of the most susceptible varieties to *P. griseola*.

Rhizoctonia solani, causal agent of foliar blight disease of *Brachiaria* was used as inoculum. Inoculum production and inoculation methods were as described in section 2.4.2.

***Plant inoculations and treatment applications*:** Seeds of a highly susceptible bean variety (Sprite) were planted in pots in the greenhouse at CIAT headquarters. Seventeen-day old bean plants (15 plants per treatment) were first sprayed with, either the fungicide benlate (500 µg/ml), crude antifungal protein preparation, or sterile water. Two hours later all the plants were inoculated with *P. griseola* conidia at a concentration of 2 x 10^{4} conidia per mL. The inoculated plants were placed in a humidity chamber for 4 days. They were then transferred to the greenhouse for development and symptom expression. Treatments with crude antifungal protein, benlate or sterile water continued every 2 days. Disease evaluations were conducted 7, 10, 12, 14 and 17 days after inoculation.

Brachiaria CIAT 36061, which is highly susceptible to *R. solani*, was used in this test. Fully developed detached leaves were used for inoculations as described in Materials and Methods under section 2.4.2.

***Treatment of P. griseola conidia with the protein Finotin*:** A conidial suspension of 2 x 10^{4} conidia per mL was diluted 10^{-2} - 10^{-5} and examined under a microscope in order to determine the right concentration with evenly distributed and separated conidia. The dilution 10^{-4} was chosen for treatment with the antifungal protein Finotin and further examination. Twenty-µl of this conidial suspension was placed on a slide and subsequently covered with a thin layer of potato dextrose agar medium. A 200-µl crude protein preparation (the same concentration that was used to spray onto bean plants) was applied on the agar. Control slides had water instead of the antifungal protein. These were placed in Petri dishes containing wet filter paper and incubated at room temperature. Pictures of conidia were taken under the microscope at 0, 2, 7, 24, 32 and 96 hours to observe the development of individual conidia.

Results and Discussion

Effect of antifungal protein Finotin on bean angular leaf spot: The crude protein extract from seeds of *C. ternatea* CIAT 20692 showed antifungal activity in vitro on the pathogen *P. griseola* (data not shown). Plants treated with the crude antifungal protein preparation consistently developed fewer angular leaf spot disease lesions than the control plants that were treated with sterile distilled water **[Figure 1; Figure 2]** . Had a purified protein been used to control the disease on bean plants, the level of disease control would perhaps have been even higher. It is interesting to note that even a crude protein extract sprayed directly onto plants provided protection against the disease. Experiments are currently in progress to control tomato diseases under field conditions and natural infections using crude protein preparations. Tomatoes are generally susceptible to a number of diseases. The purpose of these experiments is to develop a simple disease control strategy for small producers using this antifungal protein.

***Effect of antifungal protein on conidia of P. griseola in vitro*:** Conidia treated with crude protein or sterile water along with a layer of potato dextrose agar as described earlier reacted differently. Conidia failed to germinate in the presence of the antifungal protein Finotin 96 hours after treatment, whereas those treated with sterile water germinated and converted into mycelia **(Figure 3)**. Thus, one of the mechanisms of pathogen control by the protein may be by preventing fungal spore germination. However, a more detailed work on plant tissue as well as on culture is needed to fully establish the mechanism of disease control by this protein.

***Effect of antifungal protein Finotin on Brachiaria foliar blight*:** Detached *Brachiaria* CIAT 36061 leaves sprayed with crude protein extract and subsequently inoculated with *R. solani* mycelial discs developed very limited or no foliar blight lesions, whereas control leaves developed severe lesions **(Figure 4)** when evaluated 72 hours after inoculation. Although we don't intend to use the antifungal protein for direct applications in the control of foliar blight disease in *Brachiaria* (it will be impractical to do so), we are exploring the possibilities of transforming some of the endophytic microbes associated with *Brachiaria* with the gene encoding the protein.

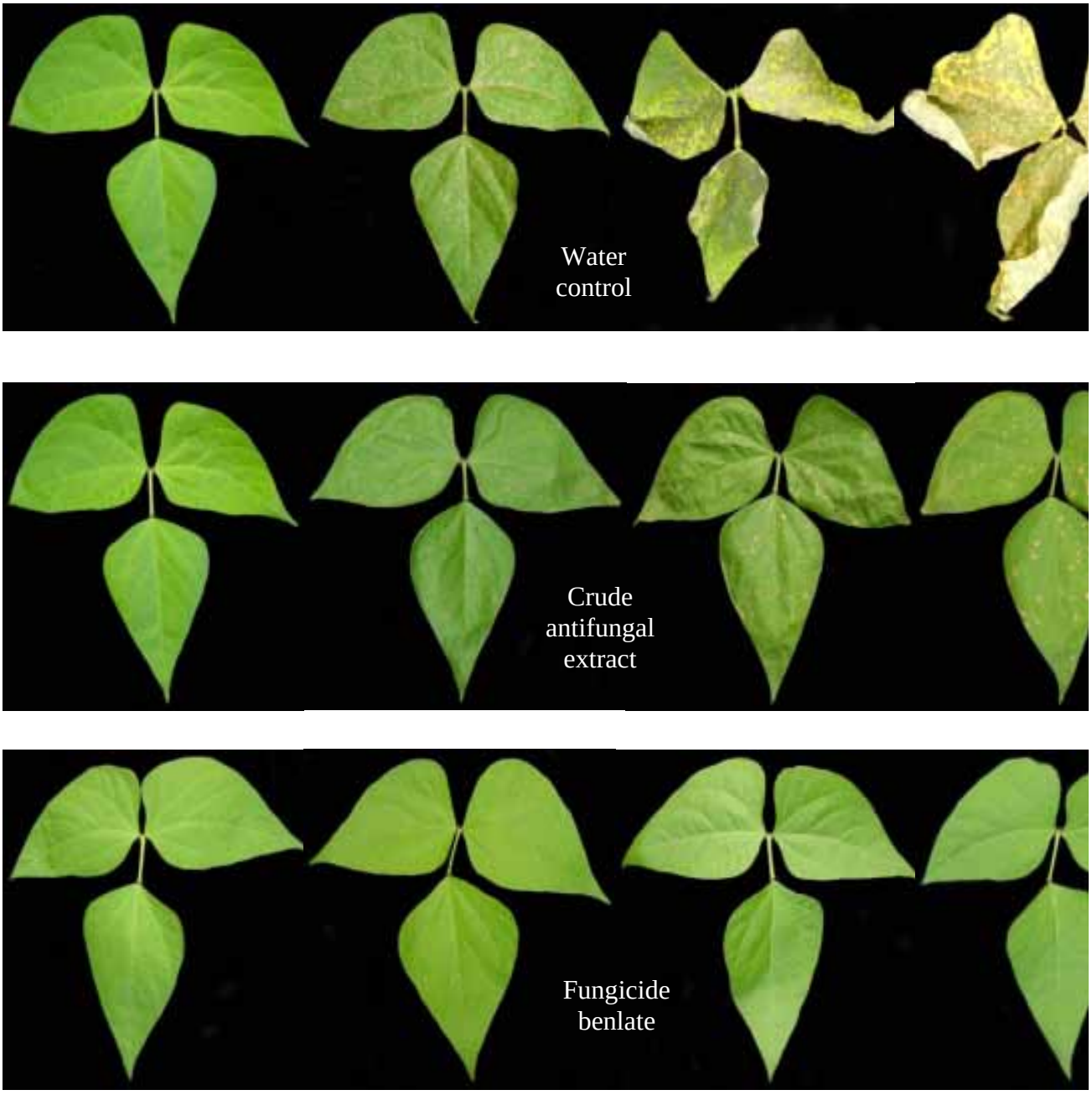

Figure 1. **Treatment of bean plants with crude protein extract from seeds of *C. ternatea* CIAT 20692 against the fungal pathogen *P. griseola*, causal agent of angular leaf spot disease. Plants treated with the crude antifungal protein preparation consistently developed fewer angular leaf spot disease lesions than the control plants that were treated with sterile distilled water.**

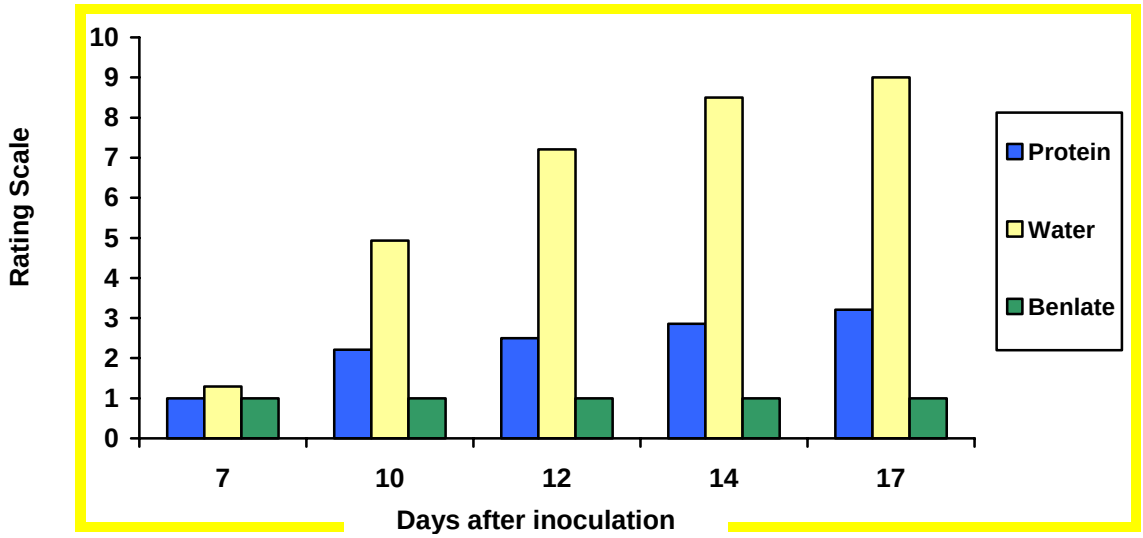

Figure 2. **Angular leaf spot disease developmen t in artificially inoculated bean plants following tr eatment w ith crude an tifungal protein preparations isolated from *C. ternatea* CIAT 20692, the fungicide benlate, or water control.**

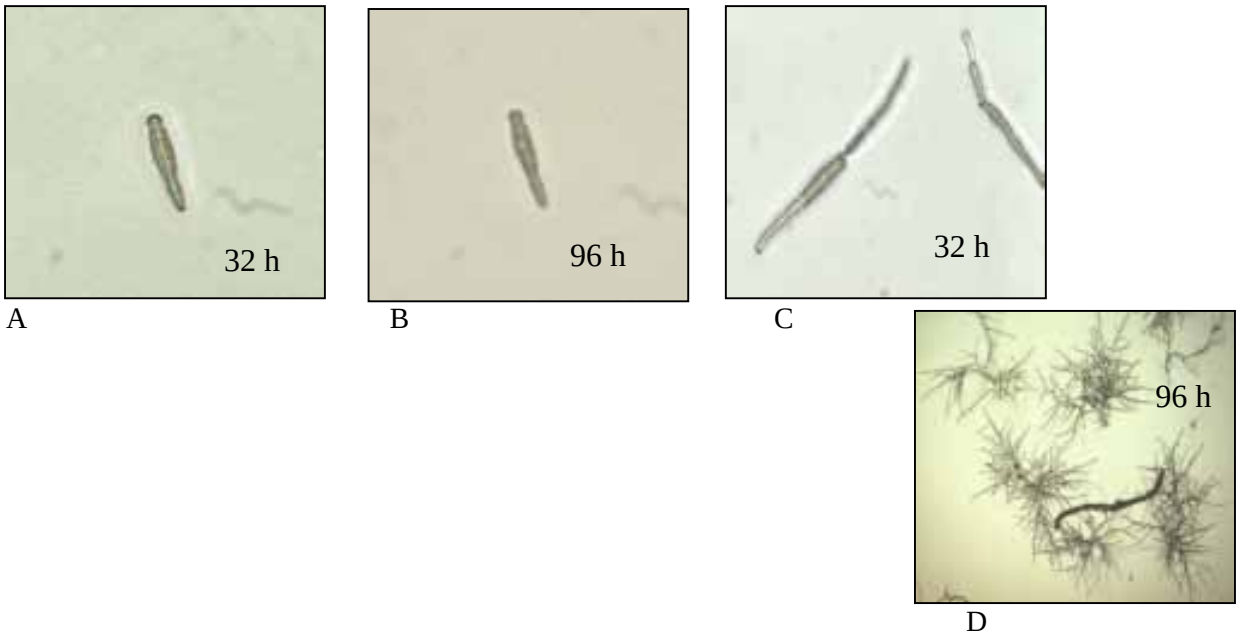

Figure 3. **Treatment of *Phaeoisariopsis griseola* conidia w ith the antifun gal protein Finotin. Conidia failed to germinate in the presence o f the antifung al protein Finotin 32 and 96 hours (A and B) aft er treatment, whereas those treated with sterile water germinated and grew into mycelia (C and D) .**

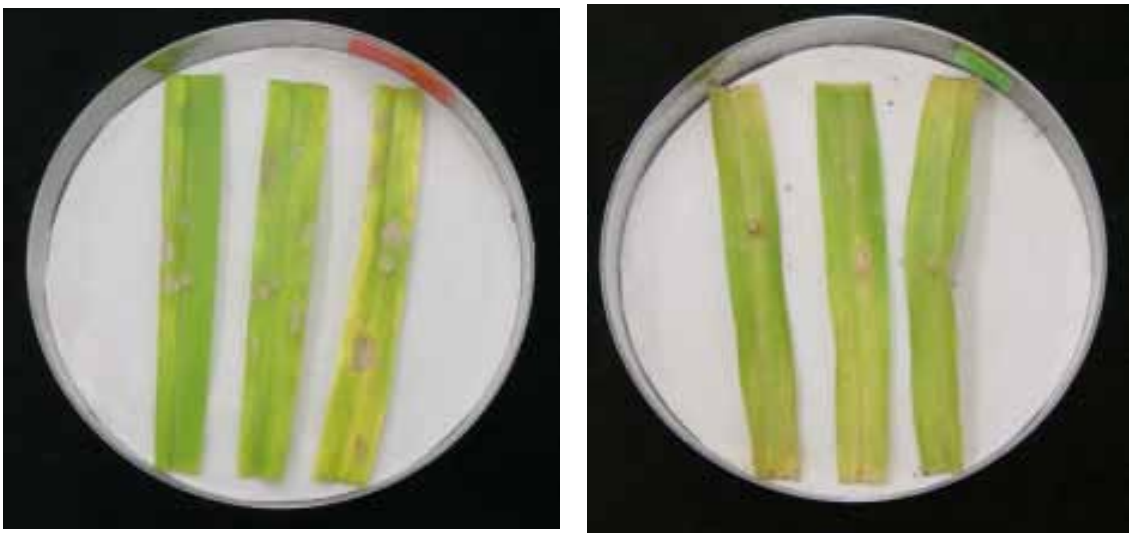

Figure 4. Detached *Brachiaria* CIAT 36061 leaves sprayed with crude antifungal protein isolated from the forage legume *Clitoria ternatea* (L.), and subsequently inoculated with *Rhizoctonia. solani* mycelial discs (right) and control leaves (left).

Contributors: Gustavo Segura, George Mahuku, Segenet Kelemu.

Activity 2. Association of bacteria with *Brachiaria* genotypes

Characterization of endophytic bacteria isolated from *Brachiaria*

Rationale

Endophytic bacteria that reside in plant tissues without causing any visible harm to the plant have been isolated from surface-sterilized *Brachiaria* tissues. The primary point of entry for many of these bacteria is the root zone, although aerial plant parts like flowers and stems may also be entries. Once inside a plant, they may either be localized at the point of entry or spread throughout. Bacterial endophytes have been reported to live within cells, in the intercellular spaces or in the vascular system of various plants.

Many plant-growth-promoting bacteria (PGPB) that include a diverse group of soil bacteria are thought to stimulate plant growth by various mechanisms such as plant protection against pathogens, providing plants with fixed nitrogen, plant hormones, or solubilized iron from the soil. Three bacterial isolates 01-36062-R2, 02-36062-H4, and 03-36062-V2 were isolated from *Brachiaria* CIAT 36062 in roots, leaves and stems, respectively, that tested positive for sequences of the nifH gene (the gene that encodes nitrogenase reductase) [IP-5 Annual Report 2003]. As stated in the 20003 Annual Report, the fatty acid analysis matched the bacterium coded 03-36062-V2 with *Flavimonas oryzihabitans* at 0.887 similarity index. *F. oryzihabitans* has been described as a plant growth promoting rhizobacterium in graminicolous plants. The analysis matched isolate 02-36062-H4 with *Agrobacterium rubi* at 0.845 similarity index. The name *A. rubi* is synonymous to *Rhizobium rubi*. The match using fatty acid data of the isolate 01-36062-R2, however, was not conclusive, matching it with *Leclercia adecarboxylata*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, at 0.879, 0.841, and 0.820 similarity index, respectively. Of these, *E. cloacae* has been described as one of the dominant endophytic bacteria isolated from citrus plants (Araújo et al., 2002. Applied and Environmental Microbiology 68:4906-4914). A nitrogen-fixing endophytic strain of *Klebsiella pneumoniae* (Kp342) has been ioslted from a nitrogen-efficent line of maize (Chelius and Triplett, 2000. Applied and Environmental Microbiology 66:783-787). This strain has been described to have a very broad host range and is capable of colonizing the interior of many plants with fewer than 10 cells in the inoculum (Dong et al., 2003. Plant Soil 257:49-59). More recently, endophytic colonization and nitrogen fixation in wheat were demonstrated upon inoculation with *Klebsiella pneumoniae* strain Kp342 (Iniguez et al., 2004. Molecular Plant Microbe Interaction 17:1078-1085).

The objective of this study is to isolate and characterize bacterial strains with potential plant growth promoting properties.

Materials and Methods

Marking bacterial cells for antibiotic resistance: An overnight culture of bacterial cells (strain 01-36062-R2) were plated on nutrient agar medium containing rifampicin (50 µg/ml) and incubated at 28 C for 48 h. Individual colonies which appeared on the medium were transferred on to freshly prepared medium containing the same concentration of rifampicin. The growing colonies were transferred on to freshly prepared medium containing 50µg/ml rifampicin. The same process was repeated until a mutant bacterium was obtained which grew the same on

rifampicin-containing medium at a concentration of 50 μg/ml as well as on medium containing no rifampicin. Dilution series of the mutant bacterium were plated on nutrient agar medium with and without rifampicin to determine that the mutant grew equally on both media. Growth curves of the mutant bacterium were also conducted in nutrient broth media with and without rifampicin. The growth of the rifampicin-resistant mutant strain was determined in comparison with that of the original isolate from which the mutant was derived. In addition, nested PCR amplifications were conducted on both the marked mutant and the original bacterial isolate to make sure that the *nifH* gene sequences can be detected in both.

Nested PCR Amplification: Three primers were used, which were originally designed by Zehr and McReynolds (1989. Appl. Environ. Microbiol. 55: 2522-2526) and Ueda, et al. (1995. J. Bacteriol. 177: 1414-1417) to amplify fragments of *nifH* genes. Amplification steps described by Widmer et al (1999. Applied and Environmental Microbiology 65:374-380) were adopted.

Inoculation of Brachiaria: Rifampicin–resistant bacterial cells were used to inoculate *Brachiaria* CIAT 36061 (Mulato) plants. Plants were inoculated with the rifampicin-resistant mutant either by injection or immersing the roots in bacterial suspension for 48 hours. In the root immersion inoculation method, roots of 19 plants were washed with sterile distilled water. The plants were then transferred to a suspension of bacterial cells (rifampicin resistant mutant derived from strain 01-36062-R2 at a concentration of optical density (OD_{600} = 0.1). The plants were removed from the suspension two days later and rinsed with sterile distilled water. They were then planted in sterile soil. Mutant bacterial cell suspensions (200 μL of OD_{600} = 0.1) were injected into stems and leaves of each plant (a total of 19 plants). Control plants were treated with sterile distilled water.

Evaluation of inoculated plants: root tissues or above ground tissues were macerated in 200 μL sterile distilled water, 3, 7, 12, 21, 26 and 75 days after inoculations. A dilution series was made and plated on nutrient agar containing rifampicin at a concentration of 50 μg/mL. The colonies were counted after 48 hours incubation at 28 C. The values were used to calculate the approximate number of colony forming units per tissue sample. The isolated bacteria were also tested with nested PCR to determine whether they were positive for *nif*H gene sequences.

Results and Discussion

Bacterial cells were re-isolated from inoculated plants (hybrid *Brachiaria* CIAT 36061; cv. Mulato) on nutrient agar medium containing rifampicin (50 μg/mL) as late as 75 days after inoculation. No bacterial cells that can grow on the rifampicin-containing media were isolated from control plants. These rifampicin-resistant bacterial colonies also tested positive for sequences of *nif*H **(Figure 1)** . In summary, we took a bacterial strain isolated from what appeared to be a nitrogen-efficient *Brachiaria* CIAT 36062 and that tested positive for *nif*H sequences, marked it for resistance to the antibiotic rifampicin at 50 μg/mL, introduced it to cv. Mulato and re-isolated it 75 days after inoculations, indicating that the bacterium was established in artificially inoculated plants of cv. Mulato.

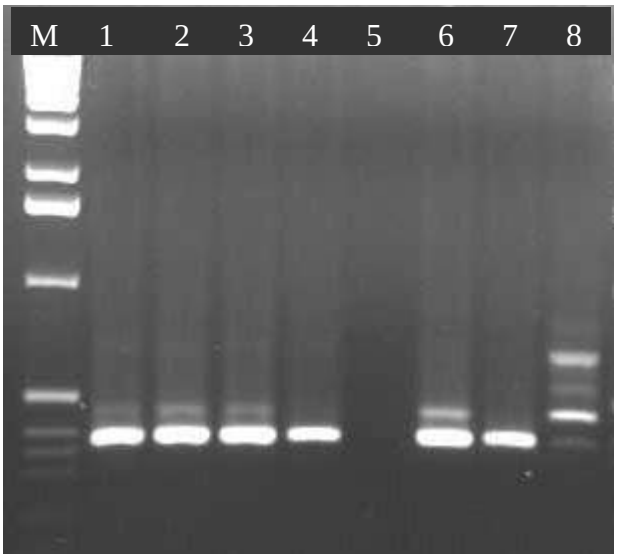

Figure 1. Nested PCR analysis of rifampicin- resistant bacterial colonies reisolated from artificially inoculated CIAT 36061 (cv. Mulato) plants, for *nif*H gene sequences. Lanes 1-4 are rifamp icin-resistant independent bacterial colonies re- isolated from Mulato plants 23 days after inoculations (lanes 1 and 2 DNA of bacteria isolated from leaves; lanes 3 and 4 isolated from roots). Lane 5 is negative control. Lanes 6 and 7 are po sitive control and DNA from original positive ba cterium from w hich rifamp icin resis tant mutants w ere derived, respectively. Lane 8 is a randomly picked bacterium. Lane M is size marker.

The rifampicin-resistant bacterial cells were isolated both from leaves and roots of inoculated plants, although the bacterial population is not evenly distributed in all the leaves. The bacterium was consistently re-isolated from root tissues. Although both inoculation methods (plant injections or root immersions) gave successful results, more bacterial cells were recovered following root immersion inoculations and thus, root immersion method is a better inoculation method. Not surprisingly, the bacterial cell population in *Brachiaria* was much lower than that observed for a plant pathogenic bacterium such as *Xanthomonas campestris* pv. *graminis*.

By introducing this strain into cv. Mulato, we can now study the effect of the bacterial strain on the growth of Mulato plants in comparison with genetically identical plants without the bacterial strain.

Contributors: Raul Sedano, Carolina Zuleta, Segenet Kelemu

Activity 3. Publications, book chapters, conferences and workshops.

Refereed Journals

Chakraborty, S., Ghosh, R., Ghosh , M., Fernandes, C. D., Charchar, M. J. and Kelemu, S. 2004. Weather-based prediction of anthracnose severity using artificial neural network models. *Plant Pathology* 53:375 –386.

Dongyi, H. and Kelemu, S. 2004. *Acremonium implicatum*, a seed-transmitted endophytic fungus in *Brachiaria* grasses. Plant Disease 88:1252-1254.

Kelemu, S., Cardona, C. and Segura, G. 2004. Antimicrobial and insecticidal properties of a protein isolated from seeds of the tropical forage legume *Clitoria ternatea* (L.). (Abstract) *Phytopathology* 94:S50.

Submitted

Kelemu, S., Changshun, J., Guixi, H. and Segura, G. 2004. Genetic transformation of the tropical forage legume *Stylosanthes guianensis* with a rice-chitinase gene confers resistance to *Rhizoctonia* foliar blight disease. *Plant Pathology*.

Accepted

Kelemu, S., Cardona, C. and Segura, G. 2004. Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea* (L.), a tropical forage legume. *Plant Physiology and Biochemistry*.

Invited Book Chapters

Chakraborty, S., Ghosh, R., Ghosh, M., Maji, A. K., White, N., Fernandes, C. D., Charchar, M. J., Ramesh, C. R. and Kelemu, S. 2004. Weather dependency of anthracnose and risk mapping. ***In:*** S.Chakraborty (editor) High-yielding anthracnose-resistant S*tylosanthes* for Agricultural systems. Chapter 20, pp 203, ACIAR, Australia.

Chakraborty, S., Fernandes, C.D., Charchar, M.J., Weeds, P.L. and Kelemu, S. 2004. *Colletotrichum gloeosporioides* diversity at centres of origin in Brazil and Colombia. In: S.Chakraborty (editor) High-yielding anthracnose-resistant *Stylosanthes* for Agricultural systems. Chapter 15, pp.165, ACIAR, Australia.

Kelemu, S., Miles, J. W. and Rao, I. M. 2004. Biotic and abiotic constraints to *Stylosanthes* production. In: S.Chakraborty (editor) High-yielding anthracnose-resistant *Stylosanthes* for Agricultural systems. Chapter 8, pp 97. ACIAR, Australia.

Conference and Workshop Proceedings

Kelemu, S. 2004. The Role of Agricultural Biotechnology in Crop Protection. Paper for the Workshop on The Ethiopian Agricultural Biotechnology Initiative, 6-8 July 2004, Addis Ababa, Ethiopia.

Other Publications (Newsletter articles)

In Press

Kelemu, S., Lascano, C., Miles, J., Rao, I. and Horne, P. 2004. Bringing an African pasture grass home: The zebra's staple, the vaquero's joy, the Bantu's hope. *Spore*.

Project Staff List

Raul Sedano, Assistant II (till 30 July, 2004)
Ximena Bonilla, Technician I
Gustavo Segura, Technician I
Javier Abello (undergraduate student)

BEAN PATHOLOGY

Activity 1. Characterizing and monitoring pathogen and insect diversity.

Determination of pathogenic variation within the common bacterial blight pathogens (*Xhanthomonas campestris* pv *phaseoli* (Xcp) and *Xhanthomonas campestris* pv *phaseoli* var *fuscans* (Xcpf))

Rationale

Different studies have reported the presence of physiological races in the CBB pathogens while others have found to the contrary. The question of physiological races within the common bacterial blight has been the subject of much discussion, with one group reporting the presence of physiological races when the CBB pathogen is challenged onto different bean genotypes while another group has found to the contrary. The significance of such findings is that the presence of physiological races signifies the gene for gene interaction, which would have profound impacts or influence as to how breeding for CBB resistance is undertaken. To date, all information shows that CBB resistance in *Phaseolus vulgaris* is quantitative in nature. In addition, this would lead to the formulation of a differential series, that can be used to rapidly characterize the CBB pathogen, identify the most effective resistance genes to use in breeding programs, as well as formulate ways to effectively deploy CBB management strategies. To test the hypothesis of the presence of physiological races in the CBB pathogen, we collected all bean genotypes that have been reported in the past to show differential response when challenged with the CBB pathogen, and inoculated these under greenhouse conditions, using isolates of a diverse origin. In addition, these results are hopped to provide insights into the co-evolution of Xcp and Xcpf with Andean and Mesoamerican gene pools, and collaborate the results of Gilbertson et al. (2004). The objective was to detect if there existed differential reaction in the interaction of Xcp or Xcpf with Andean and Mesoamerican bean genotypes.

Materials and Methods: Bacteria isolates: Bacteria isolates were selected to represent different geographical areas where bean is grown, and for which we had isolates in stock. A total of 29 isolates were selected, 15 Xcp and 14 Xcpf.

Bean germplasm: Fifty bean genotypes were used in this study, 26 belonged to the Andean gene pool and 24 to the Mesoamerican gene pool **(Table 1)**. The majority of these genotypes have previously been reported to show a differential reaction to the CBB pathogen and as having the capability to distinguish between isolates. In addition, six lines specifically developed for resistance to the CBB pathogen (VAX 1 to VAX 6) through interspecific hybridization of *P. vulgaris* x *P. acutifolius* and embryo rescue techniques were included.

Plant inoculation: Each bacterial isolate was inoculated onto the first trifoliate leaf of six plants for each genotype using the multiple needle method (CIAT, 2003), at a concentration of 5×10^7 CFU. Disease severity and progression was recorded starting 10, 13 and 17 days after inoculation using the CIAT 1-9 scale.

Table 1. Reaction of *Phaseolus* genotypes to inoculation w ith 14 i solates of *Xanthomonas campestris* pv. *phaseoli* var fu scans (Xcp f) and 15 isolates of *Xanthomonas campestris* pv. *phaseoli* (Xcp).

Genotype Gene	Pool[a]	Xcp Xc		pf	
		Incompatible[b] Co	mpatible[c] Inco	mpatible	Compatible
Nuña maní Roja	A	2	13	7	7
A 196	A	1	14	3	11
Bola 60 días	A	1	14	1	13
Burros Argentinos	A	1	14	1	13
G 76	A	0	15	0	14
Taylor	A	0	15	0	14
ICA CERINZA	A	0	15	0	14
A 475	A	0	15	0	14
A 36	A	0	15	0	14
G 5686	A	0	15	0	14
Jatu Rong	A	0	15	0	14
Ecuador 1056	A	0	15	0	14
Jalo EEP 558	A	0	15	0	14
Alubia Cerrillos	A	0	15	0	14
Mortiño	A	0	15	0	14
Bolón Bayo	A	0	15	0	14
Ecuador 299	A	0	15	0	14
G 11867	A	0	15	0	14
MCD 4011	A	0	15	0	14
MCD 4012	A	0	15	0	14
Radical San Gil	A	0	15	0	14
Frutilla Corriente	A	0	15	0	14
Coscorrón Corriente	A	0	15	0	14
Tórtolas Corriente	A	0	15	0	14
Caballero	A	0	15	0	14
Bolón Rojo	A	0	15	0	14
VAX 3	M	15	0	14	0
VAX 4	M	15	0	14	0
VAX 6	M	15	0	14	0
VAX 5	M	14	1	14	0
VAX 1	M	12	3	13	1
VAX 2	M	12	3	11	3
XAN 266	M	7	8	8	6
Guanajuato 31	M	3	12	5	9
SEA 14	M	1	14	3	11
SEA 13	M	1	14	2	12
Durango 222	M	1	14	2	12
Cejita	M	1	14	2	12
San Cristóbal	M	0	15	1	13
APN 114	M	0	15	1	13
MAM 28	M	0	15	1	13
DICTA 17	M	0	15	1	13
Flor de Mayo Bajio	M	0	15	1	13
Carioca	M	0	15	1	13
Porrillo Sintético	M	0	15	0	14
Orgulloso	M	0	15	0	14
Rió Tibagi	M	0	15	0	14
Zacatecano	M	0	15	0	14
Ojo de Cabra	M	0	15	0	14
Frijola	M	0	15	0	14

Genotype Gene	Pool[a]	Xcp Xc		pf	
		Incompatible[b] Co	mpatible[c] Inco	mpatible	Compatible
Garbancillo Zarco	M	0	15	0	14
Flor de mayo IV	M	0	15	0	14
Amarillo 154	M	0	15	0	14
México 235	M	0	15	0	14
México 309	M	0	15	0	14
BAT 41	M	0	15	0	14

[a] *Phaseolus* gene pool; A = Andean; M = Mesoamerican.
[b] Number of isolates that had a resistant response.
[c] Number of isolates that had a susceptible response.

Results an d Discussio n: Considering the Andean genotypes only, 98.7% had a susceptible response to inoculation with Xcp isolates and 96.7% were susceptible when inoculated with Xcpf isolates, revealing that Andean genotypes where equally susceptible to both Xcp and Xcpf isolates of CBB **(Table 1)** . A similar result was evident for Mesoamerican isolates when inoculated with Xcp (96.1% susceptible) and Xcpf (91.6% susceptible). These results show no significant differences in the reaction of Andean and Mesoamerican gene pools to Xcp and Xcpf isolates, revealing a lack of co-evolution of Xcp or Xcpf with bean gene pools as has been reported (Mkandawire et al., 2004). Principal component analysis showed no differences in the reaction of Andean and Mesoamerican genotypes to Xcp and Xcpf. (data not shown). Contrary to reports of differential interaction between bean and CBB isolates, no such interaction was apparent in this study **(Table 1)**. The VAX lines showed high levels of resistance to all isolates, in particular VAX 3, VAX 4, and VAX 6 **(Table 1)**.

Conclusion: The results obtained in this study do not permit us to establish host differential varieties for the CBB pathogen, as no differential interaction was observed in the interaction of bean and the CBB pathogens from different geographical areas. In addition, these results reveal a lack of co-evolution between Xcp or Xcpf with gene pools established for the common bean host. However, such a conclusion can only be made following evaluation of wild beans with the same spectrum of CBB isolates, as the genotypes that have been used in this study are improved, therefore the original host diversity might have been lost during bean improvement. We are in the process of evaluating Andean and Mesoamerican wild bean accessions.

References

CIAT 2003. Annual Report, Bean Program 2003. CIAT, Cali, Colombia.

Mkandawire, A.B.C., Mabagala, R.B., Guzmán, P., Gepts, P., and Gilbertson, R.L. 2004. Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggests pathogen coevolution with the common bean. Phytopathology 94: 593-603.

Activity 2. A specific molecu lar a ssay for Detecting a nd Differen tiating *Xanthomonas campestrsi* pv. *phaseoli* and *Xanthomonas campestri* pv. *phaseoli* var. *fuscans.*

Rationale

Common bacterial blight and fuscous blight, caused by *Xanthomonas campestris* pv. *phaseoli* (xcp) and *Xanthomonas campestris* pv. *phaseoli* var. fuscans (xcpf) respectively, are major diseases of common bean world wide. Yield losses range from 0 to 40% on susceptible cultivars. The two pathogens are seed borne, this being the principal source of inoculum and means of dissemination to new areas. One way to minimize the impact of the CBB pathogens is to ensure the distribution of disease-free seed. Current assays to identify and quantity *X. c. phaseoli* in bean tissues include plating on selective media, phage typing, immunoassay, and host inoculation. Although valuable, they are labor intensive and not sufficiently precise for routine use. We describe the development of a rapid, sensitive and specific assay for detecting the CBB pathogens in seed, and for differentiating between the two causal agents of the disease. It is hoped that this assay can find wide application in quarantine and seed certification services.

Material and Methods: Previous studies reported the presence of a Xcpf diagnostic 820 bp fragment following amplification with the RAPD primer OPG11 (Birtch et al., 1997). Amplification of representative Xcp and Xcpf isolates using this primer resulted in two fragments, a 900 bp fragment that was present in all Xcp isolates and an 820 pb present in all Xcpf isolates **(Figure 1)**. The fragments were excised form gels, cloned, DIG labeled and used as probes in Southern hybridization analysis of total genomic DNA either digested with EcoRI or not digested and PCR amplified products, to confirm specificity of these fragments to the two bacteria. Once confirmed, the fragments were sequenced and specific primers designed. The specificity of these primers for Xcp or Xcpf was tested using DNA from different bacteria (*Xanthomonas campestris* pv. *phaseoli*, *Xanthomonas campestris* pv. *phaseoli* var. fuscans, *Xanthomonas campestri* pv. *manihotis*, *Xanthomonas campestri* 36062 and *Xanthomonas campestri* 1622/16 isolated from Brachariaa , *Xanthomonas campestri* pv.o*ryzae, Agrobacterium tumefaciens, Pseudomonas fuscovaginae)*, *Phaseolus vulgaris*, and from several fungi that infect beans (*Pythium ultimum, Colletotrichum lindemuthianum, Phaeoisariopsis griseola* and *Macrophomina phaseolina).* DNA amplification was performed in a MJ Research Thermal Cycler with one cycle at 94^{o}C for 5 min, 65^{o}C for 40 s and 72^{o}C for 2 min, followed by 35 cycles at 94 ^{o}C for 1 min, 65^{o}C for 40 s and 72^{o}C for 2 min and a final cycle at 72^{o}C for 10 min. Reactions were carried out in 12.5 µl reaction volumes containing 5ng of genomic DNA, 0.5 unit *Taq* polymerase (Promega), 0.16 µM of each primer, 200 µM of each dNTP, 1x PCR reaction buffer, and 1.5 mM $MgCl_{2.}$

Utility of developed probes: The utility of the designed probes for detecting the CBB pathogens was validated by amplification of DNA from seeds collected from known infected pods and leaf tissues. In addition, bacteria DNA extracted using different methods (Mahuku, 2004) were used to test the sensitivity of this method to impurities in the PCR assay.

DNA extraction from seed : Ten seeds were washed with sterile distilled water, placed in a plastic bag and 4 ml of a salt solution (8.5 g NaCl in 1 liter of sterile distilled water) added.

Alternatively, ten seeds in a plastic bag were macerated in NaCl solution. The bags were put on a shaker (~100 rpm) at room temperature for ~18 hrs, the contents transferred to a 15 ml falcon tube, centrifuged (4000 rpm) for 1 hr at 4°C. The pellet was resuspended in 100 µl of sterile double-distilled water and a serial dilution of up to 1:500 done. One µl from each dilution was used in a 12.5 µl PCR reaction volume.

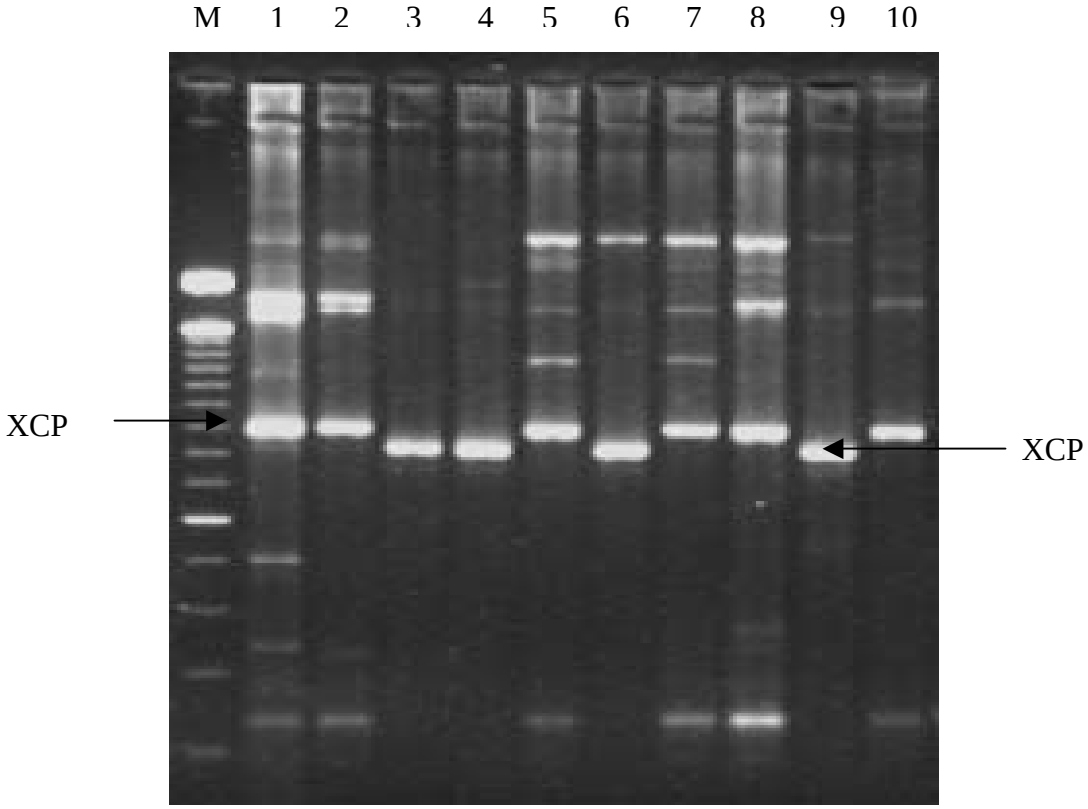

Figure 1. Amplification of *Xanthomonas campestris* pv. *phaseoli (*Xcp*)*, *Xanthomonas campestris* pv. *phaseoli* var. fuscans (Xcpf) using RAPD primer OPG11. Lanes 1, 2, 5, 7, 8, and 10 are Xcp isolates; lanes 3, 4, 6, and 9 are Xcpf isolates. Lane M is the 100 bp molecular size marker.

