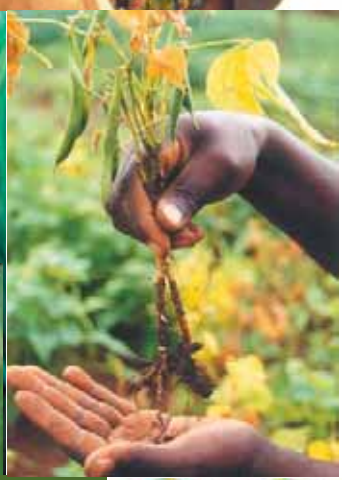
Integrated Pest and Disease Management in Major Agroecosystems


PROJECT - PE1
Annual Report 2003



CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

System - wide Programme on Integrated Pest Management

TABLE OF CONTENTS

CASSAVA AND TROPICAL FRUIT PATHOLOGY ***(1216 kb)***

Activity 1. Detection of a phytoplasma associated with cassava Frogskin Disease (FSD) in Colombia
Activity 2. Transmission of a phythoplasma affecting cassava seedlings and identification of indicator plants
Activity 3. Identification of cultural practices and strategy to control frogskin disease in cassava
Activity 4. Evaluation of the influence of the soil as a source of FSD vectors
Activity 5. Isolation and characterization of Agrobacterium tumefaciens from soil and cassava roots
Activity 6. Identification of RAMS markers to evaluate genetic diversity of selected isolates of Ralstonia solanacearum obtained from plantain
Activity 7. Evaluation of the effect of hot water treatment of stem cuttings, biocontrol agents, selection of healthy stem cuttings and fertilizers on Phytophthora Root Rot under field conditions in Cauca and Quindío. Application of fungicides will be included as control treatments
Activity 8. Use of organic matter and ash amendments as a strategy to manage cassava root rots and improve soil fertility in Tukanoan chagras in Mitú, Vaupés
Activity 9. Preparation of a brochure on disease control for farmers
Activity 10. On-farm evaluations of the effects of cover crops, green manure, organic compost, and plant extracts on Ralstonia solanacearum in Quindío
Activity 11. Developing a heat treatment and establishing a plantain nursery
Activity 12. Evaluating three doses of tetracycline to control the growth of bacteria isolated from oil palm
Activity 13. Diagnosing plant diseases and technical assistance
Activity 14. Training farmers, technicians, and extension agents in participatory research, cassava and other crops management, and disease control strategies

TROPICAL FORAGE PATHOLOGY ***(847 kb)***

Activity 1. Characterization of Xanthomona. campestris pv. graminis isolates
Activity 2. Resistance to Xanthomonas campestris pv. graminis (Xcg) in Brachiaria
Activity 3. Elucidate the role of endophytes in tropical grasses
Activity 4. Endophyte distribution in Brachiaria plants, and PCR analysis and screening of Brachiaria genotypes for endophytes
Activity 5. Effect of fungal endophytes on pathogens in planta
Activity 6. Search for nitrogen-fixing bacteria associated with species of Brachiaria
Activity 7. Bacterial endophytes isolated from Brachiaria
Activity 8. Antifungal protein isolated from seeds of tropical forage legume

BEAN PATHOLOGY ***(288 kb)***

Activity 1. Identifying common bean genotypes with resistance to angular leaf spot
Activity 2. Angular leafspot pathogen Characterized
Activity 3. Evidence that the common bean anthracnose pathogen, Colletotrichum lindemuthiuanum co-evolved with common bean gene pools
Activity 4. Characterization of Colltotrichum lindemuthianum isolates from Antioquia and Santander en Colombia
Activity 5. Nature and inheritance of angular leaf spot resistance in ALS differential genotypes and identified resistance sources
Activity 6. Inheritance of anthracnose resistance in the Andean germplasm accession G 19833
Activity 7. Allelism test for anthracnose resistance genes in the germplasm accession G 19833

RICE PATHOLOGY ***(142 kb)***

Activity 1. Characterization of Blast Pathogen Populations. Monitoring the Evolution in the Genetic and Virulence Diversity of the Blast Pathogen over time
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
Activity 3. Identification of Molecular Markers Associated with the Blast Resistance Genes Pi-1, Pi-2, Pi-33 and their Incorporation into Commercial Rice Varieties Through Backcrossing and Marker Assisted Selection (MAS)

AFRICA: BEAN PATHOLOGY ***(184 kb)***

Activity 1. *Characterization and distribution of Pythium spp causing root rots in Eastern Africa*
Activity 2. *Effect of organic amendments on bean Fusarium root rot disease and soil inoculum levels*
Activity 3. *Development and use of dilution plating method to quantify the effect of organic amendments on the inoculum levels of Pythium spp and severity of Pythium root rots*
Activity 4. *Scaling-up of IPM bean based technologies*
Activity 5. *Publications, Conferences, Workshops, Training, Students*

The Systemwide Tropical Whitefly IPM Program ***(610 kb)***

Activity 1. *Coordination*
Activity 2. *Studies on whitefly (Aleurotrachelus socialis) resistance mechanisms in selected cassava genotypes*
Activity 3. *Studies on the biology and development of biotype B of Bemisia tabaci on cassava, Manihot esculenta and the wild species, Manihot carthaginensis*
Activity 4. *Developing integrated pest management components*
Activity 5. *Refining of an action threshold for Trialeurodes vaporariorum control*
Activity 6. *Development of sampling methods for whitefly populations*
Activity 7. *Monitoring of insecticide resistance in whitefly population*
Activity 8. *Management strategies for whiteflies*
Activity 9. *Screening for virus resistance in snap beans*
Activity 10. *Whiteflies as pests and virus vectors in Middle America*

CASSAVA ENTOMOLOGY

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

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

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

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

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

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

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

Project 1 - Whiteflies

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

Objectives: Process and identify species collected in Peru, Brazil and other countries from different crops. The materials will be organized within the reference collection and registered in the data bank. Molecular techniques (RAPD-PCR) will be used in the identification and personnel from national institutions will be trained.

Methodology: Whitefly samples are sent by collaborators in alcohol in vials, and permanent mounts are made in Canadian balsam (see CIAT PE-1 2002 Annual Report for more details). Parasitoids are sent to corresponding taxonomists.

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

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

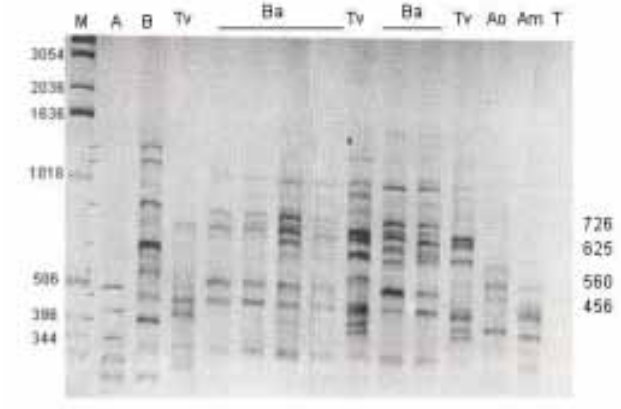


Figure 1. DNA Banding pattern obtained with the OPA-4 primer on whitefly samples from Peru and other sources. M=Marker 1 kb, line Ba (*Bemisia afer*). Other whiteflies include, Line: A (*B. tabaci* "A"); B (*B. tabaci* "B"); Tv (*Trialeurodes vaporariorum*); Tv (*T. variabilis*); Ao (*A. occidus*); Am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

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

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

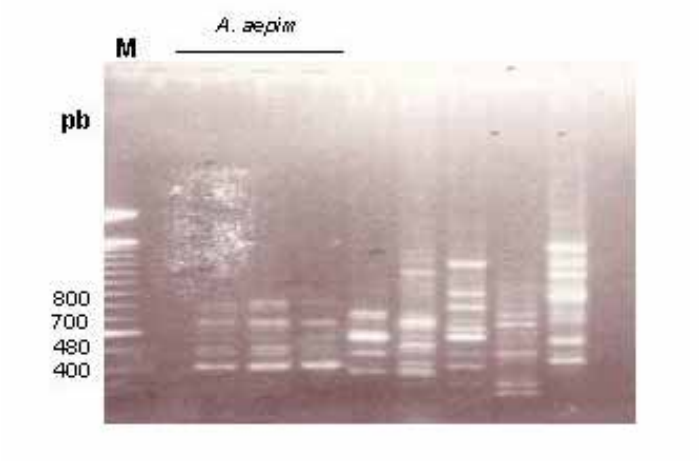


Figure 2. RAPD-PCR for whitefly samples from Bahia, Brazil.
M = Marker, lines 1-3 = *Aleurothrixus aepim* (Jacaraci, Guajer and Licinio de Almeida, Bahia); 4-8 = other whitefly species.

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

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

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

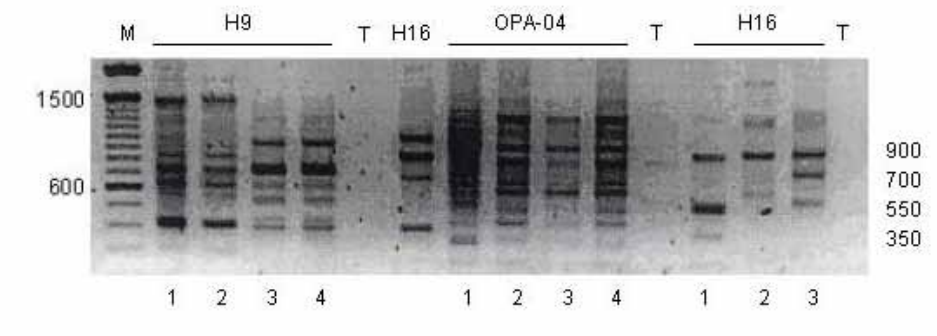


Figure 3. Banding patterns for whitefly and parasitoid species using primers H9, H16 and OPA-4, M+ marker 100 pb. Lines 1-2, *B. tabaci* (Africa); 3-4, *B. tabaci* (Colombia). On the same gel bands obtained with H16 primer. Line 1, *Encarsia nigricephala* (Valle); 2, *Eretmocerus* sp. (Quindío); 3, *Eretmocerus*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

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

Project 2 - Mite Identification

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

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

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

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

* Mites infested with the Entomopathogens *Neozygites* sp.

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

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

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

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

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

The objectives of this initial study are to:

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

Literature Search

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

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

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

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

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

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

Field Sampling and Identification

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

Figure 3

Figure 4

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

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

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

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

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

Figure 5

Figure 6

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

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

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

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

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

A. *Anastrepha striata*

B. Ovipositor

C. Wing pattern

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

A. *Anastrepha grandis*

B. Ovipositor

C. Wing pattern

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

A. *Anastrepha pickeli*

B. Ovipositor

C. Wing

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

A. *Anastrepha nunezae*

B. Ovipositor

C. Wing

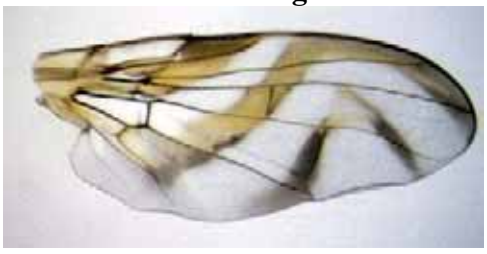


Figure 11. *A. nunezae* was found infesting zapote (*Quararibaea cordata*) especially between 900 to 1700 m.a.s.l.

Contributors: Mónica Lucía Marín, María del Pilar Hernández, Anthony C. Bellotti.

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

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

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

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

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

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

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

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

* *Scaphytopius*= (Platymetopius).

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

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

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

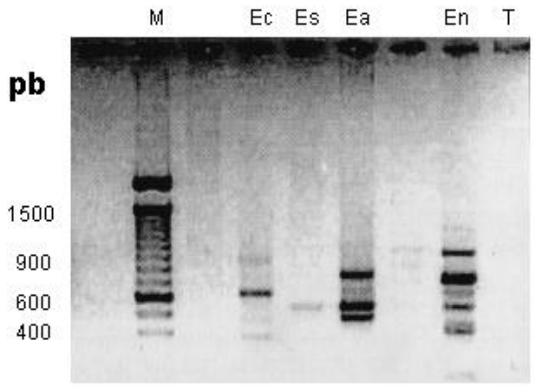


Figure 1. Band patterns obtained using RAPD-PCR with the primer H9 on samples of *Empoasca spp* from three cassava localities on the Colombian Atlantic Coast (M=marker, Lines EC=Córdoba; ES=Sucre; EA=Atlántico and EN=*Empoasca* from Guanábano, Valle

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

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

Objectives

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

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

Methodology

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

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

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

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

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

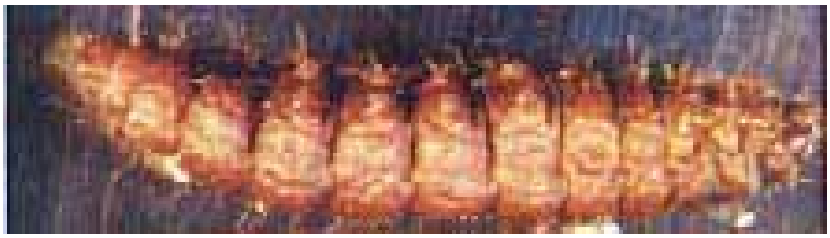


Figure 1. Larvae of *Galleria mellonella* infected with *Heterorhabditis sp*-CIAT. Photo by (Cristian Olaya, Microscopy Lab. Virology Unit, CIAT).

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

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

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

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

Two different bioassay were tested:

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

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

Nematode Species

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

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

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

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

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

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

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

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

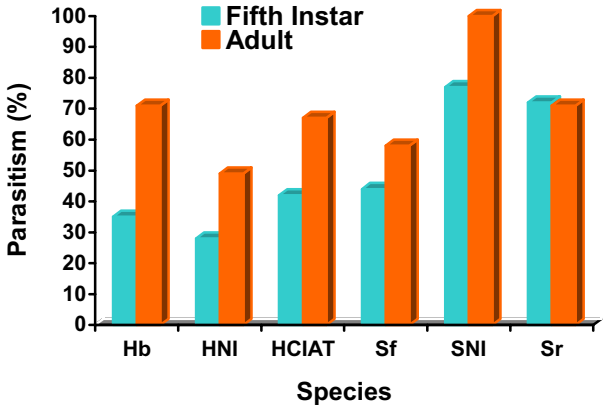


Figure 3. Parasitism of two *C. bergi* stages with six nematodes species.

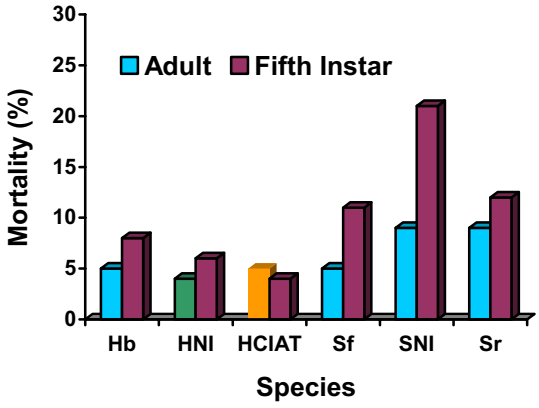


Figure 4. Mortality of two *C. bergi* stages with six nematodes species.

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

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

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

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

Three different bioassay were tested in the glasshouse:

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

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

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

Nematode Species

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

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

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

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

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

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

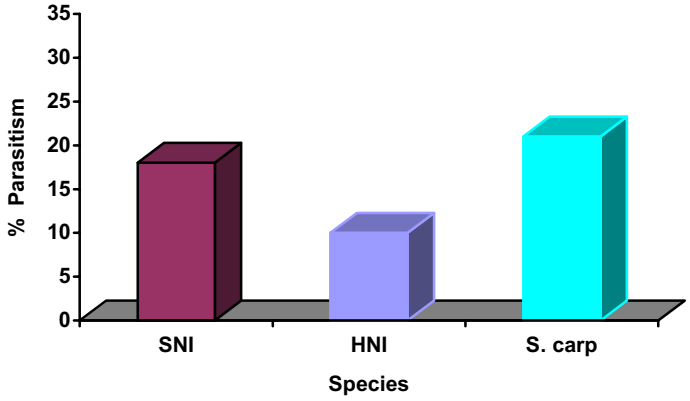


Figure 5. Parasitism of three species of EPNs species against *C. bergi* adult in glasshouse with 1000 nematodes.

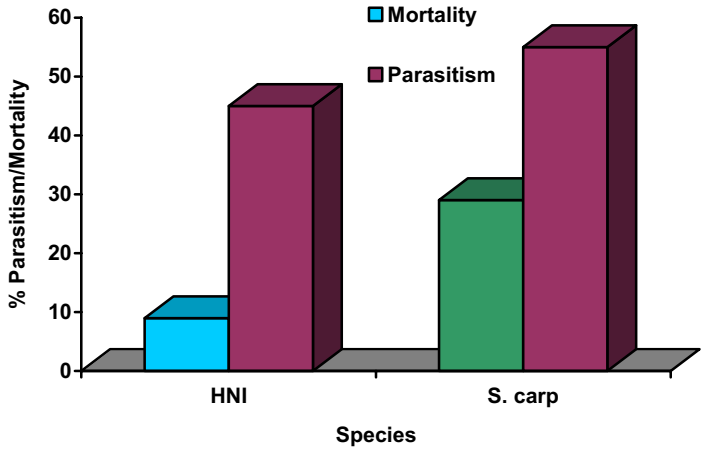


Figure 6. Parasitism and Mortality of two EPNs species against *C. bergi* adult with 25.000 nematodes in glasshouse

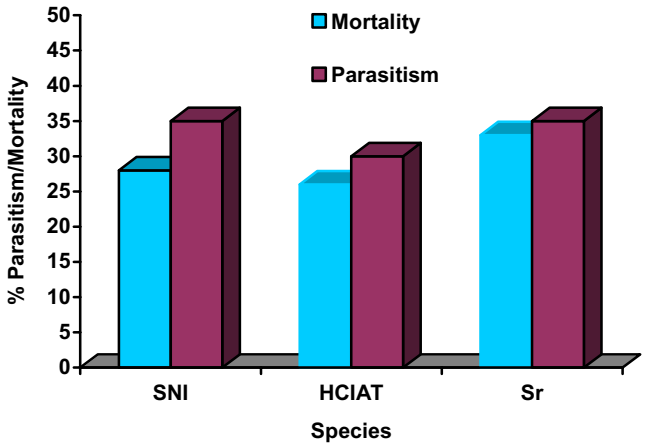


Figure 7. Parasitism and Mortality of three EPNs species against *C. bergi* adult stage in glasshouse with 100.000 nematodes

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

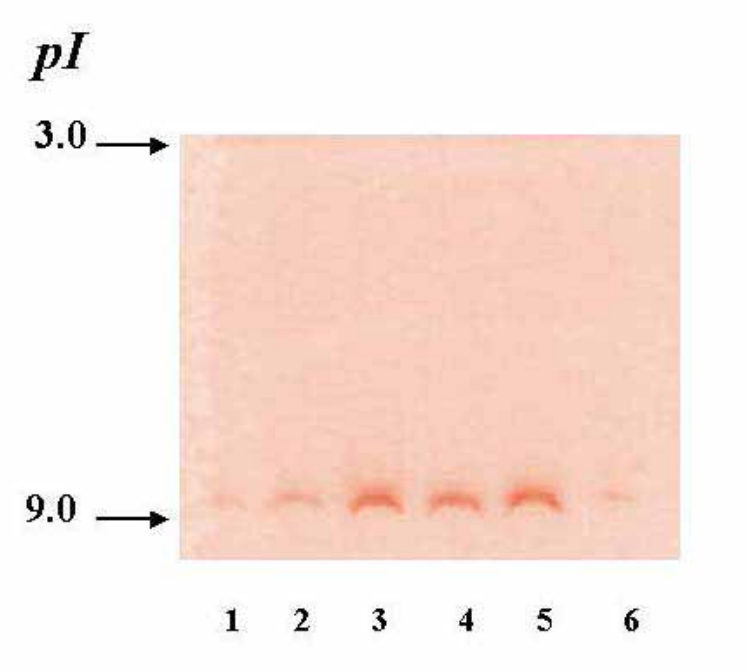


Figure 8. **Phenoloxidases of *C. bergi* infected with live nematodes. 1 y 6 control (24h); 2. SNI (24h); 3. SNI (48h); 4. HCIAT (24h); 5. HCIAT (48h).**

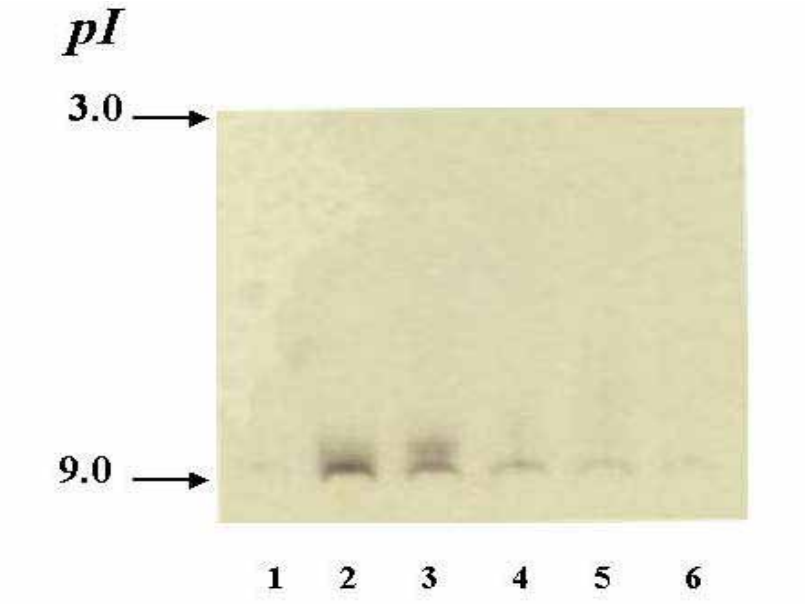


Figure 9. **Phenoloxidases of *C. bergi* infected with death nematodes. 1 y 6 control; 2. SNI (24h); 3. SNI (40h); 4. HCIAT (24h); 5. HCIAT (40h).**

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

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

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

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

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

References

Bedding R., and Akhurst R.J. 1975. A simple technique for the detection of the parasitic rhabditid nematode in soil. Nematologica 20:109-110.

Caicedo, A.M. & Bellotti, A.C. 1994. Evaluation of the potential of the entomogenous nematode *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) for the control of *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) under laboratory conditions. Revista Colombiana de Entomología, Vol 20. No. 4 p. 241-246.

Caicedo, A.M. & Bellotti, A.C. 1996. Survey of native entomogenous nematodes associated with *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) in eight Colombian sites. Revista Colombiana de Entomología, Vol 22. No1. p. 19-24.

Jarosz, J. 1998. Active resistance of entomophagous rhabditid Heterorhabditis bacteriophora to insect immunity. Parasitology 117, 201-208.

Guillespie J.P., and Kanost M.R. 1997. Biological mediators of insect immunity. Annu. Rev. Entomol. 1997, 42:611-643.

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

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

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

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

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

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

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

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

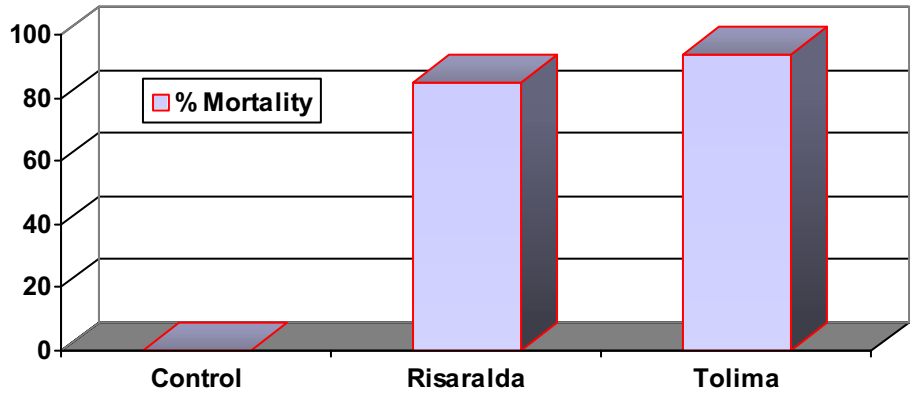


Figure 2. Cassava hornworm (*E. ello*) mortality due to field applications (300 grams/ha) of the *E. ello* baculovirus (Biovirus: BIOTROPICAL) at two locations in Colombia.

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

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

EL TIEMPO SÁBADO 23 DE AGOSTO DE 2003 1-7

TIERRAS & GANADOS

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

Bioplaguicidas para la yuca

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

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

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

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

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

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

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

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

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

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

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

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

EL GUSANO CACHÓN

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

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

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

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

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

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

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

Cassava Burrower Bug

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

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

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

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

Contributor: Carlos Julio Herrera, Anthony C. Bellotti.

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

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

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

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

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

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

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

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

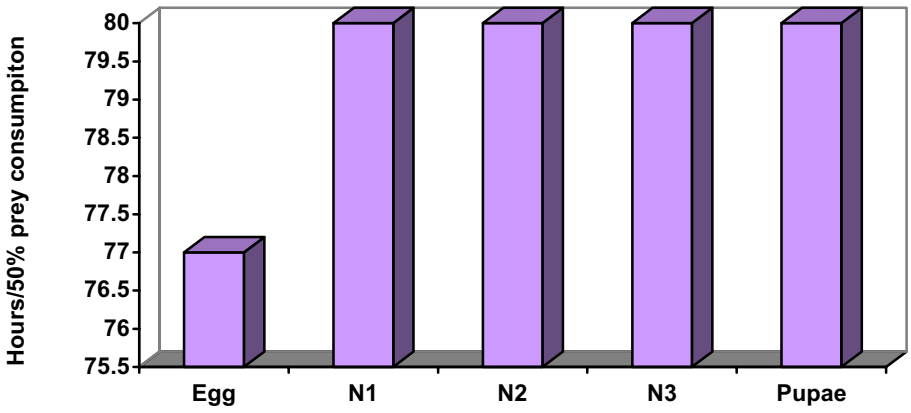


Figure 1. Consumption of *A. socialis* immatures by *C. carnea* (Neuroptera: Crysopidae) adults in laboratory studies (Duncan Multiple Comparison test at 0.05%).

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

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

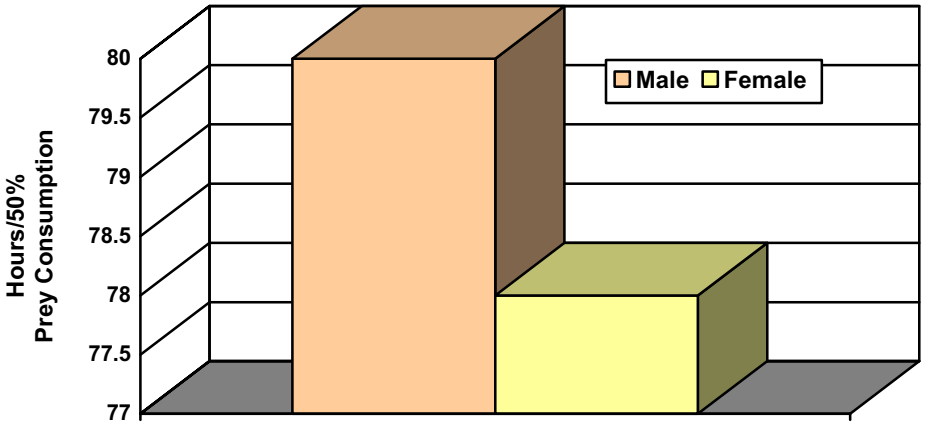


Figure 2. Consumption of *A. socialis* immatures by male and female *C. carnea* adults in laboratory studies.

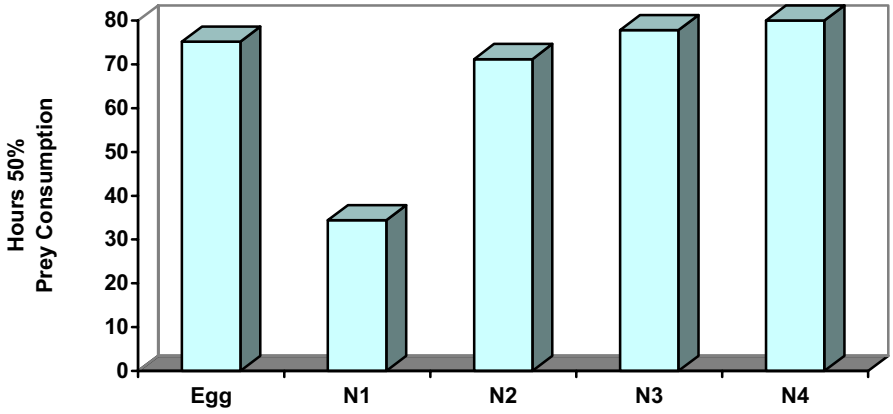


Figure 3. Consumption of *A. socialis* immatures by *C. carnea* larvae in laboratory studies (Duncan Multiple Comparison test at 0.05%).

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

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

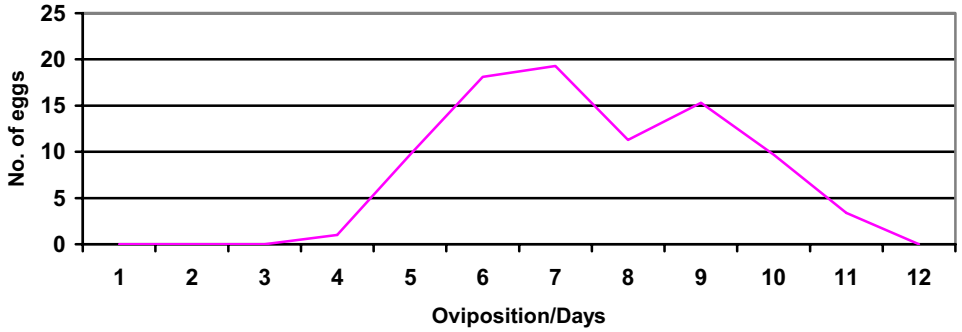


Figure 4. Oviposition of *C. carnea* adult females feeding on an artificial diet in laboratory studies.

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

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

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

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

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

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

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

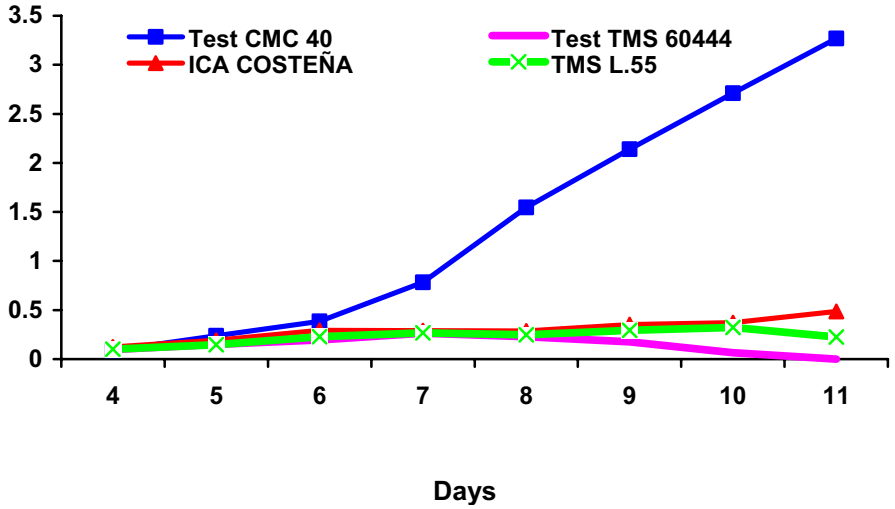


Figure 1. ***Erynnis ello*** **larval weight increments over time when feeding non-transgenic CMC 40 and TMS 60444 vs. feeding on transgenic (Bt) ICA Costeña and TMS-L.55.**

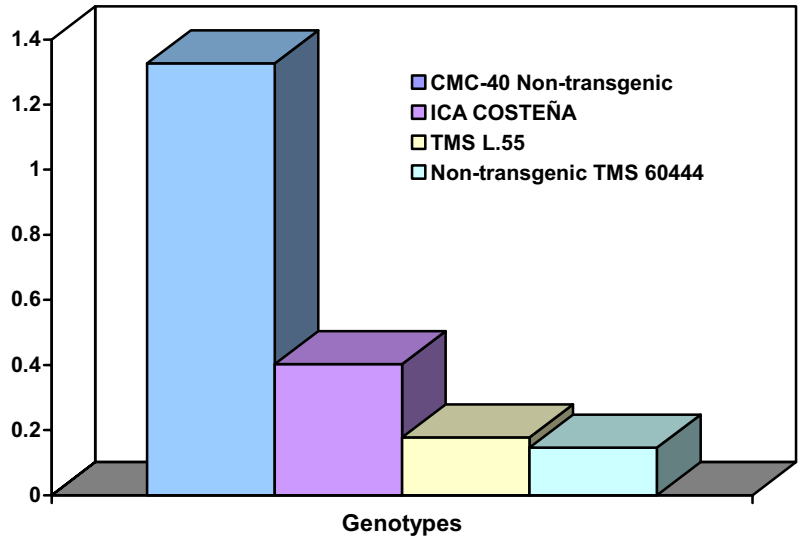


Figure 2. **Average weight gain in grams of** ***Erynnis ello*** **first instar larvae feeding on non-transgenic CMC 40 and TMS 60444 vs. larvae feeding on transgenic ICA Costeña and TMS L-55 (AOV probability at 0.05 level).**

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

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

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

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

Contributor: Carlos Julio Herrera, Anthony C. Bellotti.

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

Publications

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

DORN, B., MATTIACCI, L., BELLOTTI, A., DORN, S. 2002. Effects of a mixed species infestation on the cassava mealybug and its encyrtid parasitoids. Biological Control 27:1-10.

VARGAS, H., BOLIVAR, L., ARIAS, B. and BELLOTTI, A. 2002. Nataima-31 Variedad de yuca (*Manihot esculenta* Crantz) resistente a mosca blanca (*Aleurotrachelus socialis* Bondar) para el Valle Cálido del Alto Magdalena. Colombian Ministry of Agriculture and New Zealand Ministry of Trade and Foreign Affairs (MFAT) Corporación Colombiana de Investigación Agropecuaria (CORPOICA) Regional 6 and Centro Internacional de Agricultura Tropical - CIAT, Cali, Colombia. 400 copies. **[Information leaflets]** [Spanish].

Dorn, B., Mattiacci, L., Bellotti, A.C. & Dorn, S. 2003. Verhalten in einfachen und komplexen Systemen von tropischen natürlichen Gegenspielern. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 76 (1-2). 178.

ARIAS, B., A.C. BELLOTTI. 2003. Ciclo biológico, comportamiento e importancia económica de *Amblistira machalana* Drake (Hemiptera:Tingidae). Chinche negro de encaje, en el cultivo de la yuca *Manihot esculenta* Crantz. Revista Colombiana de Entomología 29 (2).

Publications Accepted

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

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

ALEAN, I., A. MORALES, C. HOLGUÍN, A.C. BELLOTTI. Patogenicidad de diferentes hongos entomopatógenos para el control de *Aleurotrachelus socialis* (Homoptera: Aleyrodidae) bajo condiciones de invernadero. Revista Colombiana de Entomología.

HOLGUIN, C.M., A.C. BELLOTTI. Efecto de la aplicación de insecticides químicos en el control de la mosca blanca *Aleurotrachelus socialis* Bondar en el cultivo de yuca *Manihot esculenta* Crantz. Revista Colombiana de Entomología.

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

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

Publications Submitted

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

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

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

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

Posters

BELLOTTI, A., B. ARIAS, A. BOHORQUEZ, J.VARGAS, H.L. VARGAS, G. TRUJILLO, C.MBA, M.C. DUQUE, J. TOHME. 2002. Recent advances in host plant resistance to whiteflies in Cassava. Congress of Entomological Society of America. Fort Lauderdale, FL. USA.

ARIAS, B., A.C. Bellotti, H.L. VARGAS. 2003. Nataima-31, variedad de yuca (*Manihot esculentaI* Crantz) resistente a mosca blanca, *Aleurotrachelus socialis* Bondar (Homoptera: Aleyrodidae), una contribución al manejo integrado de plagas. Memorias XXX Congreso Sociedad Colombiana de Entomología, SOCOLEN. Julio 17-19, Cali, Colombia. p. 100.

Bellotti, A.C., A. BOHÓRQUEZ, B. ARIAS, J. VARGAS, H.L. VARGAS, CH. MBA, M.C. DUQUE, J. TOHEM. 2003. Avances recientes en la identificación de genes de resistencia a mosca blanca, *Aleurotrachelus socialis* Bondar (Homoptera: Aleyrodidae) en yuca (*Manihot esculenta* Crantz). Memorias XXX Congreso Sociedad Colombiana de Entomología, SOCOLEN. Julio 17-19, Cali, Colombia. p. 99. (Available in English).

Conferences

Bellotti, A.C. 2003. Biological control in the Neotropics; a selective review with emphasis on cassava. 8th Simposio de Controle Biologic, SICONBIOL. Sao Paulo, Brazil. June 22-26, 2003.

Bellotti, A.C. and J. Tohme. 2003. Host plant resistance to whiteflies in cassava. USDA-ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL, USA. February 12, 2003.

Dorn, B., Mattiacci, L., Bellotti, A.C. & Dorn, S. Verhalten in einfachen und komplexen Systemen von tropischen natürlichen Gegenspielern. Jahrestagung der Schweizerischen Entomologischen Gesellschaft. (Comportamiento de antagonistas tropicas en systemas simples y complejos-Zurich Entomological Society). Zürich. Schweiz. 7.3.2003.

Aart van Schoonhoven, Francisco Morales, and Anthony C. Belloti. 2003. CIAT Role in Research and Management of Invasive Pests and Pathogens in the Caribbean Basin. USDA, CSREES, T-Star Sponsored Symposium Challenges and Opportunities in Protecting the Caribbean, Latin America, and the United States from Invasive Species. Caribbean Food Crops Society, 39th Annual Meeting, 13-18 July 2003, Grenada, West Indies. 15 July 2003.

Book Chapter

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

Thesis Completed

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

Thesis in Progress

Aleán, I. 2003. Evaluación de la patogenicidad de diferentes hongos entomopatógenos en el control de *Aleurotrachelus socialis* Bondar (Homoptera: Aleyrodidae) bajo condiciones de invernadero. **(**Microbiología Agrícola). Universidad Javeriana, Bogotá, CO.

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

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

Training and Consultancy Services offered during 2003

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

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

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

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

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

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

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

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

Linkages with Other CIAT Projects and with CIAT's Partner Institutions
IPRA, based at CIAT, Colombia
Instituto Agronómico de Campinas (IAC), Brazil
Instituto de Investigaciones de Viandas Tropicales – INIVIT, Cuba
Universidad Nacional de Colombia, Sede Palmira, Colombia
EMBRAPA, Cruz das Almas, Brazil
Escuela Politécnica del Ejército (ESPE), Ecuador
CLAYUCA
Conservation and Use of Tropical Genetic Resources SB-2
Improved Cassava for the Developing World IP-3
Tropical Fruits IP-6
GIS

TABLE OF CONTENTS

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 work 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. The date of 50% germination was 5 October 2002 and the date of harvest (20% moisture) was 15 February 2003. Yield was measured according to protocols of CIMMYT's office in Colombia.

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 on 3 October 2002. 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 and 40. 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 **(Figure1A)**. 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)**.

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

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)**.

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. Counting and identifying specimens 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: During the survey period of the first growing cycle (17 Oct 2002 – 29 Jan 2003) 8465 specimens were captured representing 15 orders and 5 classes of arthropods **(Tables 1, 2)**. Of these, 98.9% were identified to order and 71.9% to family. Class Collembola was the most represented, with 56.0% of all individuals evaluated. Class Chilopoda was the least abundant, with 0.2% **(Table 1)**. Of total individuals captured, 59.8% corresponded to the insecticide treatment and 40.2% to the control.

Table 1. Number of individuals and composition of arthropod classes caught in pitfall traps in second semester maize, 2002, with and without insecticide.

Class	Total	%
Arachnida	2000	23.6
Chilopoda	17	0.2
Collembola	4737	56.0
Diplopoda	32	0.4
Insecta	1679	19.8
Sum	8465	100

The orders of greatest abundance were Collembola and Acarina with 56.0 and 16.1% of all individuals captured **(Table 2)** (note: Collembola was considered at the level of both class and order). Only the order Thysanoptera exhibited a significant difference in abundance between

treatments **(Table 3)**, while only orders Acarina and Collembola had a significant difference between treatments in area under the curve **(Figures 3, 4)**. The greatest abundance of Collembola was in the insecticide treatment with 30.0% more individuals than the control. Of all Collembola collected, 94.0% of individuals were from the family Podomorpha **(Table 4)**.

Table 2. Number of individuals and composition of arthropod orders caught in pitfall traps in second semester maize, 2002, with and without insecticides.

	With insecticide		Without insecticide		Sum with and without	
Order	**Total**	**%**	**Total**	**%**	**Total**	**%**
Acarina	773	15.3	590	17.4	1363	16.1
Araneae	443	8.7	194	5.7	637	7.5
Chilopoda	6	0.1	11	0.3	17	0.2
Coleoptera	244	4.8	191	5.6	435	5.1
Collembola	3077	60.8	1660	48.8	4737	56.0
Dermaptera	9	0.2	2	0.1	11	0.1
Diplopoda	15	0.3	17	0.5	32	0.4
Diptera	29	0.6	26	0.8	55	0.6
Hemiptera	95	1.9	88	2.6	183	2.2
Homoptera	16	0.3	30	0.9	46	0.5
Hymenoptera	259	5.1	472	13.9	731	8.6
Lepidoptera	27	0.5	45	1.3	72	0.9
Neuroptera	0	0.0	1	0.0	1	0.0
Others	59	1.2	45	1.3	104	1.2
Orthoptera	9	0.2	7	0.2	16	0.2
Thysanoptera	4	0.1	21	0.6	25	0.3
Total	5065	100	3400	100	8465	100

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

Order	With insecticide	Without insecticide
Acarina	12.08±18.0 a	9.22±10.6 a
Araneae	6.92±39.2 a	3.03±12.0 a
Chilopoda	0.09±0.3 a	0.17±0.7 a
Coleoptera	3.81±7.0 a	2.98±4.9 a
Collembola	48.08±153.1 a	25.94±116.0 a
Dermaptera	0.14±0.6 a	0.03±0.2 a
Diplopoda	0.23±0.8 a	0.27±0.6 a
Diptera	0.45±1.0 a	0.41±0.9 a
Hemiptera	1.48±3.2 a	1.38±2.3 a
Homoptera	0.25±0.6 a	0.47±1.1 a
Hymenoptera	4.05±9.9 a	7.38±20.1 a
Lepidoptera	0.42±1.0 a	0.70±1.7 a
Neuroptera	0.00±0.0 a	0.02±0.1 a
Orthoptera	0.14±0.5 a	0.11±0.4 a
Thysanoptera	0.06±0.2 b	0.33±0.8 a

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 Collembola families caught in pitfall traps in second semester maize, 2002, with and without insecticides.

Family	With insecticide	%	Without insecticide	%	Total	%
Entomobryidae	85	2.8	71	4.3	156	3.3
Podomorpha	2922	94.9	1530	92.2	4452	94.0
Sminthuridae	71	2.3	59	3.6	130	2.7
Total	3078	100	1660	100	4738	100

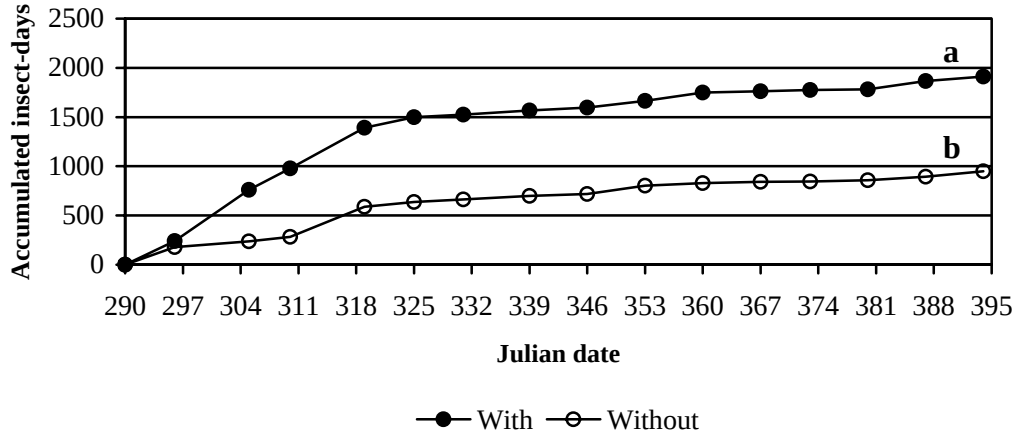


Figure 3. Area under the abundance curve for Acarina in second semester maize, 2002, with and without insecticides.

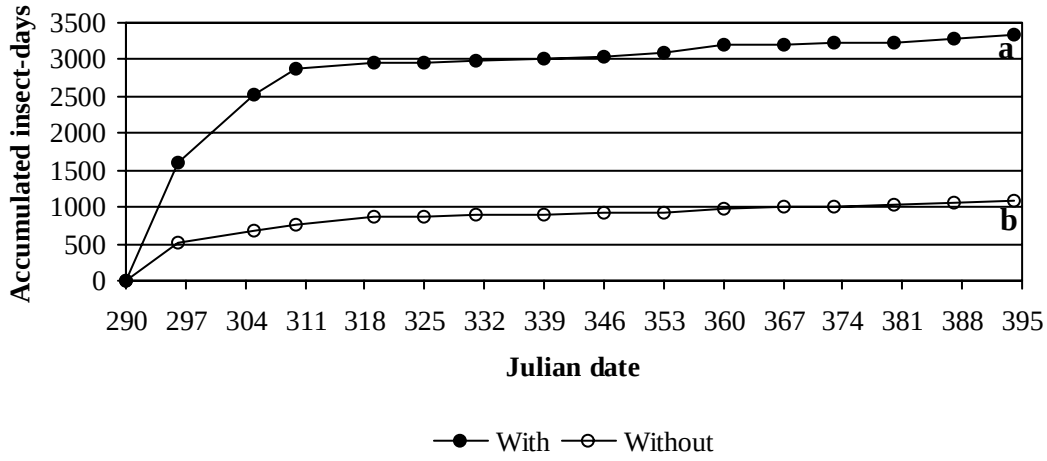


Figure 4. Area under the abundance curve for Collembola in second semester maize, 2002, with and without insecticides.

Of the 321 Coleoptera captured, 56.4% were from the insecticide treatment and 46.3% from the control. Analysis of the area under the curve showed statistically higher accumulated area for the control treatment **(Figure 5)**. The Carabidae and Cicindellidae were the most represented families, comprising 68.8 and 12.5% of all beetles, respectively **(Table 5)**; 69.1% of Carabidae and 13.3% Cicindellidae were captured in the insecticide treatment. The most represented genera of the Carabidae were *Calosoma* (especially *C. granulatum*) with 84.2% of individuals

and tribe Galeritini with 9.0%. For the family Cicindellidae all individuals corresponded to the genus *Megacephala* (*Tetracha).*

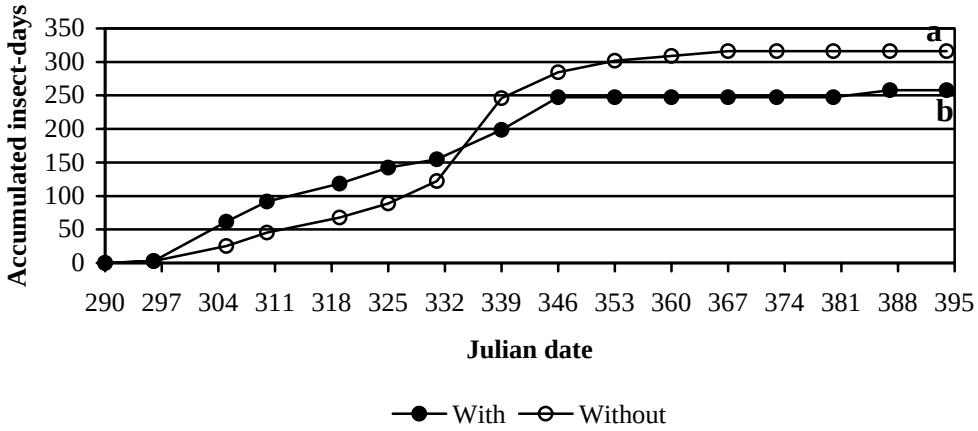


Figure 5. Area under the abundance curve for Coleoptera in second semester maize, 2002, with and without insecticides.

Table 5. Number of individuals and composition of Coleoptera families caught in pitfall traps in second semester maize, 2002, with and without insecticides.

	With insecticide		Without insecticide		Sum with and without	
Family	**Total**	**%**	**Total**	**%**	**Total**	**%**
Carabidae	125	69.1	96	68.6	221	68.8
Cicindellidae	24	13.3	16	11.4	40	12.5
Chrysomelidae	1	0.6	0	0.0	1	0.3
Cucujidae	1	0.6	0	0.0	1	0.3
Geotrupidae	2	1.1	0	0.0	2	0.6
Lycidae	1	0.6	1	0.7	2	0.6
Myxophaga	0	0.0	1	0.7	1	0.3
Nitidulidae	3	1.7	6	4.3	9	2.8
Scarabaeidae	22	12.2	16	11.4	38	11.8
Scolytidae	0	0.0	1	0.7	1	0.3
Staphylinidae	2	1.1	3	2.1	5	1.6
Unidentified	3	1.7	6	4.3	9	2.8
Total	181	100	140	100	321	100

Of the 183 individuals captured from the order Hemiptera, 51.9% were captured in the insecticide treatment **(Table 6)**. The family Pyrrhocoridae was the most represented with 95.6% of total individuals captured, all belonging to the genus *Dysdercus*.

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

Family	With insecticide	%	Without insecticide	%	Total	%
Lygaeidae	1	1.0	1	1.1	2	1.1
Pyrrhocoridae	92	97.0	83	94.3	175	95.6
Reduviidae	1	1.0	2	2.3	3	1.6
Tingidae	1	1.0	2	2.3	3	1.6
Total	95	100	88	100	183	100

The order Hymenoptera represented 8.6% of total individuals captured, with 94.5% representing the family Formicidae where 66.0% were captured in the control treatment. Analysis of the area under the curve showed significant differences in accumulated area in favor of the control **(Figure 6)**.

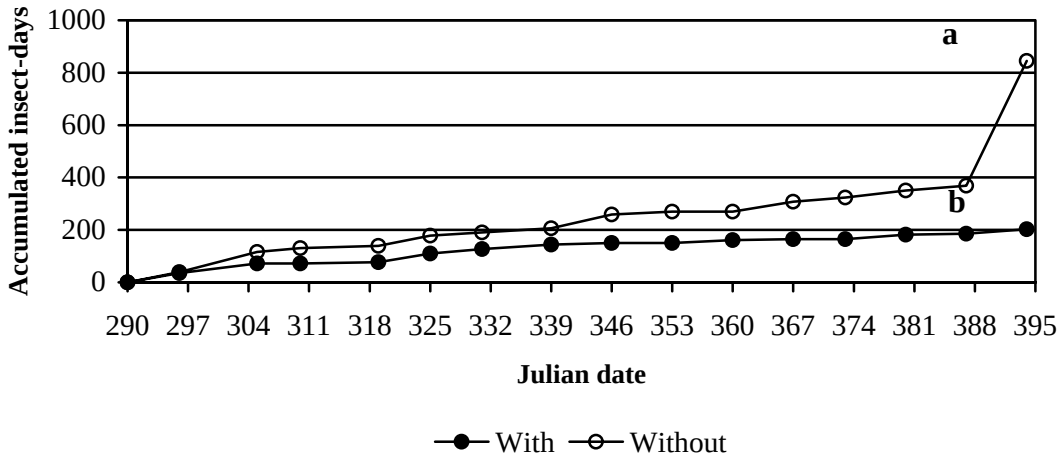


Figure 6. Area under the abundance curve for Hymenoptera in second semester maize, 2002, with and without insecticides.

Arthropod Taxonomic Diversity: Taxonomic richness, measured at the level of order, was highest in the control maize without insecticide **(Table 7)**. The Shannon, Margalef and Simpson diversity indices were all lower in the insecticide treatment versus the control. The dominance index was 1.3 times higher in the insecticide treatment; while the equity index was 1.2 times higher in the control treatment.

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

Index	With insecticide	Without insecticide
Shannon diversity index	1.30	1.58
Margalef diversity index	1.53	1.72
Simpson diversity index	0.59	0.70
Simpson dominance index	0.41	0.30
Equity index	0.50	0.59

Summary of Results

- Over the 16 evaluation dates conducted in the first growing cycle, 19.8% of individuals captured in pitfall traps belonged to the class Insecta.
- For the control and insecticide treatments, 60.8 and 48.8% of the total individuals belonged to the order Collembola, followed in abundance by the orders Acarina, Hymenoptera, Araneae and Coleoptera.
- The most abundant family of the Collembola were the Podomorpha, with 65.6% of podomorpha caught in the insecticide treatment, and 34.4% in the control.

- The most abundant species of the Coleoptera was *Calosoma granulatum*, with 58.1 and 41.9% caught in the insecticide and control treatments, respectively.
- The most abundant family of the Hymenoptera was the Formicidae, with 66.0% of ants caught in the insecticide treatment.
- Abundance differences between treatments was only detected for one (Thysanoptera) of 15 orders the first growing cycle.
- For the area under the curve, Acarina and Collembola were greater in the insecticide treatment, while Coleoptera and Hymenoptera were greater in the control treatment
- Compared to the control treatment, the insecticide treatment had a lower diversity index, higher dominance index, and lower equity index when taxonomic diversity was considered at the level of order

Bibliography

CORPOICA. 1998. Manejo Tecnológico de los cultivos de sorgo y maíz. Ibagué. 44 p.

CORPOICA. 2001. Boletín Técnico No. 34.

DOLES, J. L.; ZIMMERMAN, R. J. and MOORE, J. C. 2001. Soil microarthropod community structure and dynamics in organic and conventionally managed apple orchards in Western Colorado U.S.A. Applied Soil Ecology 18 (1):83–96.

ECA (Edafología Ciencias Ambientales). 2001. El suelo como hábitat 2001. [On-line] <http://www.unex.es/edafo/ECAL6Fauna.htm> [Consulted: 28 March 2003]

GARCÍA ROA, F. 1996. Integración de métodos para el manejo de *Spodoptera frugiperda* (J. E. Smith). In: Boletín Sanidad Vegetal: División de Sanidad Vegetal. Unidad de Proyectos de Prevención. Ed 1º. No. 13 Manejo Integrado de Plagas y Enfermedades en Maíz y Sorgo. Instituto Colombiano Agropecuario ICA, Palmira. 171 p.

JARAMILLO ACEVEDO, E. 1997. Determinación de la artropofauna bosque muy húmedo montano bajo (bmh-MB) en la Región de Monteleón, Manizales. [On-line]. Fitotecnia. No. 6. Entomología. <http://ciagrope.tripod.com/fitote06.html> [Consulted: 28 March 2003]

JORDANA BUTTICAZ, R. 1996. Ecología y aspectos funcionales de la biodiversidad en el suelo. [On-line]. Agricultura. Ecología y Desarrollo Rural. <http://www.agroecologia.net/congresos/pamplona/20.pdf> [Consulted: 30 April 2003]

KIRSTEN, P.; PULSCHEN, L.,; SAUERBORN, J. and ZEBITZ, C.P.W. 1999. Influencia de varios regímenes de uso de plaguicidas sobre la entomofauna de tomate en las tierras altas del Ecuador. [On-line]. In: Manejo Integrado de Plagas. <http://www.catie.ac.cr/informacion/RMIP/rmip54/art8-c.htm#Principales> [Consulted: 28 March 2003]

MARASAS, M.E; SARANDON, S.J and CICCHINO, A.C. 2001. Changes in soil arthropod functional group in a wheat crop under conventional and non tillage systems in Argentina. Applied Soil Ecology 18 (1):61–68.

OSPINA, JOSÉ GABRIEL 1999. Tecnología del cultivo de maíz. Santa Fe de Bogotá: Promedios. 335 p.

PARIS, V. 1979. Biología y Ecología del Suelo. Barcelona, Blume. 169 p.

POSADA, O. L. 1989. Lista de insectos dañinos y otras plagas en Colombia. 4ª. Ed. Bogotá, ICA. p. 662. Boletín Técnico No. 43.

RAW, F. 1971. Artrópodos (Excepto Acaros y Collémbolos) Barcelona, Omega. 422 p.

RODAS R.; J. A.; RUIZ N.; R. E., QUIROGA M. and R. R. 1994. Análisis del uso de los insecticidas en el cultivo de maíz (*Zea mays* L.) en el Municipio de Villaflores, Chiapas, México. In: Avances de investigación en ciencias agronómicas, Villaflores, Chiapas. V. 1, p. 37-43.

ROSS, H. H. 1956. Textbook of Entomology. New York. John Wiley and Sons. 536 p.

VELEZ, A. R. 1989. Catálogo del museo de entomología "Francisco Luis Gallego". Universidad Nacional de Colombia. Seccional Medellín. Medellín. p. 87.

ZANIN, I.; ARAYA, J. E.; and VALDIVIESO, C. 1995. Comparaciones de la entomofauna epigea en los cultivos asociados de maíz y fríjol. Investigación Agrícola vol 15 Resumen [On-line línea].
<http://www.uchile.cl/facultades/cs_agronomicas//publicacioes/iagricola/vol15/15p6.html> [Consulted: 20 March 2003]. ISSN 0304-5617.

Contributors: Jairo Rodríguez Chalarca, Daniel C. Peck, Principal Investigator (Department of Entomology, NYSAES, Cornell University), Mariluz Mojocoa, Gerson F. Vélez.

Collaborators: Anthony Bellotti, Edmundo Barrios.

Collaborating Institutions: Cornell University, Ithaca, U.S., ICA, Palmira, Colombia.

Funding: Subcontract to CIAT, Cornell University Project funded by USAID (Biotechnology and Biodiversity Interface).

SOIL PESTS - CASSAVA AND OTHER CROPS

Activity 1. Integrated control of subterranean pests in South America.

Introduction

Soil pests cause serious economic losses to many important crops in South America. Until about 20 years ago white grubs and burrower bug *Cyrtomenus bergi* were not considered important pests in Latin America. However, in the recent past outbreaks are no longer seasonally restricted as before and are present in many agro-ecosystems, such as hillsides, tropical lowlands, including savannas and forest margins in Central and South America (King & Saunders, 1984; Posada, 1993; Londoño & Pérez, 1994; Pardo, 1994; Corpoica, 1996; Shannon & Carballo, 1996). Inappropriate agricultural methods like inadequate cropping patterns, burning of harvest residues, deforestation, cultivation of marginal land, discontinuance of tillage, loss of floral and faunal biodiversity, reduction of organic material, ill-timed and excessive applications of synthetic pesticides and elimination of natural enemies of pests are considered to be the key factors responsible for the increased pest status of white grubs and *C. bergi* (Posada, 1993; Londoño, 1994). The frequent use of highly toxic soil pesticides can lead to development of resistance in pests and is additionally very often ineffective. The link between pesticide use and soil and water contamination is well documented, as well as its threat to farmers' and consumers' health (e.g. Mervyn, 1998).

Five outputs were envisaged in our project in order to accomplish the major objective, that is to identify the key pest species and to develop appropriate IPM strategies for their effective control:

I. Problem diagnosis from the farmers' perspective and identification of local existing knowledge and cultural practices.
II. Description of pest problem (taxonomic identification of the white grub species complex, rapid identification of larvae, distribution and occurrence of species, yield loss and damage estimates).
III. Characterization of potential biological control agents (search for natural enemies, identification and propagation, laboratory and field evaluation).
IV. Establishment of network for soil arthropod research (electronic compilation of relevant data, communication network, website).

I. Problem diagnosis by means of farmer interviews.

Methodology: The problem diagnosis from the farmers' perspective is a key tool for a thorough identification of the pest problem. We interviewed 172 farmers in the departments of Quindío (99), Risaralda (52) and Northern Cauca (21). Farms were identified with the help of the local Umatas, rural meetings where farmers were asked about their problems with soil pests and in an arbitral manner. On all selected farms one of the following crops were cultivated: cassava, onion, and pasture. We included cassava because this crop is of increasing importance in the coffee zone and because of CIAT's expertise in this crop. Onions have a long tradition in the hillsides of La Florida, La Bella and La Colonia, three villages close to Pereira in a range of 1600 – 2000 meters above sea level (asl).

The questionnaire focused on farmers' perception of the principal pest species and their management practices, such as use of pesticides, crop rotation, intercropping, previous and adjacent crops, degree of damage, soil management and other factors. The great majority of the persons who were interviewed were only temporary 'majordomos' or tenants and did not have sufficient background knowledge for fully answering all questions. For this reason we visited additional 29 farms near Montenegro (Quindío), where the farmers confirmed soil pests as one of the major pest problems.

Results and Discussion: Fifty-two farmers considered trips as their major pest problem, followed by the hornworm (*Erinnyis ello*) (32) **(Figure 1)**. Only 16 farmers mentioned white grubs and six *C. bergi* as a problem. In contrast, onion farmers only considered burrower bug and white grubs as important insect pests. These results indicate that farmers' perception of a pest problem is apparently related to its visibility. When harvesting, onion farmers find the burrower bug directly feeding on the onions and can thus relate directly the damage to the pest. However, in cassava *C. bergi* is not present when the root develops to a mature tuber. Likewise white grubs are not present throughout the entire growing season. Thus it is not surprising that farmers considered the trips or the hornworm as the most important pests although we rarely observed the presence of these insects. Almost no pest species were reported on pasture. We frequently observed that farmers confounded burrower bug damage with *Phytophthora* root rot.

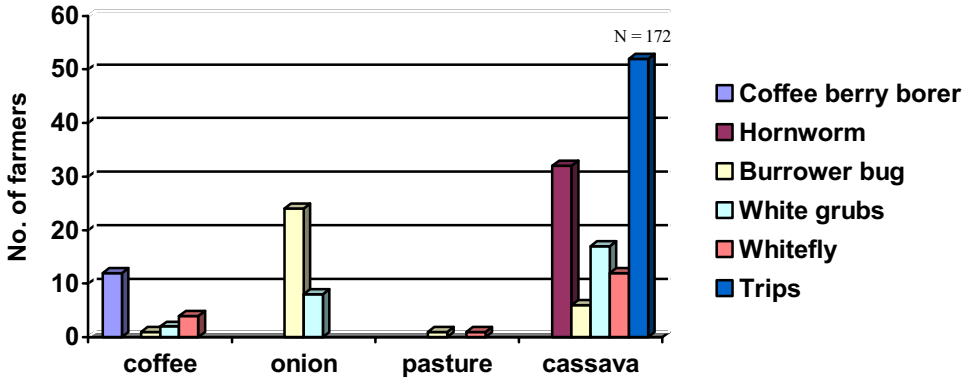


Figure 1. Farmers' perception of key pests.

Consequently only six farmers (all crops) complained about yield losses greater than 76% due to white grub attack; 15 farmers reported losses in a range of 51 – 75%, and 15 farmers estimated the losses between 25 – 50%. In the case of burrower bug only five farmers reported losses between 51 – 75% on onion **(Figure 2**).

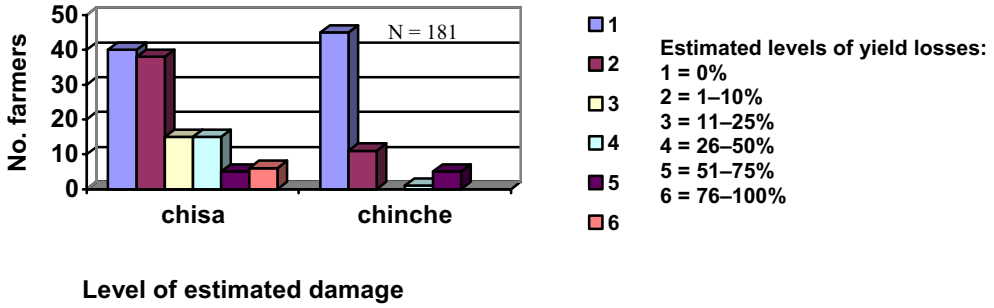


Figure 2. Estimated level of yield losses due to soil pests of cassava in Quindío, Risaralda and Cauca. The interviews were realized in the second semester off 2002.

Of the 29 farmers who recognized the presence of soil pests, 71% applied insecticides for control of white grubs and/or burrower bug; 14% used biological control and another 14% combined insecticides and with biological and organic methods (*e.g.* entomopathogenic fungi, lime, mulch). 82% were convinced that their applied treatment (chemical and/or biocontrol) is effective , only 17% denied the efficiency. Use of insecticides was the predominant control strategy (86%), and carbofuran was the most frequently used active ingredient (32%). 10% of the interviewed farmers used other strategies such as combination of onion and detergent. 5% of them applied a combination of an insecticide and entomopathogenic fungi.

Training of farmers: In order to enhance the perception of the soil pest problem together with SENA Armenia we organized a two days farmers' training workshop on identification of white grubs and burrower bugs. In December 2003 we will organize a follow-up meeting to evaluate the adoption rate of this knowledge.

II. Description of pest problem.

Methodology: In September 2002 we started stratified surveys (frequency once a month) in cassava, potatoes, onion, and pasture for soil pests to study the fluctuation (time and spatial) of scarabids. The crops were selected according to their economic importance in each region: Cassava in Northern Cauca (990 m asl) and in Pereira (1400 m asl), onion in La Florida and La Colonia in Risaralda (1660 m and 2000m asl, respectively), potatoes in North and East Antioquia (2100 - 2800 m asl) and in Cundinamarca at the Centro Agropecuario Marengo (CAM) (2547 m asl) and in Cogua (3200 m asl). Additionally, we monitored pastures in each zone because of its high susceptibility to white grub attack. First results indicate that 10 samples of 100 x 100 x 15 cm per site are an appropriate sample size. We selected this sampling method because it provides more accurate data on abundance and diversity of white grubs than the monolith (30 x 30 x 25 cm) (Pardo 2002). Moreover, we collected adult beetles at weekly intervals by means of light traps in each region (North of Cauca, Pereira, and La Florida).

Collaboration with partner institutions: In order to be able to realize stratified surveys we established the cooperation with Research Center of Corpoica "La Selva" in Rionegro (Antioquia), the National University of Bogotá and University of Caldas, Manizales. This cooperation is crucial for fulfilling the project output of identification of the pest species

complex in various agroecological zones. CIAT focused activities on surveys in the North of Cauca and gave logistic support for surveys in Risaralda such as transportation and supplies where the University of Caldas participated in forms of two undergraduate theses.

Moreover, the project collaborates with two German institutions. The Federal Institute of Biological Research (Germany), that identified fungi and other entomopathogens and Kiel University that collaborated in improving our activities with entomopathogenic nematodes.

In December 2002 we received a call from the Hacienda Córcega (1330 m asl) (Quimbaya, Quindio), one of the biggest coffee producers in this zone. White grubs had severely attacked coffee grains that were stored in sacks for seedlings next to a maize field. The administration of the hacienda collaborates in executing the field surveys and is willing to conduct research activities in order to develop control strategies. We also receive logistic support from SENA Armenia sending us the sampled material for identification.

Results

Santander de Quilichao: Until August 2003 at CIAT's experimental station in S/Quilichao we collected in soil samples pasture 2,434 larvae, 46 pupae, 114 adult scarabs, and 61 parasitoids. This corresponds to a density of 6.3 larvae, 0.2 pupae, 1.6 adults and 0.6 parasitoids per m^2. In the cassava plots we found 769 larvae, 10 pupae, 10 adult scarabs, and 13 parasitoids, corresponding to a density of 5.9 larvae, 0.1 pupae, 0.1 adults and 0.2 parasitoids per m^2.

In the light trap we collected in total 17,355 specimens, belonging to the following scarab genera: *Cyclocephala, Plectris, Dyscinetus, Aphodiinae, Anomala, Phyllophaga, Pelidnota, Lygirus, Sericini, Podischnus, Strategus,* and *Lycomedes.*

Eleven months of light trap data indicate a response of scarabids to the short but not to the long rainy seasons, with a distinct peak in flight activity around October/November but not in March-May. During October/November we collected more than 50% of our annual collection.

The most abundant scarabid genus was *Cyclocephala* (50%), followed by *Plectris* (28%), *Aphodiine* (9%), *Dyscinetus* (2.7%), *Anomala* (2.3%), *Phyllophaga* (2%), and *Lygirus* (1.8%). Predominant species were *Cyclocephala amazonica* (28.3%) and *Plectris* sp. 2 (17.4%) **(Figure 4)**. *Cyclocephala amazonica* was trapped throughout the entire observation period. In contrast, high numbers of *Plectris* sp. 2 were trapped in October and a smaller peak was recorded in June. In general, all species peaked in October.

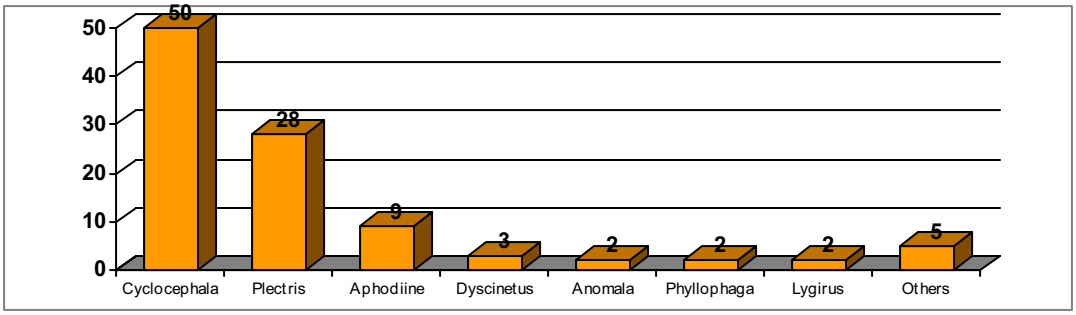


Figure 3. Most abundant Melolonthidae species (adults) collected from September 2002 to June 2003 in light traps in Quilichao.

Cyclocephala amazonia was the most abundant white grub larvae found in pasture and cassava. *Phyllophaga* sp. and *Leucothyreus* followed in pasture. The sequence was reverse in cassava where *Leucothyreus* was more abundant than *Phyllophaga* sp. *Plectris* spp., a genus of presumed economic importance was almost absent. We did not observe any damage due to white grub attacks, neither on cassava nor on pasture. *Cyclocephala amazonia* is commonly not considered as a pest. The presumed important pest genera *Phyllophaga* and *Plectris* were detected in high numbers in pasture only in January and February.

In the initial phase white grubs from Risaralda and Northern Cauca were taken to our laboratory Quindío at CIAT for adults' identification. However, we observed that the larvae development was delayed or the pupae were malformed. We hypothesized that these abnormalities were due to the fact that they were removed from their original habitat. Hence, we established an on-farm laboratory in Caldono (Cauca) where we can study their life cycle and feeding behavior. In this lab we are observing *Phyllophaga menetriesi*, *Plectris fassli, Plectris pavida, Anomala undulata, Anomala sp., Cyclocephala* pos. *lunulata* and *C. amazonia*. The farmer Manuel Trujillo and his son Huber are running this lab in participatory research manner. Both have been collaborating working many years with CIAT and have received a training in the work with white grubs.

Quindío: In the light trap on the Hacienda Córcega in Quimbaya (Quindío) we found the following white grub complex: *Phyllophaga menetriesi, Phyllophaga* sp*., Anomala cincta, A. undulata* and other species whose the economic importance is unknown. On the big cassava, coffee or maize plots *P. menetriesi* was mainly recorded. According to the administrator, it occurs primarily in December and March, causing up to 100% damage in cassava and maize. Employees reported that they found up to 20 larvae per m^2 during this period. In January and February we collected 8 and 7 larvae per m^2, respectively, in a maize field intercropped with cassava.

Risaralda: In La Florida we collected 1,934 adult scarabids between October 2002 and June 2003. The subfamily Rutelinae harbored 65% of the captured beetles, followed by Dynastinae with 26%. *Anomala cincta* and *A. undulata* were the most abundant species (**Figure 5).**

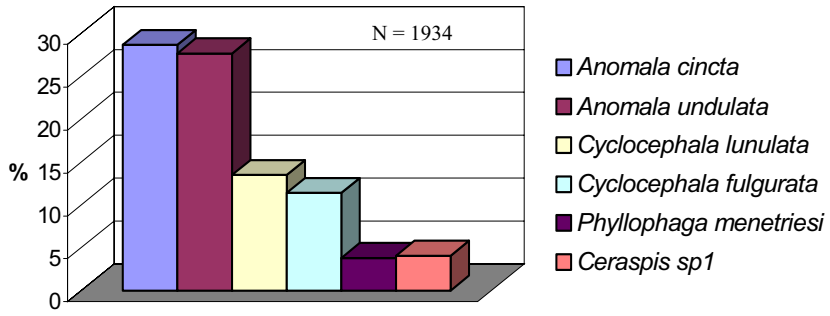


Figure 4. Scarabid adults caught in light traps in La Florida (Risaralda) from September 2002 to August 2003.

In La Colonia we collected in total 1,962 adults. *Cyclocephala fulgurata* and *C. lunulata* were the dominant species, followed by *Anomala cincta* and *A. undulata* (**Figure 6**).