Extraction of bacteria l DNA from single seeds : A single bean seed from infected pods was thoroughly washed with sterile distilled water, macerated in a plastic bag and the contents washed into a 2 ml eppendorf tube using 100 µl of NaCl solution. A plastic pestle that tightly fits the eppendorf tube was used to further macerate and homogenize the solution; the mixture was left standing for ten minutes at room temperature. The supernatant was transferred to a new tube, and centrifuged at 800 rpm for 5 minutes at 4°C. The supernatant was removed, the pellet resuspended in 100 µl of sterile distilled water and a 1 µl of a 1:100 dilution used in a 12.5 µl PCR reaction volume.

Detection level (specificity): To determine the detection level, 1 µl of the pellet was added to 100 µl of sterile distilled water and plated on YCGA medium and incubated at 28 °C. After 18-24 hrs, the number of CFUs was counted, incubated for 48 hrs, to distinguish between Xcpf and Xcp.

Results and Discussion: Amplification with OPG11 resulted in two diagnostic fragments, a 900 bp for Xcp and an 820 bp fragment for Xcpf **(Figure 1)**. Southern blot analysis revealed that these fragments were unique to Xcp an Xcpf respectively **(Figures 4A and 4B)**. A set of three primer combinations was developed; one set (xcpG11-L1/xcpG11-R1) was specific to the CBB pathogens (Xcp and Xcpf), amplifying an 800 bp **(Figure 2A)**. This primer pair did not amplify DNA from other pathogens (*Xanthomonas campestris* pv. *phaseoli, Xanthomonas campestris* pv. *phaseoli* var. fuscans, *Xanthomonas campestri* pv. *manihotis*, *Xanthomonas campestri* 36062, *Xanthomonas campestri* 1622/16, *Xanthomonas campestri* pv.o*ryzae, Agrobacterium tumefaciens, Pseudomonas fuscovaginae, Pythium ultimum, Colletotrichum lindemuthianum, Phaeoisariopsis griseola* and *Macrophomina phaseolina)* or bean DNA **(Figure 3)**. The primer pair xcpfG11-L1/xcpfG11-R1 was specific for Xcpf **(Figure 2B)**, while the primer pair xcpG11-L2/xcpG11-R1 was specific to Xcp **(Figure 2C)**. When tested on seed from infected pods of plants that had been inoculated with Xcpf under field conditions, only Xcpf was detected **(Figure 3C)**. Amplification with the CBB general primers **(Figure 3A)** revealed the presence of the bacteria, while amplification with Xcp specific primers revealed the absence of Xcp in seed **(Figure 3B)**. Cultures of the same seeds after seed washings revealed that they were infected with Xcpf, and all seeds that were negative in the PCR assay where also negative using the culturing method, showing that this assay can potentially be used as a faster method of detecting the CBB pathogens in seed. This PCR assay could detect a minimum of 5 CFU of the bacterium.

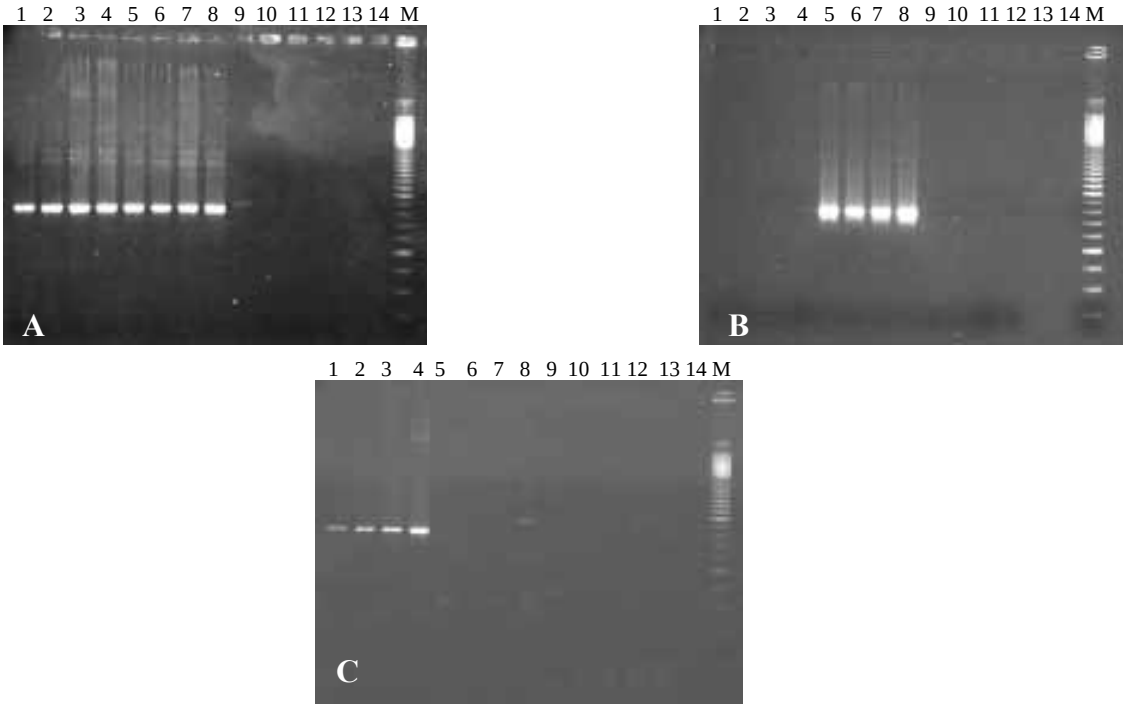

Figure 2. Specific detection of the commo n bacterial blight pathogens. Lane 1-4; *Xanthomonas campestris* pv. p *haseoli* , lane 5-8 *Xanthomonas campestris* pv. p*haseoli* var, fuscans; lane 9; *Xanthomonas campestri* pv. m *anihotis*, l ane 10; *Xanthomonas campestri* pv.o *ryzae*, lane 11; *Phaeoisariopsis griseola*, lane 12; *Colletotrichum lindemuthianum*, lane 13; *Phaseolus vulgaris,* lane 14; negative control, lan e 15; 100 pb molecular ladd er. (A) Specific detection of the CBB pathogens (Xcp and Xcpf); (B) specific detection of X cpf; and (C) specific detection of Xcp.

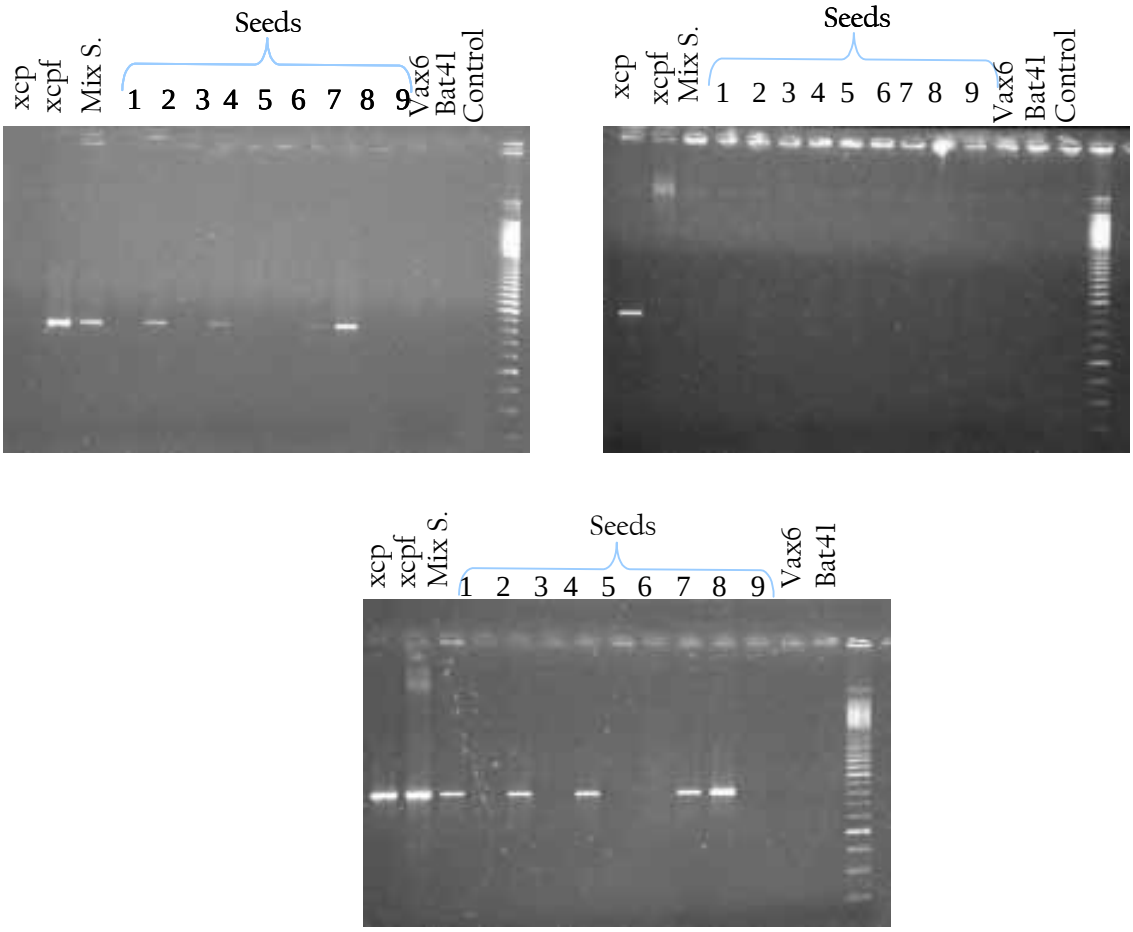

Figure 3. Detection of CBB pathogens in co mmon bean seed, collected fro m infected pods of BAT 41 inoculated w ith . *Xanthomonas campestris* pv. p *haseoli* var, fuscans (Xcpf). Lane 1 is Xcp, lane 2 Xcpf, lane 3 is DNA extracted from mixed seeds; lanes 4 individual s eeds, lane 12 is bacteria free seed from VAX 6, lan e 13, bacteria free seed from BAT 41. (A) th e CBB pathogen speifi c primers were used for PCR amplification in A; w hile (B) Xcp s pecific primers w ere used, and in (c), Xcpf specific primers were used.

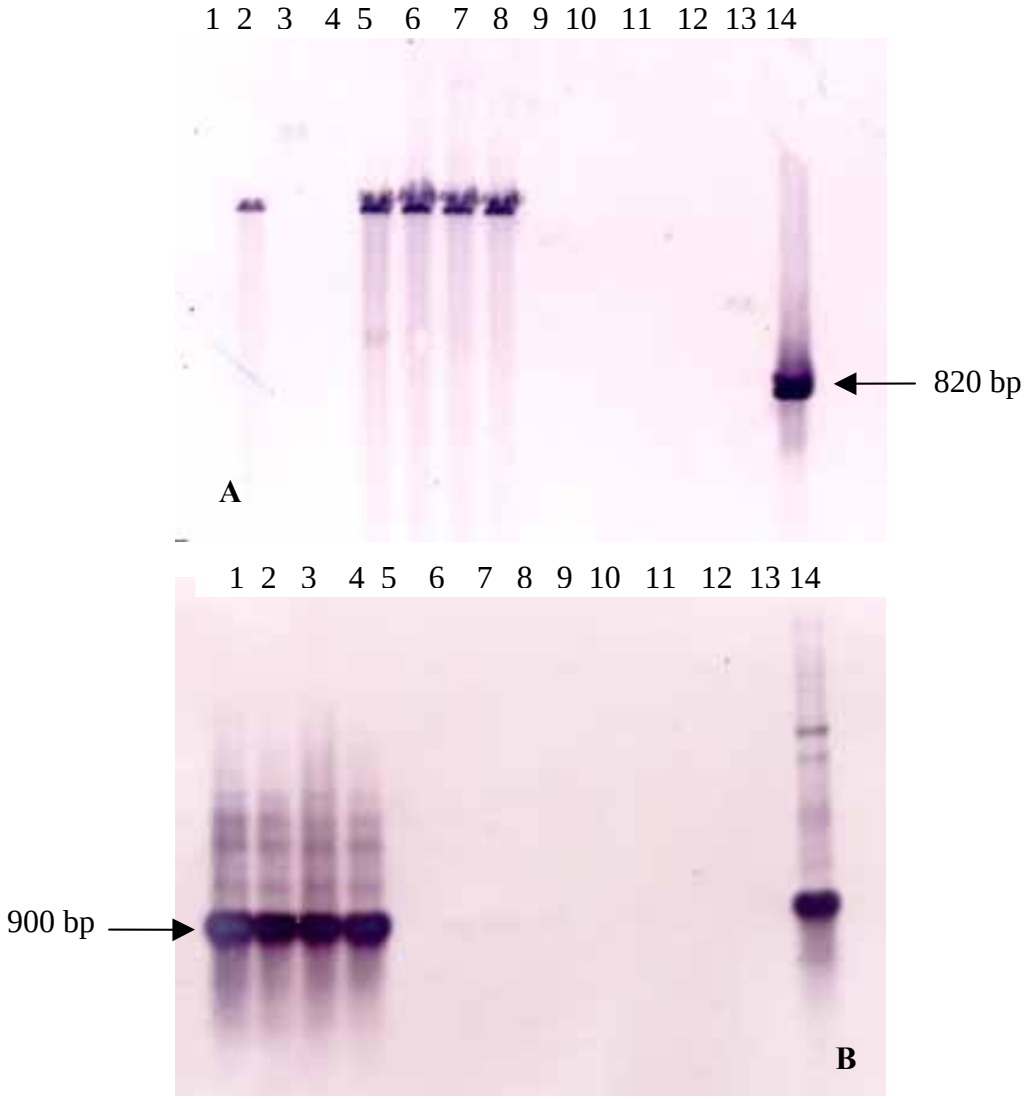

Figure 4. **Specificity of the CBB probes for *Xanthomonas campestris* pv. *phaseoli* (Xcp) and *X. campestris* pv. *phaseoli* var fuscans (Xcpf). (A): Genomic DNA was digested w ith EcoRI and hybridiz ed using the DIG-labeled Xcpf specific fragment; Lane 1, 3 4 correspon d to Xcp, lanes 2, 5, 6, 7, 8 are Xcpf isola tes; Lane 9 is *X. campestris* pv. *manihotis*,; lane 10 is *X. campestris* pv. *oryzae*; lane 11 is *Phaeoisariopsis griseola*, Lane 12 is *Colletotrichum lindemuthianum*; lane 13 is bean and lane 14 is the plasmid containing the 820 bp fragment specific to Xcpf. (B) PCR amplified DNA using RAPD primer OPG11 and hybridiz ed using the DIG-labeled Xcp specific fragmen t. of Xcp, Xc pf. Lanes 1-4 is Xcp isolates, lanes 5-8 is Xcpf; Lane 9 is *X. campestris* pv. *manihotis*; lan e 10 is *X. campestris* pv. *oryzae*; lane 11 is *Phaeoisariopsis griseola*, Lane 12 is *Colletotrichum lindemuthianum*; lane 13 is bean and l ane 14 is the plasmid containing the 820 bp fragment specific to Xcp.**

References

Birch, P.R.J.,Hyman, L.J., Taylor, R., Opio, A.F., Bragard, C., Toth, I.K. 1997. Rapd PCR-based differentiation of *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris* pv. *phaseoli* var. *fuscans.* Eur. J. Plant. Pathol. 103: 809-814.

Mahuku, G. 2004. A simple extraction method suitable por PCR-Based analysis of plant, fungal and bacterial DNA. Plant Molecular Biology Reporter 22: 71-81.

Contributors: Maria Antonia Henríquez, Monica Navia, George Mahuku.

Activity 3. Pathogenic characterization of *Colletotrichum lindemuthianum* isolates from different regions of Colombia.

Rationale

Anthracnose of common bean, caused by *Colletotrichum lindemuthianum* continues to be one of the most important diseases of common bean in Colombia, especially in high areas with cool temperatures, high rainfall and relative humidity. Farmers in these areas produce climbing beans, mainly Cargamantos, Bola roja, Radicales, Mortiño. These varieties fetch high prices in the market. Since 2002, we have been monitoring the population structure so as to determine the most prevalent races and compare this with information collected in the eighties, in the hopes of determining whether there has a shift in the pathogen population structure, introduction of new pathogen races or both.

Materials and Methods: Samples (43) were received from different departments of Colombia where anthracnose of common bean is a serious problem. A total of 43 single spore isolates were made, 16 from Antioquia, 21 from Cundinamarca, 4 from Santander and 2 from Darien in the Cauca valley **(Table 1)**. Fungal characterization on a set of 12 differential varieties **(Table 2)** established for *C. lindemuthianum* was done in the greenhouse as described previously (Mahuku et al., 2003).

Table 1. Frequency distribution of *Colletotrichum lindemuthianum* races characterized from different departments of Colombia.

	Locality				
Pathotype	Altiplano Norte	Oriente Antioqueño	Santander	Cundinamarca	Valle
1		1[e]			
3		4	4	8	1
5	2				
7				1	
11				1	
129		1			
131				1	
132		1		2	
133		1		2	
135	1				
137		1		4	
139	1			1	
141	1	1			
143					1
515		1			
641				1	
Total isolates	5	11	4	21	2

Results and Discussion: Sixteen pathotypes were identified among the 43 isolates and the most frequently characterized pathotype was 3 **(Table 1)**. This pathotype (with 17 isolates) was present in all the departments from where samples were received. All pathotypes have been described before in Colombia. The most susceptible varieties were Michelite (infected by 93% of the isolates), MDRK (58.1%), PI 207262 (46.51%), Perry Marrow (32.6%), Cornell 49242

(25.2%), and TU (4.6%) **(Figure1).** None of the isolates infected Widusa, Mexico 222, Kaboon, and G 2333, and these can potentially serve as source of anthracnose resistance. In addition, the resistance genes in Widusa and Kaboon have been well characterized and tagged, making the use of molecular markers in MAS in breeding programs involving these genotypes possible.

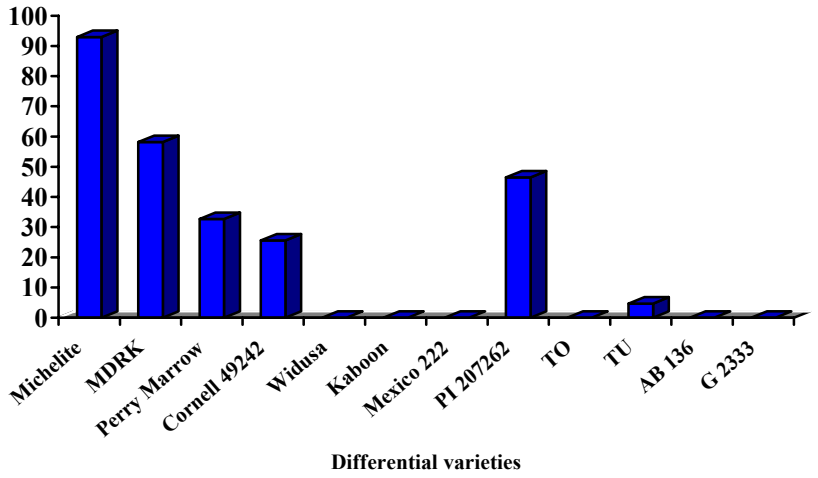

Figure 1. Percent susceptibility of anthracnose differential varieties inoculated with 43 isolates of *Colletotrichum lindemuthianum* from different zones in Colombia

Table 2. Common bean Anthracnose differential varieties and their respective identified resistance genes.

Code	Differential Variety	Gene Pool[a]	Resistance (s) gene[b]	Binary Value[c]
A	Michelite	M	?	1
B	MDRK	A	Co-1	2
C	Perry Marrow	A	Co-1^3	4
D	Cornell 49242	M	Co-2	8
E	Widusa	M	Co-1^5	16
F	Kaboon[d]	A	Co-1^2	32
G	Mexico 222	M	Co-3	64
H	PI 207262	M	Co-4^3, Co-9	128
I	TO	M	Co-4	256
J	TU	M	Co-5	512
K	AB 136	M	Co-6, Co-8	1024
L	G 2333[d]	M	Co-4^2, Co-5, Co-7	2048

a = *Phaseolus* gene pool; A = Andean; M = Mesoamerican.
b = identified resistance genes.
c =Binary value assigned for each differential variety and used for race designation.
d = differential varieties that are resistant to all isolates that have been characterized from Antioquia and Santander.

Conclusion: Kaboon and G2333 continue to be immune to isolates from Colombia. The use of the resistance genes from these genotypes in bean improvement for anthracnose resistance in the different zones of Colombia should provide complete anthracnose resistance. Kaboon carries the Co-1^2 allele while G 2333 has three resistance genes (Co-4^2, Co-5, Co-7). Molecular markers

linked to these resistance genes are available, making MAS possible in crosses involving these varieties.

References

Mahuku, G., Jara, C., Cajiao, C., and Beebe, S. 2003. Sources of angular leaf spot (*Phaeoisariosis griseola*) in common bean core collection, wild *Phaseolus vulgaris* and secondary gene pool. Euphytica 130: 303-313.

Contributors: C. Jara, J. Fory, G. Castellanos, JB Cuasquer y G. Mahuku.

Activity 4. Developing integrated pest management components.

Identification of potential biocontrol bacterial agents

Rationale

Because of the increase in the negative effects that synthetic chemicals have on the environment, it is apparent that the use of antagonistic microorganisms may be a good alternative in controlling diseases caused by pathogens with large pathogenic variability. If this is to be a viable and reliable alternative, it is important to understand why, when and how these microorganisms and their products affect the development of pathogens. Several bacteria that exhibit a biocontrol effect on some common bean pathogens have been isolated in our laboratory. Preliminary experiments revealed the antagonistic of these bacteria. Three bacteria have been of particular interest. We report the identification of these bacteria.

Bacteria identification

Bacteria 1: The bacterium was isolated from the phyloplane of common bean leaves. The bacterium is gram positive, non-motile, with large spores and produces acid in the presence of manitol, maltose and cellobiose, does not utilize urea, has the ability to utilize catalase and oxidase. Based on the biochemical and morphological analysis, this bacterium was identified as a *Bacillus*. Analysis of the partial sequence of the 16S rDNA gene revealed that this bacterium was 98% similar to *Paenibacillus polymyxa*. Based on sequence analysis of the partial 16S ribosomal gene, morphological and biochemical tests, bacterium 1 was tentatively classified as *Paenibacillus polymyxa*.

Bacterium 2: The bacterium was isolated as a contaminant from petri plates of V8 juice medium. Morphologically, this bacterium is irregular in shape, convex, translucent with colonies that are $\leq$ 1 mm. It is gram positive with small Bacillus type spores. Based on morphological and biochemical tests, this bacterium was identified as *Bacillus subtilus.* This identification was confirmed following partial sequence of the 16S rDNA gene and blast sequence search that showed that the sequence of this bacterium was 98% similar to that of *Bacillus subtilus.*

Bacteria 3 : The bacterium was isolated from the phyloplane of *Morinda citrifolia.* Morphological tests revealed that this bacterium is a gram-positive cocos with circular colonies < 1 mm, transparent and convex. Partial analysis of the 16S rDNA sequences revealed that this bacterium is 100% similar to *Gluconobacter* spp. This bacterial has been tentatively labeled as *Gluconobacter* spp.

Conclusion: Further identification and classification of the bacteria using other diagnostic tools is under way. Meanwhile, characterization of these bacteria and establishment of their biocontrol activity and range is in progress.

Contributors: Carlos Jara, Guillermo Castellanos, Maria Antonia Henriquez, George Mahuku.

Activity 5. In vitro in hibition of Colle totrichum linemuthianum by three potential biocontrol bacteria l s pecies (Paeniba cillus polimixa, Bacillus su btilus and Gluconobacter spp.).

Rationale

Biological control is an alternative sound strategy for the management of plant pathogens because it is environmentally safe while promoting build up of natural enemies, thus creating a sustainable production system. For this reason, we are involved in studies to identify and evaluate the efficacy of potential biological control agents. We report we report the effect of cell-free culture filtrates of three potential bicontrol bacteria on mycelial growth and germination of *Colletotrichum lindemuthianum* conidia.

Materials and Methods: Three potential bacteria biocontrol agents (tentatively classified as *Paenibacillus polimixa* (B1); *Bacillus subtilus* (B2); and *Gluconobacter* spp (B3)) were used in this study. The bacteria were grown on either PDA or nutrient agar, unless otherwise specified.

Inhibition of *C. lindemuthianum* grow th: To establish the effect of the bacterium on the growth of *C. lindemuthianum* , bacterium from a 48 hr culture on PDA was inoculated onto PDA medium in a circle at different distances (2, 3, 4, and 6 cm) diameters from the center of the petri plate. A plug of mycelium (4 mm diameter) cut from the edges of an actively growing *C. lindemuthianum* isolate was placed in the center of each plate. To establish the nature of the potential antifungal compound, the fungus was inoculated immediately after culturing the bacteria (0 hrs), and at varying times 24, 48 72 and 96 hrs after culturing the bacteria. There were five plates for each treatment and the experiment was repeated. Radial growth of the fungus was evaluated 7, 14 and 21 days after culturing. Control plates contained the fungus on the same media, and inoculated at the same time but without the bacteria.

Preparation of cell free culture filtra tes: Cell-free culture filtrates of the bacteria were produced by culturing the bacteria in 250 mL of nutrient broth (Difco) in 750 mL flasks and incubating at 28°C with shaking at 200 rpm until an OD_{600} of 1.1 was reached. The culture filtrate was centrifuged at 7000 g to remove bacteria cells and then the fluids were passed through a 0.22 um pore-size nylon membrane to remove residual bacterial cells. The cell-free culture filtrate either heat inactivated by 100 C for five minutes or not heated were used to prepare medium for culturing *C. lindemuthianum* and test inhibition of conidia germination.

Conidia germination assay: Cell-free culture filtrates were mixed with autoclaved and cooled PDA agar to a final concentration of 60% (v/v). *C. lindemuthianum* conidia suspension (10^6 conidia/ml) in sterile distilled and deionized water was plated on to PDA medium amended or non – amended PDA medium (control) with cell-free culture filtrates. Inoculated plates were incubated at 24°C and evaluated for the growth and development of *C. lindemuthianum*.

Results and Discussion: All bacteria were effective in inhibiting the growth of *C. lindemuthianum* **(Tables 1, 2, and 3)** , however, *Gluconobacter* spp. and *Paenibacillus polimixa* were the most effective. *C. lindemuthianum* spores plated on medium containing cell-free culture filtrates did not germinate, where as on control plates (lacking bacterial filtrates), normal fungal

growth was observed (data not shown). The antimicrobial compound is diffusible, and was more potent with time that the bacteria was allowed to establish before inoculating the fungus **(Tables 1, 2 and 3)** . The rate of fungal growth inhibition dependent on the distance that *C. lindemuthianum* was from the bacteria. This was more evident for *B. subtilis* and *Paenibacillus polimixa*. This might reflect the nature of the antimicrobial compound, which in turn, might dtermine the rate of diffusion through the medium. Conversely, this might also reflect the rate of antimicrobial production, with *Gluconobacter* producing and reaching high concentrations faster than the other bacteria. The compound produced by *Gluconobacter* appears to be in high concentrations and diffuses through the medium rapidly. More studies are under way to optimize the production media and culturing conditions, as a means of verifying this assertion.

Heating the culture filtrate destroyed the activity of the antifungal compound, produced by *B. subtilis* and *Paenibacillus polimixa* but not that produced by *Gluconobacter*. The antimicrobial compounds seem to be different. For B1 and B2 it appears that the antifungal compound is protein in nature; where as the antimicrobial compound produced by B 3, is either a heat resistant protein or some other type of compound. Further tests are needed to definitely identify the antimicrobial compounds in these bacteria.

Conclusion: Three bacteria with potential to manage fungal pathogens have been identified. Preliminary results have revealed at least two compounds; a heat susceptible and a heat stable compound. It is probable that one these compounds are protein in nature. However, this assertion needs to be confirmed. There is a need to optimize culturing conditions, and to test the efficacy of these compounds on a diverse range of plant pathogens.

Table 1. Radial growth of *Colletotrichum lindemuthianum* challenged with the potential biocontrol bacteria *Paenibacillus polimixa* inoculated at different times an d distance.

Time (hrs)	Distance of Bacteria from Center of Plate (cm)				
	2 3 4 6				Control
0	0.28	0.41	0.92	1.54	4.54
24	0.10	0.26	0.45	1.29	4.45
48	0.10	0.1	0.24	1.08	6.71
72	0.10	0.1	0.16	0.47	4.58
96	0.10	0.1	0.20	0.48	5.50

Table 2. Radial growth of *Colletotrichum lindemuthianum* challenged with the potential biocontrol bacteria *Bacillus subtilus* inoculated at different times and distance.

Time (hrs)	Distance of Bacteria from Center of Plate (cm)				
	2 3 4 6				Control
0	0.80	1.14	0.97	2.17	6.80
24	1.31	1.16	1.25	1.71	4.50
48	0.59	0.54	0.29	1.17	4.43
72	0.41	0.54	0.49	1.30	5.14
96	0.41	0.44	0.30	1.24	4.80

Table 3. Radial growth of *Colletotrichum lindemuthianum* challenged with the potential biocontrol bacteria *Gluconobacter* spp. inoculated at different times an d distance.

	Distance of Bacteria from Center of Plate (cm)				
Time (hrs)	**2 3 4 6**				**Control**
0	0.13	0.13	0.23	0.32	4.54
24	0.10	0.10	0.10	0.10	7.5
48	0.10	0.10	0.10	0.10	6.71
72	0.10	0.10	0.10	0.10	4.58
96	0.10	0.10	0.10	0.10	5.60

Contributors: Carlos Jara, Guillermo Castellanos, G. Mahuku.

Activity 6. Integrated Soil Fertility/Pest & Disease Management approaches to address root-rot problems in common beans.

Rationale

Consensus about societal demands for agricultural sustainability and biodiversity conservation has been reached in the past decade (UNCED-1992). New approaches to continuing problems, like soil degradation and soil pest and diseases, are then needed in order to achieve agricultural sustainability. Our overall working hypothesis in this study is that combining soil fertility and pest management approaches would provide a unique opportunity to exploit synergies allowing a better control of soil fertility/pest&disease limitations to crop productivity than either approach alone.

The management of organic matter is crucial to the activities of the soil biota. Use of green manures can have a multi-faceted beneficial effect on crop productivity arising from (i) protection of the soil from erosion; (ii) increased nutrient cycling; (iii) synchronized nutrient release and uptake by the plants; and (iv) increase in soil biological activity and diversity of microorganisms, which in turn can lead to minimized damage and loss from soil borne pathogens, and increased activity of beneficial microorganisms. However, different sources of green manure can have different effects on the balance between populations of harmful and beneficial organisms because they have different rates of decomposition and nutrient release as well as different impact on soil moisture and temperature that invariably affects relative population sizes. For this reason, it is important to evaluate the effect of different sources of green manure on three key functional groups of soil biota: 1) pathogens, 2) microregulators and 3) microsymbionts. We are studying the population dynamics of soil pathogenic fungi (*Fusarium*, *Sclerotium*, *Macrophomina*, *Rhizoctonia* and *Pythium*), soil nematodes (discriminated by feeding habit), soil microsymbionts (mycorrhiza, rhizobia) during cultivation of common bean in soils infested with pathogenic fungi. Evaluations were carried out by: a) directly identifying and quantifying different soil biota from functional groups mentioned above and b) indirectly, by evaluating the incidence of disease on susceptible plant genotypes, by plant infection test for native rhizobia symbiotic potential and AMF activity in soil through hyphal lengths. The relative position of these three groups in the soil food web suggests the potential for soil organic management to reduce soil pathogenic fungi populations and incidence in bean plants by change induced in soil moisture and temperature, nutrient availability and interaction with other soil organisms.

Materials and Methods: An experiment was established in CIAT's Santander de Quilichao Research Station, using a plot that has a history of high incidence of root rot pathogens. The plots were planted with a root rot susceptible bean variety A 70. Immediately after planting, the plots were covered with three green manures treatments: (1) rapidly decomposing *Tithonia diversifolia*(TTH); (2) intermediate rate of decomposition but greater soil cover due to leaf morphology by *Cratylia argentea*(CRA); (3) slow decomposing (*Calliandra calothyrsus* (CAL) at a rate of 6 ton ha^{-1}; and (4) control (no green manure added). The experiment was replicated five times. Soil samples (0-10 cm) collected during the cropping season included at least planting

and harvesting time. Samples were collected within rows and between rows, to measure the effect of the rhizosphere of bean plants on the soil biota studied.

Results and Discussion

Diversity of soil pathogenic fungi *:* Preliminary data revealed that plots receiving CRA had a significantly less fungal diversity ($p<0.05$) than plots receiving the other sources of green manure or the control **(Figure 1)**. No differences were observed between the other treatments and the control. However, since this is the second season after initiation of the experiment, it is still too early to make sound conclusions.

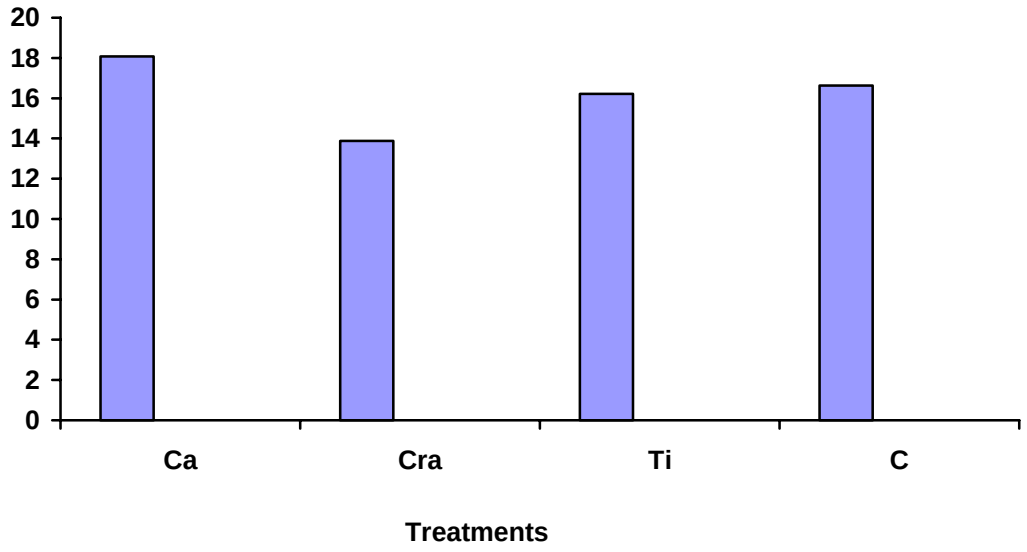

Figure 1. Diversity of soil-borne fungi in plots receiving or not receiving different sources of green manure. H represents the Shannon Wiener diversity index.

The most frequently isolated fungus was *Aspergillus* (A) in all treatments, while Macrophomina (Ma) and Rhizoctonia (R) were the least isolated fungi **(Figure 2)**. Other fungi that were isolated included *Fusarium* (F), *Penicillium* (P), *Humicola* (H) and *Mucor* (M) **(Figure 2)**. The presence of *Penicillium* is interesting, as some species of this fungus are known to solubilize phosphorus. *Humicola* is a fungus that has been found to be involved in decomposing organic matter, and this was found in abundance in plots receiving *Calliandra*. Several fungi were isolated that are currently being classified. These were tentatively placed under the "unknown" group (D). It is possible that some of these fungi could be potential biological control agents. Although *Macrophomina* has been observed in the past in high frequencies and incidence on infected plants, this fungus was not detected in the soil samples analyzed thus far. It is possible that the method of analysis that is used leads to the exclusion of this fungus, or the high incidences observed under field conditions results from seed-borne inoculum.

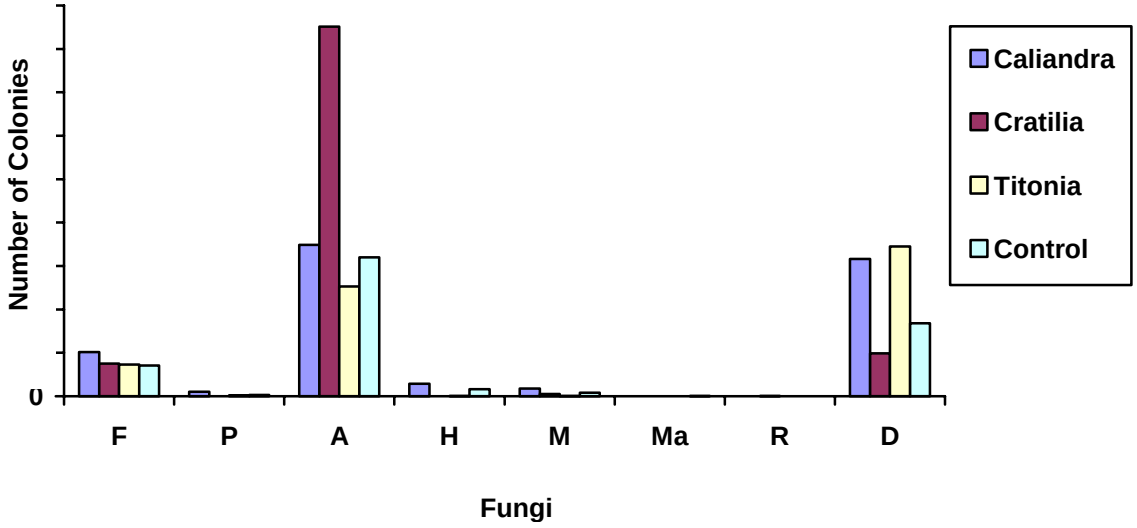

Figure 2. Frequency of different fungi isolated from plots receiving a fast, intermediate and slow decomposing green manure or the control.

Abundance of soil nematodes: Total number of soil nematodes was always higher in the row than between the rows highlighting the importance of the bean plant rhizosphere effect **(Figure 3)**. On average greater number of nematodes were found when Tithonia pruning was applied to the soil and the overall order was TTH>CRA>CON=CAL. Taxonomic identification of nematodes and classification into feeding groups is on going and should help in the interpretation of abundance trends observed.

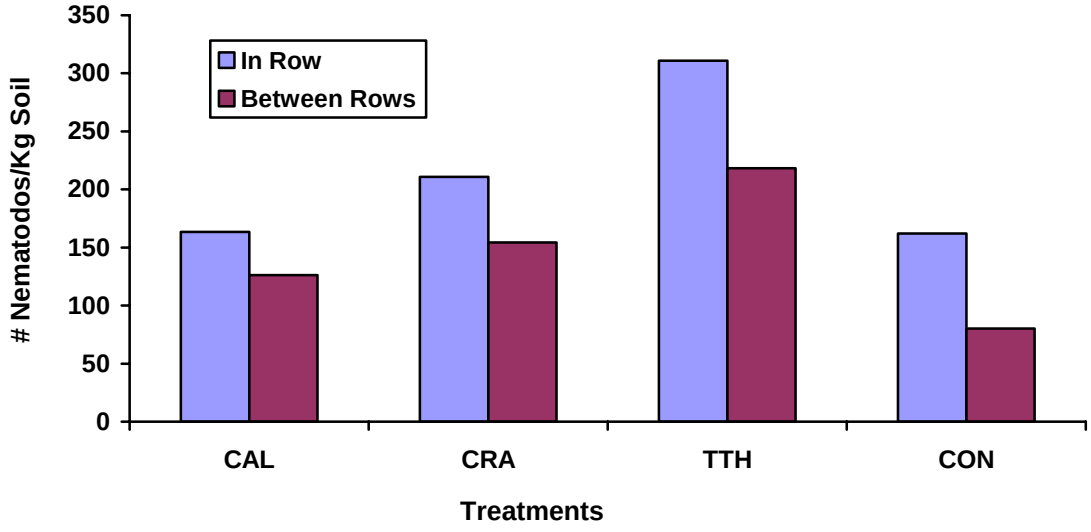

Figure 3. Total number of nematodes from plots receiving a fast, intermediate and slow decomposing green manure or the control.

Incidence of root rot pathogens: Significant differences were observed in the incidence of root rots in some treatments, when compared to the control **(Figure 4).** Application of *Calliandra*, and *Tithonia* significantly reduced disease incidence ($p<0.05$), where as a slight increase in disease incidence was observed in plots receiving *Cratylia.* . Analysis of the samples collected from these plots revealed that most of the root ort symptoms were caused by *Macrophomina phaseolina* and *Fusarium solani*, while *Rhizoctonia solani* was occasionally isolated. Significant yield increases were observed for plots treated with *Calliandra* (10%) and lowest for plots receiving Tithonia (-29%) **(Figure 4)**. Although a slight increase in yield was observed (1.2%) for plots receiving *Cratylia*, this was not significantly different from the control plots.

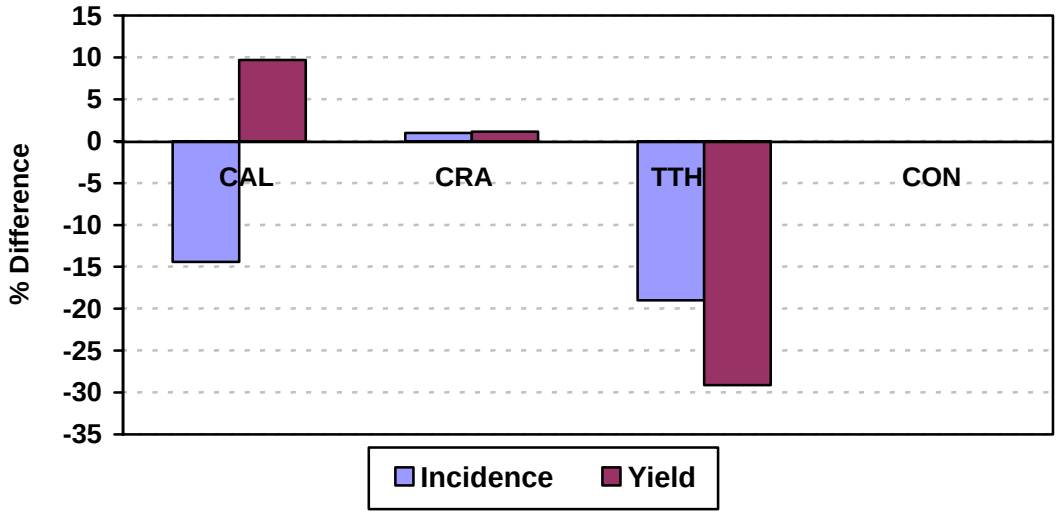

Figure 4. Incidence of root rots and yield of the bean genotype A 70, grown in plots with or without different types of green manures expressed as a percent of control treatment.

Conclusion: First results indicate that despite the relatively limited time of green manure treatments some initial trends can be identified. Compared with the control application of *Calliandra* resulted in increased bean yield, reduced incidence of root rots and low nematode abundance. In the case of *Cratylia*, there were minor differences root rot incidence, yield and nematode abundance (in row) when compared to the control. Although disease incidence was low in plots receiving *Tithonia*, bean yield was also negatively affected. Taxonomic identification of nematodes would help to understand if high nematode populations in TTH were involved in reducing bean yield. In addition, the impact of treatments on the bean plant symbiosis with mycorrhiza and rhizobia needs to be included for a more complete explanation of yield differences encountered. Nevertheless, yield differences were likely also influenced by a combination of physico-chemical factors including differences in nutrient release by the three green manure sources.

While at this early stage application of *Calliandra* seems to offer the best results we need to examine how transient or cumulative these effects are and the mechanisms of action involved. The potential exists that unknown beneficial microorganisms are promoted in the soil by green manures and thus can potentially be used to manage root rot pathogens and/or for promoting plant growth. We are currently evaluating fungi that have tentatively been grouped under the

"unknown" group for potential antagosistic effects, as well as *Penicillium* species for their ability to solubilize phosphorus.

Contributors: G. Mahuku (IP-1), E. Barrios (PE-2), Lorena Cortes (IP-1), C. Jara (IP-1), Asakawa N (PE-2), Jara C., Navia J. (PE-2).

Activity 7. Publications, book chapters, workshops.

Refereed Journals

Mahuku, G., Montoya, C., Henríquez, M.A., Jara, C., Teran, H., and Beebe, S. 2004. Inheritance and Characterization of the Angular Leaf Spot Resistance Gene in the Common Bean Accession, G 10474 and Identification of an AFLP Marker Linked to the Resistance Gene. Crop Science 44:1817-1824.

Mahuku, G. S. 2004. A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. Plant Molecular Biology Reporter 22: 71-81.

Mahuku, G.S. and Riascos, J. J. 2004. Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. European Journal of Plant Pathology 110: 253-263.

Kelemu, S., Mahuku, G., Fregene, M., Pachico, D., Johnson, N., Calvert, L., Rao, I., Buruchara, R., Amede, T., Kimani, P., Kirkby, R., Kaaria, S., and Ampofo, K. 2003. Harmonizing the agricultural biotechnology debate for the benefit of African farmers. African Journal of Biotechnology 2:394-416.

Non-refereed Journals

Riascos, J.H., Mahuku, G., and Cárdenas, H. 2004. Diversidad genética del agente causal de la antracnosis del fríjol común (*Colletotrichum lindemuthianum*) (ASCOLFI Newsletter).

Henriquez, M.A., Mahuku, G., Muñoz, J.E., Castellanos, G., y Jara, C. 2004. Determinaciones de la diversidad genética del agente causal de la mancha angular del fríjol, *Phaeoisariopsis griseola* (Sacc.) Ferraris, mediante el uso de marcadores moleculares. (ASCOLFI Newsletter).

Book Chapters

Mahuku, G.S. 2003. Angular leaf spot. In: Compendia of Bean Diseases. American Phytopathological Society, St. Paul. MN. **(In press).**

Workshop Presentations

Jara, C., Castellanos, G., Cuasquer, J.B., y Mahuku G. 2004. Determinación de la variabilidad patogénica en diferentes cepas de *Xhanthomonas capestris* pv. *phaseoli* y *Xanthomonas campestris* pv. *phaseoli* var fuscans en 56 genotipos de fríjol común. XXV Congreso de la Asociación Colombiana de Fitopatología y Ciencias afines (ASCOLFI) , Agosto 11-13 de 2004, CIAT, Palmira.

Navia, M., Mahuku, G., y Arroyave, J.A. 2004. Evaluación del proceso de infección de *Phaeoisariopsis griseola* en interacciones compartibles e incompatibles con el fríjol común, *Phaseolus vulgaris*. . XXV Congreso de la Asociación Colombiana de Fitopatología y Ciencias afines (ASCOLFI) , Agosto 11-13 de 2004, CIAT, Palmira.

Montoya, c., Mahuku, G., Henríquez, M. A., y Jara, C. 2004. Identificación de marcadores moleculares ligados a genes de resistencia a mancha angular de fríjol. . XXV Congreso de la Asociación Colombiana de Fitopatología y Ciencias afines (ASCOLFI) , Agosto 11-13 de 2004, CIAT, Palmira.

Henríquez, M.A., Mahuku, G., y Navia, M. 2004. Cebadores específicos para la detección y diferenciación de *Xanthomonas campestris* pv *phaseoli* y *Xanthomonas campestris* pv. *phaseoli* var fuscans en el fríjol común. . XXV Congreso de la Asociación Colombiana de Fitopatología y Ciencias afines (ASCOLFI) , Agosto 11-13 de 2004, CIAT, Palmira.

Students

Name	Degree	Status	University	Title
Monica Navia	BS.	Completed	Universidad del Valle	Elucidación del proceso y eventos de infección del frijol común Phaseolus vulgaris (Fabaceae) con el hongo Phaeoisariospsis griseola (Stilbaceae) agente causante de la mancha angular.
Lorena Cortes	BS.	Continuing	Universidad del Valle	Efecto de las diferentes fuentes de abono verde en el suelo sobre el manejo de hongos causantes de pudriciones en el frijol (Phaseolus vulgaris, L. Fabaceae).
Maria Antonia Henriquez	Ms.	Continuing	Universidad Nacional de Colombia, Palmira	ESTs para entender la interacción entre genotipos del frijol común (Phaseolus vulgaris) y Phaeoisariopsis griseola, el agente causal de la mancha angular.

Trips

Date	Destination	Event or Purpose
June 4, 2004	Ibagué-Tolima	Workshop to train technicians (65) on the agronomic management of snap and dry beans and integrated disease management.
June 24-25, 2004	Bogotá, D.C.	Workshop to train technicians (110) on the agronomic management of snap and dry beans and integrated disease management.
July 30, 2004	Pitalito-Huila	Workshop to train technicians (65) on the agronomic management of snap and dry beans and integrated disease management.
July 11-16, 2004	Kampala, Uganda	Discussion on the workplan to implement molecular techniques for detection of Pythium species that cause bean root rots.

Special Projects

Title	Donor	Comments	Funding Period	Total Amount
Improving rural livelihoods in Rwanda: Promoting integrated crop, disease, and pest management (ICDPM) strategies for intensification and diversification of agricultural systems.	Bilateral project for Belgium	Concept note	3 years (2005-2007)	3 million Euros
Iniciativa Peruana de Rhizobiología : Fijación biológica de nitrógeno para el establecimiento de sistemas agrícolas sustentables y el progreso de los pequeños productores del Perú.	IDRC	Rejected	5 years	CAN$ 999 625

RICE PATHOLOGY

Activity 1. Blast (*Pyricularia grisea*) and sheath blight (*Rhizoctonia solani)* diseases on rice.

Characterization of blast pathogen populations . Monitoring the Evolution in the Genetic and Virulence Diversity of the Blast Pathogen over time.

Abstract

Rice blast, the most important rice disease worldwide can be managed through genetic resistance. Continuous monitoring of the evolution leading to important changes in the genetic structure and virulence spectrum of the pathogen is very important for the identification of resistance genes and their combinations to resist those pathogenic changes and preventing resistance breakdown. Understanding this pathogen-host interaction can attain development of suitable breeding strategies for a more stable blast resistance.

Introduction

Rice blast caused by *Pyricularia grisea* Sacc. is the most important disease worldwide. Genetic resistance is the most effective way to control the disease but resistance is defeated by the pathogen shortly (1 to 3 years) after cultivar release with the exceptions of the Colombian commercial cultivars Oryzica Llanos 5 and Fedearroz 50, whose resistance lasted for at least fifteen and six years, respectively. Compatible blast interactions with these two cultivars were observed in 2004 at the Santa Rosa experiment station in Villavicencio, Colombia. This breakdown is mainly due to the continuous changes and evolution of the pathogen, which gives origin to new pathotypes compatible with the resistant rice cultivars. Continuous monitoring of blast pathogen populations in breeders as well as commercial fields is needed to detect recent changes in pathogen virulence. New pathotypes detected are used to identify resistance genes that can be introgressed into new genetic material before there is an increase in frequency of these new isolates, and therefore reducing the risks of resistance breakdown.

Materials and Methods: Rice leaves and panicles with typical blast symptoms are continuously collected from different rice lines and commercial cultivars in the pathology and breeder's plots at Santa Rosa experiment station. Blast isolates from the cultivars Oryzica Llanos 5 and Fedearroz 50 were recovered from infected samples in the laboratory and inoculated on a set of differential rice lines with different resistance genes to identify potential sources of resistance to new pathotypes. The same sample of isolates was used for determining their genetic structure using the Pot-2 PCR fingerprinting technique. More than 50 blast isolates recovered from several rice breeding lines, the cultivars Oryzica Llanos 5 and Fedearroz 50, and the cultivar Bonanza, released by the private sector in 2004 were analyzed and new pathotypes are reported in this chapter. These studies also included some isolates from the commercial cultivar Cica 8, which is not planted any more by farmers due to its susceptibility to blast. However, this cultivar had a resistant reaction in Santa Rosa in most replications planted in 2004 in the station. Cica 8 is used as a component in the mixture of the spreader rows used in our field methodologies to increase the frequency of lineage SRL-5 compatible with this cultivar.

Results an d Discussio n: All blast isolates analyzed belonged mainly to the known genetic groups SRL-6, SRL-5, SRL-4 and SRL-2 already identified in Colombia. Blast isolates recovered from the cultivars Oryzica Llanos 5 and Fedearroz 50 were lineage SRL-4 and exhibited a similar and wide virulence spectrum defeating most known resistance genes present in the rice differentials used in this study and most commercial cultivars released in Colombia in the last 20 years **(Table 1)** . These isolates however, did not reinfect Oryzica Llanos 5 and Fedearroz 50 in controlled inoculations in the greenhouse **(Table 1)** exhibiting mainly small lesions and a low disease severity. Given the fact that many isolates were recovered from these two cultivars and that greenhouse inoculations gave similar results of a low compatible interaction, we can speculate that the resistance of these two cultivars will still be durable for a longer period of time under farmers fields. We are in the process of dissecting the resistance genes present in the cultivar Oryzica Llanos 5 and results suggest both the presence of major and minor genes. Greenhouse inoculations also indicate that the resistance genes Pi-1, Pi-k, Pi-k^m, and Pi-kh, all of which are in the same region of Chromosome 11, confer resistance to these pathotypes **(Table 1)**. We have initiated a backcrossing program to incorporate Pi-1 into these two cultivars using marker assisted selection, and greenhouse and field inoculations. The rice cultivars Oryzica 2 and Cica 8, and the rice accession Tetep were also resistant to these pathotypes, probably due to the presence of one of the genes located on Chromosome 11, or a different resistance gene. Analysis of the blast samples collected from the rice commercial cultivar Bonanza released by the private sector in 2004 exhibited a similar virulence spectrum to those pathotypes recovered from Oryzica Llanos 5 and Fedearroz 50 **(Table 1)** . The recent release by the private sector of several commercial cultivars susceptible mainly to the blast genetic lineage SRL-4, has allowed the increase of this lineage in frequency. It seems that the private sector is working probably with similar germplasm obtained from different nurseries obtained from CIAT and which lack resistance genes to lineage SRL-4 such as those located on Crhomosome 11 in the region of the Pi-k locus.

A new accession (75-1-127) carrying the resistance gene Pi-9 derived from the wild species *Oryza minuta* and located on Chromosome 6 also exhibited resistance to these as well as many other pathotypes **(Table 1)**. This line has exhibited also a complete resistant reaction under field conditions in Santa Rosa after three years of evaluation. We are in the process of conducting genetic studies to determine if this resistance is indeed controlled by only one resistance gene. Our experience for many years and controlled inoculations of several thousand of isolates indicate that there are no single genes within the cultivated species *Oryza sativae* effective against all pathotypes of the blast pathogen. This wide spectrum of resistance controlled by this single gene suggests that rice blast populations carry a common avirulence gene whose products interact with the products of the resistance gene conferring resistance. The durability of the resistance conferred by this gene still needs to be demonstrated. These results also suggest that it is worthwhile looking for new resistance genes in wild rice species, which may carry different resistance genes/alleles to those present in the cultivated species and which have no coevolved with the pathogen. We will also focus on wild species originating in Latin America such as *O. glumepatula*, or the cultivated species *O. glaberrima* originated in Africa as they may carry useful resistance genes that have not been exposed to blast populations for many years as they most probably evolved from weeds to rice in Asia.

In our efforts to detect new changes in virulence in the blast pathogen population, few blast lesions observed in 2003 on the highly resistant line CT 13432-34 carrying the resistance genes Pi-1, Pi-2, Pi-33 were collected and analyzed in the laboratory in 2004. All isolates retrieved turned to be lineage SRL-4 and exhibited at least two pathotypes **(Table 1)** . Greenhouse inoculations of these two pathotypes indicated their ability to potentially defeat the three resistance genes Pi-1, Pi-2, and Pi-33. This pathotype was not recovered from the same or any other cultivar in 2004, indicating that its frequency is very low and probably its fitness is weak compared to the well-established blast populations. This hypothesis will be studied closely monitoring blast isolates collected from rice lines carrying the three genes, however, it was not possible in 2004 as all lines planted in Santa Rosa with the three genes exhibited a complete resistance to blast. We don't know at this point the relevance of this pathotype and the role it can play in breaking down the resistance conferred by the combination of these three genes as we have demonstrated in previous years. It is interesting to observe, however, that in order to lose the three avirulence genes avr-Pi-1, avr-Pi-2, and avr-Pi-33, these pathotypes had to maintain in one isolate the avirulence gene for Pi-ta^2 and in the other the avirulence gene for Pi-b **(Table 1)**, located on Chromosomes 12 and Chromosome 2, respectively. The corresponding resistance genes Pi-ta^2 and Pi-b present in the differentials F 128-1 and F 145-2 confer resistance to these isolates **(Table 1)** . These results indicate that these two resistance genes Pi-ta^2 and Pi-b will probably have to join the combination of the three resistance genes Pi-1, Pi-2, and Pi-33 to prevent a potential breakdown of the resistance genes. The presence of the Pi-ta^2 and Pi-b genes in the cultivars Oryzica Llanos 5 and Fedearroz 50 determined by us in other studies explains why these isolate did not infect severely these cultivars in greenhouse inoculations **(Table 1)** despite the fact that all other commercial cultivars were severely infected in the same test. We are in the process of analyzing more blast samples collected in 2004, both, in terms of genetic structure and virulence spectrum, to determine if this new pathotype can be recovered from other cultivars. It should be noted that these two pathotypes to defeat a large number of resistance genes had lost many avirulence genes, which may confer a deleterious effect on the pathogen affecting its fitness and establishment. This could explain the low frequency observed in 2003 and the possible absence in 2004.

Analysis of blast samples recovered from the cultivar Cica 8 yielded isolates within genetic lineage SRL-5 with a narrow spectrum of virulence **(Table 1)** . These isolates were highly specific on the cultivars Oryzica 3, Cica 8, and Tetep, which is the source of resistance of Cica 8. These isolates also defeated the resistance genes Pi-ta^2, Pi-b, and Pik^m. The low frequency observed in this lineage may be explained by the narrow spectrum of virulence present in these isolates. Cica 8 is not grown anymore commercially and this lineage is not compatible with all the actual commercial cultivars grown by farmers such as Fedearroz 50 and Bonanza. Pathogenicity tests indicate that this lineage carry many avirulence genes **(Table 1)**. In order to increase its frequency for next year evaluations of our breeding lines, it will be necessary to multiply this lineage previous to our planting and perform some artificial inoculations on the spreader rows which have Cica 8 as a component.

Our results from the last several years suggest that in order to develop a more stable blast resistance, a combination of several resistance genes is needed to resist the potential changes in virulence of the rice blast pathogen. The combination of several major resistance genes will probably have to be accompanied of some important minor or quantitative trait loci, as it is being

indicated by our studies on the dissection and analysis of the stable resistance of the cultivar Oryzica Llanos 5.

As we can see from **Table 1** , few gene combinations would confer resistance to the blast population present in the upland environment of the Llanos Orientales from Colombia. We see the urgent need to identify new genes, probably present in other rice species. We have tested for three years the resistance gene Pi-9 present in the line 75-1-127 and derived from *Oryza minuta*, finding that the gene confers complete resistance in greenhouse inoculations as well as field evaluations **(Table 1)** . We have observed high levels of field resistance in the species *O. glaberrima* that deserve attention to identify potential new resistance genes. Once more, we see the importance of having a "hot spot" site with high blast pressure and pathogen diversity, to identify the best resistance gene combinations, and to detect in advance potential changes in genetic structure and virulence in the pathogen population that could threaten cultivar resistance.

Future Activities

Blast populations will continue being analyzed for their genetic structure and virulence spectrum to determine the potential changes of the pathogen that would lead to resistance breakdown. New resistance genes and proper combinations will be identified in the cultivated as well as wild species of rice to be incorporated in our breeding program. We will analyze the importance and potential role of the new pathotypes identified in 2004 and to determine the effectiveness of resistance genes effective against those isolates. The potential importance of the resistance gene Pi-9 will be evaluated again in 2005 under field conditions and greenhouse inoculations.

Table 1. Virulence Spectrum of Rice Blast Pa thotypes Detected at the Santa Rosa Experiment Station in 2004.

Rice Line	Resistance Gene	Blast Isolate (Cultivar of Origin)							
		OLL5	OLL5	F 50	13432	13432	C 8	BZA	BZA
C 104 LAC	Pi-1				+++	+++			
C 101 A51	Pi-2	+++	+++	+++	+++	+++		+++	+++
C 101 LAC	Pi-1+Pi-33				+++	+++			
CT 13432-33	Pi-33	+++	+++	+++	+++	+++		+++	+++
CT 13432-34	Pi-1+Pi-2+Pi-33				+++	+++			
C 104 PKT	Pi-3	+++	+++	+++	+++	+++		+++	+++
C 101 PKT	Pi-4a	+++	+++	+++	+++	+++		+++	+++
C 105 TTP4 (L23)	Pi-4b	+++	+++	+++	+++	+++		+++	+++
K1	Pi-ta	+++	+++	+++	++	+++		++	+++
F 128-1	Pi-ta^2	+++	+++	+++		+++	+++		+++
Kanto 51	Pi-k				+++	+++			
Tsuyuake	Pi-k^m				+++	+++			
F 129-1	Pi-k^p	+++	+++	+++	+++	+++	+++	+++	+++
F 145-2	Pi-b	+++	+	++	+++		+++	+++	++
Aichi Asahi	Pi-a	+++	+++	+++	+++	+++		+++	+++
K 3	Pi-k^h				+++	+++			
K 59	Pi-t	+	++	++	+++	+++		+++	+++
Rico 1	Pi-k^s	+++	+++	+++	+++	+++		+++	+++
Norin 2	Pi-sh	+++	++	+	+	+++		+++	++
Nato	Pi-I	+++	+++	+++	+++	+++		+++	+++
Ou 244	Pi-z	+++	+++	+++	+++	+++		+++	+++
Toride 1	Pi-z^t	++	++	++	+++	+++		+++	+++
Commercial Cultivars									
Fanny		+++	+++	+++	+++	+++		+++	+++
Metica 1		+++	+++	+++	+++	+++		+++	+++

Rice Line	Resistance Gene	Blast Isolate (Cultivar of Origin)							
		OLL5	OLL5	F 50	13432	13432	C 8	BZA	BZA
Oryzica 1		+++	+	+++	+++	+++		++	+++
Oryzica 2					+++	+++			
Oryzica 3		+++	+++	+++	+++	+++	++		+++
Cica 7		+++	+++	+++	+++	+++		+++	+++
Cica 8					+++	+++	+++		
Cica 9		+++	+++	+++	+++	+++			+++
IR 22		+++	+++	+++	+++	+++			++
Tetep					+++	+++	+++		
Ceysvoni		++	+++	++	+++	+++			+
O. Llanos 5		+	+	+	+	+			+
Línea 2 Semillano)		+++	+++	+++	+++	+++			+++
O. Llanos 4		+	+	+++	+++	+++		++	++
O. Caribe 8		+++	++	+++	+++	+++		+++	++
O. Yacu 9		+++	+++	+++	+++	+++			+++
Fedearroz 50		+	+	++	+	+			+
75-1-127	Pi-9								

OLL5= Oryzica Llanos 5; F50= Fedearroz 50; 13432= CT 13432; C8= Cica 8; BZA= new commercial rice cultivar Bonanza.

Contributors: F. Correa, F. Escobar, G. Prado, G. Aricada.

Activity 2. Selection of rice blast resistance sources to different genetic lineages of the blast pathogen. Development of a blast nursery with potential sources of resistance.

Abstract

The frequency of blast resistant plants in F2 populations is highly dependent on the blast reaction and stability of the parents used for the development of these populations. We initiated in year 2000 the development of a nursery with potential sources of durable blast resistance. Advanced rice lines are being evaluated for at least seven seasons under high disease pressure and only highly and durable resistant lines will be maintained into the nursery. This nursery will be tested under different conditions in several countries and used as a source of parents for breeding programs in Latin America.

Introduction

The frequency of blast resistant plants observed in F2 populations in the field is highly dependent on the blast reaction and stability of this reaction of the parents used for the development of these populations. An increase in the number of susceptible F2 plants and F4 lines found in the past years in different breeding materials from CIAT and FLAR at the Santa Rosa experiment station has been observed and will be discussed in the Rice Program Annual Report for 2004. This has been related probably to the low stability of the blast resistance of the parents used in the corresponding breeding programs. We have initiated the blast evaluation over time in the field and greenhouse of several hundred advanced as well as segregating lines exhibiting desired agronomic traits to identify potential sources of blast resistance. We are developing a nursery of potential sources of blast resistance to be used as parents, and will distribute them to partners in Latin America for testing and use in their breeding programs.

Materials and Methods were followed according to those described in the Annual Reports of the Rice Project since 2001.

Results: A total of 418 advanced rice lines from different sources described in last year report were evaluated and selected at the Santa Rosa field experiment station since year 2000. The most resistant lines over the last four years are shown in **Table 1**. Most of the resistant lines with a blast score 0-3 belong to the Germplasm Bank of CIAT-FLAR **(Table 1)**. One line (CT 13937-16-1-M-M-2) exhibiting a stable blast resistance for all these years was derived from an interspecific cross with *Oryza rufipogon,* and two other lines which exhibit also a highly resistant reaction to grain discoloration come from the upland germplasm from Brazil (RIO PARAGUAY and TRES MARIAS). Several of these lines have already been used in different crosses and yielded rice lines with potential stability of their blast resistance in advance generations. These results indicate the importance of evaluating the potential donors of blast resistance for several semesters before including them in a breeding program. Selected lines with a blast score of 0-3 as well as those with an intermediate reaction with a score of 4 will be evaluated again in replicated trials in year 2005 for their inclusion in a nursery as potential donors of stable blast resistance, which will be multiplied and distributed to our partners in the Latin American region.

Table 1. Potential Progenitors for Stable Blast Resistance Exhibiting Blast Scores 1-3 in Santa Rosa Field Evaluations during Five Years, Santa Rosa 2000 - 2004.

Pedigree	Pedigree
1. FL 00518-1P-4-3P-M	10. FL00535-21P-4-3P-M
2. FL00518-14P-15-3P-M	11. FL00542-45P-8-2P-M
3. FL00518-16P-7-3P-M	12. FL00871-1P-3-1P-M
4. FL00518-23P-11-2P-M	13. CT11275-3-F4-8P-2
5. FL00447-35P-4-2P-M	14. CT11280-2-F4-12P-5
6. FL 00459-21P-2-2P-M	15. CT13937-16-1-M-M-2
7. FL 00459-21P-2-2P-M	16. RIO PARAGUAY
8. FL00530-7P-7-1P-M	17. TRES MARIAS
9. FL00530-7P-7-2P-M	

Discussion: Durability of blast resistance is in general associated with the period of time that a cultivar remains as resistant after being exposed to a targeted pathogen. Field studies conducted by CIAT at Santa Rosa demonstrated that stable blast resistance could only be identified if the lines were evaluated through the F6-F7 generation. It is possible that only after several generations of exposure that the most effective resistance genes and their combinations can be identified. These genes at the same time should correspond to those avirulence genes highly conserved in the pathogen population with lower rates of change or mutation. In order to identify resistance genes associated with durability, it is necessary to evaluate and confirm the stable resistance of the potential donors for at least seven generations. We are in the process of developing a nursery with potential donors of resistance to different pathogens. Therefore, these nurseries will be evaluated continuously for several seasons under high disease pressure in the field to assure that the resistance selected is not a escape to infection and that the lines retain their durable resistance.

Future Activities

The evaluations of advanced breeding lines will be an annual activity to assure that the selected sources retain their stable resistance to the different pathogens. The search for new blast resistance genes will continue. The pathogen population will be monitored on these resistant lines to identify changes leading to a potential breakdown of the resistance. An analysis of the parents used in the genetic crosses giving origin to rice lines with stable and durable resistance will be initiated. Genetic crosses giving origin to rice lines with potentially durable resistance will be developed on the basis of the information generated since year 2000.

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Acitivity 3. Identification of molecular markers asso ciated w ith the durable blast resistance genes in the commercial rice cultivar Oryzica Llanos 5.

Abstract

The genetic basis of the high level of durable resistance to rice blast in the cultivar Oryzica Llanos 5 is being characterized in F7 Recombinant Inbred Lines (RILs) from a cross between the susceptible cultivar Fanny and O. Llanos 5. A linkage map was constructed using 250 molecular markers: SSR, RFLP and RGAs. Eleven loci, distributed on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 11, and 12, were associated with the resistance of the cultivar. As a whole, the observed durable resistance in Llanos 5 could be the result from a combination of quantitative and qualitative resistance genes.

Introduction

Blast resistance in the Colombian commercial rice varieties has been defeated in periods of 1-3 three years after cultivar release. However, the resistance of the cultivar Oryzica Llanos 5 has been durable and has remained stable under field conditions for more than 14 years. Genetic studies have indicated the presence of at least four major genes controlling the resistance to some blast isolates. Based on the presence of avirulence genes in our blast populations we have inferred that the cultivar O. Llanos 5 carries at least 8 major genes. Studies retrieving blast isolates from the immediate parents giving origin to this cultivar and characterizing their genetic structure and virulence composition suggest that the durable resistance is associated with the pyramiding of complementary resistance genes to the different lineages of the pathogen present in those parents. It is therefore important to understand the basis of the durable resistance of this rice cultivar in order to establish a breeding strategy based on the same principle. A study to identify and localize major and minor loci genes controlling the resistance in Oryzica Llanos 5 was initiated in year 2000 in collaboration with Kansas State University using a QTL (quantitative trait loci) detection approach with multiple rice blast isolates.

Materials and Methods: Recombinant inbred lines (RIL's) of the cross between the resistant indica cultivar Oryzica Llanos 5 and the japonica susceptible cultivar Fanny have been developed to a total of almost 1000 lines. An initial set of 120 lines was inoculated with different blast isolates representing the pathogen genetic lineages SRL-1 to SRL-6 from Colombia. Inoculations and evaluations were performed at the Rice Pathology greenhouse of CIAT according to the methodology described in other annual reports. Two evaluation methods, lesion type (LT) and disease leaf area (DLA) were used to score the blast resistance. One isolate named "killer", was recovered from O. Llanos 5 and observed to be highly aggressive and have a very broad virulence spectrum. This isolate has been used to detect minor resistance genes. DNA of each of the 120 lines was extracted at Kansas State University for molecular analysis and microsatellites were used as potential markers for the identification of the resistance genes present in Oryzica Llanos 5. Blast resistance genes to each genetic lineage of the pathogen are being identified based on the phenotypic reaction and located on the different chromosomes of the rice genome. The genetic linkage map constructed from the RIL mapping population contains 250 markers including simple sequence repeats, and RFLPs. The chromosomal locations of the markers were determined using the Mapmaker program Version 2.0. Both composite interval mapping (CIM) and multiple

interval mapping (MIM) techniques were used for QTL detection using QTL Cartographer package v2.0.

Results: QTLs associated with LT and DLA were detected for 7 of the 8 isolates on rice chromosomes 1,2,3,4,5,6,8,9, and 11. QTLs with the largest effects were on chromosomes 8, 6, and 11, which explained 28%, 72%, and 42% of the genetic variation in resistance to some isolates. Other QTL explained 2 to 16 % of the genetic variance. In some of the QTL locations there are blast resistance major genes that have been reported i.e. chromosome 4,6,5,8,9, and 11. However, some of the QTL have small effects, indicating the presence of minor genes. A number of these genes are located in areas previously identified to be associated with QTL with large effects. Chromosomes 1 and 8 were found to carry important resistance factors that are not associated with previously identified resistance genes.

Discussion: The durable broad-spectrum resistance in O. Llanos 5 is associated with multiple major genes that induce resistance to different blast isolates. All of the QTLs detected in this study for isolates other than killer were for lesion type blast resistance. LT is typically associated with QTLs with large effects (major, or "R" genes). In contrast all QTL identified by killer were for DLA. None of the major genes detected in Oryzica Llanos 5 are effective against the killer isolate, but O. Llanos 5 is still highly resistant. This resistance appears to be controlled by genes with small main effects. The killer isolate apparently allows these genes to be identified. As a whole, the observed durable resistance in O. Llanos 5 could be the result from a combination of quantitative and qualitative resistance genes.

Future Activities

Evaluate the reaction of more RIL's to the blast isolates already used in previous inoculations as well as new isolates exhibiting a compatible reaction with the cultivar O. Llanos 5. Continue analysis with microsatellite markers to identify and locate more blast resistance genes. Develop near isogenic lines with blast resistance genes present in Oryzica Llanos

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Activity 4. Identification of Resistant Lines to *Rhizoctonia solani* (Sheath Blight) and Development of an Evaluating Methodology.

Abstract

A suitable greenhouse screening method and evaluation scale has been developed for identification of tolerance to the sheath blight pathogen in rice. This method allows a better differentiation between tolerance and susceptibility. Tolerance to sheath blight present in the wild species *O. rufipogon* has been successfully transferred to the cultivated species. Unknown tolerance to sheath blight has also been identified in advance rice lines of *O. sativa* with the new screening and evaluation method, and both sources of tolerance can be used in our breeding program. Tolerance to sheath blight identified in the greenhouse has been corroborated under field conditions in several rice lines. Tolerance seems to be controlled by the action of several minor genes. Increasing resistance to sheath blight seems to be possible by crossing parents with high levels of tolerance.

Introduction

The filamentous basidiomycete *Thanatephorus cucumeris* (anamorph = *Rhizoctonia solani*) is the causal agent of sheath blight of rice. This disease has increased in economic importance in most Asian countries as well as in the USA in the last 10 years. The disease is also increasing in importance in most rice growing countries of tropical Latin America where the species *R. solani* AG-1 IA seems to be the most common while the species *R. oryza-sativae* seems to be the most common in the temperate areas of South America. The disease, which has increased in incidence and severity, is most frequently controlled with the use of fungicides, however this method of control has increased the production costs of many farmers. There are not well known sources of resistance to the pathogen. We initiated in year 2000 the evaluation in the greenhouse of different rice lines in order to identify potential sources of resistance to this pathogen. The evaluated germplasm includes Colombian commercial cultivars, wild rice species, Asian and USA reported sources of resistance, and advanced breeding lines of CIAT's and FLAR Rice Projects. The wild species *Oryza rufipogon* exhibited a resistant to intermediate reaction to more than 14 different isolates in trials conducted between 2000 and 2002, while the species *O. barthii* and *O. glaberrima* were susceptible to most isolates.

Materials and Methods: Several groups of rice lines including 523 advanced lines from the interspecific cross between *O. rufipogon* x Oryzica 3, advanced elite lines from FLAR, accessions from the rice germplasm bank, and a set of progenitors and populations developed by FEDEARROZ were evaluated in the greenhouse for their sheath blight tolerance in 2004. Tolerant lines were selected and their reaction corroborated in different and replicated trials. The best lines identified were planted in the field in collaboration with FEDEARROZ (Saldaña) for determining the correlation between the two screening sites. The genetics of sheath blight tolerance to determine the possible number of genes was evaluated in the F2 generation (70-80 plants) of six different crosses.

For greenhouse evaluations, each line is planted in three replications. Each replication consists of five plants sown in a pot. Disease development is favored by applying a high dose of nitrogen

divided in several applications. Inoculation is performed when the plants are 50 days old by placing at the base of the main stem of each plant a 5 mm plug of agar +mycelium of a *R. solani* isolate grown for 4-8 days on rice polish agar. Inoculated plants are incubated under high relative humidity in a growth chamber for 12-15 days. After this period of time, the pots are removed form the chamber and placed onto greenhouse benches for five days to reduce the stress conditions and allow a period of time for recovery and expression of potential tolerance. The pots and then moved back to the high humidity conditions for another period of 10 days before evaluating their reaction.

For evaluating the reaction to sheath blight, we are considering the percentage of plant area affected, by evaluating the main tiller of each plant. The reaction score of a line is considered as the average of the 15 plants evaluated in the three replications. Percentage of plant area affected is calculated by given the following maximum values to the different leaves and stem evaluated: First leaf (flag leaf): 30%; second leaf: 15%; third leaf: 15%; fourth leaf: 10%; fifth leaf: 10%; stem: 20%. Since disease development and severity observed depends on the effect of the environmental conditions in the greenhouse, tolerant and susceptible checks are always included in each replication and used for determining the level of tolerance of the lines being evaluated.

Results: The greenhouse screening methodology including the two periods of incubation in the growth chamber under high humidity with an intermediate period of plant recovery has been suitable to detect better differences in tolerance among the rice lines evaluated. Tolerance to sheath blight observed in the wild species *O. rufipogon* has been successfully transferred to the cultivar Oryzica 3. The tolerant reaction identified was corroborated in several replicated trials **(Table 1)** and the best lines have been planted for evaluation under field conditions. Tolerance to sheath blight has also been detected in different rice lines and populations including a set of progenitors being used by FEDEARROZ in its breeding program **(Table 2)**. This tolerance has also been confirmed in several replicated trials in the greenhouse and is being confirmed under field evaluations. The tolerant reaction of several rice lines from the interspecific cross between *O. rufipogon* x Oryzica 3 and rice lines from the germplasm bank observed under greenhouse conditions has been corroborated under field conditions in evaluations performed by FEDEARROZ at the Saldaña experiment station in 2003 and 2004. These lines could be used as potential donors of tolerance to this pathogen. Tolerance to sheath blight has also been identified in several advanced elite lines from FLAR **(Table 3)**. These lines are being used, as progenitors for other desired agronomic traits, or for potential release as commercial cultivars in different countries. The tolerance to sheath blight observed in these lines is a plus since they have never been selected for this trait.

Evaluation of the F2 generation in six different crosses do not suggest the presence of major dominant gene resistance as most F2 plants exhibited a susceptible or intermediate reaction to sheath blight. Tolerance seems to be controlled by the effect and accumulation of probably several minor genes. In all crosses, rice lines with better tolerance than the two parents were identified, suggesting the effect of transgressive segregation or accumulation of several genes. These lines were present in a group of 30-50% plant area affected. The highest number of susceptible F2 plants was observed in those crosses where the commercial cultivar Fedearroz 2000 was involved. However, tolerant lines better than the two parents were identified even in the cross between Fedearroz 2000 x Remadja. Although the cultivar Pankai exhibited a higher

tolerance to sheath blight than the cultivar Remadja, the tolerance of this last cultivar seems to be better inherited. Those crosses involving Remadja yielded more tolerant lines than those crosses with Pankai.

Table 1. Selection of the most tolerant lin es to *Rhizoctonia solani* in the greenhouse evaluation of 523 advanced lines from the cross *Oryza rufipogon* x Oryzica 3.

Rice Line	Plant Area Affected % Rice	Line	Plant Area Affected %
CT14524-2-M-2-1	28.0	**Checks**	
CT14534-2-M-5-2	29.0	ORYZICA 3	58.0
CT14543-6-M-5-2	30.0	ORYZA RUFIPOGON	58.0
CT14543-10-M-3-1	25.0	PN 1	70.0
CT14544-1-M-4-3	20.0	ORYZICA 1	72.0
CT14544-12-M-4-3	28.0	FEDEARROZ 50	64.0
CT14547-6-M-4-2	27.0	COLOMBIA 1	89.0
CT14547-31-M-7-1	26.0	CT 6096-7-4-4-3-M	97.0
CT14548-34-M-2-2	28.0	PALMAR	41.0
CT14554-20-M-4-4	22.0	REMADJA	36.0
CT 14546-8-M-M-2-2-M	25.0	PANKAI	52.0
		AMAZONAS	41.0

Table 2. Selection of Tolerant Segregating Lines to *Rhizoctonia solani* in the Greenhouse Evaluation of 171 Crosses from FEDEARROZ.

Line Identification	Cros s	Plant Area Affected %
FS1R007-2	Fs 261-8-1//Pankai/Fedearroz 50	19.0
FS1R010-9	Remadja / Fedearroz 50//Fedearroz 50	22.0
FS1R021-6	FOA-16-9/FL00809-26P-5-2-M	25.0
FS1R029-5	FL00809-26P-5-2-M/Fedearroz 50	25.0
FS1R029-6	FL00809-26P-5-2-M/Fedearroz 50	20.0
FS1R029-7	FL00809-26P-5-2-M/Fedearroz 50	14.0
FS1R029-13	FL00809-26P-5-2-M/Fedearroz 50	25.0
FS1R034-2	FSR1310-1-1-1/FL00984-10P-5-2P-M	25.0
FS1R034-6	FSR1310-1-1-1/FL00984-10P-5-2P-M	25.0
FS1R034-7	FSR1310-1-1-1/ FL00984-10P-5-2P-M	23.0
FS1R034-8	FSR1310-1-1-1/ FL00984-10P-5-2P-M	19.0
FS1R037-1	FSR 1185-1-1-1/ CT 13958-13-M-2-1-M-M	23.0
FS1R037-6	FSR 1185-1-1-1/ CT 13958-13-M-2-1-M-M	22.0
FS1R038-2	LV636-1-7-4-1/ FL00984-10P-5-1P-M	25.0
FS1R038-3	LV636-1-7-4-1/ FL00984-10P-5-1P-M	18.0
Checks		
LV636-1-7-4		22.0
FOA-16-9		27.0
ORYZICA 3		37.0
O .rufipogon		38.0
Fedearroz 50		41.0
PN-1		76.0
Colombia 1		64.0

Table 3. Elite Rice Lines fro m FLAR tolerant to *Rhizoctonia solani* in Greenhouse Evaluations in 2004.

Rice Line	Plant Area Affected %	Rice Line	Plant Area Affected %
FL03186-1P-7-3P-2P-M	30.3	**Checks**	
FL03197-22P-4-1P-2P-M	36.3	PALMAR	43.0
FL03186-1P-4-2P-1P-M	48.3	ORYZICA 3	48.3
FL03186-1P-4-3P-1P-M	46.7	REMADJA	50.7
FL03191-6P-9-2P-1P-M	48.7	*Oryza rufipogon*	53.0
FL03323-5P-21-1P-1P-M	44.8	PANKAI	58.3
FL03199-26P-3-1P-2P-M	50.0	ORYZICA 1	60.7
		COLOMBIA 1	66.3
		FEDEARROZ 50	67.0
		FEFFERSON	77.0
		FL03187-12P-5-2P-3P-M	87.3

Discussion: The new greenhouse screening method is suitable for selecting tolerant lines to sheath blight. It seems that the period of plant recovery after incubation for 15 consecutive days is allowing tolerant plants to express this reaction for a better differentiation with susceptibility. Modification in the evaluation scale is also allowing a better differentiation between tolerance and susceptibility as it considers the plant as a whole (plant area affected) and not the relative position reached by a single lesion (traditional scale). There is a better correlation between the percentage of plant area affected and the visual aspect of a diseased plant. The new method for screening and evaluation differentiates better between tolerance and susceptibility. This has permitted us to identify unknown tolerance present in the cultivated *O. sativa*. Genetic studies conducted in 2004 suggest that tolerance to sheath blight can still be increased by crossing tolerant lines. Our previous studies have suggested a possible isolate-cultivar interaction with the existence of races. We are reporting this year the characterization of the genetic structure of 140 isolates of the sheath blight pathogen from Colombia and have started pathogenicity tests of the same population.