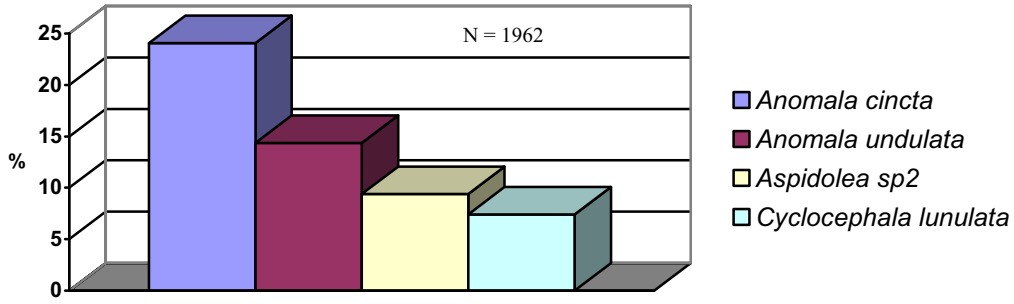


Figure 5. Scarabid adults caught in light traps in La Colonia (Risaralda) from September 2002 to August 2003.

During the surveys in onion and pasture fields we collected in total 1,949 white grub larvae. 1,255 larvae (64,4%) were collected in pasture and 694 (35.6%) in onion. The average density of white grub larvae were 10.4 and 5.7 larvae per m^2 in pasture and onion, respectively.

The white grub complex consisted of 11 species belonging to the subfamilies Melolonthinae, Dynastinae and Rutelinae. **Figure 7** shows that Dynastinae sp. 1 dominated in pasture, followed by un identified species of Macrodactylini, Melolonthinae and *Phyllophaga*. The most abundant species in onion was Dynastinae sp. 1 (284), followed by Rutelinae sp. 1. In onions we found a similar distribution, however, Dynastinae sp.1 was more dominant **(Figure 8).**

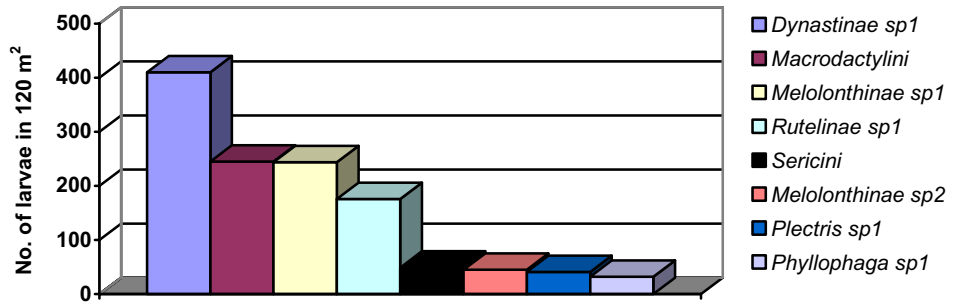


Figure 6. No. of scarabid larvae collected in pasture in La Florida from September 2002 to August 2003.

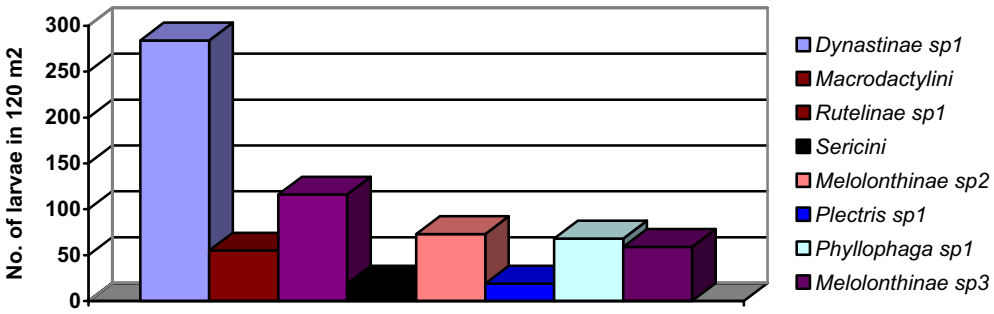


Figure 7. No. of scarabid larvae collected in onions in La Florida from September 2002 to August 2003.

In the Pereira region the most frequently collected adult scarabs in pasture were *C. lunalata* (69%). The other species were*: Anomala cincta* (20%), *A. undulata* (11%), *P. menetriesi* and *C. fulgurata* (2%). In the light trap in cassava we again found *Cyclocephala lunalata* (51%) to be the most abundant species. Other species were *A. undulata* (32%), *Anomala* sp. 1 (8%), *A. cincta* (5%). *Phyllophaga menetriesi* and *C. fulgurata* were present in the same portion as in the pasture samples (2%).

In cassava plots near Pereira we identified 1,858 larvae of eight white grub species. An unidentified species of the tribe Rutelinae was present in greatest abundance (42.4%), followed by *Cyclocephala* (18.8%) and *Plectris* sp. 2. In pasture we collected 2,834 larvae of 10 species, where *Cyclocephala* dominated (38.3%), followed by Macrodactylini (23,1%). Larvae associated with cassava showed three peaks: The first in December (Ø 12 grubs per m^2), the second in March (Ø 100 grubs per m^2) and in May (Ø 80 grubs per m^2). Highest numbers of larvae in pasture were recorded in November (Ø 27 grubs per m^2) and April (Ø 34 grubs per m^2).

Antioquia: Adult scarabs and larvae were collected in pasture and in potato fields. We installed six light traps in the municipalities of Rionegro (2,100 m asl), El Carmen de Viboral (2,258 m asl), San Vicente (2,300 m asl), La Union (2,460 m asl), Santa Rosa (2,486 m asl), Entrerrios (2,437 m asl) and San Andres de Cuerquia (1,211 m asl). Until June we revised about 80,000 specimens and separated them into 28 species.

In the savanna of Northern Antioquia (2,600 – 2,800 m asl) we have identified adults of scarab 15 species: Ancognatha scarabaeoides, A. humeralis, Cyclocephala sexpunctata, C. gregaria, Heterogomphus dilaticollis, H. schoenerri, H. chevrolati, H. rubripennis, Megaceras pos. morpheus, Astaena sp., Golofa eacus, Anomala sp., Plectris sp. and one unidentified species. Ancognatha scarabaeoides was the most abundant species (almost 37,000 specimens), followed by C. sexpunctata (18,000). In La Union, a village in the savanna of East Antioquia (2,400 – 2,600 m asl) we identified 11 species: A. scarabaeoides, A. humeralis, C. sexpunctata, C. gregaria, H. dilaticolis, H. schoenerri, A cincta, Plectris sp., Astaena sp., Isonichus sp. and Golofa sp. As in the North of Antioquia, A. scarabaeoides was the predominant species (10,000 captured adults); Astaena sp. was collected less frequently. The savanna of Northern Antioquia corresponds to a moderate cold climate zone (2,100 – 2,300 m asl). Here we collected the following 20 species: A. scarabaeoides, A. humeralis, C. sexpunctata, C. gregaria, C. fulgurata, Cyclocephala sp., Phyllophaga obsoleta, Astaena sp., H. dilaticollis, H. chevrolati, G. eacus, Isonychus sp., Plectris sp., Anomala cincta, A. pos. undulata, Anomala sp., Macrodactylus sp., Anatista lafertei, Callistetus cupricollis and one unidentified species. Again, A. scarabaeoides (5,400 specimens) dominated in abundance, followed by C. sexpunctata and P. obsoleta with 1,300 specimens captured each and Isonychus sp. with 1,000 adults trapped.

Up to now we have revised 500 m^2in potato fields. We found tuber damage in 250 m^2 of survey, but not necessarily caused by insects. We checked a total of 19,946 tubers, corresponding to 40 tubers per m^2. Of these, 8.6% showed symptoms of insect attack and 0.1% due to other reasons. White grubs caused 5.3% of the damage, followed by Guatemalan potato moth (Tecia solanivora) (3,2%), and black cutworm (Agrotis ipsilon) (0.1%). White grubs were present at an average density of 7.6 larvae per m^2. We additionally sampled 440 m^2 in Kikuyo (Pennisetum clandestinum) and found white grubs and cutworms in 50.7% and 1.6% of the area, respectively.

So far we were unable to identify larvae to species level, underlining the need for development of suitable keys for their identification.

Cundinamarca: 1,038 larvae of Ancognatha sp. and Heterogomphus dilaticollis (Dynastinae) and Clavipalpus pos. ursinus (Melolonthinae) were collected between September 2002 and May 2003 at the Centro Agropecuario Marengo (CAM, Cundinamarca). During the first four months we observed very low population densities (average of 5 larvae per m^2). In February 2003, populations increased to an average of 21.6 larvae per m^2 and maintained this density during the following two months. Variation was very high; in some cases we collected up to 168 larvae in m^2. Larvae of Ancognatha sp. and H. dilaticollis were associated with organic material and/or plant parts in decomposition, suggesting that this white grub complex does not feed on living plants or may prefer organic material to living plants.

In Cogua (3,200 m asl, Cundinamarca) we sampled from October 2002 until August 2002 in total 350 larvae of Ancognatha sp. and Heterogomphus dilaticollis (both subfamily Dynastinae). With 8.7 larvae per m^2 the greatest density was recorded in December 2002. Thereafter densities decreased to a minimum of 2.2 larvae / m^2 in July 2003. The average larval density was lower in Cogua than at CAM (8.7 larvae / m^2), indicating a preference for pasture as habitat.

In June 2003 we found in a fallow plot larvae of Clavipalpus pos. ursinus associated with decomposing plant material, suggesting that this species has a facultative saprophagous behavior as strategy for survival when principal host plants are rare.

In the light trap in pasture at CAM we collected in total 7,008 adult scarabids. Ancognatha scarabaeoides was by far the predominant species (98%); other specimens were A. ustulata and Heterogomphus dilaticollis (subfamily Dynastinae) and Manopus bigutatus (subf. Melolonthinae). The majority of the beetles was trapped in January and March, and lowest numbers were recorded in February, March and May. In Cogua we only could make three surveys. We detected five species of three genera of Dynastinae: Ancognatha scarabaeoides, A. ustulata, H. dilaticollis, Astaena pos. tarsalis, and A. vulgaris. 90% of 910 collected adults corresponded to A. scarabaeoides, corroborating the results at CAM where A. scarabaeoides strongly dominated the scarab diversity. Interestingly, larvae of Clavipalpus pos. ursinus, Manopus bigutatus, Astaena sp. and Lacioccala sp. were never found in Cogua or at CAM despite their presence as adults in the light traps.

The white grub complex in Cundinamarca shows a much more reduced species biodiversity than in other similar agro-ecological zones. In Cauca or in the North of Antioquia up to 45 species have so far been identified.

Field-collected larvae were transferred to the lab for establishing colonies. In doing so we obtained important data on biology and behavior of the larvae. For instance we can prove that Ancognatha scarabaeoides, A. ustulata and Heterogomphus dilaticollis are not obligatory pest species since all larvae of the three species developed from egg to adult on a pure diet of organic material, corroborating our field observations. When feeding larvae of these three species with carrots, they always clustered on spots where the food was already in the process of decomposition. In contrast, Clavipalpus pos ursinus always focused on fresh parts of carrots and moving to the soil surface when the food presented symptoms of decomposition.

These observations are important indicators for the potential pest status of white grubs in the Savannah of Bogotá and corroborate results from the earlier study by Ruiz and Posada (1986) who claimed that “the mixture out of soil, decomposed wood and dung were the only substrate where they complete their life cycle”.

We morphologically described larvae of Ancognatha scarabaeoides, A. ustulata, Clavipalpus pos. ursinus, H. dilaticollis and Ceraspis sp.

Discussion of survey results from the three agro-ecological zones: In general, our data show that the biodiversity of the white grubs complex is greater in warmer (S/Quilichao) and reduced colder agro-ecological zones (Andean zones of Antioquia and Cundinamarca). Another important finding is that the complex of noxious white grubs is limited. *P. menetriesi* is the most important pest species in Quindío and Risaralda. Its economical importance is well documented (*e.g.* King 1984). In Antioquia in zones over 2000 m asl the pest complex is restricted to *P. obsoleta* (Vallejo *et al*. 1998, Vallejo *et al*. 2000). In Antioquia and in Cundinamarca *Ancognatha scarabaeoides* is the most abundant white grub (up to 98%). However, our observations that this species feeds mostly on decomposing organic material seriously questions

its pest status though more data on the biology and ecology of *A. scarabaeoides* is needed. However, there is a need for a more detailed study of the feeding behavior of this insect.

III. Characterization of potential biological control agents.

Search for native entomopathogenic organisms in Cauca, Risaralda and Panama: We collected 320 soil samples in Panama (31), and in Colombia in Quindío (61), Risaralda (135), Caldas (40) and Cauca (53) and isolated entomopathogenic nematodes by means of the *Galleria* bait method **(Table 1)**.

Table 1. Sites where soil samples were collected for isolation of entomopathogenic nematodes.

Country	Department	Municipality	Date	Crop
Panamá	El Valle de Antón	Coclé	Oct-02	onion, peanuts
	Ocú	Veraguas	Oct-03	cassava
	Sioguí	Chiriquí	Oct-03	cassava
	Cerro Punta	Estac. IDIAP	Oct-03	onion
Colombia	Quindío	Quimbaya	Mar-03	cassava, plantain, maize
	Risaralda	Santa Rosa	Mar-03	onion
		Pereira	Mar-03	pasture, cassava, onion
			May-03	onion
			Feb-03	pasture, onion, cassava, peas
		Dosquebradas	Mar-03	pasture
		La Florida	Jun-03	onion
	Caldas	Manizales	Jun-03	guamo, breadfruit tree, maize, beans, cassava, avocado, plantain, mango, mandarin, lemon, passion fruit, coffee
	Cauca	S/Quilichao	Mar-03	maize, pasture, cassava

The three nematode strains collected in Panama were shown to be saprophytic. In Colombia we isolated 16 strains from samples taken in various crops, 10 of them showed lethal effects on *Galleria* in sand. Thereafter we verified these observations, following Koch's postulates. Pathogenic strains will be sent to Kiel University (Germany) for identification as soon as we will obtain the exportation permit from the Colombian authorities.

In S/Quilichao no entomopathogenic EPN or bacteria were collected but the following fungi were isolated from 850 white grubs: 3 x *Metarhizium anisopliae*, 7 x *Fusarium* sp. and 3 x *Paecilomyces* sp. The unidentified fungi will be sent to Germany for identification. *M. anisopliae* and *Paecilomyces* sp. were isolated from white grubs found in cassava, *Fusarium* sp. in pasture.

In preliminary experiments we tested *M. anisopliae*, a strain of *Paecilomyces*, and *Beauveria bassiana* against larvae (third instar) of *P. menetriesi* with 20 replicates for each strain. The white grub control was 100%. It is of interest for future work to repeat these experiments at greenhouse or field level. Moreover, other white grubs should be included in these tests.

Risaralda: In fields near Pereira and in La Florida 500 white grubs per locality were collected and brought to the lab for further studies of potential pathogens. Later, these pathogens were identified by experts of Cenicafé. 50% of the collected larvae in Pereira showed symptoms of a bacterial infection **(Table 2)**. This rate seems to be extraordinarily high since the larvae were

collected on several plots. Hence, it can be excluded that all the 250 white grubs were from a spot where bacteria were highly concentrated. Moreover, we have never observed such a high extent of bacterial infections in larvae sampled in the other surveys. Hence it is possible that the larvae collected in Pereira were contaminated in the lab.

Table 2. Frequency of various entomopathogens associated with white grubs in Pereira collected from pasture and cassava.

Entomopathogens	No. of Observations
Bacteria	
Bacillus popilliae	250
Fungi	
Metarhizium spp.	9
Beauveria bassiana	2
Trichoderma	50
Fusarium	20
Nematodes	
Mermithida sp.	2 nematodes in one grub

In La Florida we found 30 larvae attacked by entomopathogens. Eight were infected by *Metarhizium* spp., 10 by *Trichoderma* spp. and five by *Fusarium* spp. and none by bacteria.

Antioquia: We sampled in potato and pasture fields for white grubs (10 m^2 per plot) and took the larvae to the laboratory in order to identify them and their natural enemies. We observed a high mortality of the larvae due to infection by pathogens. Mortality was higher in East Antioquia than in the North. The rate of mortality is summarized in **Table 3**. After evaluation in the lab, microbial organisms turned out to be main cause for white grub mortality. The most frequent pathogens we found were the bacteria *Bacillus popilliae* and the fungus *Metarhizium anisopliae* **(Table 4)**.

Table 3. Mortality due to pathogens of collected white grub larvae sampled in three agroecological zones in Antioquia from October 2002 to June 2003.

Region	No. of grubs alive	No. of dead grubs	%
North 2600-2800 m asl	548	432	78
East 2600-2800 m asl	624	566	90
East 2100-2400 m asl	648	613	94

We collected a total of 502 white grubs from the pasture Kikuyo, in three ecological zones in Antioquia. 93.7% of the grubs taken from the cold North Antioquia (2800 m asl) died in the lab, 57.4% from the cold East (2600 m asl) and 86.7% from the West, the latter characterized by a moderate cold climate. Frequency of mortality factors were similar to the ones in potatoes, however, with an increased frequency of *Bacillus popilliae* and hymenopteran and dipteran parasitoids.

Table 4. Mortality factors of white grubs (D = Dynastinae and M = Melolonthinae) collected in three regions in Antioquia from potato fields and pasture from October 2002 to June 2003.

Mortality factors	North (2600-2800 m)		East (2600-2800 m)		East (2100-2400 m)	
	D	M	D	M	D	M
Bacillus cereus	4	0	9		2	2
Bacillus larvae	4	0	1		0	0
B. lenthimorbus	3	0	1		0	0
B. popilliae	70	1	72		30	9
B. sphaericus	10	0	6		7	0
Clostridium sp.	9	0	7		6	2
Pseudomonas sp.	0	0	1		0	0
Ma-1	9	0	241		54	1
Ma-2	0	0	2		54	0
Ma-3	0	0	0		1	1
Ma-4	3	0	0		0	0
Cladosporium sp.	0	0	0		1	0
Paecilomyces sp.	0	0	2		1	
Fusarium sp.	0	0	0		3	
Synnematium sp.	0	0	0		0	
Ectoparasite	0	0	0		2	
Endoparasite	0	0	0		2	
Protozoans	0	0	1		0	
Nematodes	2	0	0		37	
In process	2	0	0		1	
No visible reason	96	6	65		57	
Other reasons	188	16	132		174	
Total	400	23	540		432	

Cundinamarca: Approximately 100 white grub larvae showing disease symptoms were brought to the lab for further identification of the pathogens. 22% of these larvae were collected in Cogua, 32% at CAM and 46% in Subachoque. Entomopathogenic fungi were recorded most frequently (43%), followed by bacteria (40%), nematodes (21%) and protozoa (2%). The higher numbers of diseased larvae found in Subachoque and at CAM might be related to the fact that the potato fields in Subachoque had passed through a two years fallow period and at CAT samples were taken in pasture. Such conditions are possibly more suitable for both white grubs and entomopathogens than the pesticide-treated crops at Cogua.

60% of the 100 diseased larvae belonged to the genus *Ancognatha*, 33% were *Clavipalpus* pos. *ursinus* and 6% were *Heterogomphus dilaticollis*. Although the rate of natural infection was highest in *Ancognatha* spp., the mortality rate in *C. ursinus* was considerably higher compared to the other infected white grubs, possibly explaining why this white grub species is much less frequent in Cundinamarca than in other zones.
So far we have isolated 35 fungal strains that we store in a ceparium. To date the following species/strains have been identified in collaboration with our partners from BBA Darmstadt (Germany): *Metarhizium anisopliae* (4 isolates), *Fusarium* spp. (3), *Beauveria bassiana* and *Verticillium* spp. (2 isolates each) and *Aschersonia* spp. (1).

We additionally isolated 29 bacterial strains. In collaboration with Corpoica, Rionegro, we identified 15 isolates as *Bacillus popilliae*, three as *B. sphaericus*, two as *Clostridium* sp. 1, one

as *B. larvae* and one as *B. cereus*. Bacterial-infected white grubs were soft and of a white, brown, or black color. The majority of these grubs had a strong odour, probably due to fermentation caused by the bacteria.

In total 16 white grubs were attacked by, presumably entomopathogenic, nematodes and we managed to rear four strains on *Galleria mellonella*. Presently these samples are in the process of purification and identification since some of them were associated with saprophytic organisms (mites, bacteria or other nematodes). White grubs attacked by nematodes were in general transparent and of slightly reddish color. The nematodes clustered close to the spiracles or the legs. When the grubs were stored in maturation chambers, in most of the cases the nematodes were unable to pass through the cuticle. Often grubs turned black, possibly due to proliferation by other saprophytic microorganisms. In these cases it was almost impossible to recover infective nematode juveniles. We observed that the mouth of the grub cadavers is the main exit for the nematodes. We managed to identify two nematode populations belonging to the genera *Mesorhabditis* and *Steinernema*.

Discussion of recollection of entomopathogenic organisms in three agroecological zones: The poorest collection of pathogens was in S/Quilichao, the most numerous in Antioquia. The poor presence of pathogens in S/Quilichao may be explained by the dry climate. The difference in pathogen collections may be due to factors other than agroecological conditions. In Cundinamarca the survey was conducted by only one student, while Corpoica could carry out a more extensive survey. Pathogen collections in Risaralda fell between the two extremes. Therefore, it is suggested to continue the survey of soil pests affected by pathogens including the registration of climatic factors and soil components (*e.g.* organic material).

Another important task results from the fact that many collected isolates still have to be identified. Here, we can count on the collaboration with German specialists.

Tritrophic interactions between burrower bug, host plants and entomopathogenic nematodes (*Heterorhabditis megidis*): The entomopathogenic nematode *Heterorhabditis megidis* is attracted by cues from maize plants, but not by those of its insect host, the burrower bug *Cyrtomenus bergi*. These are the conclusions of a research project carried out by partners at Hannover University. The objectives of the study were:

1. To investigate the searching behavior of entomopathogenic nematodes (EPN) associated with *C. bergi.*
2. To study possible plant- and/or host-related cues that guide the EPN.

In preliminary experiments we recorded moderate levels of EPN-induced mortality (on average 32%) in adult *C. bergi*, confirming that *H. megidis* can successfully parasitize and kill burrower bugs. In our experiments we only used adult bugs and selected *H. megidis* due to its cruiser behavior. Maize was used as the host plant. Experiments were conducted in a sand-filled olfactometer, similar to the one used by Boff *et al*. (2001) **(Figure 8**).

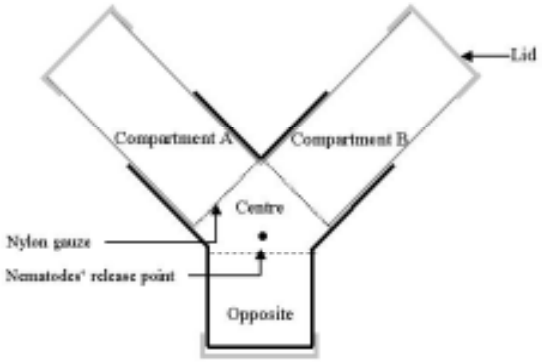


Figure 8. Sand-filled olfactometer (after Boff *et al*. 2001).

We conducted six experiments:
1. *C. bergi* vs. sand
2. Maize plants vs. sand
3. Maize plants + *C. bergi* vs. sand
4. *C. bergi* vs. maize plants
5. Maize plants + *C. bergi* vs. maize plants
6. Maize plants + *C. bergi* vs. *C. bergi*

The nematodes were significantly attracted by the maize seedlings but not by *C. bergi* adults. Interestingly the behavior of the EPN did not differ when bug-infested and non-infested plants were offered simultaneously. The EPN reacted indifferent when exposed to *C. bergi* or sand. Possible reasons for these findings are: (i) as a root-sucking insect, *C. bergi* does not produce enough cues, compared to root-chewing insects, for attraction of EPNs, and (ii) *H. megidis*, an EPN species from the Northern hemisphere, is not well adapted to *C. bergi* as a host. In ongoing experiments we are investigating the response pattern of EPN species/strains native to Colombia.

Evaluation of three native entomopathogenic nematodes against *Phyllophaga menetriesi* larvae: Third instar of larvae of *P. menetriesi* are hard for EPNs to kill. The insect's thick integument and layers of hair are probably efficient defense mechanisms against an EPN attack. Additionally, it seems that *P. menetriesi* larvae posses an efficient, yet to be identified immune response that kills EPNs after penetration. These are the main results of evaluating the virulence of three native Colombian EPN strains. The tested strains were: *Heterorhabditis* sp HNI 0100 (Cenicafé), *Heterorhabditis* sp. (CIAT) and *Steinernema* sp SNI 0198 (Cenicafé), applied at concentrations of 7,000 and 13,000 infective juveniles per ml. Penetration rate was observed after 5 and 10 days. We used *P. menetriesi* as a test species because it is one of the most harmful white grub species in Colombia. White grub larvae were collected in fields in Cauca and Risaralda. The strains from Cenicafé showed slightly higher penetration rates than the CIAT strain. Concentrations did not affect the penetration rate of all tested EPN strains. Curiously we found less EPN in the grub after 10 days than after five. Mortality was higher after 10 days in the case of HNI 0100 at both concentrations and *Heterorhabditis* (CIAT) at high concentration.

In ongoing studies we are evaluating more EPN species/strains. Moreover, younger instars of white grubs will be tested, and for this we are in the process of establishing lab colonies of several white grub species.

Why are some white grubs easier to control than others?

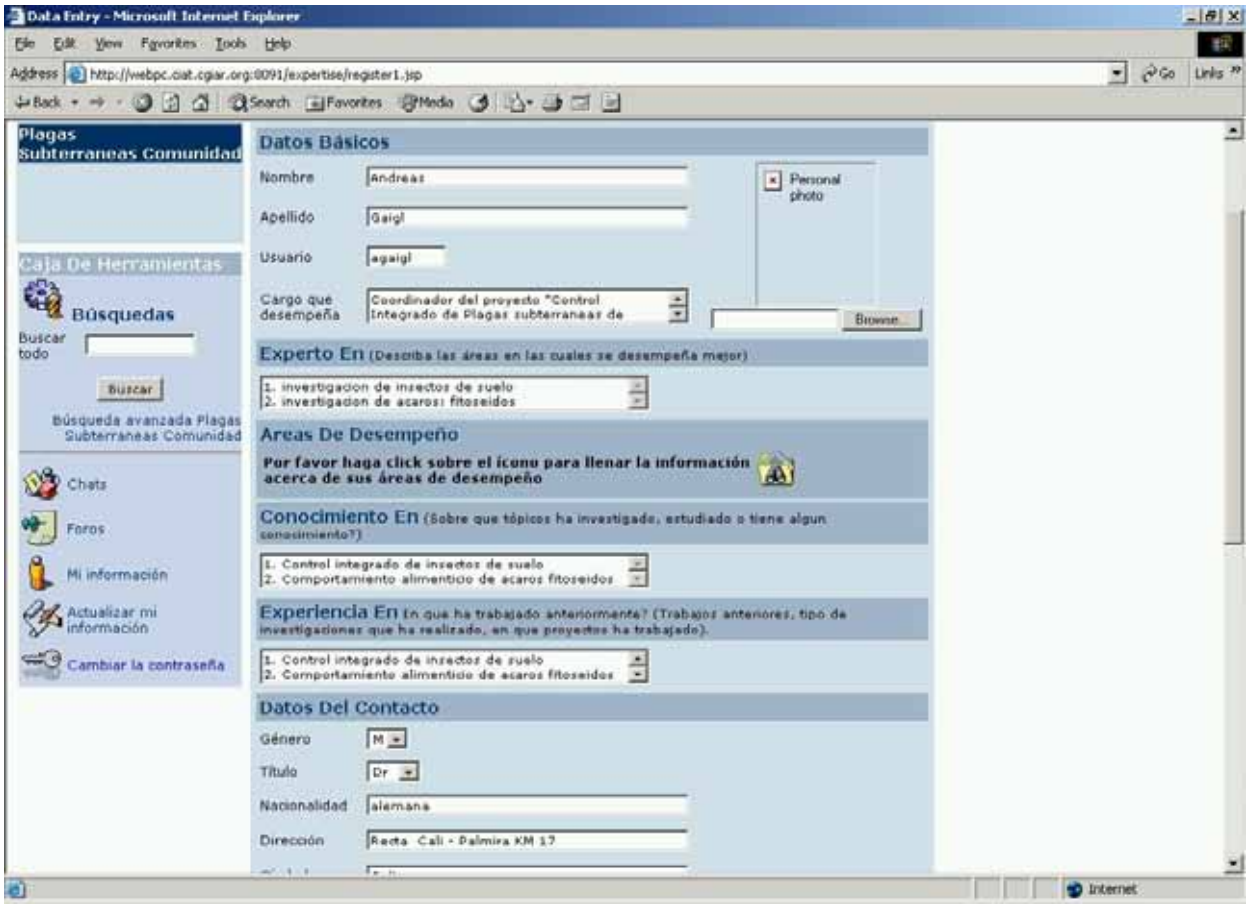
Field surveys suggested that the most susceptible targets for entomopathogens are white grubs from the subfamily Dynastinae. In contrast, Melolonthidae were less infected. The reasons for this possibly the fact that we collected much more Dynastinae than Melolonthidae larvae, thereby increasing the chance to find diseased Dynastinae. However, the are several additional factors that might be responsible for an overall reduced susceptibility of Melolonthidae to entomopathogens:

1. Physical barrier: The integument of *P. menetriesi* (Melolonthidae) is thicker than that of *Cyclocephala* (Dynastinae).
2. Resistance: We assume that *P. menetriesi* can activate an immune response that kills nematodes that have penetrated into the host.
3. Evade antagonists: We have observed during our field surveys that *Phyllophaga* spp. dwell deeper in the soil than *Cyclocephala* spp. or other Dynastinae or Rutelinae.

IV. Establishing a network of soil arthropod researchers in Latin America.

So far research on subterranean pests in Colombia as well in South America have not been properly coordinated. For this reason CIAT developed an Internet-based platform, which is easy to access, and where researchers can exchange information on soil pests, share their experience, and coordinate their activities (http://webpc.ciat.cgiar.org:8091/expertise/index.jsp).

We presented this database at the SOCOLEN congress this year. Our Internet community to date counts 35 members and we want to extend the group. For this reason the network will be presented on the Round Table of Soil Pests in Mexico in October 2003. Presently the database contains a directory of all members. However, during the next weeks we will include more data on literature and general information on subterranean pests.



Outlooks

- We will continue with the surveys in Northern Cauca and Antioquia. We decided to switch from Risaralda to Quindío where we have identified farmers who have repeatedly experienced out breaks of soil pests on their farms. They are willing to support our work by collaborating in the surveys. Moreover, next, we will conduct several participatory on-farm experiments in Quindío.
- Establishing of white grub colonies for experiments with entomopathogens.
- Testing of four fungal strains against *Cyrtomenus bergi* in the laboratory and greenhouse.
- Field experiments with commercially available entomopathogenic fungi for control of *C. bergi* (asparagus, Caldas).
- Testing intercropping practices with repellent plants against soil pests (Montelindo, Universidad de Caldas).
- Evaluation of commercial EPN products against *C. bergi* in the greenhouse.
- Evaluation of several native and exotic EPN species/strains against various white grub species in the laboratory.
- Evaluation of the economic damage (i.e. yield loss trials) of white grubs in participatory on-farm experiments (Quindío).

Theses completed or near completion

César A. Zuluaga (U. Nacional/Bogotá): Recognition of white grubs, *Cyrtomenus bergi* and their natural enemies (Cundinamarca). Completed.

Maria Paulina Quintero (Univalle): Pathogenicity of entomopathogenic nematodes in *Phyllophaga menetriesi.* Completed by the end of 2003.

Lina María Serna: (U. de Caldas): Recognition of white grubs, *Cyrtomenus bergi* and their natural enemies in Pereira. Completed by the end of 2003.

Nelly Villegas (U. de Caldas): Recognition of white grubs, *Cyrtomenus bergi* and their natural enemies in La Florida, Risaralda. Completed by the end of this year. Completed by the end of 2003

Lisa Struck (Hannover University): Tritrophic interactions between burrower bug, host plants and entomopathogenic nematodes. Completed.

Thesis underway

Germán Andrés Calverto (Universidad Autonoma): Establishment of rearing methods of whitegrubs.

Juliana Jaramillo (M.Sc., Hanover): Pathogenicity of entomopathogenic fungi against *Cyrtomenus bergi* and white grubs in semi-controlled experiments.

Ana M. Caicedo (M.Sc., Hanover): Pathogenicity of entomopathogenic nematodes against *Cyrtomenus bergi* in semi-controlled experiments (initiated in October 2003).

Anuar Morales (M.Sc., Cornell Univ.) Use of pheromones for the study of soil pests.

References

CORPOICA (Colombian Corporation of Agricultural Research). 1996. Estudios sobre biodiversidad, biología y manejo integrado de las plagas rizófagas del "complejo chisas" en Colombia. Project proposal.

KING, A.B.S. 1984. Biology and identification of white grubs (Phyllophaga) of economic importance in Central America. *Tropical Pest Management* 30: 36-50.

LONDOÑO, M. 1994. Informe Realizaciones (Corpoica). *Investigación y Desarrollo Tecnológico*. Corpoica.

LONDOÑO, M. and M. PEREZ. 1994. Reconocimiento de los enemigos naturales de la chisa o mojojoy (Coleoptera: Scarabaeidae) en el oriente antioqueño. *Rev. Colomb. Entomol*. Vol. 20: 199-206.

MERVYN, R. 1998. Pesticides - friend or foe? *Water Science & Technology* 31: 19-25.

PARDO, L.C. 1994. Escarabajos (Col.: Melolonthidae) de importancia agrícola en Colombia, pp 159-176. In: Memories of the 21st congress of the Colombian Society of Entomology (SOCOLEN), Medellin, Colombia.

POSADA, F.J. 1993. Las chisas, sus enemigos naturales y recomendaciones sobre su manejo. *Agricultura Tropical* 30: 71-79.

SHANNON, P and M. CARBALLO. 1996. Editors. *Biología y control de Phyllophaga spp. Seminario-Taller Centroamericano sobre la biología y control de Phyllophaga spp*. 23-27

de mayo de 1994, CATIE, Turrialba, Costa Rica. CATIE. Serie Técnica. Informe Técnico No. 227.

VALLEJO, F., M.-A. MORÓN, S. ORDUZ, 1998 First report and description of immature stages of *Phyllophaga obsoleta* (Blanchard) (Coleoptera: Melolonthidae) in Colombia. *The Coleopterist*. 52: 109-117.

VALLEJO, F., M.A. MORON, S. ORDUZ, 2000 Avances en el estudio morfológico del complejo chisa (Coleoptera: Melolonthidae) de Colombia XXVII Congreso de Socolen. Socolen, Medellin. pp. 307-327.

Contributors: Andreas Gaigl, Germán Andrés Calberto, Carlos Julio Herrera, Martha Londoño (Corpoica Rionegro), Elsa Melo, Anuar Morales, Luis Carlos Pardo, María Paulina Quintero, Lina María Serna (CIAT/Universidad de Caldas), Lisa Struck (Hannover University), Nelly Villegas (CIAT/Universidad de Caldas), Cesar Zuluaga (CIAT/Universidad Nacional de Bogotá).

Participants:
CIAT: Anthony Bellotti, Ana Milena Caicedo, Sonja Ximena Restrepo, Catalina Rodríguez, Rosalba Tobón, Oscar Yela, Rodrigo Zúñiga.
Corpoica: Elizabeth Meneses, Diana Acevedo.
Universidad de Caldas: Fernando Vallejo, Alberto Soto.
Universidad Nacional de Bogotá: Miguel Santiago Serrano.
Hannover University (Germany): Dr. Christian Borgemeister.
Federal Biological Research Centre for Agriculture and Forestry (BBA, Germany): Dr. Regina Kleespies, Dr. Gisbert Zimmermann.
Kiel University (Germany): Dr. Ralf-Udo Ehlers.

BEAN ENTOMOLOGY

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

Screening for sources of resistance to major insect pests

Introduction

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: *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. Bruchid nurseries are tested in the laboratory simulating normal storage conditions (20^{o} 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*.

Results and Discussion: In 2002 we finished studies on the mechanisms of resistance to *T. palmi*. Main results of this work can be summarized as follows. Antixenosis and antibiosis were studied under laboratory and field conditions. Comparisons were made between a susceptible genotype (APN 18) and four moderately resistant genotypes ('Brunca', BH-130, EMP 486, FEB 115). In multiple-choice tests, antixenosis was identified in FEB 115. The antixenotic nature of FEB 115 was confirmed in dual-choice tests under field and laboratory conditions. Life table studies **(Table 1)** showed significant differences in egg duration, survivorship of adults and immature stages, female longevity, daily oviposition rates, and total fecundity among bean genotypes, meaning that antibiosis does play a role in the resistance of beans to *T. palmi*. Based on the most important demographic parameter, the intrinsic rate of natural increase, the five bean genotypes were divided into two groups: BH-130 and 'Brunca' were less favorable for the population growth of the thrips than EMP 486 and FEB 115. FEB 115 was not antibiotic. Population growth on this genotype did not differ from that on APN 18, the susceptible check.

We also finished studies on tolerance as a mechanism of resistance to *T. palmi*. As shown in **Table 2**, resistant genotypes suffered significantly lower pod and yield losses than susceptible ones. Consistent results from both field and greenhouse tests allowed us to conclude that tolerance is indeed a mechanism of resistance to *T. palmi*. In the particular case of FEB 115, tolerance is combined with antixenosis whereas antibiosis and tolerance seem to be the mechanisms responsible for resistance in Brunca, EMP 486, and BH-130.

Table 1. Demographic parameters for *Thrips palmi* reared on five bean genotypes.

Genotype	N	Net Reproductive Rate (R_0)	Generation Time (Days)	Intrinsic Rate of Natural Increase (r_m)	Doubling Time (Days)
EMP 486	74	32.6 ± 1.9c	37.8 ± 0.1c	0.092 ± 0.001a	7.5 ± 0.12b
BH-130	70	46.1 ± 2.0a	44.8 ± 0.7a	0.085 ± 0.002b	8.1 ± 0.23a
APN 18	60	46.0 ± 1.3a	41.2 ± 1.1b	0.093 ± 0.002a	7.5 ± 0.19b
Brunca	72	29.9 ± 2.7c	40.2 ± 1.6bc	0.084 ± 0.003b	8.2 ± 0.12a
FEB 115	48	39.1 ± 0.8b	39.9 ± 0.7bc	0.092 ± 0.002a	7.5 ± 0.16b

[a] For each parameter, differences among bean genotypes were determined by SNK sequential tests, based on jackknife estimates of variance for each parameter. Means (± SEM) within a column followed by the same letter are not significantly different at the 5% level.

Table 2. Percentage losses caused by *Thrips palmi* on eight bean genotypes.

Genotype	Percentage Empty Pods	Percentage Pod Losses	Percentage Yield Reduction
Brunca (R)	13.7b	21.8ab	38.5bcd
EMP 486 (R)	7.3b	5.0b	33.9bcd
FEB 115 (R)	17.0b	16.9ab	18.8d
BH-5 (I)	14.8b	20.0ab	27.9cd
BH-60 (R)	15.7b	19.9ab	34.0bcd
EMP 514 (S)	10.7b	23.1a	50.0ab
BAT 477 (S)	17.6b	23.8a	46.4abc
APN 18 (S)	50.0a	23.8a	65.9a

[a] R, resistant; I, intermediate; S, susceptible. Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's PLSD).

Work on the identification of molecular markers for thrips resistance was also terminated. For details, please see the SB-2 report.

Contributors: A. Frei, J. M. Bueno, C. Cardona, M. Blair (CIAT), H. Gu, S. Dorn (ETH University, Zurich).

Bruchids:

Acanthoscelides obtectus

As indicated in our 2002 Annual Report, there is need to develop fertile *P. vulgaris* x *P. acutifolius* (common x tepary) bean hybrids using the tepary genotype NI576 (a genotype competent to *Agrobacterium*-mediated genetic transformation). Using a novel Double Congruity Backcross technique developed at CIAT, the Biotechnology Unit has been able to produce fertile interspecific hybrids involving NI576. Some of these crosses involve the tepary accession G 40199, an excellent source of resistance to the bean weevil, *Acanthoscelides obtectus*. In 2002 we identified several progenies containing both *P. vulgaris* and *P. acutifolius* cytoplasm with very high levels of antibiosis resistance to *A. obtectus*. Individual pre-selected seeds were reconfirmed in 2003 with excellent results **(Table 3)**. Six of the progenies tested showed an absolute level of antibiosis (zero adult emergence), comparable to that in G 25042, a *P. lunatus* accession known to be one of the most resistant genotypes ever tested for resistance to the bean weevil. Resistance in these progenies compared favorably with resistance in the *P. acutifolius*

resistant parent, G 40199. After multiplication of seeds in the greenhouse, further testing for resistance is in progress.

Table 3. Reconfirmation of resistance to *Acanthoscelides obtectus* in pre-selected segregating progenies derived from interspecific *Phaseolus vulgaris* x *Phaseolus acutifolius* crosses.

Code and Generation	Cross	No. of Seeds Tested[a]	Mean Percentage Emergence	Days to Adult Emergence
Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. vulgaris* cytoplasm				
T7 K2 F_3	V-DCBC5 x V-DCBC4	3	0.0	-
Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm				
GNVAV-2 F_4	{[(G40022 x N1576) x V5] x A3}x VS42-7	10	0.0	-
GVV-1 F_3	{[(G40022 x N1576) x V5] x A3}x VS42-7	14	0.0	-
GKX-6 F_2	A-DCBC8-2	3	0.0	-
GKA-12 F_2	A-DCBC7-2 x A6	14	0.0	-
ZXX-5 F_2	A-DCBC8-3	2	0.0	-
Checks				
G 40199	Resistant *P. acutifolius* accession	26	11.2	54.0
G 40168	Susceptible *P. acutifolius* accession	22	90.1	45.7
G 25042	Resistant *P. lunatus* accession	21	0.0	-
G 25410	Susceptible *P. lunatus* accession	20	95.0	43.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	30	95.5	32.8

[a] Pre-selected seeds were tested individually using the vial technique.

We also tested 68 F_2 and F_3 progenies derived from several different interspecifc crosses as well as intraspecific *P. acutifolius* and *P. lunatus* crosses. Seeds were tested in bulk with three repetitions per entry at a level of infestation of 2-3 larvae per seed depending on seed size. Most were susceptible. However, as shown in **Table 4**, five were selected for showing acceptable levels of resistance. Selected individual seeds were multiplied and the resulting seed will be evaluated in bulk in replicated nurseries.

Table 4. Resistance to *Acanthoscelides obtectus* in selected $F_{2,3}$ progenies derived from inter and intraspecific crosses evaluated in 2003.

Code and Generation	Type of Cross	Percentage Adult Emergence	Days to Adult Emergence
GNVA21 F_3	Intraspecific *P. acutifolius* x intraspecific hybrid *P. lunatus* x intraspecific hybrid *P. lunatus*	45.5	40.7
GKA11 F_2	Double congruent hybrid with *P. acutifolius* cytoplasm	34.3	53.5
Z99ZX6 F_2	Double congruent hybrid with *P. acutifolius* cytoplasm	34.6	58.5
V5 F_2	Intraspecific *P. lunatus* hybrid	0.0	-
VS42-7 F_2	Intraspecific *P. lunatus* hybrid	34.5	67.3
Checks			
G 40199	Resistant *P. acutifolius* accession	18.6	55.7
G 40168	Susceptible *P. acutifolius* accession	86.4	41.5
G 25042	Resistant *P. lunatus* accession	6.7	69.0
G 25410	Susceptible *P. lunatus* accession	97.8	42.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	91.1	31.4

Another set of 55 F_{2-4} progenies received from the Biotechnology Unit was tested in replicated tests for resistance to *A. obtectus* in 2003. Six double congruent hybrids with *P. acutifolius* cytoplasm and two progenies derived from intraspecific *P. lunatus* crosses showed resistance ranging from high (< 20% adult emergence) to intermediate (20-50% adult emergence) **(Table 5)**. Resistance was also expressed in terms of prolonged life cycles (up to 84 days after infestation). Reconfirmation using the individual seed testing technique is in progress.

Table 5. Resistance to *Acanthoscelides obtectus* in selected segregating progenies derived from interspecific *Phaseolus vulgaris* x *Phaseolus acutifolius* hybrids and intraspecific *Phaseolus lunatus* crosses evaluated in 2003.

Code and Generation	Type of Cross	Percentage Adult Emergence	Days to Adult Emergence
Z99ZX-1A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	18.7	74.2
Z99ZX-11A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	34.9	55.7
Z99ZX-15-2 F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	36.6	59.9
ZXTG31-4-10 F_4	Double congruent hybrid with *P. acutifolius* cytoplasm	8.3	65.0
ZXTG33-3 F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	42.1	58.2
GKVGAG-1A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	0.8	44.0
A6 F_2	Intraspecific *P. lunatus* hybrid	26.8	64.7
VS42-14 F_2	Intraspecific *P. lunatus* hybrid	5.6	84.0
	Checks		
G 40199	Resistant *P. acutifolius* accession	7.2	68.8
G 40168	Susceptible *P. acutifolius* accession	83.2	43.4
G 25042	Resistant *P. lunatus* accession	0.6	78.0
G 25410	Susceptible *P. lunatus* accession	90.0	43.8
ICA Pijao	Susceptible *P. vulgaris* cultivar	91.1	31.4

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

Zabrotes subfasciatus

The work on the development of a molecular marker for arcelin presence and resistance to the Mexican bean weevil was terminated in 2003. In 2002, single and multiple crosses using RAZ 44 and RAZ 105 as arcelin-donor parents were made. Resulting F_5 and F_6 were used to develop microsatellites (details in SB-2 Report). The resulting 854 progenies from 29 different crosses were tested for resistance to *Z. subfasciatus* (results in the 2002 Bean Entomology Report). In 2003, we reconfirmed levels of resistance of the best progenies in replicated nurseries in the laboratory. Results **(Table 6)** confirmed previous results, with 55 of the highly resistant lines showing very high levels of resistance to the Mexican bean weevil. There was absolute correspondence between the presence of arcelin and resistance to the insect.

As part of the on-going collaborative project with the University of Ghent, we tested 11 arc-5 and six arc-1 homozygous transgenic *P. acutifolius* lines. All were very susceptible to *Z. subfasciatus* in replicated tests **(Table 7)**, possibly because the arcelin gene is not expressing itself.

Table 6. Levels of resistance to *Zabrotes subfasciatus* in selected bean lines used to develop a DNA-based molecular marker for the presence of arcelin.

Previous Rating	No. of Lines Tested	Arcelin presence	Percentage of adult emergence	Days to adult emergence	Percentage seeds damaged
Highly resistant	55	Arc +	7.4 ± 0.18d	49.9 ± 0.19b	27.1 ± 0.8c
Resistant	5	Arc +	15.5 ± 0.9c	47.2 ± 0.36c	64.1 ± 3.9b
Susceptible	2	Arc –	94.2 ± 0.7b	36.8 ± 0.15d	100 ± 0.0a
RAZ 44[a]		Arc +	4.6 ± 0.9e	52.1 ± 0.81a	15.9 ± 2.1d
ICA Pijao[b]		Arc –	97.9 ± 0.16a	37.2 ± 0.09d	100 ± 0.0a

[a] Standard improved resistant check; [b] Standard susceptible check.

Means (± SEM) of 4 replications per genotype. Means within a column followed by the same letter are not significantly different at the 5% level by LSD.

Table 7. Effect of selected bean genotypes on the biology of *Zabrotes subfasciatus.*

Genotype	Days to Adult Emergence[a]	Percentage of Emergence[b]	Percentage Seeds Damaged[c]
$5a^+$ 01-01	33.7 ± 0.11hi	92.9 ± 1.4a	100.0 ± 0.0a
$5a^+$ 01-04	34.3 ± 0.51hi	72.9 ± 3.9c	78.1 ± 5.7c
$5a^-$ negative control	32.4 ± 0.18I	97.9 ± 0.7a	100.0 ± 0.0a
$5bc^+$ 176-10	37.3 ± 0.19e	96.4 ± 0.5a	100.0 ± 0.0a
$5bc^-$ 176-10	33.7 ± 0.41hi	95.9 ± 2.1a	100.0 ± 0.0a
$5bc^+$ 176-15	35.1 ± 0.19fg	96.6 ± 1.4a	100.0 ± 0.0a
$5bc^-$ 176-15	34.4 ± 0.37hi	97.3 ± 1.5a	100.0 ± 0.0a
$5bc^+$ 186-PI-2R	39.7 ± 0.23d	95.9 ± 1.4a	100.0 ± 0.0a
$5bc^-$ 186-PI-2R	34.0 ± 0.17hi	97.9 ± 1.1a	100.0 ± 0.0a
$5bc^+$ 188-PI-2R	38.8 ± 0.23de	95.8 ± 1.6a	100.0 ± 0.0a
$5bc^-$ 188-PI-2R	34.7 ± 0.19h	95.2 ± 1.3a	100.0 ± 0.0a
$Arc1^+$ 182-11	34.1 ± 0.19hi	95.1 ± 1.5a	100.0 ± 0.0a
$Arc1^-$ 182-11	32.4 ± 0.09i	94.8 ± 0.8a	100.0 ± 0.0a
$Arc1^+$ 182-6	37.1 ± 0.28ef	82.5 ± 1.6b	100.0 ± 0.0a
$Arc1^-$ 182-6	33.8 ± 0.21hi	95.4 ± 1.6a	100.0 ± 0.0a
$Arc1^+$ 186-TR	40.4 ± 0.26d	66.0 ± 4.5d	100.0 ± 0.0a
$Arc1^-$ 186-TR	38.4 ± 0.44de	82.5 ± 6.8b	91.8 ± 1.6b
TB1 wild type	33.2 ± 0.19hi	97.5 ± 0.3a	100.0 ± 0.0a
PI440795	34.2 ± 0.18hi	96.9 ± 0.4a	100.0 ± 0.0a
EMP 175	33.0 ± 0.22hi	97.0 ± 1.2a	100.0 ± 0.0a
RAZ 2	51.7 ± 1.35c	9.8 ± 3.2e	30.5 ± 7.6e
Ica Pijao 'Ghent'	33.5 ± 0.15hi	97.1 ± 0.7a	100.0 ± 0.0a
RAZ 44	50.8 ± 1.48c	6.4 ± 0.8ef	32.1 ± 5.1e
G 12882 Arc 1	51.1 ± 0.94c	7.5 ± 1.4e	26.2 ± 5.7e
G 02771 Arc 5	56.4 ± 3.79b	1.1 ± 0.3f	4.2 ± 1.3f
G 12952 Arc 4	59.5 ± 0.55a	10.8 ± 1.5e	32.1 ± 4.6e
RAZ 136	50.3 ± 0.66c	5.4 ± 0.7ef	29.7 ± 3.3e
ICA Pijao 'CIAT'	33.3 ± 0.08hi	97.6 ± 0.3a	100.0 ± 0.0a

[a] Means ± SEM of 5 replications per genotype. Means within a column followed by the same letter are not significantly different by LSD. ANOVA testing for differences among genotypes: F = 113.2; df = 27, 107; P < 0.001.

[b] Means ± SEM of 5 replications per genotype. Means within a column followed by the same letter are not significantly different by LSD. ANOVA testing for differences among genotypes: F = 121.3; df = 27, 108; P < 0.001 on data transformed to arcsine square root of proportion.

[c] Means ± SEM of 5 replications per genotype. Means within a column followed by the same letter are not significantly different by LSD. ANOVA testing for differences among genotypes: F = 193.7; df = 27, 108; P < 0.001 on data transformed to arcsine square root of proportion.

Contributors: S. Prieto, J. Valor, C. Cardona, M. Blair.

Leafhopper (*Empoasca kraemeri*)

In 2003 we screened a total of 867 bean germplasm accessions for resistance to the leafhopper. Those selected in 2002 (42) 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 111 individual plant selections in Andean crosses performed with EMP 250 as a parent. Eighteen were selected for yield testing in 2004. Other tests included progeny row testing of 147 F_3 selections made among 19 populations developed for resistance to leafhopper and BGMV. Selections in Andean types also included 33 $F_{6,}$ which were tested in 2003. Of these, 16 lines are being yield-tested at present.

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 received and tested 21 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 **Table 8**. Seven were rated as intermediate, one as resistant. None was as resistant as the resistant parents. Further testing is in progress.

Table 8. Resistance to *Empoasca kraemeri* in selected F_2 and F_3 progenies derived from interespecific *Phaseolus vulgaris* x *Phaseolus acutifolius* crosses.

Code	Pedigree[a]	Mean Damage Score	Rating
A19Y-103 F_3	V-DCBC x (G40019 x A-DCBC)	6.7	Intermediate
A36Y-42 F_3	V-DCBC x (G40036 x A-DCBC)	6.0	Resistant
A99Y-86 F_2	V-DCBC x (G40199 x A-DCBC)	7.0	Intermediate
G36NGP-3 F_2	G 40036 x A-DCBC	7.0	Intermediate
KKQ-11 F_3	V-DCBC x V-DCBC	7.0	Intermediate
A99Y-15 F_2	V-DCBC x (G40199 X A-DCBC)	6.5	Intermediate
G19NGP-3 F_2	G40019 x A-DCBC	7.0	Intermediate
G36NGP-9 F_2	G40036 x A-DCBC	7.0	Intermediate
	Checks		
G 40019	Resistant P. acutifolius accession	5.0	Resistant
G 40036	Resistant P. acutifolius accession	4.5	Resistant
G 40119	Resistant P. acutifolius accession	5	Resistant
G 40016	Susceptible P. acutifolius accession	9	Susceptible
G 40056	Susceptible P. acutifolius accession	9	Susceptible
EMP 250	Resistant EMP line	5.5	Resistant
BAT 41	Standard susceptible P. vulgaris check	9	Susceptible
ICA Pijao	Standard resistant P. vulgaris check	6.5	Resistant

[a] V-DCBC, double congruent hybrid with P. vulgaris cytoplasm; A-DCBC, double congruent hybrid with P. acutifolius cytoplasm.

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. 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 heavy (up to 7 nymphs per leaf, seasonal average) leafhopper infestation **(Table 9)**. Another set of lines derived from crosses between EMP 250 and PVA 773 or CAL 143 also performed well, certainly better than the very susceptible preferred Andean parents **(Figure 1)**. 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.

Table 9. Leafhopper resistance in selected Andean bean lines.

Line	Damage scores 50 DAP[a]	Nymphs per leaf 50 DAP	Yield (kg/ha)		Percentage loss	Susceptibility index
			Unprotected	Protected		
Lines derived from the EMP 250 x PVA 773 cross						
4-1-11	5.2	3.7	1260	2102	39.9	0.81
4-11-8	5.3	2.4	522	1715	69.0	1.21
4-11-5	5.4	4.9	973	2327	58.1	0.97
4-11-14	5.3	3.0	854	2980	71.3	1.02
4-12-9	5.7	6.8	733	2168	65.9	1.10
4-12-10	5.7	4.7	708	1378	48.5	1.12
4-17-18	5.6	2.9	909	2039	55.3	0.99
4-18-1	5.3	2.8	989	2089	52.8	0.95
4-18-5	5.1	3.3	947	1668	43.2	0.96
Lines derived from the EMP 250 x CAL 143 cross						
5-9-1	5.2	2.8	750	1930	60.9	1.10
5-14-4	5.4	5.0	645	2167	70.2	1.12
5-25-3	5.0	3.5	894	2356	61.9	1.00
5-25-6	5.4	4.7	927	2194	57.9	0.99
5-26-9	5.4	3.8	889	2041	56.3	1.00
			Checks[b]			
CAL143 (S)	6.0	1.0	370	2215	83.3	1.24
PVA773 (S)	5.9	1.4	379	2028	81.1	1.26
EMP250 (R)	5.1	3.0	1486	2847	47.6	0.79
EMP228 (R)	5.6	3.7	1123	1889	40.3	0.86
PIJAO (T)	5.0	5.6	1483	2473	40.0	0.76
LSD 5%	0.25	0.52	290.6	491.8		

[a] DAP, days after planting; [b] S, susceptible, T, tolerant, R, resistant.

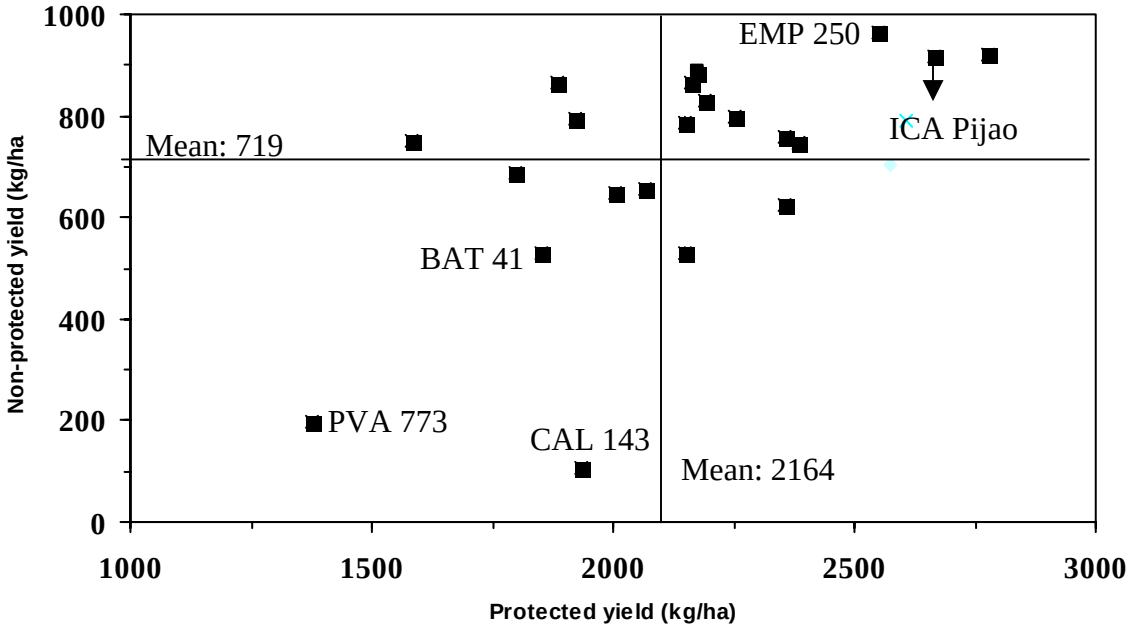


Figure 1. The relationship between protected and non-protected yield in selected Andean bean lines bred for tolerance to leafhopper. PVA 773 and CAL 143 are susceptible parents. EMP 250 is the tolerant parent. BAT 41 and Pijao (both Mesoamerican) are susceptible, and tolerant checks, respectively.

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

Tolerance to leafhopper studies

Studies aimed at measuring progress in incorporating tolerance to leafhopper continued in 2003. On this occasion we measured the response of selected EMP lines (bred for leafhopper resistance) to two levels of infestation (3 and 6 nymphs per leaf) obtained by exercising chemical control at pre-established action levels. In terms of percentage yield losses, new bred lines (the EMP 500 series) performed better at all levels of infestation than the improved checks EMP 124 and EMP 250, and better than the standard tolerant check, ICA Piajo **(Figure 2)**. 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.

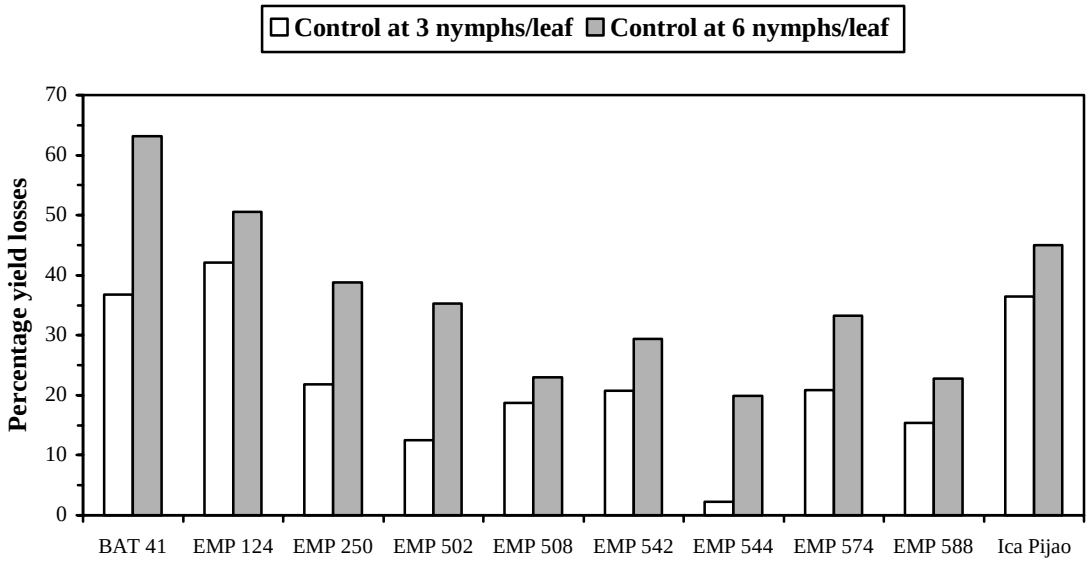


Figure 2. Yield losses in selected EMP lines and checks (BAT 41, ICA Pijao) at two levels of infestation with the leafhopper *Empoasca kraemeri.*

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

BSc, MSc or PhD candidates supervised in 2003

Juan Miguel Bueno (National University), M.Sc. Thesis on Sampling methods for whiteflies.
Maria Fernanda Montenegro (National University), B. Sc. Thesis on Management of whiteflies.
Sergio Prieto (National University), B. Sc. Thesis on resistance to Mexican bean weevil.
Andrea Frei (ETH University, Switzerland), Ph.D. Thesis on resistance to *Thrips palmi* (terminated*).*

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.

Publications

Manzano, M. R., J. C. van Lenteren, and C. Cardona. 2002. Searching and oviposition behavior of *Amitus fuscipennis*, a parasitoid of the greenhouse whitefly. J. Appl. Ent. 126: 528-533.
Manzano, M. R., J. C. van Lenteren, and C. Cardona. 2003. Influence of pesticide treatments on the dynamics of whiteflies (Homoptera: Aleyrodidae) and associated parasitoids in snap bean fields. Biocontrol 48: 1-9.

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

Frei, A., J. M. Bueno, J. Díaz, H. Gu, C. Cardona, and S. Dorn. 2003. Tolerance as a mechanism of resistance to *Thrips palmi* in common beans. Ent. Exp. et Appl. (submitted July, 2003)

Frei, A., M. W. Blair, C. Cardona, S. E. Beebe, H. Gu, and S. Dorn. 2003. Identification of quantitative trait loci for resistance to *Thrips palmi* Karny in common beans (*Phaseolus vulgaris* L.). Crop Science (submitted September 2003).

Schmale, I., F. L. Wackers, C. Cardona, and S. Dorn. 2003. Combining parasitoids and plant resistance for the control of the bruchid *Acanthoscelides obtectus* in stored beans. J. Stored Products Res. 39: 401-411.

Rodríguez, I.; Morales, H.; Cardona, C.2003. Líneas base, dosis diagnóstico y medición periódica de resistencia a insecticidas en poblaciones de adultos e inmaduros de *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) en el Valle del Cauca. Rev. Col. Entomol. 29(1): 21-27.

Rodríguez, I.; Durán, I.; Morales, H.; Cardona, C. 2003. "Líneas base, dosis diagnóstico y medición periódica de resistencia a spinosad y carbosulfan en poblaciones de adultos de *Thrips palmi* (Thysanoptera: Thripidae) en el Valle del Cauca. Rev. Col. Entomol. 29(1): 29-34.

Díaz, J.; Cardona, C.; Bueno, J. 2003. Caracterización de genotipos de fríjol (*Phaseolus vulgaris*) por su resistencia a *Thrips palmi* (Thysanoptera: Thripidae). Rev. Col. Entomol. 29(1): 35-42.

Osorio, J.; Cardona, C. 2003. Fenología, fluctuación de poblaciones y métodos de muestreo para *Thrips palmi* (Thysanoptera: Thripidae) en habichuela y fríjol. En: Rev. Col. Ent. 29 (1) : 43-50.

Bueno, J.; Cardona, C. 2003. Umbral de acción para *Thrips palmi* (Thysanoptera: Thripidae) en habichuela en el Valle del Cauca, Colombia. En: Rev. Col. Ent. 29 (1) : 51-56.

Workshops and Conferences

Rodríguez, I.; Morales, H.; Bueno, J.; Cardona, C. 2003. El biotipo B de *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) adquiere mayor importancia en el Valle del Cauca. En: Resúmenes XXX Congreso SOCOLEN Cali, 17-19 Julio de 2003. Universidad Autónoma de Occidente, Cali Colombia. p.82.

Tapia, X.; Proaño, M.; Cardona, C.; Rodríguez, I.; Poats, S. 2003. Alternativas para el manejo de la mosca blanca *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) en fríjol en las comunidades del Valle del Chota, Ecuador. En: Resúmenes XXX Congreso SOCOLEN Cali, 17-19 Julio de 2003. Universidad Autónoma de Occidente, Cali Colombia. p.83.

Bueno, J.; Rodríguez, I.; Cardona, C. 2003. Alternativas para el manejo de la mosca blanca *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) en habichuela en el Valle del Cauca. . En: Resúmenes XXX Congreso SOCOLEN Cali, 17-19 Julio de 2003. Universidad Autónoma de Occidente, Cali Colombia. p.82.

Prieto, S.; Cardona, C.; Blair, M.; Muñoz, J. 2003. Marcadores moleculares para selección por resistencia al gorgojo pintado del fríjol, *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae). En: Resúmenes XXX Congreso SOCOLEN Cali, 17-19 Julio de 2003. Universidad Autónoma de Occidente, Cali Colombia. p.13.

AFRICA: BEAN ENTOMOLOGY

Activity 1. Verification of farmers' indigenous knowledge on the influence of *Ootheca* larval feeding on bean root development in northern Tanzania.

Introduction

The bean foliage beetle, *Ootheca* spp. (Coleoptera: Chrysomelidae) is a major insect pest of beans (*Phaseolus vulgaris*) in northern Tanzania. Adult beetles feed on foliage at seedling stage causing extensive defoliation. Eggs are laid in the soil and the larvae feed on roots of beans and other legumes where they cause stunted growth and premature senescence. The yield loss ranging from 18-30% reported by Karel and Rweyemamu (1984) to be due to adult *Ootheca* on beans is an underestimate considering the total damage caused by the adults and larvae on the bean plant in the same season.

Farmers in Hai and Arumeru districts in northern Tanzania, experienced substantial bean crop losses during the 2002 growing season. Some farmers observed that bean plants were stunted, some senesced pre-matured and the number of pods per plant and seeds per pod were relatively lower in fields that were infested by *Ootheca* adults at seedling stage compared to fields that had low pest numbers. These observations prompted for investigation into the effect of *Ootheca* larvae on root development.

Materials and Methods: Adult *Ootheca* mating pairs were collected from farmers' fields in Hai district and reared in the screenhouse at Selian Agricultural Research Institute (SARI), Arusha. Single pairs were each caged in Petri dishes (9 cm diameter) filled with 15 gm loose sterilised forest soil. The loose soil served as the oviposition substrate. Two tender and fresh bean leaflets were securely placed in each dish on daily basis to provide food for the adult beetles. Oviposition was monitored and all eggs collected daily using a soft camel hair brush. The eggs were incubated at room temperature (24-27^{o}C).

Two pre-germinated bean seeds were planted in perforated plastic pots (15cm bottom diameter, 20cm high, 20cm top diameter) filled with sterilised forest soil. There were 160 pots for 4 treatments (0, 20, 40, 80 larvae per pot) that were split into two sets. Each treatment had 20 replicates. One set of pots was infested with first instar *Ootheca* larvae and the second set with second instar larvae. In the first set, newly emerged larvae (neonates) were removed from the dishes and placed in sterilised forest soil with potted bean plants at the rate of 0, 20, 40 and 80 larvae per pot. With the second set, the neonates were removed from the dishes and placed in sterilised soil with potted bean plants where they were reared to the second instar.

The first and second instar larvae were introduced into potted bean plant roots at growth stage V4 (when beans have the fourth trifoliate leaf). At the end of third instar larval stage when beans were at R7 developmental stage (plants show first pods), 10 pots were randomly drawn from each treatment. The soil was carefully removed from the roots and observations were made on the nature of larval damage. The plants were thoroughly washed with water and all roots cut at soil surface level. The clean roots were dried in the oven (at 60^{o}C overnight) and weighed to

determine root biomass dry weight. A correlation analysis was carried out between larval numbers and root damage levels.

Results and Discussion: Observations on *Ootheca* larval feeding behaviour showed that the first instars prefer to feed and bore into root nodules while the second and third instars feed by chewing up root nodules and the epidermal tissue on lateral roots. The results also showed that second instars were more damaging than first instars. However, both stages caused significant (P= 0.01) reductions in root biomass weight compared to the control. As expected, increase in the number of larvae per pot resulted in higher root damage and hence biomass loss **(Table 1)**. Correlation analysis confirmed that root damage was positively correlated to the number of larvae per pot. Although this experiment was not extended to cover the third larval stage, it is likely that third instar larvae would cause higher damage than the second instars. These results on root damage can help to explain farmers' observations in their own bean fields.

Table 1. Bean root biomass dry weight loss (%) due to *Ootheca* spp. larval feeding at Arusha, Tanzania, 2003.

Infestation level (Number of larvae per pot)	% root biomass dry weight loss due to:	
	1st larval stage	2nd larval stage
0	0.00(0.00)*	0.00(0.00)
20	9.00(1.40)	39.58(1.5)
40	32.83(1.50)	69.89(1.7)
80	43.80(1.58)	73.23(1.8)
Mean	28.97(1.12)	45.68(1.29)
LSD (0.05)	16.03(0.24)	20.02(0.22)
CV(%)	60.29(23.26)	47.77(18.87)
Correlation coefficient	+0.602	+0.781

* Figures in brackets are transformed data $(X+3/8)^{0.5}$

Reference

Karel, A.K. and Rwenyemamu, C.L. 1984. Yield losses in field bean following foliar damage by Ootheca bennigseni (Coleoptera: Chrysomelidae). Journal of Economic Entomology 77: 761-765.

Contributors: EM Minja, JKO Ampofo and HA Mziray.

Activity 2. Scale up and scaling out proven technologies through strategic alliances.

Achievements:

- Increasing farmers' knowledge about the biology and ecology of the key pests affecting their bean crops has enabled them to develop appropriate management practices
- Farming communities have been empowered to make IPM decisions with focus on indigenous knowledge systems, and to rediscover value in their traditional IPM strategies
- Individual farmers have gained confidence and have been enabled to disseminate IPM information through their participation in regional workshops
- Women farmers play key roles in the leadership of community groups and community based organisations

Scaling up participatory IPM development and promotion

Introduction

Many technologies have been developed for the management of pest problems in smallholder production systems but most have remained out of reach. Community participatory approaches, combining farmer field school and participatory approaches, are needed to increase farmer awareness of the availability of IPM technology and encourage adaptation, and to develop skills in national research and extension services.

Methods: The project on, "Participatory IPM development and promotion in Eastern and Southern Africa " supported by the DFID Crop Protection Programme, aims to scale up and scale out the approach developed by CIAT in northern Tanzania to Kenya, Tanzania and Malawi. The ECABREN and SABRN Networks have also linked their IPM subprojects to this activity and are funding the extension of the project to Democratic Republic of Congo, Madagascar, Mozambique and Sudan. Collaborative links for dissemination have been developed with the Ministry of Agriculture in Tanzania, African Highlands Initiative (AHI) at Lushoto, KARI and the Ministry of Agriculture in Kenya, and several NGOs including Farm Africa at Babati, World Vision International -WVI (Tanzania), Adventist Development and Relief Agency –ADRA (Tanzania), Concern Universal –CU (Malawi), Community Mobilization Against Desertification –CMAD (western Kenya).

Participating extension officers, adult education teachers and farmer extensionists were further trained in IPM methodologies including pest biology and ecology, and in the principles of participatory research. Traditional knowledge and available scientific information were discussed for their suitability in the management of major bean pest problems. The main principles were the use of community participatory approaches and the inclusion of traditional pest management strategies for evaluation and training. Farmer groups in collaboration with their research and extension personnel established demonstration and learning plots at target sites in western Kenya, northern and southern Tanzania and central Malawi.

The original pilot site in northern Tanzania is now fully led by farmer communities in collaboration with area based extensionists. CIAT, NGOs, private service providers and the national extension and research staff are now providing backstopping services on demand from

farmer groups through methodology and on-station research to address basic issues raised from farmers' field observations. Demonstrations, learning plots, field days, cross village and cross site visits, village information centres, seminars, radio messages and drama have been chosen by different farmer groups as their major approaches to share and exchange knowledge.

Results: Training representative farmers, village extension officers, adult education teachers, NGO personnel and community leaders have contributed to increased IPM awareness among communities within project pilot sites and beyond. Cross village and within site farmer group visits were facilitated in western Kenya as well as in northern and southern Tanzania. Cross site visits were facilitated for farmer groups in Kenya (western and south western Kenya) and Tanzania (Hai, Lushoto and Southern Highlands - Mbeya). In addition, 5 farmers, 1 extensionist (Concern Universal) and 1 research staff from Malawi were facilitated by SABRN and the IPM promotion Project to visit farmer groups in the Southern Highlands of Tanzania. In scaling out, farmer representatives from the former Participatory Plant Breeding (PPB) project in northern Tanzania (Makiba farmers in Arumeru district, Arusha region) and those involved with Farm Africa at Babati in Manyara region were facilitated to participated in one of a series of field days organised by farmer groups in Hai district. In the process, participating farmers were keen to share ideas and exchange information with other site group farmers and non-participating colleagues.