Future Activities

Characterization of the genetic resistance present in the rice populations developed from the crosses between the wild species *Oryza rufipogon* and the commercial cultivars Oryzica 3 and BG 90-2 were initiated in 2003 and continued in 2004. More than 300 advanced lines have been evaluated for their sheath blight reaction and the resistance genes are expected to be located with the use of the microsatellite markers described in **Table 4**. Molecular markers associated with sheath blight resistance genes have been reported on different regions of Chromosomes 2, Chromosome 3, Chromosome 5, Chromosome 9, and Chromosome 11. Microsatellite markers saturating these regions have been identified and used in this study. Data is in the process of analysis and will be reported in 2005. These studies should allow us to develop a breeding strategy for incorporating tolerance to sheath blight in our breeding populations of the rice project.

Table 4. Rice Microsatellites used in the identification of Molecular Markers Associated with Tolerance to *Rhizoctonia solani* in advanced Populations of th e crosses between *Oryza rufipogon* with the cultivars Oryzica 3 and BG 90-2.

Chromosome 1	**Chromosome 5**	**Chromosome 9**
Rm 323 1.0cM	Rm 164 78.7cM	Rm 316 1.8cM
Rm 272 37.3cM	Rm 146 78.7cM	Rm 285 1.8cM
Rm 24 78.4cM	Rm163 78.7cM	Rm 105 32.1cM
Rm 297 155.9cM	Rm 430 78.7cM	Rm 288 74.6cM
Rm 315 165.3cM	Rm 161 96.9 cM	Rm 278 77.5CcM
	Rm 173 99.8cM	
Chromosome 2	Rm 233B 110cM	**Chromosome 10**
Rm 154 4.8cM	Rm 87 129.2c	Rm 222 11.3cM
Rm 279 17.3cM		Rm 239 25.2cM
Rm 290 66.0cM	**Chromosome 6**	Rm 184 58.3cM
Rm 221 143.7cM	Rm 170 2.2cM	Rm 228 96.3cM
Rm 318 150.8cM	Rm 217 26.2cM	
Rm 6 154.7cM	Rm 121 43.8cM	**Chromosome 11**
	Rm 340 133.5cM	Rm 202 54.0cM
Chromosome 3	Rm 345 145.2cM	Rm 287 68.6cM
Rm 232 76.7cM		Rm 209 73.9cM
Rm 251 79.1cM	**Chromosome 7**	Rm 21 85.7cM
Rm 227 143.2cM	Rm 125 24.8cM	Rm 206 102.9cM
Rm 135 157.3cM	Rm 180 30.1cM	Rm 254 110.0cM
Rm 186 168.2cM	Rm 214 34.7cM	Rm 224 120.1cM
Rm 143 207.3cM	Rm 11 47.0cM	
Rm 114 208.2cM	Rm 346 47.0cM	**Chromosome 12**
	Rm 346 47.0cM	Rm 4 5.2cM
Chromosome 4		Rm 19 20.9cM
Rm 307 0.0cM	**Chromosome 8**	Rm 247 32.3cM
Rm 564 73.1cM	Rm 152 9.4cM	Rm 309 74.5cM
Rm 273 94.4cM	Rm 339 72.2cM	Rm 17 109.1cM
Rm317 118.3cM	Rm 210 90.3cM	
Rm 131 148.8cM	Rm 256 101.5cM	

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Activity 5. Characterization of th e Genetic Structure of the Fungus *Rhizoctonia solani* Causal Agent of the Sheath Blight Disease of Rice.

Introduction

Sheath blight, caused by the anastomosis group AG1-IA of the complex *Rhizoctonia solani* is the second most important rice disease in the world. The increase in incidence of the disease is associated with modern techniques of exploitation of the crop that favor the pathogen such as planting high yielding semi-dwarf cultivars, use of high nitrogen applications, rotation with soybean, and high seeding rates among others (Marchetti, 1983). In Colombia, the disease became important since 1990 causing yield reductions up to 50% in the cultivar Oryzica 1 (Correa-Victroia, 1992). The genus *Rhizoctonia* has a wide number of species with characteristics of growth and plant symptoms similar to sheath blight, making it difficult any taxonomic classification, field identification, epidemiological studies, and management of the disease complex. This project aims at studying the genetic structure of the sheath blight pathogen in Colombia by using several molecular markers allowing establishing species, anastomosis groups and genetic diversity within pathogen populations. We expect to get a better understanding of this host-pathogen interaction to guide our work for developing resistant cultivars and appropriate disease management strategies.

Materials and Methods: A total of 140 isolates were studied including 130 isolates from the rice pathology CIAT's collection, two control isolates of *R. oryzae-sativae,* two *R. solani* AG1-IA from dry beans, and six isolates morphologically classified as *Sclerotium* spp. The isolates were collected from 24 rice cultivars representing 23 localities in the Departments of Tolima, Huila, Valle, Casanare, and Meta. The isolates were obtained from infected leaves, stems, or from soil (four isolates) and two isolates from the Brachiaria and pasto estrella pastures were included. The isolate collection extends from 1987 to 2004.

Each isolate was grown on rice polish agar for 24 hours and sub-cultivated from hyphal tips developing monothalic cultures equivalent to monosporic pure isolates. ADN was extracted from each isolate following the protocols of the CIAT's rice pathology lab (Escobar and Correa). Each DNA sample was amplified using the primer combination ITS 3/ ITS 4 of the Internal Transcribed Spacer regions of the fungus rDNA ribosomal genes (Liu and Sinclair, 1993; White *et al,* 1990), running the amplified fragments in agarose gels and staining with ethidium bromide. Amplified products were also digested with the restriction enzymes MBOI, HinfI, EcoRI, HaeIII and Taq I (15 enzyme units per 10ul of PCR product) and a total of 42 bans were determined. Similarity analysis (Dice index) and multiple correspondence analyses were performed for each individual enzyme and on the total PCR plus the five restriction enzyme data.

Five primer combinations with reported specificity for distinguishing the species *R solani, R. oryza-sativa, and R oryzae* (Johanson *et al,* 1998) were used for the identification or diagnostic of species. These primers were developed after secuencing the regions 5.8S of the rDNA ribosomal genes of each species. Genetic variability was preliminarily determined by using the RAPD primer 91300 (Lilja *et al,* 1997).

Results: Amplification with the primers ITS3/ITS4 with no digestion produced four fragments while digestion of the amplified products with the five different enzymes yielded a total of 38 bands. Similarity analysis of the no digested products revealed four genetic groups with one, three, 127 and nine isolates per group **(Table 1)**. Genetic group # 3 was the largest and included the *R. solani* isolates from beans and the *R. oryza-sativa* controls. Group one had only one isolate collected from Valle (Jamundí), which was classified with the species-specific primers as both *R. oryza-sativae and R. oryza.* Group three included three isolates that had some specific characteristics such as small and round sclerotia, black sclerotia collected from stems, or production of white mycelium on agar media. However, the three isolates were classified as *R. solani* by the species-specific primers. Pathogenicity and anastomosis tests will be conducted to determine their association with rice. Group four had nine isolates: six had morphological characteristics on agar media similar to *Sclerotium* with an intense black color. Three of them were not classified as any of the three species pathogenic on rice with the species-specific primers, two were classified as *R. solani*, and one classified as both *R. solani/R. oryza-sativae*; another isolate recovered from a soil sample was classified as *R. solani*, and the last two isolates classified as probably *R. oryzae* with also a weak band for *R. solani* and collected from the cultivar Oryzica 3 although there was no record if from leaves or from stems.

Table 1. Number of Genetic Groups in th e Analyses of Sheath Bligh t Pathogen Populations using the Interspace ITS 3- ITS 4 Regions of the Ribosomal Genes Amplified and Digested with Five Enzymes.

Marker / Digestion	PCR ITS3-ITS4	Digestion MBOI	Digestion HinfI	Digestion EcoRI	Digestion HaeIII	Digestion Taq I	Digestion 5 Enzimas	PCR + Digestion 5 Enzymes
Groups	**4**	**7**	**8**	**5**	**6**	**5**	**14**	**14**
Number of	1	1	1	1	1	1	4	4
Isolates	3	32	2	32	31	1	2	2
Per	127	7	7		2	2	79	79
Genetic	9	1	87	3	1	127	9	9
Group		89	9	95	7	9	1	1
		8	1	9	4		1	1
		2	2		94		30	30
			31				1	1
							1	1
							1	1
							1	1
							1	1
							7	7
							2	2

Digestions of the PCR products with the five different enzymes yielded different number of genetic groups **(Table 1)**. Analysis of the data pooling results for the five restriction enzymes or adding the PCR products without digestions data to the digested data formed fourteen genetic groups **(Table 1)**. Isolates within groups 1, 2, and 4 of the PCR products without digestion were further separated into six genetic groups (Groups 6, 10, 11, 12, 13, 14, **Table 2**). Isolates classified as different to *R. solani* were left in different groups (group 6 and group 14); isolates named as Sclerotium were within group 13, which also included a soil isolate. The largest group 3 with 127 isolates was divided in 11 genetic groups. Some of the groups were formed by single isolates, which in general had some specific characteristics such as a specific site of origin,

collected from soil or stems, or originated from sclerotia. In general, the largest group 3 (after digestion) obtained after pooling all the data included isolates obtained from infected leaves and which were classified as *R solani* using specific primers **(Table 3)**. The second largest group 7 included many isolates classified as no *R. solani*, which originated mainly from infected stems of the cultivar Fedearroz 50 or from sclerotia **(Table 3)**. This group included two control isolates of *R. oryzae-sativa* obtained from Uruguay. Interestingly, these two isolates were not classified within any of the expected three species *R. oryzae, R. oryza-sativae, or R. oryza* by the species-specific primers. The two control isolates obtained from dry beans were classified within group 1 together with two isolates obtained from sclerotia or from the soil. The two bean isolates AG1-IA were classified as *R. solani* by the specific primers. Preliminary observations indicate that a single plant can be infected by more than one genetic group of the pathogen, however, it is possible that only one group, and most probably group 3 containing *R. solani* pathogenic rice is economically the most important. Studies using RAPDs also exhibited between 1 to 3 different haplotypes within a single genetic group **(Table 4)**. These variants will be evaluated in terms of their pathogenicity and aggressiveness in inoculation studies.

Table 2. Relationship between Genetic Groups Determined based on the Amplif ication and Digestion of the ITS 3/ITS 4 region of the Ribosomal Genes of the Rice Sheath Blight Pathogen.

Isolates	PCR No digest. 4 groups	Dx1 MBOI 7 groups	Dx 2 HinfI 8 groups	Dx 3 EcoRI 5 groups	Dx 4 Hae III 6 groups	Dx 5 Taq I 5 groups	Dx´s 1 to 5 14 groups	PCR + Dx´s 14 groups
VJD03A_2768_F2000	g1 (1)	g2 (1)	G1 (1)	g1 (1)	g1 (1)	g4 (1)	g6 (1)	g6 (1)
TCP88A-2063_01C	g2 (3)	g 4 (1)	g6 (1)	g4 (1)	g2 (1)	g3 (1)	g7 (1)	g10 (1)
XXX01B_2766_QR6		g 4 (1)	g6 (1)	g4 (1)	g2 (1)	g3 (1)	g8 (1)	g11 (1)
TSD04A-2854-TAI4		g 7 (1)	g 5 (1)	g4 (1)	g4 (1)	g5 (1)	g12 (1)	g12 (1)
VLCO2B_2756-01L	g3 (127)						g1 (1)	g1 (1)
Tpf00A_2793_F50							g1 (1)	g1 (1)
96B_2835_RhAG1-I-Col							g1 (1)	g1 (1)
M43 bean							g1 (1)	g1 (1)
96B_2836_RhAG1-I-C								
M43 bean								
VJD88A_1929-01M							g2 (1)	g2 (1)
VJD88A_1929-01M							g2 (1)	g2 (1)
XXX01A_2705_M F.VICT.1							g5 (1)	g5 (1)
TSD00A_2785-F50							g10 (1)	g8 (1)
TSD00A_2775-F50							g11 (1)	g9 (1)

Pathogenicity tests will be conducted by inoculating several rice cultivars with several isolates representing each one of the fourteen genetic groups and the different genetic variants (haplotypes) within a genetic group determined by RAPDs. These studies will let us know in more detail the symptomatology caused by them and their association with any part plant (leaves, stem). We will determine if the sheath blight disease observed in the fields could be a complex of several pathogens represented in the different genetic groups found, which could

infect simultaneously different plant parts. These isolates will also be grown under different artificial media to determine their phenotypic and morphological characteristics in terms of mycelia growth and sclerotia developed. We will also determine if there is any intereaction isolate-cultivar or differences in aggressiveness in different rice cultivars, which would have important implications in our breeding program for developing rice cultivars with tolerance to the sheath blight pathogen. It will be necessary to determine the best sample collection representing a wide genetic diversity of this pathosystem to conduct more detailed studies. These collections should include mainly different regions, different cultivars, different plant parts, different rice production systems (irrigated/upland), and different structures (sclerotia's size, color, shape) of the pathogen developed on the infected tissue. These studies should also include morphological and cytological observations as well as determination of the anastomosis groups and a detailed observation of the symptoms developed.

Table 3. Genetic Group and Species of some Isolates Associated with Rice Sheath Blight Symptoms.

Isolates	PCR 4 groups (No. Isolates)	Species (Johanson Markers)	Global: PCR + Digestions 14 Groups
R. oryza-.sativae			
2820 M	3 (127)	-	7
2821 M	3 (127)	-	7
Rhizoctonia Control causing sheath blight			
2399-1r	3	*R. solani*	3
2399-copy			
Oryzica Llanos 5-1			
2369-1r R. s.	3	-	3
Fedearroz 50			
2599	3	R. s.	3
2679-01	3	R. s.	3
2695	3	R. s.	3
2695-01	3	R. s.	3
2695-02	3	R. s.	3
2738-01	3	R. s.	3
2770 r3 new	3	R. s.	3
2826	3	R. s	3
2832 f	3	R. s.	3
2777	3	No R. s.	7
2778	3	R. s	7
2780 m	3	No R. s	7
2791	3	No R. s ¿?	7
2794	3	No R. s	7
2796	3	No R. s	7
2798	3	No R. s	7
2800	3	No R. s	7
2805	3	No R. s	7
2806	3	No R. s	7

Isolates	PCR 4 groups (No. Isolates)	Species (Johanson Markers)	Global: PCR + Digestions 14 Groups
2810	3	No R. s	7
2823 M	3	No R. s	7
2824	3	No R. s ¿?	7
2825	3	No R. s	7
2785	3	No R. s ¿?	8
2793	3	R. s.	1
2775	3	R. s	9
Fedearroz 2000			
2704	3	*R.s.*	3
2768	1	*R.o.s./R.o.*	6
2769	3	*R.s.*	3
Rhizoctonia Frijol			
XXX96B_2835_RhAG1-I-Col M43 frijol	3	*R.s.*	1
XXX96B_2836_RhAG1-I-C M43 frijol	3	*R.s.*	1
Rhizoctonia (Brachiaria)			
2744-01r	3	*R.s.*	3
Rhizoctonia (Pasto Estrella)			
2745	3	*R.s.*	3
Rhizoctonia (soil)			
2390	3	*R.s.*	3
2828 Saldaña	3	*No R.s.*	7
2830 campo alegre	3		7
NR1 (MER)	4		13
Sclerotium	4		13
2813		*R.s.*	
2814		*No R.s.*	
2816		*R.s.*	
2817		*No R.s.*	
2818		*R.s./R.o.s.*	
2819		*No R.s.*	

Table 4. Genetic va riation (R APD Patterns) w ithin ITS Gene tic Groups of some Isolates Associated to Sheath Blight Symptoms on Rice.

Isolate	Isolates per Plant	No. ITS Groups	RAPD Pattern No. Culti	var	Date Collection	Site Collection	Zone
1700	2	1	2	Oryzica 3	88[a]	Jamundí	Valle
1929	2	1	1	Oryzica 3	88[a]	Jamundí	Valle
1953	8	1	2	Oryzica 1	88A	Saldaña	Tolima
1954	7	1	1	Cica 4	88[a]	Saldaña	Tolima
1957	3	1	1	F3	87B	Santa Rosa	Meta
1959	7	2	3	Esparcidor	87B	Santa Rosa	Meta
2052	2	1	2	-	88A	Tolima	Tolima
2054	2	1	2	-	88A	Salda	Tolima
2059	2	1	1	-	88A	Tolima	Tolima
2062-1r	5	1	1	Cica 4 ?	88A	Ambalema	Tolima
2237	2	1	1	-	89A	Agua Chica	Cesar
2399	2	1	1	Oryzica 1	93A	Tolima	Tolima

The correspondence multiple analyses (CMA) classified the pathogen population in five genetic groups **(Table 5)**, which in general coincides with the classification obtained in the similarity analyses. Group1 with 68% of the isolates (95) included most of the isolates classified as *R. solani*. This group includes the two isolates from pastures, which had also been included in the genetic group 3 in the similarity analyses and which were classified as *R. solani*. These isolates will probably be pathogenic on rice determining two potential hosts of the pathogen. The bean isolates were also included in this genetic group, however they appeared in a different group according to their ITS regions. Group two included the 9 isolates from groups 13 and 14 of the diversity analyses being equivalent to group 4 of the non-digested PCR products. Group 3 included 32 isolates, which are mainly non-*Rhizoctonia* according to the specific primers used. Once again, the two control isolates obtained as *R. oryzae-sativae* were grouped together with the non-*Rhizoctonia* isolates in this group 3. Groups 1 and 3 of the CMA have a similar structure to groups 3 and 7 of the diversity analysis, respectively.

Group 4 included three isolates (2063, 2766, 2768-01), which have specific characteristics (black sclerotia isolated from the sheath, small-round sclerotia, and Jamundí origin, respectively). The isolate 2768 was classified as *R. oryza/R. oryza-sativae* while the other two as *R. solani.* Group 5 included only the isolate 2854, which produces a white mycelium on artificial media and no-sclerotia. This isolate was collected from the cultivar Tailandia 4 in Saldaña-Tolima in 2004, however was classified as *R. solani* by the species-specific primers.

We used reported species-specific primers (Johansen and Tuerner, 1998) to diagnose the presence of the species *R. solani*, *R. oryza-sativae*, *and R. oryza.* In many cases, *R, solani* isolates were positively identified, however, there were several pathogenic isolates that were not identified with these markers, and in other cases, the isolates were classified in more than one

species **(Table 6)**. We need to elucidate if this is a problem with the specificity of the markers or to the presence of a different pathogen. Parmeter (1967) reports that from lesions similar to sheath blight it is possible to obtain not only different strains of basydiomycetes, ascomycetes, and deuteromycetes, but also different strains of *R.* solani. Turner *et al* (1999) using these species-specific primers detected the presence of at least two of the three species in a single field or in a single plant in India. Pathogenicity tests will also help to understand this pathogen-host interaction. There were clear associations however in the grouping of isolates both in the diversity analysis as well as the CMA with the classification of isolates as *R.* solani or as no *R. solani*. These results suggest that *R.* solani is more important than the other two species in the Rhizoctonia complex in Colombia. We will repeat the test with more isolates to determine the specificity and the real potential use of these markers in the identification of species. Isolates should be collected in similar numbers from different parts of the plant as in general, isolates collected from infected leaves correspond to *R. solani.*

Table 5. Genetic Groups of Fungal Isolates Associated with Sheath Blight Symptoms on Rice based on Multiple Correspondence Analysis (MCA).

MCA Groups	Isolates Number	%
1	95	67.86
2	9	6.43
3	32	22.86
4	3	2.14
5	1	0.71

Table 6. Rice Isolates Associated with Sheath Blight Symptoms Classified as more than one Species based on Species-Specific Markers develop ed by Johansen and Turner (1998).

Isolate	Host or Classification	Species		
		R. solani	***R. oryzae sativae***	***R. oryzae***
2818	Sclerotium	+	+	-
1959-3	Esparcidor	+	-	+
2064-5	Oryzica 1	+	-	+
2752-1	None	+	-	+
2768	Fedearroz 2.000	-	+	+

These studies will allow us to determine which species is the most important economically in rice farmers fields Colombia and if there are other rice pathogens causing similar symptoms to sheath blight on the plant leaves or the lower part of the stems.

Discussion and Future Work: Establishing the genetic structure and genetic relations of the sheath pathogens of rice using the interspace ITS genetic regions of the ribosomal RNA genes together with other molecular markers is necessary to understand this host-pathogen interaction in order to be able to develop breeding strategies focused on the development of resistant cultivars and to develop an integrated management of the disease. These studies will be complemented with pathogenicity tests of the populations studied as well as morphological characteristics and determination of anastomosis groups on artificial media in the laboratory during 2005.

We should explore the possibility to develop more specific primers for the identification of all the species and forms present in Colombia and other countries in the regions as the results obtained in this study suggest that the markers reported in the literature may not be adequate enough for much diverse pathogen populations. These results also suggest the need to develop a regional project to study the sheath pathogens associated with the disease as we may be facing different pathogens/species increasing in importance in each country. Collection of isolates should include infected samples from different countries, sites, cultivars, plant parts, etc.

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AFRICA: BEAN PATHOLOGY

Activity 1. Characterization and distribution of Pythium spp associated with bean root rot in East Africa.

Rationale

Among approximately 100 known species of the genus *Pythium* include pathogenic, saprophytic and biological control groups. Our recent studies in Uganda have shown that over seven *Pythium* spp cause root rots on common beans, but their distribution and relative importance in other countries in East Africa are unknown. Characterization of *Pythium* species and their distribution is therefore considered a necessary pre-requisite in order to develop effective management strategies. However, identification of *Pythium* species using morphological or pathogenic characteristics is difficult given the large species numbers and their mixed occurrence in the soil. We have therefore continued with the characterization of Pythium spp, using molecular methods as a basis for developing simpler, accurate and rapid but reliable detection and characterization techniques. We therefore continued to characterize *Pythium* spp prevalent in Kenya and Rwanda

Methods: One hundred and thirty-four Pythium isolates obtained from root rot affected areas in Kenya and Rwanda were characterized by sequencing using the protocol of Levesque *et. al.* (1998). The DNA of isolates was amplified with universal eukaryotic primers targeting the internal transcribed spacer (ITS) regions and the 5.8S gene of nuclear ribosomal DNA. Purified template DNA was sequenced using an ABI prism automated sequencer. Sequences obtained were edited and compared to data of *Pythium* spp managed by Dr A. Levesque of the Agri-Food and Food and Agriculture Canada.

Results and Discussion: Out of 134 isolates characterized, 22 species were identified **(Table 1)**. Thirteen of these have been reported in our previous pathogen characterization studies in Uganda and Kenya but nine were new additions. All except three (*P. macrosporum, P. zingiberis, P. graminicola*) species were recovered from Rwanda with *P. ultimum* being the most frequent, followed by *P. torulosum* and *P. spinosum.* The three are pathogenic to beans. Fifteen of the 22 species were recovered from Kenya with *P. vexans* being the more frequent species, followed by *P. torulosum, P. irregular and P. ultimum.* Species distribution maps for Kenya and Rwanda are shown in **Figures 1 and 2** respectively. These results are consistent with past observations that overall *P. ultimum* is the most frequent species in the region. Pathogenicity of some of the new species is being determined to establish their role in the bean root rot problem in the region.

Table 1. Identification by sequencing of *Pythium* isolates obtained from bean growing areas associated with bean root rots in Kenya and Rwanda.

Species	Pythium Isolates		
	Kenya	Rwanda	Total
P. acanthicum	2	1	3
P. chamaehyphon	1	2	3
P. folliculosum	3	2	5
P. indigoferae	2	2	4
P. irregulare	9	1	10

Species	Pythium Isolates		
	Kenya Rw	anda	Total
P. lutarium	1	3	4
P. macrosporum	1	0	1
P. myriotylum	1	1	2
P. paroecandrum	3	3	6
P. torulosum	9	10	19
P. vexans	10	4	14
P. zingiberis	5	0	5
P. graminicola	4	0	4
P. spinosum	1	7	8
P. ultimum	5	23	28
P. arrhenomane	0	2	2
P. catenulatum	0	1	1
P. deliense	0	1	1
P. diclinum	0	2	2
P. dissotocum	0	3	3
P. rostratum	0	5	5
P. salpingophorum	0	4	4
Total	57	77	134

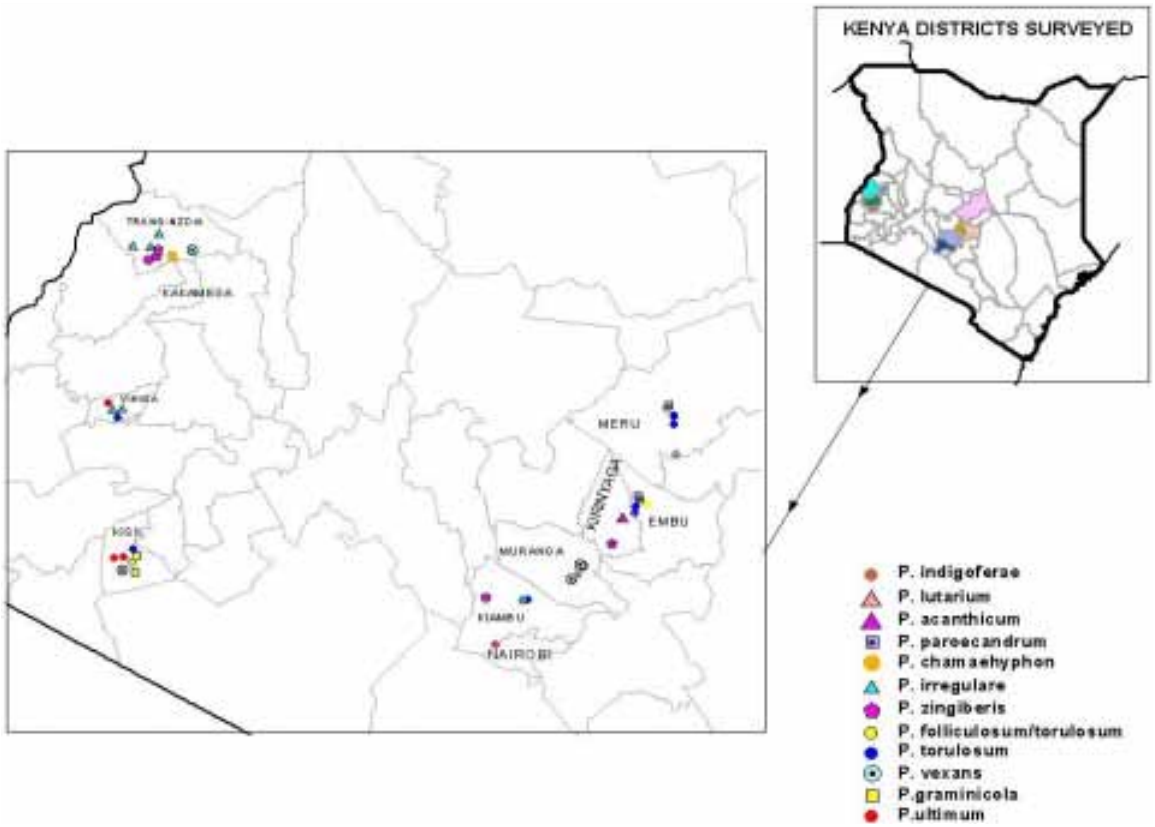

Figure 1. Distribution of Pythium species in some districts of Kenya w here bean root rots is prevalent. Characteriz ation w as ba sed on sequencing of Pythium isolates.

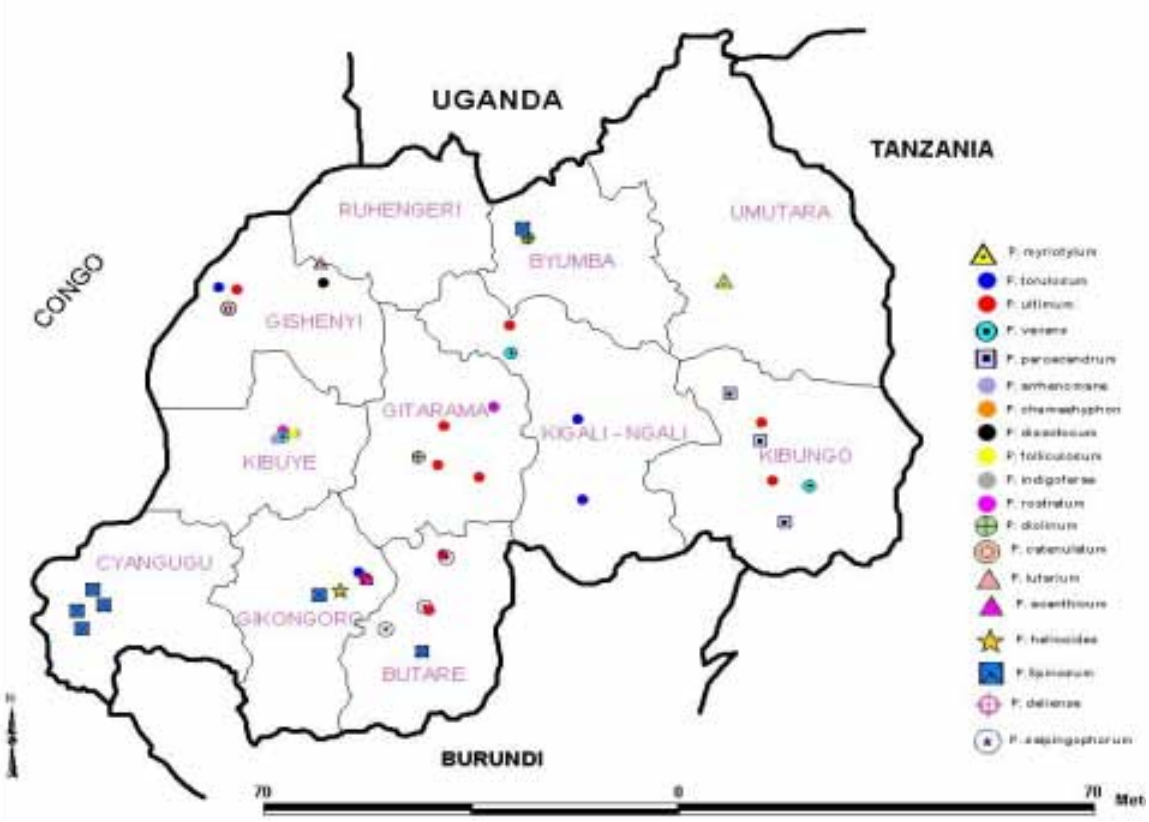

Figure 2. Distribution of Pythium species in Rwanda where bean root rots is prevalent Characterization was based on sequencing of *Pythium* isolates.

Progress towards achieving output milestones

- Pythium isolates (134) from root rot affected areas in Kenya and Rwanda were characterized by sequencing of ITS-1 region. *P. ultimum* was the most frequent occurring species followed by *P. torulosum*.
- Pythium distribution maps showing relative importance of characterized species in Kenya and Rwanda were developed.

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Activity 2. Developing integrated pest management components of root rots.

Pathogenicity of *Pythium* spp and effects of management options for root rots on crops grown in association with beans in southwest Uganda

Rationale

Beans is one of the crops grown under the intensive agricultural system in southwest Uganda. Others include sorghum, maize, sweet potatoes, Irish potatoes, bananas and peas. Crop rotation in the strict sense is rare. Dominance of crops in the field shifts according to season. Rotations commonly practiced include beans-maize-sorghum, beans-maize-beans and beans-Irish potato/maize-sweet potato (Edidah, 2003). Maize and sorghum are also intercropped with beans and/or Irish potatoes such that the bean crop appears in the field season after season. However, of all these crops, beans are most affected by root rots. In recent years this has resulted in the decline in bean production in the area. Given that some of the root rot causing pathogens (e.g. Pythium spp) are known to have a wide host range, some of the questions asked are: do crops grown in association or in rotation with beans play any role in the pathogen survival, inoculum density and severity of root rots in beans?; is bean the only crop in the system that is affected or is it simply a good indicator of the level of root rot pathogens?; to what extent are other crops in the system affected by bean pathogens?; what are the effects of management options for bean root rots on other crops? To address these questions, we initiated studies to characterize Pythium spp associated with major crops found in the bean based systems; to determine pathogenicity of some Pythium species on these crops; and to determine the effects of management options for bean root rot on crops grown in association with beans.

Materials and Methods

***Pathogenicity studies*:** Three *Pythium* species pathogenic to beans (*P. ultimum, P. chamaehyphon, P. pachycaule)* were artificially inoculated on three crops commonly associated with beans namely: sorghum, millets and maize. Autoclaved millet (100 g) was mixed with 200 ml of water in a 500-ml bottle and subsequently used to raise the fungi. After two weeks of incubation, the infested millet was mixed with pre-sterilised soil at a ratio 1:10 v/v in wooden trays. Maize, sorghum and millet were planted in two rows of twelve plants and replicated in three trays. Bean varieties CAL 96 and RWR 719 were used as susceptible and resistant checks respectively. Cumulative emergence and plant stand was recorded one week after germination. Three weeks after germination, plants were assessed for any root and shoot symptoms that may be associated with *Pythium* infection.

Effect of management practices: Four crops; beans (B), sorghum (S), maize (M), and peas (P) were subjected to four amendments i.e., farm yard manure (FYM), green manure (GM), inorganic fertilizer (NPK), fungicide (Ridomil) in farmers fields in Rubaya, Kabale district, southwest Uganda. Sorghum, maize and peas seed were obtained locally from farmers. A root rot susceptible bean variety (CAL 96) was used as a check. Farm yard manure and green manure (Crotalaria) were applied on a dry weight basis at a rate of 5t/ha and their nutrient level determined. NPK fertilizer was applied at a rate of 50 kg of N/ha. Ridomilwas applied as seed treatment (slurry) at a rate of 2.5 kg/ha. Qualitative data was obtained through field observations

and photography. Quantitative data collected included: emergence, plant stand, disease incidence and severity at different times during the growing season, plant vigor and yield parameters (dry matter poduction). Disease severity was evaluated according to a CIAT nine-point scale where 1 is resistant and 9 susceptible (Abawi and Pastor Corralles, 1990)..

Results and Discussion

Pathogenicity studies: The different *Pythium* species invoked typical root rot symptoms on susceptible bean cultivar CAL 96 in screen house studies. As expected, cultivar RWR 719 was resistant. Sorghum exhibited severe stunting and purple color on leaves. These features were more pronounced with isolate KAK 5 B (*P. pachycaule)*. Similarly, millet exhibited stunting as well as yellowing and drying of the leaf tips, unlike plants in un-inoculated control trays. Maize showed less pronounced effects characterized by reduced plant vigor and size.

Symptoms on roots of sorghum were comprised of red-black lesions and discolorations, reduced root mass and length. Millets displayed some lesions and reduced root mass. Maize exhibited little if any lesions on roots 3 weeks after emergence although root mass was relatively lower than in the control trays. Pythium was re-isolated from roots of all crops grown in infected soil.

These screen house results showed that *Pythium* species used had an effect on the different crops tested. The most affected crop was beans and then sorghum, millet and maize in that decreasing order. Maize exhibited an interesting reaction in that there was some reduction in both shoot and root mass but little necrosis on the latter. Stunting in crops is attributed to reduced capacity of roots (either due to damage or reduced amount) to support adequate water and food uptake. We can tentatively conclude from these preliminary observations that *Pythium* species pathogenic to beans cause damage to sorghum, millets and maize to varying degrees. Further investigation to elucidate these interactions is going-on.

Effects of management options on incidence and severity of root rots: The management options evaluated affected the crops in different ways. FYM and ridomil significantly reduced initial root rot infection on beans. High incidence of root rots was observed with GM and attributed to interactions between the root rot pathogens and soil micro-organisms. But FYM, GM and NPK, enhanced root (mass) growth in beans, contrary to observations in control plots.

As in screen house studies, infected sorghum plants exhibited stunted growth, purple leaves, shoot death and dark-red to black root lesions **(Figure 1)**. Significantly high incidence and severities were observed in control plots particularly 54 and 72 days after planting. Amendments reduced these effects and plant recovery was evident in plots amended with GM, FYM and NPK.

Symptoms on maize were expressed as grey lesions on roots **(Figure 2)** , stunting and poor establishment. However, incidence and severity were low indicating that maize was less affected **(Figure 3a)**. As with sorghum, amendments and particularly FYM reduced severity **(Figure 3b)** and improved plant vigor and growth.

Figure 1. (Left) Sev erely affecte d sorghum r oots (con trol plo ts) and (Right) sorghum root with prop root development in plots amended with NPK

Figure 2. Maize roots showing root lesions.

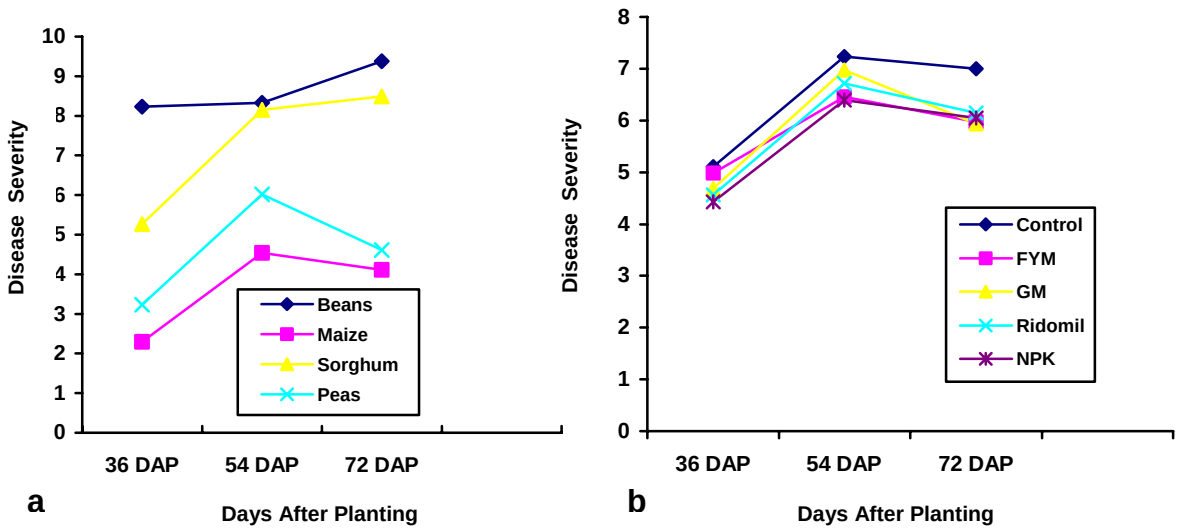

Figure 3. Disease severity in crops over the season (left), and effects of different root rot management practices on root rots over the season (right).

NPK, GM, FYM and ridomil (in sorghum) improved dry matter production (DMP) in both maize and sorghum **(Table 1).** Improved DMP in sorghum due to ridomil is probably due to its protective effect against *Pythium* species.

Table 1. The effect of different soil amendments on mean dry matter production (72 days after planting) for maize and sorghum. Rubaya, Kabale, 2004 season A.

Crop/Treatment	Dry Matter (g)	
	Maize	Sorghum
Control	106.9	18.5
Farm yard Manure	128.3	42.7
Green Manure	138.4	38.9
Ridomil	112.5	46.1
NPK	163.2	48.8
	L.s.d at $p < 0.05$ (32.20)	

Overall the different management options evaluated influenced severity of root damage and other growth parameters on crops grown in association with beans. This implies that the use these options do not only contribute in the management of bean root rots, but are also beneficial to other crops. Studies are underway to further define this contribution**.**

Progress towards achieving output milestones

- Pythium species pathogenic to beans were shown to cause damage on cereal crops grown in association with beans implying that they may be hosts of the pathogens.

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The Systemwide Tropical Whitefly IPM Program

Activity 1. Coordination.

Executive Summary

Background: The Co-ordinator's position was created by the original Task Force that conceived the Tropical Whitefly IPM Project (TWFP), to co-ordinate the various research activities conducted by the different subprojects that operate in Africa, Asia and Latin America. The TWFP was born under the umbrella of the CG System-wide IPM Programme, but Phase I was initiated thanks to the financial support of the Danish Development Agency (DANIDA) following negotiations with the Co-ordinator of the TWFP.

During Phase I, the appointed Co-ordinator followed an active global agenda to incorporate more research groups and additional donors to pursue complementary research activities, such as the search of sources of resistance against whiteflies in cassava (New Zealand AID) and against whitefly-borne viruses of horticultural crops in Asia (ACIAR).

The TWF Coordination (CIAT-based) has maintained the communication and coherence of the research conducted by the different subprojects of the TWFP in: Africa (two cassava subprojects, one of which is also financed by USAID); Asia (begomoviruses of horticultural crops, financed by ACIAR); Central America, Mexico and the Caribbean (common bean and horticultural crops in Mesoamerica); the Andean Region (common snap beans); and East Africa (horticultural crops and begomoviruses). The coordination of the TWFP has also maintained close linkages with other DFID-funded whitefly projects managed through the Crop Protection Programme (CPP), particularly in Africa and Asia, and has facilitated the interaction and exchange of sources of resistance between IITA and CIAT (cassava) and between national programs (CENTA-El Salvador and INIFAP-Mexico and AVRDC).

The Coordinator of the TWFP created and supervises the Communications Office located at CIAT, which manages the databases that contain global information on pertinent literature, whitefly research networks, collaborating scientists and technical guidelines, mainly through a Web Page created for this purpose. This office also compiles and submits technical reports produced by the various subprojects of the TWFP.

Project purpose: The DFID-funded Tropical Whitefly IPM Project (TWFP-Phase II) sought to implement sustainable pest management strategies to control the devastating yield losses caused by whiteflies and whitefly-transmitted viruses in cassava, sweet potato, common bean, tomato, sweet pepper, chilli, and other horticultural crops, and, thus, prevent hunger and famine, and ultimately improve the livelihood of resource-poor farmers in developing countries of Africa, S.E. Asia, and Latin America.

The specific objectives of Phase II of the TWFP were to : 1) Strengthen the pan-tropical whitefly research network created during Phase I by developing information management and exchange channels to disseminate research findings among project collaborators (NARIs, Universities, NGOs, Advanced Research Laboratories, Farmer Associations, Policy Institutions, and the

general public interested in these topics). 2) Undertake basic studies on whitefly population dynamics and disease epidemiology in order to understand whitefly/virus pathosystems and thus implement effective IPM strategies. 3) Select and evaluate the most promising IPM measures available to date in selected 'hot spots' identified in Phase I, in order to develop area-wide IPM packages for crops and/or cropping systems currently affected by whiteflies and whitefly/transmitted viruses. 4) Develop training materials for the last phase (III) of scaling up the dissemination of results on the most suitable IPM packages validated in the different whitefly-affected regions of Africa, Asia and Latin America.

The economic importance of whiteflies as pests and vectors of plant viruses was recognised in the late 1980s, not only by developing countries but by industrialised nations as well, including the United States, where a "National Research, Action, and Technology Transfer Plan" was conceived in 1991 to combat the 'silverleaf whitefly' (*Bemisia tabaci* biotype B), and European and Middle East countries in the Mediterranean region. Popular news media called it the 'Pest of the Century' and yield losses have been calculated in the billion of dollars, leading in some countries to famine, as in the case of Uganda, following the emergence of a recombinant variant of different viruses associated with African cassava mosaic disease. Whitefly-transmitted viruses also caused the collapse of food and industrial crops in Latin America, particularly in Central America, Mexico and the Caribbean region. Nascent industries (tomato paste) in the Dominican Republic and Haiti had to close down following the epidemics of *Tomato yellow leaf curl virus* in the 1990s. The entire industry of vegetable production for export to North America also collapsed in the 1990s due to the emergence of numerous viruses transmitted by *B. tabaci* throughout Mesoamerica.

Small-scale farmers throughout the tropics have been struggling for the last two decades to increase the income derived from their limited land resources, by diversifying their subsistence crops with more valuable crops, mainly vegetables. Unfortunately, neither national nor international agricultural research institutes have provided technical assistance to resource-poor farmers for non-traditional crops, leaving poor farmers in the hands of pesticide salesmen. As a result, production costs have increased (up to 60% of current vegetable production costs are related to crop protection), environmental and human contamination due to pesticide residues has become a serious problem, agricultural produce is rejected by international markets that test for pesticide residues, and pest problems have worsened due to the development of insecticide resistance and elimination of the beneficial bio-control fauna. The TWFP focuses on all of these production and environmental problems with the ultimate purpose of improving the livelihood of the poor rural and urban population of developing countries in the Tropics.

Coordination activities: The Coordination of the TWFP has contributed to the strengthening of whitefly management networks in developing countries, and to the exchange of information compiled from all of the accessible and grey literature sources on whiteflies. A database has been created at CIAT, which has also establish links with a complementary database created by USDA on whitefly research conducted primarily in the U.S.A. and other developed countries. A directory of professionals conducting research on whitefly-related production problems around the world has also been created.

A Web Page (www.tropicalwhiteflyipmproject.cgiar.org) has been gradually developed to make all of the available information on whitefly and geminivirus management, accessible to users around the world.

The project has a Communication Office and a communications specialist in charge of information dissemination and data management, including Geographic Information Systems (GIS) to implement more interactive channels for data reporting and sharing among users. The Co-ordinator has been involved in the improvement of the information dissemination capabilities of the TWFP, including various articles, presentations and publications that describe the work and results obtained by the TWFP and related CPP projects financed by DFID.

The main publication in the pipeline is a book containing the results of all of the diagnostic work conducted in Phase I: surveys, biological characterisation of whitefly species/biotypes, molecular characterisation of begomoviruses, description of crops affected, identification of 'hot spots' in east and west Africa, the Andean region, Central America, Mexico and the Caribbean, and socio-economic studies.

The building of the Tropical Whitefly Research Network started in Phase I, and it is now linked to all the national and international projects that conduct research on whitefly pests and related problems around the world. This network includes the International Whitefly Studies Network, managed from the U.K., which includes all of the European countries that suffer crop losses caused by whiteflies and the different viruses these insects transmit, mainly in the Mediterranean region.

The TWFP Coordinator is also involved in the strengthening of National Agricultural Research Institutions (NARIs) that have not been previously covered by the TWFP, but which have asked the TWFP for assistance to manage severe whitefly problems. This has been the case of the Andean Region, where whiteflies have recently emerged as direct pests and virus vectors, particularly in Bolivia, Perú and Colombia. The Coordinator has been invited by these Governments to observe the whitefly problems that affect crops, such as potato, common bean, tomato, and cucurbits. A special report was prepared on the whitefly problem of mixed cropping systems in the mesothermic valleys of Bolivia, where DFID has special projects. With respect to collaborating NARIs, the TWFP Co-ordination has provided training for some national program scientists, particularly in the area of molecular characterisation of plant viruses and whitefly species/biotypes, but also in the area of rural development and participatory research.

The emergence of *Bemisia tabaci* biotype B, as a vector of viruses affecting common bean and tomato in the main agricultural region of Colombia, the Cauca Valley, is primarily a consequence of unusual climatic phenomena (climate change), represented by persistent, dry conditions for two consecutive years beginning in 2001. The attacks have been so severe that snap bean production has been eradicated from the valley in the last year. The TWFP Co-ordinator organised a series of talks for tomato farmers in order to explain the nature of the problem and recommend and IPM package, which has been successful in maintaining tomato production in this region.

In 2003, the Co-ordinator received an urgent request from El Salvador to diagnose an unusual whitefly outbreak in cereals and grasses, mainly rice, sorghum, maize, and forage pastures. This

is the first time that grasses have been attacked in Mesoamerica by whiteflies. Samples were sent to CIAT where the TWFP's taxonomist, Ms. María del Pilar Hernández, identified the exotic whitefly pest as *Aleurocybotus occiduus*. This is apparently a neotropical whitefly species that has been previously reported in S.W. USA, and the Amazon region of Perú, where it was also reported on rice. The Coordinator visited the affected rice-growing area in N.W. El Salvador and confirmed the complete destruction of over 35 has of rice **(Figures 1 and 2)** and several sorghum fields.

Figure 1. Total yield loss in rice fields of El Salvador caused by the emergence of a new whitefly pest capable of attacking cereals.

Figure 2. Damage and pupae of the new whitefly pest of rice in El Salvador.

The TWFP has also provided information and advice to CPP (“Adaptive evolution within *Bemisia tabaci* and associated *Begomoviruses*: A strategic modelling approach to minimising threats to sustainable production systems in developing countries” by Frank van den Bosch and M.J. Jeger), and other international projects currently engaged in the validation of sustainable IPM practices and modelling of whitefly/geminivirus epidemics.

The Coordination of the TWFP has also facilitated six meetings of sub-project coordinators to discuss project activities and future research strategies. The last two meetings to plan Phase III took place at CABI, U.K., and CIMMYT, Mexico, with the participation of NRInternational (CABI meeting), all sub-project coordinators, and other potential participants in Phase III. Several concept notes have been prepared in anticipation of Phase III.

Outputs

In terms of Information Management and Technology, the TWFP has made a significant effort to collect all the pertinent available and 'grey' literature on whiteflies and whitefly-borne viruses published around the world. To complement this effort, the TWFP has also linked with the extensive bibliography of *Bemisia tabaci* compiled from various sources since 1995 by USDA scientists in Arizona. These extensive databases and hard copies of the original documents are still in the process of transformation into electronic documents, but can be consulted for the most part by contacting the TWFP's Information Officer at CIAT, Palmira, Colombia.

Two publications were produced to: 1) promote the integration of the TWFP and the CPP projects conducting research on whitefly pests and whitefly-transmitted viruses. This publication, "A United Effort Against a Global Pest", has been distributed in Latin America, Africa, Asia and Europe. A second publication describing the various sub-projects that integrate the Tropical Whitefly Project, its participating institutions and donor agencies, was recently published for global distribution. The Coordination of the TWFP has also facilitated six meetings of sub-project coordinators to discuss project activities and future research strategies. The last two meetings to plan Phase III took place at CABI, U.K., and CIMMYT, Mexico, with the participation of NRInternational (CABI meeting), all sub-project coordinators, and other potential participants in Phase II.

The TWFP Coordination also promoted the dissemination of information regarding information on IPM strategies and packages that have been shown to be effective for the management of whitefly pests and whitefly-transmitted viruses in Latin America. Three electronic documents on: 1) management of whiteflies as direct pests in highland crops; 2) management of whiteflies as virus vectors in mixed cropping systems in the tropics; and 3) use of physical barriers for the control of whitefly-borne viruses in horticultural crops in the tropics, have been made available through the TWFP Web Page. The TWFP has also provided information and advice to CPP (“Adaptive evolution within *Bemisia tabaci* and associated *Begomoviruses*: A strategic modelling approach to minimising threats to sustainable production systems in developing countries” by Frank van den Bosch and M.J. Jeger), and other international projects currently engaged in the validation of sustainable IPM practices and modeling of whitefly/geminivirus epidemics.

The Coordination has maintained permanent contact with all the national and international institutions involved in Phase I and new partners in preparation for Phase III: technology dissemination through farmer participatory research. The Coordination has also maintained the communication with all project partners and the donor community, since the termination of Phase II on March 31st, 2004, to assure the continuity of the project. A series of Concept Notes have been prepared to this end.

Contributions of Outputs to Developmental Impact

Poverty alleviation: This project primarily responds to the needs of resource-poor farmers in need of technical assistance to manage whitefly pests and whitefly-transmitted viruses in staple and cash crops. In the case of basic food crops, such as cassava in Africa and common bean in Latin America, whitefly management is necessary to prevent significant and even total yield losses induced by African cassava mosaic and bean golden/yellow mosaic viruses. The deployment of virus-resistant cassava and common bean cultivars in Africa and Latin America, respectively, assures the food and the regular income derived from these crops by poor farmers. The TWFP has also provided technical assistance for small-scale farmers on IPM measures designed to protect high-value vegetable crops in mixed cropping systems. Vegetable crops, such as tomato, pepper and chillies, provide resource-poor farmers with additional income in very small areas (e.g. a tenth of a hectare planted to tomato, may produce more income than 4 has of common bean or maize). Vegetable production is not possible in whitefly-stricken agricultural areas without the adoption of effective IPM practices.

Food security: The TWFP has intervened in the mitigation of a famine caused by the emergence of a new recombinant whitefly-transmitted virus of cassava in East Africa (USAID funds). This event demonstrates that whiteflies can cause food security problems. In Latin America, the damage caused by whitefly-borne viruses in common bean plantings, caused the abandonment of over a million hectares to bean production. The resulting shortage forced many Latin American countries (including Brazil, the main producer of common bean in the world) to import beans from countries as far as China. Central America, Mexico and countries in the Caribbean region have to import beans on a regular basis, because of the whitefly problem. Vegetables have become an important component of the diet in developing countries, where the cost of sources of animal protein is beyond the purchasing power of poor people.

Generation of inco me: Horticultural crops have become an important source of income for small-scale farmers in developing countries of Asia, Africa and the Americas. A hectare of tomato, produced under high whitefly/virus pressure thanks to the IPM measures implemented by the TWFP in Mesoamerica, may produce over £ 5,000 in income (as compared to a £ 100 profit obtained from a hectare of maize or common bean). Hence the emphasis of the TWFP on mixed cropping systems for food security and income generation, particularly in East Africa and Latin America. The co-ordinator of the TWFP has secured the help of AVRDC's breeders in Taiwan, to initiate genetic improvement activities for tomato and peppers in Latin America, the centre of origin of these crops.

Sustainable use of Natural Resources: The implementation of IPM measures, such as use of virus- or vector-resistant varieties, use of physical barriers, and bio-control agents, results in a

major reduction of pesticide applications. This fact has been demonstrated in the experimental trials conducted in Mesoamerica and the Andean region. The DFID-funded CPP and TWF projects are unique in combining food production with natural resource management (NRM) practices. Emphasis on NRM per se, without a food production component, has led to unimaginable levels of pesticide abuse in developing countries, in detriment of the environment (contamination of soils, water sources and the environment), public health (applicators, rural communities, and consumers of highly contaminated produce), and the beneficial fauna (bio-control organisms).

As mentioned before, the co-ordinator of the TWFP has promoted three different meetings to discuss with subproject co-ordinators, the best approach to promote the findings of the work conducted so far by the project. In general terms, the TWFP recognises the need to scale up the implementation of IPM measures found to be effective and sustainable to control whitefly pests and the viruses that these vectors transmit. To this end, the Co-ordinator has contacted the Farmer Participatory Research (FPR) and Impact Assessment Groups of the System-wide IPM Programme, to develop a joint work plan for Phase III. This objective does not preclude the need to continue some basic research activities in the area of pathogen and pest monitoring, ecology and epidemiology of whiteflies and whitefly-borne viruses, and refinement of IPM strategies. The organisation of Farmer Field Schools (FFS) and analysis of policy issues related to this project, are also contemplated.

Further studies are necessary to link small-scale farmers to markets, and to develop agro-enterprises. Area wide impact assessment studies are also needed to determine the real contribution of the TWFP to poverty alleviation in target countries. To fulfil these objectives, the TWFP has been contacting Information and Communication specialists on electronic, radio and written media to chose the most effective channels to deliver the technology generated.

One of the major obstacles to the dissemination of technology in its initial stages ('pilot sites') has been the lack of trained personnel in FPR and FFS. There is a need for qualified personnel knowledgeable in transferring IPM technology to small-scale farmers, emphasising the economic benefits of adopting IPM measures. The co-ordination and subproject leaders of the TWFP have taken initial steps to establish collaborative links with FPR specialists working in the target regions, particularly in Africa and the Andean region. In Central America, the co-ordinator and leader of the Mesoamerican subproject has made possible the training of a national program scientist in Rural Development and Farmer Participatory Research at a regional international centre (CATIE).

DFID has expressed its interest in continuing its support to the TWFP in order to disseminate the technology generated by the different subprojects in sub-Saharan Africa, South East Asia, Mesoamerica and the Andean region.

Activity 2. Technical Report: Mesoamerica.

This output is from a research project funded by the United Kingdom Department for International Development for the benefit of developing countries. The view expressed are not necessary those of DFID.

Executive Summary

Mesoamerica is the region most severely affected by whiteflies and whitefly-transmitted viruses in the world. The Mesoamerican subproject of the Tropical Whitefly IPM Project (TWFP) was conceived to help small-scale farmers manage whitefly-borne diseases in basic food and high-value horticultural crops. Whereas food security is the main concern of most resource-poor farmers, they are trying to maximise the profitability of their limited land resources by adopting high-value horticultural crops in hopes of improving their livelihoods. Unfortunately, the lack of technical assistance from national and international institutes for non-traditional crops; and endemic nature of the whitefly problem, has meant the ruin of many resource-poor farmers who have attempted to diversify their subsistence cropping systems.

We describe here the results of the validation of some of the most promising whitefly control (IPM) practices observed in Phase I, in two 'pilot sites': the Valley of Zapotitan, El Salvador, and the state of Yucatán, Mexico. Basic socioeconomic and biological data were generated in order to determine the magnitude of the whitefly problem and select suitable IPM strategies to meet the needs of small-scale farmers in this region. The *ex ante* data collected showed that most small-scale farmers have diversified their cropping systems, and that the most limiting problems are the whitefly *Bemisia tabaci* and the viruses it transmits.

In Central America, common bean has been one of the two main staples (together with maize) since pre-Columbian times. This crop was the first food staple affected by whitefly-borne viruses in this region, in the late 1970s. In El Salvador, common bean production had been nearly phased out from traditional bean growing areas, particularly during the dry months of the year, when whitefly populations/geminivirus incidence reaches a peak. The Mesoamerican subproject promoted the adoption of a new common bean line bred for resistance to the whitefly-borne *Bean golden yellow mosaic virus*, the main production problem of this legume in the region. With this new cultivar, released recently as 'CENTA San Andrés', common bean production has returned to the Valley of Zapotitán, the main supplier of common bean to San Salvador, the capital of El Salvador.

In the case of tomato and peppers, the main horticultural food crops affected by whitefly-borne viruses in this region, there is practically no crop improvement programs in Latin America (despite being the center of origin of these crops). The TWFP evaluated physical control strategies against the whitefly *B. tabaci*, namely: insect-proof nets or 'fleece', that protect susceptible annual crops during the first month of their life cycle. The use of physical barriers also contributes to eliminate pesticide abuse and, thus, food and environmental contamination in rural and urban communities. The use of microtunnels during the critical whitefly/geminivirus periods of the year, has once more made possible and profitable the production of tomatoes and peppers in El Salvador, Mexico and other neighbouring countries that are already adopting this

IPM strategy. Yields of over 40 MT/Ha have been obtained at a time when tomatoes cannot be planted due to whitefly attacks, generating profits in excess of £ 5,000/Ha.

In El Salvador, the Mesoamerican subproject addressed gender issues by incorporating women into a small project on whitefly management of a high-value, perennial horticultural crop (loroco), usually tended by women in the backyard of their homes. Preliminary results show that the IPM strategy implemented has effectively controlled the pest problems that affected this crop. Potential profits for this crop exceed £ 700/ a tenth of a Ha.

The project has placed considerable emphasis on farmer education to eliminate one of the main problems associated with whitefly pests: pesticide abuse. Pesticide abuse results in the elimination of beneficial bio-control agents, emergence of pesticide-resistant whitefly populations, increased production costs, contamination of the environment and food products for the local and export market, and chronic health problems in rural communities.

Background

Whiteflies were declared the pest of the XXth century because of the severity of the damage they inflict directly or indirectly (as vectors of plant viruses) to a multitude of important food and industrial crops around the globe. Despite considerable research conducted in developed and developing countries to control this pest, crop loss is still a common occurrence in tropical regions where small-scale farmers do not receive technical assistance, other than the biased assistance they get from agrochemical companies. This situation has led to crop abandonment, chronic poverty, considerable pesticide abuse, and high levels of food/environmental contamination in developing countries.

As mentioned before, of all the regions in the world affected by the whitefly *Bemisia tabaci*, Central America, southern Mexico and the Caribbean (Mesoamerica) constitute the region with the largest number of crops damaged by this insect, both as a direct pest and vector of an even larger number of plant viruses (geminiviruses or, more specifically, begomoviruses). **Figure 1** and **Table 1** show the areas affected by the whitefly *B. tabaci* and the numerous viruses that this insect vector transmits. These areas are usually located in the most fertile and agriculturally suitable land found between sea level and 1,000 meters of altitude in the entire region, from northern Mexico to Panama, and throughout the Caribbean Basin. Of the various food staples native to this region (*e.g.* maize, common bean, several cucurbits, tomato, sweet pepper and chillies) most have been attacked by whiteflies. Common bean, tomato, sweet pepper and chilli production has practically ceased in the main agricultural areas shown in **Figure 1**, during the prolonged dry season (November-April), due to the large whitefly populations that develop at that time of the year.

Table 2 shows the impact of whitefly-related problems in one of the main agricultural areas of El Salvador, the Valley of Zapotitán, considered the 'pantry' of the capital city of San Salvador. The abandonment of prime agricultural land due to the high incidence of whitefly-transmitted viruses, occurred in all of the Central American and Caribbean countries, and all the agricultural states of Mexico, wherever *B. tabaci* can thrive.

Table 1. Whitefly-transmitted viruses (begomoviruses) present in Middle America.

Virus Acronym		Main Region Affected
Bean calico mosaic virus	BCaMv	Mexico
Bean dwarf mosaic virus	BDMV	Nicaragua
Bean golden yellow mosaic virus	BGYMV	Entire region
Cabbage leaf curl virus	CaLCV	Jamaica
Calopogonium golden mosaic virus	CalGMV	Costa Rica
Chino del tomate virus	CdTV	Mexico
Cotton leaf crumple virus	CLCrV	Mexico, Guatemala
Cotton yellow mosaic virus	CYMV	Dominican R., Guatemala
Cucurbit leaf curl virus	CuLCrV	Mexico
Jatropha mosaic virus	JMV	Puert Rico
Malvaceous chlorosis virus	MCV	Entire region
Okra mosaic Mexico virus	OkMMV	Mexico
Papaya leaf curl virus	PaLCV	Panama
Passiflora leaf mottle virus	PLCV	Puerto Rico
Pepper golden mosaic virus	PepGMV	Mexico, C. America
Pepper huasteco yellow vein virus	PHYVV	Mexico
Pepper mild tigre virus	PepMTV	Mexico
Soybean golden mosaic virus	SGMV	Caribbean, C. America
Squash yellow mild mottle virus	SYMMoV	Costa Rica
Tobacco apical stunt virus	TbASV	Mexico
Tobacco leaf rugose virus	TbLRV	Cuba
Tomato dwarf leaf curl virus	TDLCV	Jamaica
Tomato golden mottle virus	TGMoV	Guatemala
Tomato leaf curl Nicaragua virus	TLCNV	Nicaragua
Tomato leaf curl Sinaloa virus	ToLCSinV	Mexico, C. America
Tomato mosaic Havana virus	ToMHV	C. America, Cuba
Tomato mottle Taino virus	ToMoTV	Cuba
Tomato mottle virus	ToMoV	Mexico, Caribbean
Tomato severe leaf curl virus	ToSLCV	C. America
Tomato yellow dwarf virus	ToYDV	Jamaica
Tomato yellow leaf curl virus	TYLCV	Caribbean, Mexico

Figure 1. Areas affected by whitefly-transmitted viruses in Mesoamerica.

As suggested by the data presented in **Table 2**, the main impact of whitefly-transmitted viruses took place in the 1990s, although whitefly-transmitted diseases, such as bean golden yellow mosaic, were already important food production constraints in this region prior to 1980. Different factors contributed to the exponential increase in whitefly-transmitted viruses. First, Latin America plunged into an economic depression (known as the 'lost decade' of the 1980s), caused by the mounting external debt of the region. Secondly, Latin American governments saw their traditional export crops (*e.g.* coffee, sugar, bananas) lose value relative to manufactured industrial imports, and thus resorted to non-traditional export crops (NTECs), mainly horticultural (*e.g.* tomato, peppers, cucurbits) and industrial (*e.g.* soybean) crops. Third, these changes took place at a time when the profound economic crisis and austerity measures imposed on Latin American governments by the International Monetary Fund, which resulted in the downsizing of National Agricultural Research Institutions (NARIs), which could no longer provide technical assistance to growers of NTECs. Fourth, this vacuum was rapidly filled by the agrochemical companies; which resulted in widespread pesticide abuse, and, ultimately, high levels of pesticide residues in NTECs and traditional food crops, and resistance to most commercial insecticides in whitefly populations. As a consequence, contaminated produce could not be exported and the saturation of local markets and high production costs, put an end to the hopes of small- and medium-scale farmers to improve their livelihoods by producing high-value crops.

Table 2. Evolution of land use in the valley of Zapotitan, El Salvador, during the dry season (1989-1999).

Crop 1	989	1999
Maize	465 has	780 has
Common Bean	175 has	3 has
Tomato	153 has	3 has
Pepper/Chilli	35 has	1 ha
Cucumber	64 has	68 has

From the biological point of view, two main factors further contributed to the emergence of new whitefly-transmitted viruses: first, the diversification of crops (higher number of whitefly hosts), and, secondly, the introduction of a more aggressive and prolific whitefly biotype (B) in the Americas, in the early 1990s.

Central America, Mexico and the Caribbean constitute a region greatly dependent on agricultural products to satisfy its food demand and need to generate foreign income from traditional and non-traditional export crops in order to pay an ever increasing external debt that demands more than half of the Gross Regional Product. For instance, the external debt of Central America grew from US $ 8.5 billion in 1979, to US 20.7 billion in 1985. In that year, Central America was spending over 40% of the revenues derived from the export of goods and services to pay the external debt, and this figure is even higher (>50%) today. Currently, over half of the population of Central America, Mexico and the Caribbean are considered as poor, and 58% of these poor people live in rural areas and work in farming units under 3 has. The dwindling prices of traditional agricultural commodities and the increasing demand for horticultural products in North America during the winter season, creates a potential market for most Middle American countries. When high-value crops (e.g. tomato, pepper, chilli, melon, eggplant, okra, snow pea, broccoli, etc) were introduced in traditional agricultural areas to supply the North American markets, a series of problems emerged. Most of the new crops corresponded to varieties created in temperate countries and, therefore, were not adapted to the tropical and sub-tropical conditions characteristic of Middle America. The intensive use of pesticides applied as a risk-aversion strategy, eliminated most biological control agents for the whitefly *B. tabaci*, giving rise to large whitefly populations, most of which had developed resistance to the traditional insecticides used. Pesticide abuse led to increasing levels of pesticide residues being detected in NTECs, which, together with high production costs, collapsed the agro-export business. These were the main reasons why susceptible crops, such as tomato, pepper, chilli, eggplant, okra and melon, were abandoned in many regions during the dry season.

During Phase I of the TWFP, 11 countries in Central America, the Caribbean Basin, and Mexico, were surveyed to determine the importance and socio-economic impact of the whitefly and geminivirus problems in their main agricultural areas. The survey also included case-studies in selected regions of Guatemala, El Salvador, Honduras and Costa Rica. The data collected clearly showed that every country surveyed had severe whitefly/geminivirus problems, mainly affecting

common bean (one of the two main staples in the region) and vegetables, namely tomato, sweet pepper, chillies, several cucurbits, eggplant, and industrial crops such as tobacco. The case-studies confirmed that farmers considered whiteflies as the number one production problem and the main cause for crop failure and significant economic losses.

The extensive surveys undertaken in the region, allowed the TWFP to identify the crops affected; whitefly species and biotypes involved; whitefly-transmitted viruses in the region; and the environmental factors that condition whitefly outbreaks. Moreover, the TWFP could observe all of the IPM tactics employed throughout the region and their potential contribution to whitefly/begomovirus management.

Project Purpose

The purpose of the TWFP-Mesoamerican subproject is to help small-scale farmers diversify their cropping systems and improve their livelihoods by providing technical assistance to manage whitefly-related problems affecting traditional and high-value non-traditional crops in Central America, Mexico and the Caribbean.

Once the geographic dimension, socioeconomic importance, and biological factors conditioning whitefly/virus outbreaks were analysed in Phase I, Phase II undertook the evaluation of the most promising IPM measures available, in selected 'hot spots' of Middle America. The specific purpose of these evaluations was to select IPM packages for the management of whiteflies and whitefly-borne viruses in common bean and horticultural crops in this region.

A major thrust of the project is to eliminate pesticide abuse associated with whitefly control in all crops affected, and thus reduce the levels of pesticide residues in food and horticultural crops in rural and urban areas of Middle America. Ultimately, the adoption of the IPM measures recommended by the TWFP should increase the profitability of mixed cropping systems and improve the livelihood of for resource-poor farmers.

Research Activities and Results: El Salvador

Phase II of the Mesoamerican TWF subProject included two pilot sites: the valley of Zapotitan, in El Salvador, located approximately 35 Km west of the capital city San Salvador **(Figure 2)**, at 460 m above sea level, precipitation of ca. 1,700 mm, and an average annual temperature of 27° C. This valley has an irrigation district (1,813 has) with an annual planting capacity of approximately 4,695 has (over 70% of the farming units are under 4 has), divided into three planting seasons. However, the second most important planting season (December-April) in terms of area planted (over 2,100 has), has been drastically reduced in the case of common bean and vegetable plantings, due to the whitefly/geminivirus problems **(Table 2)**. Thus, this valley, considered as the main food supplier for the capital city, has not been able to fulfil expectations, and, thus, food must be imported (e.g. In 2002, 41,416 MT of red-seeded beans worth US $ 9,404,192; and 41,418 MT of tomato, worth, 7.7 million dollars, were imported) to sustain the demand of San Salvador during the dry months of the year. The TWFP responded to internal policies adopted by the Government of El Salvador to recover this valley to food production during the dry season, by inserting the project into the national agricultural research priorities set by the Ministry of Agriculture (MAG) and its National Centre of Agricultural and Forestry

Technology (CENTA), as stated in their Strategic Plan for 2000-2004. This document reads "the official plan (called 'Alliance for Work') has the objective of increasing the production levels and productivity of the agricultural sector, so that it contributes to higher levels of employment and income, and, therefore, to reduce the existing poverty levels, specially in the rural families". The document states that "the agricultural sector of El Salvador includes over 60% of the economically active labour force of El Salvador, and population-wise, this sector represents the largest number of nationals of any of the productive sectors of the nation". The plan clearly acknowledges that: "the production of traditional crops is weakened despite improvements in their productivity, due to a decreasing price for these commodities in the international market". An states that "the comparative advantage of El Salvador and other Central American countries lies in their biodiversity and tropical climate, which permit the production of certain crops, such as fruits and vegetables, during the winter season of North American and European countries". Unfortunately, the period between November and December, when there is a demand for those products in the north, coincides with the peak of whitefly populations and begomovirus incidence in the Central American and Caribbean regions, as well as in southern Mexico. The agricultural sector of El Salvador has been decreasing its contribution to the Gross Domestic Product (-0.6%) since 2000.

Figure 2. Pilot site (red star) in the Valley of Zapotitán, El Salvador.

El Salvador was also chosen because it was represented by the largest number of institutions willing to collaborate in Phase I. These included: The Ministry of Agriculture, the National Program CENTA, the University of El Salvador, the Latin American Technical University of San Salvador, The Zapotitan Farmer Association (AREZA), private companies and NGOs.

I. Socioeconomic and biological characterisation of pilot site.

The first set of activities initiated in 2001-2002, was designed **to characterise *the ex ante* socio-economic s ituation** of these target regions. In El Salvador, a questionnaire was designed specifically for the project with the help of the Socio-Economics Unit of the Salvadorean National Agricultural Research Program (CENTA) and given to 62 family units in the valley of Zapotitan.

In the Valley of Zapotitan (3,020 has), El Salvador, as in the rest of Latin America, most of the farmers interviewed were males (96.8%); 66% of whom, have farms under 3.5 has. Common bean was the predominant crop until the late 1980s, but the high incidence of whiteflies and whitefly-transmitted viruses during the dry season, greatly reduced the area planted to this crop in Zapotitan **(Table 1)**. According to the survey, over 40% of the farmers interviewed have abandoned the cultivation of this legume staple because of problems related to the presence of the whitefly/bean golden yellow mosaic. In reference to tomato and pepper/chilli, 67% and 41% of the farmers interviewed had abandoned these crops, respectively. Pests, particularly the whitefly/virus complex, were mentioned by over 52% and 37.5% of the farmers as the main production problems influencing their decision to abandon tomato and peppers, respectively.

A total of 93% of the farmers mentioned that they had abandoned the above-mentioned crops in one of the two main seasons of the year. In the case of tomato and pepper/chilli, 79-82% of the farmers interviewed mentioned the dry season, and the whitefly/virus problems as the main cause. In the case of common bean, 49% of the farmers said that they had abandoned the cultivation of this legume during the dry season, and a similar proportion had desisted planting the crop during the rainy season. The causes for their decisions were 1) the whitefly and 2) fungal/bacterial diseases and flooding problems, respectively. Other problems mentioned, were: price fluctuations, theft and climate change. However, 32% of the farmers interviewed had problems marketing their produce. **Table 3** shows the main market outlet for the different crops analysed here.

Table 3. Main market outlets for agricultural products analysed-Zapotitan.

Crop Farm		Market Place	Local Stores	Household
Common bean	57.7%	33.5%	0%	6.9%
Tomato	18.2%	9%	54.4%	9.2%
Pepper/Chilli	52.6%	13.2%	34.2%	5.3%
Loroco	16.6%	9.1%	8.2	0.2%

Approximately 37% of the farmers interviewed derived some income from other sources, such as animal husbandry, agricultural machinery rental, commercial activities, and retirement pensions. Interestingly, only 40% of the farmers consulted, kept records of their crop production costs. In tomato, 54% of the production cost corresponds to chemical inputs, both during the dry and rainy season. Although production costs are 36% higher for tomato during the rainy season, yields are also 43% higher during this season. However, prices during the rainy season may drop as much

as 84% when compared with summer prices. *Hence, the importance of producing tomato and other high value horticultural crops during the dry season*.

These trends are similar for most agricultural products affected by whiteflies and whitefly-transmitted viruses, although price fluctuations are not as marked for traditional commodities, such as common bean. The insistence of small-scale farmers with risky horticultural crops is based on the fact that 0.1 ha of a crop like tomato, yields a net profit at least twice than that obtained from a whole ha of a subsistence crop such as common bean or maize.

Contributors: Evelyn Osorio (CENTA), James Garcia (CIAT).

Biological Characterisation: *'Investigation on the potential role of different crops as reproductive hosts of the whitefly Bemisia tabaci in the Valley of Zapotitan.'*

The main whitefly species in the lowlands and mid-altitude valleys of El Salvador has traditionally been *Bemisia tabaci*. The first report of *B. tabaci* as a pest and vector of plant viruses in El Salvador was made in 1960, affecting cotton, kenaf and common bean. In the early 1990s, the new biotype B of *B. tabaci* appears in the Caribbean and Mexico, and the TWFP starts monitoring the composition of *B. tabaci* biotypes in El Salvador. Towards the end of Phase I (1998-1999), the presence of biotype B of *B. tabaci* is detected in El Salvador, feeding on chilli (*Capsicum* spp.), loroco (*Fernaldia pandurata*), and various cucurbits (melon, watermelon, cucumber and *Cucurbita moschata*, locally known as 'ayote'). Phase II of the project has paid particular attention to the **evolution of *B. tabaci* biotypes** in El Salvador, as evidence from other countries affected by the B biotype, suggests that the original (A) biotype may be displaced from agricultural regions by the more prolific and aggressive B biotype.

In El Salvador, during phase II, a total number of 18,428 whitefly individuals were collected on different crops for further identification and population analyses. Results (166 assays) obtained until 2003 with a representative sample of whitefly individuals associated with 12 different crops (common bean, soybean, tomato, cucumber, pipian (*Cucurbita argyrosperma*), eggplant, ayote, cabbage, cauliflower, radish, watermelon and loroco, demonstrated the presence of the B biotype of *B. tabaci* in 100% of the samples assayed using the SCAR technique. However, the original (A) biotype was also present in approximately 25% of the samples tested. This year, an analyses of the biotypes found in six different crops (ayote, pipian, eggplant, chipilin (*Crotalaria* sp.), cucumber and tomato) grown in the Valley of Zapotitan, revealed the presence of only the B biotype of *B.* tabaci. These results suggest that the B biotype is gradually displacing the A biotype of *B. tabaci* in the valley of Zapotitan.

Although the collection of whiteflies and analysis of data are not finished yet, **Figure 1** provides a preliminary picture of the potential of some crops as hosts to whiteflies, mainly *B. tabaci*. Fortunately, soybean is not widely cultivated in Central America, as is the case in South America, where soybean is the most important reproductive host of the whitefly *B. tabaci*. The high population of *B. tabaci* found on common bean plants reflects more the condition of this plant species to act as a feeding host, rather than as a reproductive host of *B. tabaci.* In 2001, we observed *B. tabaci* populations of approximately 200 adults per bean plant in 2001, in Zapotitan, but even populations of 5 adult *B. tabaci* per bean plant could result in high rates of BGYMV

transmission. A high incidence of whitefly adults per plant usually results in plant death due to feeding damage and development of sooty mould.

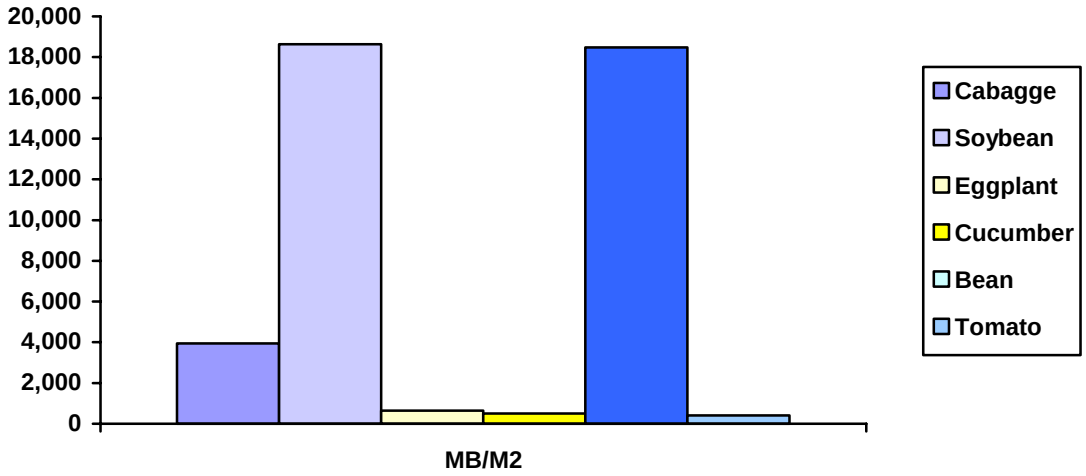

Figure 1. Whitefly adults/m2 on Selected Crops.

We have also observed very high populations of *B. tabaci* on eggplant in the Valley of Zapotitan, but there are no viruses transmitted by this whitefly species in this area. However, eggplant can be affected by sooty mould in this valley. This investigation constitutes the M.Sc. thesis research of the principal investigator at the University of El Salvador.

The **characterization and biotyping of whitefly specimens** is a continuous activity at CIAT, where hundreds of specimens are examined every year by a qualified taxonomist (Ms. María del Pilar Hernández) working part-time for the TWFProject (on USAID funds). All *B. tabaci* specimens are sent to the Virology Laboratory at CIAT for molecular biotyping, using the RAPD (Random Amplified Polymorphic DNA) and SCAR (Sequence Characterized Amplified Region). The latter technique was entirely developed at CIAT by the TWFP in order to simplify the identification of biotype B of *B. tabaci* (**Figure 2).**

These data have been geo-referenced, so that the TWFP and collaborators can monitor the composition of *B. tabaci populations*, as biotype B continues to displace the original (A) biotype **(Figure 3).**

In the case of **whitefly-transmitted viruses** in the Valley of Zapotitán, *Bean golden yellow mosaic virus* (BGYMV) was first reported in 1964, and this tentative identification was confirmed at CIAT in 1992, as the predominant BGYMV isolate in Central America and the Caribbean. In 1998, the TWFP re-confirmed the predominance of this virus in El Salvador, but demonstrated changes in its antigenic properties, probably in response to the arrival of the new *B. tabaci* biotype (B). This situation has remained unchanged, and only a broad-spectrum BGYMV monoclonal antiserum developed by CIAT and the University of Florida, is able to detect the virus.

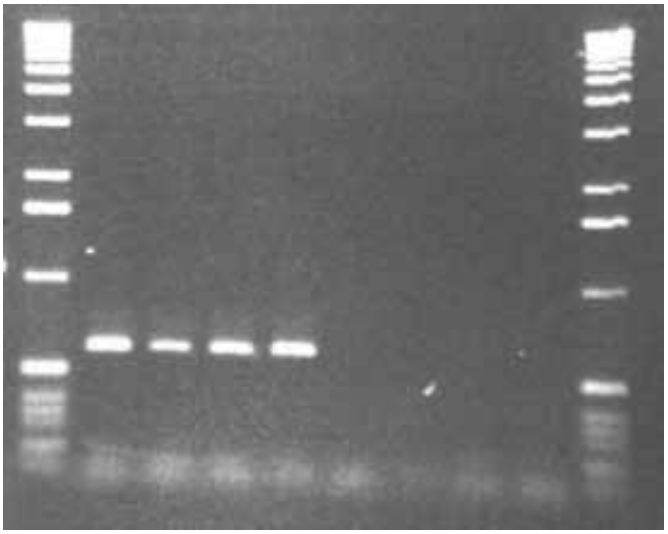

Figure 2. Sequence Characterised Amplifie d Region (SCAR) technique developed at CIAT to specifically detect bioty B of the w hitefly *Bemisia tabaci*. Extreme lanes: Molecular Markers (1Kb); Lanes 2-5: Biotype B of *B. tabaci*.