Farmer groups at different sites have demanded additional services from different stakeholders including farm inputs from NGOs and the private sector as well as information on markets, small enterprises, soil and water management, quality seed production and other technologies from CIAT and the national programmes. Other farmer groups have forged ahead and formed community based organisations (CBOs) to meet some of these demands. For example, the groups in Hai district have united to form and register their CBO (Union of Development Groups in Hai District – MUVIMAHA) while other groups in Lushoto and Southern Highlands of Tanzania have formed and registered Savings and Credit Accounts Societies- SACCOS.

All sites have strong collaboration between farmers, researchers, extension personnel and development NGOs, with regular meetings to monitor and evaluate observations in variety and other technology demonstrations and IPM learning plots. The national research and extension staff, ADRA, WVI and ZCC have collaborated in the translation of extension leaflets and handbooks while the ADRA and WVI have been involved in translating and meeting costs for printing initial copies of these materials. Additional leaflets and posters in Kiswahili were prepared. A series of farmer activity reports (17) have been also been prepared. Initial copies of the Kiswahili version of the CIAT field guide on 'Bean pests, diseases, and nutritional disorders of the common bean in Africa' are now available at CIAT Arusha and World Vision office at Hai district. All these materials have and will be distributed to village information centres at project sites in ECABREN and SABRN.

Visits by CPP, DFID, DG CIAT, CIAT Communication Unit, CIAT Africa at Kampala, Ministry of Agriculture Officials at district, region, zonal and national levels have further strengthened farmers' confidence and encouraged them to look forward to achieving their goal in alleviating poverty.

Contributors: E. Minja, K. Ampofo, H. Mziray, J. Ogecha and D Kabungo.

Collaborators: M. Pyndji, R. Chirwa, Y. Mbwana (ADRA), A. Masam (WVI), E. Ulicky (DALDO, Hai District), B. Chibambo (Malawi), JG Mowo (AHI).

Publications

Snapp, S.S., Jones, R.B., Minja, E.M., Rusike, J. and Silim, S.N. 2003. Pigeon Pea for Africa: A Versatile Vegetable – And More. HortScience, Vol 38(6): 1-7.

International Newsletters

Minja, E.M., Silim, S.N. and Karuru, O.M. 2002. Efficacy of *Tephrosia vogelii* crude leaf extract on insects feeding on pigeonpea in Kenya . International Chickpea and Pigeonpea Newsletter. Number 9:49-51.

Minja, E.M., van der Merwe, P.J.A., Ferguson, M.E. and Mviha, P.J. 2002. Screening wild *Arachis* for resistance to groundnut plant hopper *Hilda patruelis* in Malawi. International *Arachis* NewsletterNumber 22:49-51.

Conference

Minja, E.M. and Green, S.V. 2003. Armoured Bush Cricket Control – A Farmer Perspective. Paper presented at the Symposium on IPM of Armoured Bush Crickets 6-9th July 2003 at the Congress of the Entomological Society of Southern Africa, University of Pretoria, South Africa.

Workshops, Meetings and Seminars

Stakeholder workshop to monitor, review and plan for the future of the bean IPM project held at Arusha, Tanzania.

Training workshops/seminars (2 at Lushoto and 1 at Hai both in northern Tanzania) for farmer group representatives and extension officers, and for adult education teachers (1 in Kisii, Kenya).

A series of farmer group meetings/conferences and field days at project sites in Kenya, Tanzania and Malawi.

A farmer (woman) from Hai district represented project farmer groups in a regional workshop on Science and Technology in Agricultura held in Addis Ababa, Ethiopía.

Another lady farmer and village extension officer also from Hai, represented project farmer groups and village extension officers in the ECABREN regional workshop on priority setting held at Nairobi, Kenya.

Donor: DFID

Collaborating Partners:

For Bean IPM: Kenya, Malawi, Tanzania, Uganda and Rwanda; NGO partners – WV, ADRA and FA in northern Tanzania, CMAD in Kenya, and CU in Malawi).

Project Staff: CIAT Rwanda: JKO Ampofo, CIAT Arusha: EM Minja and HA Mziray).

Acronyms and Abbreviations used:
WV= World Vision, ADRA= Adventist Development and Relief Agency, FA= Farm Africa, CMAD = Community Mobilization Against Desertification and CU= Concern Universal.

FORAGE ENTOMOLOGY

Activity 1. Screening Brachiaria hybrids for spittlebug resistance.

Continuous mass rearing of spittlebug species in Palmira and Macagual

This is a continuous activity. A permanent supply of insects is essential in the process of evaluating genotypes for resistance to spittlebug. Progress made in the logistics of mass rearing of nymphs and in obtaining eggs from adults collected in the field has allowed us to screen *Brachiaria* genotypes for simultaneous resistance to six major spittlebug species: *Aeneolamia varia*, *A. reducta*, *Zulia carbonaria*, *Z. pubescens*, *Mahanarva trifiss*a, and *Prosapia simulans*. Insect material produced in our mass rearing facilities is used for greenhouse evaluations in Palmira and field evaluations in Caquetá.

Contributors: C. Cardona, G. Sotelo.

Identify *Brachiaria* genotypes resistant to spittlebug

Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species (*Aeneolamia varia, A. reducta, Zulia carbonaria, Z. pubescens*)

Introduction

The correct identification of resistant hybrids is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. This is why assessment of resistance to spittlebug received special attention in 2003. Based on results obtained in 2001 and 2002, simultaneous but independent screening for resistance to three key spittlebug species was fully implemented.

Materials and Methods: A set of 64 "pre-selected" SX x AP (sexual-by-apomictic) hybrids received from the Breeding Program were evaluated for resistance to *Aeneolamia varia, A. reducta,* and *Zulia carbonaria*. Test materials were compared with six checks fully characterized for resistance to one or more spittlebug species. 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 reached the fifth instar stage or adult emergence occurred. Plants (5 per genotype) were scored for symptoms using the damage scale (1, no 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 the 1-5 scale) and reduced percentage survival (< 30%). Those genotypes showing resistance to two or more spittlebug species were reconfirmed in replicated nurseries (10 replications per genotype per spittlebug species).

Results and Discussion: The preliminary screening revealed that 10 of the 64 hybrids showed acceptable levels of resistance to at least two spittlebug species **(Table 1)**. As in previous occasions, fewer genotypes showed antibiosis resistance to *Z. carbonaria.*

Further testing with 10 replications per genotype per insect species allowed us to identify four hybrids combining antibiosis resistance to *A. varia*, *A. reducta*, and *Z. carbonaria* **(Table 2)**. Levels of resistance in this case were comparable to those exhibited by the resistant checks CIAT 36062 and 'Marandú' (CIAT 6294).

Table 1. Sexual-by-apomicitic hybrids selected in 2003 for reconfirmation of resistance to three spittlebug species. Means of five replicates per genotype per spittlebug species.

Genotype	Damage Scores			Percentage Nymph Survival		
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*
			Hybrids			
BR02NO/0419	1.2	1.6	2.1	3.3	10.0	23.3
BR02NO/0465	1.8	1.1	1.4	30.0	0.0	20.0
BR02NO/0638	1.2	1.6	2.6	23.3	36.7	-
BR02NO/0643	1.1	1.9	3.2	3.3	40.0	-
BR02NO/0644	1.4	1.3	2.2	33.3	20.0	80.0
BR02NO/0649	1.4	1.2	1.6	16.7	3.3	53.3
BR02NO/0756	3.0	1.2	2.0	-	0.0	26.7
BR02NO/0812	2.3	1.6	2.4	-	16.7	56.7
BR02NO/1372	2.4	1.6	1.6	-	20.0	56.7
BR02NO/1485	1.3	1.7	2.5	13.3	20.0	66.7
			Checks[a]			
FM9503/4624 (T)	3.5	2.2	1.5	85.0	25.0	11.7
CIAT 6294 (R)	2.0	1.4	2.5	26.7	25.0	56.7
SX0NO/0102 (R)	1.0	1.1	1.1	0.0	0.0	0.0
CIAT 36062 (R)	1.1	1.2	1.4	1.7	0.0	16.7
CIAT 0606 (S)	5.0	4.3	4.1	95.0	80.0	68.3
BR4X/44-02 (S)	4.9	4.8	3.5	90.0	73.3	70.0
LSD 5%	1.21	1.23	0.94	28.8	22.2	23.7

[a] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Table 2. Sexual-by-apomictic hybrids selected in 2003 for high antibiosis resistance (<30% nymphal survival) to three spittlebug species. Means of 10 replicates per genotype per spittlebug species.

Genotype	Damage Scores			Percentage Nymph Survival		
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*
			Selected hybrids			
BR02NO/0419	1.0	1.1	1.8	0.0	0.0	5.0
BR02NO/0465	1.0	1.0	1.1	10.0	1.7	18.3
BR02NO/0756	1.4	1.2	1.8	18.3	3.3	21.7
BR02NO/0812	1.0	1.5	2.4	6.7	8.3	28.3
			Checks[a]			
FM9503/4624 (T)	1.6	1.8	1.9	51.7	21.7	30.0
CIAT 6294 (R)	1.2	1.9	2.7	25.0	21.7	63.3
SX01NO/0102 (R)	1.0	1.0	1.1	1.7	0.0	1.7
CIAT 36062 (R)	1.0	1.0	1.5	0.0	0.0	6.7
CIAT 0606 (S)	4.9	4.4	3.8	96.7	68.5	46.7
BRU4X/44-02 (S)	4.8	4.1	3.8	96.7	55.0	75.0
LSD 5%	0.36	0.65	0.68	16.8	17.8	26.3

[a] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species (*Aeneolamia varia, Zulia carbonaria, Z. pubescens, Mahanarva trifissa*)

Introduction

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 a technique that allows us to properly identify resistance under field conditions. Evaluating for resistance under field conditions is important because it allows us 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, eight major screening trials (six with *Zulia pubescens*, two with *Mahanarva trifissa*) were conducted in Caquetá in 2003.

Results and Discussion: In **Table 3** we highlight the results of evaluating 32 hybrids for resistance to *Z. pubescens* in comparison with six checks well known for their reaction to *Aeneolamia varia*. As in previous occasions, there was a significant negative correlation ($r = -0.452$; $P < 0.001$; $n = 2273$) between damage scores and tiller ratios. This means that damage scores are useful in predicting tiller losses resulting from intense insect damage. Selected hybrids in **Table 3** showed significantly lower damage scores and significantly higher tiller ratios than the susceptible checks CIAT 0606 and BRUZ4X/44-02.

Table 3. Field resistance to *Zulia pubescens* in selected *Brachiaria* hybrids and checks. Means of six trials, 10 replicates per genotype per trial.

Genotype	Damage scores	Tiller ratio[a]
	Hybrids	
BR00NO/1494	2.0	1.04
BR00NO/0755	2.0	0.92
BR00NO/1392	2.0	0.91
BR00NO/1032	2.1	0.89
BR00NO/0604	2.1	0.88
BR00NO/1076	2.1	0.88
BR00NO/1295	2.0	0.88
BR00NO/0036	2.0	0.87
BR00NO/0042	2.1	0.96
BR00NO/0029	2.1	0.86
	Checks[b]	
FM9503/46/024 (T)	1.1	1.04
CIAT 6294 (R)	1.1	1.04
CIAT 36062 (R)	1.1	1.01

Genotype	Damage scores	Tiller ratio[a]
CIAT 6133 (T)	1.8	0.92
CIAT 0606 (S)	3.6	0.46
BRUZ4X/44-02 (S)	3.9	0.47
LSD 5%	0.13	0.12

[a] Tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process
[b] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

The same set of 32 hybrids was evaluated for field resistance to *Mahanarva trifissa*. Results are shown in **Table 4**. Resistant hybrids exposed to *M. trifissa* performed significantly better than the checks both in terms of damage scores and tiller ratios.

Table 4. Field resistance to *Mahanarva trifissa* in selected *Brachiaria* hybrids and checks. Means of two trials, 10 replicates per genotype per trial.

Genotype	Damage scores	Tiller ratio[a]
	Hybrids	
BR00NO/0587	2.1	1.16
BR00NO/1494	2.1	1.07
BR00NO/1392	2.0	1.06
BR00NO/0106	2.0	1.01
BR00NO/0078	2.1	1.00
BR00NO/0049	2.1	0.97
BR00NO/1733	2.1	0.96
BR00NO/0235	2.1	0.96
	Checks[b]	
FM9503/46/024 (T)	1.1	0.98
CIAT 6294 (R)	1.1	1.22
CIAT 36062 (R)	1.0	1.03
CIAT 6133 (T)	1.8	0.99
CIAT 0606 (S)	3.8	0.41
BRUZ4X/44-02 (S)	4.3	0.28
LSD 5%	0.19	0.16

[a] Tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process
[b] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Contributors: C. Cardona, G. Sotelo, A. Pabón, and J. W. Miles.

Activity 2. Identify host mechanisms for spittlebug resistance in *Brachiaria.*

Mechanisms of resistance to five spittlebug species

Introduction

We have shown in previous reports that resistance to one spittlebug species does not necessarily apply to other species. We have also shown that the mechanisms of resistance vary. In 2003 we finalized the characterization of antibiosis and tolerance to *Aeneolamia reducta*, the most important species in the Caribbean zone. What follows is a summary of what we know about host plant resistance mechanisms to five major spittlebug species present in Colombia.

Materials and Methods: Several experiments were conducted and are reported herein. As test materials we used four germplasm accessions well known for their reaction to *Aeneolamia varia*: the susceptible checks CIAT 0606 and CIAT 0654 and the resistant checks CIAT 6294 ('Marandú') and CIAT 36062 (a hybrid-derived clone). These four host genotypes were also used to compare their resistance to other spittlebug species. CIAT 0654 and CIAT 36062, highly susceptible and resistant, respectively, were used in antibiosis studies. Tolerance studies were conducted with CIAT 0654, CIAT 6294, and CIAT 36062. *A. varia*, *A. reducta*, and *Z. carbonaria* were mass-reared on plants of CIAT 0654 in a screen-house. Mature eggs were used to infest test plants in the different experiments. In the case of *Z. pubescens* and *M. trifissa*, large numbers of adults were collected in the field with a sweep net and transferred to muslin cages in a screen-house to feed on potted plants of CIAT 0654. Adults were allowed to oviposit and eggs were separated from the soil. As with other spittlebug species, test plants were infested with mature eggs. All tests were conducted in a glasshouse at a mean temperature of 24^{o}C (range, 19-27^{o}C) and mean relative humidity of 75% (range, 70-90%).

To evaluate antibiotic effects, cohorts of no fewer than 900 individuals of each of the five species under study were established on each of two host genotypes well characterized for their reaction to *A. varia*: CIAT 0654 (highly susceptible) and CIAT 36062 (highly resistant). Cohorts were established by infesting 150 single-plant units with 6 eggs of the respective species per unit as described above. Following eclosion, a sample of two or three tubes per host genotype was examined daily to determine the fate of 12 or 18 individual insects. Nymphal instars and their duration were determined from measurement of the width of the head capsule of every nymph recovered (dead or alive). Survival rates were calculated. The dry weight of each nymph was recorded. Daily sampling continued until all surviving nymphs reached adulthood.

To study tolerance we initially compared the response of the susceptible CIAT 0654 and the *A. varia*-resistant CIAT 6294 (‘Marandú’) to increasing levels of infestation with nymphs of *A. varia, M. trifissa, Z. carbonaria,* and *Z. pubescens*. *A. reducta* was not included in these studies. Thirty-day-old plants of CIAT 0654 and CIAT 6294 were exposed to 0, 2, 3, 5, 7, or 10 nymphs per plant of each of the spittlebug species. The 48 host genotype- insect species-infestation level treatment combinations were randomly assigned to single-plant experimental units within 10 complete blocks. Plants were infested with mature eggs and the infestation was allowed to proceed until all nymphs were mature or adult emergence occurred. Plants were then scored for damage using the 5-point scale described above and the percentage nymphal survival recorded.

Aboveground dry weight of plants was recorded following drying in an oven at 40°C. Percent weight loss was calculated (relative to the uninfested controls). We calculated functional plant loss indexes for each infestation level based on plant weight loss and damage response. Using the same general methodology, we conducted one more tolerance test in which plants of CIAT 0654 (susceptible) and CIAT 36062 (resistant to *A. varia*) were submitted to increasing levels of infestation (0, 2, 3, 5, 7, or 10 nymphs per plant) with each of the following species: *A. varia*, *A. reducta*, *M. trifissa*, *Z. carbonaria*, and *Z. pubescens*. We used a randomized complete block design with 10 repetitions per species-infestation level-host genotype combination. Damage scores, nymph survival, and above ground plant dry weights were recorded. Functional Plant Loss Indices were calculated.

All data were analyzed using the general linear model procedure. Means were separated by least significant difference (LSD: $\alpha = 0.05$) only when the overall *F* test was significant ($\alpha = 0.05$). Percentage nymph survival was transformed to arcsine square root of proportion; percentages of dry weight loss were transformed to square root. Means and standard errors of untransformed data are presented. Antibiotic effects for the different spittlebug species were assessed by comparing nymphal instar duration and nymph weight between the susceptible and resistant host genotypes by paired *t*-test. To compare survivorship of nymphs on susceptible and resistant host genotypes, median survival times were calculated using the Kaplan-Meier test. The Cox-Mantel survival test was used to compare survival distributions on susceptible and resistant host genotypes. Tolerance to the different spittlebug species was assessed by comparing mean percentage survival, mean damage scores, and mean percentage plant dry weight loss of five infestation levels between the susceptible and resistant host genotypes by paired *t*-test within spittlebug species.

Results and Discussion: ***Antibiosis tests.*** Relative to the susceptible control, CIAT 0654, there was a significant delay in development time of nymphs of *A. varia*, *A. reducta*, and *Z. pubescens* reared on CIAT 36062 **(Figure 1)**. No such effect was found in the case of *Z. carbonaria*. Mortality of second instars of *M. trifissa* was so high, that we were unable to calculate developmental times for this species.

Nymphal survival on the resistant CIAT 36062 was least for *M. trifissa* and greatest for *Z. carbonaria*. Survival of all species was less on CIAT 36062 than on the susceptible CIAT 0654. The Kaplan-Meier survival test revealed significant effects (no overlapping confidence intervals) of the resistant genotype on the median survival times of *A. varia*, *A. reducta*, and *M. trifissa* and, to a lesser extent, *Z. pubescens* populations. Survival time of *Z. carbonaria* was not affected by the resistant genotype. Calculation of the Cox-Mantel survival statistic showed differences at the 1% level of significance between CIAT 0654 and CIAT 36062 **(Table 1)** in terms of survival rates for *A. varia*, *A. reducta*, and *M. trifissa*, and at the 5% level of confidence for *Z. pubescens* (a lower level of antibiosis). No difference was found in the case of *Z. carbonaria* meaning that there is no antibiosis to this species in CIAT 36062. This was confirmed when survival rates of *Z. carbonaria* and *Z. pubescens* on CIAT 36062 were compared. The Cox-Mantel survival test statistic (2.8) was positive and significant at the 1% level, indicating that CIAT 36062 is more favorable to *Z. carbonaria* than to *Z. pubescens*.

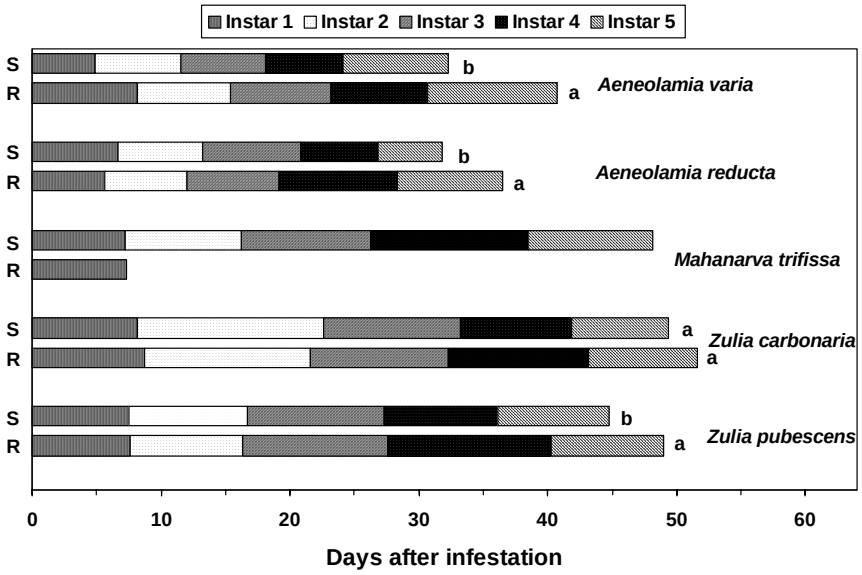


Figure 1. Duration of nymphal instars of five spittlebug species reared on susceptible (S, CIAT 0654) or resistant (R, CIAT 36062) *Brachiaria* genotypes. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within species. *Mahanarva trifissa* was not analyzed due to very high mortality of second instars.

Table 1. Survivorship parameters for nymphs of five spittlebug species reared on susceptible (CIAT 0654) or resistant (CIAT 36062) *Brachiaria* genotypes.

	Number tested		
Spittlebug species	**On CIAT 0654**	**On CIAT 36062**	**C[a]**
Aeneolamia varia	480	480	4.8**
Aeneolamia reducta	420	480	6.4**
Mahanarva trifissa	708	246	9.7**
Zulia carbonaria	744	720	1.4ns
Zulia pubescens	648	648	2.2*

**, Significant at the 1% level; *, significant at the 5% level; ns, not significant.

[a] C is the test statistic for the Cox-Mantel two-sample survival test (CIAT 0654 versus CIAT 36062).

Antibiosis to *A. varia*, *A. reducta*, and *Z. pubescens* in CIAT 36062 was also manifested by the reduced weight of surviving 4th and 5th instar nymphs, and adults. No effect on nymphal or adult weight of *Z. carbonaria* was detected. Other manifestations of antibiosis were the occurrence of minute 2nd, 3rd, and 4th instar nymphs, staggering of developmental times, and reduced spittle production by surviving nymphs. Also, we found that nymphs reared on CIAT 36062 usually leave the spittle and wander over the soil surface, eventually dying of dehydration. We found no deformation of nymphs or adults nor did we detect obvious disruptions in the molting process. The level of antibiosis resistance in CIAT 36062 clearly differs by spittlebug species and can be classified as follows: very high for *M. trifissa*, high for *A. varia* and *A. reducta*, moderate for *Z. pubescens*, and absent for *Z. carbonaria*.

Tolerance tests. CIAT 6294 expressed clear antibiosis to *A. varia* and *M. trifissa* as the mean nymphal survival of five infestation levels was significantly lower than the mean for the susceptible control CIAT 0654 **(Figure 2)**. However, survival of *Z. carbonaria* or *Z. pubescens* nymphs was high on both genotypes at all levels of infestation, indicating lack of antibiosis in CIAT 6294 to these two species. These results were consistent with those obtained in resistance reconfirmation tests. CIAT 6294 plants suffered less damage and less plant dry weight loss than the susceptible control at all levels of infestation **(Figure 3)**. As in previous studies, visual damage scores predicted biomass loss. Since survival of the *Zulia* spp. nymphs did not differ between the genotypes, we interpret the lower damage scores and lower plant dry weight losses caused by *Z. carbonaria* and *Z. pubescens* on CIAT 6294 as tolerance.

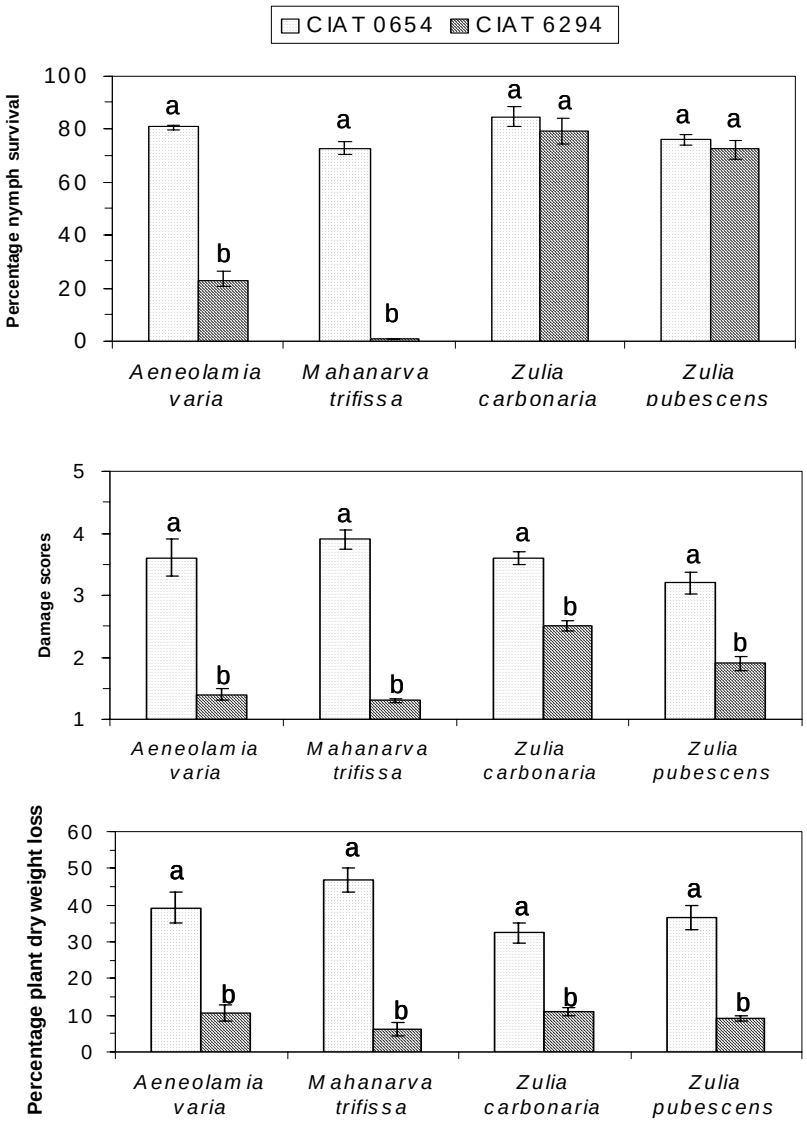


Figure 2. Response of susceptible (CIAT 0654) or resistant (CIAT 6294) *Brachiaria* genotypes to attack by nymphs of four spittlebug species. Means (± SEM) of five levels of infestation. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within spittlebug species.

At all levels of infestation, survival of nymphs on CIAT 36062 was much less than on the susceptible control for *A. varia*, *A. reducta*, and *M. trifissa*, but only moderately less for *Z. pubescens*. *Z. carbonaria* nymphs survived equally well on the two genotypes **(Figure 3)**. Thus, expression of antibiosis in CIAT 36062 was dependent on spittlebug species. CIAT 36062 suffered significantly less damage (expressed as damage scores or plant weight loss) than the susceptible control at all levels of infestation with *Z. carbonaria* **(Figure 3)**. Since *Z. carbonaria* nymphs survived equally well on both genotypes, we interpret the mechanism of resistance to *Z. carbonaria* in CIAT 36062 as tolerance.

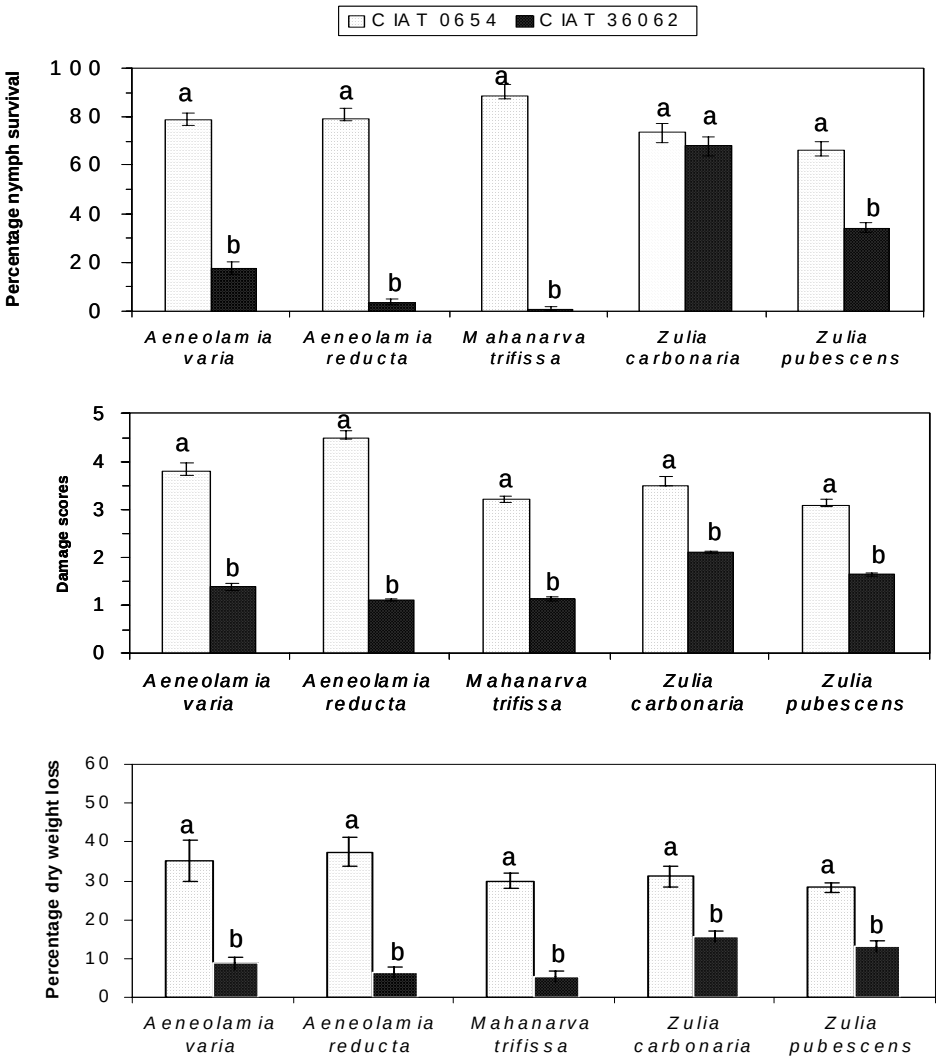


Figure 3. Response of susceptible (CIAT 0654) or resistant (CIAT 36062) *Brachiaria* genotypes to attack by nymphs of five spittlebug species. Means (± SEM) of five levels of infestation. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within spittlebug species.

We also calculated a functional plant loss index to measure tolerance to both *Z. carbonaria* and *Z. pubescens*. Losses were highest for the susceptible control, CIAT 0654, at all levels of infestation **(Figure 4)**. Losses caused by both species on CIAT 6294 and on CIAT 36062 were lower at all infestation levels. These results suggest the presence of true tolerance to *Z. carbonaria* in CIAT 6294 and CIAT 36062, true tolerance to *Z. pubescens* in CIAT 6294 and a combination of tolerance coupled with antibiosis as mechanisms of resistance to *Z. pubescens* in CIAT 36062.

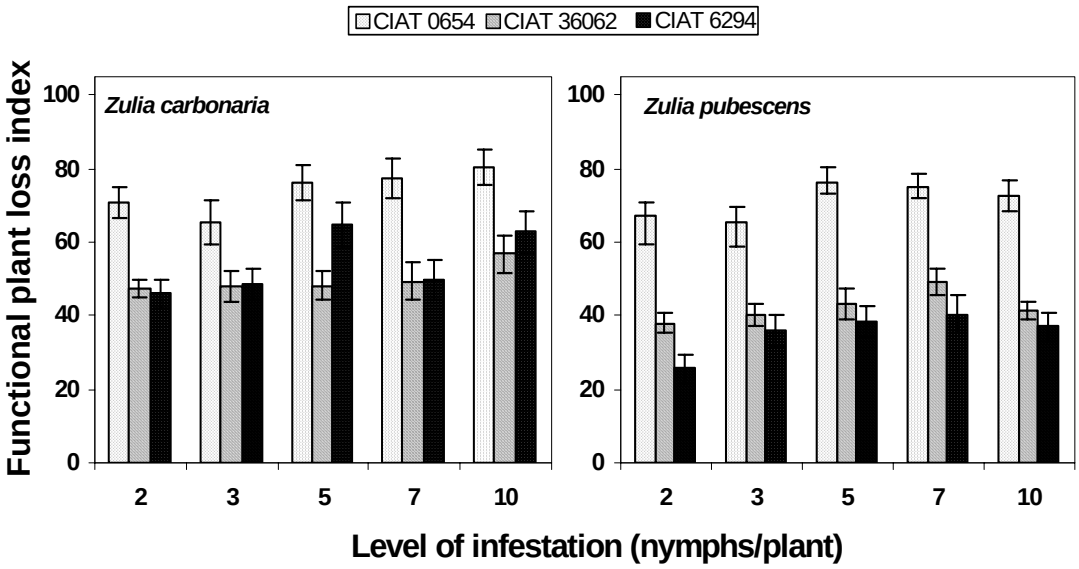


Figure 4. Functional plant loss indices (percentage) for susceptible (CIAT 0654) or resistant (CIAT 36062, CIAT 6294) *Brachiaria* genotypes exposed to five levels of infestation with each of two spittlebug species.

Contributors: C. Cardona, G. Sotelo. A. Pabón, P. Fory, and J. W. Miles.

Activity 3. Effect of mixed infestations on resistance expression in selected *Brachiaria* genotypes.

Introduction

As explained in the 2002 Annual Report, we can identify spittlebug nymphs with absolute precision by means of RAPD-PCR DNA analysis or by comparison of esterase banding patterns. Using these techniques we have been able to detect mixed infestations in commercial fields and to measure percentage survival of different species when mixed infestations by two or more species occur. This in turn has allowed us to study how different species combinations affect resistant expressions in selected resistant or susceptible genotypes.

Materials and Methods: In 2003 we measured the effect of single species infestation as opposed to mixed infestations by infesting plants with eggs of two or more spittlebug species in different proportions. The infestation was allowed to proceed until adult emergence occurred. Plants were then scored for damage and the surviving nymphs were collected and identified to species level by comparison of esterase banding patterns or, in some cases, by RAPDs-PCR analysis. Percentage survival was calculated for each spittlebug species.

Results and Discussion: We will highlight results of studies on the effect of mixed *Aeneolamia reducta* - *Zulia carbonaria* infestations. These are two of the most important spittlebug species present in Colombia. As shown in **Figure 1**, when the resistant genotype CIAT 36062 is exposed to *Zulia carbonaria* alone or when *Z. carbonaria* predominates in the mixture, damage scores increase so that the genotype is classified as intermediate resistant rather than resistant. This was not the case with the hybrid SX01NO/0102, the most resistant hybrid tested to date for resistance to five spittlebug species.

Most important, we detected significant and differential antibiosis effects **(Figure 2)** when mixed populations of *A. reducta* and *Z. carbonaria* in different proportions were used to infest plants of the resistant genotypes CIAT 36062 and SX01NO/0102 **(Figure 2)**. At all levels of infestation, survival of *A. reducta* on both resistant genotypes was significantly reduced to levels below the cut-off point for resistance rating (< 30%). On the contrary, the survival of *Z. carbonaria* nymphs on CIAT 36062 was significantly higher, in some cases well above the 50% level used to classify genotypes as susceptible. The hybrid SX01NO/0102 showed intermediate resistance to *Z. carbonaria* at two of the levels of infestation tested. Again, these findings emphasize the need to characterize resistance to as many species as possible and illustrate the need to breed for multiple antibiosis resistance.

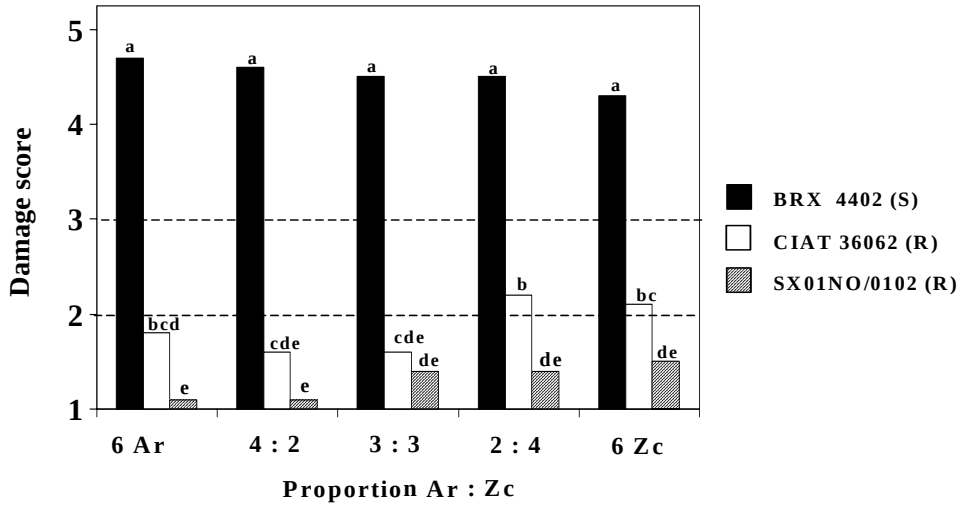


Figure 1. **Damage scores recorded on susceptible (S) and resistant (R) *Brachiaria* genotypes exposed to individual or simultaneous attack by nymphs of *Aeneolamia reducta* (Ar) or *Zulia carbonaria* (Zc). Dotted lines represent cut-off points for resistance (< 2) and intermediate ratings (2 -3) in a 1 - 5 damage score scale. Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.**

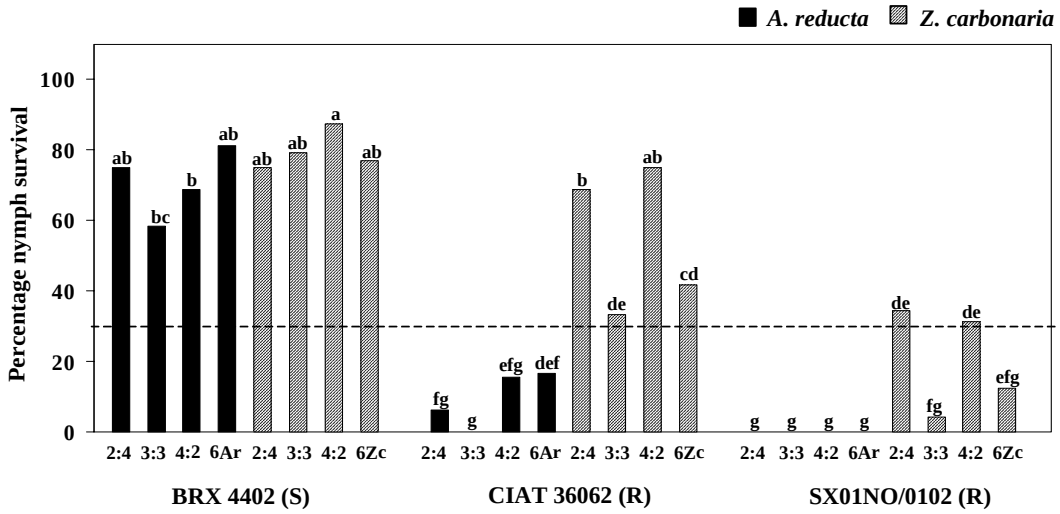


Figure 2. **Levels of antibiosis (reduced percentage nymph survival) detected when plants of susceptible (S) or resistant (R) *Brachiaria* genotypes were infested with *Aeneolamia reducta* or *Zulia carbonaria* or combinations thereof (*A. varia*: *Z. carbonaria*). The dotted line represents the cut-off point for resistance rating (< 30% percentage survival). Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.**

Contributors: A. Pabón, G. Sotelo, and C. Cardona.

Activity 4. Interactions between strains of five spittlebug species and resistance expression in selected *Brachiaria* genotypes

Introduction

The protective properties of insect-resistant cultivars may be overcome by the development of resistance-breaking strains of a given insect species. These are insect populations that possess an inherent genetic capability to overcome plant resistance. Typically, biotypes develop as a result of selection from the parent population in response to exposure to the resistant cultivar. It may occur that the genetic capability of an insect to overcome resistance is so great that resistance is nullified before the resistance cultivar is grown in a large geographical area. This is why it is important to obtain information on the reaction of resistant genotypes to as many geographical strains of a given insect pest as it is possible. We initiated a series of experiments aimed at measuring the response of resistant cultivars to populations of *A. varia*, *A. reducta*, *Z. pubescens*, *Z. carbonaria*, and *M. trifissa* collected in several different areas of Colombia. For the first time, we also generated information on resistance to *Prosapia simulans* (Walker).

Materials and Methods: All trials were conducted using test materials well known for their reaction to *Aeneolamia varia*. We evaluated the susceptible checks CIAT 0606 and CIAT 0654, the resistant checks CIAT 6294 and CIAT 36062, and two new sexual hybrids SX01NO/0102 and SX01NO/0233 classified as highly resistant to *A. varia* in previous studies. The *A. varia*-CIAT colony combination was used as the standard check in all trials. Screening for resistance was conducted using standard methodologies. Plants were infested with six eggs per plant of the respective spittlebug species-geographical combination and the infestation was allowed to proceed without interference until all nymphs reached the fifth instar stage or adult emergence occurred. Plants (20 per genotype) were scored for symptoms using the damage scale (1, no damage; 5, plant dead) developed in previous years. Percentage nymph survival was calculated.

Results and Discussion: We have conducted four trials. At this point, we will highlight results obtained with geographical strains of *A. varia* and *Z. pubescens*. We will also report on our first-ever screening for resistance to *P. simulans*.

The reaction of six genotypes to attack by nymphs of three strains of *A. varia* is shown in **Table 1**. No significant genotype x strain interaction was detected for damage scores or percentage nymph survival, meaning that resistance ratings did not change when the genotypes were exposed to different strains of *A. varia*. Similarly, no significant genotype x strain interaction was detected when susceptible and resistant genotypes were exposed to attack by nymphs of *Z. pubescens* **(Table 2)**.

Table 1. Reaction of selected *Brachiaria* genotypes to strains of *Aeneolamia varia* from two geographical areas of Colombia.

Origin of strain	Genotypes[a]					
	BRUZ4X-44-02	CIAT 0606	CIAT 6294	CIAT 36062	SX01NO/ 0102	SX01NO/ 0233
	Damage scores					
Florencia, Caquetá	3.9b	3.8b	1.2a	1.3a	1.1a	2.0a
V/vicencio, Meta	4.2a	3.7b	1.3a	1.1a	1.0a	1.3b
CIAT colony	4.9a	4.6a	1.3a	1.1a	1.0a	2.2a
Mean	4.3A	4.0A	1.3C	1.2C	1.0C	1.8B
	Percentage nymph survival					
Florencia, Caquetá	75.8b	86.6a	17.5b	0.0c	2.5b	26.7b
V/vicencio, Meta	90.8a	71.1b	35.8a	9.2a	6.7a	14.2c
CIAT colony	86.1a	87.1a	19.8b	4.7b	6.3a	40.7a
Mean	84.2A	81.6A	24.4B	4.6C	5.2C	27.2B

Means of 20 replicates by genotype by insect strain. For each variable, 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.

Table 2. Reaction of selected *Brachiaria* genotypes to strains of *Zulia pubescens* from three geographical areas of Colombia.

Origin of strain	Genotypes					
	BRX 44-02	CIAT 0606	CIAT 6294	CIAT 36062	SX01NO/ 0102	SX01NO/0233
	Damage scores					
Darién, Valle	3.9a	4.2a	2.0a	1.0b	1.1a	1.5b
Popayán, Cauca	3.8a	3.3b	2.1a	1.2ab	1.1a	1.3b
S. José de Fragua, Caquetá	4.3a	4.1a	2.1a	1.4a	1.3a	2.6a
Mean	4.0A	3.9A	2.1B	1.2C	1.2C	1.8B
	Percentage nymph survival					
Darién, Valle	71.6a	55.9ab	34.1b	2.8b	0.9c	4.6b
Popayán, Cauca	44.3b	45.5b	47.5a	11.6a	6.7b	5.0b
S. José de Fragua, Caquetá	74.1a	69.0a	34.2b	8.8a	21.6a	47.2a
Mean	63.3A	56.8A	38.6B	7.7D	9.7D	18.9C

Means of 20 replicates by genotype by insect strain. For each variable, 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.

Table 3 summarizes results of our first screening for resistance to *Prosapia simulans*. Susceptible (CIAT 0606, BRX-44-02) and resistant genotypes (CIAT 6294, CIAT 36062, SX01NO/0102, and SX01NO/0233) differed for damage scores for all spittlebug species tested. *P. simulans* caused more damage than *A. varia* and *M. trifissa* on the resistant genotype CIAT 6294 (‘Marandú’). Using our resistance classification, CIAT 6294 would be classified as resistant to *A. varia* and *M. trifissa* (damage scores: 1-2) but intermediate to *P. simulans* (damage scores: 2.1-3.0). SX01NO/0233 was intermediate to all three species tested. Survival of nymphs of *A. varia* and *M. trifissa* was significantly lower on the *A. varia*-resistant genotypes than on the susceptible controls CIAT 0606 and BRX-44-02 **(Table 3)**. Survival of *P. simulans* nymphs was significantly higher on CIAT 6294 than on the other resistant genotypes suggesting that antibiosis resistance to this species is absent in 'Marandú'. Using our resistance classification, CIAT 6294 would be classified as susceptible (> 50% survival) to *P. simulans*. The relatively

low levels of damage caused by *P. simulans* on CIAT 6294 could be the result of tolerance to this species.

Table 3. Response of selected *Brachiaria* genotypes to attack by nymphs of three spittlebug species.

Spittlebug species	Spittlebug species		
	Mahanarva trifissa	***Aeneolamia varia***	***Prosapia simulans***
	Damage scores		
BRX-44-02	4.2aA	4.9aA	4.3aA
CIAT 0606	3.5bB	4.6aA	4.3aA
CIAT 6294	1.2cB	1.3cB	2.4bA
CIAT 36062	1.1cA	1.1cA	1.3cA
SX01NO/0102	1.0cA	1.0cA	1.5cA
SX01NO/0233	2.1bcA	2.2bA	2.3bA
	Percentage nymph survival		
BRX-44-02	55.0aB	86.1aA	90.8aA
CIAT 0606	36.0aC	87.1aA	79.2bA
CIAT 6294	0.0bC	19.8cB	65.0bA
CIAT 36062	0.0bB	4.7dA	14.2cA
SX01NO/0102	0.0bA	6.3dA	6.7dA
SX01NO/0233	34.5aA	40.7bA	5.0dB

Means of 20 replicates by genotype by insect species. For each variable, 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.

Contributors: C. Cardona, G. Sotelo, and A. Pabón.

Activity 5. Mechanisms of resistance to adults of five spittlebug species and sub-lethal effects of antibiosis on adults of spittlebug

Introduction

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 is known about possible direct effects of antibiotic genotypes on the biology of adults. Even less is 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*.

Materials and Methods: Initially, we conducted two experiments aimed at measuring how feeding on an antibiotic genotype affects the biology of adults of *A. varia*. Later on, we initiated a comprehensive series of experiments aimed at determining whether antibiosis to nymphs has an adverse effect on the biology of resulting adults. For this, a number of life tables will be constructed. Treatment combinations are shown in **Table 1**.

Table 1. Treatment combinations to study possible sub-lethal effects of intermediate and high levels of nymphal antibiosis on adults of *Aeneolamia varia*.

Nymphs reared on:	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
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 susceptible to *A. varia*. CIAT 6294 and CIAT 36062 show intermediate and high levels of antibiosis resistance to nymphs of *A. varia*, respectively.

Results and Discussion: This work is in progress. Results will be presented in full in 2004.

Contributors: P. Sotelo, G. Sotelo and C. Cardona.

Publications

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. (accepted with minor revisions September 25, 2003).

Workshops and Conferences

Cardona, C., G. Sotelo and A. Pabón. 2003. Primer registro del barrenador de tallos *Apinocis subnudus* (Buchanan) (Coleoptera: Curculionidae), importante plaga del pasto pará en el Valle del Cauca. Memoria, XXX Congreso de la Sociedad Colombiana de Entomología, Cali, Colombia, 17-19 Julio, 2003. p. 84.

Pabón, A., C. Muñoz and C. Cardona. 2003. Diferenciación de ninfas de cinco especies de salivazo (Homoptera: Cercopidae) mediante marcadores moleculares. Memoria, XXX Congreso de la Sociedad Colombiana de Entomología, Cali, Colombia, 17-19 Julio, 2003. p. 14.

Pabón, A., G. Sotelo and C. Cardona. 2003. Resistencia de dos híbridos de *Brachiaria* spp. al ataque combinado de cuatro especies de salivazo (Homoptera: Cercopidae). Memoria, XXX Congreso de la Sociedad Colombiana de Entomología, Cali, Colombia, 17-19 Julio, 2003. p. 97.

Cardona, C. 2003. Resistencia en *Brachiaria* spp. a cinco especies de salivazo: métodos, mecanismos, avances. Curso de Pastos en los Llanos Orientales, Junio 16-20 junio, 2003. Estación Experimental La Libertad, Corpoica, Villavicencio, Meta.

Cardona, C. 2003. Resistencia en *Brachiaria* spp. a cinco especies de salivazo: métodos, mecanismos, avances. Taller "Nuevos Avances en el Desarrollo de Gramíneas y Leguminosas Tropicales", Mayo 22-23, 2003. Estación Experimental Turipaná, Corpoica, Montería, Córdoba.

Pabón, A., C. Cardona and G. Sotelo. 2002. Resistencia de un híbrido de *Brachiaria* spp. al ataque combinado de tres especies de salivazo (Homoptera: Cercopidae). XLVII Foro Entomológico, Universidad Nacional de Colombia, Palmira. September, 2002.

Sotelo, G. and C. Cardona. 2002. Reseña histórica de las evaluaciones del género *Brachiaria* a la respuesta de resistencia al salivazo de los pastos. XLVII Foro Entomológico, Universidad Nacional de Colombia, Palmira. September, 2002.

Fory, P., C. Cardona and G. Sotelo. 2002. Caracterización de la resistencia a tres especies de salivazo (Homoptera: Cercopidae) en un híbrido de *Brachiaria* spp. resistente a *Aeneolamia varia*. XLVII Foro Entomológico, Universidad Nacional de Colombia, Palmira. September, 2002.

Contributors: César Cardona, Guillermo Sotelo, Gilberto Córdoba, Reinaldo Pareja, William Mera.

Students:

Alejandro Pabón (National University), B.Sc. Thesis on resistance to spittkebugs.

Paola Sotelo (Universidad del Valle), B.Sc. Thesis on resistance to spittlebug.

Collaborators: J. W. Miles, Personnel in the Breeding Section, CORPOICA – Macagual.

VIROLOGY

Activity 1. Resistant varieties for cassava frogskin disease.

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. During the three last cycles, around 50 lines have been evaluated for their agronomic characteristics and resistance to other pests. All these lines are rated as tolerant and have remained infested with CFSD at least seven 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. This line reacts like Secundina and when it is affected with CFSD, the plant develops mosaic leaf symptoms. This meant that it was easy to assure that the sources of inoculum were indeed affected with CFSD. Originally five plants from each line were inoculated by grafting with the CFSD affected stem cuttings of line 5460-10. In the last three years between 44 and 50 lines were grown in randomize block design of 4 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 rating scale used was 1 for no symptoms, 2 for very mild symptoms, 3 for moderate symptoms, and 4 for severe symptoms. The ratings, of 30 lines and their yields during the last three years, are summarized in table 1. These are the best cassava lines in the CIAT cassava core collection for resistance to cassava frogskin disease and that yield well in the conditions at the CIAT experiment station at Santander de Quilichao, Cauca, Colombia. There are eight lines from Peru, four from Colombia, and only two from Brazil. Almost 50% of the lines selected came from Countries where CFSD is endemic. One odd result is the there are four lines from Malasia. It is suspected that they share common resistant parents.

There is ample resistance in the cassava germplasm for cassava frogskin disease. 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. Some lines do have more disease in some years, but this is expected given that in cool condition, there is a tendency for greater expression of the root symptoms. After eight 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.

What needs to be done

The 30 varieties reported in these trials are resistant to CFSD and many have adequate yields under the conditions tested. 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. These resistant varieties can be tested using participatory selection, and this should give the farmers a method to reduce economic losses due to CFSD and select cassava that meets there criteria of agronomic and utilization traits.

Additional trials are needed to determine if the resistance will be effective at higher elevations. We are looking for participatory farmer groups to test these materials in the Department of Cauca.

In Countries where CFSD is endemic, screening local varieties that have little or no disease should be done. It is probable that many will be resistant varieties.

It is time to start a study to understand the genetics of resistance to CFSD. The same populations that can be used to determine the genetics of resistance can be used to develop molecular markers for the resistance. This will be useful for countries where the disease is not common, but there is demand for resistance materials.

Active breeding programs that incorporate CFSD resistance should be started in Colombia, Brazil, Costa Rica and other countries where the disease is endemic. Resistant varieties are needed to minimize losses due to CFSD.

Table 1. The best lines in the CIAT core collection for resistance to CFSD.

Variety	2000-2001		2001-2002		2002-2003		3 years
	Symptoms	Yield	Symptoms	Yield	Symptoms	Yield	Average Yield
M Per 183	1.00	3.95	1.02	5.50	1.00	3.07	4.17
M Per 438	1.00	3.95	1.00	2.69	1.00	1.79	2.81
M Chn 2	1.00	3.32	1.00	2.16	1.00	1.69	2.39
M Mex 95	1.03	2.79	1.04	2.35	1.00	2.00	2.38
M Per 213	1.00	2.70	1.00	2.16	1.00	2.11	2.32
M Bra 886	1.08	2.32	1.50	2.56	1.08	1.71	2.20
M Ecu 68	1.00	1.18	1.00	1.91	1.00	3.15	2.08
M Col 634	1.00	2.54	1.19	2.04	1.29	1.56	2.04
M Mal 50	1.00	3.13	1.00	1.58	1.08	1.38	2.03
M Per 431	1.00	2.12	1.00	1.86	1.00	1.98	1.99
M Gua 78	1.00	1.97	1.20	1.63	1.04	2.21	1.93
M Col 1468	1.03	2.21	1.30	1.83	1.33	1.52	1.85
HMC 1	1.00	1.72	1.23	1.62	1.25	1.69	1.68
M Bra 325	1.00	2.22	1.00	1.68	1.25	1.12	1.67
M Per 209	1.00	1.99	1.12	1.91	1.00	1.08	1.66

Variety	2000-2001		2001-2002		2002-2003		3 years
	Symptoms	Yield	Symptoms	Yield	Symptoms	Yield	Average Yield
M Cr 59	1.13	2.00	1.06	1.59	1.20	1.43	1.60
M Per 243	1.00	1.29	1.00	1.49	1.06	1.98	1.59
M Mal 24	1.00	1.91	1.04	1.66	1.08	1.65	1.55
M Gua 41	1.05	1.67	1.00	1.39	1.06	1.56	1.54
M Mex 80	1.00	1.40	1.07	1.82	1.06	1.04	1.42
M Per 184	1.72	1.28	1.54	1.87	1.24	0.86	1.34
M Mal 13	1.00	1.16	1.02	2.16	1.00	1.77	1.31
M Ind 26	1.04	1.96	1.11	1.14	1.00	0.35	1.15
M Cr 79	1.23	1.71	1.23	1.22	1.21	0.50	1.14
M Mal 38	1.03	0.95	1.07	1.38	1.14	1.06	1.13
M Col 2157	1.00	1.13	1.00	1.14	1.04	0.77	1.01
M Per 377	1.03	1.07	1.00	0.96	1.00	0.73	0.92
M Par 163	1.03	1.47	1.09	0.48	1.23	0.58	0.84
M Bol 1	1.00	0.89	1.00	0.77	1.03	0.63	0.76
M Mex 102	1.00	0.73	1.00	0.43	1.00	0.74	0.63

Activity 2. Further studies to associate a reolike virus in *Manihot esculenta* affected with cassava frogskin disease.

Detection of a Genomic Segment of Cassava Frogskin Virus

In last years report, we reviewed the evidence for a reolike virus in cassava. This included multiple double stranded RNA species, virus-like particles and cDNA clones that have homology with rice ragged stunt virus. This virus appear to be associated with CFSD but we asserted that additional research was needed before reaching a definite conclusion. This year, we have made a plant by plant analysis of the dsRNA products using both the cassava frogskin virus (CFSV) Segment (S)5 and the CFSV S1 cDNA clones. These are extractions that do not involve amplifying either RNA nor DNA. With the CFSV S5 clone, the results are consistent and nothing is detected in the healthy plants while a dsRNA product of approximately 3000 nucleotides is found in the CFSD affected plant. This product is specific and hybridizes with the CFSV S5 clone **(Figure 1)**. In these experiments, we used CFSD infected and healthy plants of the varieties Secundina and CMC40 (M Col 1468). These varieties demonstrate two distinct types of plant reactions. Secundina is highly susceptible and develops mosaic symptoms on the leaves as well as the typical root symptoms. CMC40 is a tolerant variety that never has leaf symptoms and the root symptoms are general mild.

This is approximately the size expected of the genomic S5 segment of a reo-virus. The rice ragged stunt virus (RRSV) S5 segment is 2682 nucleotides and shares amino acid homology with the CFSV S5 sequence. The detection of the CFSV S5 genomic segment is further evidence of the presence of a reolike virus in cassava. Since it could only be detected in the CFSD affected plants, this is additional evidence that the virus is associated with the disease.

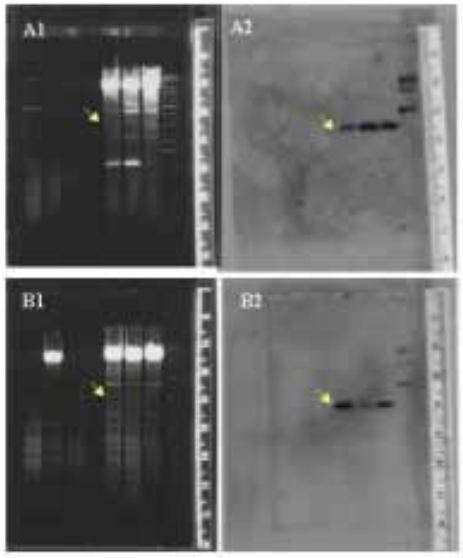


Figure 1. Double stranded RNA from healthy (lanes 1-3) and CFSD affected (lanes 5-7) cassava of the variety CMC40 (A1) and Secundina (B1). The hybridization of the dsRNA using the cDNA CFSV S5 clone for CMC40 (A2) and Secundina (B2). Lane 8 is a molecular weight marker. Each lane represents an individual plant.

Detection of the CFSV Segment 1

In the dsRNA extractions and hybridization, detection of the CFSV Segment 1 proved to be inconsistent. It was never detected in any health plants but was not consistently detected in the CFSD affected plants. When a band was detected, it appeared to be the same product that was detected by the CFSV S5 clone. It is known that the resolution of the dsRNA segments is fairly poor in agarose gels and that several "single bands" in agarose resolve into two or three products in polyacrylamide gels. In one experiment using polyacrylamide gel, the CFSV S5 segment was detected by hybridization but the CFSV S1 segment was not. The Segment 1 is detected from dsRNA extractions using specific primers designed from the CFSV S1 clone by reverse transcriptase PCR. PCR products of the expected size were amplified in the CFSD infected plants of Secundina and CMC40 but not in the healthy controls.

In one experiment, the primer CFSV S1 forward was used to prime the reverse transciptase reaction to produce cDNA. This was followed by PCR using the primers CFSV S1 forward and the CFSV S5 reverse. A PCR product of approximately 700 nucleotides was amplified and it hybridizes with the CFSV S1 clone, but does not hybridize with the CFSV S5 clone. This is evidence that the Segment 1 and Segment 5 are distinct genomic segments, and over 50 cDNA clones were produced and they are being analyzed. When the 700 nucleotide RT-PCT product is sequenced, it will be determine more information will be available on the genome of CFSV.

Diagnostic Method for the Detection of CFSD

Using dsRNA extraction followed by hybridization with the CFSV S5 is proposed as a diagnostic method. The dsRNA technique is too variable to be used for diagnostic purposes, but adding a specific hybridization greatly increases the confidence of this method. The clone CFSV S5 has proven to consistently detect CFSV and it is only detected in CFSD affected plants.

The limitations of this diagnostic technique are that the costs involved in the extraction and hybridization are moderately high per sample, and 5 grams of plant tissue are needed for each dsRNA extraction. The diagnosis can be done in two days, and this is a major advantage over the grafting with Secundina, which is the current standard detection method. Additional testing is underway to assure that all the different sources of CFSD are detected using this method.

Conclusions: Progress has been made in the characterization and detection of CFSV. This year, there has been additional characterization of the reo-like virus that is infecting cassava. The consistent detection of the CFSV using both specific hybridizations and RT-PCR S5 are steps forward in the association of the virus and the disease. Since plants produce few dsRNA, this type of extraction is appropriate for the partial purification of the genomic segments of the virus. Now that CFSV cDNA clones are available and can be used to identify specific products, the dsRNA technique has been a reliable detection method. This diagnostic method while relatively expensive will increase the confidence the testing required by other countries to receive CIAT cassava lines.

Until recently, CFSD was controlled by phyto-sanitary methods. With the extensive testing and identification of resistant varieties, we are prepared to recommend control strategies that are based on germplasm.

Contributor: Lee A. Calvert, Maritza Cuervo, Ivan Lozano, Natalia Villareal, and Jose Arroyabe.

CASSAVA AND TROPICAL FRUIT PATHOLOGY

Activity 1. Detection of a phytoplasma associated with cassava Frogskin Disease (FSD) in Colombia

Objective

To confirm the presence of a phytoplasma associated with cassava frogskin disease (FSD).

Introduction

Frogskin disease (FSD) is an important disease affecting cassava roots, whose causal agent remained unknown for many years. FSD has been reported with increasing frequency in Colombia, Brazil, and Venezuela. In Colombia, for example, incidences of up to 70% have been recorded in commercial fields in the production areas of Valle del Cauca, Cauca, Meta, and the North Coast. Disease symptoms consist of small, longitudinal fissures distributed throughout the root. As the roots increase in diameter, the fissures tend to heal, giving the injuries a lip form. Root cortex or epidermis presents a cork-like appearance that is peels off easily. Depending on the severity of symptoms, the depth and number of lesions increase until the root becomes deformed.

This study evidences the existence of an association between FSD and phytoplasma. By applying molecular tools and microscopy, phytoplasma was successfully detected in FSD-infected cassava roots, leaf midribs, petioles, and peduncles.

Methodology

Plant tissue. Roots, stems, petioles, and leaf midribs of both FSD-infected and healthy cassava plants, grown in the field and greenhouse, were processed.

Microscopic analysis. Small pieces of tissue, about 1 mm × 2 mm, were excised and then fixed in 2%-3% glutaraldehyde/0.1M phosphate buffer. The samples for electron microscopy were prepared by ultra thin section (60-90 nm) and viewed with a transmission electron microscope.

DNA extraction. Total DNA was extracted as described by Gilbertson *et al,*1991.

Nested PCR analysis. The primer pairs P1/P7 or R16mF2/R16mR1 were used for the first amplification, with an annealing temperature of 55°C. For the nested PCR, diluted (1:30) PCR products were used for amplification, with the primer pair R16F2n/R16R2 at an annealing temperature of 50°C. PCR products were analyzed by electrophoresis on 1.5% agarose gel.

RFLP analyses. The amplified PCR products were digested with the restriction endonucleases *Taq* I, *Rsa* I, and *Alu* I. The restriction products were analyzed by electrophoresis on 5% polyacrylamide gel.

Cloning and DNA sequencing. Purified PCR products were 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.

Grafting. Cassava cuttings from the highly susceptible genotype Secundina were grafted on cassava infected plants.

Results: The presence of phytoplasma in different plant tissues of affected plants was confirmed by electron microscopy **(Figure 1)**. The specific primers R16mF2/R16mR1 and R16F2n/R16R2 were used in a nested PCR assay to detect phytoplasma. Nested PCR revealed 1.3 kb fragments in root, stem, and leaf samples from symptomatic plants **(Figure 2)**. No fragments were obtained from healthy plants.

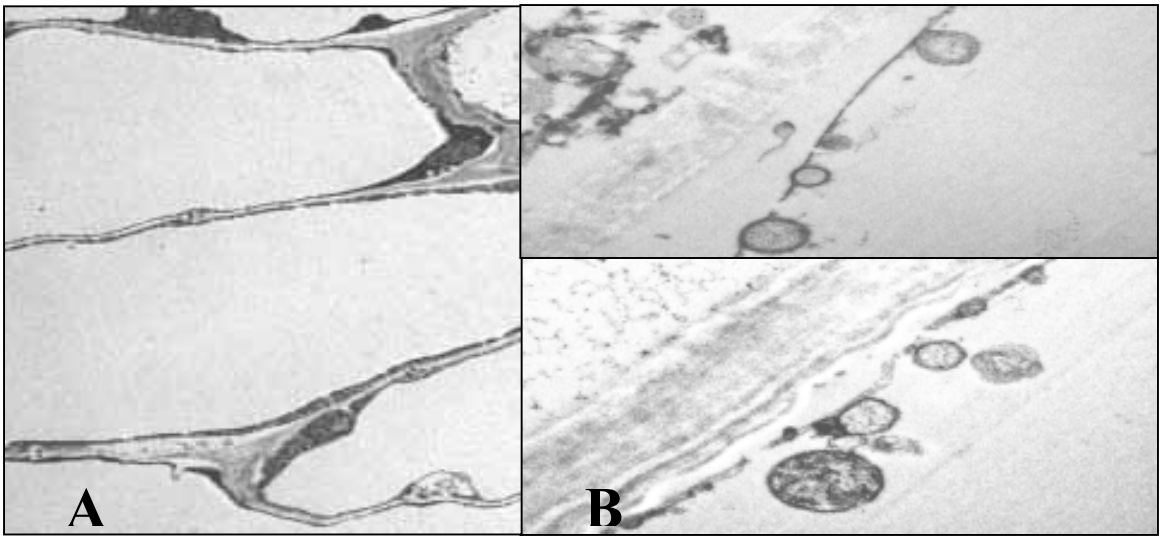


Figure 1. Electron microscopy of healthy (A) and infected (B) cassava tissue.

Phytoplasma was also detected by PCR in the leaves of grafted stem fragments on infected plants under greenhouse conditions, indicating successful transmission of the pathogen. Sequence analysis of a cloned fragment revealed that the cassava phytoplasma was similar to the Chinaberry yellows phytoplasma (GenBank acc. no. AF495657, 16SrXIII Mexican periwinkle virescence group) and Cirsium white leaf phytoplasma (GenBank acc. no. AF373106, 16SrIII X-disease group), both with a sequence homology of 100% and 99% in two partial fragments with a total of 1.01 kb **(Figure 3)**.

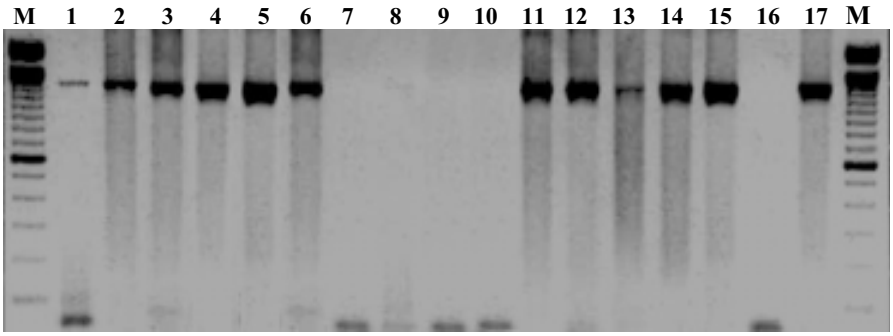


Figure 2. **A 1.3-kb fragment was amplified from diseased samples by. Lanes 1-2, infected stems; 3-4, infected petioles; 5-6, infected leaf midribs; 7, healthy roots; 8, healthy stems; 9, healthy petioles; 10, healthy leaf midribs; 11-12, infected roots; 13-14, infected stems; 15 and 17, periwinkle (*Catharanthus roseus*); lane 16, negative control; and lane M = 100 pb DNA marker.**

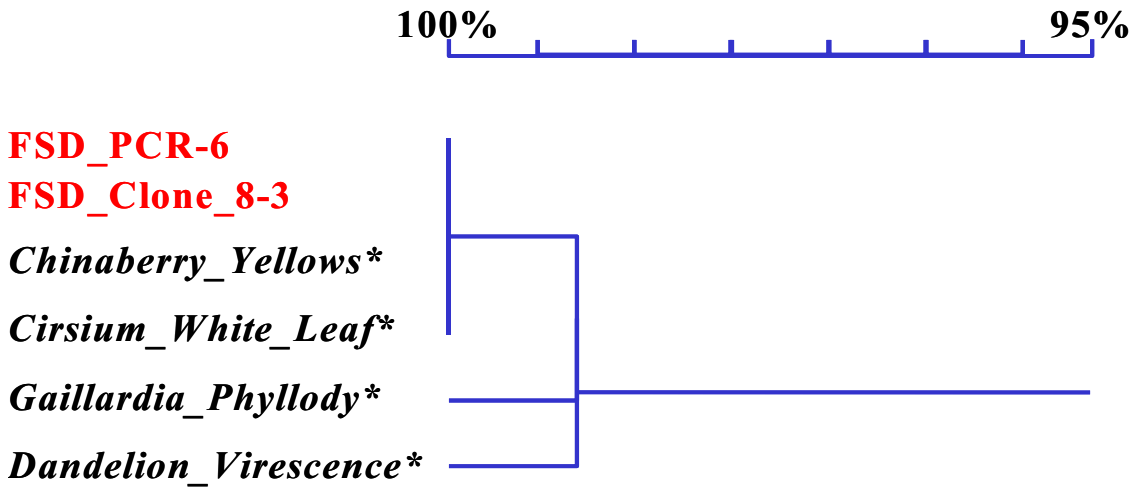


Figure 3. **Homology tree of 16S rRNA sequences from 6 phytoplasmas, including the sequences from cloned and direct PCR fragments obtained from cassava. * = GenBank accession.**

Digestion with *Taq* I, *Rsa* I, and *Alu* I of amplified products of different samples showed similar restriction patterns **(Figure 4)**.

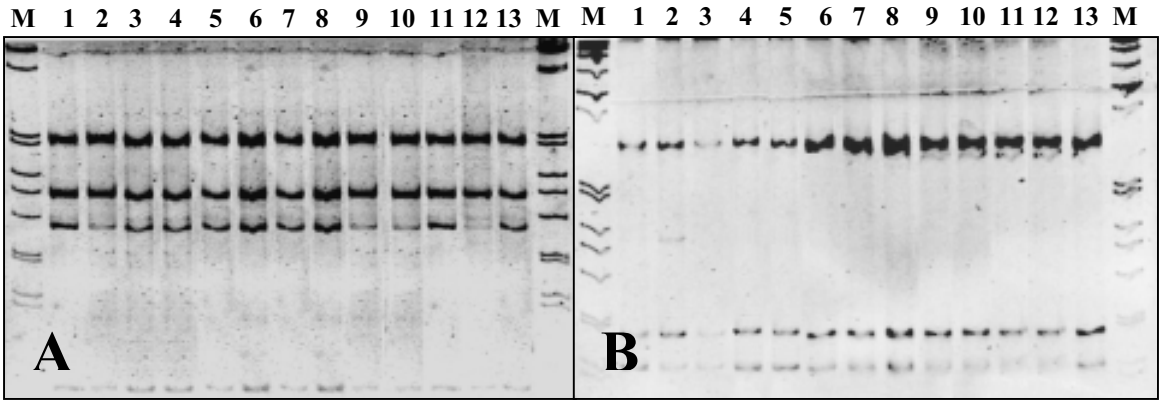


Figure 4. Restriction enzyme analysis of 16S rDNA after PCR amplification with primer pair R16F2n/R2, using the endonucleases *Rsa* I (A) and *Alu* I (B). Lane M = 1-Kb DNA marker.

Conclusions: Phytoplasma was successfully detected in all FSD-infected tissues by electron microscopy, and nested PCR techniques. Among the methods used in this study, PCR was the most sensitive for detecting, identifying, and classifying phytoplasma**.** Sequence homology from a cloned fragment, obtained from an infected cassava plant, was 100% similar to the Chinaberry yellows phytoplasma and 99% similar to that of Cirsium white leaf. This is the first report of a phytoplasma being associated with FSD in cassava. These results allow us to infer the possible role played by the phytoplasma in this disease. Future research will involve the evaluation of additional samples with other groups of enzymes as well as sequence analysis to classify the phytoplasmas. Experiments are underway to achieve remission of symptoms with the antibiotic oxychlortetracycline. Other research topics will include the development of specific primers for pathogen detection, vector identification, and classification of phytoplasmas associated with FSD.

References

Deeley, J., W.A. Stevens, and R.T.V. Fox. 1979. Use of Dienes' stain to detect plant diseases induced by MLOs. Phytopathology 69**:**1169-1171.

Dyer, A.T. and W.A. Sinclair. 1991. Root necrosis and histological changes in surviving roots of white ash infected with mycoplasma-like organisms. Plant Dis. 75:814-819.

Gilbertson, R.L., M.R. Rojas, L.D. Russel, and D.P. Maxwell. 1991. The use of the asymetric polymerase chain reaction and DNA sequencing to determine genetic variability among isolates of bean golden mosaic geminivirus in the Dominican Republic. J. Gen. Virol. 72:2843-2848.