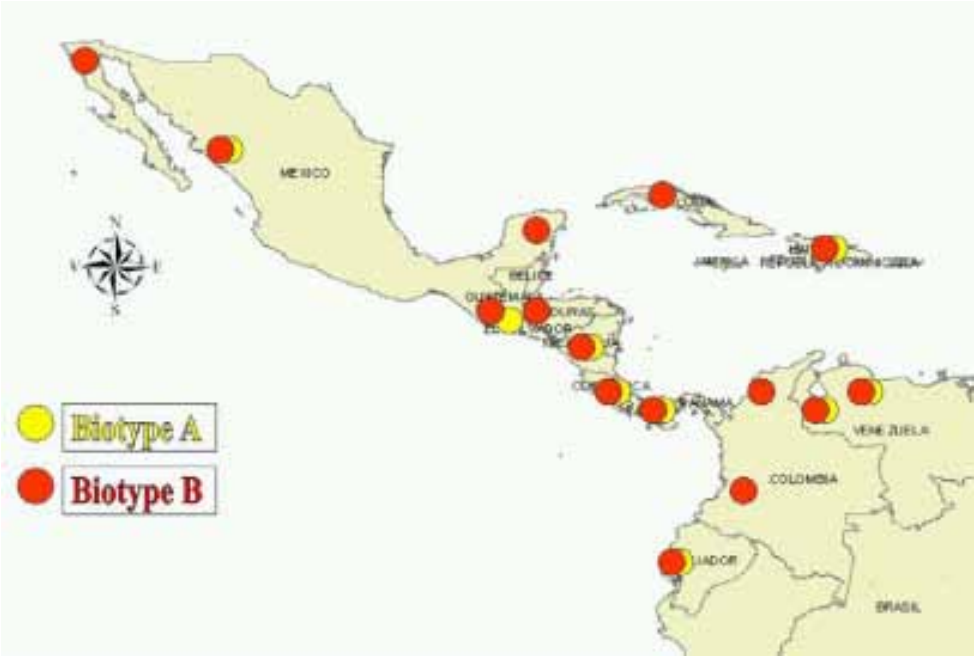

Figure 3. Distribution of *B. tabaci* biotypes A (yellow) and B (red) in Mesoamerica.

Contributor: M.Sc. Leopoldo Serrano Cervantes, Departamento de Protección Vegetal, Unidad de Estudios Post-Grado, Facultad de Ciencias Agronómicas, Universidad de El Salvador

Molecular characterisation of begomoviruses.

During Phase II, other whitefly-transmitted viruses have been detected in peppers, tomato, and loroco (*Fernaldia pandurata*). Different infected samples of these crops have been assayed by PCR, cDNA cloning, and partial sequencing for identification **(Table 4A)**. Some diseased plant samples were shown to be infected by potyviruses, probably transmitted by aphids **(Table 4B)**. This project has achieved the first characterization of viruses affecting loroco in Central America, which made possible the implementation of simple IPM measures to control these viruses, their vectors and pests, such as the whitefly *B. tabaci*.

Table 4A. Begomoviruses identified in horticultural crops in the valley of Zapotitan, El Salvador.

Crop	Begomoviruses
Tomato	Tomato dwarf leaf curl virus
Tomato	Tomato severe leaf curl virus
Pepper	Pepper golden mosaic virus

Table 4B. Other viruses detected (Poty-/Cucumo-viruses).

Tomato	Tobacco etch virus
Pepper	Pepper mottle virus
Loroco	Loroco mosaic potyvirus
Loroco	Loroco foliar distortion cucumovirus

Whitefly population dynamics are also under study and analysis since 2001-2002, when large populations were present on most crops. Whitefly populations were only moderate in the 2002-2003 due to late rains and cold fronts ('nortes') at the end of 2002 **(Figure 4).**

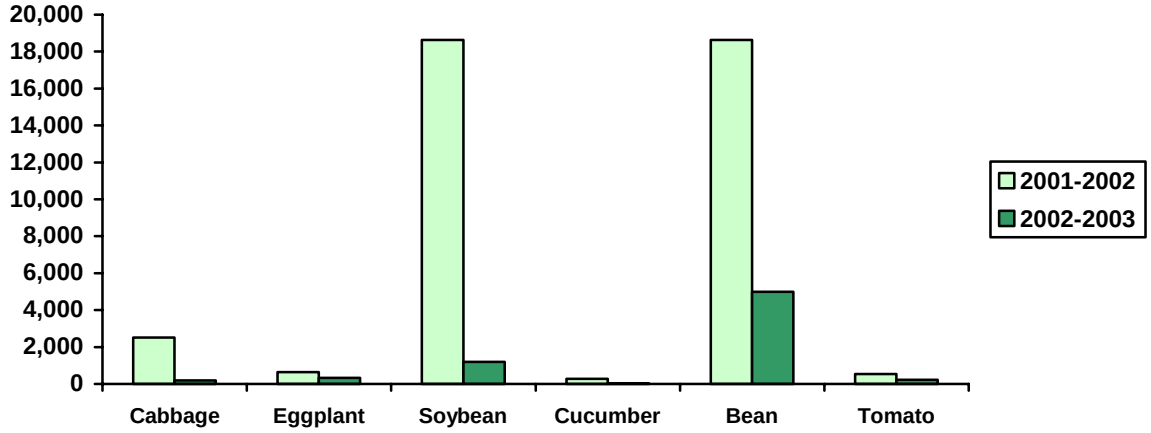

Figure 4. Seasonal variation in whitefly populations – Zapotitan.

Contributors: Francisco J. Morales and Ana Karina Martinez (CIAT).

II. Implementation of IPM measures.

Common bean: '*Recovery of common bean production in the Valley of Zapotitán'*.

Justification: Until 1985, the Valley of Zapotitán was the main common bean production area to satisfy the demand of the capital city of San Salvador, to consume mainly during the months of April, May and June, which come after the end of the prolonged dry season (November-March). The increasing incidence of *Bean golden yellow mosaic virus* (BGYMV), transmitted by the whitefly *B. tabaci*, gradually led to the abandonment of common bean production in this valley during the dry season. Although common bean is produced throughout Central America, the Salvadoran market demands a unique red-seeded bean type ('Rojo de Seda') only produced in this country. Thus common bean imports from neighbouring countries, did not satisfy the consumers and common bean prices and consumption fell (from 12 to 8 kg/*per* capita) since 1985. In 1990, the collaborative project (PROFRIJOL) between CENTA and CIAT, led to the selection of a BGYMV-tolerant common bean variety (CENTA-Cuzcatleco). However, the commercial characteristics of this new variety were not adequate and, consequently, its market price was relatively low. Moreover, the BGYMV resistance of CENTA-Cuzcatleco has been breaking down even during the rainy months of the year, which has further contributed to its rejection by local farmers due to its high protection costs. Hence, the TWFP and CENTA initiated activities towards the identification and validation of new improved common bean genotypes for the San Salvador market.

Research plan: A promising red-seeded common bean line possessing high levels of BGYMV resistance and adequate commercial characteristics was identified in field trials of materials developed by Dr. Juan Carlos Rosas, breeder of the Pan American School (ZAMORANO) in Honduras using parental materials selected through the PROFRIJOL project. The line selected, EAP 9510-77, was planted in September 2001, in five plots of 2,000 sq/m each, to cover the five districts of the Valley of Zapotitán. Half of the area was planted to the local susceptible common bean landrace, 'Rojo de Seda', and the other half with the new EAP line. The plots were planted and evaluated with local farmers in each district. The treatments consisted of minimum inputs: seed treatment (imidacloprid) and herbicide (Prowl). Yield was estimated per plant and per plot **(Table 5)**.

Table 5. Comparative yield (kg/ha) of a new virus-resistant breeding line and the preferred local common bean landrace in the valley of Zapotitán, El Salvador.

Zone 1		2	3	4	5	Average
Year	2001	2001	2002	2002	2003	2004
Virus Inc.	8	8	4	4	6	6
Rojo de Seda	120	150	350	408	230	**251.6**
EAP 9510-77	810	890	1.250	1.400	910	**1,052**

A demonstration plot was planted in 2001 in order to show farmers the superior yielding capacity of the new line EAP 9510-77, as compared with the previous cultivar CENTA-Cuzcatleco (DOR 364) and the preferred landrace 'Rojo de Seda' **(Figures 5 and 6)**. DOR 364 was a CIAT-bred,

virus-resistant cultivar released over a decade ago, and although its seed colour was more purple than red, it was widely planted in various Central American countries. This cultivar is on its way out because of its increased susceptibility to BGYMV and dark red colour. The EAP line has a combination of different sources of BGYMV-resistance and better seed colour. Given the clear preliminary results obtained in the first series of evaluation sites, which demonstrate that it is possible to grow common bean during the dry season (November-March) in the Valley of Zapotitán using minimum inputs, line EAP 9510-77 was evaluated at the national level by CENTA with complementary funding from DFID/PROFRIJOL/CRSP-USAID

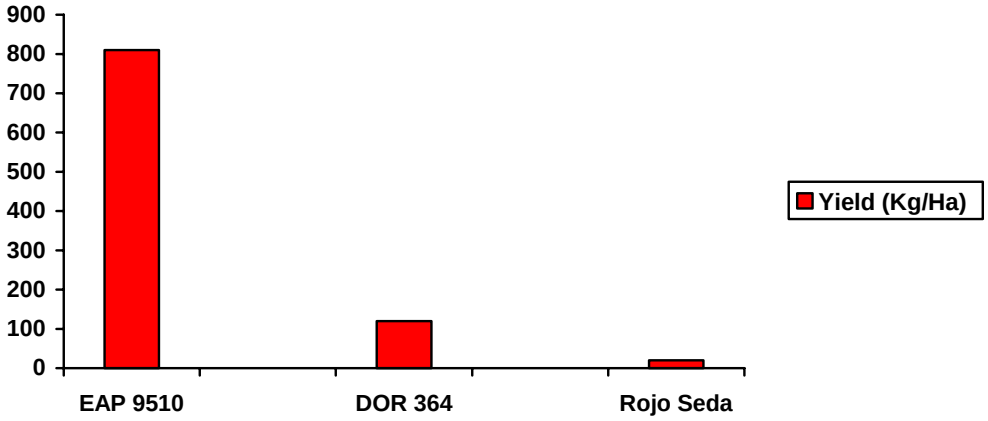

Figure 5. Yield (Kg/Ha) of selected Common Bean Cvs in El Salvador.

Figure 6. Comparison of th e new EAP common bean line w ith the local lan drace 'Rojo de Seda,' under BGYMV pressure in the field.

A **case study** was conducted with 60 farmers in the western (6), central (23), para-central (22), and eastern (9) regions, during the second semester of 2003. Only 3 of the 60 farmers interviewed were women, which reflects the cultural characteristics of farming in Latin America. The age range of the farmers interviewed was 30-81 years, with 60% of the farmers being older than 50 years. This finding illustrates the migration of young people from rural to urban areas in search of jobs in commerce, industry and maquila, all activities that show positive growth in

recent years, as well as an increase in minimum wages. Interestingly, 92% of the farmers interviewed were literate, although only 22% reached secondary school. Over 70% of the farmers owned their farms and 58% lived in the farm. 75% of the farmers do not have access to credit and most farmers have incomes between US $ 1.50 and 3.00/day.

The area of the validation plots varied according to the capabilities and willigness to collaborate of the participating farmers, from 200 sq/m to 1,750 sq/m for the new line, and from 200 sq/m to 2,598 sq/m for the local check (red-seeded cultivar chosen by the farmer).

Farmers also differ in relation to the cropping system used: monoculture (42%), association (20%) and relay (38%). The most popular common bean cultivars are: Rojo de Seda (30%), followed by two BGYMV-resistant cultivars (CENTA 2000 and DOR 585). 80% of the participating farmers registered higher yields with the new improved common bean EAP line. Only in the central region approximately 20% of the farmers concluded that they preferred their traditional bean cultivar. 62% of the farmers manifested that the new line had superior disease resistance qualities as compared to their own cultivars. 33% could not tell any difference (mainly those that already grow virus-resistant cultivars, such as CENTA 2000 and the DOR lines), and 5% concluded that the new material was more susceptible. However, it was later shown that the susceptibility of the new line was to 'web blight', a fungal disease present in isolated areas of El Salvador. 83% of the farmers considered that the commercial characteristics of the EAP line as excellent. The remaining 7% thought that their local material was better (mainly the local landrace 'Rojo de Seda' which is highly susceptible to BGYMV and cannot be grown in the dry season even under heavy chemical protection).

The most important result of this survey is that 87% of the farmers that planted the new improved bean line were willing to adopt it. This figure was almost 100% in areas affected by the whitefly-transmitted BGYM virus. Of all the seed obtained by the collaborating farmers, 37% was used for household consumption, 32% was saved as seed for the next planting, and 27% was sold to generate income.

Table 6 shows the statistical analysis of the different variables evaluated in order to determine the level of acceptance of the new line EAP 9510-77.

Table 6. Main variables that determine the adoption of a new bean cultivar.

Variable Co	efficient	Standard Error	t-Statistic	Probability
Intercept	0.141624	0.260788	0.543063	0.5895
Growth Habit	0.14294	0.120277	1.188416	0.2403
Vegetative Cycle	0.115406	0.075519	1.528176	0.1328
Yield	-0.115342	0.087253	-1.321933	0.1922
Disease R	-0.166001	0.099686	-1.665248	0.1021
Pest Resistance	0.101755	0.097583	1.042755	0.3021
HumidityTolerance	0.053299	0.049807	1.070111	0.2897
Market Price	-0.057198	0.054633	-1.04696	0.3002
Acceptance	0.693283	0.156329	4.434778	0.0001
R^2	0.376418	Mean dep. var.		0.881356
Adjusted R^2	0.276645	S.D. dep. var.		0.326145
St. Error Regress.	0.277387	F value		3.772743
Residual S.C.	3.847183	Probab (F)		0.001584

Model: Varietal Adoption = 0.141624 + 0.14294 contall + 0.115406 cveg-0.115342 rend-0.166001 resenf + 0.101755 respla + 0.053299 tolhum-0.057198 sale + 0.693283

Table 7 shows the superior yielding capacity of the new material EAP 9510-77 in the selected regions where it was evaluated, in relation to the local cultivar.

Table 7. Results of the validation trials of EAP 9510-77 in 4 regions of El Salvador.

Region EAP	9510-77	Local cvar. Yield	Difference	Percentage
West	1815 kg/ha	1312	503	27.7
Central	1259 kg/ha	951	308	24.5
Para-Central	1088 kg/ha	875	213	19.6
East	1171 kg/ha	901	270	23.0
National Av.	1240 kg/ha	952	288	23.2

The line EAP 9510-77 was officially released in November 2003 as the new variety 'CENTA San Andres'. In the District of Zapotitán, the TWFP (DFID) has financed two field days for 83 farmers (including 18 women), and 18 technicians, in order to promote the new variety.

Contributor: Ing. Carlos Atilio Perez (CENTA).

Collaborators: Agents (3) of the Zapotitán Extension Agency (CENTA) under the coordination of Ing. Mario Aragón.

Horticultural crops: '*Management and control of whiteflies using physical barriers to protect tomato and pepper crops in the Valley of Zapotitán, El Salvador*'.

The Ministry of Agriculture and CENTA had manifested the need to recover tomato production in El Salvador, in order to reduce the increasing amount of tomato imports required to satisfy the internal demand. As seen in **Table 1** and as stated by most farmers interviewed for the *ex ante* case study conducted in El Salvador, vegetable production simply became unviable because of the whitefly problem. Two decades ago, the Valley of Zapotitán contained over 280 hectares of tomato, but due to the whitefly problem, the area was reduced to only 35 has in 2000. This situation has forced the Salvadoran government to import 41,418 MT of vegetables in 2002.

The main strategy evaluated in the Valley of Zapotitán during Phase II, was the use of physical barriers (**microtunnels**) for tomato and peppers, using different types of mesh (fleece). Initially, the experimental design contemplated a series of five replications (in the five zones into which the irrigation district of Zapotitán is divided) in paired plots. The evaluation variables selected were: production cost, yield and net benefit. During, the first evaluation conducted at the onset of the dry period in 2001, the experimental design suffered modifications due to various constraints. First, the importation of the material to make the microtunnels was difficult because anti-insect nets were considered a luxury item in El Salvador, which significantly increased the price of the net and prolonged its nationalization. Secondly, some farmers modified the design of the microtunnels to prolong the protection period for the transplanted tomato seedlings, which left some chilli rows uncovered for lack of this imported material. Third, some participating farmers did not control weeds inside some microtunnels, which eventually reduced yields below the

economic threshold, and had to be discarded. However, the remaining evaluation plots allowed farmers to appreciate the clear benefits of using microtunnels, in terms of making possible the cultivation of susceptible horticultural crops under high whitefly/virus pressure **(Figure 7)**. This picture shows the effect of high whitefly/virus incidence (as it occurred in the dry season of 2001/2003): the complete destruction of the uncovered rows of tomato. Even the rows protected with the net for over 30 days suffered significant yield loss. In this evaluation, uncovered tomato plants died from the early virus infection, whereas tomato plants protected for up to 30 and 60 days produced 12.8 and 60 MT/ha, respectively. In a second trial, the covered tomato produced 55 MT/ha, whereas the uncovered control produced 15 MT/ha under chemical (imidacloprid) protection **(Table 8)**. The national average during the rainy season is 20 MT/ha.

Cultural practices and physical barriers to the whitefly vector, were complemented with pesticide reduction tactics to lower the amount of pesticide residues in horticultural products for the local markets, and their negative impact on the environment and production costs.

Figure 7. Effect o f covering to mato plan ts for 30 and 60 days after transplant as compared to the uncovered control.

Continued

Table 8. Results of physical barriers against whitefly-transmitted viruses in tomato rows protected 30 and/or 60 days without and with chemical protection.

Treatment 1	Treatment 2	Incidence 30 days	Incidence 60 days	Severity 60 days	Yield TM/Ha
Without insecticides	Uncovered	100%	100%	5	0 C
	Covered 30 days	0%	100%	3	12.8 B
	Covered 60 days	3%	6%	2	60.0 A
With insecticides	Uncovered 30 days	100%	100%	4	15.0 B
	Covered 30 days	0%	30%	2	55.0 A

Due to the promising results obtained, five demonstration plots were established in the irrigation district of Zapotitán **(Tables 8A-C.)**. Due to the high cost of establishing the plots, only one plot was established with each of five different farmers. Each plot was formed by three rows of tomato plants and other three rows of pepper plants sown in 1 m wide/20 m long beds, 1.20 m apart from their centre point. The distance between plants was 50 cm, for a final density of 16,600 plants per hectare. One row of both tomato and pepper plants was cover with a polypropilene net (Agryl), one with a more expensive but resistant net (tricot), and one row was left uncovered as control. The pepper variety was 'Nathalie' and the tomato variety chosen was 'Sheriff'. The seedlings were raised under screenhouse conditions to avoid early virus infection (first IPM measure). Soil preparation, fertilization and weed control was done according to farmers' practices. A month later, the test plants were transplanted in the fields with only one application of a systemic insecticide (imidacloprid), and the two protected rows were covered. The net was taken off a month later when the plants were already starting their reproductive stage. Viral symptoms were scored using a 0-4 scale (no symptoms-no production), 30 and 60 days after transplant. The fruits were harvested and weighed at the end of the test. Two of the plots were lost to fungal and bacterial diseases because they were planted late into the rainy season. The following are the results obtained in the three surviving plots.

Table 8.A. Results of physical protection against whitefly-transmitted viruses in tomato and pepper plot established in farm no. 2, District of Zapotitán.

Crop	Treatment	30 Days After Transplant		60 Days After Transplant		Yield (MT/Ha)
		Incidence	Severity	Incidence	Severity	
Tomato	Uncovered	50%	1	100%	2	6.61
	Agryl	0	0	60%	2	16.38
Pepper	Tricot	0	0	65%	2	15.87
	Uncovered	30%	1	75%	2	7.51
	Agryl	0	0	50%	2	17.63
	Tricot	0	0	50%	2	19.73

Table 8.B. Results of physical protection agai nst w hitefly-transmitted viruses in tomato and pepper plot established in farm No. 3, District of Zapotitán.

Crop	Treatment	30 Days After Transplant		60 Days After Transplant		Yield (MT/Ha)
		Incidence	Severity	Incidence	Severity	
Tomato	Uncovered	50%	1	100%	2	5.5
	Agryl	0%	0	100%	2	13.7
	Tricot	0%	0	100%	2	15.6
Pepper	Uncovered	30%	1	100%	2	4.6
	Agryl	0%	0	20%	2	13.48
	Tricot	0%	0	40%	2	14.4

Table 8.C. Results of physical protection agains t w hitefly-transmitted viruses in tomato and pepper plot established in farm no. 3, District of Zapotitán.

Crop Treatme nt		30 Days After Transplant		60 Days After Transplant		Yield (MT/Ha)
		Incidence	Severity	Incidence	Severity	
Tomato	Uncovered	5%	1	50%	2	10.2
	Agryl	0%	1	40%	1	25.0
	Tricot	0%	1	40%	1	19.0
Pepper	Uncovered	25%	1	50%	2	11.0
	Agryl	0%	1	10%	1	15.0
	Tricot	0%	1	10%	1	16.3

Contributors: Jose Maria Garcia, and Juana E. Pérez Mancía, CENTA.

Breeding for resistance to whitefly-transmitted begomoviruses.

In the case of **tomato** and **peppers**, there is practically no crop improvement in Latin America, despite the fact that this region is the centre of origin of these crops. Towards the end of Phase II, limited additional resources became available to the Tropical Whitefly Project, which allowed us to evaluate in Mesoamerica, some tomato genotypes selected by the Asian Vegetable Research Development Centre (AVRDC) of Taiwan, as sources of resistance to whitefly-transmitted viruses that occur in Asia. Dr. Peter Hanson, tomato breeder of AVRDC, and the Coordinator of the TWFP, personally evaluated the potential sources of resistance in the two pilot sites: Zapotitán, El Salvador and Yucatán, Mexico. The materials **(Table 9)** were evaluated between January and June 2003 in Zapotitan, according to a complete randomised block design, with three replications.

As mentioned above, these tomato genotypes had been selected in Taiwan (AVRDC) for their resistance to Old World tomato begomoviruses (whitefly-borne), such as *Tomato yellow leaf curl*

virus (TYLCV) and *Tomato leaf curl virus*, which are completely different from the New World begomoviruses that attack tomato in the Americas, with the exception of TYLCV, which was introduced in the Caribbean Region in the 1990s. However, the lines bred in Florida (FLA), USA, were originally selected for their resistance to New World begomoviruses, before being shipped to Asia. The North American begomoviruses probably originated in South America, and then moved into the Caribbean region.

Table 9. Tomato genotypes selected by AVRDC as potential sources of resistance to New World begomoviruses.

Genotype Orig	in	Provider
TY 52	LA 1969 (L. chilense)/Tyking	D. Zamir, Israel
FLA 456-4	LA 2779 (L. chilense)	J. Scott, USA
FLA 505	LA 1969 (L. chilense)	J. Scott, USA
FLA 478-6-3-0	LA 1938 (L. chilense)	J. Scott, USA
FLA 653-3-1-0	LA 2779 (L. chilense)/ Tyking	J. Scott, USA
H 24	L. hirsutum f. sp. glabratum	G. Kalloo, India
TLB 111	H 24	AVRDC
TLCV 7	H 24	AVRDC
CLN 2026 D	Susceptible check	AVRDC
Trnity Pride	National cultivar	Seminis
Sheriff	Local cultivar	Harris Moran

Table 10 shows that there are two entries: FLA 456-4 and FLA 505 that behaved as resistant to the viruses present in the screening location chosen in the Valley of Zapotitan. Serological tests performed at CIAT, confirmed the presence of whitefly-transmitted viruses in susceptible materials, with the exception of the line FLA 478-6-3-0, which was infected by an aphid-borne virus. So, it is possible that this material has resistance to begomoviruses.

Whereas the purpose of this experiment was to detect possible sources of resistance and not to select commercial cultivars, the virus-resistant FLA 505 line possessed large, round fruits suitable for fresh (salad) consumption. These fruits were very sensitive to manipulation. FLA 456-4 had small, firm fruits but an orange colour, which is not suitable for the Central American market that prefers medium-sized, red tomatoes. These traits can be corrected in a breeding program. The remaining lines were readily infectected and suffered considerable yield losses, as seen in **Table 11**. It is possible that the affected FLA lines were susceptible to other viruses (probably aphid-borne), as suggested before according to preliminary serological tests.

Table 10. Field screening of tomato genotypes selected by AVRDC as potential sources of resistance to begomoviruses in Zapotitan, El Salvador.

Genotype	Incidence	Mosaic	Curling	Stunting	Malform
TY 52	100%	2	2	4	3
FLA 456-4	0%	1	1	1	1
FLA 505	0%	1	1	1	1
FLA 478-6-3-0	20%	2	2	1	2
FLA 653-3-1-0	100%	1	1	1	1
H 24	100%	2	3	4	3
TLB 111	100%	2	4	4	3
TLCV 7	100%	2	4	4	3
CLN 2026 D	100%	2	3	3	2
Trinity Pride	100%	2	3	3	2
Sheriff	100%	2	4	3	3

Scale: 1 = no symptoms; 5 = maximum symptom expression.

Table 11. Evaluation (yield) of tomato genotypes selected by AVRDC as potential sources of resistance to begomoviruses in Zapotitan, El Salvador.

Genotype	Yield (MT/Ha)
TY 52	0.4 C
FLA 456-4	28.0 A
FLA 505	11.0 B
FLA 478-6-3-0	3.7 B
FLA 653-3-1-0	1.4 C
H 24	0.5 C
TLB 111	0.3 C
TLCV 7	0.1 C
CLN 2026 D	0.5 C
Trinity Pride	0.7 C
Sheriff	0.3 C

Contributors: Juana E. Pérez Mancía (CENTA), Peter Hanson (AVRDC).

Loroco: '*Whitefly and virus control in a horticultural crop usually managed by women in their backyards, with high income potential'*.

Loroco (*Fernaldia pandurata*) is a local vegetable (flower buds are harvested) that has reached unexpectedly high prices due to external demand from Salvadoran and Guatemalan migrants working in the US as well as from local restaurants and U.S. fast-food chains operating in Central America. This crop is tended mostly by women in their backyards **(Figure 8)** , and provides a significant amount of income for resource-poor households. A hectare of loroco has the potential to produce a net profit of US $ 15,000 in its third year of cultivation. Unfortunately, this native crop usually lasts about a year due to whitefly and virus attacks **(Figures 9 and 10)**.

Figure 8. Loroco plot under shade in the Valley of Zapotitán.

The TWFP decided to conduct some preliminary tests with this crop considering its potential contribution to poverty alleviation. To this end, three plots were located in the Valley of Zapotitán, each experimental plot occupying and area of 1,000 square meters, divided in two treatments, the traditional planting system (500 sq/m) , and the improved system (500 sq/m) under shade (covered with palm leaves), as shown in **Figure 11a, b**.

Figure 9. Whitefly attack on loroco.

Figure 10. Whitefly and virus damage to loroco (see damaged buds).

Supports were spaced in a 4X4m pattern and plants were transplanted at 2X2 m distances. After the first year, the buds were harvested once a week. As part of an IPM package, the planting material for both treatments was produced under insect-proof conditions. The distance between the support stakes was 4 meters, and the distance between plants was 2 meters. The variables studied were: plant vigour, disease incidence, and yield. It must be taken into account, that loroco is a perennial crop, which develops its maximum yielding capacity after the second year of planting. The 2003 plots were transplanted in May, and first evaluated in February 2004. None of the experimental plots were allowed to be colonised by whiteflies, using a mild detergent whenever these insects were observed to colonise the test plants. No insecticides were used in these experiments.

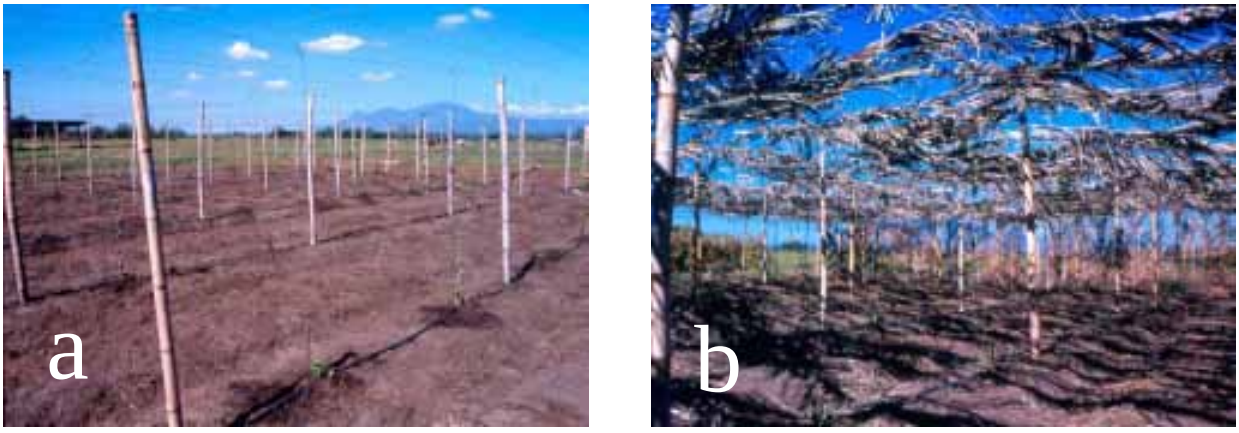

Figure 11. Traditional (a) and co vered (b) lo roco plots evaluated fo r virus incidence and yield.

In the traditional loroco planting system (uncovered), viral disease incidences > 50% were observed in the three plots two months after planting, whereas the loroco plants under shade had average disease incidences < 10% in all three experimental plots **(Table 12)**.

Nine-month loroco plants grown under the traditional open system, showed an average viral disease incidence of 70%, whereas the loroco plants grown under shade had an average viral disease incidence of 12% **(Table 12)**. Plant vigour under the uncovered and covered systems was 7 and 2, using a 1-9 scale, where 1 meant normal plant vigour and absence of symptoms **(Figure 12)**, and 9 was a systemically infected plant, showing malformation **(Figure 13)**.

Table 12. Preliminary results of loroco trials in the valley of Zapotitan.

Plot	Treatment	Virus Incidence		Yield (Lbs)
		3 Months	9 Months	12 Months
1	Uncovered	68%	85%	70
	Covered	10%	20%	120
2	Uncovered	60%	75%	80
	Covered	9%	12%	140
3	Uncovered	30%	50%	110
	Covered	7%	5%	200

Figure 12. Loroco leaves under shade showing no virus symptoms.

Harvesting of loroco buds for both treatments was initiated in June 2003, and 8 months later, yields for the protected plots were 43.5% higher than those for the traditional planting system **(Table 12)**. This difference may have been larger, should we have used unprotected planting material, as was the case in the past. Moreover, the yield of the unprotected loroco plants will probably start declining after the first year as a result of the high incidence of viral disease. These are preliminary but encouraging results for this ethnic crop. Loroco has also shown to be a non-traditional export crop. In 2000-2001, El Salvador exported over 23 metric tons of this crop. A pound of loroco sells for US $ 10.oo in the U.S. market, primarily to Salvadoran and Guatemalan ex-patriates.

Figure 13. Virus-infected loroco leaves in the traditional system.

Contributor: Estela Escamilla (CENTA).

Research activities and results: Yucatán, Mexico

The second pilot site was the State of Yucatán, Mexico, characterized by its traditional agricultural practices since pre-Columbian times and, more recently, by a continuous struggle on the part of small-scale farmers to diversify their agriculture with high-value horticultural crops, in hopes of improving their livelihoods.

The first set of activities initiated in 2001-2002, was designed to characterise ***the ex ante*** **socio-economic situation** of these target regions. In Yucatan, Mexico, a study was already in progress when the project started, and the CPP-TWFP partially supported this study in order to use the data for the *ex ante* analysis. In Yucatán, there has been a clear trend from traditional 'milpa' (shifting maize-bean-squash cultivation) to horticultural production among small-scale farmers of Mayan origin. This trend pursues the same basic objective of maximising profits from limited land resources. An *ex-ante* study was conducted in Yucatan among resource-poor farmers, based on different models of integrated agricultural production systems, considered by different authors as the most sustainable for the American humid tropics. Production systems included: traditional food staples, fruit crops, vegetables, animal husbandry and forestry. In the case of traditional crops, such as maize, the strategy was to increase their productivity through the use of improved varieties and better agronomic practices (e.g. drip irrigation, fertilisers). Backyard animals, such as pigs and sheep were added to these production systems successfully. Fruit crops, such as citrus, papaya and banana, and tree species were also included, although their perennial nature did not significantly contributed to the preliminary evaluation of the economic benefits of these integrated production systems.

The document (in press) entitled “Integrated Production Modules for Low-Income Producers in the Yucatan Peninsula”, addresses the need to change their traditional monoculture systems (e.g.

maize, rice, henequen) for mixed cropping systems that includes basic grains, vegetables, fruit crops, animals and forestry. The study focused on the development of the Farmers' Family Production Units (UPFC), which group 80% of the small-cale farmers in the region. The TWFP was particularly interested in the module: 'Production of Vegetables, Fruit Crops, and Basic Grains'. This study was conducted by Arnulfo Gómez, Luis Miranda, and José Tun Dzul of INIFAP-Mocochá. The last investigator is part of the TWFP-INIFAP group of collaborators. The study unit had four hectares, planted to tomato, chillies, watermelon, cucumber and 'calabacita' (small squash). The labour force consisted of the farmer, four sons, and three labourers. The *ex ante* income of this farm was approximately US $ 5,000. The innovations introduced in this module to maximise profits were: 1) drip irrigation (which is being promoted by the TWFP in conjunction with the microtunnel technology), 2) production of basic grains for food security, particularly during the rainy period when vegetables suffer from phytosanitary problems related to the high humidity characteristic of the rainy season. And 3) production of crops with short (vegetables), medium (bananas, papaya), and long (citrus, trees) production cycles.

The results of this study over three years, showed that the drip irrigation system is a good investment because it reduced the cost of labour in 70% for that task. The initial investment can be recovered in the five years of life that the irrigation system is supposed to last. The maize and cowpea planted for consumption, ended up being sold in the market in the green stage (immature) because the production of vegetables failed due to whitefly-related problems. This problem affected the entire Peninsula of Yucatán. The fruit crops, particularly papaya, produced over 18 MT/Ha. The forestry component was at an early stage to contribute to the total farm income at the time of the first analysis. Ultimately, the system was not profitable due to the whitefly problem (Cost/Benefit < 1.0 = 0.4) and collapse of the horticultural component. The authors concluded that "the project was severely affected by the emergence of viruses transmitted by whiteflies, which caused severe yield losses in the horticultural crops selected, mainly in tomato and chilli, the primary crops of this module. Should the whitefly problem been managed, this production module could have produced a net return on the capital invested of > 32%.

Biological characterisation of pilot site.

A thorough analysis of the ecology of the whitefly *Bemisia tabaci* in the Yucatán Peninsula was conducted in 2001-2002. This study describes the horticultural areas of the state, where the TWFP works; maps the distribution of *B. tabaci* in Yucatán; and describes the dynamics of whitefly populations according to environmental factors (rainfall and temperature) that favour whitefly/virus outbreaks **(Figure 14)**. This information has been used to produce 'whitefly risk maps' for every month of the year and horticultural area in the region **(Figure 15)**, which has greatly aided the TWFP in the selection of 'hot spots' and implementation of IPM measures. As in most regions of Central America, the increase in whitefly populations occurs between the months of December and May.

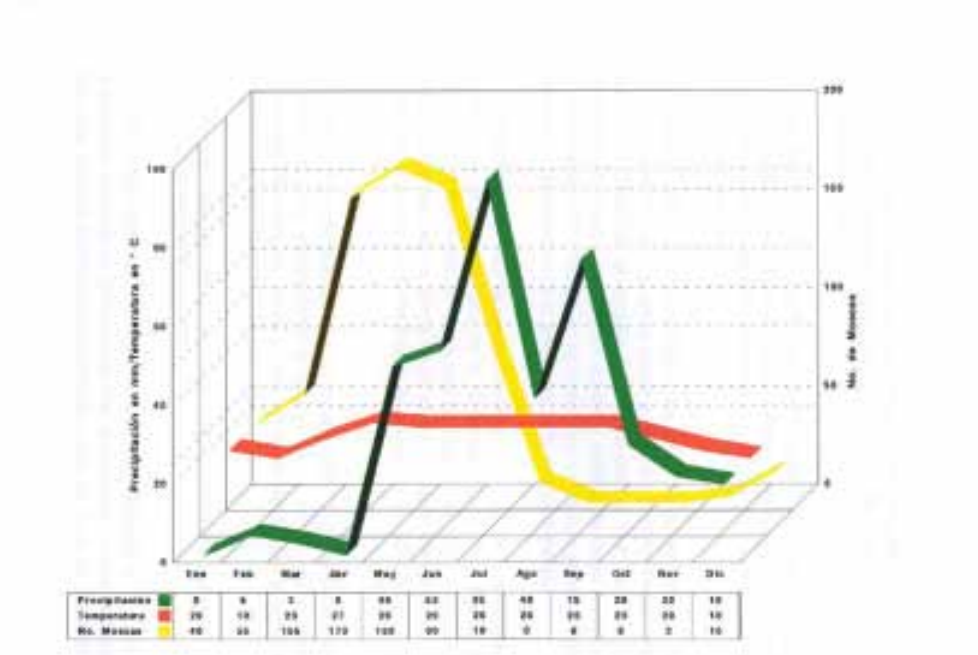

Figure 14. Whitefly population dynamics a nd related environmental factors in th e Yucatan Peninsula, Mexico.

The main crops chosen in Yucatan were tomato and chilli, primarily the 'Habanero' chilli grown for its high quality in this region and exported to the rest of the country. Given the high rate of transmission of the begomoviruses detected, such as *Pepper golden mosaic virus, Pepper huasteco yellow vein virus, Tomato mottle virus* and *Tomato yellow leaf curl virus,* it was assumed that the main whitefly vector was *B. tabaci*. However, it was not known whether the B biotype had already emerged in Yucatan until samples were analysed at CIAT last year **(Figure 16)**.

These tests confirmed the presence of the original biotype (A) of *B. tabaci,* and some unidentified biotypes. Only one tomato sample yielded a possible individual with characteristics of biotype B of *B. tabaci*. It is thus evident that the B biotype is present in Yucatan, albeit in low frequency.

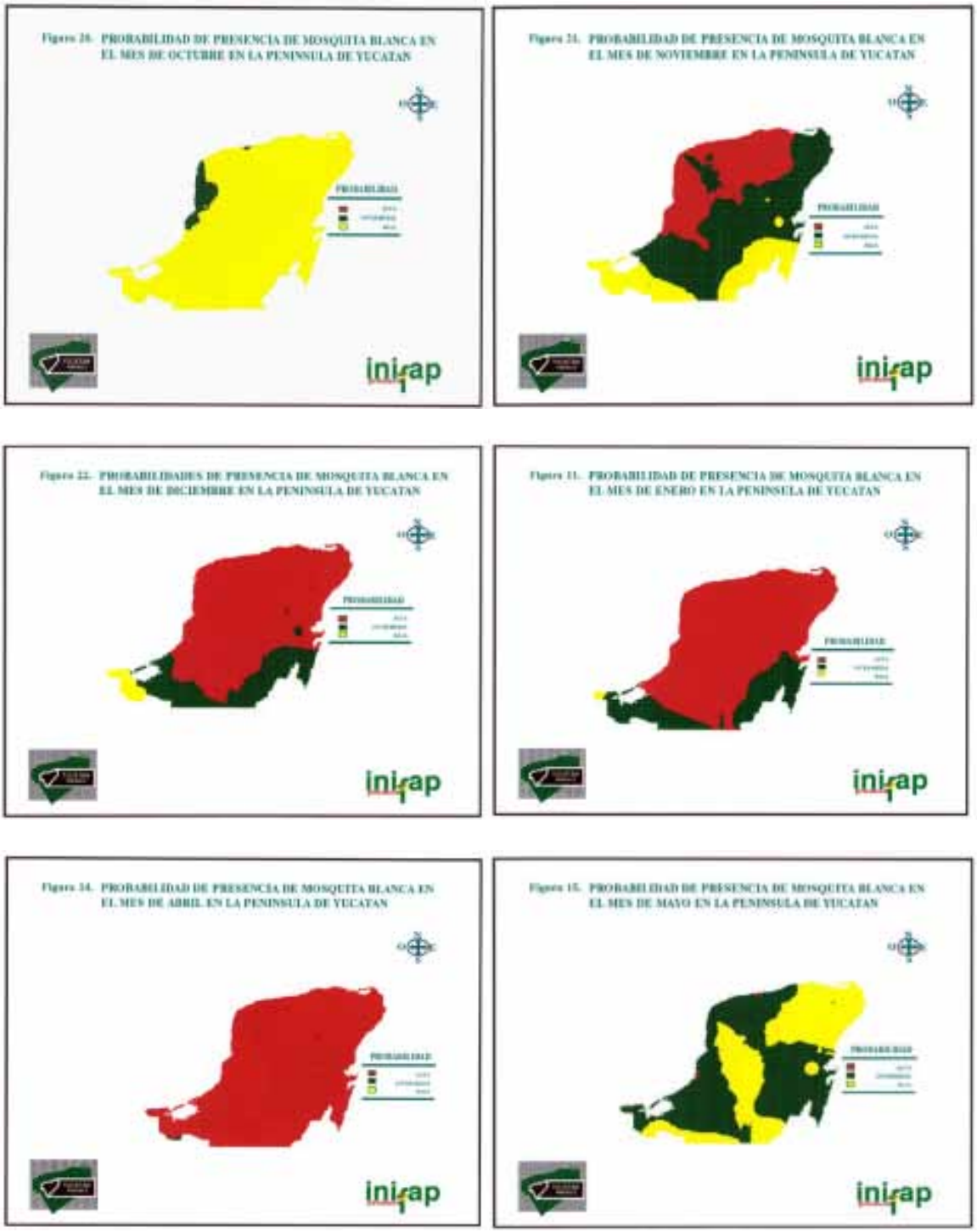

Figure 15. Whitefly/geminivirus risk probability in selected months of the year according to climatic parameters in the Yucatán Peninsula, Mexico.