Activity 2. Transmission of a phythoplasma affecting cassava seedlings and identification of indicator plants.

FSD symptoms recede after chlortetracycline treatment

Objective

To determine the causal agent of FSD in the cassava crop.

Experiment 1:

Materials and Methods: Plantlets from 10 Catumare- and 10 Manzana-affected field plants (Rozo, Palmira, Valle del Cauca, Colombia), and 20 disease-free plants of the same varieties (Montenegro, Quindío, Colombia) were treated by chlortetracycline.

For all experiments, the following precautions were included. Stakes were selected at harvest time to ensure FSD was present. CIAT virologists indicated that roots were affected by FSD according to symptoms. The stakes were planted in pasteurized soil, free of FSD, in plastic pots (10") or bags placed in isolated glass- or screenhouses at CIAT-Palmira (except Experiment no. 3). All plants were maintained in anti-aphid cages, and healthy Secundina plants were included. This was to monitor the presence of vectors. These plants did not show any symptoms during the experiments. Plants and cages were fumigated perodically, rotating the following products: Vertimec® 1.8% CE (0.5 cc/l of commercial product, abamectin), Malathion® (malathion, 1 cc/l of commercial product), Sistemin® (dimethoate, 3 cc/l of commercial product), and foliar fertilizers. All results presented in this report were checked by a CIAT Virology Specialist to make sure symptoms were caused by FSD.

Stem cuttings, with the medulla previously perforated with a drill, were immersed in a solution of 1500 ppm of tetracycline (750 ppm prepared based on a liquid solution and 750 ppm on capsules) during 10 min. on planting day. Plants of each treatment were planted at CIAT-Palmira in a glasshouse (temperature and RH: minimum 19 °C and 31%, maximum 28 °C and 98%), and in a screenhouse (temperature and RH: minimum 20 °C and 26%, maximum 39°C and 98%). All stem cuttings were maintained in different cages and other precautions were taken to avoid infection among plants. During 3 months, the soil was watered monthly with the same tetracycline solution (200 ml/plant). Plantlets were treated twice a week with 1500 ppm of tetracycline by foliar applications. After 3 months, the dose was reduced to 1000 ppm. Twenty plantlets (Manzana and Catumare) were also included from plants affected with FSD, and 20 plantlets from healthy plants, without FSD.

The plants were evaluated periodically to detect symptoms on leaves. After 4 months, stems of germinated plants were grafted with Secundina. Grafts were made directly on plants or through rootstock cuts, maintained in deionized water.

Results: Table 1 shows results obtained after grafting with Secundina.

Table 1. Effect of applications of tetracycline on phytoplasm of FSD-infected cassava Plants.

Variety	Origin of plant material	Place	Applications with chlortetracycline	No. of FSD-affected plants by grafting with Secundina[a]
Catumare		Screenhouse		3 (3)
Manzana		Screenhouse		3 (3)
Catumare		Glasshouse	Yes	5 (5)
Manzana	FSD-affected plants	Glasshouse		5 (5)
Catumare		Screenhouse		2 (2)
Manzana		Screenhouse		3 (3)
Catumare		Glasshouse		4 (5)
Manzana		Glasshouse		4 (5)
Catumare		Screenhouse		0 (4)
Manzana	FSD-free plants	Screenhouse		0 (3)
Catumare		Glasshouse	No	0 (5)
Manzana		Glasshouse		0 (5)

[a]No. of plants analyzed in parentheses.

All plantlets from affected plants in the field showed foliar symptoms in the Secundina grafts. No plantlet from the field of healthy plants showed symptoms in Secundina grafts on Catumare or Manzana. The effectiveness of obtaining grafts in the screenhouse was less than in the glasshouse. However, foliar symptoms were observed in both. Foliar applications of tetracycline do not reduce the incidence or severity of FSD.

Phytoplasm transmission to cassava (Secundina) plants susceptible to FSD, produced *in vitro*, and free of disease was successful through grafting.

Experiment 2:

Materials and Methods: Two foliar applications of chlortetracycline (1000 ppm, liquid form] were made weekly during 6 weeks to plants of SM 1219-9 and La Reina that showed leaf symptoms indicating FSD infection. Plants were located in a glasshouse (temperature and RH: minimum 19 °C and 31%, maximum 28 °C and 98%) at CIAT-Palmira. The stakes of these plants were obtained from FSD-affected plants (Jamundí, Valle del Cauca, Colombia).

Results and Conclusions: We observed that the affected leaves (curling and mosaic) remained affected through the applications (six plants). The new leaves also showed a severity similar to the affected leaves of plants (five) untreated with chlortetracycline. It is concluded that foliar applications with a high dosage of chlortetracycline do not inhibit leaf symptoms related to FSD.

Experiment 3:

Materials and Methods: Cuttings of Secundina genotypes, M Bra 383 and La Reina, from affected plants from the glasshouse (cuttings with FSD leaf symptoms) or from the screenhouse (cuttings without FSD leaf symptoms) were taken from plants in plastic pots. After cutting the true leaves of the cuttings, they were rooted in deionized water at different doses of chlortetracycline (injectable form, capsules can cause greater levels of intoxication of the plant, and are less effective against FSD). The cuttings were incubated in a laboratory with a controlled

temperature system (min. 20 °C, max. 25 °C), and 12-h alternate periods of light and darkness. The high humidity (66%-98%) was achieved through the use of closed boxes for the rooting of the cuttings.

Results and Conclusions: Table 2 presents the treatments used and results obtained.

Table 2. Effect of tetracycline on cassava frogskin disease (FSD)-affected cuttings.

Genotype	Source	Dose of chlor-tetracycline (ppm)	No. cuttings affected by FSD and total no. cuttings analyzed[a]
Secundina	Screenhouse, asymptomatic plants (foliar); these were previously in a glasshouse and expressed leaf symptoms	0	4 (4)
		2.5	2 (3)
		5	4 (4)
		10	4 (4)
		25	3 (6)
		50	0 (3)
	Glasshouse, plants affected with leaf symptoms	0	5 (5)
		2.5	5 (5)
		5	5 (5)
		10	5 (5)
		25	1 (5)
		50	0 (5)
M Bra 383	Glasshouse, plants affected with leaf symptoms from field plants with FSD-affected roots	0	5 (5)
		2.5	5 (5)
		5	5 (5)
		10	5 (5)
		25	3 (5)
		50	2 (5)[b]

[a]Evaluated 26, 32, and 40 days after initiation of chemical treatment. No. of plants analyzed given in parentheses.
[b]Two cuttings without FSD, one affected cutting, two cuttings with no leaf formation.

The inhibition of leaf symptoms caused by FSD was successful in two experiments using a dosage of 50 ppm chlortetracycline. The leaves of affected plants treated with 0 ppm of tetracycline showed presence of phytoplasm through nested PCR. Similar tests should be carried out with a greater number of cuttings. The cuttings of the variety La Reina did not show FSD foliar symptomology despite being infected and showing foliar symptoms in a glasshouse where cuttings were obtained to establish the experiment.

Cuttings treated with gentamicin (50 ppm) did not form leaves to adequately evaluate the antibiotic's effect on FSD. A treatment with 10 g/l of sugar to reduce the effect of phytotoxicity of chlortetracycline on the formation of leaves and roots did not function because it increased rotting of the stems by microorganisms.

Cuttings place in continuously oxygenated deionized water (by means of an air pump) with (50 ppm) and without chlortetracycline did not reduce the effect of phytotoxicity on the plants. Plants without the antibiotic rapidly (3 weeks) formed roots and leaves. Plants with the chemical treatments formed leaf primordials and rooting callus, but because of the phytotoxicity, no true leaves were formed.

In preliminary trials treating cuttings with 50 ppm of gentamicin, inhibition of FSD foliar symptoms was not observed.

Experiment 4:

Stakes with one, two, or three leaf buds from FSD-affected plants (Jamundí) of the variety SM 1219-9 were treated during 15 min. with 0, 25, and 50 ppm of tetracycline or gentamicin respectively, and planted in sterile sand under screenhouse conditions at CIAT-Palmira.

The substratum is humidified daily with the antibiotics (renewed every 3 days). This trial is in progress. Transplanting is scheduled to containers with sand, and applying nutritive solutions, constantly adding the antibiotics to obtain plants with roots developed sufficiently large to observe the effect of chlortetracycline on FSD. The treatments with 50 ppm of chlortetracycline and gentamicin inhibit germination of the leaf buds; 25 ppm permits germination, and adequate plant development.

Acknowledgments

- Robert Zeigler, Kansas State University
- Jaime Eduardo Muñoz, Universidad Nacional de Colombia, Palmira
- CIAT-Virology Unit facilitated the Secundina grafting, and Tulio Rodríguez performed the indexing.
- Agrovelez S.A. (Jamundí, Valle).

Activity 3. Identification of cultural practices and strategy to control frogskin disease in cassava.

Objective

To develop a methodology that includes a process for disinfecting cassava stakes of FSD.

Experiment 1:

Before planting, infected cassava stakes (M Bra 383, harvested at Jamundí, Valle del Cauca) were treated with thermotherapy. Temperature in the glasshouse ranged from 19 °C to 28 °C (although the temperature sporadically rose above 25 °C in the anti-aphid cage), and RH from 31% to 98%. Plants were evaluated periodically to detect FSD symptoms in leaves. Table 1 presents results.

Table 1. Effect of four hot water treatments on germination of stem cuttings and frogskin disease (FSD).

Pretreatment	Main treatment	Germination of stem cuttings (no. of cuttings)[a]	Disease incidence of FSD according to foliar symptoms (%)
None	None	5	100
54 °C during 5 min	54 °C during 10 min	4	50
56 °C during 5 min	56 °C during 10 min	2	50
58 °C during 5 min	58 °C during 10 min	3	0
60 °C during 5 min	60 °C during 10 min	1	0

[a]For each treatment six cuttings were used.

Stakes of the variety M Bra 383, treated with hot water for 15 min at 60 °C, reduced germination severely. Germination rates after 54 °C was highly acceptable. Plants treated at 58 °C or 60 °C apparently were clean of FSD, therefore the following experiment was designed with the main objective to improve plant vigor of treated plants to realize analysis for presence of FSD.

Experiment 2:

Fourteen cassava genotypes (M Chn 2, HMC 1, M Arg 2, M Bra 325, M Bra 829, M Bra 839, M Bra 856, M Bra 882, M Bra 886, M Col 634, M Col 1178, M Col 1468, M Cub 74, and M Per 16), infected by FSD, were harvested at CIAT-Santander de Quilichao. Thirty-two hot water stake treatments in combination with Agrodyne®SL (13.20 g/l iodine polietoxi-polipropoxi-poloetoxi–ethanol complex, 1.59 g/l iodic acid, Electroquímica West S.A., Medellín, Colombia) were tested for their effect on germination of stem cuttings and presence of FSD, applying two methods of detection. All stem cuttings were planted in plastic bags, and maintained isolated in an anti-aphid cage outdoors.

Germination of treated stem cuttings was better outside the glasshouse (temperature and RH: minimum 20 °C and 30%, maximum 38 °C and 96%) than inside, because stakes are less affected by saprophytic fungi. Stems of germinated plants (about 3 months old) were grafted onto Secundina. Table 2 presents results.

Table 2. Effect of hot water treatment and iodine on germination rates of stakes from cassava infected with frogskin disease (FSD).

Pretreatment		Main treatment		No. ungermi-nated stakes	No. germi-nated stakes	Germin-ation of stakes (%)	No. of plants with positive response to phytoplasm (PCR) or FSD (indexing), and no. of plants analyzed	
Without Agrodyne	With Agrodyne	Without Agrodyne	With Agrodyne				Nested PCR	Grafting with Secundina
-	-	-	25°C/5 m [c]	0	6	100.0	2 (2)	1 (2)
-	-	-	25°C/1 h [c]	0	12	100.0	1 (2)	1 (1)
No treatment	-	No treatment	-	1	13	92.9	0 (1)	
-	-	-	25 °C/5 m [d]	1	5	83.3	0 (2)	
49 °C/1 h	-	60 °C/10 m	-	6	16	72.7	1 (2)	0 (2)
-	-	60 °C/5 m	-	3	8	72.7	0 (2)	
55 °C/10 m	-	60 °C/10 m[b]	-	2	4	66.7	0 (1)	
-	60°C/5 m[c]	-	60 °C/10 m [c]	1	2	66.7		
-	-	-	49 °C/1 h [c]	6	4	40.0	1 (2)	
-	-	60 °C/10 m	-	6	3	33.3	0 (1)	1 (1)
60 °C/5 m	-	60 °C/10 m	-	24	11	31.4	1 (2)	
55 °C/10 m	-	60 °C/10 m	-	9	3	25.0	1 (1)	
-	-	-	60 °C/5 m [c]	3	1	25.0	1 (1)	
60 °C/5 m	-	60 °C/12.5 m	-	7	2	22.2	1 (2)	1 (1)
55 °C/10 m	-	60 °C/12.5 m[b]	-	4	1	20.0	0 (1)	
-	-	60 °C/20 m	-	6	1	14.3	0 (1)	0 (1)
60 °C/5 m	-	60 °C/15 m	-	9	1	10.0	0 (1)	
55 °C/10 m	-	60 °C/12.5 m	-	9	1	10.0		
60 °C/5 m	-	60 °C/10 m[b]	-	6	0	0.0		
60 °C/5 m	-	60 °C/12.5 m[b]	-	6	0	0.0		
55 °C/10 m	-	60 °C/15 m	-	8	0	0.0		
55 °C/10 m	-	60 °C/15 m[b]	-	5	0	0.0		
60 °C/5 m	-	60 °C/15 m[b]	-	5	0	0.0		
55 °C/10 m	-	60 °C/17.5 m	-	7	0	0.0		
55 °C/10 m	-	60 °C/17.5 m[b]	-	4	0	0.0		
60 °C/5 m	-	60 °C/17.5 m	-	8	0	0.0		
60 °C/5 m	-	60 °C/17.5 m[b]	-	5	0	0.0		
49 °C/1 h	-	60 °C/20 m	-	10	0	0.0		
60 °C/5 m	-	60 °C/20 m	-	10	0	0.0		
60 °C/5 m	-	60 °C/30 m	-	10	0	0.0		
-	-	60 °C/30 m	-	2	0	0.0		
49 °C/1 h	-	60 °C/30 m	-	10	0	0.0		

[a]Agrodyne®SL (13.20 g/l iodine complex polietoxi-polipropoxi-poloetoxi–ethanol, 1.59 g/l iodic acid, Electroquímica West S.A., Medellín, Colombia), 1 ml/l Inex A. [b]6 hours after pretreatment. [c]1.5 ml/l Agrodyne. [d]3 ml/l Agrodyne.

The treatment of stakes with Agrodyne without thermotherapy does not affect germination. The use of hot water at 60 °C up to 10 min reduces germination, but the plants obtained are possibly

healthier. At this temperature, the use of iodine can be included in the hot water treatment. It is unclear whether phytoplasm can be definitely inactivated through thermotherapy. A trend was observed that treatment is more important during a relatively long period, rather than at an extremely high temperature.

The most effective treatment is 49 °C during 1 hour (pretreatment) followed by a main treatment at 60 °C for 10 min, without using Agrodyne. The germination achieved with this treatment was most acceptable (72.7%, 17 stakes germinated). Cleaning was demonstrated as effective through indexing with Secundina.

Experiments 3 and 4:

Stakes with one, two, or three leaf buds of the SM 1219-9 genotype affected by FSD (Agrovelez, Jamundí), were treated at 60 °C during 5 min (pretreatment), and the following day at 60 °C during 10 min (main), and planted in sterile sand in a screenhouse (temperature and RH: minimum 20 °C and 26%, maximum 39 °C y 98%) at CIAT-Palmira. This treatment was carried out with 1 ml of Agrodyne/l in hot water. The treatment was too strong, and affected germination of leaf buds. No plant germinated, although callus was present in the stakes. In another experiment, without Agrodyne, the temperature for the pretreatment was lowered to 55 °C (treatment 1) and to 50 °C (treatment 2), and for the main treatment to 60 °C (treatment 1) and 55 °C (treatment 2), but without promising results because no stake germinated.

Stakes of a leaf bud without heat treatment did not germinate either; stakes with two or three leaf buds germinated satisfactorily. Experiments are being scheduled with a treatment of leaf buds at 49 °C, with a duration between 30 and 60 min.

Acknowledgements

We thank CIAT-Virology for facilitating the Secundina grafts, and Tulio Rodríguez for carrying out the indexing. We also thank Agrovelez S.A. (Jamundí, Valle) for access to infected field materials, and James George (Central Tuber Crops Research Institute, Kerala, India) for suggestions concerning use of short stem cuttings.

Activity 4. Evaluation of the influence of the soil as a source of FSD vectors.

Objectives

1. To evaluate the soil as a possible source of microorganism vectors of FSD.
2. To evaluate whether the presence of aerial vectors is related with dissemination of the disease.

Materials and Methods: In the municipality of Sincelejo (Sucre), Chochó basin, the possible influence that the soil may have as a source of FSD vectors is being evaluated. In the region, the disease occurs most frequently in lots where the presence of FSD has been reported previously, unlike nearby lots that remain disease free. For the trial, the variety M Tai 8 was chosen as presenting high susceptibility to FSD, and being one of the commercial genotypes most cultivated in the region, facilitating the obtaining of seed from lots where the disease has never occurred. Treatments were:

- Healthy plants in the screenhouse,
- Diseased plants in the screenhouse,
- Healthy plants outside the screenhouse, and
- Diseased plants outside the screenhouse.

Two muslin cages were constructed, 1.80 m in height, one for healthy plants (10.5 m long x 5 m wide), and one for affected plants (4.5 m long x 5 m wide). The rest of the trial was planted outside the screenhouse.

An experiment design was used of divided plots with three repetitions. The experiment unit for healthy plants inside the screenhouse, consisted of a plot of nine plants, distributed in three furrows of three plants each planted at 1 m x 1 m. For the diseased plants, three plants were planted for each repetition.

Outside the cage, the same experiment design was kept—three plots of healthy and diseased plants, with two furrows for 11 healthy plants and eight diseased.

For the seed cut, the machete was disinfected in a solution of 1% sodium hypochlorite. As part of the management, insecticide was applied, 1 week Sistemin® (dimethoate, 3 cc/l of commercial product), and the following week Malathion® (Malathion, 1 cc/l of commercial product). Application was only made within cages, and to half the outside plots, so that of the three repetitions outside, half received insecticide, and the other half was conserved without application. A barrier of the commercial genotype M Ven 25 was planted between the two to also evaluate the effect of aerial vectors, or whether the transmission is carried out through some agent of the soil.

Evaluations of the treatments will be made at time of harvest, observing incidence and severity in the roots.

Activity 5. Isolation and characterization of *Agrobacterium tumefaciens* from soil and cassava roots.

Objectives

1. To isolate *Agrobacterium tumefaciens* from soil samples and cassava roots.
2. To evaluate the pathogenicity of strains isolated from several cassava varieties.
3. To characterize isolates of *A. tumefaciens* pathogenic on carrot disks using PCR with specific primers.

Materials and Methods

***Isolation of* Agrobacterium tumefaciens.** To isolate *A. tumefaciens*, samples were initially taken of both healthy and diseased cassava roots and of the soil where diseased plants were sown.

Of each soil sample, 100 grams were weighed and dissolved in 100 ml sterile distilled water (SDW), agitated for 30 minutes, and then left to settle for another 30 minutes. Four serial dilutions in 9 mL of 0.75% NaCl were performed based on this first mixture (base solution) and 0.1 mL of each was planted on DIM (D1) media, which is selective and differential for *Agrobacterium* sp. isolates. The Petri dishes were incubated at 30 ºC for 24 hours. Colony-forming units (CFUs) growing on D1 were counted and those of yellowish-orange color were selected and planted per isolate on the same media for purification.

The healthy and diseased roots collected were washed with deionized water and processed by separating the plant tissue in 4 layers depending on their distance to the phloem (layer 1 = external, layer 4 = near the phloem). The portion obtained from each layer was washed with deionized water for 15 minutes, disinfected in 50% alcohol and then excess alcohol was eliminated with SDW. Smaller portions of each sample were cut and placed on D1 medium and then incubated at 30ºC for 48 hours. *Agrobacterium* colonies of characteristic color (yellowish orange) in the D1 medium were selected and purified on this media.

Pathogenicity test. Bacterial colonies isolated from the soil and cassava roots **(Table 1)** were planted on nutritive agar with sucrose (5%) and incubated at 30ºC for 48 hours.

To evaluate pathogenicity on carrot disks, fresh tubers were washed in deionized water, then submerged in sodium hypochlorite (5%) for 10 minutes and subsequently washed with SDW. Tubers were finally dried with sterile towels and small carrot disks were cut and placed in sterile Petri dishes.

The colonies obtained were inoculated by puncturing around the vascular cambium of the carrot disks and incubated for 3 weeks at 25ºC in moist chambers. Each strain was inoculated by duplicate and gall formation around the vascular cambium was observed on a daily basis.

Carrot disks inoculated with SDW were included as negative check, and *A. tumefaciens* isolate 1182 as positive check.

Two tissue-culture grown varieties Secundina and M Bra 383 were used to test the pathogenicity of strains isolated from cassava. The in vitro plants were planted on sterile soil, kept in cages to avoid the presence of insects as possible disease vectors, and fertilized weekly by intercalating the following three NPK fertilizers: 15-15-15, Coljap Producción 5-15-30, and Coljap Desarrollo 30-7-6, produced by Industria Agroquímica S.A., Bogotá, Colombia. Inoculation was carried out 1 month afterwards. Strains selected for a pathogenicity test in cassava were isolated from cassava roots (isolates 23, 24, and 27) with typical symptoms of frogskin disease and from soil samples of infected cassava crops (isolates 28, 29, 30, 33, and 35B).

For inoculation, a bacterial suspension of 1x108 CFU/mL (0.5 absorbancy) of the strains was prepared to inoculate the soil, roots, or base of corona. The soil was inoculated by adding the bacterial suspension to the surface. To inoculate the roots, plants were removed from the soil, their roots washed and tips cut, after which they were submerged in bacterial suspension for 30 minutes. Plants were then re-planted and placed within the cage. To inoculate the base of the corona, a puncture was made diagonally in this part of the plant using a 1-mL syringe to inject the inoculum. Once the inoculation was finished, all plants were placed in the cages and kept in a closed growth chamber, with 12 hours daylight, at a constant temperature of 20-25ºC and 80% relative humidity, thus favoring the colonization of the pathogen. Each month the roots were evaluated under the stereoscope to determine any changes in morphology.

Molecular characterization of isolates. The DNA was initially extracted from 43 strains isolated from soil and roots that resulted positive in pathogenicity tests on carrot disks and the check strain of *A. tumefaciens* (1182). Extraction was performed following the Boucher *et al.* (1985) protocol and the concentration of all strains was adjusted to 20 ng/µL to be amplified with the specific primers VCR/VCF paired with the Vir C region of the Ti plasmid of *A. tumefaciens* (Sawada, 1995). The Vir C region forms part of the Ti plasmid virulence genes that measured the transfer of plasmid T-DNA to the plant where an excessive proliferation of cells occurs, causing one or more galls to form. Each PCR reaction was carried out in 25-µL final volume, with final concentrations of 0.2 mM dATP, dCTP, dGTP, and dTTP; 1.5 mM $MgCl_2$; 1.5 U *Taq* polymerase; 0.1 µM of each primer; 1X *Taq* polymerase buffer; and 100 ng template DNA. For the reaction of the negative check, the DNA was replaced by SDW. The PCR was performed in a MSJ-Research PTC-100 thermal cycler with the following amplification program: 2.5 min at 95°C; 40 cycles of denaturation for 1 min at 95°C, pairing for 1 min at 55°C, and extension for 2 min at 72°C; and a final extension for 7 min at 72°C.

The amplification product was visualized in 2.0% agarose gel with TBE 0.5X buffer dyed with ethidium bromide (1 µL disolution at 10 mg/ml for 100 ml agarose gel). A 100-bp molecular weight marker was used to estimate the size of the amplified fragments.

Results

Pathogenicity test. Fifty-five strains with typical *A. tumefaciens* morphology were initially isolated from soil and cassava roots in D1 media. All were submitted to pathogenicity tests on carrot disks, where gall formation was observed 2 weeks after inoculation **(Figure 1)** with 43 of the isolated strains **(Table 1)**.

The pathogenicity test in cassava has not yet shown changes in root morphology. Inoculated plants have been periodically examined and no apparent changes have been observed in the roots. To promote plant development and root enlargement, all inoculated plants were transplanted to large pots and will be monitored monthly until roots reach a size in which any morphological change resulting from disease caused by the inoculated strains can be observed by the naked eye.

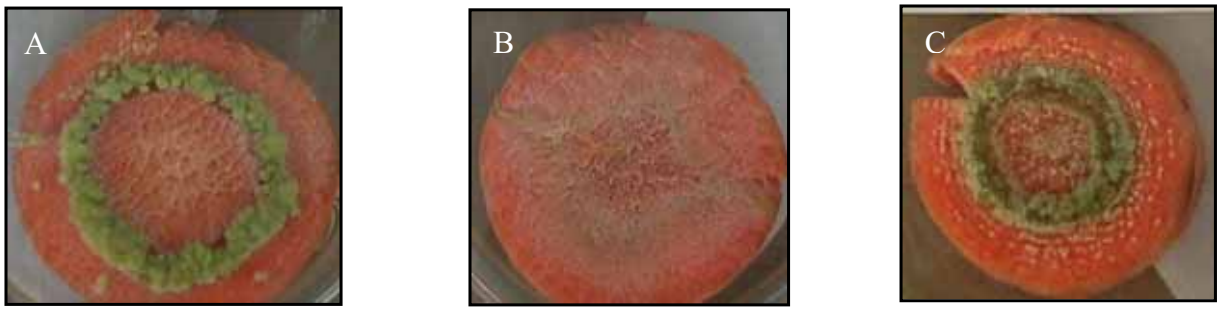


Figure 1. Gall formation on a carrot disk. A. Positive control (*A. tumefaciens* strain 1182). B. Negative control (sterile distilled water). C. Isolate 23 (root, genotype CM 2772-3).

Once the pathogenicity test was performed, the DNA of positive strains was extracted and quality visualized. The concentration of all strains was adjusted in 20 ng/µL and was amplified with the specific primers VCR/VCF. A 730-bp band was observed in the check strain 1182 and in most of the amplified strains **(Figure 2)**.

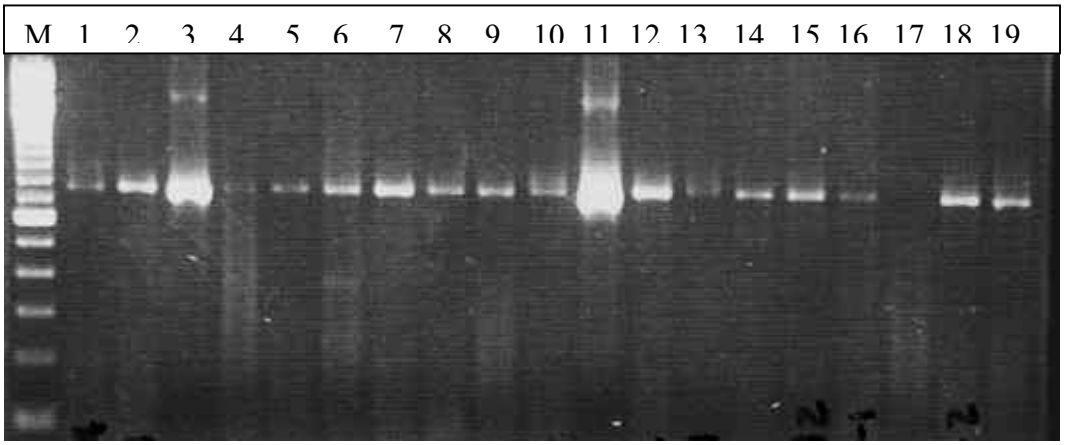


Figure 2. Amplification of DNA of *A. tumefaciens* strains obtained with the specific primers VCR/VCF from samples of infected cassava roots and soil. Lane M = 100-bp marker; lanes 1-19 = *Ralstonia solanacearum* (18, 23, 24, 26B, 27, 28, 30, 32, 33, 40, 1182, 21, 10, 11, 15, 19, 1, 6); lane 17 = negative control.

Table 1 presents the results obtained in the pathogenicity test and the amplification of strains with VCR/VCF primers.

Table 1. Pathogenicity of *A. tumefaciens* isolates and DNA amplification with specific primers (Vir C).

Isolate no.	Source	Origin	Identification	Pathogenicity 1	Pathogenicity 2	Specific DNA amplification
1	Root 1	Growth chamber	Petri dish 1	-	+	+
2	Root 1	Growth chamber	Petri dish 2	+	-	+
3	Root 2	Growth chamber	Petri dish 1	+	+	+
4	Root 2	Growth chamber	Petri dish 2	+	+	+
5	Root 2	Growth chamber	Petri dish 3	+	+	+
6	Root 4	Growth chamber	Petri dish 2	+	+	+
7	Root 4	Growth chamber	Petri dish 3	+	+/-	+
8	Root 1, diseased	Santander de Quilichao	Layer 1	+	+	+
9	Root 1, diseased	Santander de Quilichao	Layer 2	+	+	+
10	Root 1, diseased	Santander de Quilichao	Layer 3	+	+	+
11	Root 1, diseased	Santander de Quilichao	Layer 4	+	+	+
12	Root 2, diseased	Santander de Quilichao	Layer 1	+	+	+
13	Root 2, diseased	Santander de Quilichao	Layer 2	+	+	-
14	Root 2, diseased	Santander de Quilichao	Layer 3	+	+	+
15	Root 2, diseased	Santander de Quilichao	Layer 4	+	+	+
16	Sterilized soil	Quindío	Dil 10^1	-	+/-	+
17	Soil of a healthy crop (Catumare)	Quindío	Dil 10^2, 2nd camping	+/-	+/-	+
18	Root (Manzana)		Layer 2	+/-	+/-	+
19	Root (GM 309-7)		Layer 1	+	+	+
20	Root (GM 309-7)		Layer 2	+/-	+/-	+
21	Root (GM 309-7)		Layer 4, colony 1	+	+	+
22	Root (GM 309-7)		Layer 4, colony 2	+	+	+
23	Diseased root (CM2772-3)	CIAT	Layer 4	+	+	+
24	Diseased root (MBRA 383)	Palmira	Layer 1	+	+	+
25	Diseased root (Catumare)	Palmira	Layer 4	+	+	+
26A	Diseased root (Manzana)	Palmira	Layer 1	+	+	
26B	Diseased root (Manzana)	Palmira	Layer 2	+	+	+
27	Diseased root (Venezolana)	Sincelejo, Sucre	Layer 4	+	+	+
28	Soil of a diseased crop	Jamundí, Valle	No. 2, colony 2	+	+	+
29	Soil of a diseased crop	Jamundí	No. 3, colony 2	+	+	-
30	Soil of a diseased crop	Jamundí	No. 4, colony 1	+	+	+
31	Soil of a diseased crop	Jamundí	No. 5, colony 1	+	+	
32	Soil of a diseased crop	Santander de Quilichao	No. 1, colony 2	+	+	+
33	Soil of a diseased crop	Sincelejo	No. 1, colony 1	+	+	+
34A	Soil of a diseased crop	Sincelejo	No. 2, colony 1	+	+	+
34B	Soil of a diseased crop	Sincelejo	No. 2, colony 2	+	+	+
35A	Soil of a diseased crop	Sincelejo	No. 3, colony 1	+	+	-
35B	Soil of a diseased crop	Sincelejo	No. 3, colony 2	+	+	+
36	Soil of a non-diseased crop (Catumare)	Quindío	Colony 1	+	+	+
37	Soil of a non-diseased crop	Quindío	Colony 2	+	+	+

Isolate no.	Source	Origin	Identification	Pathogenicity 1	Pathogenicity 2	Specific DNA amplification
	(Catumare)					
38	Soil of a non-diseased crop (Catumare)	Quindío	Colony 3	+	+	+
39	Soil of a non-diseased crop (Catumare)	Quindío	Colony 4	+	+	-
40	Soil of a non-diseased crop (Manzana)	Quindío	Colony 1	+	+	+
41	Soil of a non-diseased crop (Manzana)	Quindío	Colony 2	+	+	
42	Soil of a non-diseased crop (Manzana)	Quindío	Colony 3	+	+	+
43	Soil of a non-diseased crop (Manzana)	Quindío	Colony 4	+	+	+
1182	Agrobacterium tumefaciens	Biotechnology Project-CIAT		+	+	+
Ti plasmid	Agrobacterium tumefaciens	Miniprep				+

References

Boucher C; Barberis P; Trigalet A; Demery D. 1985. Transposon mutagenesis of *Pseudomonas solanacearum:* isolation of Tn5-induced avirulent mutants. J Gen Microbiol 131:2449-2457.

Sawada H; Ieki H; Matsuda I. 1995. PCR detection of Ti and Ri plasmids from phytopathogenic Agrobacterium strains. Applied and Environmental Microbiology. February, p. 828-831.

Activity 6. Identification of RAMS markers to evaluate genetic diversity of selected isolates of *Ralstonia solanacearum* obtained from plantain.

Objective

To detect and characterize *Ralstonia solanacearum* isolates by PCR and RAM analyses.

Methodology

Isolates. A total of 107 *R. solanacearum* isolates were obtained from diseased plants, soil and water samples from different regions of Colombia by isolation on TZC medium (Kelman 1954). Only Gram-negative isolates, positive to oxidase and KOH, were used for PCR.

DNA extraction and PCR analysis. The DNA extraction of 87 isolates was performed as described by Boucher *et al.* (1985). The other 20 isolates were evaluated using whole cells by boiling a colony resuspended in 100 µL sterile distilled water (Seal *et al.*, 1993). The OLI1 and Y2 primers used by Seal *et al.* (1993) were used to amplify DNA from all isolates. Each PCR reaction was performed in 25-µL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 µL 10X *Taq* polymerase buffer; 1.5 mM $MgCl_2$; 0.5 U *Taq* polymerase; 0.5µM primer; and 100-150 ng template DNA or 5 µL boiled culture. Amplification was carried out in a MSJ-Research PTC-100 thermal cycler programmed for 2 min at 96°C; 50 cycles of denaturation for 20 s at 94°C, annealing for 20 s at 68°C, and extension for 30 s at 72°C; and final extension for 10 min at 72°C.

RAM analysis. A total of 107 isolates were evaluated with seven RAM primers (ACA, CCA, CGA, AG, CT, GT and TG). ACA, CCA, and CGA were useful to evidence polymorphisms between isolates. Each PCR-RAM reaction was performed in 12.5-µL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 µl 10X *Taq* polymerase buffer; 1.5 mM $MgCl_2$; 0.5 U *Taq* polymerase; 0.5 µM primer; and 50-100 ng template DNA. Amplification was performed in a MSJ-Research PTC-100 thermal cycler programmed for 5 min at 95°C, 37 cycles of denaturation for 30 s at 95°C, annealing for 50 s at 49°C (ACA primer), 55°C (CCA primer) or 62°C (CGA primer), and extension for 2 min at 72°C; and final extension for 7 min at 72°C.

Results: Results showed that, by specific OLI1-Y2 PCR amplification, 36 of the 107 isolates **(Table 1)** evaluated can be classified as *R. solanacearum*, confirmed by the presence of the 288-bp band similar to the check isolates **(Figure 1)** included in the evaluation.

High genetic polymorphism was observed among the *R. solanacearum* isolates from different localities, using the primers ACA, CCA, and CGA. Despite the fact that the primers ACA and CGA were useful to detect polymorphisms among strains, the CCA primer amplified DNA of all isolates and the products obtained showed clear bands **(Figure 2)**. Groups formed by strains from the same habitat (plant tissue, soil, or water) were observed.

The dendogram evidences high genetic polymorphisms among isolates.

In addition, two different groups were formed. Isolates R22 and R18 (obtained from water) were grouped with isolates g175, g214, g216, which had previously identified by other scientists as *R. solanacearum.* Both groups consist of isolates obtained from soil and plants.

Table 1. List of *Ralstonia solanacearum* isolates obtained from different sources by PCR amplification.

Isolate no.	Source
1, 2, 9, 10, 11, 79	Pseudostem, plantain affected by Moko
18	Water, source of a stream
22	Water
48, 53, 56	Soil, 0-30 cm
47	Soil, 30-60 cm
59	Soil, 5 m below focus, 0-30 cm
50, 54, 62, 63	Soil, 10 m below focus, 0-30 cm
68, 69	Soil, focus treated a year ago, on the surface
70, 81	*Emilia sanchifolia* (weed)
73	Black nightshade (*Solanum nigra*)
78	Pulp with borer, near focus
Dapa 2, Dapa 4	Soil of Heliconia plants affected by Moko
Heliconia 2, 11, 12	Heliconia plant, affected by Moko
G176, g214, g216, g217, g218	Check strains from collection

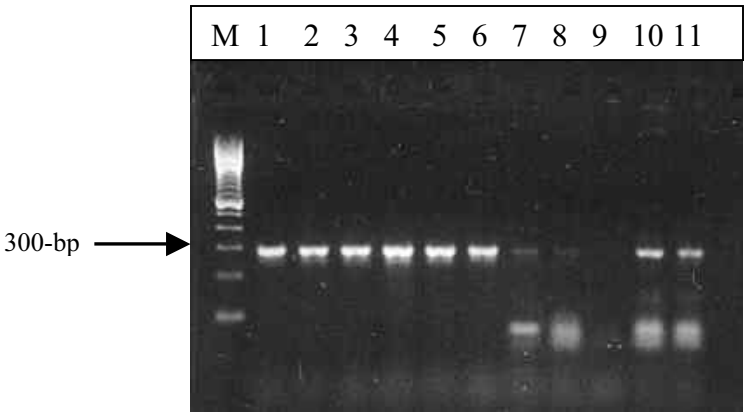


Figure 1. PCR patterns from genomic DNA of different strains, using OLI1 and Y2. Lane M = 100-bp marker; lanes 1-8 and 10–11 = *Ralstonia solanacearum* isolates; lane 9 = negative control (without DNA).

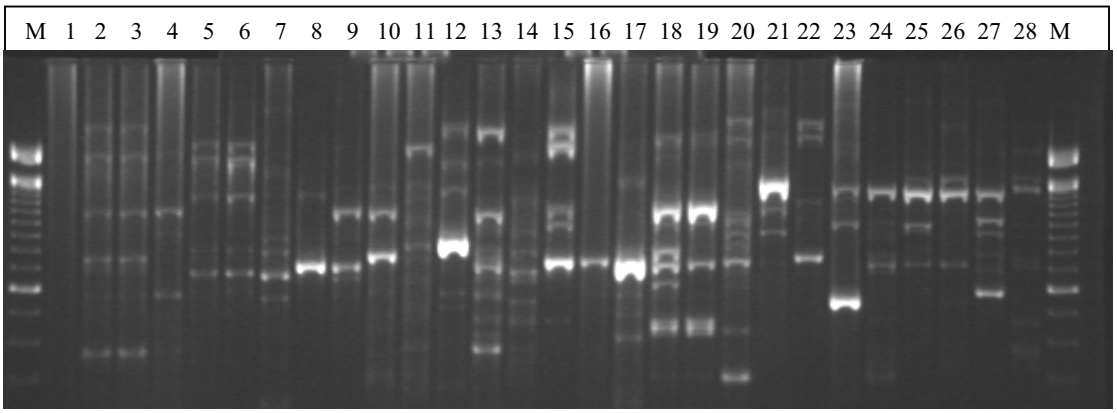


Figure 2. **RAM patterns from genomic DNA of different strains of *Ralstonia solanacearum* with the CCA primer. Lanes M = 100-bp marker; lane 1 = negative control; lanes 2-28 = *Ralstonia solanacearum* (1, 2, 3, 9, 10, 11, 18, 22, 47, 48, 50, 53, 54, 56, 59, 62, 63, 68, 69, 70, 73, 78, g175, g214, g216, g217, g218).**

Activity 7. Evaluation of the effect of hot water treatment of stem cuttings, biocontrol agents, selection of healthy stem cuttings and fertilizers on *Phytophthora* Root Rot under field conditions in Cauca and Quindío. Application of fungicides will be included as control treatments.

Cauca

Methodology: To evaluate the effect of five control practices on *Phytophthora*, which induce root rot in cassava, experimental plots were established on a farm in the Municipality of Caldono (Cabuyal Village District), Department of Cauca, Colombia, in April 2002. The farmer is indigenous and belongs to the Interinstitutional Consortium for Sustainable Agriculture in Hillsides (CIPASLA, its Spanish acronym).

At planting stakes were grouped for use in five treatments, which were then evaluated for their effect on the incidence and severity of root rots in the harvested roots of each group. The types of control are described in Table 1. For all treatments, stakes of the regional cassava variety Algodona (M Col 1522) were used, and chicken manure was incorporated into the soil at 2.5 t/ha. The experimental design was randomized complete blocks, with two replicates and 44 plants per treatment. As checks other cassava genotypes that had previously given high yields in field experiments were planted in the same plot, with 38 plants per genotype. These genotypes were CM 7438-14, M Bra 383, SM 1053-23, SM 1058-13, and SM 1937-1. All plots were planted in association with beans.

Results: In this trial, the heat treatment did not affect stake germination **(Table 1)**. No root and stem rot was observed. In spite of fertilization with chicken manure the root yield was low. *Trichoderma* enhanced yield dramatically, 65% compared to tradicional farmer's practice. By training farmers to use this technology and by analysis of soil samples, yields in this part of Cauca will increase.

Quindío

Methodology: Different control practices for *Phytophthora* spp. were evaluated for disease incidence and severity, and for yield in one field trial at "La Elena" Farm, Municipality of Montenegro. The trial was planted in November 2002 with the local variety Chiroza (M Col 2066). Treatments were as follows:

1. Integration of the following practices:
   a. Selection of high quality stem cuttings, including 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 then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil around the stake, 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.

Table 1. Effect of different control practices to manage root-rot on germination, Caldono, Department of Cauca, Colombia.

Treatment	Germination (%)[a]	Yield (T/ha)[b]
Algodona (M Col 1522)		
Stake selection[c]	96.6	4.0
Thermotherapy[d]	93.3	5.7
Traditional farmer's practice	95.3	5.1
Trichoderma strain 14PDA-4[e]	88.6	8.4
Chemical control[f]	98.9	6.0
Check varieties		
CM 7438-14	100.0	
M Bra 383	100.0	Will be harvested at the end of Octubre 2003.
SM 1053-23	94.6	
SM 1058-13	100.0	
SM 1937-1	97.4	
Average	96.5	5.8

a. 30 days after planting.
b. 16 months after planting.
c. Stakes were selected for their stake quality.
d. Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.
e. Strain 14 PDA-4 of the fungus *Trichoderma* sp. which attacks root rot fungi (*Phytophthora* spp.), was used to make a suspension of 1 x 10^6 conidia/mL. Planting stakes were then inoculated by immersing in the suspension for 10 min. The suspension was also applied to the soil near the base of each plant.
f. Planting stakes were immersed for 5 min in Ridomil® (metalaxyl) at 3 g/L of water.

All plots were fertilized 45 days after planting with 500 kg/ha of the fertilizer mix Nitrax®, DAP, and KCl, applied at a rate of 1:2:2. A randomized complete block experiment design was used with three replicates and 55-60 plants per treatment.

Results: No differences in germination of stem cuttings were observed. *Phytophthora* sp. was successfully isolated from infected roots of trial plants. Chiroza can be considered as highly susceptible to Phytophthora root rot. The field experiment will be harvested in 2004. This year more than 2,500 Colombian farmers and researchers have visited this experiment to learn, first-hand, how to grow cassava and manage root rot diseases affecting this crop.

Acknowledgements
- Silverio González, Special, La Tebaida (Quindío).
- Rosy Santacruz, Universidad de Quindío, Armenia (Quindío) Biogreen, Palmira.
- Bolívar Muñoz and Adelmar Chucue, Interinstitutional Consortium for Sustainable Agriculture in Hillsides (CIPASLA), Pescador and Caldono (Cauca) respectively SENA and ICA, Armenia.

Activity 8. Use of organic matter and ash amendments as a strategy to manage cassava root rots and improve soil fertility in Tukanoan chagras in Mitú, Vaupés.

Objectives

To evaluate the effect of amendments of ash and organic matter, cutting selection and association with cowpea, on root rot incidence, and cassava yield.

Materials and Methods: The effects of applying ashes (200 g/plant), organic matter (200 g/plant) obtained from dead leaves taken from forest soil surface, and an ash:organic matter mixture at a 1:1 ratio on the yield of a native cassava crop were evaluated using a farmer participatory research approach. The selection of cuttings and the associated cropping of cassava with cowpea (*Vigna unguiculata*) were also compared with traditional management practices. The test site was a Tukano indigenous community located in Mitú (Vaupés, Colombia), where women grow cassava as a second-cycle crop in small plots of slash-and-burn agriculture with decreased soil fertility, called *chagras* in the northern Amazon rainforests. Cassava yields and root rot percentage were recorded.

Results: The incorporation of ashes mixed with organic matter, organic matter alone, and ashes alone into the soil increased cassava yields to 10, 8.41, and 8.38 t/ha, respectively, while the selection of cuttings produced cassava yields of 5.13 t/ha and the associated cropping of cassava/cowpea, without soil amendment, cassava yields of 4.99 t/ha. Crops under traditional management practices yielded 5.57 t/ha **(Table 1)**.

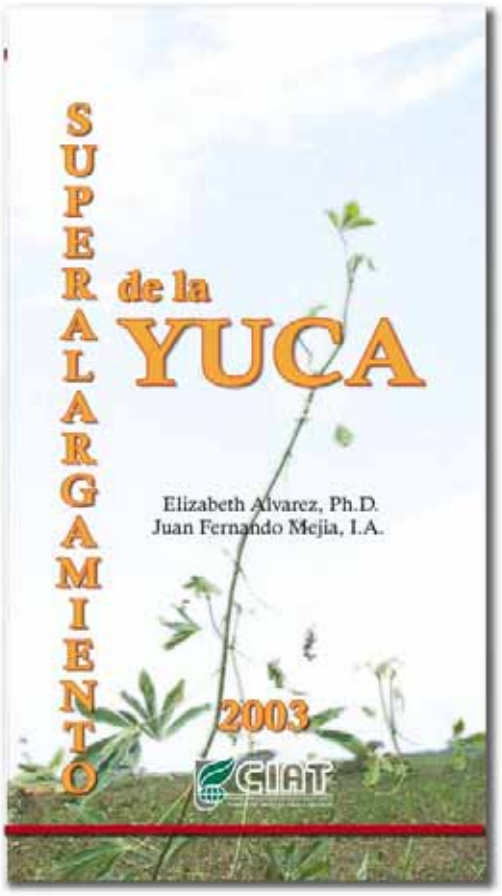
Table 1 shows root rot reduction from 21.97% to zero, when ash mixed with organic matter was incorporated into the soil, and cowpea was associated with cassava.

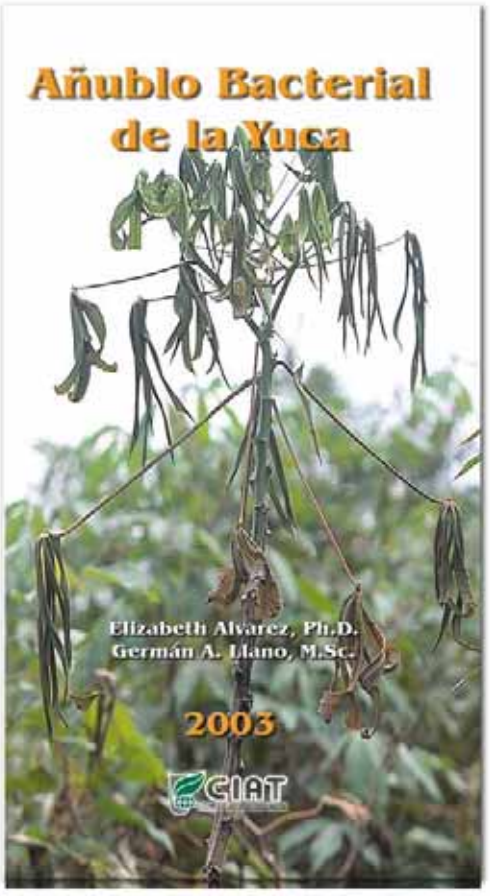
Table 1. Effect of the organic matter, ash, cutting selection and association with cowpea, on cassava yield and root rot severity in Mitú (Vaupés, Colombia).

Treatment	Yield (T/ha)	% Root Rot
Ash	8.38	1.89
Organic matter	8.41	7.81
Ash + organic matter	10.00	0.00
Cutting selection	5.13	3.10
Association with cowpea	4.99	0.00
Traditional management	5.57	21.97

Activity 9. Preparation of a brochure on disease control for farmers.

The following informative brochures were designed to help train farmers how to prevent and manage superelongation disease (SED) and bacteriosis (CBB), two diseases that seriously affect cassava production.





Activity 10. On-farm evaluations of the effects of cover crops, green manure, organic compost, and plant extracts on *Ralstonia solanacearum* in Quindío.

In close collaboration with plantain producers of Quindío, Colombia, field experiments were designed to validate the efficiency of velvet bean (*Mucuna pruriens*) and crotalaria (*Crotalaria juncea* L.) as control of *R. solanacearum* (Kelaniyangoda 1997). The incorporation of these cover crops into the soil aims to disinfect hotspots of plantain plants affected by Moko or banana bacterial wilt. In addition, Calfos (10% assimilable phosphorus, 48% calcium, 1% magnesium, and 1% trace elements), lime extract (residual product of CICOLSA, Armenia, Quindío) and plantain lixiviate are being tested because these products demonstrated good control of *R. solanacearun* in vitro (see CIAT annual report 2002).

During 2003, farmers and researchers planted velvet beans and crotalaria to obtain seed for future trials on Moko-free farms. As a result, this material can be multiplied to satisfy the high demand for this type of seed of farmers interested in evaluating the efficiency of both crops in controlling Moko.

The practices to be studied, as well as different combinations, were determined during field days held with farmers, representatives of the private sector, and staff of official entities. The advances achieved during these events resulted in the establishment of eight trials.

- Field trial (no. 1) established on a disease-free farm in Montenegro, Quindío, to train farmers.
- A complete block design was used. A farmer established another trial (no. 2) in the region, on his own initiative.
- A field trial (no. 3), arranged in three complete blocks, was established in a Moko hotspot (Armenia).

These trials involved the following treatments:

Trial no.	Treatment
1	No treatment (farmer-managed).
2	ICA's recommended Moko control methodology (with several modifications), which includes use of ditches, application of Roundup (glyphosate) and Basamid (dazomet), application of formol to the soil, and coverage with a piece of clear plastic.
3	ICA's methodology, but without the application of formol nor coverage with plastic. Included use of ditches; planting of crotalaria and velvet beans (intercropped); application of Roundup, plantain lixiviate, lemon juice, orange juice, and Calfos. Several products were periodically applied, alternating different products or practices. When velvet beans and crotalaria reach flowering, their leaves and stems were incorporated into the soil and then both cover crops were replanted.

Two more trials (no. 4 and 5) were established on another farm in La Tebaida (Quindío). These aimed to disinfect the Moko-contaminated soil. On each farm, 10 plots (7.5 m^2 per plot) were marked in form of a circle (radius = 5 m) around a production unit initially infected with Moko. The procedure was as follows:

- Prepare a ditch the width (30 cm) and depth (50 cm) of a garden spade, on the outside edge of the hotspot.
- Build a filtration well the size of a barrel (55 gallons).

- Keep the land out of the ditch.
- Apply Roundup to all lots.
- Eliminate three healthy plants around Moko-diseased plants by injecting a solution of Roundup at 20% (3 injections per plant), according to ICA's recommended methodology.

Each plot received a specific treatment, which included cost analysis of materials (Quindío, 2003):

1. Crotalaria: Planted at 20 cm between furrows, in a continuous line within the furrows and parallel to the exterior row of the circle.
2. Velvet beans: Planted, one seed per hole, at 50 cm between plants. A machete is used to keep the growth of velvet bean within plot limits. Cut plant material is incorporated into the soil.
3. Calfos: 5 tons/ha applied to the soil; reapplied at 4-month intervals (this schedule may vary among farmers). Cost = US$5/100-$m^2$ hotspot.
4. Farmer check: No treatment.
5. Formol: Holes 0.4 m deep made with a garden spade (or hole digger), leaving 0.5 m between holes; 750 ml of a formol:water (1:5) solution applied per hole. The entire plot was then covered with a piece of plastic. Cost = US$70/100-$m^2$ hotspot. Although CIAT has continuously recommended to not apply formol because of its high toxicity for humans, both ICA and INIBAP recommend its use.
6. Fresh coffee pulp: A 20-cm layer was applied. Once the pulp has dried in the field, another application is made. Cost = US$30/100 m^2.
7. Plantain lixiviate: 20 L applied per plot with a water can; three applications per year at 4-month intervals. Cost = US$10/100-$m^2$ hotspot.
8. Basamid: A dose of 0.2 kg incorporated per plot (250 kg/ha) and immediately covered with a piece of transparent plastic. Cost = US$60/100-$m^2$ hotspot.
9. Solid Fulvan, an organic fertilizer based on the fermentation of cardboard residues, press cake, and other wastes produced by sugar mills (Biogreen, Palmira, Colombia): 4 kg incorporated into the plot (5 t/ha); three applications per year, at 4-month intervals. Cost = US$5/100-$m^2$ hotspot.
10. Combination: Calfos, velvet beans, crotalaria, and solid Fulvan (see above). Direct contact of Calfos (or Huila phosphorous) with the seed should be avoided. Calfos and solid Fulvan should be applied three times per year, at 4-month intervals.

Two field trials (no. 6 and 7) were established nearby trials no. 4 and 5 in La Tebaida and Montenegro as negative checks.

The youngest stems of 30 production units were tagged and the following treatments applied:

- Manual weeding of the production unit.
- Application of a mixture of 1.8 kg Calfos and 2 kg solid Fulvan around the production unit.
- Planting of crotalaria around the production unit, 1 furrow in line, using a planter.
- Elimination of the terminal part of the raceme or acorn and hermetical bagging of inflorescence (closed, white plastic bag).

The check treatment involved 30 production units to which nothing was applied. Bags were left open.

Trial results are currently being evaluated. Several farmers who participated in the project have adopted the following practices:

- Use of Calfos or Huila phosphate.
- Use of iodine to disinfect tools, for example Agrodyne®SL (13.20 g/L iodine polyethoxi-polypropoxi-poloethoxi–ethanol complex, 1.59 g/L hydriodic acid, produced by Electroquímica West S.A., Medellín, Colombia) at 20% (manufacturer's dose was lower).
- Planting of Crotalaria.
- Application of fresh coffee pulp to Moko-diseased soil.

Farmers can prevent Moko occurring in their plantain crops by applying phosphorus, as evidenced in the comparison of different disease hotspots with disease-free lots. Tissue and soil analyses are needed, however, before this practice can be recommended as a disease management practice.

In Dapa (municipality of Yumbo, Valle del Cauca), the advance of Moko in a Heliconia crop for export was detained in a farmer-managed trial (no. 8), with CIAT's technical assistance. After a previous diagnosis in which *R. solanacearum* was isolated in TZC medium, the following measures were applied, in chronological order:

- Use of iodine (for example Agrodyne at 20%) to disinfect working tool, boots, and hands.
- Installation of a tray with Calfos, covered with a thin sheet of zinc, to disinfect boots or shoes before entering and leaving the diseased plot.
- Injection of both diseased and healthy plants within the diseased plots with Roundup at a concentration of 20%. Roundup was also applied to eliminate weeds.
- Fencing off of the three Moko-diseased plots with barbed wire.
- Digging of ditches around the diseased plots.
- Application of formol to the soil and to diseased plants, according to the above methodology.
- Coverage of Roundup-treated plants and ditches around the plots with a piece of transparent plastic.

Plants in production near the treated plots remained healthy.

Activity 11. Developing a heat treatment and establishing a plantain nursery.

An experiment was carried out at CIAT, with the participation of a plantain farmer of Valle del Cauca, with Moko-diseased suckers (Dominico Harton) to determine the optimal temperature for inactivating *R. solanacearum.* **Table 1** summarizes the treatments used.

Table 1. Temperature and duration of the test treatments.

Status of plants at the beginning of the experiment, temperature, and duration of heat treatment	Total no. of plants	Percentage plants[1]			
		Good growth, healthy	Retarded growth, but healthy	Severely affected by Moko	Dead
Moko-diseased suckers					
No treatment	10	0	10	0	90[2]
45°C/30 min	12	33	17	17	33[3]
47°C/30 min	12	42	0	17	42[3]
53°C/30 min	11	9	0	9	82[3]
Healthy suckers					
No treatment	5	80	20	0	0

1. Two months after heat treatment and planting.
2. Possibly by Moko.
3. Possibly by heat therapy.

In tandem the farmer carried out a series of experiments on his farm. After experimenting with a temperature of 51°C (30 minutes), the temperature was lowered to 49°C and the water:sucker ratio was changed from 2:1 to 3:1 (140 L water and 2 sacks of suckers, weighing 400 g on average) in a 50-gallon barrel heated with firewood instead of a gas burner. The temperature in the middle of the suckers reached 45°C. Losses in the commercially managed nursery, covered with saran, were minimal (10%). The farmer is planning to treat 4000 suckers.

Another farmer established a nursery in La Tebaida (Quindío), with a quarterly production of 5000 Moko-free Dominico Harton suckers, treated at 49°C for 30 to 60 minutes, as phytosanitary practices. All treated suckers germinated. Propagation material obtained in this nursery will be used to establish nurseries in other departments, such as Caquetá, Cauca, and the Amazon region.

Activity 12. Evaluating three doses of tetracycline to control the growth of bacteria isolated from oil palm.

Materials and Methods

Isolation of bacteria. The UNIPALMA plantation sent four small oil palms. Shoot, meristem, and root tissues were washed with deionized water for 15 minutes, then disinfected in 50% alcohol, excess alcohol being removed with SDW. Small pieces of each tissue were cut and placed on nutritive agar with sucrose (5%) and incubated at 30ºC for 24 hours. All possible colonies presenting different morphology and color were selected. Each isolate was cultured on the same medium for subsequent purification.

Antibiogram. Selected colonies were exposed to 3 doses of tetracycline (100, 500, and 1000 ppm) to select the concentration of antibiotic that inhibits microbial growth. Selected colonies were then mass planted in nutritive agar and 3 small filter paper disks, each moistened with a different dose of tetracycline, were placed on the surface of the Petri dish. Colonies were then incubated for 24-48 hours at 30ºC. The doses of antibiotic required to inhibit each isolate were determined.

Pathogenicity test. Each isolate was dyed using gram stain. The meristems of 3-month-old oil palms were inoculated with those isolates observed under the microscope as small gram (-) coccobacillus. The reaction of each was evaluated on a monthly basis.

Results: A total of 24 different bacterial colonies were isolated from oil palm. The results of the antibiogram and the pathogenicity test are indicated in **Table 1**. The bacteria evaluated were not pathogenic in oil palm.

Table 1. Growth inhibition depending on the dose of chlortetracycline applied.

					1st evaluation[1]			2nd evaluation[1]			
Isolate no.	Tissue	Palm no.	Colony no.	Gram	100	500	1000	100	500	1000	Patho-genicity
1	Root		1 (yellow)	-	+	++	++	+	++	++	-
2	Root		2 (white)	-	-	-	-	-	-	-	-
3	Shoot	3	1	+	+	++	++	+	++	++	-
4	Shoot	3	2	+	++	++	++	++	++	++	-
5	Shoot	3	3	-	+	++	++	+	++	++	-
6	Shoot	3	4	+	-	-	-	-	-	-	-
7	Shoot	3	5	-	-	-	-	-	-	-	-
8	Meristem	3	1	+	+	++	++	+	++	++	-
9	Meristem	3	2	-	+	+	+	+	+	+	-
10	Meristem	3	3	-	+	+	+	+	+	+	-
11	Meristem	2	1	-	+	+	+	+	+	+	-
12	Root		1 (white)	-	+	++	++	+	++	++	-
13	Shoot	4	1	-	-	-	-	-	-	-	-
14	Shoot	4	2	-	+	++	+++	+	++	+++	-
15	Root	3	1	-	+	++	++	+	++	++	-
16	Root	3	2	-	-	-	-	-	-	-	-
17	Root	3	3	+	+	++	++	+	++	++	-
18	Root	3	4	-	-	-	-	-	-	-	-

Isolate no.	Tissue	Palm no.	Colony no.	Gram	1st evaluation[1]			2nd evaluation[1]			Patho-genicity
					100	500	1000	100	500	1000	
19	Root	3	5	+	+	++	++	+	++	++	-
20	Root	3	6	+	+	++	++	+	++	++	-
21	Root	1	1	-	-	-	-	-	-	-	-
22	Root	1	2	-	-	-	-	-	-	-	-
23	Root	3	7	-	++	+	++	++	+	++	-
24	Root		3 (red)	-	-	-	-	-	-	-	-

1. Inhibition reaction on a scale of "-" to "+++": "-" = without inhibition halo; "+++" = large inhibition halo.

Activity 13. Diagnosing plant diseases and technical assistance.

Bacteriological and fungal diagnoses were performed on different samples obtained from farmers and institutions (see **Table 1**).

Table 1. Bacteria and fungi isolated from different crops and identified at the CIAT's Cassava Pathology Laboratory (Palmira, Colombia).

Location	Host plant	Disease	Detection method	Microorganism identified
Pereira	Onion	Nematodes	Isolation and direct observation under a light microscope	Nematodes
Ciat	Vanilla	Root and stem rot	Isolation and direct observation under a light microscope	*Pythium* sp.
Cajibío and La Cumbre	*Solanum quitoense*	Stem rot	Direct observation under a light microscope	*Phytophthora* sp.
Ciat	Cassava	Phytophthora root rot	Isolation and direct observation under a light microscope	*Phytophthora* sp.
San Pablo (Bolívar)	Cassava	Root rot	Isolation and selective media	Pythiaceae
Atlántico and CIAT	Cassava	Dry stem rot	Isolation and direct observation under a light microscope	*Diplodia manihotis*
San Pablo (Bolívar)	Cassava	Dry stem rot	Isolation and direct observation under a light microscope	*Botryodiplodia theobromae*
Dapa	*Heliconia* var. *estricta*	Moko	Isolation and selective media	*Ralstonia solanacearum*
Cali and Bitaco	Marjoram	Rust	Direct observation under a light microscope	*Puccinia rubsaameni*
Palmira	*Dendrobium*		Isolation and direct observation under a light microscope	*Colletotrichum*, *Alternaria*, and *Fusarium*

An *in vitro* bioassay to evaluate the effect of fungicides on *Colletotrichum* sp. isolates from blackberry showed that Molto EC (NA)® (Propiconazol + Prochloraz) was the most effective among the nine products evaluated.

At a farm in Sevilla (Valle del Cauca), a *Phytophthora*-resistance inducing agent was injected in avocado stems at doses of 3 cc/injection of 50% commercial product (Nutriphyte®, phosphorous acid: 43.4% P_20_5, potassium hydroxide, and potassium citrate: 40.3% K_2O).

Farmers say that disease incidence has been reduced with the application of the inductive agent, among several integrated management practices, such as disinfection after pruning and prevention of lesions during weeding.

Activity 14. Training farmers, technicians, and extension agents in participatory research, cassava and other crops management, and disease control strategies.

Training

- September 2002. Alejandro Corredor, Universidad de Caldas, Manizales. Taxonomy of *Phytophthora palmivora*.
- September – December 2002. Students from Universidad del Valle. Diana López y Adriana Arenas. Pathogenicity of *Agrobacterium tumefasciens* in carrot and "Coralito".
- October 3 2002. 15 students from Universidad San Buenaventura. Molecular tools for plant disease research.
- Ocober 28, and November 18 2002. 62 students from Universidad de Nariño. Molecular characterization of *Sphaerotheca pannosa*, *Xanthomonas axonopodis* and *Sphaceloma manihoticola*; integrated disease management; molecular tools for plant disease research.
- November 26, 2002. Universidad Nacional, Medellín. Three M.S students of Biotechnology. Molecular tools used in cassava pathology program.
- December 2002. Two research assistants from CENICAFE working on phytoplasma and biocontrol agents.
- March 14, 2003. Advisory for cassava assessment in Guainía, participatory research with indigenous and settlers.
- April 1, 2003. Pedro Molina, Miembro de la Comisión Evaluadora del Programa Redes de Asociación Cooperativa, Ministerio de Ciencia y Tecnología, Venezuela. Integrated cassava disease management.
- April 2, 2003. Universidad del Cauca. Professor Andrés Torres. Information on cassava pathology.
- April 4, 2003. Information on cassava diseases incidence in Valle del Cauca. Carlos Enrique Gómez and Greicy Andrea Sarria. ICA Palmira.
- April 22-May 2, 2003. Carlos Alberto Galvis. Cenicafé. Training in detection of Phytoplasma in coffee.
- May 8, 2003. Ten students from Universidad San Buenaventura. Diseases management and molecular techniques.
- May 20, 2003. Seven breeders form Cuba, Uganda, Thailand, and India. Cassava resistance to pathogens.
- June – September 2003. Technical assistance to Arvey Benavides, for *Phytophthora* management in lulo (*Solanum quitoense*).
- July 29–31, 2003. Gira tecnológica en cultivos de yuca con productores asociados y el equipo de trabajo de la Compañía Agroindustrial Yuquera. San Pablo (South of Bolívar Department).
- August 12, 2003. Rebecca Lee and Santiago Fonseca. Ceniflores (Asocolflores). Ecological practices for flowers disease management.
- August 15, 2003. Mario Pareja. Fundación Chemonics. Cassava disease management.
- July-Sepember, 2003. Hugo Martínez, Fundación Universitaria de Popayán (Ecology). Advisory for production of *Pleurotus ostreatus* y *Pleurotus sajor caju*
- January-September, 2003. Sandra Patricia Fajardo Daza and Rosa Judith Aranda Muelas, Universidad Nacional de Colombia, Ingenieria Agroindustrial, Palmira. Advisory for the production of *Pleurotus ostreatus*.

- September 8 2003. Most important cassava diseases. Ministry of Agriculture from Nigeria and eight attendants.

Workshops

October – November 2002. Four workshops with indigenous women from communities Macaquiño, Urania, Cachivera and Cucura communities, in order to create local agricultural research comitees. 87 participants.

June 9 – 13, 2003. Technicians and producers of plantain. Course: Integrated management of Moko disease in plantain. 23 participants.

Seminars

Detección de un Fitoplasma Asociado con la Marchitez Letal de Palma de Aceite (*Elaeis guineensis*) en Colombia. CIAT. Oct 30, 2002. E. Alvarez.

Development of ecological practices to manage *Phytophthora* root rot of cassava (*Manihot esculenta*) E. Alvarez, J. B. Loke, G. A. Llano. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 133.

Control of bud rot in oil palm, *Elaeis guineensis*, using resistance inducers. E. Alvarez, G. A. Llano, M. C. Feris, M. L. Hernández and S. M. Rodríguez. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 179.

Characterization and classification of *phytoplasmas* associated with oil palm (*Elaeis guineensis*). E. Alvarez and J. L. Claroz. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 284.

Detección de marcadores microsatélites asociados con la resistencia al Añublo Bacterial de la yuca (*Manihot esculenta* Crantz) en Colombia. P. X. Hurtado, E. Alvarez, M. Fregene and G. A. Llano. Presented at XXIV Congress of ASCOLFI. June 25 – 27, 2003. P. 25.

Caracterización genética y patogénica de *Colletotrichum* spp. agente causal de la antracnosis en guanábana (*Anona muricata*) en el Valle del Cauca. C.A Ospina and E. Alvarez. Presented at XXIV Congress of ASCOLFI. June 25 – 27, 2003. P. 6

Detecting the phytoplasm-frogskin disease association in cassava (*Manihot esculenta* Crantz) in Colombia. E. Alvarez, J.F Mejía, J.B Loke, L. Hernández, and G.A Llano. Phytopathology 93 (6): S4. Poster presented at APS annual meeting August 9 -13. Charlotte, NC, USA.

Detecting SSR markers associated with resistance to cassava bacterial blight (CBB) in Colombia. E. Alvarez, P. X. Hurtado, M. Fregene and G. A. Llano. Phytopathology 93 (6): S4. Poster presented at APS annual meeting August 9 -13. Charlotte, NC, USA.

Congress

8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand. Control of powdery mildew in roses by applying lixiviated plantain rachis compost. E. Alvarez, C. Grajales, J. Villegas and J. B. Loke. Poster presented at 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand, 2 - 7 February 2003. Vol 2: 272.

XXIV Congreso Nacional de Fitopatología, held by ASCOLFI, Armenia. June 25-27. Enfermedades de yuca y perspectivas de manejo sostenible. E. Alvarez. Conference presented at Congreso Nacional de Fitopatología, held by ASCOLFI, Armenia. June 25, 2003

APS annual meeting. August 9 - 13. Charlotte, NC, USA.

Publications

Alvarez, E., Mejia, J.F., and Valle, T.L. 2003. Molecular and pathogenicity characterization of *Sphaceloma manihoticola* isolates from south- central Brazil. Plant Dis. 87: 1322-1328.

E. Alvarez, J. B. Loke, S. Rivera y G. A. Llano Genética de la resistencia a pudrición causada por *Phytophthora tropicalis* en dos poblaciones segregantes de yuca (*Manihot esculenta* Crantz).. Revista Fitopatología Colombiana Vol 26 (2): 61 - 66. Cali, Colombia.

Identificación de genes análogos de resistencia a enfermedades en yuca, *Manihot esculenta* Crantz y su relación con la resistencia a tres especies de *Phytophthora*. M.Sc. thesis. Palmira, Colombia: Universidad Nacional de Colombia. 119 pp. 2003

Two brochures on management strategies of Cassava Bacterial Blight and Superelongation Disease.

Submitted

Assessment of integrated management practices of cassava root rots, by participatory research with indigenous from Mitú, colombian noreast amazon. Agren. Agricultural research and extension network.

Hurtado, P.X., Alvarez, E., Fregene, M. Búsqueda de genes análogos de resistencia asociados con la resistencia al añublo bacterial de la yuca. Revista Fitopatología Colombiana.

Hurtado, P.X., Alvarez, E., Fregene, M. Llano, G.A. Detección de marcadores microsatélites asociados con la resistencia a *Xanthomonas axonopodis* pv. *manihotis* en una familia de yuca (BC1). Revista Fitopatología Colombiana.

Concept Notes and Proposals

- Identification of a phytoplasm associated to Lethal Wilt in oil palm. Palmar del Oriente, Palmas de Casanare and Palmeras Santana. US$29.500. **Approved**
- Caraterización molecular de un fitoplasma que esta afectando el cultivo del lulo en Colombia. ICA. US$4.000. **Approved**
- Detección y manejo de microorganismos asociados con cuero de sapo en yucca. Ministerio de Agricultura y Desarrollo-Colombia. Project cost: US$104.500. Funds requested: US$45.000. **Approved**
- Avance en el desarrollo de tecnologías de cultivo y postcosecha para una producción rentable y sostenible de guanábana (*Anona Muricata* Lynn) Fase 2: FONDO DE COOPERACION ESPAÑOLA. Funds requested US$27.000. **Approved.**
- The mechanisms behind disease resistance in cereals. Presented with Royal Veterinary and Agricultural University (KVL, Denmark) and CORPOICA (Colombia). Presented to the Cereals Comparative Genomic initiative. Cost: US$260.000. Funds requested: US$200.000.
- Generación de nueva tecnología para el manejo de Mildeo Velloso en rosa y la caracterización genética de su agente causal, *Peronospora sparsa*, en Colombia. ASOCOLFLORES/CENIFLORES. Project cost: US$238.000. Funds requested: US$119.000.
- Desarrollo de medidas de manejo del Moko (*Ralstonia solanacearum*), para evitar la destrucción de las plantaciones de plátano en Colombia. Presented with ICA and CORPOICA to ASOHOFRUCOL (Colombia). Project cost: US$188.200. Funds requested: US$117.200.

- Medidas Tendientes a Evitar que el Moko (*Ralstonia solanacearum*) Destruya las Plantaciones de Plátano y Banano en Colombia. Presented to the Ministerio de Agricultura y Desarrollo de Colombia. Funds requested: US$71.428.

Theses for Master of Sciences degree

Germán A. Llano. 2003: Identificación de genes análogos de resistencia a enfermedades en yuca, *Manihot esculenta* Crantz y su relación con la resistencia a tres especies de *Phytophthora*. M.Sc. thesis. Palmira, Colombia: Universidad Nacional de Colombia. 119 pp.

Paula X. Hurtado: Evaluación de marcadores microsatélites y genes análogos, asociados a la resistencia de yuca a *Xanthomonas axonopodis* pv. *manihotis*. For a Master of Biology with emphasis in Plant Molecular Biology. Universidad de los Andes—Bogotá.

Theses for Master of Sciences degree in progress

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 of Plant Breeding.

Personnel – Staff: Elizabeth Alvarez, Germán A. Llano, John B. Loke, César A. Ospina, Juan Fernando Mejía, Juan Fernando Mejía, Lina María Tabares, Zulma Zamora, Tatiana Espítia, Herney Rengifo, Paula Hurtado.