Continued

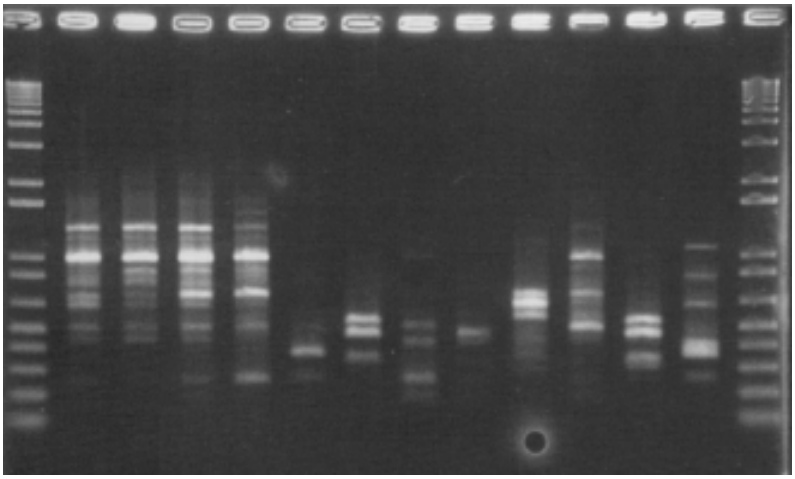

Figure. 16. SCAR´S for the identification of whiteflies from Yucatán. Lanel 1 = molecular marker (1Kb). Lanes 2 and 3 = Whieflies from mint (*B. Tabaci*). Lanes 4 and 5 = whiteflies from Tamaulipas. Lanes 6–10 whiteflies from Yucatan, Lanes 7 and 8 corr espond to biotype B of *B. tabaci*, Lane 9 corresponds to *T. vaporariorum*. 11 = *B. tabaci* control. Lane 12 = Biotype B of *B. tabaci*. Lane 13 = *T. vaporariorum*. Lane 14 = Molecular marker (1Kb).

The last sequences obtained for tomato begomoviruses found in Yucatan, indicate that *Tomato mottle virus* was the predominant virus in the pilot sites selected. Aphid-transmitted viruses, particularly potyviruses, have also been detected in pepper tomato and cucurbit samples (**Table 13)**. Yucatan is also the only region in Continental Latin America, where an Old World virus, *Tomato yellow leaf curl virus* (TYLCV) has been reported in tomato. Specific PCR assays with tomato samples from Yucatan confirmed the presence of this monopartite begomovirus, mixed with bipartite begomoviruses as well **(Figure 17)**.

Table 13. Serological assay of selected plant samples from Yucatan, Mexico.

		GEMINIVIRUS POTY VIRUS				CMV	
Plant Sample	**Locality**	**Atc. M. 4C1-3f7 Absorbance* Abs**		**AGDIA orbance** Abs**		**Atc. M. 5ª9-1F9-1D5 orbance***	
Ya-ax-ak	Hunucmá	0.08	-	0.93	+	0.13	+
Chilli habanero c/s	Hunucmá	0.08	-	0.02	-	0.002	-
Chilli habanero línea 11	Mocochá	0.09	-	0.08	-	0.01	-
Tomato	Dzán	0.25	+	0.41	+	0.06	-
Negative Control		0.01	-	0.06	-	0.008	-
Positive Control		1.22	+	2.00	+	0.39	+

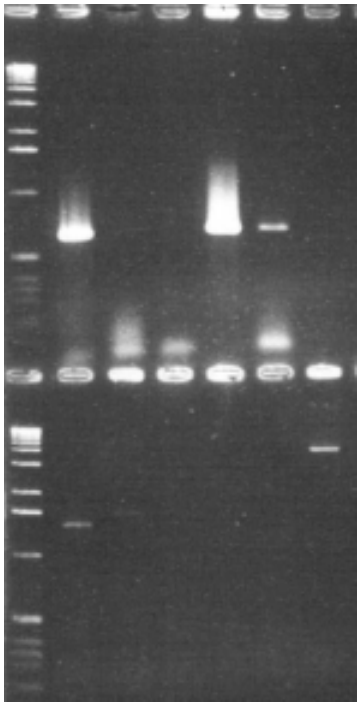

Figure 17. PCR assay for TYLCV. Lane 1 = 1 Kb molecular marker. Lane 2 = Positive control for TYLCV. Top gel: Lanes 3-7 Tomato samples from Yucatan. Bottom gel: Lanes 3 – 7: same samples assayed for bi-partite begomoviruses (sample 7 was positive).

The presence of both Old World (TYLCV) and New World begomoviruses in Yucatán, complicate the breeding for resistance to these different viruses. Fortunately, the potential sources of resistance to begomoviruses sent by AVRDC **(Table 8)**, had been originally evaluated and selected for Old World begomoviruses, including TYLCV. These materials were evaluated in three different localities of the Yucatan Peninsula, Mocochá, Yaxhoom and Uxmal. The first locality had very low virus incidence (after Hurricane Isidore) and was used to evaluate the agronomic characteristics of the imported germplasm. The second and third localities had medium and high virus pressure, respectively, and provided good data on the potential of these entries as sources of resistance. In Yaxhoom, FLA 456-4 was the best entry (no symptoms of viral infection), followed by FLA 478-6-1-0, FLA 653-3-1-0 and FLA 505. In Uxmal, FLA 456-4 was again the most resistant entry, but all of the FLA lines behaved well. The remaining materials did not produce.

Based on these results, FLA 456-4 was selected as parental material to improve susceptible local tomato cultivars, such as 'Maya'. The crosses were made at AVRDC, Taiwan, by Dr. Peter Hanson, and segregating (F2-F4) lines **(Table 14)** were sent to Yucatan. **Table 15** shows the results of this evaluation.

As it can be concluded from **Table 15**, the resistance to begomoviruses present in FLA 456-4, was not transferred to the segregating materials evaluated in Yucatan, and the lineswere susceptible to early blight. A new set of crosses is being prepared now at AVRDC to exploit the high levels of resistance found in the FLA tomato lines.

Table 14. Tomato materials at different levels of inbreeding and r ange from F2 to F4, derived from crosses involving FLA 456-4 as a source of begomovirus Resistance.

Entry Generation		Previous	Quantity
CLN2714-7	F2	21606-7	50 semillas
CLN2714-117	F2	21606-117	50 semillas
21602-21	F3		30 semillas
21602-40	F3		30 semillas
21602-76	F3		30 semillas
21602-94	F3		30 semillas
21602-105	F3		30 semillas
21602-175	F3		30 semillas
21602-264	F3		30 semillas
21602-3	F3		30 semillas
21602-56	F3		30 semillas
21602-90	F3		30 semillas
CLN2674-129-27-11	F4	22995-2	50 semillas
CLN2674-129-27-11	F4	22989-11	50 semillas
CLN2674-138-9-30	F4	22995-30	50 semillas
CLN2679-199-9-14	F4	23027-14	50 semillas
CLN2679-199-9-26	F4	23027-26*	50 semillas
CLN2679-199-12-8	F4	23028-8	50 semillas
CLN2679-199-16-29	F4	23030-29*	50 semillas
FLA456-4	check		50 semillas

Table 15. Results of the field evaluation of segregating tomato materials derived from crosses with FLA 456-4 in Yucatán.

Material	No. plants	%/virus	Severity	% E. blight
CLN2714-7	29	96	4	75
CLN2714-117	18	97	5	80
21602-21	25	99	5	66
21602-40	22	92	5	78
21602-76	16	89	5	90
21602-94	24	100	5	92
21602-21	6	100	5	67
21602-105	7	100	5	77
21602-175	24	100	5	86
21602-3	17	98	5	66
21602-56	8	100	5	87
CLN2679-199-12-8	18	97	5	98
CLN2679-199-16-29	11	92	5	49
CLN2674-129-27-11	2	100	5	76
21602-264	2	100	5	88
CLN2674-138-9-2	5	100	5	67
FLA-456-4	60	25	2	30

A survey of cultivated and wild hosts of *Bemisia tabaci* in horticultural farms of northern Yucatan, revealed the existence of 58 wild and 14 cultivated plant species. The wild species belong to 22 different botanical families, and the cultivated species to three major families: *Leguminosae* (*Vigna unguiculata*), *Cucurbitaceae* (*Cucurbita, Citrullus* spp.)and *Solanaceae* (*Lycopersicon, Capsicum, Solanum, Nicotiana* spp.). **Table 16** shows the distribution of the whitefly-transmitted viruses found in Yucatan by the coordinator of this project in Mexico, Dr. Raul Diaz-Plaza (INIFAP) in the various host plants identified.

Table 16. Ecology of begomoviruses* in Yucatan, Mexico.

Host PHY	VV	PepGMV	TYLCV	ToMoV
Malvaceae	-	+	+	+
Leguminosae	+	-	+	-
Euphorbiaceae	+	-	-	-
Cucurbitaceae	+	-	+	-
Convolvulaceae	-	-	+	-
Amaranthaceae	+	-	+	-
Solanaceae				
Tomato	+	+	+	+
Sweet pepper	+	+	+	+
Habanero pepper	+	+	+	+

* PHV = *Pepper huasteco yellow vein virus*; PepGMV = *Pepper goldem mosaic virus*; TYLCV = *Tomato yellow leaf curl virus*; and ToMoV = *Tomato mottle virus.*

Contributors: Dr. Raul Diaz-Plaza, Ing. M.Sc. Genovevo Ramirez, INIFAP-Mocochá.

IPM measures implemented in Yucatán

The INIFAP group in Yucatán has been working on whitefly control since the Peninsula was severely affected by this pest and the viruses it transmits in 1989. Yucatan was the first place where the microtunnel technology was adopted by small-scale farmers in Latin America **(Figure 18)**.

Figure 18. Chilli production under microtunnels in the state of Yucatán (1999).

This strategy worked efficiently until new chemical products appeared in the market for whitefly control (mainly the new neonicotinoids; e.g. imidacloprid). The salespeople from the agrochemical companies that market these products, convinced farmers to switch to chemical control instead of dealing with nets, which require more intensive labour and higher production costs, Thus, at the onset of the TWFP-Phase II in Yucatán, most producers of horticultural crops had abandoned the use of anti-whitefly nets and reverted to chemical control using imidacloprid. A preliminary survey of the area affected by whiteflies in the state of Yucatán, clearly showed that imidacloprid was not controlling all the viruses transmitted by insects in either tomato or peppers **(Figure 19)**.

The national program scientists of INIFAP-Yucatan were already conducting tests on IPM practices to control the whitefly *B. tabaci* when the project started. Their main strategy was the use of natural barriers (e.g. maize, cucumber, eggplant) and intercropping chillies with other plant species (mostly horticultural species, such as mint, leeks, basil and coriander) of economic value, previously shown to possess some whitefly-repellent properties.

Figure 19. Effect of microtunnels on tomato production in Yucatán, Mexico.

Whereas the main objective of the project was to show small-scale farmers that although the use of nets implies higher production costs, these are offset by the higher yields and quality obtained, the TWFP decided to merge the two strategies (natural barriers/repellent plants and microtunnels). These experiments were evaluated during the first year of the project in three different localities (Mocochá, Hunucma and Yaxhoom), and clearly showed that these strategies do not work. Basically, even under moderate whitefly pressure, virus incidence was approximately 55% in both the control (traditional planting) and plots protected by live barriers and inter-planted with whitefly-repellent crops **(Table 17)**.

Table 17. Effect of the association of chilli and whitefly-repellen t plants on w hitefly-borne virus incidence in Hunucmá, Yucatán.

	Virus Incidente (%)			
Treatment	**Rep. 1**	**Rep. 2**	**Rep. 3**	**Rep. 4**
Coriander	33	45	43	55
Mint	44	45	53	58
Basil	54	60	62	56
Leek	56	55	72	43

F.V. G.	L.	S.C.	C.M.	FOBS.	$F_{0.05}$	$F_{0.01}$
Treatment	4	498	124.5	**2.07 N.S.**	3.06	4.89
Error	15	899.75	59.98			
TOTAL	19	1,397.75				

The microtunnel work was initiated in November 2002, three months after hurricane Isidore had caused considerable damage in the Yucatan Peninsula. One of the effects of hurricanes, is the destruction of small wildlife, mainly insects. Consequently, the whitefly pressure during the first months of 2003, when the effect of the microtunnels had to be evaluated, was extremely low, obliterating the differences between the uncovered and covered controls (less than 5% begomovirus incidence).

The microtunnel trial was established again in the last quarter of 2003, in the locality of Dzán. The trial was conducted in a commercial rather than experimental field of 1.5 has, because nine farmers decided to pool their resources to finance the trial. Having had disappointing results with some farmers that did not take good care of the experimental trials recommended by the project, we decided to let them conduct the trial, as long as they followed the instructions for the correct use of the technological package. This IPM package consists of: 1) tomato seedlings produced under screen to avoid the early infection of seedlings grown outdoors; 2) one application of a systemic insecticide to seedlings two days before transplanting; 3) cover the seedlings immediately after transplant with the screening material (Agribon, Agryl, Tricot, etc); 4) a second and last systemic insecticide application two days before removing the net cover (approximately a month after transplanting). It must be taken into account that tomato and pepper growers in whitefly-infested areas usually make over 30 insecticide applications until harvest time.

The collaborating farmers covered approximately one hectare, alternating covered and uncovered rows in hopes of using the covered row as a barrier for the whitefly. **Table 18** shows the results obtained.

These results were obtained at a time of moderate virus pressure, and, moreover, the uncovered control had been properly protected in the nursery and field using the chemical protection scheme recommended by the TWFP. The intercropping of covered and uncovered rows probably helped reduce disease severity, but it did not prevent virus infection and plant damage **(Figure 19)**. Had these farmers followed their own agronomic practices in a year of high whitefly/virus pressure, total crop failure may have occurred in the uncovered treatment.

Table 18. Efect of microtunnels (Agribon) on tomato production in Dzán, Yucatan.

Variable Unc	overd	Covered
Plant height	55 cm	90 cm
Harvest time	80 dat*	87 dat
Virus Incidence	85%	4%
Disease severity (0-4)	1 (28%), 2 (23%), 3 (34%)	1 (3%), 2 (1%)
Yield	35 MT/Ha	45 MT/Ha
Fruit quality (1-3)**	1 (40%), 2 (30%), 3 (30%)	1 (60%), 2 (25%), 3 (15%)
Net Profit	US $ 14,200	US $ 19,163

* dat = days after transplant; ** 1 = best quality/higher price

An increase of 25-26% in yield and net profit (ca. US $ 5,000) under these conditions is significant, because this additional income pays for the cost of the microtunnels, including materials and labour (Total cost/Ha = US $ 2,000). Moreover, microtunnels increase the quality of the produce and control aphid-borne viruses, which are not controlled by any insecticide. Last, microtunnels protect the investment of resource-poor farmers in times of high whitefly/virus pressure (crop insurance), when insecticides do not prevent significant losses and even total crop failure.

Contributors: José de la Cruz Tun Dzul, Felipe Santamaría B., Raul Diaz-Plaza, INIFAP-Mocochá.

Outputs

The case studies and socio-economic analyses conducted in El Salvador, Yucatán and previously in other Central American countries by the TWFP, clearly show that small-scale farmers are trying to diversify their traditional cropping systems with high-value crops to maximise the income derived from their limited land resources.

These surveys also show that the whitefly *Bemisia tabaci* and the viruses it transmits (begomoviruses) are the main factors responsible for the significant yield losses suffered by traditional and non-traditional susceptible crops alike. These problems have occurred at a time when most Latin American governments had to reduce public spending (including agricultural research and extension), in order to pay their ever- increasing external debts. Consequently, small-scale farmers who had started to grow non-traditional (e.g. vegetables) crops without any technical assistance, had to protect their investment according to the recommendations of the only technical personnel reaching them: the salespeople working for agrochemical companies. At the same time, internacional agricultural research centres were forced to change the focus of their research from food production to natural resource management. Some of the many negative consequences of the lack of technical assistance to small-scale farmers has been: crop failure, need to import food, higher unemployment rates, increased poverty, and extreme levels of pesticide abuse. The latter has caused severe environmental contamination and widespread health

problems in rural and urban populations. Thus, it must become clear to policy makers that shifting resources from food production-oriented research to research in natural resources and sociology work in rural areas, is extremely counterproductive in the absence of sustainable crop production components. Farmers may be organised, but unless their current crop production problems are solved, they cannot either feed themselves or improve their livelihoods.

Common bean has been one of the two main food staples of the Mesoamerican diet (together with maize) since pre-Columbian times. Without the active common bean breeding program that the Bean Programme of CIAT initiated in the 1980s, Mesoamerica would not be eating beans during almost half of the year, due to the whitefly/virus problems that affect this region every year during the dry season. Unfortunately, the shifting of research priorities at CIAT has considerably reduced the capacity of its Bean Project (not a Programme any more due to its reduced size) to produce more begomovirus-resistant common bean lines for this region. Fortunately, the breeder of the Pan American School in Honduras, Dr. Juan Carlos Rosas, has continued to improve CIAT's materials to create new common bean cultivars. The TWFP was lucky to find in El Salvador a highly promising breeding line (EAP 9510-77) that was commercially acceptable, and highly virus-resistant, to help evaluate it and promote its adoption in order to incorporate this variety into an IPM package that allowed small-scale farmers to cultivate common beans once more during the dry months of the year. This is the first time that an IPM package has been delivered together with the virus-resistant variety. Failure to do so in the past, has resulted in the breakdown of improved, virus-resistant lines, such as CENTA-Cuzclateco (DOR 364), which has broken down due to the constant pressure of viruliferous whiteflies and misuse of pesticides.

During the duration of the project, a total of 35 plots of EAP 9510-77 (later released officially as 'CENTA San Andrés') were established nation-wide in El Salvador, with yields ranging between 1,085 and 1,200 kg/ha. The higher yields can be explained by the planting of these bean plots during the end of the rainy season (August-November), when whitefly populations are at its lowest level. Planting at this time is required to register a new cultivar in El Salvador. This new variety yielded over 700 kg/ha under the very severe whitefly (>200 adult whiteflies/plant) and begomovirus incidence that occurred in the 2001-2002 dry season, with only one application of insecticide (as compared to more than 10 applications made by local farmers in the valley of Zapotitán). The local landrace 'Rojo de Seda' and one of the old improved cultivars, 'CENTA Cuzcatleco' yielded under 100 kg/ha under these conditions. The insecticide recommendation was later increased to two applications in seasons of very high whitefly incidence, in order to increase yields to the yielding capacity of this improved variety (1-1.4 MT/Ha).

The new bean line was also subjected to cooking and tasting tests with both male and female farmers. All farmers accepted the new line because of its shorter cooking time (60 min vs. 70 min for 'Rojo de Seda'); thick broth and good taste. From the economic point of view, the net benefit for the EAP line was US $ 908.15/ha vs. US $ 786.81/ha for the latest commercial cultivar released in El Salvador (CENTA 2000), during the August-November period. During the peak whitefly season, CENTA 2000 cannot be grown without multiple insecticide applications, whereas the EAP line would yield a net profit in excess of US $ 500/ha, with only two applications of insecticide, and production
costs ranging between US $ 80-100/ha. The statistical analysis was done using 'paired plots' and their entire area (500 m2) as the sampling unit. Yields are expressed as kg/ha, and their

significance was calculated by using the Student 't' test. The potential impact of this new bean variety for El Salvador amounts to over 33 million dollars, assuming a total bean area of 64,000 has, a single planting, and the current minimum price of US $ 520/MT. CENTA San Andrés is expected to be adopted in other Central American countries that consume red-seeded common beans, such as Honduras, Nicaragua and Costa Rica.

The objective of bringing common bean back into cultivation in the Valley of Zapotitan, during the December-April dry season, is already a reality. This past summer season (November 2003-March 2004) approximately 35 has of CENTA San Andrés were planted in Zapotitán. CENTA is actively multiplying seed of this new cultivar for further distribution in Zapotitán and El Salvador. CENTA San Andrés has the best commercial and virus-resistance characteristics of any red-seeded common bean cultivar ever produced in Latin America.

Food security is an important concern of this project and most developing countries, but traditional crops, such as common bean, are not going to take any small-scale farmer or country out of poverty because of their relatively low market value. Fruit and horticultural crops, on the contrary, make possible the generation of substantial income in small land areas. For instance, a hectare of maize or beans does not produce more than US $ 200-300 per growth cycle, whereas a tenth ($1/10^{th}$) of a hectare planted to tomato or chilli may produce over US $ 1,000 per planting. The Mesoamerican subproject of the TWFP has chosen two major horticultural crops: tomato and peppers (sweet peppers and chillies) to develop IPM packages that can be used by small-scale farmers to diversify their traditional cropping systems (not to replace them) and, thus, increase the profitability of their limited landholdings.

As in the case of common bean, the most viable strategy would be to use virus-resistant tomato and pepper varieties, but despite their Latin American origin, these crops have not been improved genetically to withstand all of the production problems that affect these crops in this region. So far, production of tomatoes and peppers in Latin America has only been possible due to the excessive use of pesticides, that make these products unsuitable for the European or North American markets, but not for the local market where the extremely toxic pesticide residues these products contain, are not detected.

In 2002-2003, a new project was implemented in El Salvador by the Financial Transactions Report Analysis Centre (FINTRAC) and the Centre of Investment, Development and Export of Agribusiness (IDEA). Fintrac's primary mission is to "increase the productivity and sales of our clients in a sustainable fashion. This involves incorporating small-scale producers to local, regional and global supply chains through innovative technical interventions in the field, as well as market analysis and linkages with commercial buyers". One of the "innovative technical interventions in the field" was the adoption of the microtunnel technology promoted in this area by the Tropical Whitefly Project financed by DFID. The site chosen by FINTRAC in El Salvador, was San Juan Opico, a few kilometers away from the main pilot site (Zapotitan/Ciudad Arce) of the TWFP in that country. The project started with 16 farmers in San Juan de Opico and approximately 33 has of land planted to different horticultural crops, mainly tomato and peppers protected inside microtunnels **(Figures 20 and 21)**. The goal of this project was to cover 280 has last year, and it has been operating successfully for two years in El Salvador and Honduras. Another project that has shown interest in the TWFP's work in El Salvador, and particularly in the micro-tunnel technology, is the Swisscontact-Helvetas Consortium in Honduras. Their

mission is to “help the development of small- and medium-sized enterprises in the processing and marketing of agricultural products”.

Figure 20. Adoption of the micro-tunnel techno logy by independent small-scale farmers in San Juan Opico, El Salvador.

Figure 21. Tomato grower in San Juan Opico, El Salvador, showing results obtained with the micro-tunnels recommended by the TWFP.

This project plans to expand soon into Nicaragua. More recently, in the Cauca Valley of Colombia, where whitefly-transmitted viruses are affecting snap bean and tomato production, a

group of horticultural farmers called ‘mesa agriculture’ has also contacted the TWFP for technical assistance with the micro-tunnel technology. This information is already available to interested users through our Web Page, and a hard copy of this publication is being prepared as well.

The microtunnel technology is also being adopted by independent farmers in the pilot sites of Zapotitán **(Figure 22)** El Salvador, and Yucatán, Mexico **(Figures 23 and 24)**. The situation in Yucatán is being carefully monitored, as small-scale farmers begin to see the advantages of using the micro-tunnels again. This project can be very influential in counteracting the biased ‘technical assistance’ provided by the agrochemical companies/distributors in this and other regions of Middle America.

Figure 22. Adoption of micro-tunnel technology in Zapotitán, El Salvador.

Figure 23. Adoption of micro-tunnels by independent farmers in Yucatán, Mexico.

Figure 24. Vegetable production in Yucatán using micro-tunnels.

The project is also interested in following up the socio-economic impact of implementing simple IPM measures for pest management in loroco in El Salvador, particularly within the concept of 'backyard' or 'peri-urban' agriculture with a gender focus. From the biological point of view, that is, pest control, the work conducted so far has shown that the IPM measures implemented are effective and sustainable. The collection of data continues in the pilot site in order to let the crop reach the most productive stage at the end of this year.

The TWFP is very interested in making the best use of the considerable amount of information gathered on the identification of whitefly species and biotypes, viruses transmitted by *Bemisia* and *Trialeurodes* species, crops affected, and temporal and spatial patterns of whitefly/virus spread, to develop reliable disease forecasting methods. The purpose of developing this pest/disease prognostic capacity would be to alert farmers to environmental conditions suitable for the development of high whitefly populations. The following is an abstract of a paper published in *Virus Research* by Francisco J. Morales and Peter Jones of CIAT. This is the most complete analysis on the ecology of *Bemisia tabaci* in tropical America. "Whitefly-transmitted geminiviruses are the most important constraint to common bean and horticultural crop production in the lowland tropics, particularly in Latin America. Currently, over 30 distinct species of geminiviruses transmitted by the whitefly *Bemisia tabaci* attack common bean, tomato, pepper, cucurbits and other horticultural crops in the lowlands and mid-altitude valleys of the American tropics and subtropics. A climate probability model (FloraMap) was obtained using 304 geo-referenced locations where *B. tabaci* and geminiviruses cause significant damage. *Clustering of the 304 points produced a simple model with two climatic variables: a dry season of at least 4 months with less than 80 mm of rain, and a mean temperature of the hottest month above 21º C.* A modified Koeppen climate classification showed that 55% of the geminivirus-affected localities are in theTropical Wet/Dry region; 21.6 % in the Tropical and Subtropical Dry/Humid climates, and the remaining locations belonged to the wet Equatorial and Trade Wind Litoral climates. These findings are expected to help implement sustainable Integrated Pest Management practices in mixed cropping systems and different environments throughout the tropics". **Figure 25** shows the distribution of the points and the areas of Latin America where *B.*

tabaci and the begomoviruses that this whitefly species transmits, may cause significant yield losses.

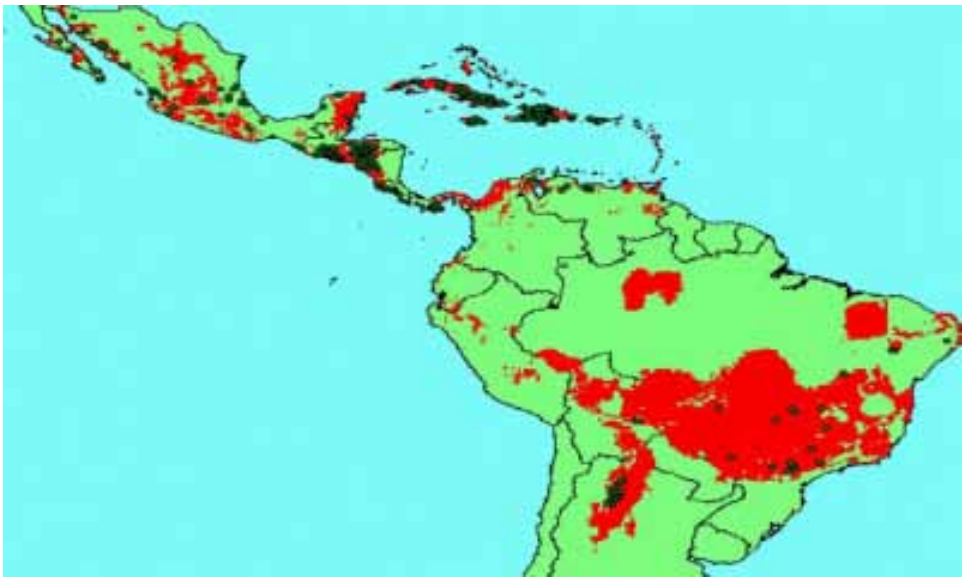

Figure 25. Distribution of hot spots (green points) and risk areas (in red) for *Bemisia tabaci* and whitefly transmitted begomoviruses.

Achievements and Obstacles

This subproject has demonstrated beyond any doubt that it is possible to manage pests and diseases as difficult and severe as those associated with the whitefly *Bemisia tabaci*, in agricultural regions where farmers have had to abandon the cultivation of susceptible basic food and cash crops. It has taken us three years to bring common bean, tomato, pepper and chilli production back to the Valley of Zapotitán (our pilot site) and other horticultural areas of El Salvador and Yucatán, Mexico, but it has been done and the small-scale farmers who have adopted the IPM methods recommended and validated by the project, have greatly profited from this work. The challenge of this subproject was not to increase productivity by 10 or 30%; whitefly transmitted viruses have the potential to cause total or significant (>50%) yield losses on all crops attacked almost every year during the prolonged dry season. Therefore, farmers prefer not to plant at all, forgoing the opportunity to gain badly needed income from their limited land resources for almost half of the year, and take their products to the market when the demand and prices are higher because of the winter season up north and the lack of internal production to supply the demand for food at that time of the year.

These achievements have not come easy for different reasons. First, most national agricultural research institutions (NARIs) in Latin America have been so badly affected by the chronic economic situation of the region, since the 1980s, that there are very few national scientists to work with or, worse yet, trained or motivated enough to conduct field experiments and work with small-scale farmers. Often, there are not enough vehicles to supervise the work, and the few vehicles available spend more time on the repair shop that on the road. The best and most experienced scientific personnel has retired or left their institutions, and the young replacements

are not well trained, Extension personnel are used to delivering ready-made products that farmers know how to use without much additional information (e.g. a new variety and a list of pesticides available in the market). They do not knot how to promote more complex IPM packages that require farmer training and education on the principles of IPM and direct and indirect economic benefits derived from the adoption of IPM practices. There is also a constant rotation of personnel at NARIs from task to task, often in response to the pressure of large-scale growers on the Ministry of Agriculture. And, finally, the timely utilisation of funds is hindered by the bureaucracy that characterises official institutions in developing countries.

Despite these obstacles, the outputs proposed by the project have been achieved as mentioned earlier. The subproject has shown that it is possible to grow common bean during the dry season of the year, without the need to apply insecticides every day or every other day as it is the belief among farmers due to the whitefly problem. We have shown that the virus-resistant new cultivar only requires a couple of insecticide applications to produce above the national average, but farmers get nervous when they are not applying pesticides every day. Obviously, the constant pressure of the pesticide companies on farmers has a lot to do with pesticide abuse in the region. This is an aspect to be dealt with in the next phase of the project, particularly in Yucatán, where small-scale farmers are just beginning to realise that they have should not have given up the use of micro-tunnels in order to rely exclusively on new, costly pesticides against whiteflies.

The promotion of physical barriers has been very successful to bring tomato and pepper production back during the dry season because they have seen that plants are not affected by vectors or viruses during their critical growth period, and the yield and quality of the produce results in higher prices and income. This technology has been practically 'stolen' by other projects and private organizations and has already been successfully used outside the pilot sites on a larger scale. Unfortunately, they have adopted only the physical materials (micro-tunnels) but not the IPM package that complements these physical barriers. The subproject needs to work on these aspects with the small- and medium-scale farmers who have already adopted this technology without all the complementary information.

All the previous sociological and biological work has provided enough material to understand the whitefly problem. This subproject and on-going CPP projects that have benefited from these data, are beginning to analyse all of this information in order to understand the ecology and epidemiology of whiteflies and whitefly-borne viruses with a view to implementing more rational and sustainable IPM measures. The current CPP Project ("Adaptive evolution within *Bemisia tabaci* and associated *Begomoviruses*: A strategic modelling approach to minimising threats to sustainable production systems in developing countries" by Frank van den Bosch and M.J. Jeger, is currently analysing the effect of the IPM measures implemented in the Mesoamerican subproject on whitefly/begomovirus control, particularly regarding their long-term consequences. This exercise has been possible thanks to the biological data provided by this subproject to the main CPP investigators.

Contribution of Outputs to Developmental Impact

The Tropical Whitefly project is a rare example of an agricultural research project that addresses a concrete food production problem and, at the same time, demonstrates the potential to make a substantial contribution to food security (recovery of abandoned areas for food production),

poverty alleviation (increased income from limited land resources), improved health standards (minimum pesticide use), and environmental sustainability (discourage migrant agriculture).

The Mesoamerican subproject of the TWFP has filled a large vacuum created in the past two decades by the drastic reduction in technical assistance to small-scale farmers, caused by a chronic regional economic crisis (external debt) that has affected both national and international agricultural research institutes.

The focus of the Mesoamerican subproject on mixed cropping systems, including both staple and high-value crops, responds to small-scale farmers' attempts to diversify their cropping systems in order to maximise the productivity and profitability of their scarce land resources.

The Mesoamerican subproject has inserted its research activities in the research agenda of the national agricultural research programs it has worked with, thus making sure that our respective research agendas coincide on the basic need to produce food in a sustainable manner.

The Mesoamerican subproject of the TWFP has implemented sustainable IPM technology that helps small-scale farmers produce more food and improve their livelihoods, but with minimum use of pesticides. Thus, the project has the potential to benefit the environment by reducing pesticide use. In fact, pesticide abuse has been an increasingly important problem ever since the focus on crop improvement was changed through pressure from environmentalist groups to natural resource management, unfortunately independently of any food production component. In the absence of technical assistance or concrete food production technology, small-scale farmers can only protect their investment through the intensive use of pesticides, thus, poisoning themselves, their families, their agricultural products, the environment and, finally, all the unaware consumers of heavily contaminated produce in developing countries that do not have food safety standards. Natural resource management projects without a food/crop production component, alleviate neither poverty nor hunger in developing countries.

A recent study by DFID claims that it takes US $ 11,000 to take a single person out of poverty in Latin America. This figure probably represents the radical change that has taken place in agricultural research priorities in Latin America since the 1990s, from crop improvement to natural research management. With proper technology and a viable crop production component, the TWFP has shown that a resource-poor farmer can overcome poverty with an additional investment of less than US $ 1,000. The economic feasibility of this strategy has been demonstrated in the Central American sub-project, where both the private sector and the Government of El Salvador have invested successfully for the past two years in agricultural projects aimed at increasing horticultural production, by facilitating credit to small-scale farmers using the technology promoted by the TWFP. These outputs, namely virus-resistant varieties and sustainable IPM technologies, are already in the hands of farmers in our current pilot sites.

Now, we have to scale up these IPM packages to reach all the regions affected by whiteflies and whitefly-transmitted viruses in the tropics. However, much more crop improvement efforts are necessary to improve horticultural crops, mainly tomato, sweet and hot peppers and cucurbits, for resistance to the whitefly/virus pests specific to each region, particularly in Latin America. Most of this work could be carried out by the International Agricultural Research Centres (IARCs), if the supporting industrialised nations realised that, without a long-term, solid crop improvement

program at these centres, poverty alleviation will continue to be an impossible goal. Also, over-reliance in biotechnology at the expense of the traditional genetic improvement field work that IARCs used to do in their most productive years, has greatly slowed down the process of crop improvement rather than accelerate it, as was expected.

Follow-up Action

Phase III of the TWFP-Mesoamerica will focus on technology dissemination, impact assessment and policy issues.

In the case of common bean, the subproject needs to assess the socio-economic impact of the work done so far in the pilot sites. We need to document how fast is the new cultivar, CENTA San Andrés, going to be adopted in the valley of Zapotitán, in El Salvador and neighbouring countries. How much are small-scale farmers going to benefit from having this new variety, and the opportunity to grow it throughout the year. How much are we going to reduce production costs because of the possibility of reducing pesticide applications to 10-20% of the current application volumes. This point, however, requires more participatory work with farmers, which is contemplated in Phase III. Finally, how much is the Government saving in food imports and how is the urban consumer benefiting from increased bean production in the region.

In the case of horticultural crops, the technology needs to be disseminated following a true farmer participatory approach, and through farmer field schools. The main objective of this approach is to explain to small-scale farmers the benefits of the physical control methods and the biological principles behind the IPM measures recommended, rather than to teach the method itself. In those areas where farmers have been using the method for three years, we will conduct an impact assessment exercise. Linked to this particular activity, we have the possibility of study the impact of policy (e.g. intervention of the Salvadoran and Mexican Governments to reduce the cost of the nets used for physical control, and/or provide loans for small-scale farmers who want to adopt this technology).

The loroco case in El Salvador will be followed up into the third year of production before an impact assessment study is conducted. The dissemination of technology for this and previous crops will be channelled through different communication media: publications, radio, television, and electronic media in order to reach as many potential users as possible. Fortunately, the TWFP has already worked in all of the countries affected by whiteflies and viruses in this region, which facilitates the dissemination of information through known channels.

One of the past research activities with the most potential impact is the development of whitefly-transmitted virus-resistant tomato germplasm. Segregating populations are already in the hands of two national programs (CENTA-El Salvador and INIFAP-Mexico). This work is proceeding with the collaboration of AVRDC. This is the first time that we have identified sources of resistance to whitefly-borne viruses of tomato in Latin America.

A Concept Note has been drafted, which describes the proposed approaches to deliver these technological breakthroughs to small-scale farmers. Two previous meetings organised by the System-wide IPM Programme have served as the channel to link up with other SP-IPM groups,

particularly with the specialists on Farmer Participatory Research (lead by CABI), in order to agree on the best approach to execute Phase III.

Publications and Trainings

CIAT. 2003. "A United Effort against a Global Pest: Helping Poor Farmers Reduce Crop Losses and Grow more Food in a Sustainable Way " A5 Colour brochure. 10pp. [English] (D)

CIAT "Tropical Whitefly IPM Project: Collaborative Research to solve a Global Problem" Colour brochure 20pp.

Morales, F.J. 2003. The Whitefly Trialeurodes vaporariorum as a potential Constraint to the Development of Sustainable Cropping Systems in the Mesothermic Valleys of the Bolivian Highlands, August 2003, CIAT, Colombia, 11pp. (C)

Morales, F.J. 2003. Promoting economic growth in small farm communities of El Salvador through suitable pest management, p.8, Agricultural Research and Extension Network, No.47, January 2003, ISSN 0951-1865. (D)

Morales, F.J.; Martinez, A.K.; Velasco, A.C. 2003. New outbreaks of begomoviruses in Colombia. Fitopatol. Col. 26: 75-79. (A)

In Press

Morales, F.; Jones, P. The ecology and epidemiology of whitefly-transmitted Viruses in Latin America. Virus Research. (A)REPORTED AS IN PRESS IN PPR1 SEP 03.

Morales, F.J. Integrating IPM and Sustainable Livelihoods in Central America. *In:* Pachico, D. (ed.). Scaling up and out: Achieving widespread impact through agricultural research. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. NB. On 20.1.04 PL forwarded an internal CIAT email dated 5 Sep 03 saying: "Still a way to go and annual reports intervening - so 2004 the likely publication time".

Internal Reports

Morales, F. 2003. The Systemwide Tropical Whitefly IPM Program. In: CIAT (Centro Internacional de Agricultura Tropical). Integrated Pest and Disease Management in Major Ecosystems: Annual Report, Project PE-1. Cali, CO. p. 224.

CIAT in Perspective 2001–2002: From Risk to Resilience. Rebuilding El Salvador's granary through integrated management of whiteflies. Cali, CO. p. 32.

Morales, F. 2002. The Systemwide Tropical Whitefly IPM Program: From Phase I to Phase II. In: CIAT (Centro Internacional de Agricultura Tropical). Integrated Pest and Disease Management in Major Ecosystems: Annual Report, Project PE-1. Cali, CO. p. 238.

Anderson, P. 2001. Systemwide Project on Integrated Sustainable Management of Whiteflies as Pests and Vectors of Plant Viruses in the Tropics. In: CIAT (Centro Internacional de Agricultura Tropical). Integrated Pest and Disease Management in Major Ecosystems: Annual Report, Project PE-1. Cali, CO. p. 208.

Other Dissemination of Results: Training

La Mosca Blanca como transmisora de enfermedades virales. (Field guide for Farmers and Extension personnel). Tropical Whitefly IPM Project.

Morales, F. 2004. Whiteflies (Homoptera:Aleyrodidae) As Virus Vectors. Presentation 15th IPPC, Beijing, China (Abstract).

Control Físico de Mosca Blanca (Bemisia tabaci) para el manejo de enfermedades virales en cultivos horticolas (Field guide for Farmers and Extension personnel) Tropical Whitefly IPM Project.

Perez, Juana E. 2004. Manejo y control de mosca blanca utilizando microtuneles en los cultivos de tomate y chile dulce. CENTA-CIAT.

Escamilla, E. 2004. Uso de coberturas para el manejo de afidos en el cultivo de Loroco. CENTA-CIAT.

Perez, Juana E. (2004) Evaluación de lineas autofecundadas e híbridas de tomate por su resistencia a virus transmitidos por mosca blanca. CENTA – CIAT.

Betancourt, M. De J.; Pérez Cabrera, C.A. 2004. Parcelas Demostrativas de Producción de Frijol con la Variedad CENTA San Andrés en el valle de Zapotitán. CENTA – CIAT.

The Central American sub-project supervised on M.Sc. Thesis, University of El Salvador, and trained three scientists from tha NARIs of El Salvador, México, and one from the Univ. of El Salvador, at CIAT.

Listing and reference to key datasets generated:

Morales, F. 2004. Socioeconomic Survey. El Salvador. Access. Tropical Whitefly IPM Project.

Morales, F. 2004. Begomovirus Characterization DB. El Salvador/Mexico. Word Document. Tropical Whitefly IPM Project.

Morales, F. 2004. Bemisia tabaci biotypes. El Salvador and Mexico DB. Word Document. Tropical Whitefly IPM Project.

Activity 3. Developing integrated pest management components.

Monitoring of the changing situation with whitefly populations in the Andean zone

Rationale: Continuous monitoring of changes in whitefly populations and species composition in target areas is one of the most important objectives of the DFID-funded project on Sustainable Management of Whiteflies. This is needed to develop appropriate management systems and, if necessary, to modify existing systems so as to be able to cope with new situations.

Materials and Methods: In 2004 we processed a total of 105 whitefly samples (adults and pupae) collected in the Cauca Valley and northern coast regions of Colombia. Samples were taken from beans, snap beans, cucurbits, tomatoes and several other annual crops. We used RAPD techniques (primer OPA-04) to identify pupae and adults. Identification was based on morphological characteristics of pupae and comparison between RAPD patterns in samples brought from the field with those of existing mass rearings of different whiteflies maintained at CIAT **(Figure 1)**.

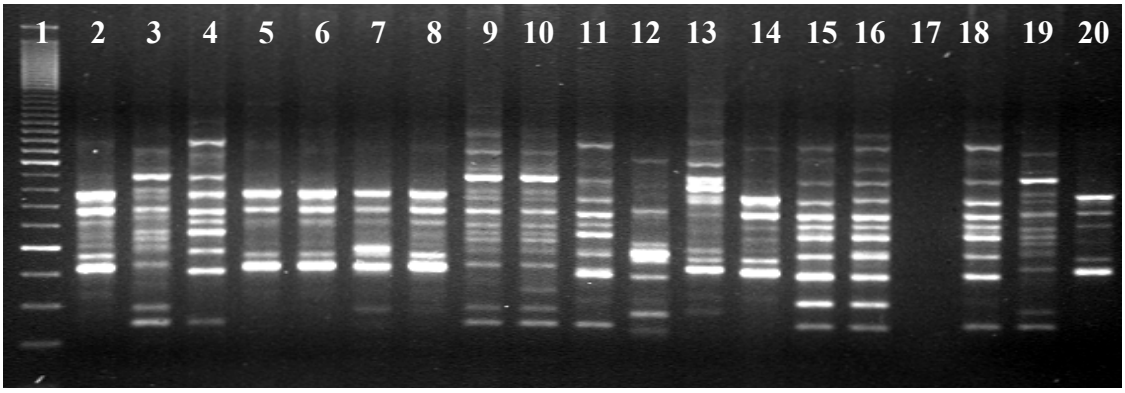

Figure 1. RAPD's for whitefly adults and pupae collected in the Cauca Valley. Amplifications using the OPA-04 primer. 1, DNA molecular marker (100pb); 2, *T. vaporariorum* from reference rearing maintained at CIAT; 3, *B. tabaci* biotype A from reference rearing; 4, *B. tabaci* biotype B from reference rearing; 5-8, adults (5-6) and pupae (7-8) of *T. vaporariorum* colleted in Darién on beans; 9-10, *B. tabaci* A adults collected on soybeans in Jamundí, 11, *B. tabaci* biotype B collected on soybeans in Jamundí; 12, parasitized pupa of *B. tabaci* collected on soybeans in Jamundí; 13-14, *T. vaporariorum* adults on beans in Jamundí; 15-16, *B. tabaci* biotype B pupae on beans in Jamundí; 17, free; 18, *B. tabaci* biotype B from reference rearing; 19, *B. tabaci* biotype A from reference rearing); 20, *T. vaporariorum* from reference rearing.

Results and Discussion: Analysis of 105 samples taken in 24 locations in the Cauca Valley (Colombia) showed that 42% of the whiteflies collected belonged to the B biotype of *Bemisia tabaci*, the most aggressive form of whitefly known to date. This biotype was found affecting snap beans, tomatoes, cucumber, melon, soybeans, pepper, tobacco, and grapes. As in 2003, we found that the B biotype is now occupying niches previously reserved to the A biotype or to *T. vaporariorum*. As shown in **Figure 2**, species composition in the Cauca Valley has changed

drastically in the past seven years. In 1997, *T. vaporariorum* was by far the most important species, representing 73% of the samples taken while the A biotype represented 15% of samples analyzed. At present, the A biotype is difficult to find (1.6% of samples), *T. vaporariorum* represents 11% of the samples and the B biotype is the predominant species with 42% of the samples. Up to 39% of crop samples examined were affected by a combination of *T. vaporariorum* and the B biotype of *B. tabaci*.

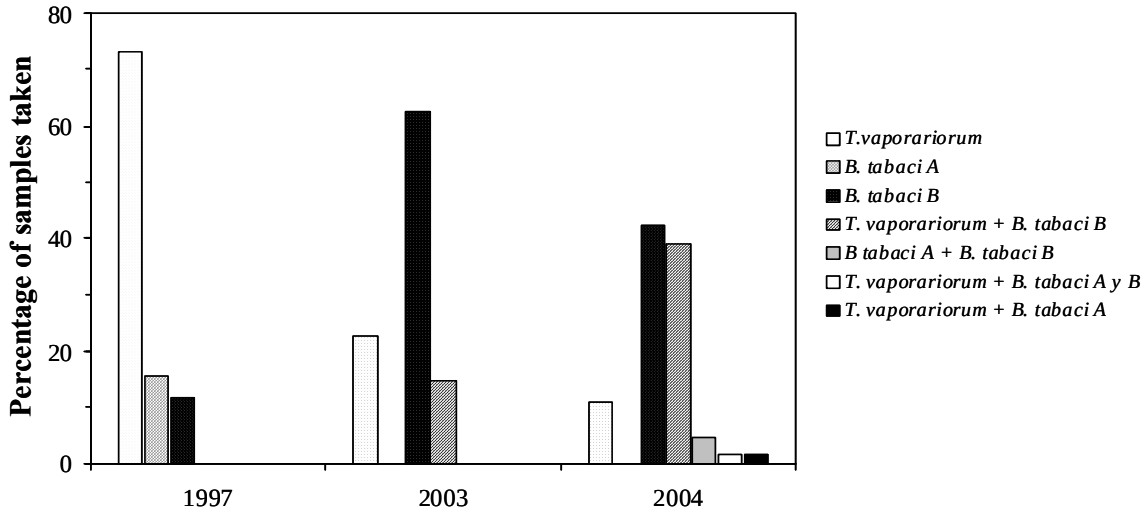

Figure 2. Changes in whitefly species composition in the Cauca Valley of Colombia (1997-2004).

Detailed monitoring of species composition on snap beans in the Pradera reference site revealed that at higher altitudes (1270-1840 m) *T. vaporariorum* is still the dominant species **(Figure 3)**. At altitudes ranging from 975 to 1120 m, most individuals collected in the Pradera region belong to the B biotype of *B. tabaci* attacking different crops either alone (33.3% of samples taken) or in combination with *T. vaporariorum* (53.4% of samples). The B biotype is an aggressive form of whitefly that is causing all the serious problems described in our 2003 Report. In snap bean growing areas, it has become the causal agent of a physiological disorder known as pod chlorosis, which renders the produce useless. Most serious, it has become a very effective vector of a geminivirus that is devastating snap beans in the region.

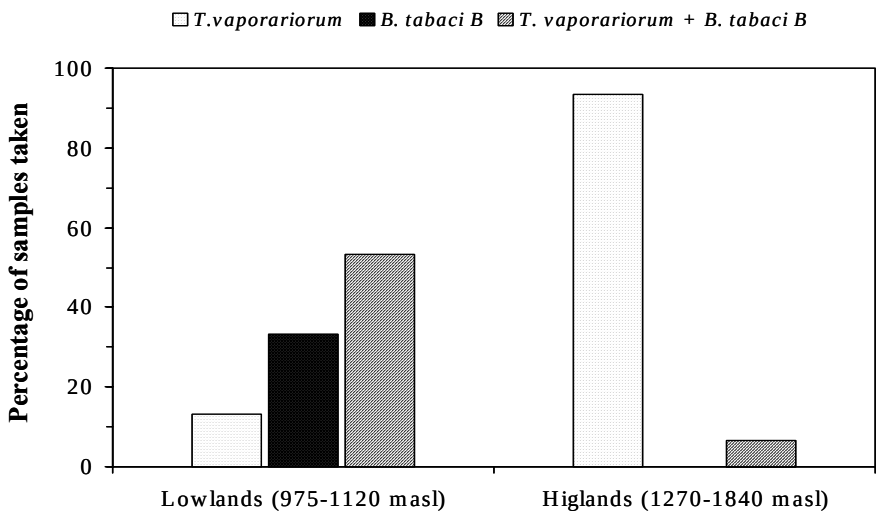

Figure 3. Whitefly s pecies composition in the Pradera (Cauca Valley) reference s ite; 2004 survey. masl, meters above sea level.

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Monitoring of insecticide resistance in whitefly populations

Rationale: Monitoring of insecticide resistance is another major objective of the DFID-funded project on Management of Whiteflies in the Tropics. Both major whitefly species and their biotypes in the Andean zone are the targets of excessive use of insecticides. This is reflected in ever increasing levels of resistance to insecticides and difficulties in control. The main purpose of a continuous monitoring of insecticide resistance is to develop alternative management strategies that will help to overcome resistance or delay the onset of this phenomenon.

Materials and Methods: In 2004 we established base-line data for five insecticides commonly used to control adults of the B biotype of *B. tabaci*: monocrotophos, carbofuran, carbosulfan, bifenthrin, and imidacloprid. These data will serve as the basis to establish diagnostic dosages for the species. These in turn will be used for periodic monitoring of resistance levels.

Using previously established diagnostic dosages for nymphs, we tested populations of whiteflies in the Cauca Valley in Colombia. Adult resistance levels were monitored under field conditions by means of the insecticide-coated glass vial technique. Resistance of first instar nymphs was measured using the foliage dipping technique. Systemic novel insecticides (mostly neonicotinoids) were tested using the petri dish technique (see 2003 Annual Report).

Results and Discussion: In general, it can be said that nymphal populations of both *T. vaporariorum* and *B. tabaci* biotype B are still susceptible to the insect growth regulators buprofezin and diafenthiuron and to imidacloprid, a novel neonicotinoid **(Table 1)**. . However, reduced responses to buprofezin in the Pradera site deserve further monitoring.

Table 1. Response (percentage corrected mortality) of nymphs of *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B to three insecticides in three consecutive growing seasons. Cauca Valley (Colombia). Diagnostic dosages in ppm.

Race	Percentage corrected mortality[a]		
	2001 B	2002 B	2003 B
Trialeurodes vaporariorum			
		buprofezin (16 ppm)	
'CIAT'[b]	98.4 a A[b]	100.0 a A	97.6 a. A
La Cumbre	100.0 a A	100.0 a A	100.0 a A
Pradera	87.0 b A	77.4 a A	81.4 b A
		diafenthiuron (300 ppm)	
'CIAT'	98.2 a A	100.0 a A	96.2 b A
La Cumbre	92.6 a B	97.8 a A	100.0 a A
Pradera	88.6 a A	93.9 a A	90.5 c A
		imidacloprid (300 ppm)	
'CIAT'	100.0 a A	98.3 a A	93.7 a A
La Cumbre	92.8 b A	93.2 a A	99.0 a A
Pradera	84.9 b A	92.6 a A	93.2 a A
***Bemisia tabaci* biotype B**			
		buprofezin (16 ppm)	
'CIAT'	---	98.4 a A	96.9 a A
Rozo	---	80.6 b A	87.8 b A
La Unión	---	100.0 a A	87.7 b B
Santa Helena	---	100.0 a A	92.2 b B
		diafenthiuron (300 ppm)	
'CIAT'	---	100.0 a A	91.7 a B
Rozo	---	100.0 a A	91.5 a B
La Unión	---	98.2 a A	91.7 a B
Santa Helena	---	100.0 a A	95.1 a B
		imidacloprid (300 ppm)	
'CIAT'	---	91.1 b A	98.3 a A
Rozo	---	89.3 b A	90.1 b A
La Unión	---	100.0 a A	89.1 b B
Santa Helena	---	100.0 a A	98.6 a A

[a] For each species and product, means within a column followed by the same lowercase letter and means within a row followed by the same uppercase letter are not significantly different at the 5% level by LSD. Each species and product were analyzed separately.

[b] A susceptible strain of B. tabaci biotype B maintained at CIAT.

Future work on integrated pest management of whiteflies as pests of beans and snap beans in the Andean zone should include studies on the relative efficiency of the two most important parasitoids affecting whitefly populations in the region: *Encarsia nigricephala* and *Amitus fuscipennis*. Given the excessive use of insecticides, it is important to know what is the present response of these natural enemies to some of the most commonly used insecticides. The base-line data in **Table 2** should in the future serve as the basis for possible development of insecticide tolerance in populations of these natural enemies, an optional strategy for management of the whitefly problem.

Table 2. Response[a] of adults of *Encarsia nigricephala* and *Amitus fuscipennis* to different insecticides.

Insecticide	No. of Individuals tested	CL_{50} (CL 95%)[b]	CL_{90} (CL 95%)	χ^2	b ± EEM	$P > \chi^2$
			E. nigricephala			
methamidophos	400	0.67 (0.440 – 0.900)	4.04 (2.910-6.800)	1.72	1.64 ± 0.24	0.19 ns[c]
methomyl	400	0.00915 (0.004 – 0.015)	0.062 (0.044 – 0.110)	0.56	1.54 ± 0.30	0.45 ns
carbosulfan	400	0.09 (0.060 – 0.120)	0.38 (0.280 – 0.670)	0.22	2.04 ± 0.38	0.64 ns
cypermethrin	400	0.65 (0.040 – 1.880)	11.09 (5.680 – 21.65)	0.57	1.04 ± 0.26	0.45 ns
			A. fuscipennis			
bifenthrin	400	0.023 (0.005 – 0.427)	0.171 (0.118 – 0.276)	1.00	1.47 ± 0.33	0.31 ns
carbofuran	400	0.074 (0.050 – 0.097)	0.380 (0.286 – 0.576)	0.70	1.80 ± 0.24	0.40 ns

[a] Values of CL_{50} y CL_{90} in µg of active ingredient/ vial.
[b] Confidence limits at 95%.
[c] ns, not significant at the 5% level.

Comparison of toxicological responses of the whitefly and their parasitoids indicate that all of the insecticides tested are much more toxic to the parasitoids than to the whitefly **(Table 3)** with up to 100-fold higher tolerance in the herbivore. Nevertheless, the data show that both natural enemies studied do possess innate mechanisms of defense against toxic substances, which may be exploited by continuous mass rearing and selection for higher levels of tolerance followed by mass releases in the field. As such, resistant strains of one or both parasitoids would become management components in an integrated pest management system.

Table 3. Comparative responses of the whitefly *Trialeurodes vaporariorum* and its parasitoids *Encarsia nigricephala* and *Amitus fuscipennis* to differen t insecticides.

Insecticide	$_{50}$ *T. vaporariorum* CL	$_{50}$ Parasitoid	Response Ratio
	E. nigricephala		
methamidophos	5.30[a]	0.670	7.91
methomyl	0.25[a]	0.009	27.77
carbosulfan	1.80[b]	0.090	20.00
cypermethrin	37.0[a]	0.650	56.92
	A. fuscipennis		
bifenthrin	2.40[b]	0.023	104.35
carbofuran	1.97[a]	0.074	26.62

[a] As determined by Cardona et al. (2001).
[b] As determined by Rodríguez et al. (2003).

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Management strategies for whiteflies

Rationale: Whiteflies have become the target of excessive pesticide use by snap bean and dry bean farmers in the Andean zone. A management system for whiteflies that contribute to reduce pesticide use has been developed and tested with farmers in Colombia and Ecuador (see 2002 and 2003 Annual Reports). In 2004 we tested other alternatives to further reduce the need for toxic insecticides and initiated diffusion of technology activities at both sites in Colombia and Ecuador.

Materials and Methods: Two large-scale trials were conducted in areas of the Pradera reference sites where *T. vaporariorum* is still the predominant species. We compared different approaches for whitefly control based upon judicious and less detrimental use of chemicals. Seed treatments and drench applications of novel systemic insecticides were compared with the timing of foliar applications of conventional (less costly) products, in some cases with applications based upon pre-established action thresholds developed in previous experiments (see 2002 and 2003 Annual Reports). These treatments were compared with farmers' practices. These trials were used as demonstration plots for farmers in the area.

Results and Discussion: As in previous trials, and as compared with farmers' practices, alternative management strategies based on judicious timing of applications and use of action thresholds resulted in yields that did not differ from those obtained by farmers with their traditional management approaches **(Table 4)**. Crop appearance, damage (sooty mold) levels, and final produce quality (as judged by farmers attending field days) did not differ either. Use of systemic insecticides as seed dressing and proper timing of foliar applications resulted in higher benefit/cost ratios with a 60-70% in the amount of applications made per cropping cycle.

Table 4. Yields (tons/ha) and economic returns obtained with different approaches for control of the greenhouse whitefly *Trialeurodes vaporariorum* in Pradera, the reference site.

Treatment	Yield (Tons/ha)		Benefit/Cost Ratios	
	Trial 1	Trial 2[a]	Trial 1	Trial 2[a]
Seed treatment with imidacloprid followed by two foliar applications of conventional insecticides at pre-established action thresholds.	11.1a[b]	11.9	1.43	1.77
Seed treatment with imidacloprid followed by three foliar applications of conventional insecticides at pre-established crop growth stages.	10.7a	11.5	1.38	1.62
Farmers' practices (6-7 foliar applications of conventional insecticides).	9.3a	11.9	1.14	1.65

[a] Un-replicated demonstrative trial. No statistical analysis performed.

[b] Means followed by the same letter are not significantly different at the 5% level by LSD.

These trials were used to initiate diffusion of technology activities in the area. A field day was organized in collaboration with ICA and the Municipal Technical Assistance Unit. Attendance was good (76 people, 15 of them women). Farmers were informed on the purposes of the demonstration plots and received training on whitefly biology and safe management of insecticides. Farmer's schools activities were initiated with 12 farmers who received training on whitefly sampling, safe management of pesticides and use of action thresholds for rational whitefly control. Diffusion activities will be strengthened if the second phase of the special project on whiteflies is approved.

Screening for virus resistance in snap beans

Rationale: In some areas, the B biotype of *Bemisia tabaci* has become a vector of a new viral disease on snap beans. There is urgent need to develop virus-resistant snap bean varieties in order to replace 'Blue Lake', the highly preferred but extremely susceptible commercial variety.

Materials and Methods: In collaboration with the Virology Unit and the Bean Breeding section, we screened 238 genotypes (snap beans and dry beans) for resistance to the new virus. We used three replications per genotype. The nursery was established in a hot spot area (La Tupia, Pradera) with high incidence of the disease. Materials were rated 43 days after planting for virus symptoms using a 1 - 5 visual scale (1, no apparent damage; 5, severe damage).

Results and Discussion: Twenty-three genotypes (9.7%) were rated resistant (damage scores 1-2); 39 (16.4%) were intermediate (2.1-3 scores), and 176 (73.9%) were susceptible (>3 in damage scores). Best materials in two consecutive trials are shown in **Table 5**. Some of these materials are well-known sources of resistance to BGMV.

Table 5. Response of selected snap bean s and dry beans genotypes to a new virus disease transmitted by *Bemisia tabaci* in the Cauca Valley of Colombia.

Identification	Pedigree and Genealogy	Damage Scores[a] 2003	2004
EMP 496	EMP 250[A 769 x {(A 429 x XAN 252)F1 x (V 8025 x Pinto UI 114)F1}F1]F1	1.3	1.3
DICTA 113		1.0	1.3
MN 13942-22	(TLP 35xG 21212)F1 x ICTA LIGERO/-MC-1P-MQ-MC-3C-MC-MC	1.0	1.3
DOR 476		1.0	1.7
Tio Canela 75		1.3	1.7
DOR 390		1.3	1.7
BAT 304		1.0	1.7
EAP 9020-14		1.0	1.7
MR 14143-28	(RAB 651x Tio Canela 75)F1 x (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-6C-MC-MC	1.7	1.7
MN 13942-22	(TLP 35 x G 21212)F1 x ICTA Ligero/-MC-1P-MQ-MC-11C-MC-MC	1.0	1.7
MEJ 1-197	ICA Pijao x (ICA Pijao x G35877)F1/-(NN)P-(NN)Q-(NN)P	1.3	1.7
EAP 9504-30B		1.0	2.0
9653-16B-3		1.0	2.0
A 429		1.0	2.0
G 35172		1.7	2.0
MR 14143-28	(RAB 651 x Tio Canela 75)F1 x (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-3C-MC-MC	1.0	2.0
MR 14143-28	(RAB 651 x Tio Canela 75)F1 x (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-4C-MC-MC	1.3	2.0
MN 13942-22	(TLP 35 x G 21212)F1 x ICTA Ligero/-MC-1P-MQ-MC-1C-MC-MC	1.0	2.0
MN 13942-22	(TLP 35 x G 21212)F1 x ICTA Ligero/-MC-1P-MQ-MC-5C-MC-MC	1.0	2.0
URG 401	(G 35649 x G 3807) x G35023	1.7	2.0
URG 215	BAT 338 x G 35252	1.3	2.0
MEJ1-121	((ICA Pijao x G 35172) x ICA Pijao)F1/-19P-(NN)P(F8)-(NN)P	1.3	2.0
MEJ1-253	ICA Pijao x (ICA Pijao x G 35877)F1/-(NN)P-(NN)Q-(NN)P	1.3	2.0
G 5746		3.0	5.0
Blue Lake[b]		5.0	5.0

[a] On a 1-5 visual scale (1, no damage; 5, severe damage).
[b] Susceptible commercial check.

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Publications

Book Chapters-In Press

Anderson, P.; Bellotti, A.; Cardona, C.; Hanson, P.; Legg, J.; Morales, F.; Riis, L. 2004. Sharing Methods, Comparing Results. ***In:*** Whiteflies and whitefly-borne viruses in the tropics: Building a knowledge base for global action.

Cardona, C. 2004. An Introduction. ***In:*** Whiteflies and whitefly-borne viruses in the tropics: Building a knowledge base for global action.

Cardona,C.; López-Avila Aristóbulo; Valarezo, Oswaldo. 2004. Colombia and Ecuador. ***In:*** Whiteflies and whitefly-borne viruses in the tropics: Building a knowledge base for global action.

Cesar Cardona, Francisco Rendón, Issaura Rodríguez, and Aristubolo López-Avila. 2004 Insecticide Resistance in Populations of Trialeurodes vaporariorum (Westwood) and Bemisia tabaci (Gennadius) in Colombia and Ecuador. ***In:*** Whiteflies and whitefly-borne viruses in the tropics: Building a knowledge base for global action.

Cesar Cardona, Aristobulo López-Avila, and Oswaldo Valarezo. 2004. Whiteflies as pests of annual crops in the tropical highlands of Latin America. Conclusions and Recommendations. ***In:*** Whiteflies and whitefly-borne viruses in the tropics: Building a knowledge base for global action.

Anderson, P.; Morales, F.; Bellotti, A.; Cardona, C.; Hanson, P.; Legg, J.; Riis, L. 2004. Conclusions and Recommendations. ***In:*** Whiteflies and whitefly-borne viruses in the tropics: Building a knowledge base for global action.

Activity 4. Identification of genomic regions responsible for conferring resistance to whitefly in cassava.

Introduction

Whiteflies are one of the most serious pest and disease vectors that affect agricultural production around the world. In cassava (*Manihot esculenta* Crantz), whitefly can causes between 70 to 80 percent of yield loss. The most important source of resistance genes was a genotype MEcu-72 (Arias, 1995). Due to the importance of whiteflies as a pest, it is necessary to know about the nature of genes that confer resistance in genotypes like MEcu-72. For this purpose we are using F1 segregation and the genetic expression of the cross MEcu-72 (resistant genotype) x a very susceptible genotype (MCol-2246) and molecular markers. This would help to accelerate selection of resistant materials to the whitefly and also to isolate resistant genes. It is hypothesized that these resistant genes may also be effective against other whitefly species, especially *Bemisia tabaci*, the species that is a vector of ACMD, a virus that causes severe crop losses in Africa and Asia. Whitefly resistant genotypes (such as MEcu 72) from the neotropics are displaying resistance to *B. tabaci* in greenhouse trials being carried out by NRI in the UK (CIAT Progress report 2003).

The application of molecular genetic analysis for cassava breeding has been limited compared to others crops. Recently progress has been made in the development of genomic and bioinformatics tools to increase our knowledge of cassava genome structure and cassava gene function. Expressed Séquense Tag (EST) provides an immediate and productive method of gene discovery. In cassava a total of 14168 ESTs were obtained in CIAT and Perpignan Universite (Lopez, et al, submitted), of these 105 have SSRs, for which we designed primers.

Materials and Methods: For the present work we have used the F1 cross (family CM 8996, 276 individuals) between MEcu-72 (as the resistance parent) and MCol-2246 (as the susceptible parent) elite cassava cultivars from Ecuador and Colombia, respectively. The parents and their offspring were evaluated in the field in two places: Nataima (Tolima) and Santander de Quilichao (Cauca). With this evaluation we intend to identify the gene segregation in the offspring and select the resistant and susceptible materials. Both parents were evaluated with 343 cassava SSRs (Simple Sequences Repeat) (Mba et al, 2001) including 156 cDNA SSRs developed (Mba et al, submitted). We are using AFLPs (Vos, et al, 1995) and to find markers associated with resistance for mapping and ultimately cloning the resistant genes. We are using silver staining to visualize the allelic segregation of the markers.Cassava RGAs primers were done in the parentals and the polymophics were mapped in the F1.

We designed primers SSRs from ESTs sequences using the software Primer 3 and these SSR were amplified in the parentals and the polymorphics were mapped in the F1.

Results

Field evaluation

The field evaluation showed high pressure being exerted by the *A. socialis* in Nataima (2003 and 2004), and Santander de Quilichao (2004) where test materials had high damage ratings;

however, some materials had lower levels of damage in the evaluations. We can conclude that these genotypes show a resistance level similar to parental MEcu 72. These evaluations were analyzed with the molecular markers to find putative associations.

SSRs from ESTs

We designed 51 pairs of SSRs primers **(Table 1)** of which 29 were polymorphics for the cross **(Figure 1).**

Table 1. SSRs from ESTs primers designed.

No.	EST Name	Motif	No. Repeat	No.	EST Name	Motif	No. Repeat
1	cn1375-1	atgg	5	27	cn1304-1	atg	9
2	cn1004-1	tatt	6	28	cn1351-1	aga	10
3	cn1098-1	aga	6	29	si.03.G1.5-1	cca	10
4	cn1388-1	tct	6	30	gi17923193gbBM260153.	taa	11
5	cn1457-1	agc	6	31	rni.06.I21.5-1	tct	11
6	cn255-1	tcc	6	32	si.02.O10.5-1	aat	11
7	cn416-2	gat	6	33	cn1635-1	aag	12
8	cn44-1	tta	6	34	m.01.H14.5-1	tc	12
9	cn700-3	ttc	6	35	si.01.E12.5-1	tc	12
10	c.04.C18.5-3	atg	7	36	cn1460-1	ag	13
11	c.05.I1.5-1	tct	7	37	cn1498-1	at	13
12	cn1186-1	ttc	7	38	cn1587-1	ata	13
13	cn2269-1	tgg	7	39	cn2418-1	ag	13
14	cn393-1	cat	7	40	m.04.K18.5-1	ct	13
15	cn732-1	aag	7	41	aflp_28-2	ga	15
16	cn764-2	tca	7	42	m.06.H4.5-1	ct	15
17	gi17922797gbBM259765.1BM259765-2	aag	7	43	rni.06.N9.5-1	at	15
18	m.04.K21.5-1	ctt	7	44	cn1009-1	ct	16
19	m.05.I2.5-1	ttc	7	45	cn1722-1	tct	17
20	rni.06.N10.5-1	tta	7	46	m.05.L3.5-1	ag	17
21	rni.09.D10.5-1	ctg	7	47	cn47-1	ct	18
22	si.03.B22.5-1	tct	7	48	m.09.N13.5-1	ct	18
23	cn1131-1	tcc	8	49	gi17922797gbBM259765.1BM259765-1	agc	19
24	m.10.J19.5-1	gat	8	50	m.08.G23.5-1	at	20
25	m.11.K5.5-1	gat	8	51	cn1880-1	at	29
26	rni.05.L17.5-1	tga	8				

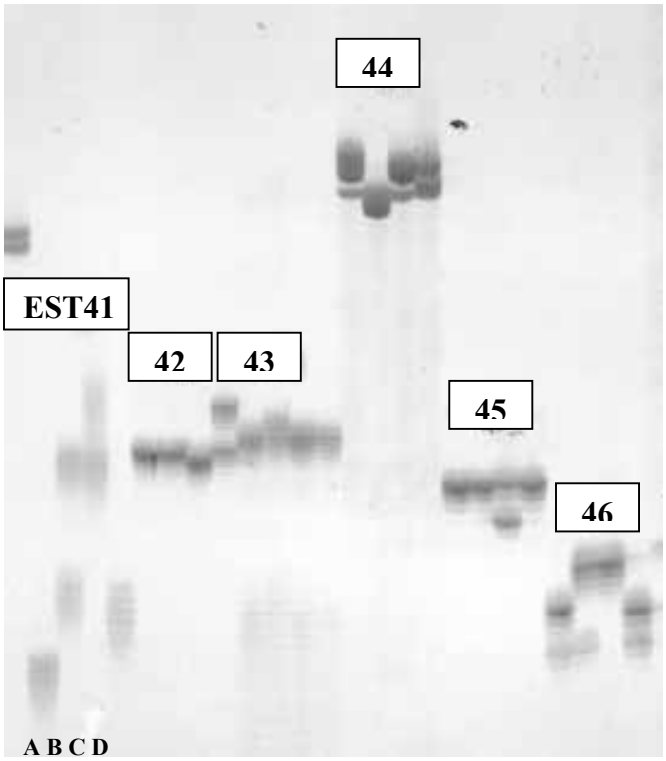


Figure 1. SSRs from ESTs in parentals, A is MNig2, B is CM21772 (both parentals used in ESTs), C is MEcu 72 and D is MCol 2246.

Mapping

For the construction of a linkage map 246 markers were analyzed, 103 SSRs, 2 RGAs, 121 AFLPs and 20 SSRs from ESTs. A genetic linkage map of cassava was constructed with 111 markers segregating from the heterozygous female parent (MEcu-72) of an intraspecific cross **(Figure 2A and 2B)** . The map consists of 20 linkage groups, which represent the haploid genome of cassava. These linkage groups span is 879.8 cM and the average marker density is 1 per 7,9 cM. The position of 111 markers, shown in the **Figure 2**, on the framework (LOD = 25 and tetha (θ) = 25) molecular genetic map of cassava. Map distances are shown in Kosambi map units and analyzed by Q gene. So far, 41 SSRs markers were mapped on the cassava framework map (Fregene et al, 1997), the other 70 markers are new. The molecular data are being analyzed using QTL packages (Q gene) to determine linkages between the SSR, RGAs and AFLPs markers and the phenotypic characterization.

Association between Molecular Markers and Resistance

The molecular data are being analyzed using QTL packages (Qgene) and Simple Linear Regression at the 5% level was done using SAS. Putative associations were found between molecular markers and the field phenotypic characterization (65 markers SSRs, RGA and AFLPs, shown by *bold* in the **Figure 2A-2B**). We observed that SSRY39 marker anchored in the linkage group K are associated with the resistance.

Figure 2A. Location of putatives QTL's (identified by colors) for partial resistance to whitefly in three localities on the female (MEcu-72) derived framework map. These results are based on Qgene analysis. Distances in centimorgans (cM) and significance levels are indicated on the right. The most significant markers are indicated in bold and blue square show the Linkage Group K which is localized a putative QTL in the marker SSRY39 (identified by *bold*).

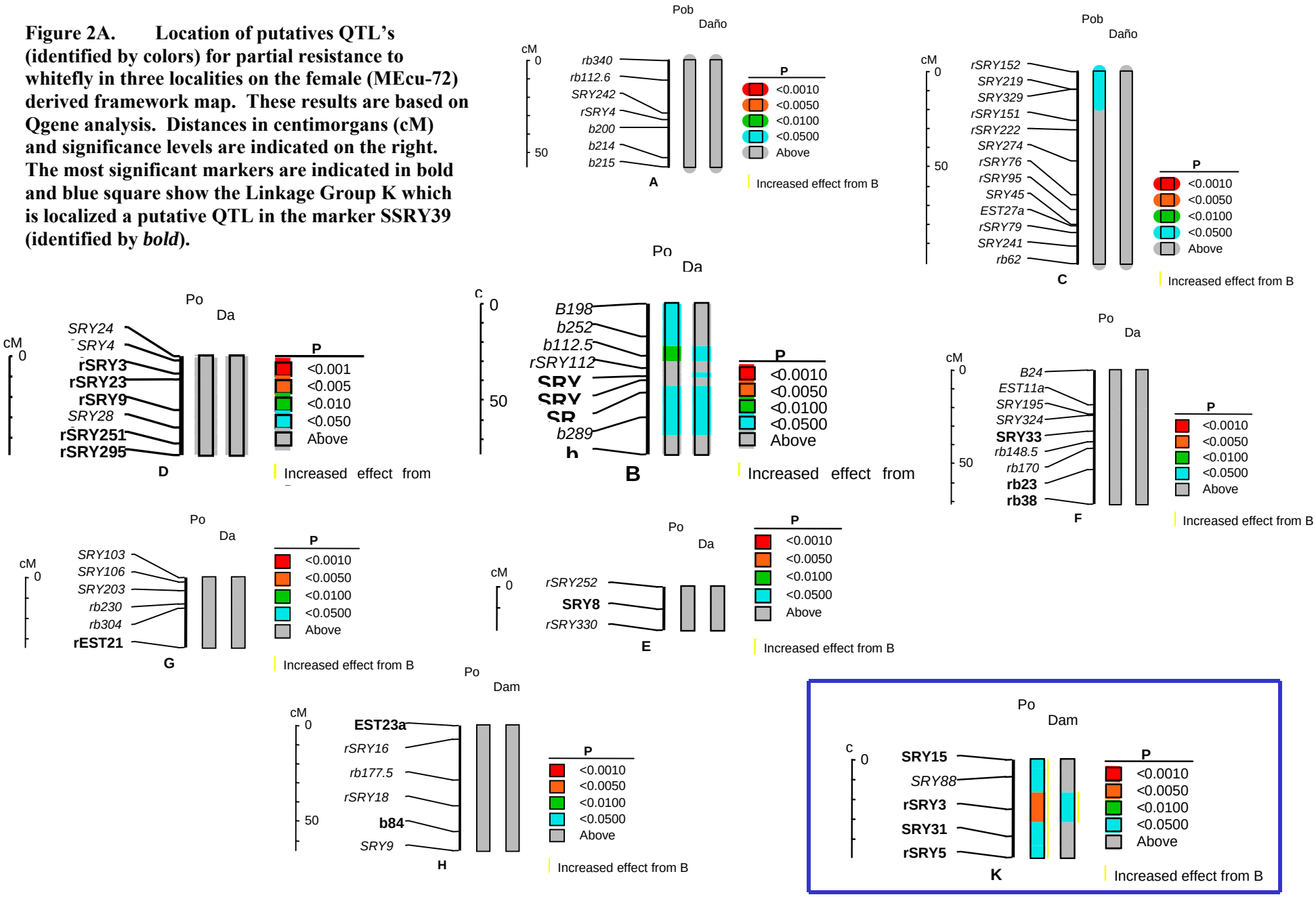
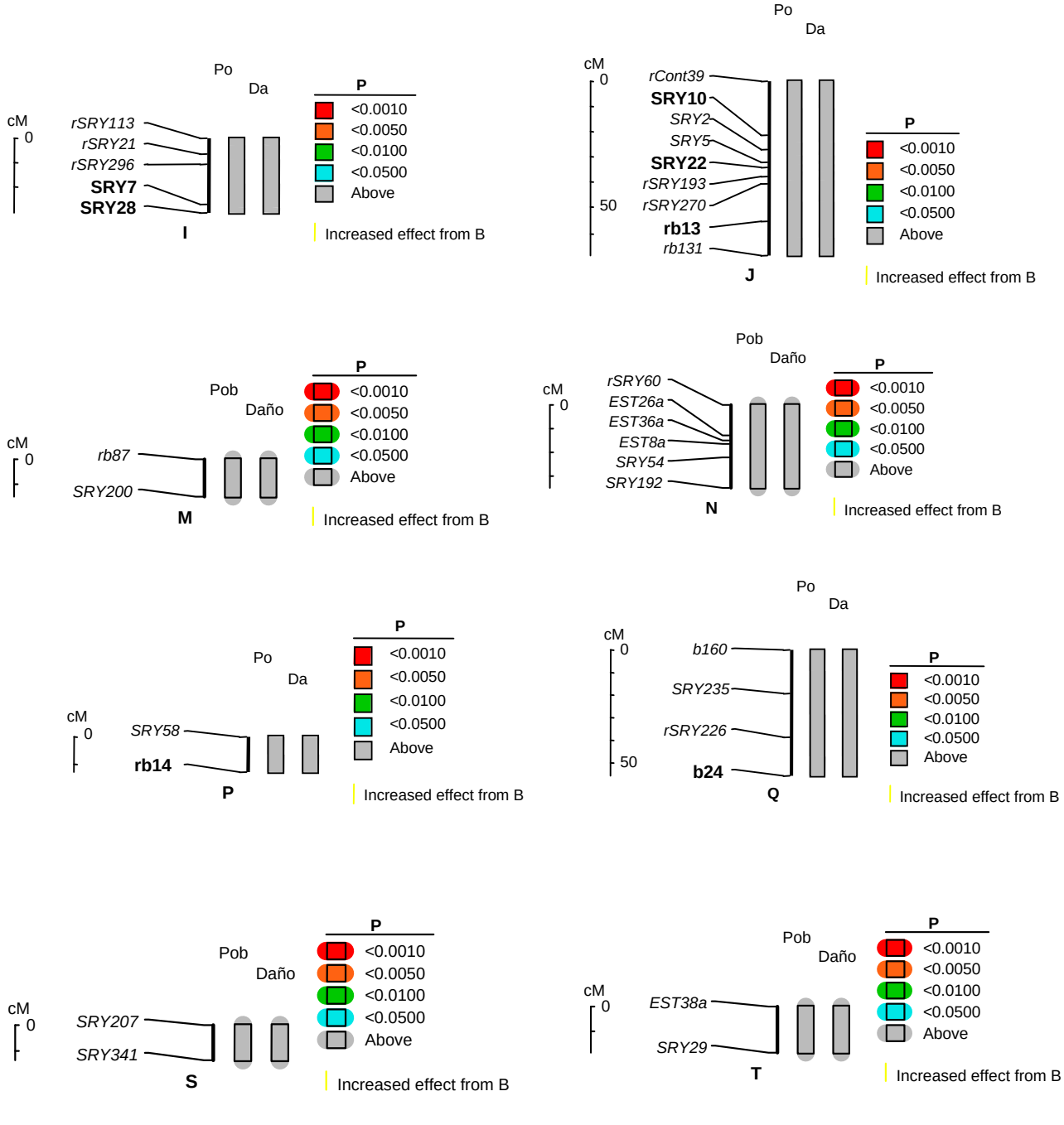


Figure 2B. Location of putatives QTL's (identified by colors) for partial resistance to whitefly in three localities on the female (MEcu-72) derived framework map. These results are based on Qgene analysis. Distances in centimorgans (cM) and significance levels are indicated on the right. The most significant markers are indicated in bold.



Ongoing Activities

- Saturation of linkage map of MEcu 72, using SNPs.
- Design of SCARs for marker-assisted selection.
- QTL analysis for whitefly resistance.
- Subtractive hybridization of the amplicon MEcu 72 (tester) and MCol 2246 (driver), during which amplified portions of differentially expressed genes are enriched and common sequences are depleted.
- Cloning and screening of the resulting products of expressed sequences during the defense response of MEcu 72 to whitefly attack.
- Microarray of clones in order to identify differentially expressed sequences.

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