Students and Technicians:

Universidad de los Andes—Bogotá:
Paula Ximena Hurtado
Universidad Católica de Manizales:
Samira Moreno
Colegio Bolívar: Cristina Londoño
Universidad del Valle: Sonia Ossa
Universidad de Caldas— Manizales:
Alejandro Corredor
Universidad de los Llanos—Villavicencio:
Sandra Milena Rodríguez
Técnico Agroforestal, Mitú, Vaupés:
Gabriel Paiva

Linkages with CIAT's Partner Institutions

ASOCOLFLORES
CLAYUCA
COLCIENCIAS
Corporación BIOTEC
Instituto de Investigaciones de Viandas Tropicales (INIVIT, Cuba)
IPRA (based at CIAT, Colombia)
Secretaría de Agricultura del Vaupés (at Mitú)
UMATAs (Mitú, Caicedonia, La Tebaida, and Montenegro)
Universidad Nacional de Colombia—Palmira (Valle del Cauca, Colombia)
Universidad de los Andes, Bogotá

Donors

Instituto Nacional de Investigación y tecnología Agraria y alimentacion INIA
Hacienda San José (Palmira)

ICA
Ministerio de Agricultura y Desarrollo Rural
Palmar del Oriente
Palmas de Casanare
Palmeras santana
PRONATTA

Collaborators-Local
CENICAÑA (Drs J. Victoria and F. Angel)
CLAYUCA (based at CIAT, Dr B. Ospina)
CORPOICA—Bogotá (Dr Jairo Osorio)
CORPOICA "La Libertad" (Villavicencio, Dr. A. Tapiero)
CORPOICA—Palmira (Dr G. Aya)
Corporación BIOTEC (Dr J. Cabra; B. Villegas)
Corporación para el Desarrollo Sostenible del Norte y Oriente Amazónico (CDA, Vaupés, Dr E. Polo and R. Peña)
CRIVA—Consejo Regional Indígena del Vaupés (Mitú)
ICA—Quindío and Valle (Drs E. Vargas, F. Varón, C. A. Montoya and C. Huertas)
Palmar del Oriente
Palmas de Casanare
Palmeras Santana
Secretaría de Agricultura del Vaupés (at Mitú, Dr G. Arbeláez)
Special (La Tebaida, Mr S. González)
UMATAs (Drs O. Holguín, L. Muñoz, and W. Ospina)
Unillanos—Villavicencio
Universidad Católica de Manizales
Universidad de Caldas—Manizales
Universidad de los Andes—Bogotá
Universidad del Valle (Cali)
Universidad Nacional de Colombia—Palmira

International
Cooperative Research Center for Tropical Plant Protection, University of Queensland, Brisbane, Australia (A. Drenth)
Iowa State University (Dr T. Harrington, Dr. T. Pepper)
Kansas State University (Drs S. H. Hulbert and R. Zeigler)
Michigan State University (Dr K. Lamour)
The Royal Veterinary and Agricultural University (Copenhagen, Denmark, Dr D. Collinge)

TROPICAL FORAGE PATHOLOGY

Activity 1. Characterization of *Xanthomona. campestris* pv. *graminis* isolates.

Introduction

We have previously reported a bacterial wilt disease of *Brachiaria* and its casual agent, (Zuleta et al., 2002. Manejo Integrado de Plagas y Agroecologia 64:41-47). *Xanthomonas campestris* pv. *graminis* infects a number of cultivated forage grasses. Some of the first symptoms are chlorotic/necrotic stripes along the leaves. As the disease advances, the whole leaf may die. Under severe conditions, the whole plant may turn yellow and die. Another typical symptom is wilting and curling of leaves without any discoloration or lesions, which result in quick plant death.

We have demonstrated that the pathogen is seed transmitted and is also transmitted vegetatively (AR-2001). Although the disease is not economically important to date, it is important for quarantine purposes.

Sixty-seven isolates of *X. campestris* pv. *graminis* have been collected from sites in Colombia from various genotypes of *Brachiaria* in order to determine pathogenicity and genetic diversity.

Families of repetitive DNA sequences such as repetitive extragenic palindromic (REP), enterobacterial repetitive intergenic consensus (ERIC), and box elements (BOX), which are present in all prokaryotes can be used for bacterial fingerprinting. Polymerase chain reactions (PCRs) based on these repetitive sequences, collectively designated as rep-PCR, have been used to assess variation among pathovars as well as to differentiate strains of the same pathotype of *Xanthomonas* species. In this study, we used rep-PCR with REP, BOX and ERIC primers to evaluate the genetic diversity of *X. campestris* pv. *graminis* isolates.

Materials and Methods

***Bacterial isolates*:** A total of 67 independent colonies of X*anthomonas campestris* pv. *graminis* were collected from naturally infected species of *Brachiaria* at Carimagua, Santander de Quilichao, Popayán, and Palmira. Leaves were cut into small pieces (approximately 1 $cm^{2)}$ and surface-sterilized in1% NaOCl solution for 2 min and in 70 % ethanol for 1 min. They were then rinsed with sterile deionized water, and macerated in sterile water. A dilution series of the macerated suspension was plated on nutrient agar for selection of independent bacterial colonies. Their pathogenicity was confirmed by inoculating a susceptible material (hybrid *Brachiaria* CIAT 36062). Selected colonies were grown in nutrient broth with shaking (200 rpm) at 28 ^{o}C. They were stored in 30% glycerol at -20 ^{o}C for use in further studies. Two isolates (CIAT 46 and CIAT 469) of *X. axonopodis* pv. *manihotis* were included as control.

***DNA isolations*:** Bacterial cells were grown overnight in Luria broth medium in a shaker (200 rpm) at 28^{o}C. Cells were collected in microcentrifuge tubes by centrifugation (8000 rpm for 10 min) and discarding the supernatant. The cells were re-suspended in 600-µl TE (50mM Tris, pH

8 and 50mM de EDTA) and stored at –20 °C for 10 min, and subsequently thawed at room temperature. Sixty-µl of a freshly prepared lysozyme (10 mg/mL in 25 mM Tris pH 8) and a 6-µl RNase (stock concentration 10mg/ml) were added to the cell suspension and incubated 15 min at room temperature and transferred to ice for another 15 min. A 120-µl STEP solution (0.5% SDS, 50mM Tris pH 7.5 and 280-µg of proteinase) was added and incubated at 37 °C for an hour. Subsequently, 216-µl of ammonium acetate solution (7.5 M concentration) was added and mixed well. The solution was precipitated with phenol: chloroform: isoamyl alcohol (25:24:1) The supernatant was treated twice with equal volume of chloroform: isoamyl alcohol (24:1). The DNA was then precipitated with isopropanol and centrifuged. The DNA pellet was washed with 70% ethanol, air-dried and re-suspended in 100-µl sterile distilled water.

***DNA amplifications*:** The following primers were used: 1) ERIC (enterobacterial repetitive intergenic consensus sequence) ERIC-1R: 5'ATG TAA GCT CCT GGG GAT TCA C 3', ERIC –2: 5'AAG TAA GTG ACT GGG GTG AGC G 3'; 2) BOXA1R (Box element sequence): 5' CTA CGG CAA GGC GAC GCT GAC GCT GAC G 3'; 3) REP (repetitive extragenic palindromic sequence) REP1R-I: 5' IIII CGI CGI CAT CIG GC 3',
REP2-I : 5' ICG ICT TAT CIG GCC TAC 3'

Each 25-µ reaction mixture contained 30 ng template DNA, 3mM $MgCl_2$, 1.2 (for BOX) and 2 (for ERIC and REP) pmol each primer, 200-µM each of the four dNTPs, 1 U of Taq-DNA polymerase, 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 0.1% Tritón X-100 and 10% (v/v) DMSO (dimethyl sulfoxide).

Amplifications were performed in an automated thermocycler (MJ Research Inc, MA) with an initial denaturation (3 min at 94 °C), followed by 35 cycles of denaturation (30 s at 92 °C, annealing ((1 min at 50 °C for ERIC and BOX; at 40 °C for REP), and extension (8 min at 65°C), with a final extension (10 min at 65°C).

RAPD-PCR: Amplifications were carried out with 7 primers from Operon Technologies, Inc. with codes and sequences as follows OPA-01 (5'- CAGGCCCTTC –3'), OPA-02 (5'-TGCCGAGCTG –3'), OPA-03 (5'-AGTCAGCCAC-3'), OPA-04 (5'- AATCGGGCTG-3'), OPAJ-11 (5'-GAACGCTGCC-3'), OPC-02 (5'-GTGAGGCGTC-3'), OPD- 03 (5'-GTCGCCGTCA-3'). The reaction had a total volume of 20-µl with 30 ng DNA, 3-mM $MgCl_2$, 0.5-µM primer, 0.26-mM of mixture of dNTPs, 50-mM KCl, 10-mM Tris-HCl (pH 8.8), 0.1% Tritón X-100 and 1 U Taq-DNA polymerase. Amplifications were performed in an automated thermocycler (MJ Research Inc, MA) with an initial denaturation (2 min at 94 °C), annealing (5 min at 28 °C), denaturation (1 min at 94 °C) followed by 45 cycles of denaturation (20 s at 92 °C), annealing (1 min at 35 °C), and extension (1 min at 72 °C), with a final extension (7 min at 72 °C).

***Data analysis*:** rep-PCR fingerprints were converted to binary form (presence =1; absence = 0) and similarity coefficients for pairs of strains were calculated NTSYS (Numerical Taxonomy and multivariate Analysis system) version 2.02 (Exeter Software), using SIMQUAL with the Dice coefficient and were subjected to unweighted pair group method (UPGMA) cluster analysis. The same data matrix was subjected to multiple correspondence analysis (MCA) and analyzed using CORRESP Procedure of SAS/STAT Software.

Results and Discussion: Multiple correspondence analysis of the combined data matrix generated using REP-PCR, ERIC-PCR and BOX-PCR resulted in 3 groups with an average similarity index of 78% **(Figure 1)**. Isolates *Xc* 44 and *Xc* 45 that were collected in Carimagua appear to be clonal. The same was true with isolates *Xc* 49 and *Xc* 50 that were collected in Palmira.

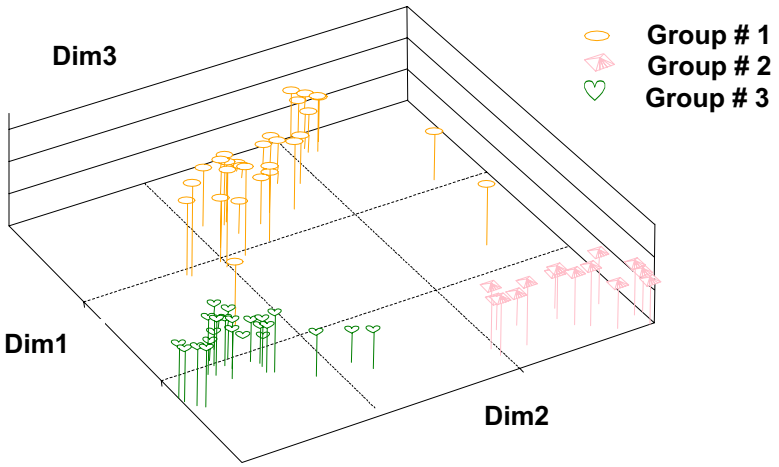


Figure 1. Correspondence Analysis (MCS) by combining the rep-PCR fingerprints of 69 isolates of *Xanthomonas* obtained with each of the two primers REP and ERIC, and BOX primer.

Group 1 consists of 30 isolates with an average similarity index of 52%. Group 2 had 15 isolates with a 50% similarity index. The two control isolates from cassava were clustered within group 2. Group 3 consisted of 24 isolates and had a high similarity index of 89%.

Multiple correspondence analysis of the RAPD data set resulted in three groups of isolates as well **(Figure 2)**. The first dimension clearly separated group 2 from groups 1 and 3, whereas dimensions 2 and 3 did not differentiate any of the groups well. Group 1 contained 40 isolates with 84% similarity index. Group 2 consisted of 25 isolates that were all collected in Palmira. Group 3 had only 4 isolates.

Both RAPD and rep-PCR generated multiple bands. However, correlation between these two techniques was low (r = 0.36). RAPD data did not differentiate the two isolates of *X. axonopodis* pv. *manihotis*. The same was the case with ERIC-PCR. However, with BOX primers, as well as the combined data of BOX and REP-PCR separated the two distinct isolates from those of *X. campestris* pv. *graminis*. These results hint that rep-PCR can be used to develop rapid diagnostic tools for *Xanthomonas* pathovars. In addition, both RAPD and rep-PCR revealed genetic diversity among isolates of *X. campestris* pv. *graminis*. In light of this, caution has to be taken in germplasm movement in order not to transfer new isolates of this seed-borne pathogen from one location to another.

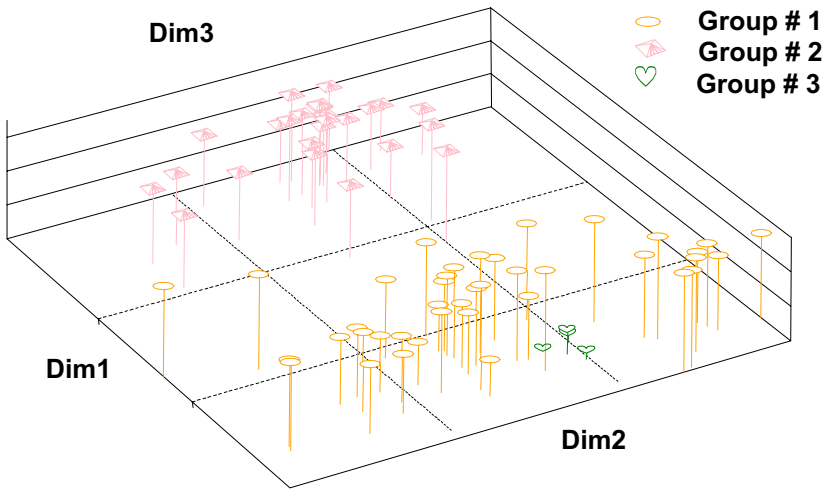


Figure 2. **Multiple Correspondence Analysis (MCS) of RAPD-PCR data conducted with 69 *Xanthomonas* isolates.**

Contributors: M. Rodriguez, S. Kelemu.

Activity 2. Resistance to *Xanthomonas campestris* pv. *graminis* (*Xcg*) in Brachiaria.

Introduction

Breeding host plants for disease resistance is the most important, cheapest and practical method of disease control. The financial, environmental and social benefits of using resistant cultivars of important crops are big. By combining the genes for resistance from various different genotypes, a formidable host resistance should be built against evolving races of the pathogen. The serious obstacle to this could be the variability encountered in the pathogen.

Although, there is currently no breeding program in IP-5 to combat this disease, we examined some genotypes of *Brachiaria* for their reaction to *X. campestris* pv. *graminis*. The objective of this study is to identify some sources of resistance as well as to evaluate important genotypes of *Brachiaria*.

Materials and Methods

Inoculum preparation and plant inoculation: Bacterial cells from a single colony of each isolate were grown in tubes containing freshly prepared nutrient broth (Difco), and incubated with shaking at 200 rpm, 28 C, overnight. Bacterial cells were collected by centrifugation at 4,000 rpm for 20 minutes. The medium was removed and bacterial cells re-suspended in sterile distilled water and adjusted to an optical density of OD_{600} = 0.1. Sterilized scissors were immersed in the bacterial suspension and used to cut leaves of *Brachiaria* plants. Leaves of control plants were cut with scissors immersed in sterile distilled water. All plants were placed in humidity chambers maintained at 27 C and RH of 70% for 48 hours. They were then moved to a growth chamber at 28-30 C and photo- period of 12 hours, or in the green house until symptoms were expressed.

Plant evaluation: Selected *Brachiaria* accessions and hybrids were evaluated for their reactions to *X. campestris* pv. *graminis*. Plants that showed any visible wilt symptoms within 15 days after inoculations were rated as susceptible (S), and those that maintained "healthy" appearance were rated resistant (R).

Results and Discussion: Thirteen *Brachiaria* genotypes, BRO-02-193, BRO-02-415, BRO-02-445, BRO-02-465, BRO-02-968, BRO-02-1045, BRO-02-1405, BRO-02-1474, CIAT 16322, CIAT 26110, CIAT 26990, CIAT 36061, 36062, were tested for their reaction to *X. campestris* pv. *graminis*. Seventeen isolates of the pathogen were used to inoculate each of the genotypes. Three genotypes, CIAT #16322, 26110 and 26990, showed no disease symptoms after inoculations with each of the 17 isolates. CIAT 36062 was the most susceptible of the genotypes evaluated, being infected with 16 of the isolates.

Isolates of *X. campestris* pv. *graminis* that infect *Brachiaria* exhibit a wide range of genetic diversity. Pathogenic variation reveal that a wide range of pathotypes exist within the pathogen population. It is encouraging to note that high levels of resistance exist in *Brachiaria*, and it is possible to combine the available resistance in a breeding program.

Contributors: M. Rodriguez and S. Kelemu.

Activity 3. Elucidate the role of endophytes in tropical grasses.

Endophyte seed transmission studies in *Brachiaria*

Introduction

Brachiaria is a pan-tropical genus of grasses with about 100 species. The fungus *Acremonium implicatum* can develop an endophytic association that is mutually beneficial with *Brachiaria* species.

DNA from isolates of *A. implicatum* was amplified using 10-base random primers. Primer OPAK 10 (Operon Technology Inc.) amplified bands including a 500-bp product common to all of the isolates tested. This fragment has been cloned and sequenced. Based on this sequence data, several primers were designed and synthesized. A primer pair designated P1 (5'-TTCGAATGATAAGGCAGATC-3' and P4 (5'-ACGCATCCACTGTATGCTAC-3') amplified a 500-bp product with template DNA from isolates of *A. implicatum* in pure cultures and in tissues of *Brachiaria* infected with *A. implicatum*. No amplification product was detected in plants free from *A. implicatum* or using DNA of non-endophytic fungi or the bacterium *Xanthomonas campestris* pv. *graminis*, a pathogen of species of *Brachiaria* (Kelemu et al., 2003. Molecular Plant Pathology 4: 115-118).

This primer pair was used to conduct seed transmission studies in plants with and without *A. implicatum*. We report here the results of *A. implicatum* transmission studies in seedsand seedlings of *Brachiaria*. Preliminary data have been reported in IP-5 annual report 2002. The primer pair amplified a 500-bp product with template DNA of seeds harvested from *A. implicatum* infected *Brachiaria* plants, but no amplified products were observed with DNA of seeds from endophyte-free plants.

Materials and Methods

Endophyte elimination: The fungicide Folicur® was used to generate endophyte-free *Brachiaria* clones. Twenty or more plantlets were propagated from a mother plant naturally or artificially infected with the endophyte. Half of these plantlets were soaked in a solution of 0.6 mL/L of Folicur® (250 g a.i./L) for 6 h to eliminate the endophyte, and the other half were left untreated to serve as controls. All plantlets were individually planted in small pots and placed in the greenhouse. Plants were examined 4-6 weeks after treatment for the presence or absence of *A. implicatum*.

DNA isolations: Fresh mycelia of endophyte isolates cultured on PDA plates, endophyte-infected or endophyte-free plant leaves, or seeds were collected and macerated in liquid nitrogen for genomic DNA isolation. Genomic DNA was extracted using the DNeasy™ Plant Mini Kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

PCR Amplifications: Specific primers P1 (5'-TTCGAATGATAAGGCAGATC-3') and P4 (5'-ACGCATCCACTGTATGCTAC-3') were used in the PCR reactions. Amplifications were carried out in a Programmable Thermal Controller (MJ Research, Inc.), programmed with 44 cycles for genomic DNA of endophyte pure cultures or plant leaves, and 54 cycles for DNA

from *Brachiaria* seeds, of a 30 sec denaturation step at 94°C (3 min for the first cycle), follwed by 1 min at 65°C, and primer extension for 1 min (10 min in the final cycle) at 72°C. The amplification products were separated by electrophoresis in a 1.0% agarose gel (Bio-Rad), stained with ethidium bromide and photographed under UV lighting.

Seed samples were collected from plants confirmed to be endophyte-infected or endophyte-free using the PCR tests with template DNA isolated from plant tissues, and fungal endophyte isolation on culture media.

Results and Discussion: *Acremonium implicatum* forms a symbiotic endophytic association with at least some of the economically important *Brachiaria* species. We sought to ascertain whether endophytic *A. implicatum* could be seed-transmitted in *Brachiaria*. Twenty tillers were vegetatively propagated from a single, endophyte-infected mother plant. Ten tillers were treated with the fungicide Folicur® to eliminate the endophyte while the remaining ten tillers were untreated. Seeds were harvested individually from these genetically identical plants, with or without the endophyte. Some of the seeds were germinated and seedlings grown in the glasshouse. A polymerase chain reaction (PCR)-based method developed previously uses a pair of endophyte-specific primers to amplify a single DNA fragment of about 500 bp. DNA both from remnant seeds and from 2-month-old seedlings was amplified with these primers to detect presence of the endophyte. The diagnostic DNA fragment was consistently amplified in DNA of seeds harvested from the endophyte-infected plants and DNA from seedlings grown from seeds harvested from endophyte-infected plants, but not from seeds or seedlings originating from fungicide treated endophyte-free plants. We conclude that *A. implicatum* can be transmitted through seeds.

The primer pair, P1/P4, allows the precise and rapid detection of *A. implicatum* in *Brachiaria* plants and permits a differentiation between endophytic and non-endophytic fungi (Kelemu et al., 2003. Molecular Plant Pathology 4: 115-118).

A single band of about 500-bp in all examined isolates of *A. implicatum* was amplified. Endophyte-containing and endophyte-free plants were also consistently differentiated using this primer combination (data not shown). Seeds were collected from plants whose tissue samples were used as well as other plants. All seed DNA from endophyte-containing plants had a 500-bp amplified product. No amplification product was detected with seed DNA from endophyte-free plants **(Figure 1)**.

Seedlings generated from seed samples of endophyte-containing and endophyte-free plants had consistently tested positive or negative, respectively, for the diagnostic 500-bp amplified product **(Figure 2)**. From these results, we concluded that *A. implicatum* maintains its symbiotic association with species of *Brachiaria* through seed transmission.

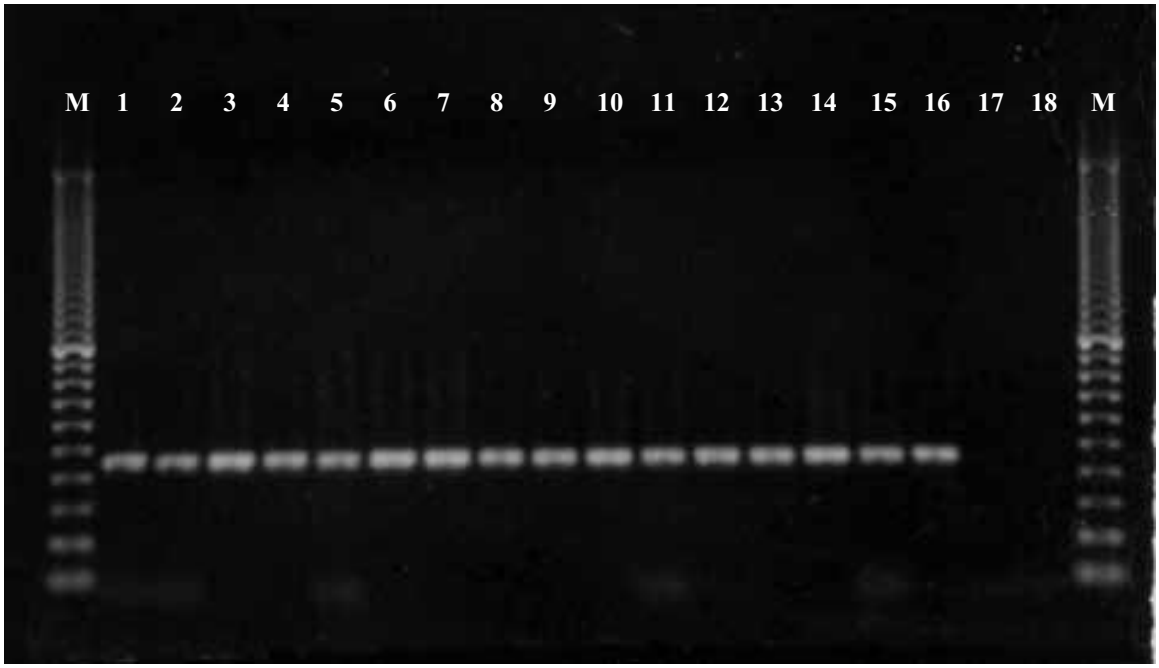


Figure 1. **Specific detection of *Acremonium implicatum* in seeds harvested from endophyte-infected *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1/P4. Lanes 1-16, template DNA extracted from seeds of endophyte infected *Brachiaria* hybrids SX99/3488 (8), SX99/0275 (14), BR99NO/4132 (22), FM9201/1873 (29), BR99NO/4015 (37), BR99NO/4132 (39), *B. decumbens* CIAT 606 (42), BRUZ4X/4402 (44), FM9201/1873 (48), SX99/0731 (52), *B. brizantha* CIAT 16320 (32a), FM9503/S046/024 (45), *B. brizantha* CIAT 26110 (15), *B. brizantha* CIAT 6780 (56), *B. brizantha* CIAT 6780 (68), and *B. brizantha* CIAT 6780 (111), respectively. Lanes 17,18, DNA extracted from seeds of endophyte-free plants of *B. brizantha* CIAT 16320 (32-25) and *B. brizantha* CIAT 16320 (32-29); lanes M, 100-bp ladders. *B. brizantha* CIAT 26110 (15), *B. brizantha* CIAT 6780 (56), *B. brizantha* CIAT 6780 (68), *B. brizantha* CIAT 6780 (111) were artificially infected. All others were naturally infected.**

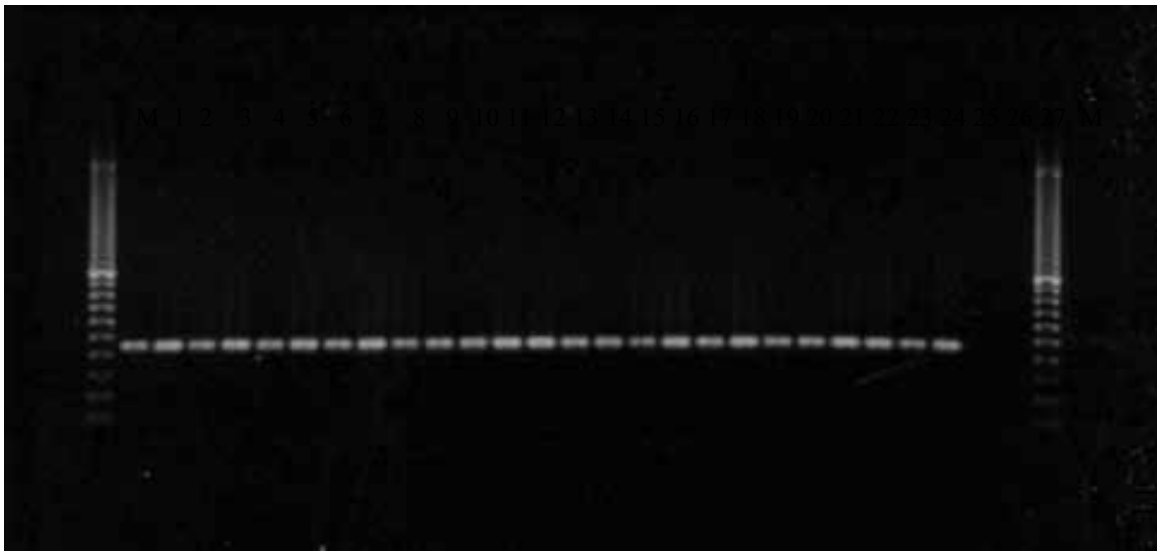


Figure 2. **Specific detection of *Acremonium implicatum* in seedlings generated from seeds of endophyte-infected and endophyte-free *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1/P4. Lanes 1-7, seedlings from seeds harvested from naturally endophyte-infected plants SX99/3488 (8), BRN99NO/4132 (22), BRN99NO/4132 (39), *B. decumbens* accession CIAT 606 (42), BRUZ4X/4402 (44), FM9201/1873 (48), SX99/073 (52), respectively; lanes 8-17, seedlings generated from seeds of ten artificially infected *B. brizantha* CIAT 26110 (15) plants; lanes 18-25, seedlings generated from seeds of eight naturally infected *B. brizantha* CIAT 16320 (32) plants; lanes 26 & 27, seedlings generated from seeds of two endophyte-free *B. brizantha* CIAT 16320 (32-25) plants; lanes M, 100-bp ladders.**

Contributors: H. Dongyi and S. Kelemu.

Activity 4. Endophyte distribution in *Brachiaria* plants, and PCR analysis and screening of *Brachiaria* genotypes for endophytes.

Introduction

Endophytic fungi often develop a systemic association with their hosts. Several reports demonstrated that endophytic fungi, such as *Epichloë* and *Neotyphodium*, could be distributed in leaf sheaths, leaf blades, stems, roots, seeds and embryos of their grass hosts.

Although endophytes infect their hosts systemically, the concentration of hyphae is not uniform throughout parts of infected plants. Some parts of endophyte-infected plants can even be endophyte-free. Using tissue staining and culturing methods, endophytic fungus *A. implicatum* was observed in leaf sheaths and seeds of *Brachiaria*. These two methods, however, are time consuming and unreliable for endophyte distribution studies in different parts of the plant, especially where fungal mycelia are sparsely distributed. We have developed a rapid and sensitive PCR-based method for specific detection of *A. implicatum* in tissues of *Brachiaria* (Kelemu et al., 2003. Molecular Plant Pathology 4: 115-118). We used this method to determine the distribution of *A. implicatum* in various parts of *Brachiaria* plants.

Materials and Methods

DNA isolation: Leaf sheaths, leaf blades, stems, roots, seeds, embryo and endosperm of seeds were collected from endophyte-infected or endophyte-free plants and macerated separately in liquid nitrogen for genomic DNA isolation. Genomic DNA was extracted using the DNeasy™ Plant Mini Kits (QIAGEN, Valencia, CA).

PCR amplifications: Composition of PCR reactions (20 mL) were 1x PCR buffer, 3mM $MgCl_2$, 0.25mM dNTPs, 0.5 μM primer P1 and P4, 1U Taq DNA polymerase, and 30ng template DNA. Amplification cycles were programmed in a Programmable Thermal Controller (MJ Research, Inc.) as follows: step 1, 94°C 3min; step 2, 94°C 30 sec; step 3, 65°C 1 min; step 4, 72°C 1 min; step 5, go to step 2 for 44 cycles (for genomic DNA of leaf sheaths and leaf blades) or 54 cycles (for genomic DNA from stems, roots, seeds, embryos and endosperms); then 72°C 10min. The amplification products were separated by electrophoresis in a 1.0% agarose gel (Bio-Rad), stained with ethidium bromide and photographed under UV lighting.

The distribution of *A. implicatum* in plant tissues was determined by the presence or absence of a diagnostic 500-bp amplification product.

Results and Discussions: A diagnostic 500-bp amplification product was observed in all examined leaf sheaths, leaf blades, stems, and roots of *Brachiaria* plants infected with *A. implicatum* **(Figures 1-4)**. The amplification product was also detected in whole seeds, embryo, and endosperm of seeds (data not shown). These results indicate that *A. implicatum* is distributed in the plant parts described above. Johnson et al. (1985, Plant Disease 69:200-202) described the concentration and distribution of Epi*chloë typhina* in tall fescue individual plants with decreasing order in leaf sheaths, seeds, crows, stems, leaf blades, and roots. Because amplifications with template DNA from leaf sheaths, leaf blades and fresh seeds generate the diagnostic 500-bp product with just 45 cycles, as opposed to 55 cycles with DNA from roots and stems, it is likely

that mycelial concentrations and distributions in *A. implicatum*/*Brachiaria* associations have a similar trend as those reported in Epi*chloë typhina*/ tall fescue. There was no obvious difference in sensitivity with genomic DNA from leaf blades and sheaths, although isolations on culture media is more routinely and successfully done from leaf sheaths than leaf blades.

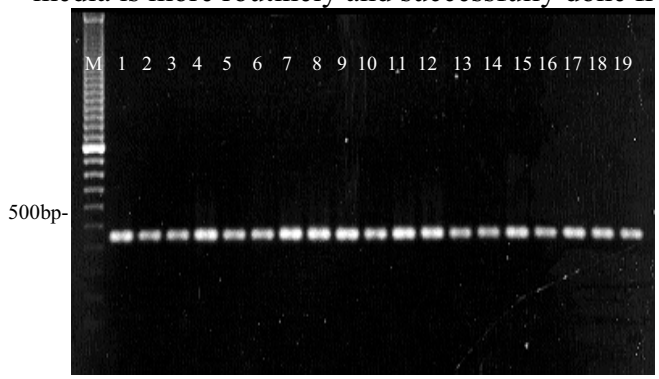


Figure 1.

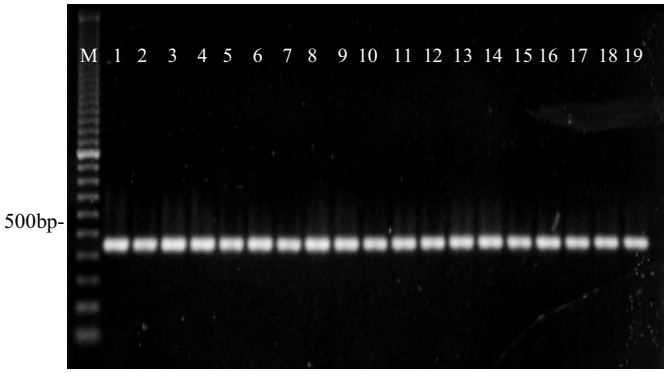


Figure 2.

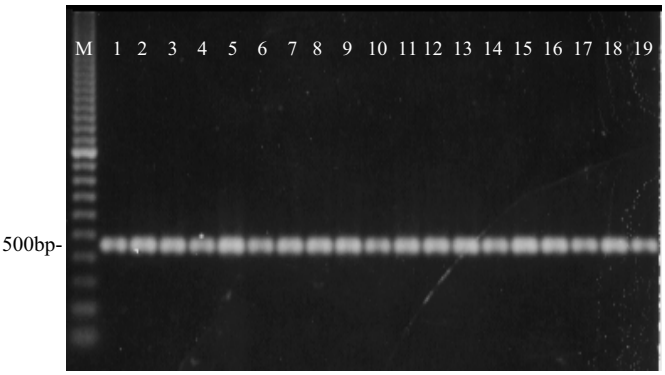


Figure 3.

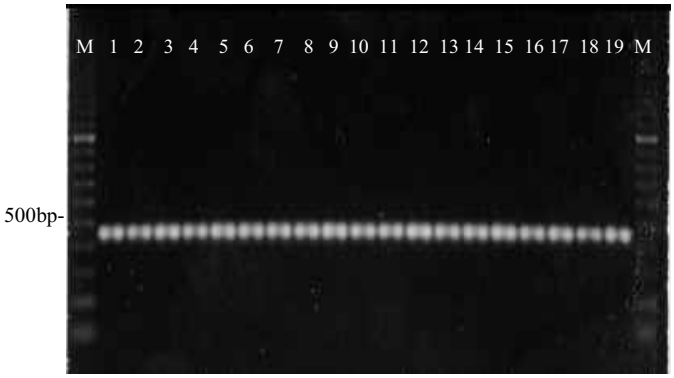


Figure 4.

Figures 1-4. Specific detection of *Acremonium implicatum* in leaf blades (Figure 3), leaf sheaths (Figure 4), stems (Figure 5), and roots (Figure 6) collected from endophyte-infected *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1/P4. Lanes 1~19: *Brachiaria* hybrid plants SX99/3488 (8), SX99/0275(14), BR99NO/4132 (22), SX99/1513 (23), FM9201/1873 (29), BR99NO/4015 (37), BR99NO/4132 (39); *B. decumbens* accession CIAT 606 (42); *B*. hybrids BRUZ4X/4402 (44), FM9201/1873 (48), SX99/0731(52), FM9503/S046/024 (19); *B. brizantha* accession CIAT 16320 (32a); *Brachiaria* hybrid FM9503/S046/024 (45), SX99/2341(47); *B. brizantha* accession CIAT 26110 (15), *B. brizantha* 6780 (56), *B. brizantha* 6780 (63), *B. brizantha* 6780 (111), respectively. *Brachiaria* hybrid FM9503/S046/024 (19), *B. brizantha* CIAT 16320 (32a), *Brachiaria* hybrid FM9503/S046/024 (45), *Brachiaria* hybrid SX99/2341(47) were naturally infected with isolates we have previously characterized. Plant *B. brizantha* CIAT 26110 (15), *B. brizantha* CIAT 6780 (56), *B. brizantha* CIAT 6780 (63), and *B. brizantha* CIAT 6780 (111) were artificially infected with an isolate (EB 6780(201) of *A. implicatum.* All remaining plants were naturally infected with yet to be isolated and characterized strains.

Contributors: H. Dongyi, T. Sakai, S. Kelemu.

Activity 5. Effect of fungal endophytes on pathogens *in planta.*

Introduction

Several *in vitro* studies have demonstrated that *Acremonium* endophytes and *Epichloë typhina* cultures exhibit antifungal activity. White and Cole (1985, Mycologia 77:487-489; 1986, Mycologia 78:102-107) reported that an *Acremonium* spp. from *Festuca*, *A. coenophialum* (now renamed *Neotyphodium coenophialum*) from tall fescue, and *A. lolii* (renamed *N. lolii*) from perennial ryegrass inhibited mycelial growth of seven different fungi including *Rhizoctonia* spp. in culture. Siegel and Latch (1991, Mycologia 83:529-537) examined the effect of a series of isolates of A*cremonium sp., E. typhina, Phialophora*-like sp. and *Gliocladium*-like sp. on mycelial growth of several grass pathogens in agar culture. Their results indicate that individual isolates of the same species differed in their growth inhibition activities of grass pathogens.

Although many endophyte isolates show antifungal activities *in vitro*, there are only a few reports on resistance to pathogens conferred by endophytes *in planta*. Reduction of tall fescue seedling density due to *Rhizoctonia zeae* was inversely correlated with endophyte (*N. coenophialum)* infestation level of the seed lot (Gwinn and Gavin, 1992, Plant Disease 76:911-914). Plant protection by *E. typhina* against *Cladosporium phlei*, the causing pathogen of purple leaf spot of timothy grass, was reported (Greulich et al. 1999, Ann. Phytopathol. Soc. Jpn. 65:454-459).

Apart from providing direct resistance to fungal pathogens, endophytes can reduce the spread of viral diseases by deterring insect vectors such as the aphid *Rhopalosiphum padi.*

Drechslera sp. and *Rhizoctonia solani* are the most important pathogens of species of *Brachiaria*. Our earlier results showed that *A. implicatum* infected plants had fewer and smaller disease lesions caused by *Drechslera* sp. than did genetically identical endophyte-free plants (Kelemu et al., 2001, Canadian Journal of Microbiology 47:55-62). Some genotypes of *Brachiaria* are resistant to *R. solani*. We speculate that *A. implicatum* may contribute to some of this resistance to foliar blight disease caused by *R. solani*.

Materials and Methods

Culture maintenance: All endophytic or pathogenic fungi were cultured and maintained as described by Kelemu et al. (2001, Canadian Journal of Microbiology 47:55-62).

Antifungal extractions from endophyte cultures: Mycelia/conidia were collected from 27 colonies (about 20 mm in size) of *A. implicatum* isolate EH32a grown on potato dextrose agar (PDA). This was macerated in 50 mL sterile distilled water and centrifuged at 12000 rpm for 30 minutes. The supernatant was lyophilised and re-suspended in 9 mL sterile distilled water. This extract was then filter sterilized using 0.22 μm pore-size nylon membranes. The 9 mL filtrate was then divided into 3 parts of 3 mL each. The first part was heat treated at 100 ºC for 20 minutes. The second portion was treated with pronase (2.0 mg/mL final concentration) and incubated at 37 ºC for 4 hours. The third portion was left in its natural state as control.

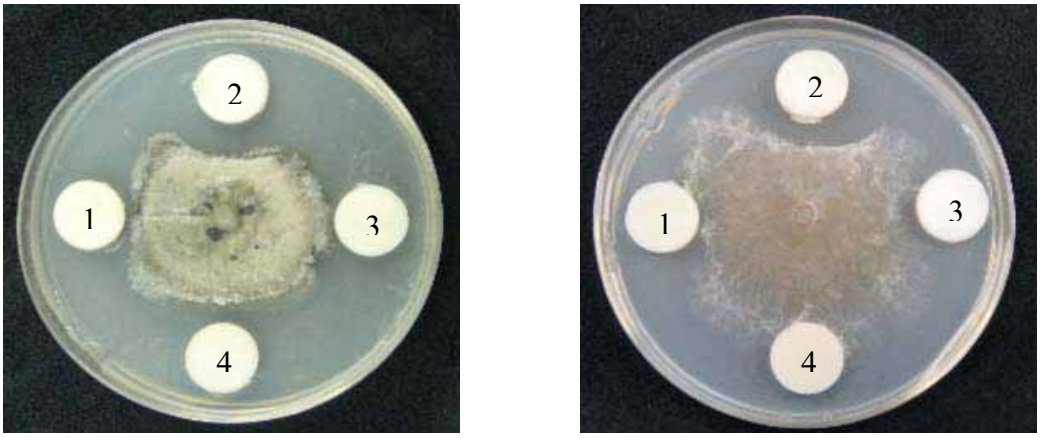
Antifungal activity tests: Filter paper discs were soaked with 400-µl endophyte mycelial/conidial extract prepared as described above. These were placed on PDA-containing petri dishes individually inoculated with *Drechslera* sp. and *R. solani* as shown in **Figures 1a and b.** These were incubated at 28 ºC in the dark for 3 –5 days.

Plant inoculation and disease evaluations: Young tillers from genetically identical endophyte-infected and endophyte-free *B. brizantha* CIAT 6780 or CIAT 16320 were transplanted individually in pots. Plants were inoculated with mycelial agar discs removed from actively growing *R. solani* cultures, by placing the discs in contact with the plant stems just above the soil level and wrapping them with parafilm to secure the contact. Inoculated plants were maintained at high relative humidity in the greenhouse. The upward progression of disease spread and symptoms was measured as distance from the inoculation point.

Results and Discussion: In *in vitro* inhibition tests, most of the 11 *A. implicatum* strains showed antifungal activities although they differ in the inhibition zone area they generated (data not shown). Strains EB 6780(501) and EH 32a showed strong inhibition to both *Drechslera sp.* and *R. solani.*

With *in vivo* tests, endophyte-infected *B. brizantha* CIAT 6780 and CIAT 16320 plants showed more resistance (exhibited as slower upward disease progression) to foliar blight disease than their endophyte-free counterparts at the early stages of infection (7 days after inoculation). Using the Harsfall-Barratt visual rating system (1945. Phytopathology 35:655), disease severity was 4% and 25% on CIAT 16320 and *B. brizantha* CIAT 6780, respectively; as opposed to 25% and 39% on their endophyte-free counterparts, respectively. We concluded that *A. implicatum* contributes to *Rhizoctonia* foliar blight resistance in these two genotypes of *Brachiaria*. It is also important to note that those isolates that exhibited strong inhibitory activities in *vitro* contributed to *in planta* resistance.

Extracts from *A. implicatum* strain EH32 showed strong inhibition to *Drechslera sp* and *R. solani* **(Figures 1a and 1b)**. Extracts treated with heat or pronase lost their antifungal activity. Further extensive studies are needed to determine the nature of the antifungal activity in *A. implicatum*.



a **b**

Figure 1. Growth inhibition of *Drechslera* spp. (a) and *Rhizoctonia solani* (b) by cell-free culture extracts of *Acremonium implicatum* strain EH32a. Filter paper discs 1-3 were soaked with cell-free extracts of *Acremonium implicatum* strain EH32a. Filter paper discs # 1, #2, #3, and # 4 were soaked with heat-treated extracts, extracts in their natural state, extracts treated with pronase, and sterile distilled water, respectively.

Contributors: H. Dongyi, S. Kelemu.

Activity 6. Search for nitrogen-fixing bacteria associated with species of *Brachiaria.*

Introduction

Nitrogen fixation is conducted by phylogenetically diverse groups of prokaryotes. Evidence on nitrogen fixation by rhizospheric bacteria associated with grass roots was first presented in the tropics (Döbereiner and Day, 1976. Associated symbioses in tropical grasses: characterization of microorganisms and nirogen-fixing sites. In: W. E. Newton and C. J. Nyman ed. Proc. of the 1st International Symposium on nitrogen fixation, Washington State Univ. Press, Pullman, pp. 518-538). Tropical forage grasses and grasslands could be ideal for investigating associations with nitrogen fixing bacteria because of their perennial nature and low chemical inputs including fertilizers. The main objectives of this initiative are to: 1) look for endophytic and rhizospheric bacteria responsible for nitrogen fixation in association with species of *Brachiaria*, 2) identify and characterize both plant growth promoting and nitrogen-fixing bacteria that also result in healthier plants.

Because nitrogen fixation is performed by diverse groups of prokaryotic organisms, detection of a marker gene which is unique and is required for nitrogen fixation may be useful to conduct our studies. The *nifH* gene (encodes nitrogenase reductase) has been used with a number of PCR primers that amplify the gene from microbes and other samples by a number of researchers.

Materials and Methods

***Bacterial isolates*:** Isolates of the genera *Rhizobium* of *Bradyrhizobium* were used as positive controls. A bacterium which was consistently isolated from *Brachiaria* CIAT 36062 in 1999, and which we suspected might have a role in fixing nitrogen was included in the test. An isolate of *Xanthomonas campestris* pv. *graminis* (isolate 1015), the causal agent of bacterial wilt of species of *Brachiaria*, was used as a negative control. Bacterial isolates include the following: 1) *Bradyrhizobium* 3101 isolated from forage legume *Centrosema* (Colombia), 2) *Bradyrhizobium* 2469 isolated from forage legume *Desmodium* (Colombia), 3) BR97-155 CBT, unidentified bacterium isolated from *Brachiaria* BR97-155 (Colombia), 4) 16445 CBT, unidentified bacterium isolated from *Brachiaria* CIAT 16445 (Colombia), 5) 16497 CBH, unidentified bacterium isolated from *Brachiaria* CIAT 16497 (Colombia), 6) FM97-383 CACT, a bacterium isolated from *Brachiaria* FM97-383 (Colombia), 7) *Rhizobium* 668 isolated from *Phaseolus vulgaris,* 8) BR97-1371, a bacterium isolated from *Brachiaria* CIAT 36062 (Colombia), 9) *Xanthomonas campestris* pv. *graminis* isolated from *Brachiaria* 1015.

***DNA extractions from bacteria*:** DNA extraction was conducted using a modified protocol based on combinations of standard methods, which includes growing bacterial cells in liquid media LB (tryptone 10g, yeast extract 5g, NaCl 10g, 10 ml of 20% glucose in 1 L of distilled water), treatment of cells with a mixture of lysozyme (10 mg.ml in 25 mM Tris-Hcl, ph 8.0) and RNase A solution, and extraction of DNA with STEP (0.5% SDS, 50 mM Tris-HCl 7.5, 40 mM EDTA, proteinase K to a final concentration of 2mg/ml added just before use. The method involves cleaning with phenol-chloroform and chloroform/isoamyl alcohol and precipitation with ethanol. The quality of DNA was checked on 1 % agarose gel.

DNA isolations from soil samples: A protocol described by Porteous et al. (1997. An improved method for purifying DNA from soil for polymerase chain reaction amplification and molecular ecology applications. Technical note. Molecular Ecology. 6: 787-791) was used to isolate DNA from soil. The method in general involves lysis of microbial cells, sonication, precipitation, and various steps of cleaning.

Plant DNA extraction: A method described by Dellaporta et al (1983. A plant DNA mini-preparation: version II. Plant Molecular Biology Reporter 1: 19-21)

Nested PCR Amplification: Three primers were used, which were originally designed by Zehr and McReynolds (1989. Use of degenerate oligonucleotides for amplification of the *nifH* gene from the marine cyanobacterium *Trichodesmium thiebautii*. Appl. Environ. Microbiol. 55: 2522-2526) and Ueda, et al. (1995. Remarkable N2- fixing bacterial diversity detected in rice roots by molecular evolutionary análysis of *nifH* gene sequences. J. Bacteriol. 177: 1414-1417), to amplify fragments of *nifH* genes. Amplification steps described by Widmer et al (1999. Analysis of *nifH* gene pool complexity in soil and litter at a douglas fir forest site in the Oregon cascade mountain range. Applied and Environmental Microbiology 65:374-380) were adopted.

Results and Discussion

DNA extraction from bacterial cells: DNA extracted from bacterial cells is shown in Figure 1.

Figure 1. DNA isolated from: 1) *Bradyrhizobium* 3101, 2) *Bradyrhizobium* 2469, 3) unidentified bacterium BR97-155 CBT, 4) unidentified bacterium16445 CBT, 5) unidentified bacterium 16497 CBH, 6) unidentified bacterium FM97-383 CACT, 7) *Rhizobium* 668, unidentified bacterium. BR97-1371, 9) *Xanthomonas campestris* pv. *graminis* 1015.

Nested PCR amplifications: Amplified products of approximately 370-bp size were amplified with template DNA from nitrogen-fixing bacteria *Rhizobium* and *Bradyrhizobium*, as well as from those randomly picked bacterial colonies isolated from *Brachiaria* CIAT 16445, *Brachiaria* CIAT 16497, and *Brachiaria* FM97-383 **(Figure 2)**. Template DNA from a randomly picked

bacterial colony from *Brachiaria* CIAT 36062 amplified a product with approximately 210-bp size **(Figure 2)**. No amplification products were observed with DNA from the pathogen *X. campestris* pv. *graminis* **(Figure 2; lane 9)** and with that of a bacterium isolated and selected from *Brachiaria* BR97-155 **(Figure 2; lane 3)**.

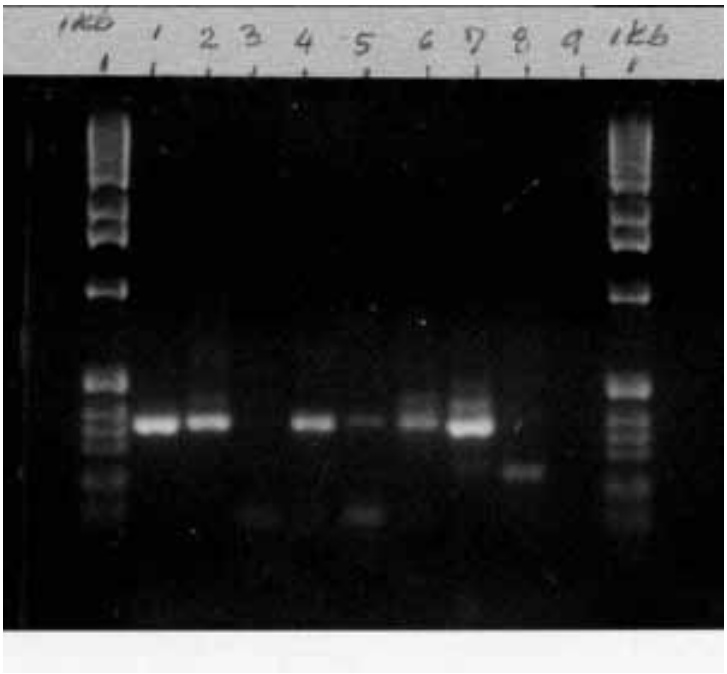


Figure 2. Nested PCR amplification products with three primers of sequences of *nifH* gene. Lanes 1-9, *Bradyrhizobium* 3101, *Bradyrhizobium* 2469, BR97-155 CBT, 16445 CBT, 16497 CBH, FM97-383 CACT, *Rhizobium* 668, BR97-1371, *Xanthomonas campestris* pv. *graminis* 1015 (negative control), respectively. Size markers 1kb ladder.

Brachiaria hybrid CIAT 36062 (BR97-1371) is of particular interest because of its maintenance of green color in the absence of nitrogen input. We have plants of this hybrid in pots in the glasshouse for the last 4 years with no application of nitrogen fertilizer, but are still green. We, therefore, concentrated on this hybrid and isolated independent bacterial colonies from roots, leaves, stems, and soil around the plant roots. Roots were sectioned into three parts: superficial (next to stems), middle and bottom parts. Pieces plant tissues were surface sterilized, macerated in sterile distilled water and plated on nutrient agar for bacterial isolations. Cells from individual bacterial colonies (random colony selection was based on colony color and morphology) were transferred to fresh nutrient agar for further increment. Nested PCR amplification with DNA of these colonies resulted in various size products ranging between 200-1000 bp sizes **(Figure 3).** Two colonies **(Figure 3; lanes 7, 15)** gave no amplification products. Some bacterial colonies isolated from the bottom part of the root, the leaf and stem generated strong amplification products with the same size as that produced by nitrogen-fixing bacteria used as positive controls **(Figure 3)**.

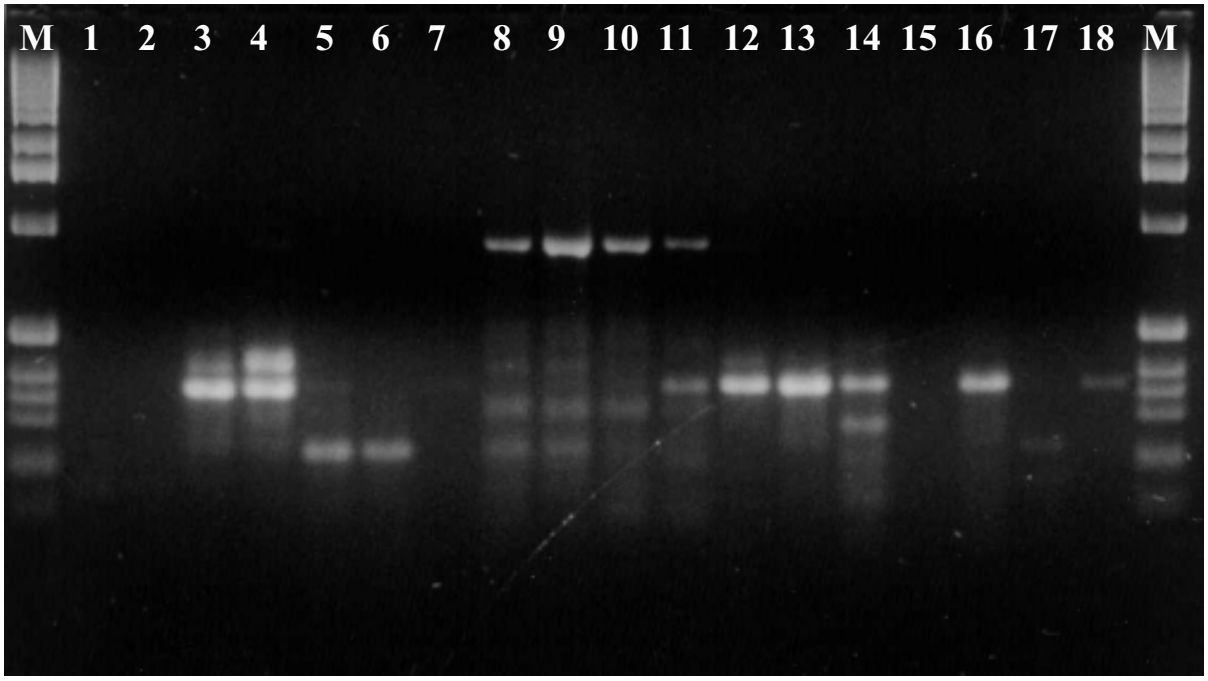


Figure 3. Nested PCR amplification products with three primers of sequences of *nifH* gene. Lane 1) negative control with no template DNA, 2) *Xanthomonas campestris* pv. *graminis* (negative control), 3) *Bradyrhizobium* 3101 (positive control), 4) *Rhizobium* 668 (positive control); lanes 5-7, bacteria isolated from the top part of the root (next to the stem) of *Brachiaria* CIAT 36062 – colonies 1, 2, 3, respectively1; lanes 8-10, bacteria isolated from the middle part of the root – colonies 1, 2,3, respectively; lanes 11-13, bacteria isolated from the bottom tip of the root – colonies 1, 2, 3, respectively; lanes 14-18, bacteria isolated from leaf -1, stem -B1, stem -A2, stem -A3, stem –C, respectively; M=1Kb

DNA isolated from soil samples taken from the surface, middle and bottom part of the potted plants all generated strong amplification products with the same size as those produced by nitrogen-fixing bacteria used as controls **(Figure 4)**.

In this study, the application of nested PCR amplifications of the *nifH* gene provided us with the first clue that there are bacteria associated with *Brachiaria* hybrid CIAT 36062 involved in nitrogen fixation. These bacteria exist in higher concentration around/in the roots and in the soil around the plant roots than in the leaves and stems. Using these preliminary results as a basis, we intend to conduct more detailed studies to understand the association and to exploit its field application.

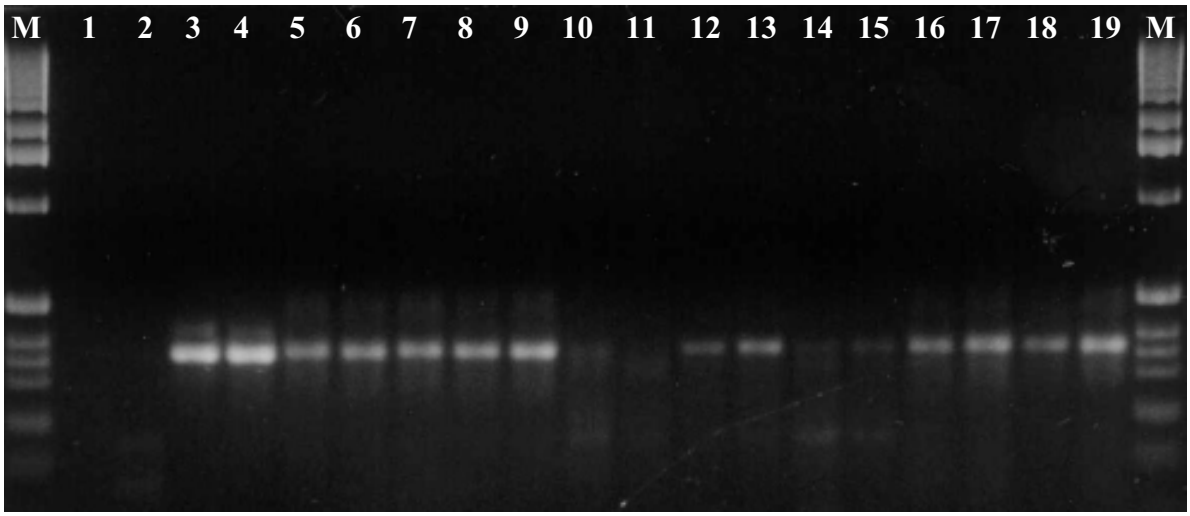


Figure 4. **Nested amplifications of the *nifH* gene from soil samples of pots, where *Brachiaria* CIAT 36062 plants have been growing for four years, and bacterial cultures. Lane 1, control with no template DNA; lane 2) *Xanthomonas campestris* pv. *graminis* 1015 (negative control); lane 3) *Bradyrhizobium* 3101 (positive control), 4) *Rhizobium* 668 (positive control); lanes 5-19, surface soil-1, surface soil-2, middle-level soil-1, soil from bottom part of pot-1, soil from bottom-part of pot-2, leaf of CIAT 36062 plant 1, leaf of CIAT 36062 plant 1, leaf of CIAT 36062 plant 2, leaf of CIAT 36062 plant 2, leaf of CIAT 36062 plant 3, leaf of CIAT 36062 plant 3, root of CIAT 36062 plant 1, root of CIAT 36062 plant 3, root of CIAT 36062 plant 3, respectively. Total microbial and plant DNA was extracted from the plant tissues for amplification. M=1Kb ladder.**

Contributors: C. Zuleta, R. Sedano, S. Kelemu.

Activity 7. Bacterial endophytes isolated from *Brachiaria.*

Introduction

Endophytic bacteria are bacteria that reside in plant tissues without causing any visible harm to the plant. These bacteria can be isolated from surface-sterilized plant tissue or extracted from internal plant tissue. Different bacterial species have been isolated from a single plant. Although the primary point of entry for many of these bacteria is the root zone, aerial plant parts like flowers and stems may also be entries. Once inside a plant, they may be localized at the point of entry or spread throughout. They have been reported to live within cells, in the intercellular spaces or in the vascular system.

Soil bacteria of the genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* are of great agricultural importance, because of their ability to fix atmospheric nitrogen in a symbiosis association with legumes. Populations of rhizobia can survive in the soil as saprophytes in the absence of legumes. In recent years, the natural habitat of rhizobia was extended to the roots of gramineous plants. *Rhizobium leguminosarum* bv. *trifolii* was reported to exist inside the roots of rice plants grown in rotation with clover in Egypt (Yanni et al. 1997. Plant Soil 194:99-114), without forming root nodules or nodule-like structures. Various other N_2-fixing endophytic bacteria, known as plant growth-promoting rhizobacteria (PGPR), such as *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in sugarcane, *Azoarcus* spp. in Kallar grass (*Leptochloa fusca*), and *Azospirillum* spp. in maize and rice have been reported. *Herbaspirillum seropedicae* has also been found in association with maize, sorghum and other gramineous plants. Sugarcane plants inoculated with a wild type strain of *A. diazotrophicus* had a higher nitrogen content those inoculated with a *nif* mutant strain or uninoculated controls in nitrogen-deficient conditions.

The objectives of this study were: 1) to isolate nitrogen-fixing endophytic bacteria associated with *Brachiaria*; 2) to identify these bacteria; 3) to characterize them using *nif* gene primers.

Materials and Methods

***Bacterial isolation*:** Leaf, stem and root tissues of *Brachiaria* CIAT 36062 (grown in pots in the green house) were collected and cut into 3-5- cm long sections (roots were first washed in tap water before sectioning them). They were then surface sterilized in 1% NaOCl for 2 min, in 70% ethanol for 1 min, and rinsed 3 times in sterile distilled water. The tissues wee separately macerated in 1-mL sterile distilled water in mortar and pestle. Fifty-µl of the macerated solution was spread uniformly on agar nutrient medium (Difco Lab., Detroit, MI) and incubated at 28 ºC until bacterial colonies appeared.

***DNA isolation*:** DNA extraction was conducted using a modified protocol based on combinations of standard methods, which includes growing bacterial cells in liquid media LB (tryptone 10g, yeast extract 5g, NaCl 10g, 10 ml of 20% glucose in 1 L of distilled water), treatment of cells with a mixture of lysozyme (10 mg.ml in 25 mM Tris-Hcl, ph 8.0) and RNase A solution, and extraction of DNA with STEP (0.5% SDS, 50 mM Tris-HCl 7.5, 40 mM EDTA, proteinase K to a final concentration of 2mg/ml added just before use. The method involves

cleaning with phenol-chloroform and chloroform/isoamyl alcohol and precipitation with ethanol. The quality of DNA was checked on 1 % agarose gel.

Nested PCR Amplification: Three primers were used, which were originally designed by Zehr and McReynolds (1989. Use of degenerate oligonucleotides for amplification of the *nifH* gene from the marine cyanobacterium *Trichodesmium thiebautii*. Appl. Environ. Microbiol. 55: 2522-2526) and Ueda, et al. (1995. Remarkable N2- fixing bacterial diversity detected in rice roots by molecular evolutionary análysis of *nifH* gene sequences. J. Bacteriol. 177: 1414-1417), to amplify fragments of *nifH* genes. Amplification steps described by Widmer et al (1999. Analysis of *nifH* gene pool complexity in soil and litter at a douglas fir forest site in the Oregon cascade mountain range. Applied and Environmental Microbiology 65:374-380) were adopted.

***Bacterial identification*:** Three bacterial colonies (codes 36062-H4 [isolated from leaf]; 36062-R2 [isolated from root]; 36062-V2 [isolated from stem]) that tested positive for *nif* were sent to Microbial ID, Newark, DE for identification. The company's identification is based on Similarity Index which expresses how closely the fatty acid composition of the unidentified sample compares with the mean fatty acid composition of the strains used to create the library entry. An exact match of the fatty acid composition results in a similarity index of 1.000.

Results and Discussion: The three bacterial isolates 36062-R2, 36062-H4, and 36062-V2 consistently isolated from *Brachiaria* CIAT 36062 in roots, leaves and stems, respectively, tested positive in nested PCR amplifications **(Figure 1)**. 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 (Luz, W.C., http://www.ag.auburn.edu/argentina/pdfmanuscripts/luz.pdf, accessed on 05 August, 2003). The analysis matched isolate 02-36062-H4 with *Agrobacterium rubi* at 0.845 similarity index. The name *A. rubi* is synonymous to *Rhizobium rubi* (Young et al. 2001. Int. J. Syst. Evol. Microbiol. 51:89-103). 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).

Future research will include: 1) isolation and characterization of more endophytic bacteria and identifications based not only on fatty acid composition, but also on morphology and DNA based; and 2) the role of these bacteria in plant development.

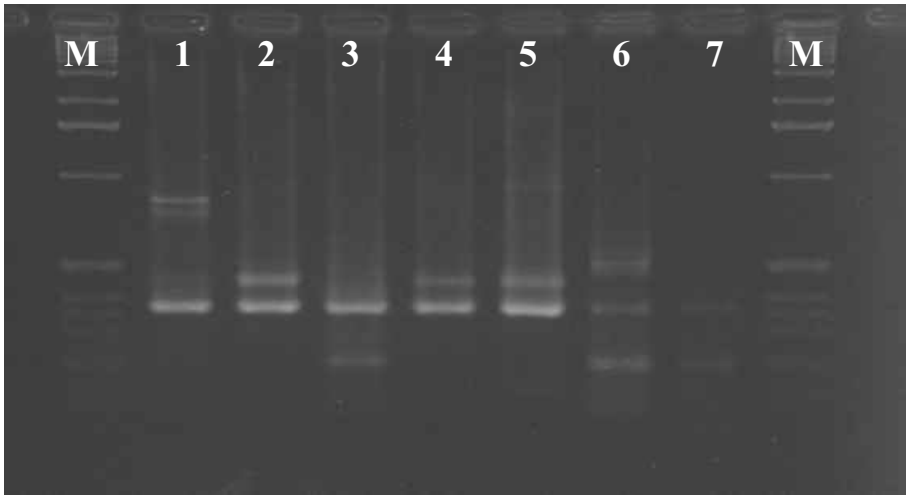


Figure 1. **Nested PCR amplifications, using *nif* gene primers, with template DNA of bacterial isolates. Lanes1-3 bacterial isolates 36062-H4, 36062-R2, 36062-V2, isolated from leaf, root and leaf sheath tissues of *Brachiaria* CIAT 36062, respectively. Lane 4, positive control *Bradyrhizobium* isolate 2469; lane 5, positive control *Rhizobium* isolate 49; lane 6, negative control *Xanthomonas campestris* isolate 1015; lane 7, nested PCR cocktail control; lanes M, 1 kb-ladder.**

Contributors: C. Zuleta, R. Sedano, S. Kelemu.

Activity 8. Antifungal protein isolated from seeds of tropical forage legume.

Introduction

An array of plant defense mechanisms can be triggered upon wounding or perception of microorganisms, including the synthesis of proteins and peptides that have antifungal activity. Like plants, bacteria, insects, fungi, and mammals synthesize a number of antifungal proteins and peptides (small proteins). Plant seeds use strategies to germinate and survive in the soil that is densely inhabited with 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.

In this study, we examined a number of tropical forage legume seeds for antifungal properties. Among those examined, *Clitoria ternatea* (L.) seeds exhibited strong antifungal activity on the test fungus *Rhizoctonia solani in vitro*. Among other traits, *C. ternatea* is: 1) adapted to a wide range of soil conditions; 2) drought resistant; 3) practically free of diseases and pests.

We report here the isolation, purification, and characterization of a peptide from seeds of *C. ternatea* with antifungal, antibacterial and insecticidal properties.

Materials and Methods

***Biological materials*:** *C. ternatea* CIAT 20692 samples were initially obtained from germplasm collection maintained by CIAT's genetic resources unit. Once antifungus activity was determined, we planted the remaining seeds on field plots at CIAT headquarters in Palmira for large quantities of seed production. The test fungus *Rhizoctonia solani* originally isolated from *Centrosema pubescens* CIAT 5596 was maintained as air-dried sclerotia produced on potato dextrose agar (PDA).

***Seed extraction*:** Seeds (3 g) of *C. ternatea* CIAT 20692 were surface sterilized in 70% ethanol (4 min), in 2.5% NaOCl solution for 15 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 30 mL sterile distilled water with sterile mortar and pestle. The macerated solution was filtered through several layers of cheese cloth. The filtrate was then centrifuged in Eppendorf tubes (1 mL) at 13,000 x g for 30 min. The supernatant was used to determine antifungal activity bioassay.

***Antifungal activity bioassay*:** Three thick filter (#7) paper discs were placed on PDA containing petri dishes. A 300-µl seed extract filtrate was carefully applied onto one of the filter paper discs, where as sterile distilled water (300-µl) was pipetted onto the second filter paper. A single sclerotium of *R. solani* was then placed in the center of the plate and incubated at 28 ºC. Evaluations were made after two days of incubation.

***Determination of the nature of active extract*:** The extract was either treated with pronase (2 mg/mL final concentration) and incubated at 37 ºC for 2 h; or heat treated at 100 ºC for 5 min.

***Protein gel electrophoresis (IEF and SDS-PAGE)*:** Samples were cleaned and concentrated (typically 10-fold) by ultrafiltration with Centricon-3 membrane tubes (3,000-molecular-weight cutoff; Amicon). They were then analyzed by isoelectric focusing (IEF) in ultra-thin-layer polyacrylamide gels (Serva Fein-biochemica GmbH & Co). The samples were loaded in triplicates on the same gel, leaving enough space between them for cutting the gel in three equal parts once the run was complete. One was stained with Coomassie Brilliant Blue R250 to visualize the proteins; the second was neutralized in a buffer and then lightly coated with PDA by pouring a warm PDA before it solidified; the third was neutralized and then over-imposed on the Coomassie-stained triplicate and gel areas corresponding to individual stained protein bands were cut out for further antifungal activity tests.

Samples were also analyzed by SDS-PAGE (separating gel: 12% total acrylamide, 0.3% bis-acrylamide; stacking gel: 4% total acrylamide, 0.2% bis-acrylamide).

***Isolation and purification of antifungal protein*:** Proteins were extracted from10 g seeds macerated in 100 mL sterile distilled water for protein purification. The macerated suspension was filtered through several layers of cheese cloth and centrifuged at 13,000 x g for 30 minutes. The supernatant was deprived of low-molecular-weight solutes by ultrafiltration with Centricon-3 and then concentrated by lyophilization. The lyophilized powder was re-suspended in ($1/10^{th}$ of the original volume) sterile distilled water. The sample was resolved by preparative granulated bed isoelectric focusing (Bio-Rad Laboratories) with pH range of 3.5-9.5, and according to the manufacturer's instructions. The gel was divided into approximately 0.7 cm wide sections, which were scooped out and placed in microcentrifuge tubes. Proteins were eluted by centrifuging the fractions in microcentrifuge tubes.

***Insect rearing and feeding tests*:** Tests were conducted with two species of bruchids that are key pests of stored beans around the world: the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman), and the bean weevil, *Acanthoscelides obtectus* (Say). Techniques to maintain insect cultures of the bruchids were identical to those described by Cardona et al. (1989. J. Econ. Entomol. 82: 310-315). All experiments were conducted at 27^{o}C and 70% RH in a controlled environment chamber.

To test for possible insecticidal effects of the protein on both bruchid species, "artificial" seeds were prepared with flour of the commercial, highly susceptible bean variety 'ICA Pijao'. Artificial seeds were prepared by following, without modifications, the technique devised by Shade et al. (1986. Environ. Entomol. 15: 1286-1291) for the cowpea weevil, *Callosobruchus maculatus* (F.). Briefly, beans were soaked, the testae were removed and the flour was dried and milled. The flour was then reconstituted in Teflon molds, lyophilized, then hydrated at room temperature. Artificial seeds were coated with gelatin and infested as if they were intact.

The purified protein was mixed with flour of 'ICA Pijao' at different concentrations (0, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, and 5% w/w). Infestation procedures were as follows: for *Z. subfasciatus,* seeds were infested with at least eight pairs of bruchids per seed. After five days, seeds were examined under a dissecting microscope and 5-6 eggs were left per seed by destroying the excess with a needle. For *A. obtectus*, seeds for each protein concentration were infested with 5-6 neonate larvae per seed. Larval penetration was subsequently checked to guarantee correct mortality counts. All artificial seeds were individually evaluated in glass vials.

Percent adult emergence and days to adult emergence until the last insect emerged were the parameters recorded, although insect survival has been expressed in terms of percent mortality. At the end of the trial, when no more adult emergence occurred, the instar of dead larvae within the seed was determined by measuring the width of the head capsule after dissection of the seeds.

Statistical analysis: Concentration-mortality responses were estimated by means of probit regression analysis (SAS Institute1989). The Statistix package (Analytical Software 2000) was used for analysis of variance performed with data on days to adult emergence

Results and Discussion

***Antifungal activity*:** The crude extract from seeds of *C. ternatea* CIAT 20692 showed strong antifungal activity on the test fungus *R. solani* **(Figure 1)**. This activity could be eliminated by treatment with Pronase E **(Figure 2)**, indicating that the active compound is a protein. The activity was heat stable **(Figure 3)**. Seeds release this heat stable proteinaceous antifungal compound after mechanical disruption of their seed coat **(Figure 4)** or after germination (data not shown).

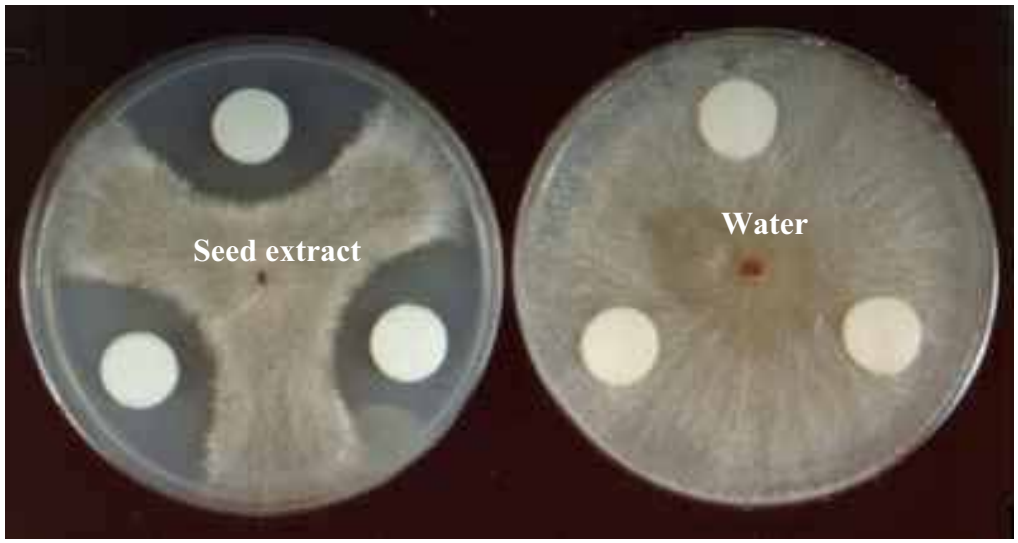


Figure 1. Growth inhibition of *Rhizoctonia solani* by seed extract from *Clitoria ternatea* CIAT 20692 on potato dextrose agar plates incubated at 28 ºC for 2 days. Seed extract filtrate (300-µl) was applied on each of the three filter paper discs, whereas the control plate had discs with equal volumes of sterile distilled water. A single sclerotium of *R. solani* was placed on the center each plate.

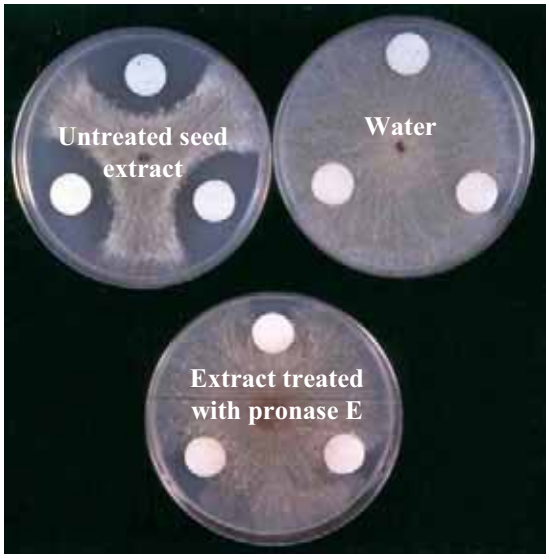


Figure 2. **Elimination of antifungal activity of extracts from seeds of *Clitoria ternatea* CIAT 20692 after treatment with pronase E. A single sclerotium of *R. solani* was placed on the center each plate containing potato dextrose agar and incubated at 28 ºC for 2 days. Seed extract filtrate (300-µl), either treated with pronase E or untreated, was applied on each of the three filter paper discs, whereas the control plate had discs with equal volumes of sterile distilled water.**

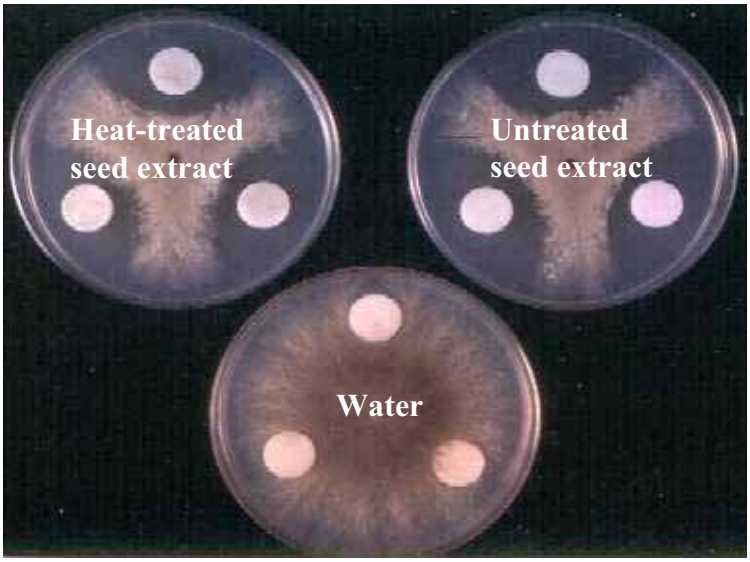


Figure 3. **Heat stability of antifungal activity of extracts from *Clitoria ternatea* CIAT 20692 seeds after boiling for 5 min. A single sclerotium of *R. solani* was placed on the center each plate containing potato dextrose agar and incubated at 28 ºC for 2 days. Seed extract filtrate (300-µl), either boiled or untreated, was applied on each of the three filter paper discs, whereas the control plate had discs with equal volumes of sterile distilled water.**

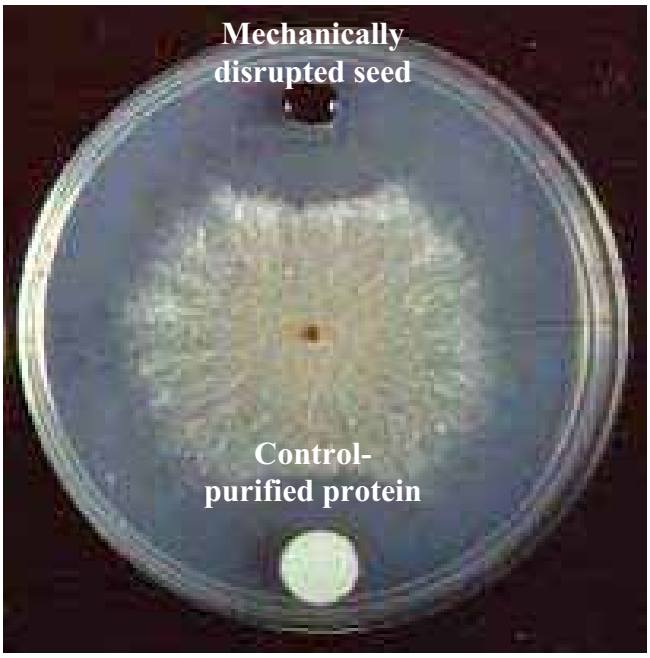
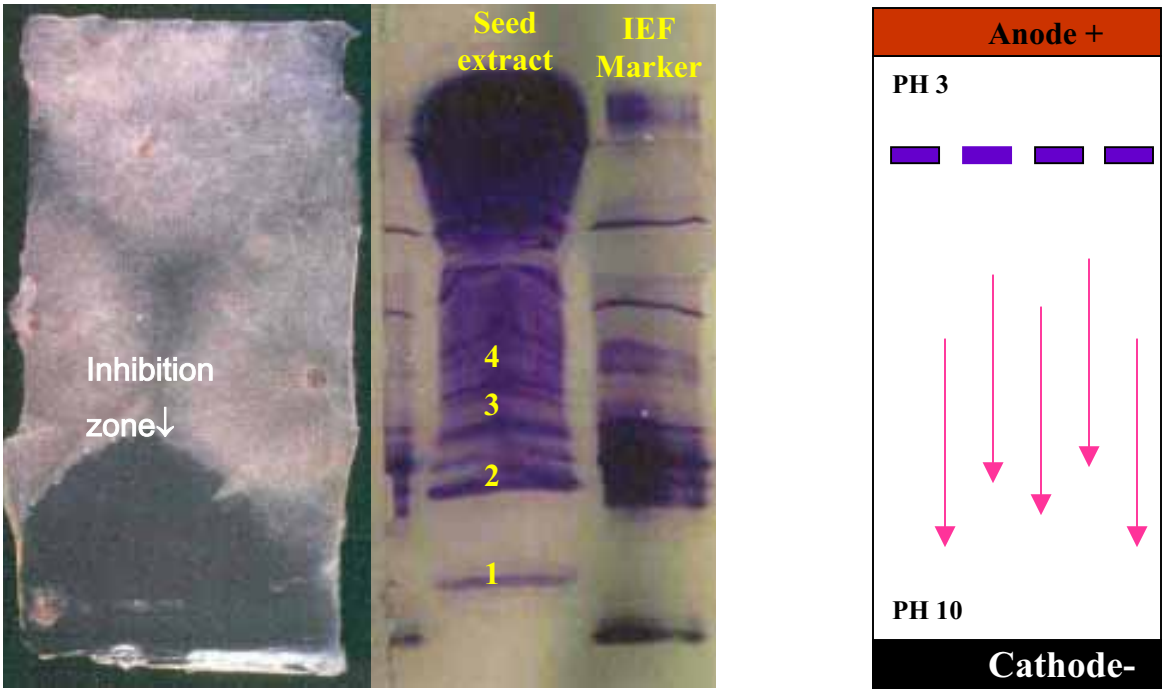


Figure 4. Seeds release an antifungal compound after mechanical disruption of their seed coat. Note that a single seed can release a compound with substantial antifungal activity against the test fungus *Rhizoctonia solani*.

***Identification of antifungal protein and purification*:** Resolving the seed extract by isoelectric focusing gel revealed a number of proteins. Thus, identifying the specific protein (s) responsible for the antifungal activity seemed a daunting task. To reduce the complexity of that task and to facilitate the identification of the specific protein of interest, we created a new protocol which involves: 1) resolving the seed extract by an IEF gel, 2) neutralizing the gel to eliminate the pH gradient, 3) lightly and uniformly coating the gel with warm PDA, 4) inoculating the gel/PDA composition with *R. solani* sclerotia, 5) wrapping it with Saran wrap to avoid loss of moisture and incubating it at 28 ºC for 2 days. This protocol, in fact, had greatly facilitated our task. *R. solani* grew uninhibited in the large portion of the gel/PDA composition, but was inhibited in the area where proteins with alkaline pI run.The specific antifungal protein was identified by cutting out ultra-thin-layer polyacrylamide gel areas corresponding to individual protein bands in a duplicate Coomassie-stained gel. The sliced gels were each macerated in 100-µl sterile distilled water in Eppendorf tubes and used for antifungal activity. The results of these tests show that a highly basic protein **(numbered 1 in Figure 5)** was responsible for the antifungal activity **(Figure 6)**.



IEF gel replica coated with PDA and inoculated with *Rhizoctonia solani*

Coomassie blue-stained IEF gel for selection of protein bands

IEF gel pH gradient

Figure 5. Extracts of *Clitoria ternatea* seeds resolved by isoelectric focusing (IEF) gel. *Rhizoctonia solani* growth inhibition zone indicated that the protein (s) responsible for antifungal activity were in the alkaline part of the gel. A triplicate IEF gel was superimposed on an identical Coomassie-stained IEF gel. Gel areas corresponding to the stained bands 1-4 were cut out for identification of the antifungal protein.

The peptide was well separated from the other proteins on IEF gels, making the purification procedure using the preparative granulated bed isoelectric focusing a relatively easy task. Five of the fractions showed activity with decreasing intensity starting from the highly alkaline pI (isoelectric point) **[Figure 7]**. The active protein which was mostly recovered in fraction 1 was named Finotin. Both SDS-PAGE **(Figure 8)** and IEF gels (data not shown) showed that fraction 1 is pure and free of other proteins.

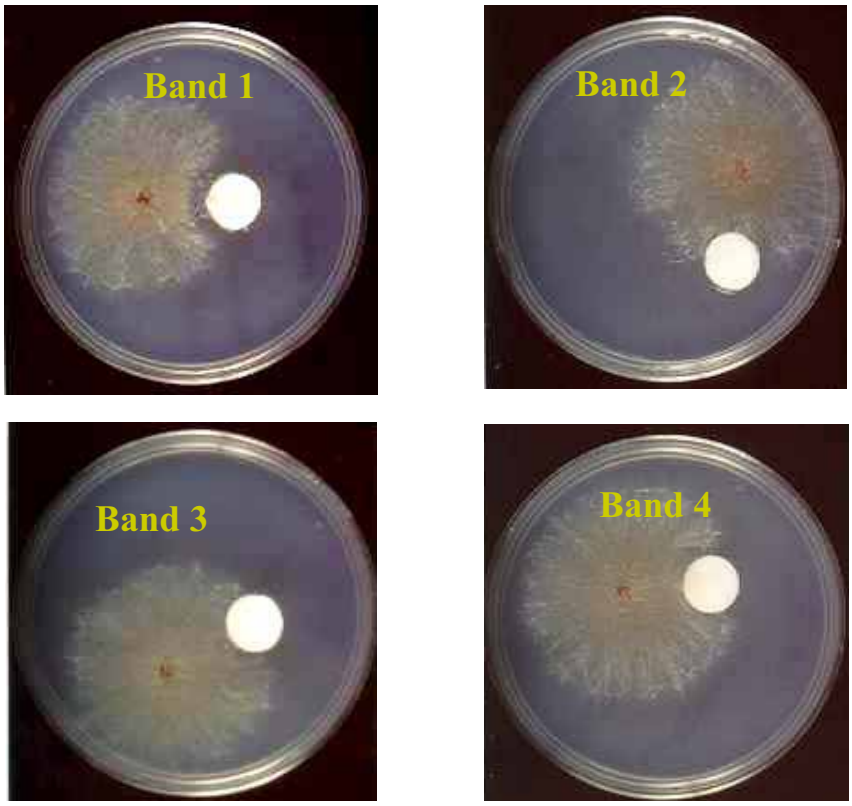


Figure 6. **Identification of specific antifungal protein band. The protein band with the most alkaline pI (isoelectric point) numbered 1 in Figure Finotin 4 demonstrated growth inhibition of the test fungus *Rhizoctonia solani*.**

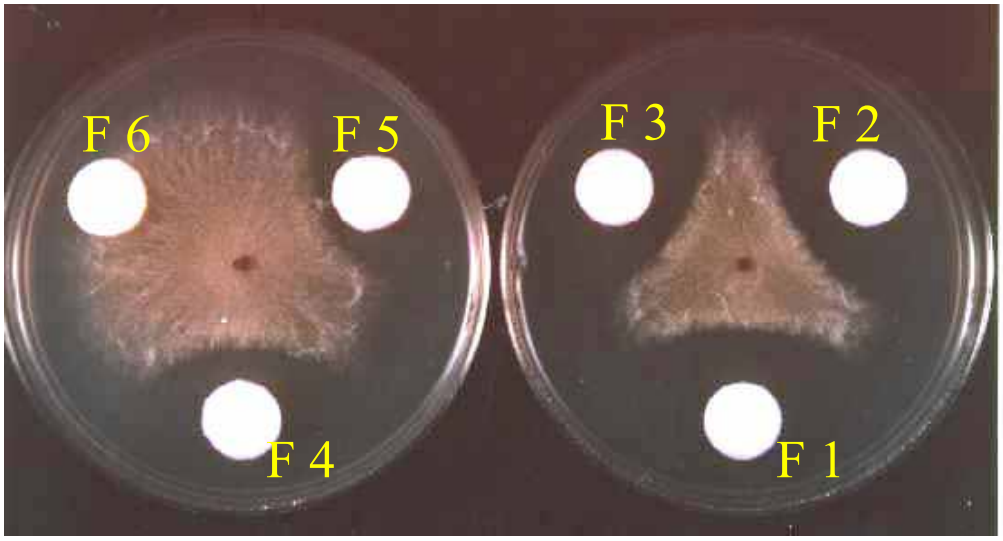


Figure 7. **Purification of an antifungal protein from seeds of *Clitoria ternatea* using preparative granulated bed isoelectric focusing. Five fractions scooped from the gel starting from the alkaline part of the gel as # F1 demonstrated antifungal activities, with F5 having the least growth inhibitory activity against *Rhizoctonia solani*.**

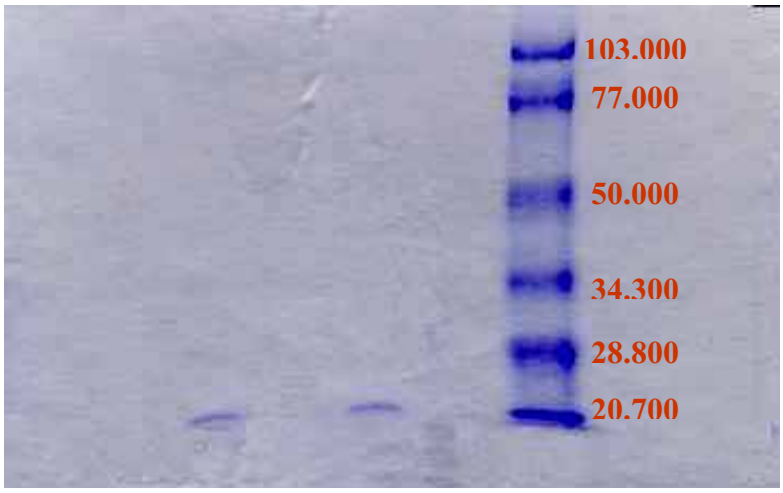


Figure 8. SDS-PAGE analysis of purified protein fraction from *Clitoria ternatea* seeds.

***Antifungal/antibacterial activity*:** Finotin was active against a number of fungi and a bacterium pathogenic on common beans. It inhibits fungi pathogenic on a number of plants, including rice, beans, *Brachiaria*, and *Stylosanthes*, that we tested so far. *Colletotrichum lindemuthianum* and *Xanthomonas axonopodis* pv. *phaseoli* from common beans; *Lasiodiplodia theobromae* and *Colletotrichum gloeosporioides* from *Stylosanthes* spp; *Helminthosporium* spp. and *Pyricularia grisea* from rice; and *Rhizoctonia solani* from *Brachiaria* were all inhibited in growth by Finotin in culture. We have yet to determine the mode of action of Finotin.

***Insecticidal activity*:** Mortality on artificial seeds prepared with the susceptible background bean flour ('ICA Pijao') was very low (less than 3%) for both *Z. subfasciatus* and *A. obtectus*. Enrichment of artificial seeds with increasing levels of the test protein led to an increase in mortality reaching maximal levels (100% larval mortality) at the dosage of 5% in the case of *Z. subfasciatus* and 1% in the case of *A. obtectus*. Probit analysis **(Table 1)** showed that the protein is highly toxic to both bruchid species with LC_{50} values that can be considered low (less than 2%). The LC_{50} value for *A. obtectus* (0.36%) was ca. four times lower than that for *Z. subfasciatus* (1.21) meaning that the protein is more toxic to *A. obtectus*. The protein is very toxic to first instar larvae of both bruchid species: dissection of infested seeds revealed that up to 75% of the larvae did not reach the second instar stage.

Concentration responses in terms of days to adult emergence are shown in Table 2. Developmental times of those few insects that survived the different protein concentrations were prolonged. There was a definite dosage response: the higher the dosage the longer the developmental time. This is further proof of the toxicity of the protein to both bruchid species.

Table 1. Toxicological responses of bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus* to a purified protein (Finotin) isolated from seeds of *Clitoria ternatea.*

No. Tested	LC_{50} (95% FL)[a]	LC_{95} (95% FL)	Slope ± SEM	χ^2
		Zabrotes subfasciatus		
147	1.21 (0.99 - 1.47)	2.88 (2.17 - 5.21)	4.3 ± 0.88	2.78
		Acanthoscelides obtectus		
155	0.36 (0.28 - 0.43)	0.77 (0.61 - 1.28)	4.9 ± 0.96	0.84

[a] FL, fiducial limits.

Table 2. Effect of increasing concentrations of a purified protein (Finotin) isolated from seeds of *Clitoria ternatea* on the biology (days to adult emergence) of the bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus*

Protein Concentration (% w/w)	Days to Adult Emergence	
	Zabrotes subfasciatus	***Acanthoscelides obtectus***
0.0[1]	43.1e	34.4c
0.0625	45.0e	33.8c
0.125	51.5d	35.2c
0.25	55.6c	49.4b
0.5	57.3c	63.4a
1.0	72.7b	N.E.
2.0	80.0a	N.E.
5.0	N.E[2].	N.E.

Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD. ANOVA on data testing for differences among dosages (protein concentrations). For *Z. subfasciatus*: $F = 106.9$; df = 6,21; $P < 0.001$; for *A. obtectus*: $F = 184.9$; df = 4,16; $P < 0.001$.

[1] Background flour prepared with 'ICA Pijao'

[2] N.E., no adult emergence, 100% larval mortality.

Finotin showed growth inhibitory effects against fungi, bacteria and insects. This wide range of activity strongly suggests that this peptide is an important component of the natural defense system of *C. ternatea*. The preferential release of this peptide during seed germination or seed damage can contribute to the protection of the emerging seedlings from soil-borne pathogens. Interestingly, the expression of this peptide is not restricted to seeds, but it also occurs in other parts of *C. ternatea* such as roots upon drought stress in greenhouse tests (data not shown). However, it is not clear whether this peptide plays any role in drought tolerance, a trait which *C. ternatea* has. This and other potential roles played by Finotin are yet to be determined once the gene encoding Finotin is isolated.

Contributors: G. Segura, C. Cardona, S. Kelemu.

Publications

Refereed Journal Articles

Kelemu, S., Dongyi, H., Guixiu, H. and Takayama, Y. 2003. Detecting and differentiating *Acremonium implicatum*: developing a PCR-based method for an endophytic fungus associated with the genus *Brachiaria*. Molecular Plant Pathology 4(2): 115-118.

Kelemu, S. and Dongyi, H. 2003. Endophytic fungus *Acremonium implicatum* is seed transmitted in *Brachiaria* spp. (Abstract). Phytopathology 93:S43.

Kelemu, S. and Mahuku, G. 2003. Harnessing Biotechnology to Improve Food and Forage Legumes: The Case of Plant Disease Resistance. Paper for the Second National Workshop on Food and Forage Legumes, 22-27 September 2003, Addis Ababa, Ethiopia.

Weeds, P.L., Chakraborty, S., Fernandes, C.D., Charchar, M. J. d'A., Ramesh, C.R., Kexian, Y. and **Kelemu, S**. 2003. Genetic Diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centers of origin and utilization. Phytopathology 93:176-185.

Submitted

Chakraborty, S., Ghosh, R., Mukherjee, M., Fernandes, C. D., Charchar, M. J. and **Kelemu, S.** 2003. A comparison of artificial neural network and multiple regression analysis for weather-based prediction of anthracnose.

Kelemu, S., Mahuku, G., Fregene, M., Pachico, P., 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 Biotechnology Journal.

Conference Papers

Kelemu, S. and Mahuku, G. 2003. Harnessing Biotechnology to Improve Food and Forage Legumes: The Case of Plant Disease Resistance. Paper for the Second National Workshop on Food and Forage Legumes, 22-27 September 2003, Addis Ababa, Ethiopia.

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.** 2003. Weather dependency of anthracnose and risk mapping. In: S.Chakraborty (editor) High yielding anthracnose resistant Stylosanthes for Agricultural systems'

Kelemu, S., Miles, J. W. and Rao, I. M. 2003. Biotic and abiotic constraints to *Stylosanthes* production. **In:** S.Chakraborty (editor) High yielding anthracnose resistant Stylosanthes for Agricultural systems'

Other Publications

T Amede, E Amézquita, J Ashby, M Ayarza, E Barrios, A Bationo, S Beebe, A Bellotti, M Blair, R Delve, S Fujisaka, R Howeler, N Johnson, S Kaaria, **S Kelemu**, P Kerridge, R Kirkby, C Lascano, R Lefroy, G Mahuku, H Murwira, T Oberthur, D Pachico, M Peters, J Ramisch, I Rao, M Rondon, P Sanginga, M Swift and B Vanlauwe. 2003. BNF: A key input for integrated soil fertility management in the tropics. (A working group paper).

Staff: Raul Sedano, B.Sc, Research Assistant II. Martin Rodriguez, B.Sc., Research Assistant III, Ximena Bonilla, B.Sc., Research Assistant III (part time), Carolina Zuleta, Research Assistant III, Tomoko Sakai, Visiting Scientist, Japan Overseas Cooperation Volunteers (JOCV), JICA, Japan, Gustavo Segura, Technician II, Alvaro Baena, Technician II, Dario Viveros, Technician III.

Donors: The Ministry of Foreign Affairs, Japan.

Collaborators: Japan International Cooperation Agency (JICA). Dr. C. Schardl, University of Kentucky, USA.

BEAN PATHOLOGY

Activity 1. Identifying common bean genotypes with resistance to angular leaf spot.

Introduction

The angular leaf spot pathogen, *Phaeoisariopsis griseola*, maintains a high level of variability, and as a result, screening for sources of resistance, identification and confirmation of resistant genotypes is a continuous activity. During 2003, we screened 87 recombinant inbred lines derived from crossing G 19833 (Andean) with DOR 364 (Mesoamerican) with different *P. griseola p*athotypes, representing the two major groups of *P. griseaola* (Andean and Mesoamerican pathotypes). The objective of this study was to identify lines that combined resistance to Andean and Mesoamerican *P. griseola* races in recombinant inbred lines (RILs) derived from a DOR364 x G 19833 cross. It was hopped that the identified lines will have the widest range of seed color and types to take advantage of the wide variability for seed type and color found in common bean. In addition, we also evaluated under greenhouse conditions advanced lines from the Mesoamerican breeding group with the most virulent pathotype (63-63) of Mesoamerican origin. It is hopped that these lines could be used as parents to improve ALS resistance in common bean.

Materials and Methods: Seven *P. griseola* races **(Table 1)** were used to inoculate plants under greenhouse conditions. These races included the most virulent and widely distributed races that we have in our collection. Inoculum production, plant inoculations and maintenance were done as described previously (CIAT 2002). Plants were evaluated starting 8 days after inoculation, using the standard procedure according to the CIAT - 9-class scale described by Schoonhoven y Pastor-Corrales (1987).

Results and Discussion: 33 recombinant inbred lines from the DOR 364 x G 19833 cross were identified that combined resistance to both Andean and Mesoamerican pathotypes of *P. griseola* **(Table 2)**. In previous evaluations, some of these lines were identified as having good levels of resistance to 6 races of *Colletotrichum lindemuthianum* **(Table 1)**. These materials combine resistance against Andean and Mesoamerican races of *P. griseola* and *C.lindemuthianum*, they therefore, from a very import potential source of multiple resistance to the two most important common bean pathogens. These materials have resistance to bean golden mosaic virus, and they should be evaluated with other races of a diverse origin to establish their suitability for managing the ALS disease of common bean.

Conclusions: The common bean lines reported here constitute very important sources of resistance for *P. griseola* and *C. lindemuthianum*. It is important that these materials are evaluated first under greenhouse conditions with isolates from different regions where ALS is an important pathogen, and other important pathogens like common bacterial blight, root rot causing pathogens etc. in order to identify lines that combine resistance to the greatest number of bean pathogens. Furthermore, the identified resistant materials should be included in an ALS nursery and distributed to our partners for evaluations under prevailing field conditions in their regions.

Table 1. Virulence phenotypes of *Colletotrichum lindemuthianum* pathotypes (races) used to evaluate recombinant inbred lines derived from a DOR 364 (Mesoamerican) x G 19833 (Andean) cross.

	Anthracnose differential cultivars*											
Pathotype	Michelite	MDRK	Perry Marrow	Cornell 49242	Widusa	Kaboon	México 222	PI207262	TO	TU4	Ab 136	G 2333
385	+							+	+			
3481	+			+	+			+	+	+	+	+
521	+			+						+		
39	+	+	+			+						

* Andean genotypes are MDRK, Perry Marrow, Widusa and Kabbon.

Table 2. Reaction of 87 recombinant inbred lines derived from a DOR 364 (Mesoamerican) x G 19833 (Andean) cross to races of *P. griseola* and *C. lindemuthianum.*

	Phaeoisariopsis griseola races							
Identification	31-19	63-59	63-63	15-55	31-47	63-0	15-0	*Anthracnose*
BT 20454- 1-1-1-1-M-M	2.3	2.0	2.8	2.1	2.3	1.0	1.9	2.4
BT 20454- 2-1-1-1-M-M	3.3	3.7	3.5	2.7	4.0	1.0	1.0	1.7
BT 20454- 9-1-1-1-M-M	3.6	5.9	3.5	2.8	2.6	1.0	1.0	4.4
BT 20454-14-1-1-1-M-M	3.0	4.0	2.3	2.6	1.1	1.0	1.0	3.8
BT 20454-18-1-1-1-M-M	1.1	2.4	1.5	1.8	1.0	1.0	2.5	1.4
BT 20454-21-1-1-1-M-M	4.3	2.2	4.8	4.8	2.0	1.0	1.0	1.2
BT 20454-22-1-1-1-M-M	4.0	3.1	4.4	3.1	2.6	1.0	1.0	2.5
BT 20454-26-1-1-1-M-M	4.9	5.4	2.4	3.8	1.8	2.3	1.0	2.7
BT 20454-30-1-1-1-M-M	2.0	1.0	2.0	1.5	2.4	1.0	2.0	1.3
BT 20454-33-1-1-1-M-M	3.2	1.0	2.7	1.9	2.4	1.0	1.0	2.3
BT 20454-39-1-1-1-M-M	4.0	4.1	4.7	3.5	3.5	1.0	2.0	2.7
BT 20454-40-1-1-1-M-M	2.0	3.9	3.2	3.0	2.4	4.5	2.0	3.3
BT 20454-42-1-1-1-M-M	4.0	4.3	2.8	4.5	2.9	1.0	1.3	3.0
BT 20454-52-1-1-1-M-M	2.0	2.2	2.3	1.7	2.2	1.0	1.0	1.5
BT 20454-53-1-1-1-M-M	2.8	2.1	2.5	2.6	2.5	1.0	1.5	1.0
BT 20454-59-1-1-1-M-M	3.7	4.7	2.3	3.5	3.0	1.1	2.6	1.5
BT 20454-63-1-1-1-M-M	2.0	3.2	2.0	1.3	2.0	1.0	1.0	1.3
BT 20454-69-1-1-1-M-M	3.6	4.8	2.7	1.8	2.2	1.0	1.0	2.1
BT 20454-70-1-1-1-M-M	4.8	3.2	3.0	2.0	3.7	1.0	1.3	2.1
BT 20454-74-1-1-1-M-M	3.0	4.4	3.5	2.4	3.8	1.6	4.7	3.0
BT 20454-75-1-1-1-M-M	3.6	3.9	2.0	1.2	1.7	1.0	1.1	2.0
BT 20454-77-1-1-1-M-M	3.0	2.4	2.0	1.5	2.0	4.7	5.1	3.6
BT 20454-78-1-1-1-M-M	4.4	3.0	2.0	1.3	1.8	1.1	1.8	2.4
BT 20454-79-1-1-1-M-M	1.5	1.8	1.3	1.0	1.0	1.1	1.2	3.2
BT 20454-81-1-1-1-M-M	2.2	3.1	3.2	2.1	2.4	1.3	1.5	2.6
BT 20454-85-1-1-1-M-M		3.9	4.0	2.6	3.0	1.0	1.1	5.0
BT 20454-88-1-1-1-M-M	3.0	2.8	2.5	1.9	1.8	1.6	1.0	4.3
BT 20454-91-1-1-1-M-M	3.2	3.1	3.7	1.5	1.7	1.0	1.0	2.7
BT 20454-93-1-1-1-M-M	1.5	3.1	1.7	1.2	1.3	1.1	3.8	1.6
BT 20454-94-1-1-1-M-M	3.1	4.2	3.2	2.3	2.2	1.0	1.0	1.1
BT 20454-95-1-1-1-M-M	3.4	4.0	3.8	1.4	1.5	1.6	1.0	2.
BT 20454-100-1-1-1-M-M	2.5	3.6	2.7	1.8	2.5	2.6	5.2	3.9
BT 20454-106-1-1-1-M-M	2.5	3.5	2.0	2.4	1.5	1.0	1.0	2.5

Contributors: G. Mahuku, C. Jara, G. Castellanos.

Activity 2. Angular leafspot pathogen Characterized.

Random Amplified Microsatellite diversity in the common bean angular leaf spot pathogen, *Phaeoisariopsis griseola*

Introduction

With the intensification of common bean production, the incidence of the angular leaf spot disease has been on the increase, with new reports of devastation of bean production being reported every season. ALS is now wide spread in all parts of Africa, Central America, South America (Bolivia and Brazil), and the Caribbean (mainly Haiti). High genetic variability has been reported almost in all areas where the disease is a major problem, yet no sexual reproduction has been reported and preliminary analysis of the genetic population have all pointed to the fact that this pathogen is exclusively asexual. To better understand the origin and maintenance of this high genetic variation, we undertook a study to look at the molecular basis of this genetic variation, and try to elucidate its mechanism. It was hopped that this information would shed light into the evolutionary history of this pathogen and its potential to change. This information would be valuable in breeding for resistance against this pathogen and in the deployment of identified resistance genes.

Materials and Methods: A total of 808 *Phaeoisariopsis griseola* isolates from Africa, Latin America and Carribean where characterized using 5 random amplified microsatellite primers (RAMS). Population subdivion analysis was done using the POPGENE program. Genetic similarity between two isolates was calculated based on Dice's coefficient with the SimQual program of NTSYS-pc Version 1.8, and a dndrogram constructed with the help of the unweighted pair grouping by mathematical averaging (UPGMA) methods using the SAHN and TREE programs in NTSYS. Multiple correspondence analysis was used to assign isolates to groups. Haplotype diversity within and between MCA groups was calculated as described by Nei (1973). In addition, analysis of molecular variance (AMOVA) was used to partition the total genetic variation observed into that ascribed to differences between Andean and Mesoamerican *P. griseola* groups and that arising from differences of isolates within a group. Isolates had been classified into Andean and Mesoamerican based geographical origin. Nei's analysis of gene diversity in subdivided populations was used to estimate the genetic identity and genetic distances between *P. griseola* Andean and Mesoamerican groups.

Results and Discussion: Sixty (60) polymorphic fragments were generated by RAMS primer among the 808 *P. griseola* isolates. Cluster and MCA identified 395 haplotypes among 808 isolates and these were separated into two major groups **(Figures 1 and 2)**. Group 1 contained all Mesoamerican isolates, irrespective of their origin, while group 2 contained all Andean isolates. Within each group, isolates clustered according to geographical origin, with Andean isolates from Africa clustering together as did isolates from Latin America **(Figures 1 and 2)**. A similar trend was evident within the Mesoamerican group, where three groups were evident, one composed of isolates from Africa, a second of isolates from Bolivia and Brazil and a third of isolates from Central America **(Figures 1 and 2)**. Mesoamerican isolates were more diverse, compared to Andean isolates. High levels of genetic variability (H = 0.93) were observed within each group.

The total gene diversity within the total P. griseola population was estimated to be 0.2936 $\pm$ 0.0277, while it was 0.2307 $\pm$ 0.1828 in the Andean group and 0.2469 $\pm$ 0.1825 in the Mesoamerican group. The genetic identifty between Andean and Mesoamerican isolates was high (0.856) while the genetic distances between the two subgroups was 0.1555. Significant geographical differentiation (Gst = 0.402) was observed within the Andean sub-group, where the genetic identity between Andean isolates from Africa and Latin America was 0.7313, while the genetic distance between the two populations was 0.313. Although the geographical differentiation within Mesoamerican isolates from Latin America and Africa (Gst = 0.3499) was lower than that between Andean isolates, it was highly significant. The genetic identity between Mesoamerican isolates was 0.7922 while the genetic distance was 0.2330. These result reveal a significant amount of geographical differentiation within the two major sub-groups of *P. griseola*. When isolates were divided into Andean

Conclusion: These results confirm previous reports (Mahuku et al., 2002) of host and geographical specialization within *P. griseola*, revealing that host specialization is apparently the dominant factor in shapping the population structure of the ALS pathogen, *P. griseola*. The high levels of genetic variabiulity within each group reveal the mixing of pathotypes, possibly through movement of scontaminated seed or through parasexual reproduction. These results have major implications in common bean improvement for reisstance to P. griseola, by revealing that mixing Andean and Mesoamerican host resistance genes might afford lasting resistance to this pathogen.

References

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321-3323.

Mahuku, G. S., M. A. Henríquez, J. Munõz, and R. A. Buruchara. 2002. Molecular Markers Dispute the Existence of the Afro-Andean Group of the Bean Angular Leaf Spot Pathogen, *Phaeoisariopsis griseola.* Phytopathology *92:* 580-589.

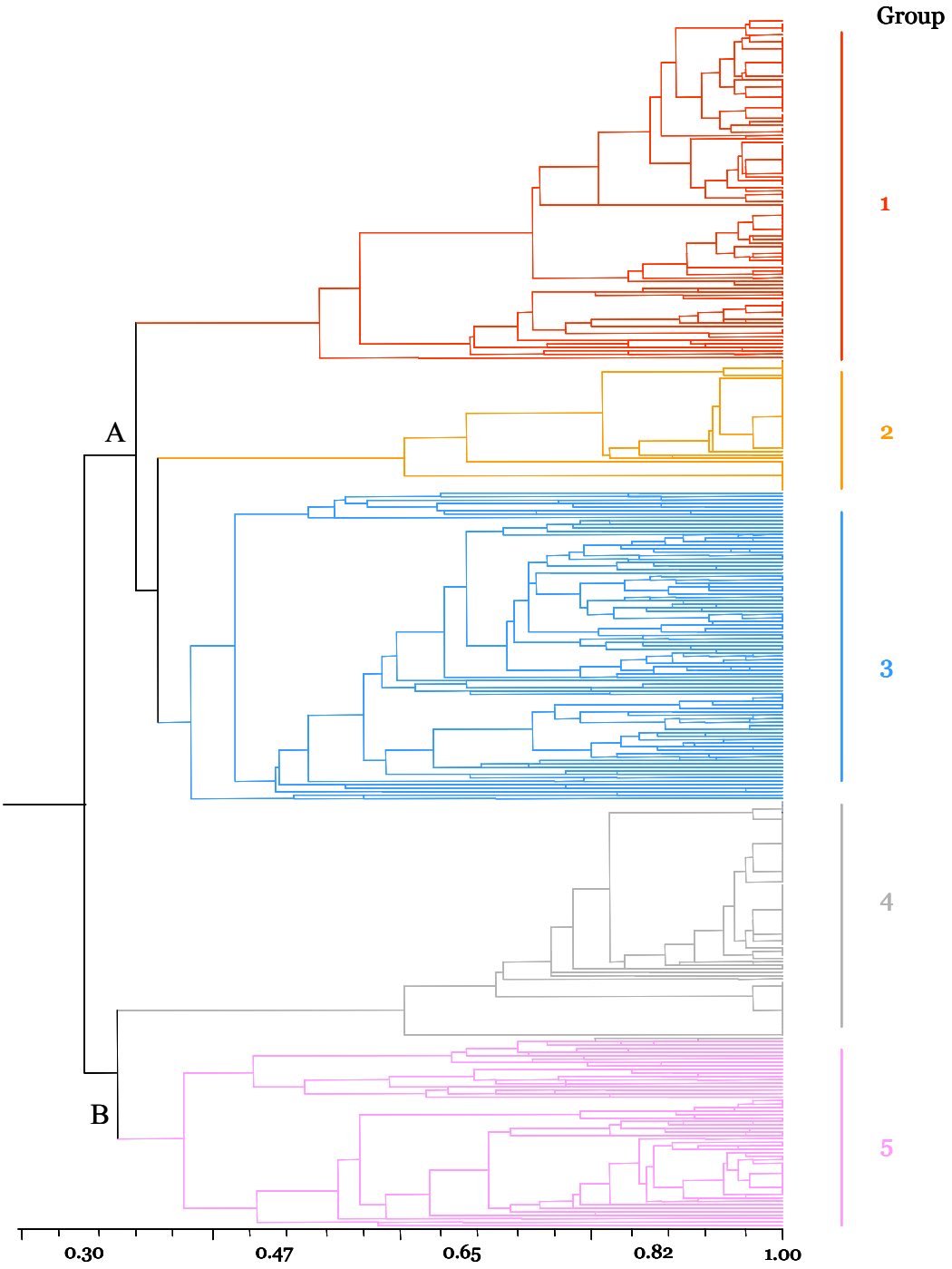
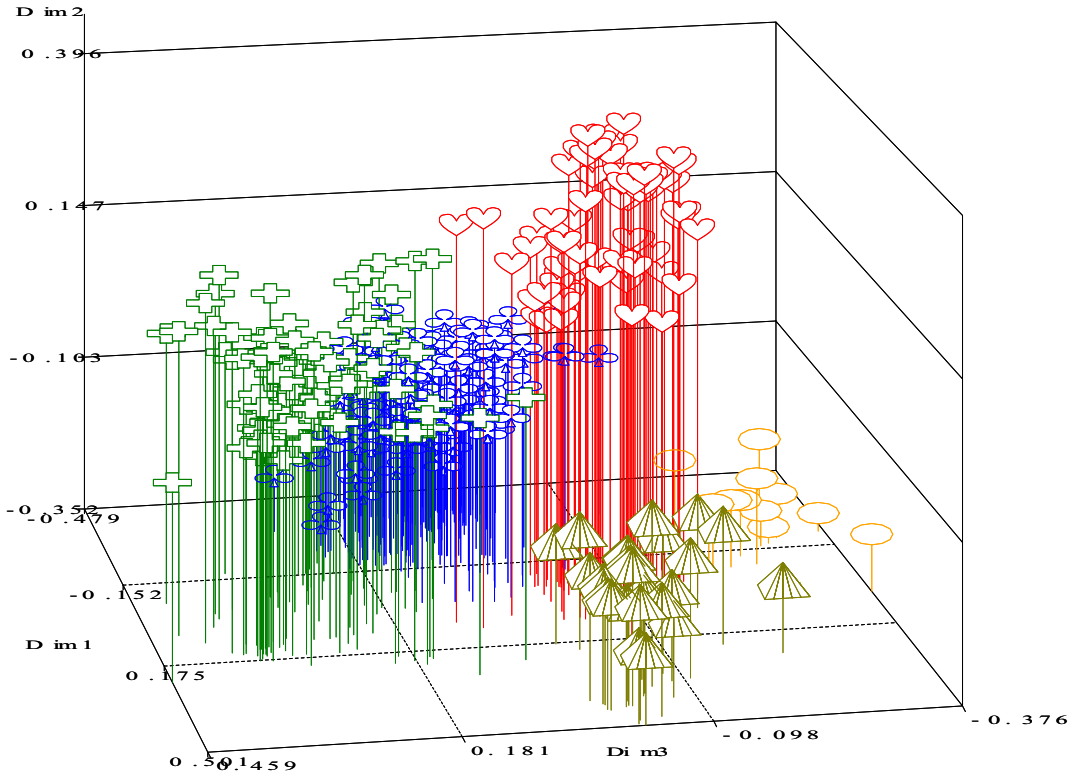


Figure 1. **Dendrogram of 808 *Phaeoisariopsis griseola* isolates plotted using data from combined RAMS data and generated using UPGMA in NTSYS. Cluster A represents Mesoamerican isolates from Brazil and Bolivia (group 1), Africa (group 2), and Central America (group 3). Cluster B represents Andean isolates from Africa (group 4) and Latin America (group 5).**



Group 1 – Mesoamerican isolates from Africa

Group 2 – Andean isolates from Africa

Group 3 – Mesoamerican isolates from Brasil and Bolivia

Group 4 – Mesoamerican isolates from Central America

Group 5 – Andean isolates from Colombia and Ecuador

Figure 2. **Three dimensional graph based on multiple corresppondence analysis of RAMS data and plotted using the spin platform of JMP program in SAS. Symbols indicate position of isolates within each cluster.**

Activity 3. Evidence that the common bean anthracnose pathogen, *Colletotrichum lindemuthiuanum* co-evolved with common bean gene pools.

Introduction

In 2002, we evaluated 40 wild and cultivated Phaseolus vulgaris genotypes of Andean and Mesoamerican genotypes using 35 *Colletotrichum lindemuthianum* isolates collected from Andean and Mesoamerican regions. Analysis of this data showed that wild beans were able to differentiate isolates into Andean and Mesoamerican subgroups, while cultivated or the established *C. lindemuthianum*, differential varieties could not clearly show this differentiation (CIAT, 2002). To further confirm the subdivion of *C. lindemuthianum* isolates into groups structured along gene pools defined for the commonbean host, we used molecular techniques (AFLP and RAMS) to characterize the 35 *C. lindemuthianum* isolates used in this study. If *C. lindemuthianum* coevolved with common bean gene pools, we would expect population subdivision analysis using molecular markers to differentiate isolates primarily into two groups that will be structured or closely so, with the two gene pools of the common bean. In addition, there should be significant levels of genetic differentiation between these groups. This information is important as it reinforces the hypothesis that pyramiding Andean and Mesoamerican anthracnose resistance genes into the same cultivar would potentially afford effective and lasting resistance.

Materials and Methods: Thirty-five *C. lindemuthianum* isolates from the bean pathology collection were used in this study **(Table 1)**. Isolates selected were either collected from Andean and Mesoamerican bean genotypes and exclusively from Andean and Mesoamerican regions. Andean isolates came from Ecuador, Colombia and Peru, and only those isolated from large seeded bean cultivars of Andean origin, while Mesoamerican isolates were selected from Costa Rica and Mexico, and were isolated from small seeded cultivars of Mesoamerican origin. Isolate recuperation, culturing, spore production, inoculum preparation and inoculations were done as described previously (Pastor-Corrales et al., 1993).

Production of mycelium and DNA extraction

Colletotrichum lindemuthianum mycelium was produced in liquid V8 juice medium as described earlier (Mahuku et al., 2002). DNA quality and concentration was determined by electrophoresis in 0.7% agarose gels, and DNA was quantified using a fluorometer (Hoefer® DyNA Quant 2000, Pharmacia Biotech, USA) and adjusted to a standard concentration of 5 ng/µl in 0.1 x TE buffer.

RAMS analysis

Seven random amplified microsatellite (RAMS) primers [(CA)n, (AG)n, (GT)n, (TG)n, (CT)n, (CGA)n, (CCA)n, (ACA)n] were used to amplify DNA from all *C. lindemuthianum* isolates. RAMS PCR reactions were carried out in 12.5 µl volumes essentially as described by Mahuku et al. (2002). The amplification product was electrophoresed in 1.2% agarose gels containing 0.17 µg ml^{-1} ethidium bromide and visualized under UV light. Gel images were captured using the Eagle Eye II gel documentation system (Strata gene) and band position was determined using the Quantity One scientific Software, Version 4 (BIO-RAD).

Table 1. Origin, year of collection and characteristic of 35 *Colletotrichum lindemuthianum* isolates used in this study.

Isolate	Origin	Host variety pool of collection	Race	Molecular group
100CRI	Puriscal Costa Rica	G 11389	1433	1
106CRI	Pejivalle, Costa Rica	Sacapobres CRS 12-1-1	1025	1
154CRI	J. Diaz Costa Rica	Criojjo CRG 4-4-1	137	1
173CRI	Turrubares, Costa Rica	Criollo CRS 52-4-1	89	1
178CRI	Mora-Tabarcia, Costa Rica	Vainica CRS 57-5-1	2001	1
41CRI	Sta. Ma. Sota, Costa Rica	Chileno	1497	1
42CRI	S. Rafael, Costa Rica	Huetar (CR 29)	3545	1
45CRI	Zapote de Z, Costa Rica	Criollo	1481	1
63CRI	Puriscal, Costa Rica	MUS 173	1435	1
28MEX	Texaco, Mexico	Amapola del camino	73	2
49MEX	Zarco; Tepatitlan Mexico	Garbancillo	5	2
13PER	Celedin-Cajamarca, Peru	Sorochuco	131	3
28ECU	Loja-Saraguro, Ecuador	Criollo	4	3
4PER	Mollepata-Cusco, Peru	Rojo mollepata	8	3
57PER	Huanbocancho, Peru	Pintado rojo	7	3
77ECU	Azuay, Valle Ecuador	Bola Amarilla	65	3
80ECU	Canton Nabon Ecuador	Bola Amarilla	6	3
82ECU	Canton-Nabon Ecuador	Bola Amarilla	129	3
84PER	Mollepata, Cusco Peru	AFR 354	7	3
89PER	Taray, Cusco Peru	Nuna Cheche local	3	3
102ECU	Chimborazo Ecuador	Silvestre-113	9	4
136 ECU	Santa Catalina Ccuador	Nuna angel poroto	133	4
224COL	Cundinamara , Colombia	Silestre	1	4
238COL	Darien, Colombia	Bolon Royo	521	4
241COL	Popayan-Cuca, Colombia	La victorie	3	4
304 COL	Rio Negro, Colombia	Cargamanto	13	4
19MEX	Zacatecas, Calera Mexico	Venezuela No. 2	1093	5
39MEX	Caeajal Mexico	A-8418-2	1088	5
59MEX	Durango Mexico	Garbancillo	129	5
70MEX	Rio Grande, Mexico	Flor de mayo	453	5
78MEX	Altos de Jalisco, Mexico	Mex 87-29-1	448	5
8MEX	FCO Madero, Mexico	G 1339	1097	5
92MEX	Calpan, Puebla Mexico	Bayo	393	5
98MEX	El Horno, Chapingo Mexico	Flor de mayo bajio	1089	5

AFLP analysis

AFLP fingerprints were generated based on the method of Voss et al. (1995), using the Life Technologies AFLP® Analysis System I. Genomic DNA (250 ng) was restricted with EcoRI and MseI (2.5 U each), and *EcoRI* and *MseI* adapters were subsequently ligated to the digested DNA. The adaptor-ligated DNA was pre-amplified with AFLP primers each having zero or one selective nucleotide, the pre-amplified DNA was diluted (1:10) and an aliquot was used for selective amplification in 12.5 µl reaction volumes with various combinations of EcoRI and MseI primers having two or three selective nucleotides at the 3' ends (E-NNN / M-NNN). The reaction products were resolved on 6% polyacrylamide gels, and the bands were detected using silver nitrate staining. Gel images were scanned and band position was determined using the Quantity One scientific Software, Version 4 (BIO-RAD).

Statistical Analysis
RAMS and AFLP markers were scored as either present (1) or absent (0) of a band. A matrix of present and absence of a band was contracted and the genetic distance between two isolates was calculated based on Dice's coefficient using SAS statistical package (SAS version 6, 1989). The similarity matrix was used to construct dendrograms with the unweighted pair grouping by mathematical averaging (UPGMA) methods using the SAHN and TREE programs in NTSYS (Rohlf, 1994). Multiple correspondence analysis was used to assign isolates to groups. Correlation between RAMS and AFLP lineages were determined using the MXCOMP option of NTSYS and Spearman's Rank Correlation Coefficients, and because RAMS and AFLP data were highly correlated, the data from the two were combined and subsequently analyzed as a single data set. In addition, analysis of molecular variance (AMOVA) was used to partition the total genetic variation observed into that ascribed to differences between Andean and Mesoamerican *C. lindemuthianum* groups and that arising from differences of isolates within a group. Isolates had been classified into Andean and Mesoamerican based on the genotype the isolates were collected from and the geographical origin. Nei's analysis of gene diversity in subdivided populations was used to estimate the genetic identity and genetic distances between Andean and Mesoamerican groups of *C. lindemuthianum*.

Results and Discussion: Of the 20 primer pair combinations tested on a set of 6 isolates from different geographical regions and classified as Andean or Mesoamerican based on virulence analysis, three (E-AGG/M-CTA; E-AAC/M-CAA y E-AAG/M-CAG) were selected for further characterization of all *C. lindemuthianum* isolates. A total of 112 polymorphic bands were generated by the three AFLP primer pair combinations, while the seven RAMS primers generated a total of 69 polymorphic fragments.

Cluster analysis separated isolates into two major groups with an average similarity index of 64% **(Figure 1)**. More variation was observed within the Mesoamerican group (0.72) compared with the Andean group(0.76) **(Figure 1)**. Multiple correspondence analysis divided isolates into five groups, groups 1, 2 and 3 constituted of isolates from the Mesoamerican region while isolates in groups 4 and 5 constituted of isolates from the Andean region **(Figure 2)**. Within the Mesoamerican group, there was a clear distinction between isolates collected from Costa Rica and those collected from Mexico, with more variation observed among isolates from Mexico. These two countries were chosen because it is in these two countries that anthracnose is a major problem. Significant genetic differentiation (Gst= 0.301), was observed among Andean and Mesoamerican *C. lindemuthianum* isolates. The average gene diversity was slightly higher for the Mesoamerican group (h = 0.2845 $\pm$ 0.1791) than in the Andean sub-group (h = 0.2029 $\pm$ 0.1964). The average gene diversity in the entire *C. lindemuthianum* population was 0.3524 $\pm$ 0.1417. Nei's unbiased measure of genetic identity between Andean and Mesoamerican *C. lindemuthianum* isolates was 0.7240, and the genetic distance between the two populations was 0.3230. AMOVA showed that 42% of the total genetic variation observed was due to differences between isolates in different groups compared to isolates within a group (58%). Although a significant amount of the total variation was ascribed to differences between isolates in a group, a larger portion was ascribed to differences between isolates in different groups, revealing that significant genetic differentiation does exist between Andean and Mesoamerican isolates of *C. lindemuthianum*. Nei's analysis of genetic diversity, AMOVA, cluster and multiple correspondence analysis all showed that the genetic structure exhibited by *C. lindemuthianum* is

congruent with the gene pools defined for its common bean host. Therefore, we can conclude that *C. lindemuthianum* coevolved with gene pools that have been defined for its common bean host.

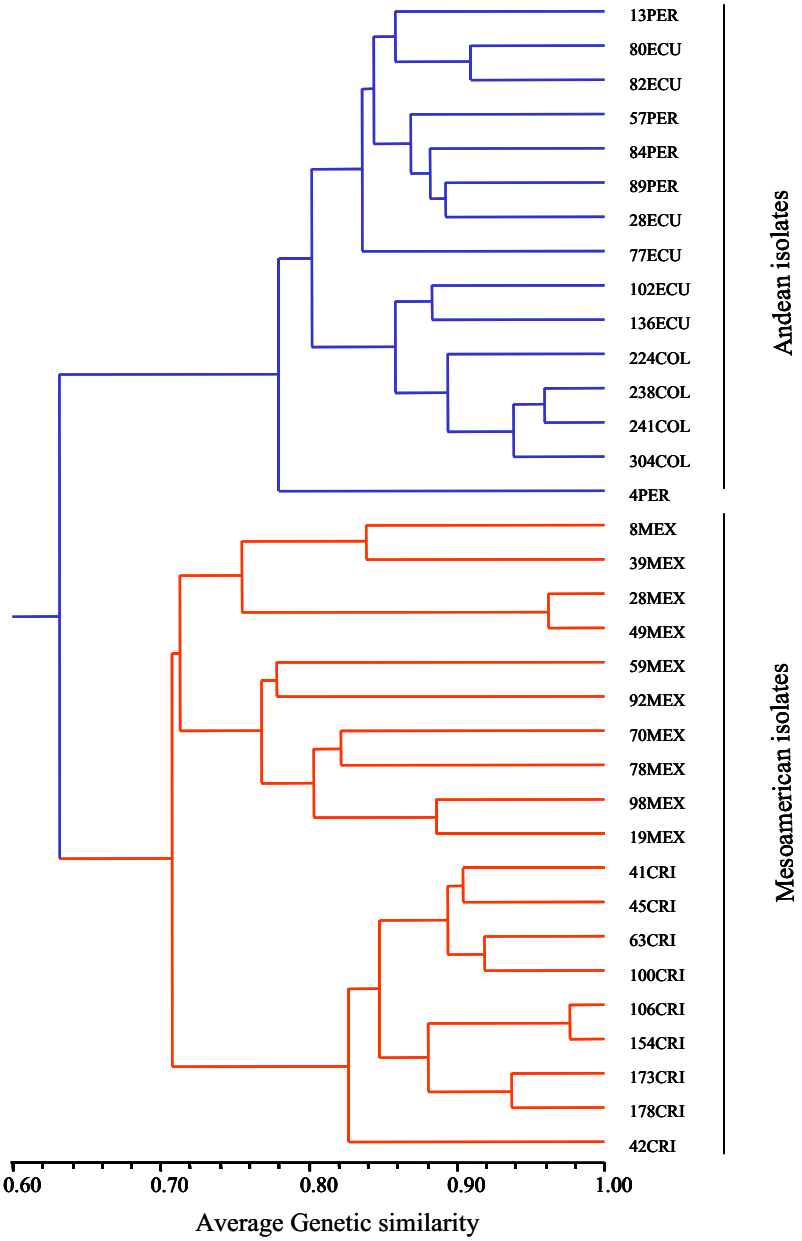


Figure 1. Dendrogram of *Colletotrichum lindemuthianum* isolates based on UPGMA methods using the SAHN and TREE option in NTSYS program with similarity coefficients calculated from combined AFLP and RAMS data. Isolates collected from Andean cultivars are shown in red and those from Mesoamerican cultivars and region are shown in blue.

Conclusion: The results reported here, combined with virulence analysis of the same isolates on 40 wild bean and land races (CIAT, 2002), clearly demonstrate that *C. lindemuthianum* exhibits a genetic structure that is congruent with gene pools defined for common bean. Therefore, *C. lindemuthianum* co-evolved with the gene pools defined for common bean.

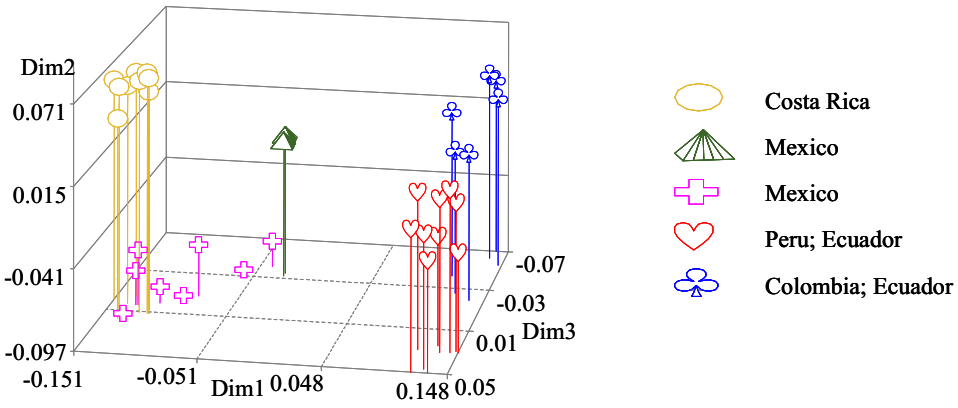


Figure 2. Three dimensional graph based on multiple corresppondence analysis of combined RAMS and AFLP data for *Colletotrichum lindemuthianum* and plotted using the spin platform of JMP program in SAS. Symbols indicate position of strains within each cluster.

References

Centro Internacional de Agricultura Tropical (CIAT). 2001. Bean Improvement Program Annual Report. Cali, Colombia. pp. 95-101.

Centro Internacional de Agricultura Tropical (CIAT). 2002. Bean Improvement Program Annual Report. Cali, Colombia. pp. 173-180.

Mahuku, GS, Henríquez, MA, Munõz, J and Buruchara, RA. 2002. Molecular Markers Dispute the Existence of the Afro-Andean Group of the Bean Angular Leaf Spot Pathogen, *Phaeoisariopsis griseola.* Phytopathology 92: 580-589

Pastor-Corrales, MA, Otoya, MM and Maya, MM. 1993. Diversidad de la virulencia de *Colletotrichum lindemuthianum* en Mesoamerica y la región Andina. Fitopatología Colombiana 17: 31-37.

Rohlf, FJ. 1994 NTSYS-PC: Numerical Taxonomy and multivariate analysis Syst, Version 1.80. New York: Exeter Software.

SAS Institute. 1989. SAS/IML Software: Usage and reference, Version 6, 1st edn. Cary, NC: SAS Institute.

Voss, P., R. Hodgers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Freijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407-4414.

Contributors: George Mahuku, Carmenza Montoya, Maria del Carmen Hernández and Juan Cuasquer.

Activity 4. Characterization of *Colltotrichum lindemuthianum* isolates from Antioquia and Santander en Colombia.

Introduction

Anthracnose of common bean caused by *Colletotrichum lindemuthianum* is the most important diseases in the departments of Antioquia and Santender, Colombia. Common bean genotypes previously identified as good sources of resistance have become susceptible. In an effort to understand and elucidate the current shift in pathogenisis, a study was initiated in 2002, to look at the composition of the *C. lindemuthianum* population in these regions. This year, more isolates were collected from different production fields to increase the number of isolates tested and verify whether the change in the composition observed last year would be reflected in the new collections. The objectives was to establish whether a there was a change in the pathotype composition and structure, attempt to ascertain the sources of the new pathotypes and identify representative pathotypes that can be used to screen potential sources of resistance. This would insure that resistance genes or gene combinations that give the widest activity against the current *C. lindemuthianum* population are deployed and or used in the breeding programs.

Materials and Methods: Sixty-five *C. lindemuthianum* isolates were isolated from samples collected from farmers' fields in the departments of Antioquia and Santander, Colombia, using previously described methods (CIAT, 2002). Sixty of the isolates were characterized on a set of 12 international anthracnose differential genotypes as described previously (CIAT, 2002). In addition, 5 isolates from Darien and Popayan were also characterized.

Results and Discussion: Twenty races were identified among 42 isolates collected from Antoioquia, while 7 races were identified among the 18 isolates from Santander **(Table 1)**. Only one race (1024) characterized from Santander was not found in Antioquia. The most frequent races were 1 and 133, and race 395 was the most virulent , infecting 5 of the anthracnose differential varieties. Five new races were characterized in Santander while 10 new races wre identified in Antioquia. In addition, some races that were previously characterized were not detected. The most resistant differential varieties were Widusa, Kaboon, Tuand G 2333, which were not infected by any of the races characterized **(Table 2)**. These differential varieties have well characterized resistance genes and they can be used as sources of resistance. In addition, molecular markers for the use of some of these genes are available, and marker assisted selection can be used to speed the process of introgressing these resistant genes into susceptible but preferred varieties.

Conclusion: The activity to characterize *C. lindemuthianum* isolates from Colombia is continuing under a subproject with CORPOICA. Preliminary results have showed a shift in race structure of *C. lindemuthianum*. A total of 15 new races were characterized, while ten races that were previously identified were not detected, showing a shift in the races structure of *C. lindemuthianum* in this region. More samples have been received following the initiation of the growing season and a complete picture of the race structure will be obtained following completion of the project. Meanwhile, the new races are being used to inoculate potential sources of resistance. The differential varieties G 2333, Kaboon and Widusa have consistently

been resistant to many of the races from these two regions and these are very good sources of resistance for these two departments in Colombia.

Table 1. Common bean Anthracnose differential varieties and their respective identified resístanse genes.

Code	Differential Variety	Gene Pool[b]	Resistance(s) gene[c]	Binary Value[d]
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[e]	M	Co-4^2, Co-5, Co-7	2048

b = M=Mesoamerican; A= Andean
c = identified resistance genes
b=Binary value assigned for each differential variety and used for race designation
e= differential varieties that are resistant in Antioquia and Santander

Table 2. Frequency distribution of *Colletotrichum lindemuthianum* races characterized from different departments of Colombia.

Race	Altiplano North	Oriente Antioqueño	Santander	Darién	Popayán
0	1		3		
1	2	4			2
3	2	1	6		
4	3	2			
6				1	
7	1		1		
65		1			
129		2	1		
131	2	2			
133	6	6	2		
135	1	1			
136		1			
141	2		1		
387	1			2	
395	1				
1024			4		
	22	20	18	3	2

Contributors: C. Jara, J. Fory, G. Castellanos, G. Mahuku, G. Santana (CORPOICA).

Activity 5. Nature and inheritance of angular leaf spot resistance in ALS differential genotypes and identified resistance sources.

Introduction

Elucidating the nature and inheritance of resistance to angular leaf spot of common bean is one of the activities that has gained precedence in the Pathology activities. Over the past several years, sources of resistance to the angular leaf spot pathogen have been identified, and sufficiently characterized using different pathotypes of *P. griseola* from distinct geographical areas. A total of 19 good sources of resistance were identified following greenhouse and field characterization in different areas. To date, the genetics of resistance has been identified in only four of these sources of resistance. To effectively exploit the diversity in bean genes to combine and pyramid useful genes, sufficient characterization of the genetics of resistance is necessary. Determining and understanding the inheritance of ALS resistance in the most promising accessions, bred and differential lines would facilitate deployment of the identified resistance genes, breeding for ALS resistance, tagging the genes and identifying molecular markers for use in MAS. This is an on going study to understand the nature of inheritance of ALS resistance in common bean, with the ultimate objective of developing molecular markers that can be used to aid the transfer of resistance to well-adapted market class type bean.

Materials and Methods: Populations (F1, F2, and F1 backcrosses to resistant and susceptible parents) were made using the variety Sprite or A 36 as the susceptible parents. Populations were developed as reported previously (CIAT 2002). Green house disease evaluations, data analysis were done as described previously (CIAT 2002). Evaluations for disease severity were assessed using a CIAT 1 – 9 scale, where 1 represents no visible symptoms and 9 = severe symptoms and disease expression. Ratings of 1 to 3 were considered resistant and ratings > 4 as susceptible. Area under disease progress curves was calculated to assign genotypes to resistance and susceptibility classes. Several different genetic hypotheses were tested for each population using a Chi-squared test in the SAS program.

Results and Discussions: The observed segregation ratios from F_1, F_2, and back cross to resistant (BC1-R) and susceptible (BC1-S) parents revealed that both dominant and recessive genes, with and without epistasis conditions resistance to *P. griseola* in common bean. The resistance of Mexico 54 to race 31-55 is conditioned by 2 recessive interacting genes (epistasis). The resistance in PAN 72 and Don Timoteo to inoculations with races 15-0 and 62-0 is conditioned by a single dominant gene **(Table 1)**, while that of G 20743 to race 63-63 is due to a single recessive gene. Two dominant genes with epistasis condition the resistance of G 2858, Flor de Mayo, G 5686.

Conclusions: As previously reported for other genotypes, the results reported show the complex nature of inheritance of resistance to *P. griseola*. Major genes (whether recessive or dominant) are involved in conferring resistance to ALS and the complex segregation patterns observed in Montcalm and Amendoim reveal a possibility that minor genes might be involved as well. In addition, several of the sources of resistance and differential varieties carry more than one resistance gene that show different forms of epistatic interactions. It is essential that these genes are separated and tagged, for them to be introgressed into market class type beans.

Table 1. Nature and inheritance of angular leaf spot resistance in some differential varieties and selected resistant sources.

Source	Generation	Observed	Expected	X^2	Interpretation	Conclusion
Mex 54	F1	20:69				Partially
	F2	3929:92	4:3:9	0.95	2 rec. genes	recessive
	BC-P1	32:24	1:1	0.28	1 rec. gene	resistance due to
	BC-P2	28:86	1:3	0.91	Tendency toward rec. resistance	two genes
PAN 72	F1	137:0			Dominant	Dominant
	F2	47:15	3:1	0.88	1 dom. gene	resistance
	BC-P1					due to one gene
	BC-P2	17:16	1:1	0.86	1 dom. gene	
G2858	F1	54:37			Partially dom.	Two recessive
	F2	96:125	7:9	0.93	2 dup. rec. genes	duplicate genes
	BC-P1	27:15	3:1	0.10	2 dup. Rec genes	
	BC-P2	14:29	1:3	0.25	2 dom genes	
Flor de Mayo	F1	74:54			Partial dom. w/ het. 40% S	Two epistatic
	F2	54:33	9:7	0.32	2 dom. genes	recessive genes
	BC-P1	93:11			1 dom w/ het. 40% S	
	BC-P2	13:33			1 dom w/ het. 40% S	
G5686 x Sprite	F1	40:25			partially dom w/ 38% S	2 interacting
	F2	69:25:43	9:3:4	0.21	2 dom. genes + IA	genes
	BC-P1 (res)	18:4	3:1	0.46	Additive?	
	BC-P2 (sus)	4:64				
G5686 x A 36	F2	52:68	7:9	0.93		2 interacting genes
Amendoim	F1	0:136			Recessive	Recessive
	F2	13:194	1:15	0.96	2 rec. genes	resistance
	BC-P1	5:87	1:15	0.74	3 rec. genes	due to 2 genes
	BC-P2	0:58			Rec. resistance	
Montcalm	F1	6:49			Additive or partially rec.	2 interacting
	F2	24:214	7:57	0.63	2 rec. genes	genes (possibly
	BC-P1	5:48	1:15	0.33	3 rec. genes	complementary)
	BC-P2	3:19	1:3	0.21		
Timoteo	F1	59:3				Dominant resistance
	F2	89:40	3:1	0.12	1 dom. gene	due to 1 gene
	BC-P1	28:3			Dom, = P1	
	BC-P2	17:17	1:1	1.0	1 dom. gene	
G10431	F1	2 :38	0 :1			2 recessive genes
	F2	11:154	1:15	0.83	2 rec. genes	
	BC-P2					
G20743	F1					A single
	F2	30:61	1:3	0.08	1 rec. gene	recessive gene
	BC-P2					

Contributors: G. Mahuku, J.Fory, G. Castellanos, C. Jara, H. Teran, S. Beebe**,** M. Blair.

Activity 6. Inheritance of anthracnose resistance in the Andean germplasm accession G 19833.

Introduction

The genotype G 19833, a common bean germplasm accession from the highlands of Peru, was identified as a good source of anthracnose resistance. G 19833 is resistant to Andean and Mesoamerican races of *Colletotrichum lindemuthianum*, including races that overcome the resistance in the germplasm accession G 2333, which carries three anthracnose resistance genes *Co-4*^{2}, *Co-5* and *Co-7*. Previous attempts to elucidate the nature of anthracnose resistance in G 19833 were confounded by the variety used in the cross, DOR 364, which is known to have resistance against Andean races of *C. lindemuthianum*. Because of epistatic effects of the resistance genes in DOR 364 and G 19833, the nature of inheritance of anthracnose resistance could not be conclusively established. In this study, a universally susceptible variety, La Victorie, with no known anthracnose resistance gene was used as the susceptible parent.

Materials and Methods: G 19833 (anthracnose resistant) was crossed with La Victorie (anthracnose susceptible) to generate F_1, F_2 populations, and backcross populations to resistant and susceptible parents. 200 F2 plants, 100 plants derived from a backcross to resistant parent and 50 plants for backcross to a susceptible parent were independently inoculated with 4 *C. lindemuthianum* races (race 1, 7, 73 and 3481) comprising Andean and Mesoamerican races. Seeds were planted in flats and grown under greenhouse conditions for 10 days until seedlings had reached the full-expanded primary leaf stage. Seedlings of each accession were sprayed with spore suspension (1 X 10^4 spores mL^{-1}) until runoff on the stem and both surfaces of the cotyledons. After inoculation, plants were maintained in high humidity (>95%) at approximately 22C, with a 12 h light/dark cycle for 8 days. Disease was rated 10 days after inoculation using a CIAT 1 to 9 severity scale (Schoonhoven and Pastor-Corrales, 1987).

Results and Discussion: Evaluation of 200 F2 individuals with race 3481 showed a segregation ratio of 1: 3 (resistant : susceptible), and a 1:1 segregation ratio of the backcross population to the resistant parent, G 19833, a 0:1 segregation ratio to the susceptible parent La Victorie and a 1:0 reaction of the F1 individuals, suggestive of a single recessive gene conditioning resistance to this race **(Table 1)**. A segregation ratio of 7:57 was observed in 200 F2 plants inoculated with the Andean race 1, indicative of two or three complementary recessive genes conditioning resistance to this race **(Table 1)**. A segregation ratio of 7 (resistant): 9 (susceptible) was indicative of two duplicated recessive genes conditioning resistance of G 19833 to *C. lindemuthianum* race 7. When an additional 200 F2 plants were inoculated with race 73, a 9:7 segregation was observed, indicating that two dominant genes conditioned anthracnose resistance in G 19833 to race 73. This was confirmed in the segregation of the F1 (1:0) and backcross populations **(Table 1)**. The results obtained in this study reveal that more than a single gene conditions resistance of G 19833 to *C. lindemuthianum*. These genes can either be dominant or recessive, depending on the *C. lindemuthianum* race being used. In general, recessive genes condition resistance to Andean races, while both recessive and dominant genes can condition resistance to Mesoamerican races.

Conclusions: The results reported here show the complex nature of anthracnose resistance in G 19833, where different genes condition resistance to different races. At least three recessive and two dominant genes condition anthracnose resistance in this genotype. These results support the QTL analysis of RILS derived from a G 19833 x DOR 364 cross, where resistance to Andean races was localized on a different linkage group to that conditioning resistance to Mesoamerican races. G 19833 contains both major and minor genes conditioning resistance to *C. lindemuthianum.* The nature of the resistance gene depends on the classification of the pathogen race used.

Contributors: G. Mahuku, C. Jara, J. Fory, G. Castellanos, H. Teran, S. Beebe.

Table 1. Reaction of F1 and F2 plants derived from crossing G 19833 x La Victorie and the backcross to resistant and susceptible parents to inoculation with Andean and Mesoamerican races of *Colletotrichum lindemuthianum*.

Race	Generation	Observed Ratio	Expected Ratio	X^2	P	Comments
1	F1	2 R : 17 S				
1	F2	17 R: 179 S	7:57	1.03	0.3098	1 dominant, 2 complementary recessive genes
1	BC-R	29 R: 64 S				
1	BC-S	0 R : 50 S	0:1			
7	F1	8 R : 8 S	1:1			
7	F2	77 R: 120 S	7:9	1.741	0.187	2 recessive genes, duplicated
7	BC-R	88 R: 11 S				
7	BC-S	0 R : 50 S	0:1			
73	F1	19 R : 0 S				
73	F2	112 R : 86 S	9:7	0.008	0.928	2 dominant genes, duplicated
73	BC-R	100 R: 0 S				
73	BC-S	17 R : 32 S				
3481	F1	2 R : 17 S				
3481	F2	49 R: 145 S	1:3	0.008	0.933	1 Recessive gene
3481	BC-R	49 R: 49 S	1:1			
3481	BC-S	0 R : 50 S	0:1			

[a] M = Mesoamerican; A =Andean.
[b] Generation F2; RC = Backcross to G 19833.

Activity 7. Allelism test for anthracnose resistance genes in the germplasm accession G 19833.

Introduction

Only one anthranconse resistance gene of Andean origin (Co-1) has been characterized. Several alleles of this gene have been defined in different Andean anthracnose differential varieties; Kaboon (Co-1^2), Perry Marrow (Co-1^3), Michigan Dark Red Kidney (Co-1) and Widusa (Co-1^5) and sources of resistance AND 227 (*Co-1^4*). Given the small number of Andean genes that have been identified and characterized, there is a need to look for other Andean anthracnose resistance genes for use in breeding programs while avoiding the deployment of only a single gene, a situation that is not desirable. G 19833 has been observed to be highly resistant to isolates that infect the varieties that carry different alleles of the *Co-1* resistance loci. This study was carried out to test the independence of the resistance gene(s) in G 19833 from the *Co-1* gene and establish if the resistance gene(s) in G 19833 were the same or different from the *Co-1* alleles in the cultivars Michigan dark red kidney, Kaboon and Perry Marrow (Melotto et al, 2000).

Materials and Methods: Two hundred F2 plants derived from crossing G 19833 x Kaboon, G 19833 x Perry Marrow and G 19833 x Michigan Dark Red Kidney (MDRK) were inoculated independently using three C. lindemuthianum races (race 1, 73, and 3481) **(Table 1)**. All bean varieties are resistant to these three races. Bean seedlings with fully expanded primary leaves were sprayed with the conidia suspension ($1.2x10^6$ conidia/ml) until runoff on the stem and both surfaces of the cotyledons. Inoculated plants were incubated in a chamber at 22ºC and 90-100% relative humidity with 12 h light/dark cycle. Each isolate was evaluated separately. Plants were scored 8 days after inoculation using a 9-class scale described by Schoonhoven y Pastor-Corrales (1987). A plant with no visible symptoms or with only a few, very small lesions mostly on the primary leaf veins was scored as resistant (rating 1 to 3). A plant with numerous small or enlarged lesions, or with sunken cankers on both the lower sides of leaves and the stems was recorded as susceptible (rating 6.1 to 9). A plant with a rating score of (3.1 – 6) was considered as intermediate.

Results and Discussion: Inoculation of the G 19833 x MDRK, Perry Marrow and Kaboon with race 3481 revealed that G 19833 contains resistance genes that are different from the ones carried by MDRK, Perry Marrow and Kaboon **(Table 2)**. The resistance gene in G 19833 is different from the one in MDRK (3:1), and has two genes that are different from the ones Perry Marrow (15:1) and at least 3 genes segregating in the G 19833 x Kaboon cross (63:1).

Inoculation of the F2 populations using *C. lindemuthianum* race 73 revealed that three genes were segregating (63:1) in the G 19833 x MDRK cross and in the G 19833 x Perry Marrow cross (61:3), while in the G 19833 x Kaboon, two independent genes were segregating (15:1). Therefore, the resistance genes conditioning anthracnose resistance in G 19833 to race 73 are different from the ones in Kaboon, Perry Marrow and MDRK, and at least three genes are involved in conditioning resistance.

When these populations were inoculated with race 1, a 9:7 segregation ratio was observed for the G 19833 x MDRK cross; a 57:7 for the G 19833 x Perry Marrow cross and a 15:1 for the G 19833 x Kaboon cross, revealing that at least three independent genes are segregating.

Conclusion: The segregation ratios observed reveal that G 19833 carries resistance genes that are different from the ones in MDRK (*Co-1*), Perry Marrow (*Co-1*^{3}) and Kaboon (*Co-1*^{2}). The 3:1 segregation ratio reveal that at least one of the resistance gene is an allele to *Co-1* locus.

Table 1. Gene pool, described anthracnose resistance genes and disease reaction of bean cultivars inoculated with different races of *C. lindemuthianum*.

Bean cultivar	Gene Pool	Known Gene	Colletotrichum lindemuthianum races			
			1	7	73	3481
MDRK	A	Co-1	R	S	R	R
Perry Marrow	A	Co-1^3	R	S	R	R
Kaboon	A	Co-1^2	R	R	R	R
G 19833	A		R	R	R	R

Table 2. Test for the independence of the resistance genes in F2 populations derived from crossing G 19833 with Kaboon, MDRK and Perry Marrow

Cross	Race	Obs (R:S)	Esp	X^2	P	Coment
G19833xMDRK	1	128:70	9:7	5.62	0.02	duplicated recessive genes
G19833xMDRK	73	197:3	63:1	0.01	0.74	3 independent genes
G19833xMDRK	3481	153:47	3:1	0.240	0.62	1 dominant gene
G19833xPM	1	175:25	57 :7	0.50	0.48	3 genes, 2 are complementary
G19833xPM	73	192:8	61:3	0.21	0.65	3 genes, 2 dominant, 1 recessive
G19833xPM	3481	186:14	15:1	0.19	0.66	2 independent dominant genes
G19833xKAB	1	184:12	15:1	0.01	0.94	2 independent dominant genes
G19833xKAB	73	187:13	15:1	0.02	0.88	2 independent dominant genes
G19833xKAB	3481	196:4	63:1	0.25	0.62	3 independent dominant genes

Contributors: G. Mahuku, C. Jara, J. Fory, G. Castellanos, H. Teran, S. Beebe.

Publications

Mahuku, G.; Jara, C.; Terán, H., and Beebe, S. 2003. Inheritance of angular leaf spot resistance in selected common bean genotypes. *In*: BIC (Bean Improvement Cooperative). Annual report. East Lansing, MI, USA. v. 46, p.151 –152.

Mahuku, G.S.; Jara, C.E.; Cajiao V., C.H.; and Beebe, S. 2003. Sources of resistance to angular leaf spot (*Phaeoisariopsis griseola*) in common bean core collection, wild *Phaseolus vulgaris* and secondary gene pool. Euphytica 130: 303-313.

Mahuku, G.S.; Jara, C.E.; Cajiao V., C.H.; Beebe, S. 2002. Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. Plant Dis 86(12):1383-1387. The American Phytopathological Society.

Mahuku, G., Jara, C., Cuasquer, J.B. and Castellanos, G. (2002) Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. Plant Pathology 51:594-604.

Submitted

Mahuku, G.S., and Riascos, J.J. (2003) Virulence and Molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. European Journal of Plant Pathology.

Mahuku, G., Montoya, C., Henríquez, M.A., Jara, C., Teran, H., and Beebe, S. 2003. 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.

Trips and attendance at meetings

Participate as a resource person in a Marker assisted selection workshop in Kampala, Uganda.

Training, students and courses

Three scientists (Senora Ligia Dense; Juliana Rojas, Ana Maria Serralde and Patricia Hernández) from CORPOICA in Bogotá were trained (1 week) in Técnicas para la conservación de microorganisimos a larga plazo).

A student, Monica Navia Urrutia (Universidad del Valle, Cali) has started work on infection process of Phaeoisariopsis griseola.

Maria Antonia Henríquez started her MSc thesis with the Universidad National, sede Palmira

Workshop and Conference

Jara, C., Castellanos, G., and Mahuku, G. 2003. Comparación a través del tiempo del agente causal de la antracnosis del frijol ((*Colletotrichum lindemuthianum)* en los departamentos de Antioquia y Santander. XXIV Congreso de la Asociación Colombiana de Fitopatologia y Ciencias afines (ASCOLFI) , Junio 25-27 de 2003, Armenia.

List of Collaborating Partners

Ing. Gloria Esparanza Santana, CORPOICA Rionegro, Antoquia, Colombia
Ing. Carlos Manuel Araya, National University, Heredia, Costa Rica
Dr Andre Levesque, Agiculture and Agri-Food Canada, Ottawa
Dr. Jean-Pierre Busogoro, Agricultural University of Gembloux, Belgium

RICE PATHOLOGY

Activity 1. 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 after cultivar release with the exceptions of the Colombian commercial cultivars Oryzica Llanos 5 and Fedearroz 50. This breakdown is mainly due to the continuous changes and evolution of the pathogen, which gives origin to new pathotypes compatible with the new rice cultivars. Continuous monitoring of blast pathogen populations in breeders 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 in the pathology and breeder's plots at Santa Rosa experiment station. Blast isolates recovered from the infected samples in the laboratory are 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 is used for determining their genetic structure using the Pot-2 PCR fingerprinting technique. More than 100 blast isolates recovered from several rice lines were analyzed in 2003 and new pathotypes are reported in this chapter.

Results and Discussion: All blast isolates analyzed belonged to the known genetic groups SRL-6, SRL-5, SRL-4 and SRL-2 already identified in Colombia. The most common pathotypes found **(Table 1)** were #1 (SRL-4) and # 2 (SRL-5). These isolates were recovered from several cultivars. Pathotypes identified as #1 induced in 2003 more typical blast lesion on cultivars Fedearroz 50 and Oryzica Llanos 5. This susceptible reaction was also observed in greenhouse inoculations, although the disease severity observed was around 10% of leaf area affected. Resistance genes effective against this pathotype are Pi-1 and Pi-K^h as had been reported in year 2002. The resistance gene Pi-1 is being incorporated in the commercial cultivar Fedearroz 50 through marker assisted selection, greenhouse inoculations and field evaluations.

In our efforts to detect new changes in virulence in the pathogen population, few blast lesions observed in the highly resistant line FL 00147-8P-6-15P were collected and analyzed in the laboratory. All four isolates retrieved turned to be lineage SRL-4, however three of them belonged to a different pathotype identified as # 3 **(Table 1)**, and the fourth isolate as pathotype #1. Greenhouse inoculations of pathotype #3 indicated for the first time in many years the ability of an isolate to potentially defeat the three resistance genes Pi-1, Pi-2, and Pi-33. This pathotype was recovered from only one cultivar, indicating that its frequency is very low. Besides, the cultivar giving origin to this pathotype exhibits a highly resistant reaction 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, this pathotype had to maintain the avirulence gene for Pi-ta^2 **(Table 1)**. The corresponding resistance gene Pi-ta^2 present in the differential F 128-1 confers resistance to this isolate **(Table 1)**. These results indicate that the resistance gene Pi-ta^2 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 gene in the cultivars Oryzica Llanos 5 and Fedearroz 50 explains why this isolate did not infect severely these cultivars in greenhouse inoculations **(Table 1)**. We are in the process of analyzing more blast samples collected in 2003, both, in terms of genetic structure and virulence spectrum, to determine if this new pathotype can be recovered from other cultivars, including few lesions observed in the near isogenic lines from the cross CT 13432 carrying the three resistance genes Pi-1, Pi-2 and Pi-33.

Our results from the last several years, and of this particular year, 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 will be discussed later in this chapter in the analysis of the stable resistance of the cultivar Oryzica Llanos 5.

As we can see **(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 two 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 2003 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 2003 under field conditions and greenhouse inoculations.

Table 1. Virulence Spectrum and Frequency of Rice Blast Pathotypes Detected at the Santa Rosa Experiment Station in 2003.

Rice Line	Resistance Gene	Pathotypes (frequency %)							
		1 (60)	2 (23)	3 (5)	4 (4)	5 (2)	6 (2)	7 (2)	8 (2)
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	+++		+++	+++	+++			+++
F 124-1	Pi-ta	+++	+++	+++	+++	+++	+++	+++	+++
F 128-1	Pi-ta^2	+++	+++		++		++	+	
F 80-1	Pi-k	+++	+++	+++	+	+++	++		+
F 98-7	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		+++		+++	+++	+++			+++
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								

Contributors: F. Correa, F. Escobar, G. Prado, G. Aricapa.

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 later in this chapter. 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 Report of the Rice Project for 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 in 2003. The most resistant lines over the last three 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**). 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 as will be shown later in this chapter. These results indicate the importance of evaluating the potential donors of blast resistance for several semesters before their inclusion 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 2004 for their inclusion in a nursery as potential donors of stable blast resistance. We had already reported in previous years the high resistant reaction of the japonica lines to the grain discoloration pathogens. It should also be noted the resistant reaction of the rice cultivars from Surinam such as Ciwini and Eloni which can be incorporated in our breeding programs as they might help to broaden the genetic diversity of our rice germplasm.

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.

Table 1. Potential Progenitors for Stable Blast Resistance Exhibiting Blast Scores 1-3 in Santa Rosa Field Evaluations during Four Cycles, Santa Rosa 2000 - 2003.

Pedigree	Pedigree
1. FL 00478-29P-23-3P	22. FL00478-29P-5-1P-M
2. FL 00518-16P-8-2P	23. FL00518-14P-15-3P-M
3. CNAx5013-13-2-2-4-B	24. FL00518-23P-11-2P-M
4. CT11275-3-F4-8P-2	25. FL00530-29P-4-2P
5. CT11280-2-F4-12P-5	26. FL00530-7P-7-1P-M
6. CT11891-2-2-7-M	27. FL00535-21P-4-3P-M
7. CT13394-5-6-M-M-1	28. FL00542-45P-8-2P-M
8. CT13449-M-3-1-M	29. FL00585-26P-1-2P-M
9. CT13449-M-8-2-M	30. FL00595-12P-10-4P-M
10. CT13458-M-3-2-M-M	31. FL00595-25P-9-3P-M
11. CT13458-M-3-3-M-M	32. FL00837-8P-5-2P-M
12. CT13458-M-3-4-M-M	33. FL00871-1P-3-1P-M
13. CT13464-M-10-1-M-M	34. FL00871-1P-5-2P-M
14. CT13937-16-1-M-M-2	35. HUALLAGA INIA
15. CT13937-16-2-M-M-2	36. IRAK 13
16. CT13937-16-2-M-M-3	37. LINEA 30
17. CT13937-16-3-M-M-4	38. PROGRESO
18. CT13941-11-1-M-M-4	39. PURG-2\0\0\1>27-1
19. CT13943-10-2-M-M-3	40. RIO PARAGUAY
20. CT8222-7-6-2P-1X	41. SAN MARTÍN 83
21. FL00447-35P-4-2P-M	42. TRES MARIAS

Contributors: F. Correa, D. Delgado, G. Prado, G. Aricapa.

Activity 3. Identification of Molecular Markers Associated with the Blast Resistance Genes Pi-1, Pi-2, Pi-33 and their Incorporation into Commercial Rice Varieties Through Backcrossing and Marker Assisted Selection (MAS).

Abstract

Farmers often choose blast susceptible varieties because they have high yields and grain quality. To sustain these characteristics, farmers have to spray fungicides, including several applications per planting season, increasing their production costs and the possibilities of contaminating the environment and/or affecting the human health. Incorporation of blast resistance genes to these varieties would make them more cost effective and ecologically sustainable. The combination of the blast resistance genes Pi-1, Pi-2, and Pi-33 confers resistance to all known blast pathogen populations of Colombia. We are identifying molecular markers associated with different resistance genes and initiated a backcrossing program assisted by these markers to introduce these resistance genes into several popular Latin American rice varieties.

Introduction

Farmers adopt faster rice varieties that have high yields and excellent grain quality. The characteristic such as resistance to diseases is highly desirable but not enough to make a variety successful. Inconsistent yields because of diseases are enough to cause varieties to be discarded by farmers. Varieties without durable blast resistance become more susceptible every year, and they need more applications of fungicides. In seasons favorable for rice blast, even fungicides may not be sufficient to prevent substantial losses. Farmers would like these varieties to be blast resistant.

We have initiated a backcrossing program in order to introduce blast resistance genes into some of those susceptible rice cultivars, which still play an important role in the economy of many rice farmers and regions of Latin America. The resistance genes being incorporated into the commercial varieties are Pi-1, Pi-2, and Pi-33 as they confer resistance to all the pathogen population in Colombia and probably the Latin America region based on their reaction to other blast populations of the region. The BC1F1 and BC1F2 breeding populations derived from the crosses between several Latin American rice varieties and four rice lines used as sources of the three resistance genes have been developed for the identification of heterozygous lines carrying the three resistance genes. Materials and Methods were described in the 2001 Annual Report of the Rice Project. Greenhouse inoculations with appropriate avirulence genes were performed in 2003 for the selection of resistant plants to perform the BC2. Additionally, BC1F2 populations were planted at the Santa Rosa experiment station for selection of resistant plants and backcrossed to the corresponding recurrent parents. A total of 56 microsatellites or PCR based markers relatively close to the resistance genes Pi-1, Pi-2 and Pi-3 were identified from different publications or databases and tested for their association with these genes.

Results: Near isogenic lines carrying any of the three resistance genes, combination of any two genes, and combination of the three resistance genes Pi-1, Pi-2, and Pi-33 were developed based on controlled inoculations in the greenhouse and evaluations under field conditions in 2002 in our Santa Rosa experiment station. The only isogenic lines exhibiting a leaf and panicle blast

resistance in 2002 and 2003 were the four lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33. However, some plants within these lines exhibited few blast samples in 2003, which are being used in the laboratory for pathogen isolation and for further analysis of the genetic structure and virulence spectrum of the pathogen. Most of the lines carrying one or two genes died before panicle development both in 2002 and 2003.

The four isogenic lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33 with a high leaf and panicle blast resistance were used for the development of the BC1F1 populations of fourteen Latin American rice varieties and the BC2F1 of four of them following the procedure described in the Rice Annual Report of year 2002. Greenhouse inoculations of the BC1F1 and the BC2F1 were performed with particular blast isolates carrying the corresponding avirulence genes and resistant plants selected for the production of the BC2 and BC3 populations. Field evaluations of the BC1F2 at the Santa Rosa experiment station were also performed under field conditions and resistant plants selected to perform the BC2F1. A total of 56 molecular markers (Gene Bank web site) close to the three blast resistance genes (37 for Pi-1, 11 for Pi-2, 8 for Pi-33) were identified and tested for their gene association in near isogenic lines carrying single or combinations of the three genes. Six markers were identified highly associated with the presence of the resistance gene Pi-1, while one marker was associated with the absence of Pi-1 exhibiting a band only in the susceptible lines; three markers were associated with Pi-33, and one with Pi-2 **(Table 1)**. Most of the other markers did not amplify at the conditions tested of 50C and 55C for the annealing temperature and will be tested under different conditions. The eleven markers associated with the three resistance genes were tested in replicated trials on 38 isogenic lines carrying different combinations of the three resistance genes and on 19 rice cultivars including those Latin American varieties used in our backcrossing program. The markers associated with Pi-1 and Pi-2 seem to be suitable to follow the introgression of these genes into the commercial cultivars, while the markers for Pi-33 failed in discriminating the presence or absence of the gene in susceptible and resistant cultivars according to greenhouse inoculations. Efforts will be made to develop closer markers associated with the three genes. The number of backcrosses will depend on the recovery of the desired agronomic traits of the recurrent rice varieties.

Discussion: The combination of the three resistance genes Pi-1, Pi-2, and Pi-33 in a single near isogenic line exhibited in years 2002 and 2003 a high level of leaf and neck blast resistance when exposed under high blast pressure in a blast pathogen population with pathotypes compatible with the combination of any two of these three resistance genes. It seems then that the blast pathogen is able to lose any of the three avirulence genes or any combination of two avirulence genes, but not the three of them in a single isolate. It is possible that losing avirulence genes for one of these three resistance genes might affect some fitness parameters such as competitiveness among pathotypes with different avirulence gene composition, and therefore this parameter needs to be measure under natural conditions of infection by collecting blast samples from different near isogenic lines carrying different combinations of the resistance genes. Few blast samples collected from lines carrying the three resistance genes were collected in 2003 and will be analyzed for their genetic and virulence structure to determine its potential importance.

Future Activities: Evaluation and selection of the BC2F1 populations and development of the BC3F1 of several backcrosses between Latin American rice varieties and near isogenic lines carrying the resistance genes Pi-1, Pi-2, and Pi-33. Molecular markers as well as inoculations

with appropriate blast isolates will be used for the identification of the rice lines carrying the three resistance genes. Field studies will be carried out to determine the possible association of the lost of an avirulence gene with parameters of pathogenic fitness such as pathogen competitiveness. These activities will be carried out by collecting blast isolates from near isogenic lines with different combinations of the three blast resistance genes and comparing the frequencies of the different avirulence gene combinations present in the pathogen population with the expected frequencies based on the resistance genes of each line. Genetic distances between the molecular markers and the resistance genes will be determined using a set of 283 near isogenic lines.

Table 1. Identification of Microsatellite Markers Associated with the Resistance Genes Pi-1, Pi-2, and Pi-33 using a set of Near Isogenic Lines.

Marker Identification		Resistance Gene	Primer Sequence
RM 1233*I	Forward	Pi-1	TTCGTTTTCCTTGGTTAGTG
	Reverse		ATTGGCTCCTGAAGAAGG
RM 7654*A	Forward	Pi-1	CAAAAGTCTGACCGTTTACC
	Reverse		TAAGAGACGGAAGAGTGAGC
RM7654*H	Forward	Pi-1	CTCATGGTTGTGTCGTGGTC
	Reverse		GTGCAGTGCCAGTGGTACG
RM 7654-2	Forward	Pi-1	GTGTCGTGGTCGTAACTTG
	Reverse		TAAGAGACGGAAGAGTGAGC
RM 6094	Forward	Pi-1	TGCTTGATCTGTGTTCGTCC
	Reverse		TAGCAGCACCAGCATGAAAG
RM 5926	Forward	Pi-1	ATATACTGTAGGTCCATCCA
	Reverse		AGATAGTATAGCGTAGCAGC
RM 224	Forward	Pi-1	GATCGATCGATCTTCACGAGG
	Reverse		TGCTATAAAAGGCATTCGGG
RM 527	Forward	Pi-2	GGCTCGATCTAGAAAATCCG
	Reverse		TTGCACAGGTTGCGATAGAG
RM 409	Forward	Pi-33	CCAATCATTAACCCCTGAGC
	Reverse		GCCTTCATGCTTCAGAAGAC
RM 483	Forward	Pi-33	CTTCCACCATAAAACCGGAG
	Reverse		ACACCGGTGATCTTGTAGCC
RM 72	Forward	Pi-33	CCGGCGATAAAACAATGAG
	Reverse		GCATCGGTCCTAACTAAGGG

Contributors: F. Correa, D. Tharreau (CIRAD), C.P. Martinez, G. Prado, F. Escobar, J. Fuentes.

AFRICA: BEAN PATHOLOGY

Activity 1. Characterization and distribution of *Pythium* spp causing root rots in Eastern Africa.

Introduction

In the development of management strategies for Pythium root rot, characterization of *Pythium* species associated with the disease and determination of their distribution in the region is important. Besides the information is useful as a basis for developing rapid detection and characterization techniques for *Pythium* spp pathogenic to beans. Last year we showed that there are Pythium species other than those commonly known and reported before, that are pathogenic to beans. This indicates the need to assess pathogenicity of species associated with beans to establish their potential role in the root rot problem. We therefore continued with the characterization Pythium spp, using molecular methods, carried out pathogenicity studies and developed species distribution maps for Uganda.

Methods: Last year we grouped Pythium isolates into several RFLP groups using PCR/RFLPs analysis. On the basis of the groupings we selected representative candidates for sequencing. Sequencing of PCR fragments were done using an ABI prism automated sequencer. The sequences obtained were edited and compared to known *Pythium* species from the *Pythium* database managed by Dr A. Levesque.

To determine pathogenicity of species characterized on the basis of sequencing, seed of susceptible and resistant cultivars CAL 96 and RWR 719 respectively, were planted in trays containing soil infested with each of the isolates representing new characterized species. The soil was maintained under conditions, which favour pathogen establishment and disease development. Plants were evaluated after 4 weeks using the method described above (under section 2.1.1).

Results and Discussion: Thirty isolates characterized by sequencing were grouped into 12 Pythium species **(Table 1)**, 7 of which were new additions (*Pythium zingiberum, P. indigoferae, P. paroecandrum, P. conidiophorum, P. chamaehyphon, P. graminicola, P. perrilum)* to those characterized last year giving a total of 19 species that we have so far associated with bean samples collected from areas in Kenya, Uganda, and Rwanda where root rots is a serious problem.

In pathogenicity studies we only considered isolates representing the 7 species identified. Isolates belonging to *P. ultimum* var *ultimum*, *P. spinosum, P. graminicola*, and *P. paroecandrum*, tested, caused severe root rot on susceptible bean cultivar CAL 96. However, RWR 719 remained resistant. The pathogenicity of *P. graminicola*, and *P. paroecandrum* species is a demonstration of the potential of these species in causing root rots on beans. Their importance depends on their distribution and levels of inoculum in the soil. The fact that some of the species pathogenic to beans are known pathogens of other crops (maize or sorghum) in the existing cropping system indicate the probable role of crops intercropped or grown in rotation

with beans, in contributing to the importance of root rots. Studies are being initiated to establish the role and significance of other crops in bean based system in contributing to the current status of bean root rots.

Table 1. Identification and classification of *Pythium* isolates collected from bean growing areas in Uganda, Kenya and Rwanda

Species	Number of isolates	Comments
Pythium ultimum	5	
Pythium irregulare	1	
Pythium spinosum	4	
Pythium torulosum	2	
Pythium vexans	1	
Pythium zingiberum	4	
Pythium indigoferae	4	
Pythium paroecandrum	1	
Pythium conidiophorum	3	
Pythium chamaehyphon	1	
Pythium graminicola	3	
Pythium perrilum	1	

Species distribution maps **(Figures 1a, b, c)** show that *P. ultimum* var *ultimum* is the most frequently recovered isolate in the three regions (Kabale, Kisoro and Sironko) surveyed in Uganda. This was closely followed in Kabale by *P. salpingophorum*, in Kisoro by *P. torulosum* and in Mbale & Sironko by *P. vexans*. It is significant to note that, in addition to *P. ultimum* , *P. salpingophorum*, and *P. torulosum* where shown to be pathogenic to beans as well.

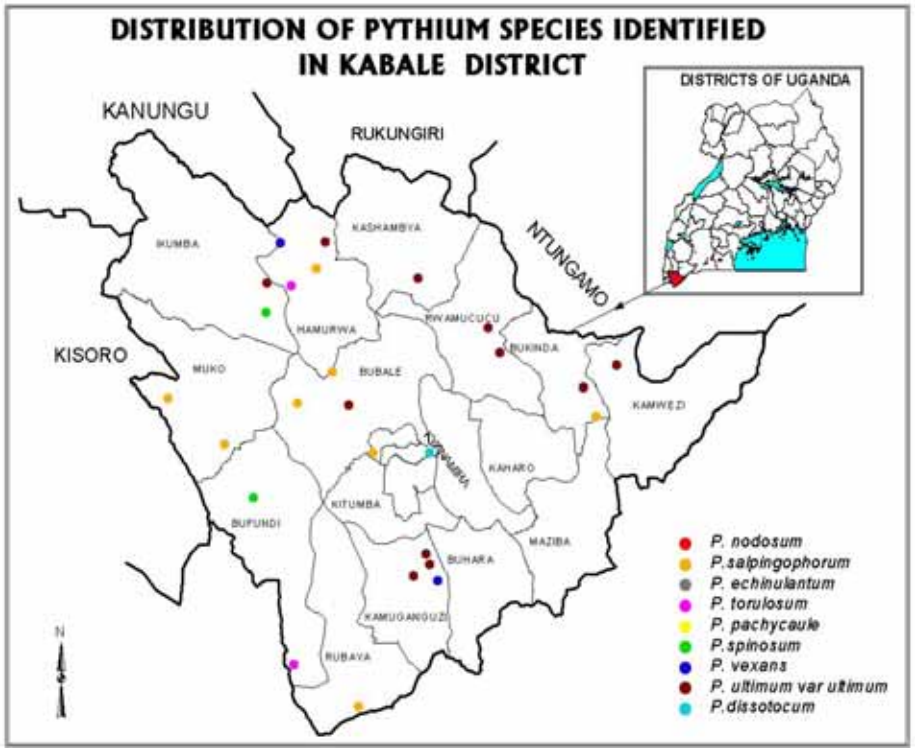


Figure 1a.

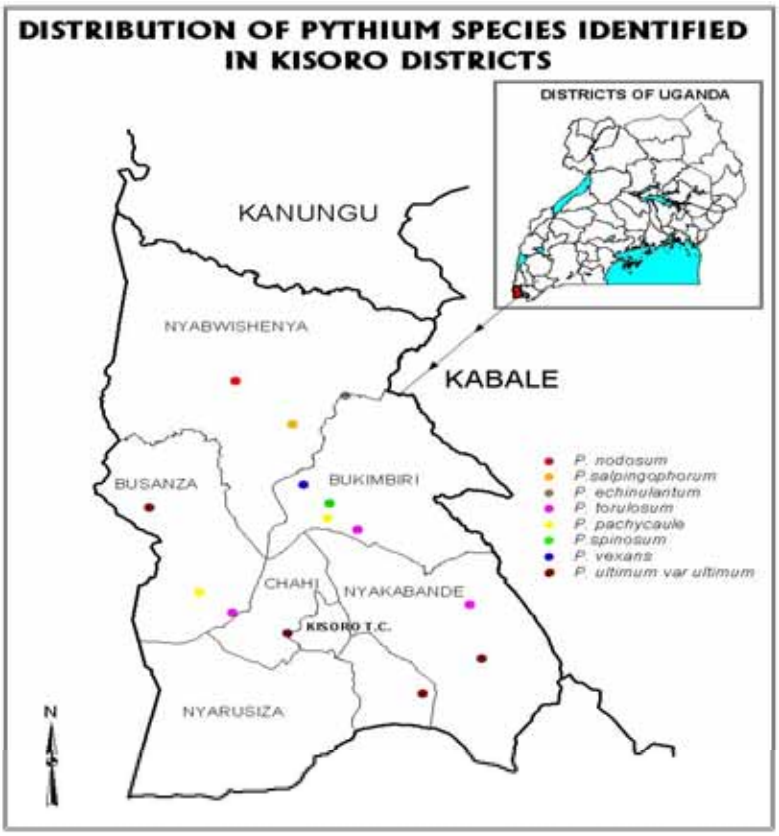


Figure 1b.

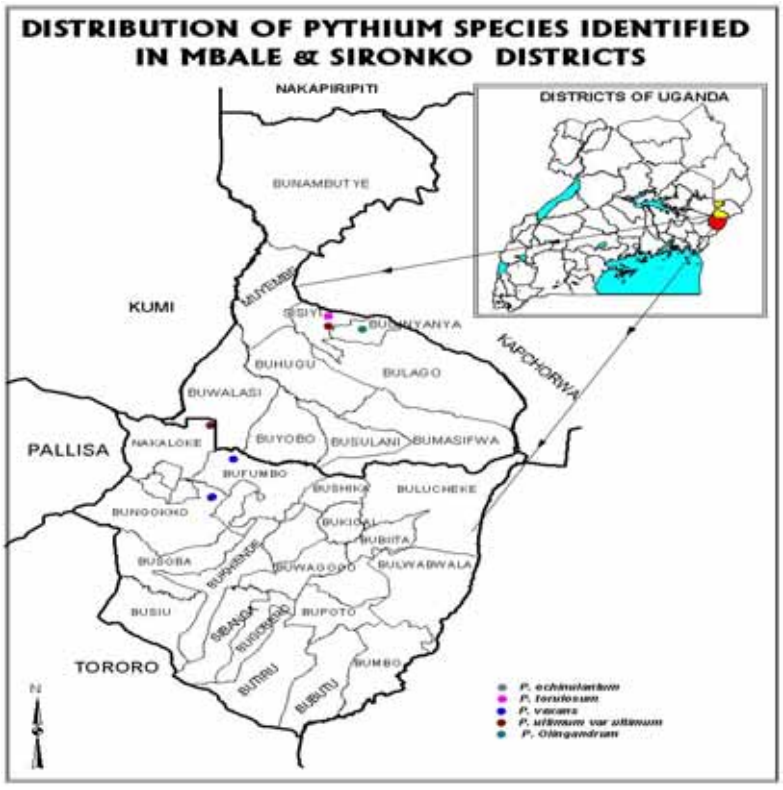


Figure 1c.

Contributors: J. Mukalazi (graduate student), R. Buruchara, S. Mayanja, G. Mahuku, F. Opio.

Collaborators: J. Carder, N. Spence, E. Adipala.

Progress towards achieving output milestones

- Characterization of thirty Pythium isolates by sequencing of IT1 region was done. Two species not considered to be pathogenic to beans were found to cause disease on beans.
- Pythium distribution maps showing relative importance of characterized species in Uganda was developed. Maps for Kenya and Rwanda will be done when characterization is complete.

Activity 2. Effect of organic amendments on bean Fusarium root rot disease and soil inoculum levels.

Introduction

Following the development of a quantification method for *F. solani* f.sp *phaseoli,* inoculum in the soil, we initiated studies last year to evaluate the effects of organic amendments on disease severity, pathogen population and plant growth. Preliminary results we obtained showed that different organic amendments have different effects on the three parameters. The objectives of the current studies were to confirm these observations and to further validate the quantification method as a basis for evaluating more IDM options.

Materials and Methods: Trials were set up both in the screenhouse and in the field in southwest Uganda. In the screenhouse, soils in wooden trays were infested with 3000 conidia of *F. s.* f. sp. *phaseoli* per gram of soil. Infested soil was then amended with farmyard or *Calliandra* green manures and left for about 2 weeks before planting K20 (susceptible) and RWR 719 (resistant) bean varieties. A similar trial was set up in the field in southwest Uganda with a history of root rot disease. In both the field and screenhouse trials, organic amendments were made at a rate of 10t/ha. Progress of root rot and soil inoculum levels were monitored at two weeks intervals.

Results and Discussion: In both the screenhouse and field trials, root rot severity was suppressed by *Calliandra* green manure, while being enhanced by farmyard manure. Similarly, the pathogen inoculum levels were highest in farmyard manure, followed by the control, and least in green manure amended soils. On the contrary, dry matter production (yield) was highest in farmyard manure amended soil. Whereas *Calliandra* green manure reduced *F. s.* f. sp. *phaseoli* inoculum levels and disease severity, farmyard manure increased both **(Figures 1a, b)**. These results confirm previous observation (CIAT, 2002). Even though farmyard manure resulted in the increase of dry matter production and grain yield, its effect on increasing inoculum is a negative contribution in the long-term management Fusarium root rot. There is therefore need to identify soil amendments or combinations that reduce soil inoculum population and disease severity but at the same time improve soil fertility and yield.

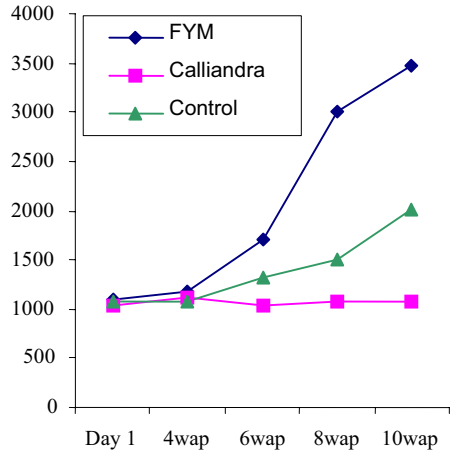


Figure 1a. Changes in soil inoculum levels of *Fusarium solani* f. sp. *phaseoli* in soils amended with farmyard manure or with *Calliandra* spp green manure, Kabale, Uganda, 2003.

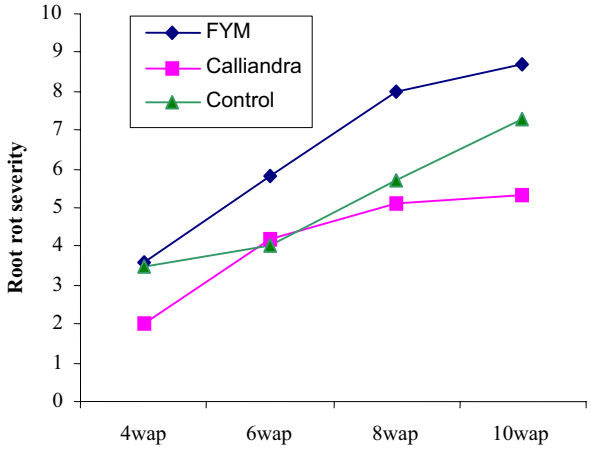


Figure 1b. Progress of Fusarium root rot disease on beans growing in soil amended with farmyard manure or with *Calliandra* spp green manure. Kabale, Uganda, 2003.

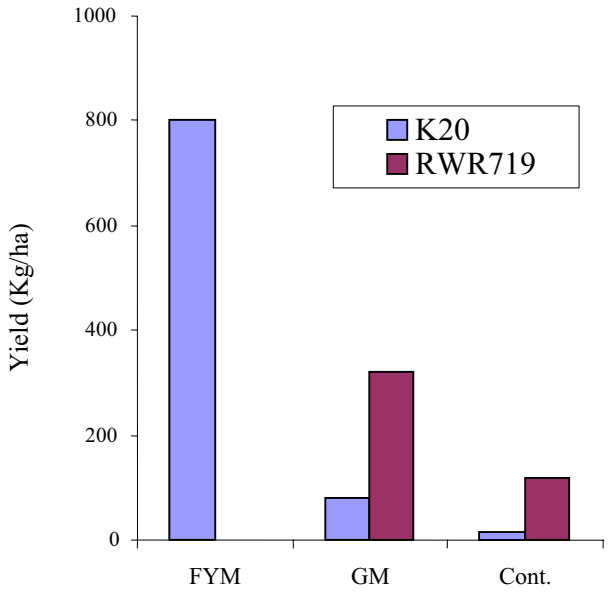


Figure 2c. Effect of organic amendments on yield of K 20 and RWR 719, susceptible and resistant bean varieties to *F. s.* f. sp. *phaseoli* respectively, Kabale, Uganda, 2003.

Contributors: G. Tusiime (graduate student), R. Buruchara, F. Opio

Collaborators: J. Carder, N. Spence, E Adipala.

Activity 3. Development and use of dilution plating method to quantify the effect of organic amendments on the inoculum levels of Pythium spp and severity of Pythium root rots.

Introduction

Population levels of soil borne pathogens influence incidence and severity of root rots. Some of the management efforts focus on reducing inoculum below economically damaging threshold levels (Buruchara and Scheidegger, 1993). Disease severity is not a good measure of the effect of management practices on soil pathogen populations because environmental factors and stage of plant growth play a critical role in disease expression and severity. There is, therefore, a need to develop a protocols to identify and quantify the effect and relative value of different management practices on inoculum levels of pathogenic *Pythium* spp. This however requires development of procedures to quantify soil inoculum. Excellent selective media exist for studying *Pythium* spp (White, 1988) and which could permit use of dilution plating in quantifying inoculum level of the fungi in the soil. The limitation of using dilution plating is that it does not distinguish between pathogenic and non-pathogenic species of *Pythium*. This study was therefore undertaken to develop a dilution-plating method in combination with molecular detection techniques to assess inoculum levels of *Pythium* spp pathogenic to beans. We hereby report studies to develop and assess the dilution plating procedure**.**

Materials and Methods: We used corn meal agar (CMA) amended with 30 mg/L of rifamycine and 75mg/L of pimaricin in 9-cm diameter petri-dishes. Soil artificially infested with Pythium ultimum var ultimum isolate (MS 41) pathogenic to beans (variety CAL 96), was used in the validation of the dilution plating procedure. Two grams of infected soil was dissolved in 100 ml of sterile distilled water in a 200 ml flask. Four soil samples from infected bean fields were also included for comparisons. Flasks were shaken on orbital shaker for 3 hours at 160 rpm. For each of the sample 0.7 ml of the suspension was evenly spread on plates (6 per sample) containing CMA and later dried in the flow isolation bench before they were covered and incubated at room temperature (20-26^{0}C) for 1-2 days.

Colonies growing on media were checked after about 20 hrs in the first day to score for the fast growing *Pythium*, and then the 2nd and 3rd day for slow ones, especially those from naturally infected field soils. The mean number of colonies per plate was relatively uniform and ranged between 5 - 8 for artificially inoculated soil, and 3 - 5 for naturally inoculated field soil. Colony forming units per gram of soil were later computed to estimate *Pythium* population in the soil as 350 - 570 and 210 – 350 cfu/gram, for artificially and naturally infected soils, respectively. Uniform colony types were observed for samples of the artificially inoculated soil, but 2 – 3 types of colonies were on plates containing samples from the naturally infected field soils suggesting occurrence of different *Pythium* species. Samples of different colonies were sub-cultured for further identification using molecular techniques. The colony types and numbers were found to be consistent for all the twelve sets tested. This method of *Pythium* quantification and detection of *Pythium* in soil was adopted for the subsequent studies on evaluation of the effects of soil amendments on *Pythium* population levels reported below.

Screenhouse trials: Seed of bean varieties CAL96 (susceptible) and RWR 719 (resistant) to Pythium root rot were sown in wooden trays containing soil that had been artificially infested with isolate MS 61 (*P. ultimum* var *ultimum*) to give pathogen concentrations of between 200 and 300 cfu.g^{-1}. Farmyard manure and *Calliandra* green manure were incorporated into the soils at rates of 10 tons/ha. Pythium populations were monitored every two weeks from the date of amendment incorporation through harvesting time using the dilution plating protocol (above). Disease progress in the two varieties was also recorded every two weeks.

Field trials: A field trial similar to the screenhouse one was set up to examine the effects of soil amendments under field conditions. Green manure from *Calliandra* spp or farmyard manure (each at 10 tons.ha^{-1}) was incorporated into randomly chosen plots. The 'control' plots received no amendments. Two weeks after the amendments had been incorporated, beans of the same two varieties (CAL 96 and RWR 719) were planted. Soil samples were collected every two weeks and Pythium populations were quantified. Disease progress in the two varieties was also recorded every two weeks

Results and Discussion: In the screenhouse where *P.ultimum* var *ultimum* was the only pathogen present our studies showed that inoculum population (in all treatments) tended to increase between the 4th and 6th week of the experiment and then declined over the remaining 10 to 8 weeks **(Table 1)**. Population numbers were also affected by the addition of organic amendments; both types causing some increase relative to the control. These relative changes in pathogen populations were mirrored by similar changes in disease severity ratings over the first 6–8 weeks but not thereafter. Farmyard manure caused a slight increase in disease in the susceptible variety. There, disease severity continued to increase throughout the duration of the experiment despite the decreases in pathogen population.

Table 1. Effect of organic amendments on population (cfu per g of soil) changes of *Pythium ultimum* var *ultimum* over a period of 14 weeks using two bean varieties (CAL96-susceptible and RWR 719-resistant), in the screnhouse, Kawanda, Uganda.

		Wks after plating							
Variety	**Soil amendment**	**Start**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
CAL 96									
	Green manure	305.0	381.0	643.0	745.0	533.0	595.0	614.0	436.0
	Farmyard manure	262.0	333.0	476.0	745.0	640.0	716.0	597.0	566.0
	Control	283.0	309.0	333.0	438.0	267.0	376.0	531.0	459.0
RWR 719									
	Green manure	250.0	286.0	452.0	519.0	348.0	524.0	511.0	336.0
	Farmyard manure	309.0	405.0	571.0	1038.0	634.0	745.0	556.0	633.0
	Control	336.0	452.0	690.0	343.0	318.0	745.0	412.0	276.0
LSD (P= 0.05)		140.1	239.1	268.0	347.3*	169.6**	353.7	455.2	356.0
CV (%)		11.5	6.9	7.9	23.1	4.9	8.1	36.4	31.0

LSD: * and ** significant at 5 and <1% respectively. Values without star are not significant at 5%

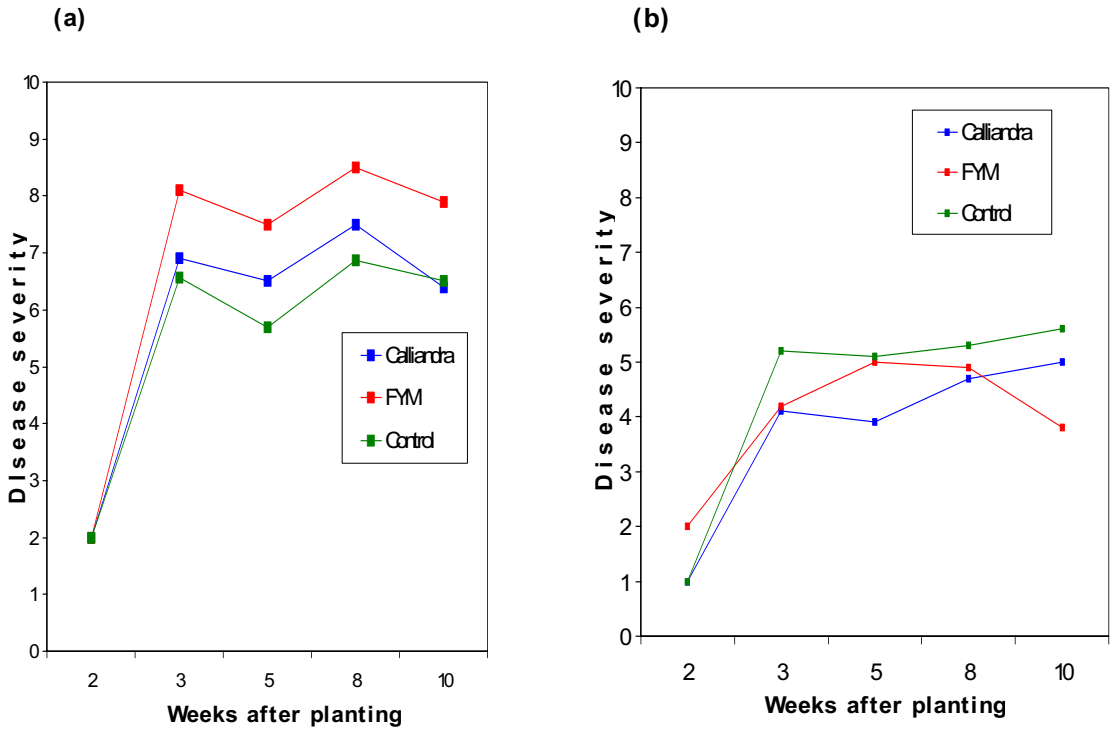


Figure 1a, b. Disease progress in the susceptible variety CAL 96 (a) and the resistant variety RWR 719 (b) and the effect of soil amendments in the screenhouse at Kawanda.

Table 2 show that population numbers of Pythium spp under field conditions was relatively lower, constant throughout the experiment and generally unaffected by the amendments. This is in contrast to the continued increase in disease severity observed **(Figure 2a, b)**. The differences seen between the screen house and field experiments may well be due to more complex interactions expected in a field situation compared to the more controlled screenhouse environment. Furthermore, only *P. ultimum* var *ultimum* was present in the screen house experiment whereas a vast range of microorganisms and Pythium spp are likely to be found in field soils. It is however not possible to correlate population of Pythium with severity as the inoculum may well have different species some of which may not be pathogenic or may be acting synergistically. Distinction of the different species isolated from field samples is underway using molecular methods. This will permit assessing the effects of organic amendments on the population of pathogenic species and also correlate the later with disease severity. The dilution plating method only enabled us to assess total Pythium population in soil. However, the method is limited if the interest is on specific (e.g. pathogenic) types. Efforts are underway to develop real time molecular-based quantitative assay for important pathogenic Pythium species.

Table 2. **Effect of organic amendments on population (cfu / g of soil) changes of *Pythium* spp over a period of 14 weeks using two bean varieties (CAL96-susceptible and RWR 719-resistant), under field condition, Kabale, Uganda.**

		Wks after plating							
Variety	**Soil amendment**	**Start**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
CAL 96									
	Green manure	131.0	155.0	231.0	107.1	192.8	174.0	128.5	121.4
	Farmyard manure	107.0	107.0	226.0	95.2	109.5	138.0	126.1	176.1
	Control	124.0	155.0	181.0	126.1	102.3	150.0	147.6	119.0
RWR 719									
	Green manure	102.0	107.0	126.0	102.3	164.2	114.0	149.9	90.4
	Farmyard manure	114.0	145.0	131.0	107.1	114.2	188.0	142.8	123.8
	Control	90	143.0	143.0	90.4	142.8	164.0	145.2	107.1
LSD (P= 0.05)		79.4	97.5	11.7	57.6	76.6	105.7	45.2	67.0
CV (%)		11.3	27.2	13.0	18.3	14.0	10.2	12.4	7.3

LSD: * and ** significant at 5 and <1% respectively. Values without star are not significant at 5%

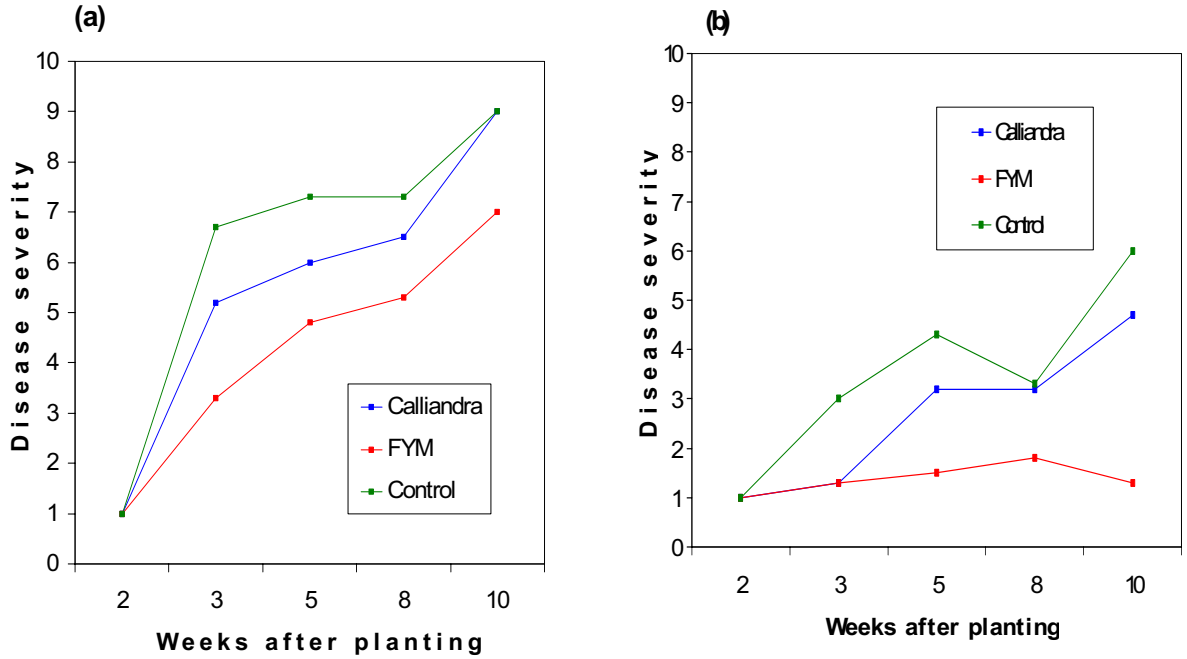


Figure 2a, b. **Disease progress in the susceptible variety CAL 96 (a) and the resistant variety RWR 719 (b) and the effect of soil amendments in the field at Kabale.**

Contributors: J. Mukalazi (graduate student), R. Buruchara, F. Opio, M. Ugen.

Collaborators: J. Carder, N. Spence, E. Adipala.

Activity 4. Scaling-up of IPM bean based technologies.

Introduction

Various traditional pest management technologies have been developed by different bean based farming communities in eastern, central and southern Africa. Some of these communities have also participated in the development of improved pest management technologies with researchers and extension agents. Most of these technologies have remained at the pilot sites where they were developed. While more technologies are being developed, dissemination approaches that combine farmer field school and participatory processes are been used at pilot sites in Kenya, Tanzania, Malawi, Rwanda and Uganda to increase awareness and adaptation of the availability of IPM technologies as well as develop capacity and skills in national research and extension service provision. Efforts are now required to scale up and scale out the approaches and processes to the wider audience.

Methods: The bean IPM project on participatory development and promotion in eastern and southern Africa, aims at scaling up and scaling out the approach developed by CIAT in northern Tanzania to Kenya, Malawi, Rwanda, Uganda and the southern highlands of Tanzania. The ECABREN and SABRN Networks have also linked their IPM subprojects to this activity and are funding the extension of project activities to Madagascar, Mozambique, Sudan and the Democratic Republic of Congo. Collaborative links have been established with National Ministries of Agriculture, NGOs and some CBOs (World Vision- WV, Adventist Development and Relief Agency -ADRA, Concern Universal- CU, Farm Africa - FA and Community Mobilisation Against Desertification- CMAD) as well as other community service providers (seed companies, religious organizations, general traders, etc.).

Participating farmer group representatives, village and district extension officers, adult education teachers, health and community leaders were trained in IPM approaches and processes (pest biology and ecology, principles of participatory approaches and community group dynamics. Farmer groups in collaboration with research and extension agents established bean seed multiplication gardens of pest tolerant genotypes (bean stem maggots and root rots) near their homesteads during the short rain season and under irrigation (September- January depending on location). The harvested seed was used to establish learning and demonstration plots as well as individual farmer fields during the long rain season (December– June depending on location). Site activities were led by farmer groups and meetings were planned and chaired by farmers. CIAT and the national researchers back stopped farmer activities and responded to farmer demands channeled through the extension service.

Results: Farmer empowerment was strengthened and scaled up at project sites in Tanzania, Kenya and Malawi. Project plans and activities in Kisii and Rachuonyo (Kenya), Hai, Lushoto and Mbeya (Tanzania) and Dedza (Malawi) were led by farmer groups (both men and women holding key positions in decision making at group, village and district levels). Farmers demanded different services depending on the location. Such services included training in various aspects including the search for market information, soil fertility and livestock management, facilitation in cross site visits, supply of farm inputs and credits, etc. Farmers have also demanded to be facilitated to disseminate their IPM message to the wider audience. It was

through this initiative that two Hai district women farmers each participated in two different regional workshops (one on science and technology in agriculture and another on bean network priority setting). Farmers groups in Hai were assisted by the district and World Vision to form and registered their association (MUVIMAHA- Muungano wa Vikundi vya Maendeleo Wilaya ya Hai, i.e. Union of Development Groups in Hai District). MUVIMAHA will facilitate members with different services including farm inputs, savings and credits, market information, etc.

Farmers in Kenya and Tanzania were facilitated by the project and other stakeholders to conduct cross site visits within their countries. New farmer groups have been formed in both countries. Representative farmers from western Kenya (Kakamega) toured project sites in southwestern Kenya (Kisii). Farmers from Arumeru and Babati districts visited Hai while farmers from Mbeya visited Mbozi district farmer groups. Five farmers from Malawi (2 men, 3 women) were facilitated by SABRIN and the project to conduct a learning, sharing and exchange visit to farmer groups in the southern highlands of Tanzania.

Additional extension materials were prepared (leaflets, posters, farmer activity reports and proceedings of project evaluation workshop). Four leaflets and two posters are in Kiswahili. The translation, editing and printing of the pest handbook was completed. New village information centres (VICs) were set up by farmer groups and community leaders in Kenya and Tanzania and were stocked with extension and other relevant materials. Other ministries (e.g. Education and Health) have supported and contributed to setting up VICs.

Contributors: E. Minja, H. Mziray, K. Ampofo, J. Ogecha, D. Kabungo and C. Madata.

Collaborators: M. Pyndji (ECABREN), R. Chirwa (SABRIN), E. Ulicky (Hai district), F. Ngulu (SARI), B. Chibambo (CU), A. Masam (WV), Y. Mbwana (ADRA), D. Ngowi (FA).

Progress towards achieving output milestones

- A bioassay method developed to quantify soil inoculum of *F. solani* f.sp *phaseoli* offers a opportunities to assess the effects of IDM options on pathogen population.
- Dilution plating method that allows quantitative measurement of total Pythium population was developed. But its utility will be enhanced if combined with fast detection procedures.
- Quantification studies of *F. solani* f.sp *phaseoli* and Pythium species show that organic amendments may have a positive effect on the crop growth and yield while having a negative influence (increase) on soil inoculum. Efforts will continue to identify management approaches that would increase plant growth but reduces pathogen populations.

Activity 5. Publications, Conferences, Workshops, Training, Students.

Refereed Journals

Wagara, I. N., Mwangómbe, A. W., Kimenju, J. W., Buruchara, R. A., Jamnadass, R. and Majiwa, P.A.O. 2003. Genetic diversity of *Phaeoisariopsis griseola* in Kenya as revealed by AFLP and group-specific primers. Submitted to Journal of Phytopathology

Conferences

1. Buruchara, R.A., Bua, B., Otsyula, R. Opio, F. and Kimani 2003. Sources of resistance in common bean (*Phaseolus vulgarism* L.) to Pythium root rot caused by *Pythium spp* in eastern and central African highlands
2. Buruchara, R. A. 2003. Integrated Disease Management In Food Legumes. Presented at the Second National Review Workshop on Food and Forage Legumes 22 – 26 September 2003, Addis Ababa, Ethiopia.
3. Macharia, R. D., P.M. Kimani, R. Buruchara and J. W. Kimenju. 2003. Breeding red mottled and red kidney bean cultivars resistant to anthracnose, angular leaf spot and tolerance to low soil fertility. Presented at the Afr. Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.
4. Kimani, P.M., R. Buruchara, R. Otsyula, G. Rachier and R.D. Macharia. 2003. Breeding bean cultivars resistant to angular leaf spot, root rots and low soil fertility in East and central Africa. Presented at the Afr. Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.
5. Musoni, A., Kimani, P.M., R.A. Buruchara, R.A., R.D. Narla and I. N. Wagara. 2003. Breeding marketable climbing beans resistant to angular leaf spot, Pythium root rot, anthracnose and Fusarium wilt. Presented at the Afr. Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.
6. Wagara, I. N., A. W. Mwang'ombe, J. W. Kimenju, R. A. Buruchara and P.M. Kimani. 2003. Pathogenic variability in *Phaeoisariopsis griseola* and response of bean germplasm to different races of the pathogen. Presented at the Afr. Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.
7. Otsyula, R., R. Buruchara, P. Rubaihayo and P. M. Kimani. 2003. Inheritance of resistance to Pythium root rots in bean genotypes (Phaseolus vulgaris L.). Presented at the Afr. Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.
8. Mukalazi, J., Adipala, E., Buruchara, R., Carder, J., Opio, F. and Spence, N.J. Variation and identification of *Pythium* species associated with bean root rot disease in Uganda.
9. Tusiime, G., Buruchara, R., Adipala, E., Carder, J., Spence, N. and Opio, F. 2003. Variation of *Fusarium solani* from beans with root rot symptoms inferred from AFLP analysis, pathogenicity and DNA sequences.
10. Namayanja, A. R. Buruchara, P. Rubaihayo, S. Mayanja. 2003. Validating the utility of angular leaf spot resistance linked markers for marker assisted breeding in common bean. Presented at the Afr. Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya

Graduate student supervision
Completed M. Sc

Annet Namayanja – M.Sc, Makerere University, Uganda: Inheritance And Marker Assisted Selection For Angular Leaf Spot *(Phaeoisariopsis griseola*) Resistance in Common Bean" at Kawanda, Uganda.

Continuing

Geoffrey Tusiime – PhD, Plant Pathology, Makerere University, "Variation and detection of *Fusarium solani* f.sp *phaseoli* and quantification of soil inoculum in common beans".

Julius Mukalazi, PhD, Plant Pathology, Makerere University, Pathogenic variation and quantification of Pythium species of beans in Uganda".

Stephen Mayanja, MSc, Plant Pathology, Makerere University, "Characterization of pathogenic diversity of *Phaeoisariopsis griseola* in Uganda"

Reuben Otsyula, Plant Breeding, Makerere University. "study of inheritance and development of root rot (Pythium) resistant varieties using marker assisted selection in common beans"

Clare Mukakunzi, PhD Plant Pathology/Breeding University of Natal. "Breeding beans (*Phaseolus vulgaris* L.) for resistance to Fusarium root rot (*Fusarium solani f*.sp *phaseoli*) in Uganda"

Augustine Musoni, Plant Breeding, University of Nairobi: "Breeding for resistance to Fusarium wilt"

Wagara Isabel: "Molecular and virulence characterization of *Phaeoisariopsis griseola* and reaction of bean germplasm races of the ALS pathogen.

Mathias Zulu, Plant Pathology, University of Zambia. "Bean anthracnose *(colletotrichum lindemuthianum)* study and determination and distribution of races prevalent in Zambia"

Workshops and Conferences

1. Biofortification Planning Meeting, 12-18 Feb Kisumu, Kenya
2. Wider impact planning workshop, March, Kawanda, Uganda
3. Participatory Plant breeding Workshop, March 2003, Kawanda, Uganda
4. PABRA Steering Committee Meeting, 26-29 May 2003, Kabale, Uganda.
5. Good Seed Initiative Workshop, 4 - 7 June Morogoro, Tanzania.
6. ECABREN problem analysis workshop, July 2003, Naivasha, Kenya
7. ECABREN stakeholders priority setting workshop, 13- 19 July 2003, Nairobi, Kenya
8. Participatory breeding Planning Meeting, 15-16 Sep 2003, Kawanda, Uganda.
9. The Second National Review Workshop on Food and Forage Legumes, 22-26 September 2003, Addis Ababa, Ethiopia.
10. Biofortification Challenge Program-Bean Planning Meeting, 1-4 Oct, 2003, Naivasha, Kenya.
11. SABRN Steering Committee meeting, 7 –11 October 2003. Potchefstroom, South Africa.
12. African Crop Science Conference, 13-17 Oct 2003, Nairobi, Kenya.

Regional travel

1. Rwanda –March, June and September 2003.
2. Tanzania – April, June
3. Malawi – March 2003

4. Uganda, - Kabale, May, June
5. Zambia - March 2003
6. Colombia – PRGA meeting, 30 Sept –3 July 2003
7. Kenya – February, April, May, July, September.

Proposal/Concept notes developed

1. "Scaling up and scaling out bean IDPM promotion activities including pest tolerant and improved high yielding bean genotypes" and extension phase to DFID.
2. "Bean root rot disease management in Uganda" extension phase to DFID.
3. "Strengthening Research & Development for Increased and Sustainable Agricultural Productivity, Improved Food Security and Nutrition, and Income of Rural and Urban Populations in East and Central Africa" for ECABREN to USAID via ASARECA.

Project submitted and funded

Donor	Project	Duration of current funding
CIDA (Canada)	Pan-Africa Bean Research Alliance	2000-2002
HRI (from DFID)	Epidemiology of bean root rots	2000-2003
Rockefeller Foundation	Genetic Improvement of Bush and Climbing Beans	2001-2003
SDC (Switzerland)	Pan-Africa Bean Research Alliance	1998-2001 2001-2004
USAID (USA)	Eastern and Central Africa Bean Research Network	1998-2003

ECABREN: activities reported here are supported financially by the member Governments of the ASARECA region, and by CIDA, SDC and USAID as the donors to PABRA.

STAFF LIST

Senior staff: **Buruchara**, Robin, PhD, Plant Pathologist / PABRA Coordinator (stationed in Kampala, Uganda), Eli Minja, PhD, IPM specialist, (Stationed in Arusha Tanzania), Kwasi Ampofo, PhD, Coordinator ATDT project (stationed in Butare, Rwanda)

Research Assistants:

Suleiman, Sebuliba, Field Assistant (Breeding) (stationed in Kampala, Uganda).
Mayanja, Stephen, BSc, Research Assistant (Biotechnology Lab) (stationed in Kampala, Uganda).
Mukankusi, Clare, BSc, MSc Research Assistant (Pathology) (study leave in S.Africa).
Manzi, Geoffrey, BA, Research Assistant (Root Rot) (stationed in Kabale, Uganda - left).
Acam, Catherine, Dip. Research Technician (Breeding) (stationed in Kampala Uganda).
H.A. Mziray, BSC, Research Assistant (Stationed in Arusha Tanzania)..

Collaborators Partners:

National Programs: Democratic Republic of Congo, Ethiopia, Burundi, Rwanda, Uganda, Kenya, Tanzania, Sudan, Madagascar, Malawi, Zimbabwe, Leshoto, Mozambique, Republic of South Africa, Swaziland, Angola, Zambia.

***Universities*:** University of Nairobi, Makerere University, Moi University, Bunda College, University of Alemaya, Sokoine University, University of Zimbabwe

NGO: World Vision International -WVI (Tanzania), AfriCare - Uganda, Catholic Relief Services, Resource, ADRA-Tanzania, Farm-Africa, Adventist Development and Relief Agency – ADRA (Tanzania), Concern Universal –CU (Malawi) and Community Mobilization Against Desertification –CMAD (western Kenya). Plan International-Malawi; Pelum; Lay Volunteers International Agency and Christian Council of Tanzania,; Harvest Help, CARE International; Rural Farm Alternative Organization,- Kenya.

***CBO and Regional Institutions*:** ASARECA, Food, Agriculture and Natural Resources,

Governmental Institutions: Zonal Communication Centre (ZCC), in the Ministry of Agriculture and Fisheries, Tanzania.

***Europe Institutions*:** Horticultural Research International (HRI)(UK), NRI (UK), Agri-Food and Food Canada (Canada).

Institutional Abbreviations

AHI	African Highlands Ecoregional Programme (led by ICRAF)
ARC/GCRI	Agricultural Research Council, Grain Crops Research Institute, South Africa
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
AU	Alemaya University, Ethiopia
CARE	(International NGO in Ethiopia, Rwanda, Uganda)
CIDA	Canadian International Development Agency
CU	Concern Universal, Malawi
DARTS	Department of Agricultural Research and Technical Services, MoA, Malawi
DRD	EARO Ethiopian Agricultural Research Organization
ECABREN	Eastern and Central Africa Bean Research Network
FOFIFA	Centre National de la Recherche Appliqué au Développement Rural, Madagascar
HRI	Horticultural Research Institute (UK)
IACR	Rothamsted (UK)
ICIPE	Centre for Research in Agro-Forestry
IBFA	Ikulwe Bean Farmers Association (Uganda)
INERA	Institut National des Etudes sur la Recherche Agronomique, DR Congo
ISAR	Institut des Sciences Agronomiques du Rwanda
KARI	Kenya Agricultural Research Institute
MoA	Ministry of Agriculture
MU	Makerere University, Uganda
NARO	National Agricultural Research Organisation, Uganda
NARS	National agricultural research system
NGO	Non-governmental organization
NRI	Natural Resouces Institute (UK)
PABRA	Pan-Africa Bean Research Alliance

RF	The Rockefeller Foundation
SABRN	SADC Bean Research Network
SACCAR	Southern African Centre for Cooperation in Agricultural and Natural Resources Research and Training
SADC	Southern Africa Development Council

The Systemwide Tropical Whitefly IPM Program

Activity 1. Coordination.

CIAT has the responsibility to coordinate the Systemwide Tropical Whitefly IPM Program (SP-IPM- TWFP), and its various sub-projects in the Americas (described as activities in this section of the annual report), Southeast Asia and subSaharan Africa. These projects are funded by different donors, namely: The United Kingdom Department for International Development (DFID); the United States (USAID) and New Zealand (NZAID) Agencies for International Development; and the Australian Center for International Agricultural Research (ACIAR). The various subprojects that constitute this global effort are currently coordinated by scientists from IITA, CIAT and AVRDC. The International Potato Center (CIP) has participated in the past and currently holds the Chair of the Systemwide IPM Program.

One of the main Coordination activities has been the integration of the various Crop Protection Programme (CPP) projects that target whiteflies as pests and vectors of plant viruses in the Tropics. The whitefly CPP projects that lie outside the coordination of the TWFP, operate in Southeast Asia and East Africa, and target crops, such as cassava, sweet potato and vegetables. The TWFP also targets cassava and vegetables in other areas of the tropics, including their center of origin, and includes the common bean as a major food crop threatened by whiteflies and whitefly-transmitted viruses in the Americas. The TWFP has also been entrusted with the responsibility of supporting the development of new projects on the epidemiological implications of the IPM strategies being promoted by the TWFP.

The TWFP Coordination is also trying to expand its target area, primarily in the Andean region. At present, only Colombia and Ecuador receive technical assistance, but Peru and Bolivia are also suffering the effects of severe whitefly outbreaks in different crops, including potato, tomato and sweet potato. The coordinator of the TWFP responded to a request from DFID and the Bolivian Foundation for the Promotion and Research of Andean Products (PROIMPA), to evaluate the whitefly problem in the mesothermic valleys of the departments of Cochabamba and Santa Cruz. The main whitefly pest in these valleys was identified as *Trialeurodes vaporariorum* and the main crops affected were: common bean, potato and tomato. This whitefly species is not a vector of the main group of viruses transmitted by the whitefly *Bemisia tabaci*, but it has the capacity to kill susceptible plants through its feeding damage and release of substances (honeydew) that promote fungal growth (sooty molds). This type of severe damage was observed in some common bean fields **(Figure 1)** and, to a lesser extent, in potato.

The TWFP is approaching the last stage of Phase III, in which the most promising IPM strategies identified during Phase I, were tested in pilot sites. Hence a meeting of all subproject coordinators from the Americas, Africa and Asia will take place at the end of this month (September) in order to define research/technology dissemination priorities for Phase III. The emphasis of the meeting will be on Farmer Participatory Research; Communication Technology and Knowledge Management.

Figure 1. **Direct (feeding damage) and indirect (sooty mold) damage caused by *Bemisia tabaci* in common bean.**

Figure 2. **Farmer field school organized in El Salvador to disseminate information on IPM practices to control whiteflies and whitefly-transmitted viruses.**

Activity 2. Studies on whitefly (*Aleurotrachelus socialis*) resistance mechanisms in selected cassava genotypes.

As direct feeding pests and virus vectors, whiteflies cause major damage in cassava based agroecosystems in the Americas, Africa and, to a lesser extent Asia. The largest complex of whitefly pests on cassava is in the Neotropics, where 11 species are reported. Eight whitefly species are reported feeding on cassava in Colombia. *Aleurotrachelus socialis* is the major species on cassava in Northern South America (Colombia, Ecuador, Venezuela) while *Aleurotrachelus aepim* predominates in Brazil and *Bemisia tabaci* in Africa and parts of Asia. In whitefly surveys on cassava in Colombia, approximately 92% of the species population is *A. socialis*. For this reason, *A. socialis* receives most of the research effort, especially in the identification of whitefly resistant cassava genotypes and the development of resistant varieties.

The first symptoms of whitefly damage are manifested by curling of the apical leaves and yellowing, necrosis and abscission of lower leaves. This results in plant retardation and considerable reduction in root yield if feeding is prolonged. Damage and yield losses of this type are common with *A. socialis* and *A. aepim.* There is a correlation between duration of whitefly attack and yield loss, which has been recorded as high as 79% in prolonged (11 months) attacks and on susceptible cultivars. Cassava farmers will respond to whitefly attack with frequent applications of toxic chemical pesticides. Pesticide use is costly, often causes environmental contamination, a hazard to human health and may not provide effective control.

Stable host plant resistance (HPR) offers a practical long-term low cost solution for maintaining reduced whitefly populations. Although whitefly resistance in agricultural crops is rare, several good sources of resistance have been identified in cassava and high-yielding, whitefly resistant cassava hybrids are being developed. At CIAT we are systematically evaluating the cassava germplasm bank of more than 6000 accessions. The clone MEcu 72 has consistently expressed the highest level of resistance and is being employed in a breeding scheme to develop whitefly resistant hybrids (see CIAT 2002, IP-3 Annual Report). Additional cultivars expressing moderate to high levels of resistance in field trials include MEcu 64, MPer 335, MPer 415, MPer 317, MPer 216, MPer 221, MPer 265, MPer 266 and MPer 365.

The objective of these present studies is to evaluate several selected genotypes for mechanisms of resistance to *A. socialis* under controlled growth chamber conditions.

Methodology: The genotypes selected for evaluation were MEcu 64, MPer 273 and MPer 334; CMC 40 was the susceptible control and MEcu 72 the resistant control. All genotypes have been field evaluated during numerous trials at CORPOICA, Nataima (Tolima). As previously mentioned MEcu 72 has consistently shown resistance to *A. socialis* and in laboratory controlled resistance mechanisms evaluations, resulted in a 72% mortality, a lower oviposition rate, longer development time and reduced size. CMC 40 supports high *A. socialis* populations and low mortality. In field trials MPer 273, MPer 334 and MEcu 64 genotypes showed low to moderate *A. socialis* populations and few damage symptoms. Four *A. socialis* development parameters were evaluated, mortality/survival, duration of the life cycle, nymphal development size, (Antibiosis), and ovipositional preference (Antixenosis). This was done in two separate experimental designs.

1. Antibiosis Experiments: This was done in two parts; in the first, test plants were infested and evaluated by using *A. socialis* adults harvested directly from the greenhouse maintained colony being reared on the susceptible CMC 40 **(Figure 1A)**. The second evaluations were done by first preconditioning *A. socialis* on the selected test genotypes for two generations. These individual colonies on the five aforementioned genotypes were reared in wooden, nylon mesh lined cages (1m x 1m x 1m) in the greenhouse **(Figure 1B)**. All antibiosis experiments were carried out in the growth chamber (28±1°C, 60-70% RH, 12 hrs. light) by measuring the life cycle development of *A. socialis* as the aforementioned resistant and susceptible genotypes. Cassava plants were grown in plastic pots and were 4 to 5 weeks of age at infestation. Plant infestation was accomplished by introducing 20 whitefly adults into small leaf cages, supported by plastic straws **(Figure 2)**. Each leaf cage has a small lateral opening and with the aid of a pasteur aspirator, *A. socialis* adults are encouraged to enter the leaf cages. Five leaf lobes were infested on each plant (total 180) during a 4-hour period with 3600 adults **(Figure 3A)**. *A. socialis* adults were allowed to oviposit for 24 hours, thereby assuring a uniform population. Leaf cages and adults were then removed and egg infested plants were placed in the growth chamber **(Figure 3B)**. Each leaf lobe was sequentially numbered to assure accurate data collection on each of the tested genotypes.

Figure 1. Antibiosis experiments. (A) Greenhouse colony of *Aleurotrachelus socialis* on CMC 40 (not-preconditioned); (B) *A. socialis* pre-conditioned and reared in nylon-meshed cages on resistant and susceptible genotypes.

Figure 2. Thirty-day cassava plants grown in plastic pots and with attached leaf cages conditioned for *Aleurotrachelus socialis* infestation.

Figure 3. (A) Cassava plants conditioned for *A. socialis* infestation; (B) cassava plants infested with *A. socialis* eggs in the growth chamber (28±1°C, 60-70% RH, 12 hrs light).

To determine the biological cycle of *A. socialis* on resistant and susceptible genotypes, 200 eggs are selected per plant, and an "infestation map" was designed so that daily evaluations of immature development can be recorded for instar changes, growth characteristics and survival/mortality. Daily evaluations were done with the aid of a stereomicroscope on the leaf undersurface. The potted plants, fastened to an iron support rod that allows upward-downward movement for optimal positioning, are inverted for easy observance. A rubber disk inserted at the base of the plant stem at the soil line prevents soil loss or plant movement and injury when the potted plants are invested **(Figure 4)**.

Figure 4. Inverted cassava plants fastened to an iron support rod allowing easy observance of *Aleurotrachelus socialis* development stages with the use of a stereomicroscope.

The differences in duration of biological stages, time of development, morphological measurements of immature stages and adult dry weight were analyzed using the Ryan-Einot-

Gabriel-Welsch Multiple range test (REGW). The rate of survival and relationship between sexes was analyzed with the Chi-Square (X^2) test.

Morphological measurements were done by removing 10 individuals per leaf lobe (40 individuals total per genotype) and taking measurements of the 2nd and 3rd instar nymphs and the pupal stage. A stereomicroscope with a digital dispositive for micro measurement (Wild MMS 225/MMS 2535) **(Figure 5)**.

Figure 5. Digital micrometric measuring devise to determine morphological size of *Aleurotrachelus socialis* immatures.

Dry weight of adult whiteflies was done by placing well-developed pupae in the small leaf cages to prevent adult escape upon hatching. Sexing was done under the stereomicroscope using adult anal morphological characteristics to separate male and females. Captured adults from each of the tested genotypes were placed in plastic vials with cotton stoppers and dried in a Blue-M stove at 37°C for 72 hours. These were weighted on a CAHN C-30 microbalance, sensitive to 1 μg.

2. Antixenosis Experiments. These experiments compared and determined the ovipositional and feeding preferences of *A. socialis* on the five genotypes. One potted plant of each genotype was randomly placed in a 1m x 1m x 1m wooden, nylon meshed lined cage. Each 30-day-old plant contained only three leaves, numbered in descending order from the top, middle and lower portions of the plant. This design allowed measurement of both total and vertical plant preference of oviposition. All plants were of equal height and distributed in a circular fashion to provide each genotype with an equal chance for oviposition **(Figure 6)**. Five hundred *A. socialis* adults of the same age and randomly selected from the whitefly colony being reared on CMC 40, were introduced into the center of each cage. Recorded data was logarithmically transformed (Log H+1) and significant differences were determined using the Ryan-Einot-Gabriel-Welsch multiple F test. The variables were, 1) the number of whiteflies perched on each genotype at 24 and 48 hours after infestation, and 2) the number of eggs oviposited on each genotype after 48 hours. A visual count of perched adults was accomplished by carefully opening each cage without disturbing plants and whitefly adults. Egg counts were made under the stereomicroscope. The evaluation was done 3 times with four repetitions using a randomized block design in the growth room (28±1°C, 60-70% RH and 12 hr. light).

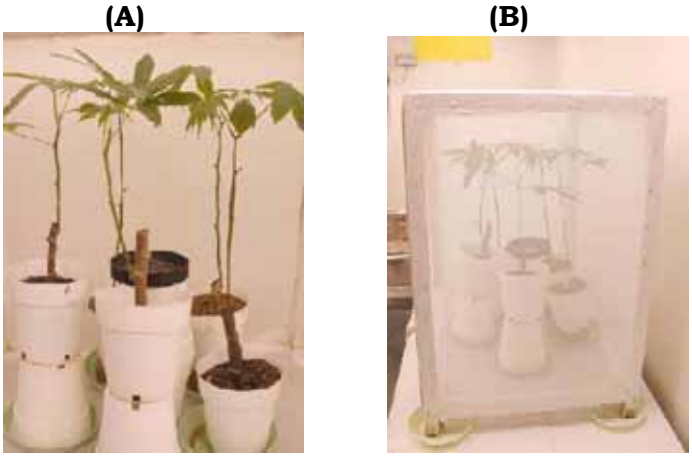


Figure 6. Cassava genotypes (Resistant and Susceptible) placed in nylon meshed cages and infested with 500 *A. socialis* adults for free choice ovipositional preference evaluations in the growth chamber.

Results

1. Antibiosis: No preconditioning.

A. socialis individuals (adult infestation directly from the greenhouse colony = un-preconditioned) feeding on MEcu 64 and MPer 334 had a significantly longer development period than on the other genotypes **(Table 1)** with 36.8 and 36.4 days respectively. MEcu 72 and MPer 273 resulted in a duration of 35.2 and 33.6 days, while CMC 40 the susceptible control had a significantly more rapid development of 32.7 days. The duration of the egg stage ranged from 10.1 (CMC 40) to 11.1 (MEcu 64) and difference between genotypes were significant **(Table 2)**. The greatest differences occurred in the first nymphal instar. Most rapid development occurred on CMC 40 (4.9 days) and longest on MEcu 64 (6.4 days); MPer 334, MEcu 72 and MPer 273 had first instar duration of 6.1, 6.1 and 5.6 days respectively **(Table 2)**. Significant differences between genotypes also occurred in the 2nd and 3rd nymphal instars but they were not as dramatic as in the first instar. The duration of the pupal stage ranged from 9.6 days (CMC 40) to 10.5 days (MPer 334). The relationship between sexes was approximately 1:1 in all of the genotypes evaluated **(Table 1)**.

Table 1. Average development time of *Aleurotrachelus socialis* (non-preconditioned) feeding on five cassava genotypes (resistant and susceptible) in the growth chamber.

Genotypes	n.	Average ± SD	Sex Relation
MEcu 64	63	36.8 ± 2.09 a1	1.0 : 1.0
MPer 334	45	36.4 ± 2.21 a	1.1 : 1.0
MPer 273	94	33.6 ± 1.55 c	0.7 : 1.0
MEcu 72	62	35.2 ± 2.56 b	1.2 : 1.0
CMC 40	152	32.7 ± 1.65 d	1.3 : 1.0
			X^2 : NS^2

1. Ryan-Einot-Gabriel-Welsch Multiple Range test. Columns with the same letter are not significantly different at the 5% level.
2. Independent Test. Female/Male sex relation is 1:1 in all genotypes.

Table 2. Duration of *Aleurotrachelus socialis* developmental stages on whitefly resistant and susceptible genotypes (non-preconditioned) (*n*=200).

Genotypes	Egg	Nymph 1	Nymph 2	Nymph 3	Pupae
MEcu 64	11.1 ± 0.57 a[1]	6.4 ± 1.08 a	4.3 ± 0.71 a	5.1 ± 0.66 a	10.4 ± 0.93 a
MPer 334	10.7 ± 0.47 b	6.1 ± 1.08 b	4.3 ± 0.95 a	5.0 ± 0.94 a	10.5 ± 1.07 a
MPer 273	10.4 ± 0.49 c	5.6 ± 1.01 c	3.7 ± 0.64 b	4.4 ± 0.63 b	10.0 ± 0.84 b
MEcu 72	10.6 ± 0.58 b	6.1 ± 1.05 b	4.4 ± 0.63 a	4.6 ± 0.96 b	9.9 ± 0.87 b
CMC 40	10.1 ± 0.50 d	4.9 ± 0.85 d	3.8 ± 0.59 b	4.4 ± 0.54 b	9.6 ± 0.83 b

1. Ryan-Einot-Gabriel-Welsch Multiple Range (F) test. Columns with the same letter are not significantly different at the 5% level.

2. Antibiosis: With preconditioned *A. socialis.*

Development time for *A. socialis* in this experiment was significantly longer when reared on MEcu 64 (34.5 days) compared to the other genotypes. It was shortest on CMC 40 (31.8 days) and intermediate for the remaining three genotypes **(Table 3)**. Nymphal duration was longest during the first instar; MEcu 64 was longest (6.3 days) and CMC 40 was shortest duration (5.0 days). The remaining three genotypes, MPer 334 (5.6 days), MPer 273 (5.5 days) and MEcu 72 (5.7 days) were significantly different from the susceptible genotype CMC 40 **(Table 4)**. Differences in development duration in the second and third instars were not as dramatic as in the first instar. Duration of the pupal stage ranged from MEcu 64 (10.5 days), the longest, to CMC 40 (9.5 days) the shortest and the remaining genotypes, intermediate **(Table 4)**. Results in this experiment were similar to those in the un-preconditioned experiment, however, the values were lower or of shorter duration, indicating that preconditioning *A. socialis* effects development time.

Table 3. *Aleurotrachelus socialis* development time on cassava genotypes (resistant and susceptible) during preconditioning phase.

Genotypes	N	Average ± SD	Sex Relation
MEcu 64	96	34.5 ± 1.94 a[1]	1.0 : 1.0
MPer 334	124	33.0 ± 1.76 bc	0.9 : 1.0
MPer 273	127	32.8 ± 2.22 c	1.3 : 1.0
MEcu 72	127	33.5 ± 1.82 b	0.8 : 1.0
CMC 40	140	31.8 ± 1.61 d	1.5 : 1.0
			X^2 : NS[2]

1. Ryan-Einot-Gabriel-Welsch Multiple Range test. Columns with the same letter are not significantly different at the 5% level.

Table 4. Duration of *Aleurotrachelus socialis* development stages on whitefly resistant and susceptible genotypes (*n*=200) (preconditioning phase) in the growth room.

Genotypes	Egg	Nymph 1	Nymph 2	Nymph 3	Pupae
MEcu 64	9.7 ± 0.54 b[1]	6.3 ± 1.34 a	4.2 ± 0.86 a	4.2 ± 0.70 a	10.5 ± 1.05 a
MPer 334	9.4 ± 0.54 c	5.6 ± 0.92 b	3.8 ± 0.83 b	4.0 ± 0.52 a	10.4 ± 1.04 a
MPer 273	10.0 ± 0.72 a	5.5 ± 0.99 b	3.8 ± 0.77 b	4.0 ± 0.61 a	9.7 ± 1.21 bc
MEcu 72	9.7 ± 0.62 b	5.7 ± 1.11 b	4.2 ± 1.01 a	4.1 ± 0.58 a	10.0 ± 1.02 b
CMC 40	9.6 ± 0.71 b	5.0 ± 0.87 c	3.8 ± 1.02 b	4.1 ± 0.614 a	9.5 ± 0.95 c

1. Ryan-Einot-Gabriel-Welsch Multiple Range test. Columns with the same letter are not significantly different at the 5% level.

A. socialis survival on resistant genotypes (MEcu 64, MEcu 72, MPer 334 and MPer 273) is significantly lower than on the susceptible check, CMC 40 **(Figures 7 and 8)**. First instar nymphs are the most effected; they have difficulty adhering to the leaf undersurface and initiating feeding on resistant genotypes. This is not a problem on the susceptible genotype CMC 40, where establishment and feeding readily occur **(Figure 7A)**. In the two experiments *A. socialis* survival remained the same (76 and 75% survival) **(Figure 8)**. Without precondition *A. socialis* survival on MPer 344, MEcu 72, MEcu 64 and MPer 273 were 22.5, 31.0, 31.5 and 47.0% respectively. For preconditioned *A. socialis*, the results were similar but the rate of survival was higher for all of the resistant genotypes **(Figure 8)**. For example, in the first experiment MEcu 64 survival was 31.5%, wile in the second it was 48.0%. In both experiments the resistant genotypes had a significantly lower survival rate than the susceptible genotypes (P=0.05). These results indicate that constant rearing of *A. socialis* on resistant genotypes may reduce the effectiveness of the resistant factors. This will play a role in the deployment of resistant cultivars in field plantings.

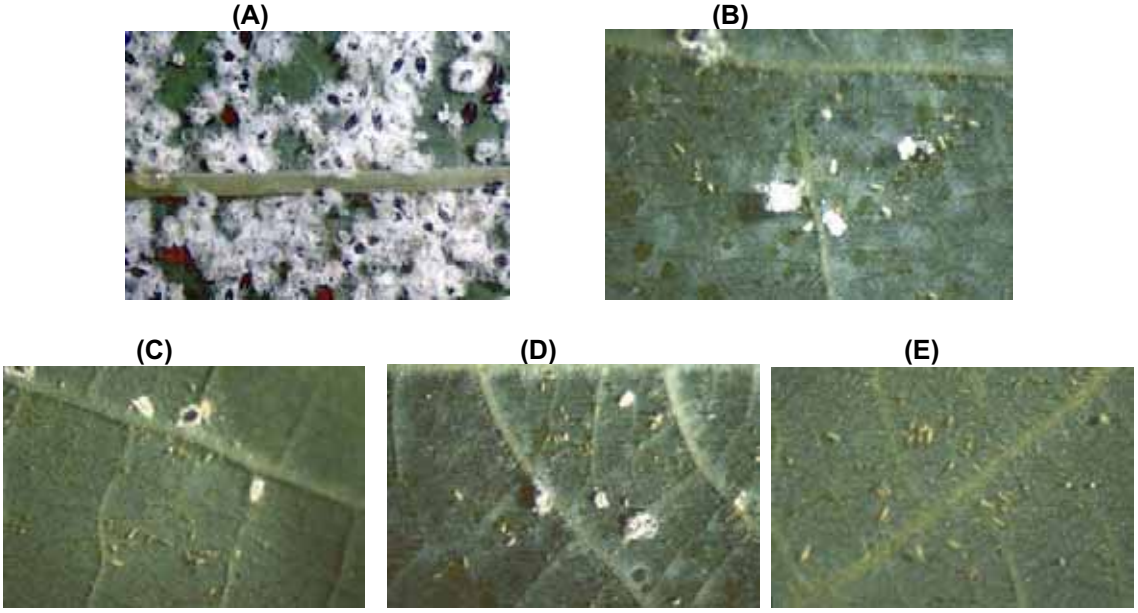


Figure 7. ***Aleurotrachelus socialis*** **nymphal survival on cassava (A) Susceptible control CMC 40; (B) Resistant control, MEcu 72; (C) MPer 273; (D) MPer 334; (E) MEcu 64.**

Morphological measurements of *A. socialis* feeding on resistant and susceptible genotypes show that 2nd and 3rd instar nymphs and pupae were significantly longer on CMC 40 than on the resistant genotypes **(Figure 9)** (P=0.05). The results for width were similar although differences were not always significant. *A. socialis* adult dry weight was significantly lower when feeding on MEcu 64, followed by MPer 334, MPer 273 and MEcu 74 (P=0.05) **(Figure 10)**. All resistant genotypes were significantly lower than the susceptible check, CMC 40, for both the non-preconditioned and preconditioned *A. socialis*.

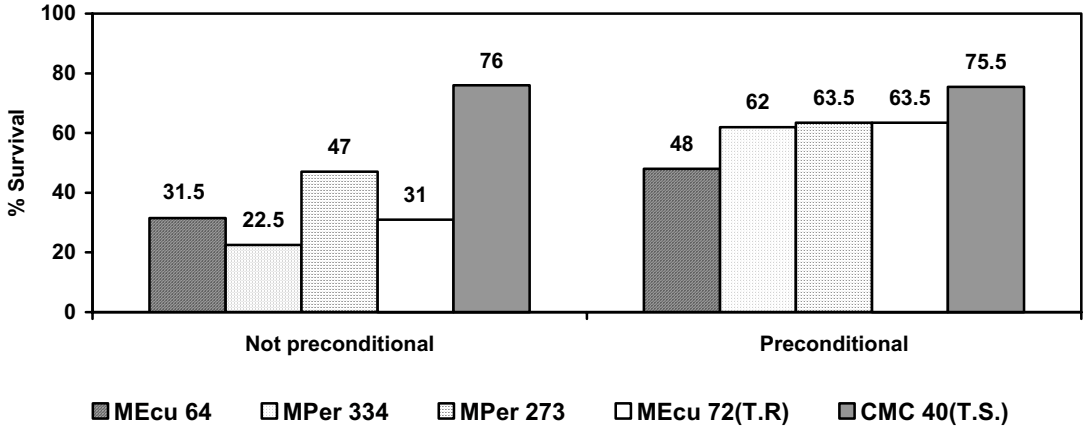


Figure 8. Percent survival of *Aleurotrachelus socialis* feeding on five cassava genotypes (resistant and susceptible) in the growth chamber (28±1°C, 60-70% RH, 12 hrs. light).

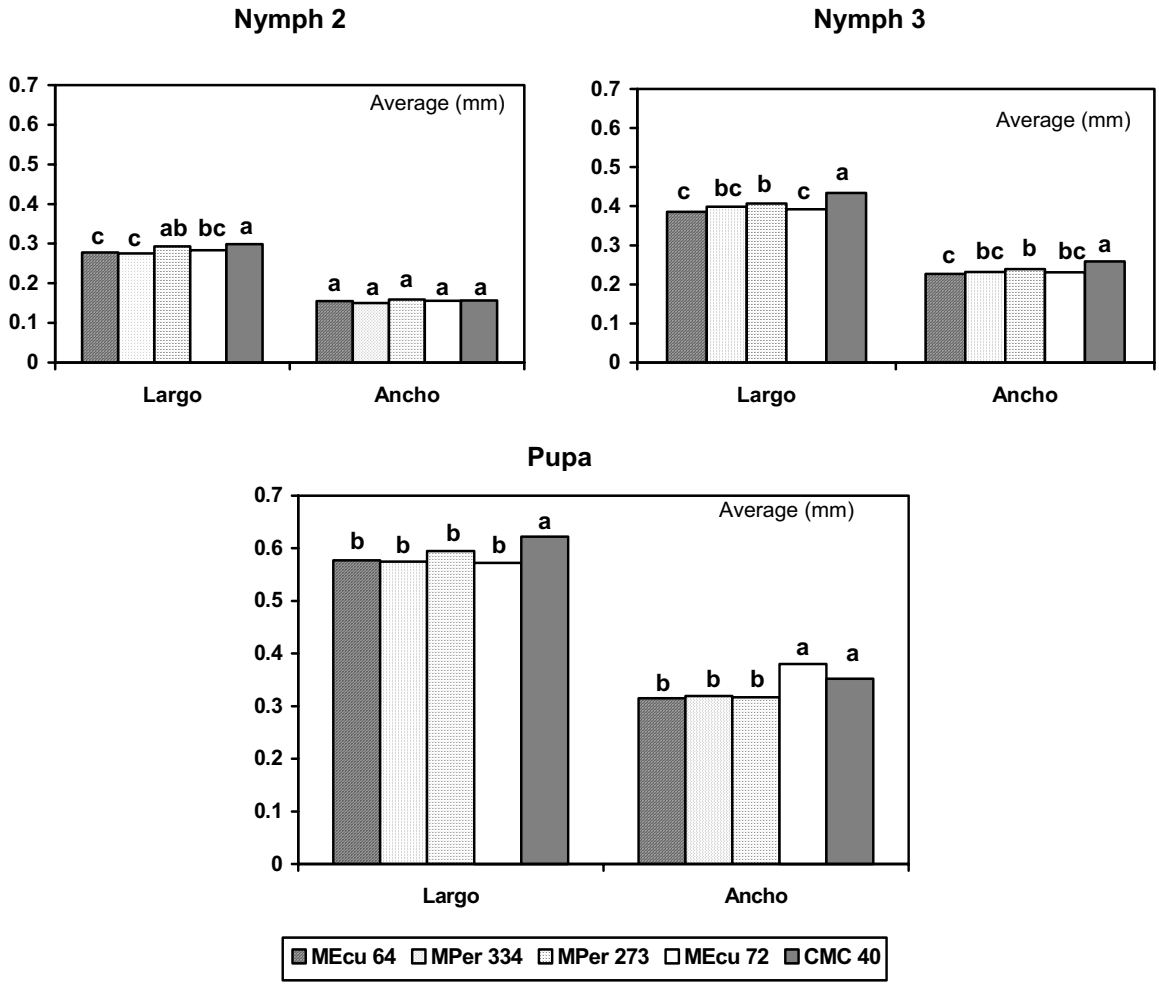


Figure 9. Morphological measurements of *Aleurotrachelus socialis* 2nd and 3rd instar nymphs and pupal stage on five cassava genotypes in the growth chamber.

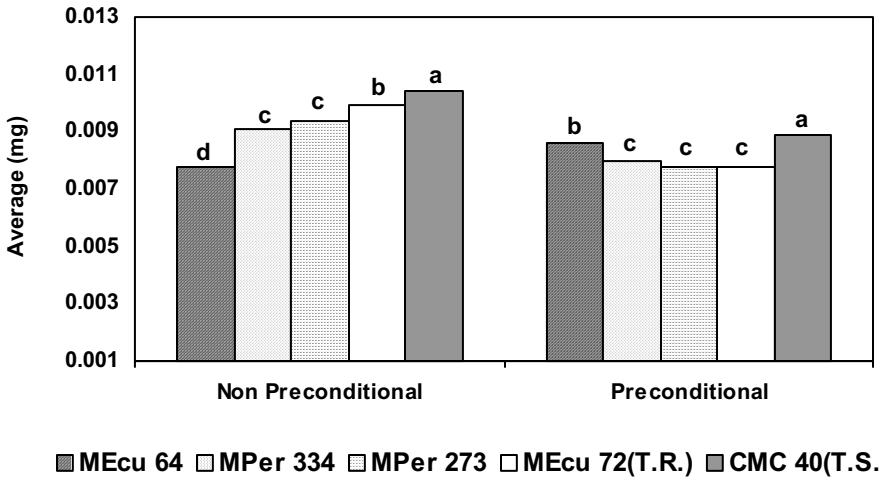


Figure 10. Dry weight of *Aleurotrachelus socialis* adults reared on five cassava genotypes (2 experiments) in the growth chamber.

3. Antixenosis: Free choice feeding preference.
Under a free choice evaluation where *A. socialis* adults were offered five randomly placed genotypes, a significantly higher feeding preference occurred on CMC 40 **(Figure 11)** (P=0.05). There was no significant difference among the remaining resistant genotype, although feeding was lowest on MEcu 64 (the data was logarithmically transformed (X+1)). An interaction was noted between experiment, time (hour) and leaf, where the time of evaluation influenced results on the first leaf, where preference for *A. socialis* feeding was the same at 24 and 48 hours. Leaf one, or the upper most leaf, was the most preferred for feeding **(Figure 12)** in all three experiments, for all genotypes. There was no significant difference in feeding preference on leaves 2 and 3, but in general, feeding activity was higher during the initial 24-hour period **(Figure 12)**.

Oviposition was effected by genotype. Oviposition on MEcu 64 was significantly lower (P=0.05) than on the susceptible check (CMC 40) in all three experiments **(Figure 13)**. In experimental 1, all resistant genotypes were significantly lower than CMC 40; however in experiment 2, only MEcu 64 was significantly lower, and in experiment 3, both MEcu 64 and MEcu 72 were lower **(Figure 13)**. Total oviposition was significantly higher on the upper leaf in all three experiments **(Table 5)**; 75% of the eggs were oviposited on the upper leaf, 15% on the second and 10% on the third leaf.

The combined results for feeding and ovipositional preference and those for mortality and nymphal development indicate that MEcu 64 along with MEcu 72 are the most *A. socialis* resistant genotypes.

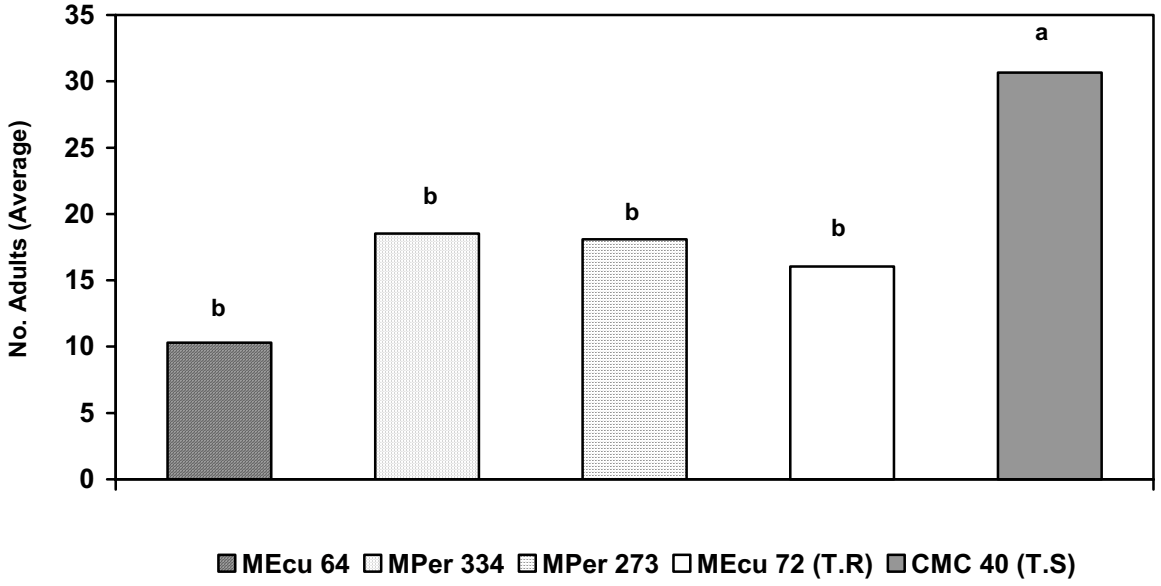


Figure 11. Free choice *Aleurotrachelus socialis* feeding trials on five cassava genotypes (3 leaves per plant and 3 repetitions over a 48hr. period.

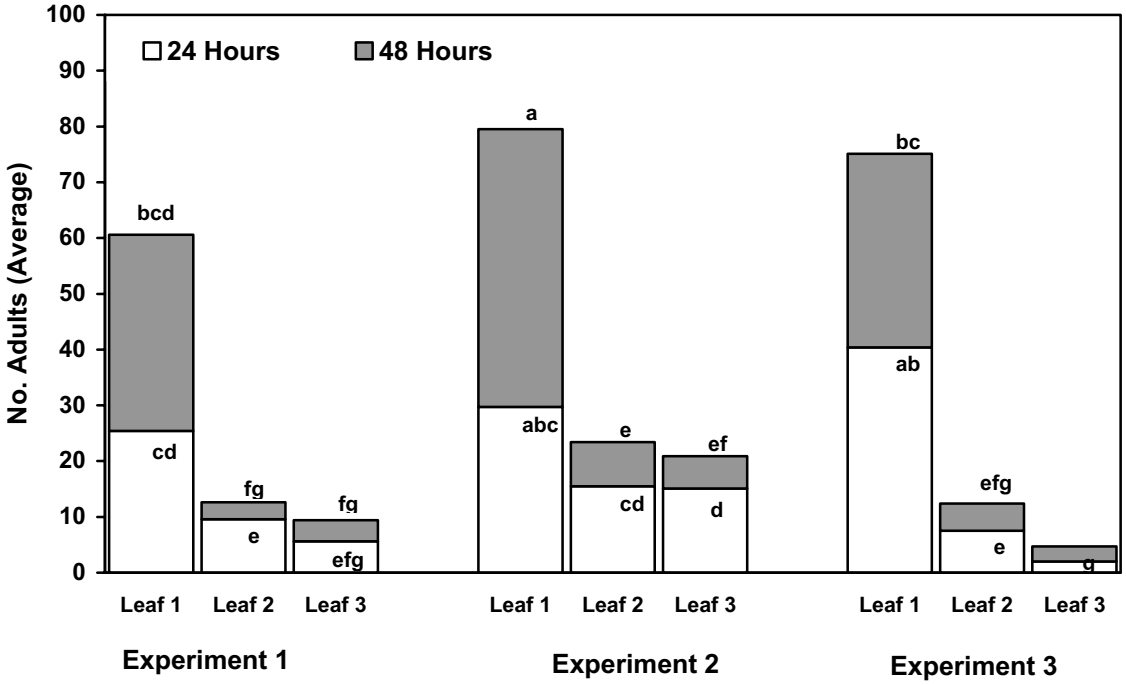


Figure 12. Free choice *Aleurotrachelus socialis* feeding preferred trials on five cassava genotypes on three leaves per plant during a 48-hour period.

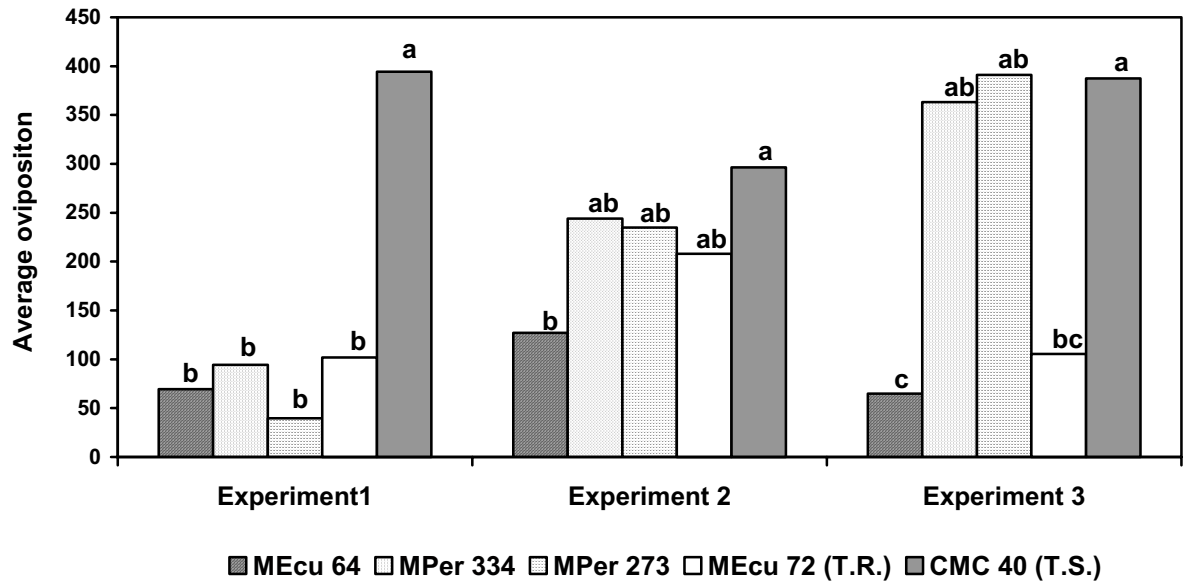


Figure 13. Free choice ovipositional preference of *Aleurotrachelus socialis* on five cassava genotypes (three experiments).

Table 5. *Aleurotrachelus socialis* ovipositional distribution on three cassava leaves of five genotypes in free choice trials.

Leaf Position	Hour	Experimental 1	Experimental 2	Experimental 3
1	48	335.3 a[1]	418.8 a	661.0 a
2	48	50.0 b	135.7 b	93.2 b
3	48	46.6 b	111.7 b	32.9 c

1. Ryan-Einot-Gabriel-Welsch Multiple Range (F) test. Columns with the same letter are not significantly different at the 5% level. Analysis with transformed data. Log (x=1).

Contributors: Miller J. Gómez, A.C. Bellotti.

Collaborators: Myriam C. Duque, Claudia M. Holguín, Bernardo Arias, Diego F. Múnera.

Activity 3. Studies on the biology and development of biotype B of *Bemisia tabaci* on cassava, *Manihot esculenta* and the wild species, *Manihot carthaginensis*.

Whiteflies are major pests of cassava in the Americas, Africa and Asia. Several species are involved; *Aleurotrachelus socialis* predominates in Northern South America (Colombia, Venezuela and Ecuador), while *Aleurothrixus aepim* is the major species in Brazil. *Bemisia tabaci*, a pantropical species prevails in Africa and parts of Asia (i.e. India) where it is the vector of Africa Cassava Mosaic virus (ACMV) and related viruses. Until the early 1990's, *B. tabaci* biotypes found in the neotropics did not feed on cassava, and it has been speculated that the absence of ACMD in the Neotropics may be related to the inability of *B. tabaci* to colonize cassava. Biotype B of *B. tabaci*, has been collected feeding on cassava in the neotropics. However, recent research at CIAT indicates that cassava is not a very successful host (see CIAT Pest and Disease Management Annual Report, 2002, pp. 25-35). *B. tabaci* feeding on beans (*Phaseolus vulgaris*) was successfully transferred to cassava only after completing several generations on other Euphorbiaceae species such as *Euphorbia pulcherrima* (Poinsettia) and *Jatropha gossypiifolia* (Jatropha). However mean longevity, female fecundity, oviposition and adult survival were low when compared to other whitefly species feeding on cassava.

This present study evaluates the potential of *B. tabaci* to adapt to wild *Manihot* species, such as *M. carthaginensis* and compares this to the development of *B. tabaci* on cultivated cassava *Manihot esculenta*, variety MCol 2063.

Methodology: Life table parameters of *B. tabaci* were evaluated in the growth chamber on potted plants of *M. carthaginensis* and the cassava variety MCol 2063. *B. tabaci* longevity, fecundity, development time, survival and demography were calculated. *B. tabaci* populations originated from a colony maintained on *Jatropha gossypiifolia* (Euphorbiaceae), in screened cages (1m x 1m x 1m) for 9 generations (25±5°C, 70±5% RH and 12/12hr. photoperiod). Longevity and fecundity were evaluated by placing 40 pairs (1m x 1f) of recently emerged Biotype B of *B. tabaci* adults in small leaf cages (2.5cm diameter x 2.0cm deep) on test plants. Every 48 hours adults were moved to another leaf area and this was repeated until all (40) females died. When males died, they were replaced until female mortality occurred. Fecundity was estimated by counting eggs oviposited by each female during the 48 hour period; longevity was estimated by the maximal survival of each female.

Development time and survival were studied by placing 50 adults (25 males + 25 females) in the small leaf cages and allowed to feed on the leaf undersurface for 6 hours. Adults were then removed and 200 eggs were selected to evaluate development time from egg to adult and record nymphal survival and sex ratio. Life tables for *B. tabaci* were calculated (Price, 1975) using net reproduction rate (R_o), generation time (T), intrinsic growth rate (r_m) of the population and employing the formula:

$\sum \exp(-r_m x) 1 x m x = 1$

where: x = age

lx = age specific survival

mx = proportion of female progeny from female x

For the calculated values of r_m the corrected age of x + 0.5 were used (Carey, 1993).

Results: The longevity of *B. tabaci* on *M. carthaginensis* and *M. esculenta* (MCol 2063) were similar. It was two days longer on *M. carthaginensis* (12 days) than on *M. esculenta* (10 days) **(Figure 1)**. By the end of 6 days 65% of the females on *M. carthaginensis* and 82.5% of the females on *M. esculenta* had died. The average longevity on the two genotypes differed significantly (Student-Newman-Keuls P<0.05, after K-Wallis P<0.0001).

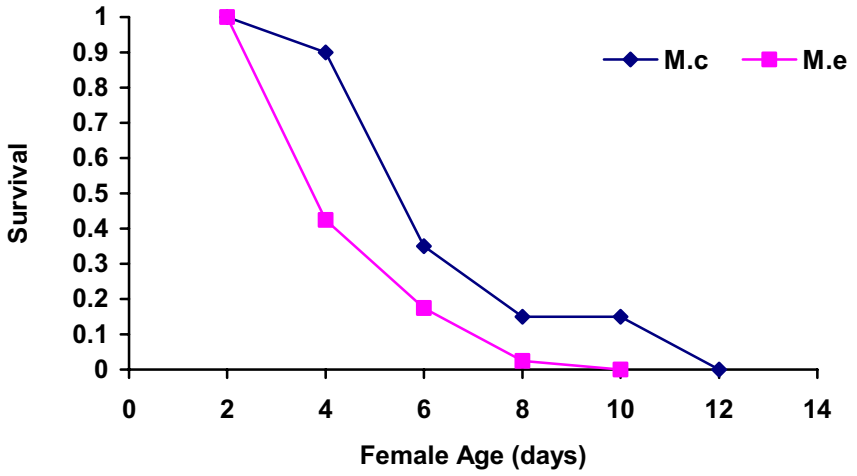


Figure 1. Survival curves of Biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Oviposition occurred readily on both genotypes but the range was greater on *M. esculenta,* although the difference was not significant (K-Wallis P<0.0001, followed by Student-Newman-Keuls P <0.0.5) **(Figure 2, Table 1)**. The mean ovipositional rate was significantly higher on *M. esculenta* (eggs per female/2 days). All females of *B. tabaci* initiated oviposition within 48 hours of eclosion on both genotypes. On *M. esculenta* 72% of the total oviposition occurred during this 48-hour period while only 35.5% occurred on *M. carthaginensis*. These results indicate a preference of *B. tabaci* to oviposit on *M. esculenta.* Highest oviposition on *M. esculenta* occurred on day 2, while on *M. carthaginensis* it was on days 4 to 6.

B. tabaci development time was significantly lower or faster on *M. carthaginensis* than on *M. esculenta* **(Table 2)**. The development time or life cycle on *M. esculenta* was 11 days (44.4 days) longer than on *M. carthaginensis* (33.3), indicating a more rapid adaptation of the immatures when feeding on *M. carthaginensis*. Taking into consideration that fecundity was higher on *M. esculenta* (8.6 eggs vs. 5.3) **(Table 1)** and combines this with the faster development time on *M. carthaginensis*, it results in the intrinsic growth rate (r_m) to be the same for both genotypes **(Table 2)**. These results indicate that populations of Biotype B of *B. tabaci*, in spite of a higher fecundity on *M. esculenta*, will be of equal growth rates on both genotypes.

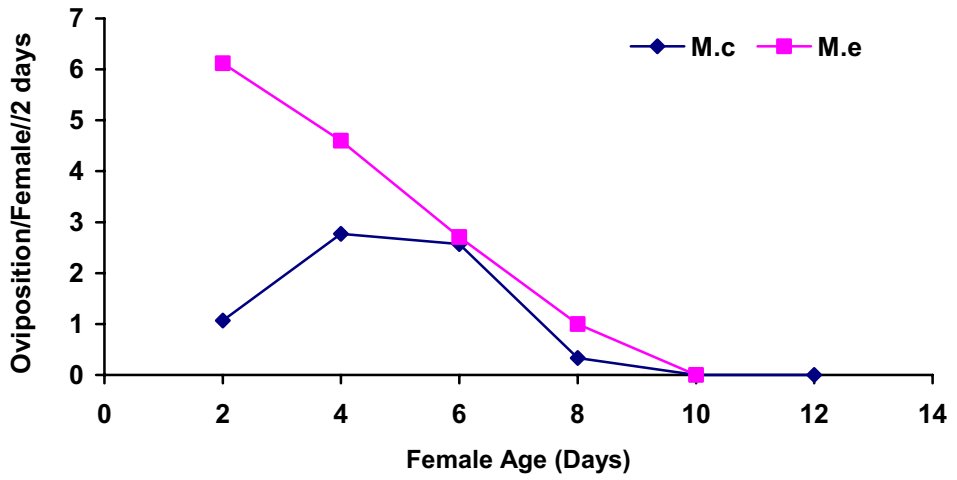


Figure 2. Reproduction curves of Biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Table 1. Average longevity, fecundity and ovipositional rate (eggs/female/2 days) of Biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Parameters	*M. carthaginensis*	*M. esculenta*
Average Longevity	5.1 a	3.25 b
Range	2-12	2-10
No insects	30	40
Average Fecundity	5.35 a	8.6 a
Range	1-35	1-41
Average Oviposition Rate	1.05 a	2.64 b
Range	0.25-3.6	0.5-8

Figures followed by different letters across columns indicate significant differences (Kruskal-Wallis) $P<0.001$, followed by Student-Newman-Keuls $P<0.05$).

Survival rates were significantly higher on *M. carthaginensis* **(Table 2)**. Results show that of 200 eggs oviposited on *M. carthaginensis,* 120 or 60% survived to the adult stage, while only 55 eggs (36%) survived to adulthood on *M. esculenta* **(Figure 3)**. Immature survival is a good indication of the eventual ability of a biotype to develop on a genotype. These results indicate that *M esculenta* (MCol 2063) is not an optimal host for Biotype B of *B. tabaci* **(Figure 3)**.

Significant differences in the net reproductive rate were obtained between the two genotypes. It was estimated that at the end of a generation, populations of Biotype B of *B. tabaci* would multiply 8.6 times on *M. esculenta* (MCol 2063), three times greater than on *M. carthaginensis* **(Table 2)**. This can be explained by total reproduction was less on *M. carthaginensis*. One generation of *B. tabaci* on *M. carthaginensis* is 35.6 days vs. 44.8 on *M. esculenta*. These results indicate that *B. tabaci* can complete 10 generations per year on *M. carthaginensis* and eight on *M. esculenta*. Population growth of *B. tabaci* was the same on both genotypes **(Table 2)**. The difference in development time was a more important criterion for the population increase of *B. tabaci* on *M. carthaginensis*, than were the differences in ovipositional rate. Population increases of *B. tabaci* on *M. esculenta* were more influenced by changes in reproduction rate. It

should be noted that the high rate of oviposition of *B. tabaci* on *M. esculenta* can be independent of subsequent development of the immature stages.

Table 2. Demographic parameters of biotype of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Parameter	*M. carthaginensis*	*M. esculenta*
Development time (d)	33.3 a	44.41 b
Rate of survival (%)	60 a	27.5 b
Proportion of females (%)	50.6	50.9
Intrinsic rate of increase (r_m)	0.048	0.048
Net reproductive rate (Ro) ∑lxmx	5.35	8.63
Generation time (T)	35.6	44.76
Days to duplicate population Ln2/ r_m	14.4	14.4

Development time: different letters across columns indicate significant differences (K-Wallis P<0.0001, followed by Student-Newman-Keuls P<0.05). Rate of survival: (χ^2=29.9, 1df, P< 0.0001).

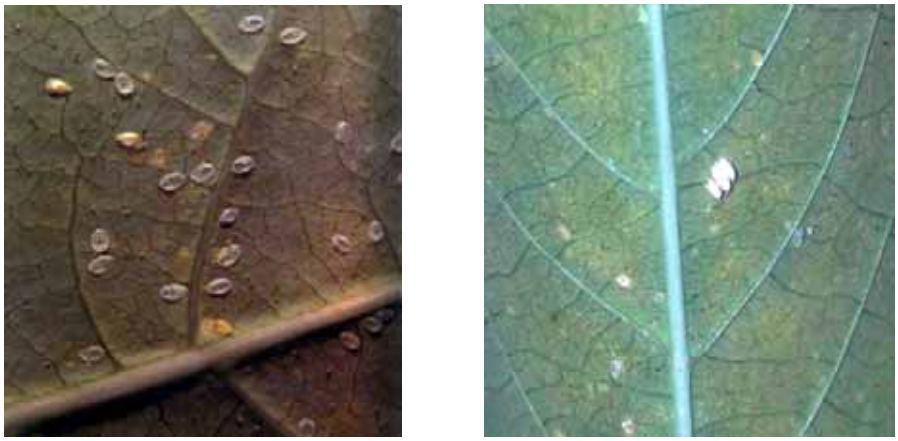


Figure 3. Pupal capsules, pupae and adults of biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

It can be concluded that Biotype B of *B. tabaci* can successfully develop on both *M. esculenta* (MCol 2063) and *M. carthaginensis*. In this case, however, it should be noted that these populations of *B. tabaci* had already adapted to related Ephorbiaceae, Jatropha, prior to being evaluated on the two aforementioned genotypes. Previous research has shown that when the *B. tabaci* populations originate on an unrelated genotype, such as beans (*P. vulgaris)*, they do not readily adapt to *M. esculenta*. These results, however, do provide evidence that biotype B of *B. tabaci* can adapt to Wild *Manihot* species as well as the cultivated species, *M. esculenta* and represents a potential threat to cassava production in the Neotropics.

Contributor: Arturo Carabalí.

Activity 4. Developing integrated pest management components.

Monitoring of the changing situation with whitefly populations in the Andean zone

Introduction

In order to develop appropriate management systems, there is need to monitor the ever-changing situation with species and biotype composition in whitefly-affected areas. This is one of the most important objectives within the DFID-funded project on Sustainable Management of Whiteflies.

Materials and Methods: As in previous years we used RAPD techniques (primer OPA-04) to identify pupae and adults of whiteflies collected throughout the two target areas: the Cauca Valley in Colombia and the Chota Valley in Ecuador. Samples were processed at CIAT. 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.

Results and Discussion: Analysis of 123 samples taken in 23 locations in the Cauca Valley (Colombia) showed that 63% of the whiteflies collected belonged to the B biotype of *Bemisia tabaci*, the most aggressive form of whitefly known to date. This biotype was first recorded in Colombia in 1997. It has very rapidly dispersed to many different areas and is now occupying niches previously reserved to the A biotype or to *T. vaporariorum*. Species composition in the Cauca Valley has changed drastically in the past six 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 can not be found (displaced by the B biotype?), *T. vaporariorum* represents 21% of the samples and the B biotype has become the most important whitefly in the Cauca Valley representing 63% of the samples analyzed **(Figure 1)**. This change has been most dramatic in certain areas like Pradera where *T. vaporariorum* was the key species. Virtually all specimens analyzed in this area belong to the B biotype of *B. tabaci* **(Figure 2)**, which is affecting many different crops.

The now predominant B biotype has caused many serious problems in agricultural areas of the Cauca Valley: uneven ripening of tomatoes, silver leaf disorder on squash, and sooty mold on cotton. 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)**.

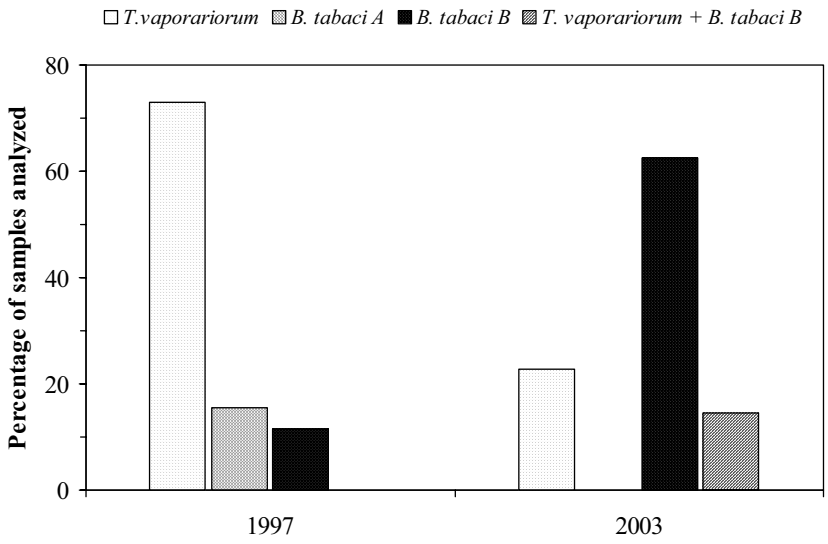


Figure 1. Changes in whitefly species and biotype composition in the Cauca Valley of Colombia (1997-2003).

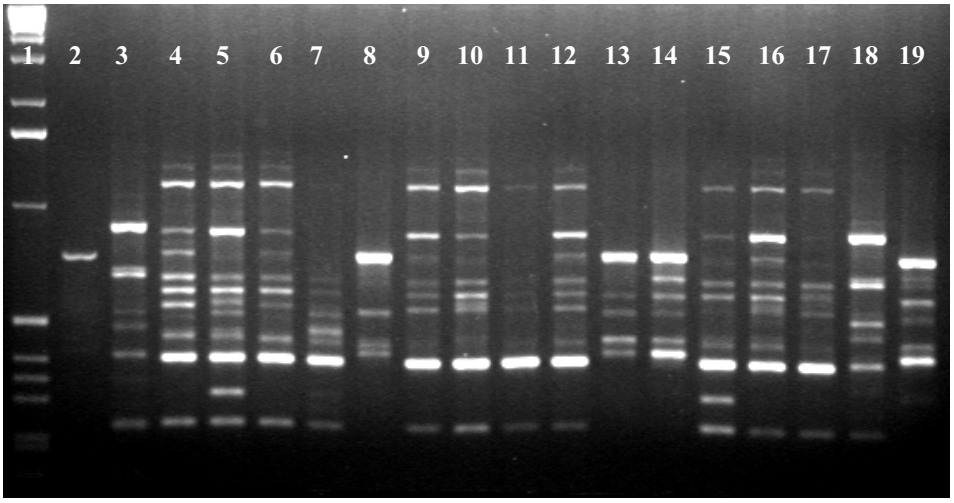


Figure 2. RAPD's for whitefly adults collected in the Cauca Valley (La Tupia, Pradera). Amplificaction using the OPA-04 primer. 1, DNA molecular marker (1 Kb); 2, *Trialeurodes vaporariorum* from reference rearing maintained at CIAT; 3, *Bemisia tabaci* biotype A from reference rearing; 4, *B. tabaci* biotype B from reference rearing; 5 - 7, *B. tabaci* biotype B collected on snap beans; 8, *T. vaporariorum* on snap beans; 9 -12, *B. tabaci* biotype B on squash; 13 - 14, *T. vaporariorum* on tomato; 15 - 16, *B. tabaci* biotype B on tomato; 17, *B. tabaci* biotype B, reference rearing; 18, *B. tabaci* biotype A, reference rearing ; 19, *T. vaporariorum* reference rearing.

Figure 3. "Pod (A) and plant (B) malformation induced by a new whitefly-borne virus in snap beans"

Activity 5. Refining of an action threshold for *Trialeurodes vaporariorum* control.

Introduction

We have successfully developed an action threshold for effective control of *Trialeurodes vaporariorum* on snap beans and dry beans. As shown in previous reports, economic control of the whitefly is achieved when an efficient insecticide is applied when first instar nymphs occur on lower leaves of the plant. To further refine this action threshold and make it user-friendlier, we have added a quantitative estimate of populations.

Materials and Methods: We used the same methodology on snap beans and dry beans. Using a 4 x 4 Latin square design we compared the following treatments: 1, control of first instar nymphs when nymphs occupy less than 30% of the leaf area; 2, control of first instar nymphs when nymphs occupy 30-60% of the leaf area; 3, 1, control of first instar nymphs when nymphs occupy more than 60% of the leaf area; 4, untreated check. Insect populations, damage levels, yields and costs were recorded and analyzed.

Results and Discussion: Highest increases in yield were obtained when chemicals were applied when first instar nymphs appear and occupy less than 30% of the leaf area **(Table 1)**. This means that the action threshold established in previous years can now be enunciated as follows: level 3, when first instar nymphs appear on lower leaves of the plant and occupy less than 30% of the leaf area.

Table 1. Yields responses of snap beans and dry beans to effective chemical control of *Trialeurodes vaporariorum* at varying levels of infestation with first instar nymphs.

Control of First Instar Nymphs	Yields (t/ha)		Yield Increase (%)[a]	
	Snap Beans	Beans	Snap Beans	Beans
When first instars occupy < 30% of leaf area	15.2 a[2]	1.06 a	52.0	14.0
When first instars occupy 30 - 60% of leaf area	11.4 b	1.01 a	14.0	8.6
When first instars occupy > 60% of leaf area	11.2 b	1.02 a	12.0	9.7
Untreated check	10.0 b	0.93 a	----	----
C. V. (%)	11.3	9.6		

[a] With respect to the untreated check

Means within a column followed by the same letter are not significantly different at the 5% level by LSD.

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

Activity 6. Development of sampling methods for whitefly populations.

Introduction

The development of appropriate sampling methods for whitefly nymphs and adults is another major objective within the DFID-funded project on Sustainable Management of Whiteflies in the Tropics. Sampling insect populations to estimate infestation levels is essential in any pest management system. Whiteflies pose special sampling problems compared with larger insects given their gregarious habits, small size, and large numbers.

Materials and Methods: we have conducted six field trials aimed at developing sampling methods for nymphs of *T. vaporariorum* on both snap beans and dry beans. Main objectives were: a) To quantify nymphal and adult populations at different crop growth stages; b) To determine the dispersion pattern of populations within the plant; c) To calculate sample sizes; d) To compare different methods of sampling; e) To develop an efficient sequential sampling plan to be used in an integrated management scheme.

Results and Discussion: We will only highlight a few of the results obtained in snap beans. The work on dry beans will be reported in 2004. As shown in **Table 1**, the spatial distribution of nymphal populations on snap beans follows a natural sequence of events: adult colonization, followed by oviposition, followed by establishment of nymphs on the leaves. This is why adults and eggs are more abundant on the upper part of the plant, while third instars and pupae (fourth instars) are more commonly found on the lower part of the plant (stratum 1). We found that all stages are aggregated. For example, nymph populations follow Taylor's Power Law and adjust very well to a negative binomial distribution **(Table 2)**, meaning that populations are clumped. This has important implications for sampling purposes.

Table 1. Number per leaflet of adults and immature stages of *Trialeurodes vaporariorum* on snap beans. Plants were divided into four strata for sampling purposes.

		Days After Planting									
Whitefly stage	**Stratum**	**7**	**14**	**21**	**28**	**35**	**42**	**50**	**57**	**63**	**70**
Adults	1	18.5	11.3	15.3	15.7b[a]	4.9c	2.7c	8.6c	4.1c	1.1d	0.7d
	2				47.7a	13.8b	3.9b	6.5c	3.5c	1.7c	2.5c
	3					35.1a	11.7a	14.9b	7.2b	4.0b	10.3a
	4						6.5b	67.8a	39.8a	6.2a	5.4b
Egss	1	62.2	46.7	76.9	42.3b	33.1b	0.9c	0.0d	0.04d	0.0c	0.09c
	2				154.3a	63.9a	17.7a	4.0c	1.9c	0.2c	4.3b
	3					36.2b	8.8b	31.7b	29.4b	3.6b	2.3b
	4						5.0c	54.0a	56.3a	19.6a	17.8a
Nymphs	1		5.5	9.4	45.0a	39.8a	51.8a	11.8a	3.4c	0.7c	1.8d
	2				0.01b	6.3b	12.8b	14.9a	10.4b	5.8b	10.6c
	3					0.0b	0.0c	0.3b	18.9a	19.7a	11.9b
	4						0.0c	0.04b	4.3c	16.6a	37.6a

Whitefly stage	Stratum	Days After Planting									
		7	14	21	28	35	42	50	57	63	70
Pupae	1					1.9a	16.1a	36.2a	21.5a	5.9a	5.0a
	2					0.0b	0.0b	0.3b	1.6b	3.9b	10.6a
	3					0.0b	0.0b	0.0b	0.0b	0.9c	10.9a
	4						0.0b	0.0b	0.01b	0.0c	7.0a

[a] Each stage analyzed separately. Means within a column followed by the same letter are not significantly different at the 5% level by LSD.

Table 2. Dispersion indexes for nymphs of *Trialeurodes vaporariorum* on snap beans, calculated by means of regression (Taylor's Power Law) and possible fit to a negative binomial distribution. Snap bean plants were divided into four strata for sampling purposes.

		Regression (Taylor's Power Law)				Negative Binomial Distribution			
Strata	DAP[a]	b	r^2	Pr > F	Interpretation	x^2c	df	Pr > F	Interpretation
1	14	1.70	0.95	**	Clumped	13.6	18	ns	Clumped
1	21	1.52	0.77	**	Clumped	13.9	21	ns	Clumped
1	29	1.60	0.88	**	Clumped	35.6	32	ns	Clumped
1	35	1.65	0.99	**	Clumped	44.5	28	ns	Clumped
2	35					14.4	5	ns	Clumped
1	42	1.12	0.69	**	Clumped				
2	42	0.99	0.86	**	Regular	27.7	18	ns	Clumped
1	50	0.93	0.44	NS	Regular	36.3	25	ns	Clumped
2	50	1.59	0.96	**	Clumped	27.1	25	ns	Clumped
3	50					0.9	2	ns	Clumped
1	57	1.57	0.70	**	Clumped	13.5	12	ns	Clumped
2	57	2.08	0.96	**	Clumped	18.1	20	ns	Clumped
3	57	1.62	0.91	**	Clumped	17.4	16	ns	Clumped
4	57	1.79	0.98	**	Clumped	4.4	6	ns	Clumped
1	63	2.06	0.92	**	Clumped	4.0	4	ns	Clumped
2	63	2.20	0.88	**	Clumped	30.3	19	ns	Clumped
3	63	1.71	0.84	**	Clumped	23.7	31	ns	Clumped
4	63	1.14	0.60	*	Clumped	32.2	24	ns	Clumped
1	70	1.66	0.98	**	Clumped	9.8	7	ns	Clumped
2	70	1.82	0.92	**	Clumped	25.4	18	ns	Clumped
3	70	1.77	0.96	**	Clumped	32.4	21	ns	Clumped
4	70	1.01	0.57	*	Clumped	39.0	36	ns	Clumped

[a] DAP = days after planting.
ns = Non significant.
*,** = Significant at 5 and 1%, respectively.

Data obtained from aggregation studies was then used to calculate sample sizes **(Figure 1)** and to develop sequential sampling methods that will be useful in the implementation of integrated management systems for whiteflies on beans and snap beans.

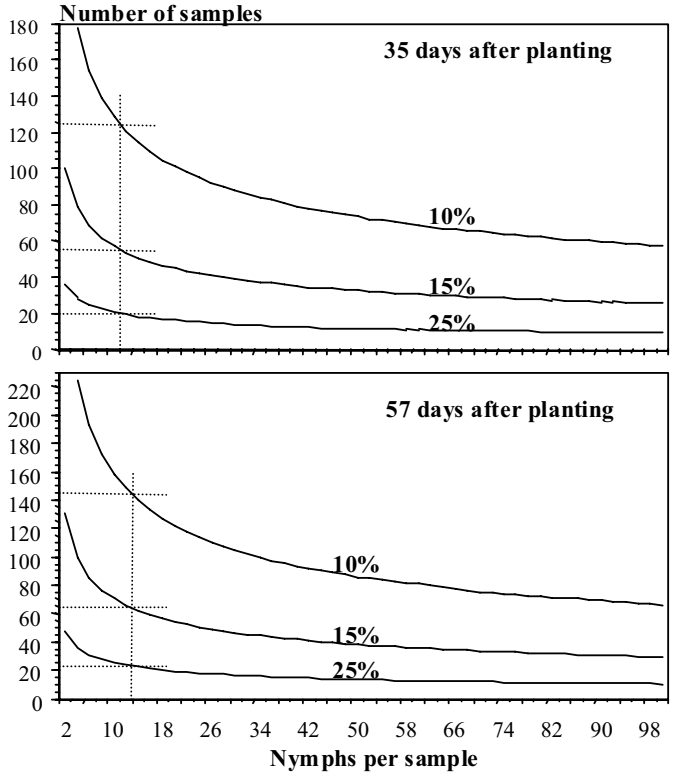


Figure 1. Sample sizes at different precision levels for nymphs of *Trialeurodes vaporariorum* on snap beans.

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

Activity 7. Monitoring of insecticide resistance in whitefly populations.

Introduction

Monitoring of insecticide resistance levels in whitefly populations is one of the major objectives of the DFID-funded Project on Sustainable Management of Whiteflies. Given the fact that insecticides will continue to be an essential method of control of whiteflies, periodic monitoring of resistance becomes an important step in the design of appropriate insect pest management strategies.

Materials and Methods: In 2003 we used the baseline data and diagnostic dosages that were established in 2001. Using the diagnostic dosages for both adults and nymphs, we tested populations of whiteflies in the Cauca Valley in Colombia and in the Chota Valley in Ecuador. 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 2001 Annual Report).

Results and Discussion: Resistance of *T. vaporariorum* adults and nymphs was measured in two critical locations in Colombia and in five locations in Ecuador. In general, the situation has not changed, with populations showing high levels of resistance to organophosphates, some susceptibility to certain pyrethroids, and susceptibility to carbamates like methomyl. With some differences among locations, there is a general trend for conventional insecticides (widely used by farmers) to be less efficient for whitefly control. *T. vaporariorum* nymphs in both Colombia and Ecuador are, in general, still susceptible to insect growth regulators like buprofezin and diaphenthiuron and to novel insecticides like the neonicotinoids imidacloprid and thiamethoxam.

Since the B biotype of *B. tabaci* has suddenly become the key pest in the Cauca Valley, we will highlight results of insecticide resistance monitoring with this species. Adults of this biotype showed high levels of resistance to methamidophos, monocrotophos, carbofuran, and bifenthrin. With some variations, adult populations showed intermediate resistance to carbosulfan and cypermethrin **(Table 1)**. Fortunately, both nymphs and adults of this biotype are still susceptible to neonicotinoids and insect growth regulators **(Table 2)**.

Table 1. Response (percentage corrected mortality) of adults of *Bemisia tabaci* biotype B to conventional insecticides in two consecutive growing seasons. Diagnostic dosages were tested using the insecticide-coated glass vial technique.

Races	Percentage Corrected Mortality[a]			
	2002B	2003B	2002B	2003B
	methamidophos (32 µg/vial)		monocrotophos (300 µg/vial)	
'CIAT'[b]	93.9 a A	94.4 a A	94.8 a A	95.3 a A
Rozo	32.1 b A	18.2 b B	74.7 b A	68.4 b A
Santa Helena	16.8 bc A	17.8 b A	54.4 c A	46.1 c A
La Unión	11.2 c B	22.3 b A	26.1 d A	30.2 d A
	methomyl (2.5 µg/vial)		carbofuran (5 µg/vial)	
'CIAT'	100.0 a A	97.4 a A	96.0 a A	93.9 a A
Rozo	99.4 a A	85.0 ab B	64.8 b A	53.1 b A
Santa Helena	78.1 b A	87.1 b A	24.6 c B	42.1 b A
La Unión	65.1 c A	78.4 b A	21.6 c B	44.0 b A
	carbosulfan (100 µg/vial)		cypermethrin (500µg/vial)	
'CIAT'	94.8 a A	93.4 a A	93.9 a A	93.4 a A
Rozo	88.7 a A	77.5 b B	48.3 c A	39.6 c A
Santa Helena	89.7 a A	70.7 b B	88.7 a A	71.2 b B
La Unión	47.4 b A	53.4 c A	68.5 b A	74.8 b A
	cyalothrin (500 µg/vial)		bifenthrin (5 µg/vial)	
'CIAT'	94.3 a A	91.2 a A	94.3 a A	96.4 a A
Rozo	81.0 b A	60.2 c B	28.8 b A	12.6 d B
Santa Helena	90.6 ab A	78.5 b A	25.2 b A	21.6 c A
La Unión	84.3 b A	84.5 ab A	36.7 b B	49.8 b A

[a] 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 product was analyzed separately.

[b] A susceptible strain of B. tabaci biotype B maintained at CIAT.

Table 2. Response (percentage corrected mortality) of adults and nymphs of the B biotype of *Bemisia tabaci* to novel insecticides in the Cauca Valley region of Colombia. Adults tested in two consecutive seasons. Nymphs tested during the 2003-growing season.

Race	Adults				Nymphs		
	imidacloprid (40 ppm)		thiamethoxam (200 ppm)		buprofezin (16 ppm)	diaphenthiuron (300 ppm)	imidacloprid (300 ppm)
	2002	2003	2002	2003			
Rozo	81.9bB	97.3aA	97.3aA	99.5aA	80.6 b	100.0 a	89.3 b
S. Helena	91.9abA	93.4abA	100.0aA	96.4aB	100.0 a	100.0 a	100.0 a
La Unión	87.4 bA	90.7 bA	97.9aA	91.4bB	100.0 a	98.2 a	100.0 a
'CIAT'	96.3aA	97.9aA	99.5aA	98.9aA	98.4 a	100.0 a	91.1 b

Each product within each semester was analyzed separately. For adults, means within a column followed by a lowercase letter and within a row followed by an uppercase letter are not significantly different at the 5% level by LSD. For nymphs, means within a column followed by the same lowercase letter are not significantly different at the 5% level by LSD.

Contributors: I. Rodríguez, H. Morales, C. Cardona.

Activity 8. Management strategies for whiteflies.

Introduction

Whiteflies have become the object of excessive pesticide use by snap bean and dry bean farmers in the Andean zone. With the body of knowledge acquired in previous years, it is possible to develop whitefly management systems that will at least contribute to reduce pesticide use. We now report on trials conducted to develop ways to reduce pesticide use on snap beans in Colombia and on dry beans in Ecuador

Materials and Methods: Several trials were conducted in the reference sites (Pradera in Colombia, Chota in Ecuador). We compared different approaches for whitefly control based on 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. These treatments were compared with farmers' practices and, in some cases, with untreated checks. In Ecuador, where the project has entered the diffusion stage, large-scale demonstrative plots were used to conduct the trials. Damage levels, insect populations, quality of produce, yields, and benefit/cost ratios were recorded and analyzed.

Results and Discussion: In Colombia (snap beans) alternative management strategies resulted in yields that did not differ from those obtained by farmers with their traditional management approaches **(Table 1)**. Crop appearance, damage (sooty mold) levels, and final produce quality did not differ either. Use of novel systemic insecticides as seed dressing or in drench application resulted in higher benefit cost ratios with a substantial reduction in the amount of applications per crop cycle **(Table 1)**. The use of systemic insecticides in application as drench or seed dressing also had a less detrimental effect on populations of the nymphal parasitoid *Encarsia nigricephalla*, the most important natural enemy of whiteflies in the region **(Figure 1)**.

Table 1. Snap bean yields and benefit/cost ratios obtained with different alternatives for management of the whitefly *Trialeurodes vaporariorum* in the Pradera region of Colombia.

Treatment[a]	No. of applications	Yield (t/ha)[b]	Benefit /cost ratio
Trial 1			
Seed dressing + conventional insecticides 28 and 43 DAP	3	18.4a	3.0
Drench + AT with conventional insecticides	2	17.4a	2.8
AT with conventional insecticides	2	17.5a	3.0
Conventional insecticides at 28 and 43 DAP	2	17.0a	2.9
Farmer's practices	6	13.1b	1.9
Check	0	9.0c	1.7
Trials 2 and 3			
Seed treatment + AT with conventional insecticides	3	13.6a	2.0
Drench + Conventional insecticides at 18 and 42 DAP	2	12.1a	1.7
Farmer's practices	6	12.6a	1.6
Check	0	8.7b	1.4
Trial 4			
Seed treatment + conventional insecticides 34 DAP	3	10.7a	1.7
Seed treatment + AT with conventional insecticides	3	12.7a	2.0
Farmer's practices	7	11.8a	1.6

[a] Seed dressings and drench treatments with neonicotinoids; AT, action threshold; DAP, days after planting

[b] Means within a column followed by the same letter are not significantly different at the 5% level by LSD.

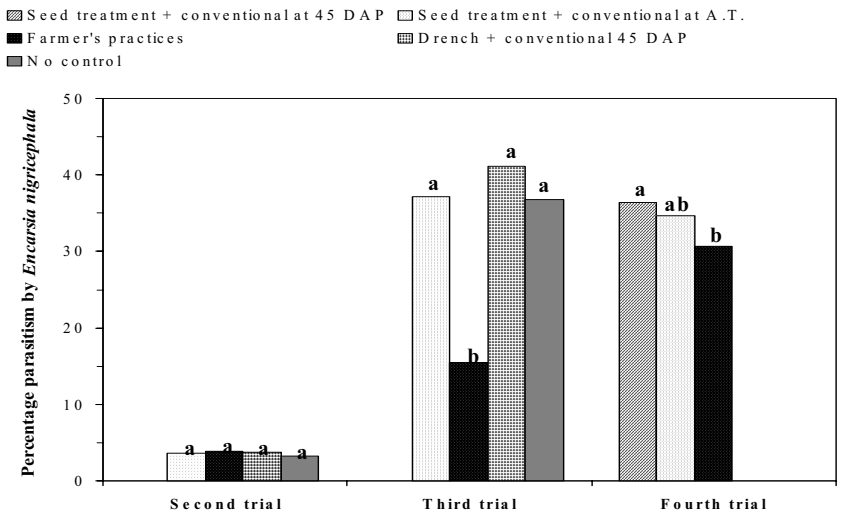


Figure 1. Impact of different whitefly management strategies on parasitism of *Trialeurodes vaporariorum* nymphs by *Encarsia nigricephala*. Bars with the same letter do not differ at the 5% level (LSD).

Trying to find alternatives to insecticides, we tested several entomopathogens. Two commercial formulations of *Verticillium lecanii* and two of *Beauveria bassiana* were completely ineffective against whitefly adults or nymphs.

In the Chota region of Ecuador, two consecutive demonstrative trials showed that integrated management approaches result in high yields of beans of excellent pod quality comparable to those obtained by farmers with traditional (conventional) approaches to whitefly control **(Table 2)**.

Table 2. Whitefly damage levels, quality of produce and dry bean yields obtained in large scale demonstrative trials conducted in the Chota region of Ecuador. Comparison of two management strategies of the greenhouse whitefly, *Trialeurodes vaporariorum.*

Treatment	Damage (sooty mold)[a]		Quality of pods[b]		Yield (kg/ha)
	30 DAP	60 DAP	30 DAP	60 DAP	
Conventional	2.3 a	2.6 a	3.6 a	3.3 a	1327.7 a
MIP	2.0 a	3.3 a	3.6 a	3.3 a	1328.3 a
Coefficient of variation (%).	18.8	36.0	19.2	21.2	17.6

[a] n a 1-5 visual scale (1, no damage; 5, very serious damage.
[b] n a 1-5 visual scale (1, very poor; 5, excellent).
DAP, days after planting.
Means within a column followed by the same letter do not differ at the 5% level of significance by LSD.

Contributors: I. Rodríguez, J. M. Bueno, X. Tapia, C. Cardona.

Activity 9. Screening for virus resistance in snap beans.

Introduction

As indicated above, there is a new situation in the Cauca Valley of Colombia where 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 120 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 55 days after planting for virus symptoms using a 1 - 9 visual scale (1, no apparent damage; 9, severe damage).

Results and Discussion: Twenty-seven genotypes were rated resistant (damage score 1-4). These included well-known sources of resistance to BGMV and eight promising snap beans breeding materials **(Table 1)**. Others were either intermediate (67 genotypes) or susceptible (21). Reconfirmation of resistance is in progress.

Table 1. Response of snap beans and dry beans genotypes to a new virus disease transmitted by *Bemisia tabaci* in the Cauca Valley of Colombia.

Identification	Pedigree and genealogy	Damage score [a]	Rating
BAT 304		1.0	R
DOR 390		1.0	R
Tío Canela		1.0	R
MD 2324 (gene w12)		1.0	R
A 429 (gene bgm 1)		1.0	R
G 35171		1.0	R
G 35172		1.0	R
DOR 364		1.0	R
DOR 476 (w12 and bgm 1)		1.0	R
Porrillo Sintético		1.0	R
RM-35		1.0	R
DICTA 113 (gene w12)		1.0	R
FEB 212 (gene bgm 1)		1.0	R
EAP 9510-77		1.3	R
ASC 75		2.0	R
ICA Pijao		2.7	R
DOR 482 (w12 and bgm 1)		2.7	R
ASIN 13266-20	G 685 x (ASC 73 x ICTA Hunapú)F1/1P-7P-1P-1P-1P-3P-5P	3.0	R
SB 14565-1	HAV 129 x (G17723 x (G 685 x (ASC 73 x ICTA Hunapú)F1)F1)F5/-4P-1P	3.0	R
ASIN 13266-20	G 685 x (ASC 73 x ICTA Hunapú)F1/1P-7P-1P-1P-1P-3P-5P-1P	3.7	R
RMC-35		3.7	R
SB 14565-4	HAV 129 x (G17723 x (G 685 x (ASC 73 x ICTA Hunapú)F1)F1)F5/-1P-2P	3.7	R

Identification	Pedigree and genealogy	Damage score [a]	Rating
ASIN 13266-20	G 685 x (ASC 73 x ICTA Hunapú)F1/1P-7P-1P-1P-1P-3P-4P	3.7	R
Red Kloud		3.7	R
ASIN 13266-20	G 685 x (ASC 73 x ICTA Hunapú)F1/1P-7P-1P-1P1P-3P-5P-4P	4.0	R
SB 14565-3	HAV 129 x (G17723 x (G 685 x (ASC 73 x ICTA Hunapú)F1)F1)F5/-1P-1P	4.0	R
SB 14565-1	HAV 129 x (G17723 x (G 685 x (ASC 73 x ICTA Hunapú)F1)F1)F5/-4P-5P	4.0	R
Top Crop [b]		9.0	S
'Blue Lake' [c]		8.0	S

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

Contributors: J. M. Bueno, M. Castaño, H. Terán, C. Cajiao, F. Morales, S. Beebe, C. Cardona, M. Blair.

Activity 10. Whiteflies as pests and virus vectors in Middle America.

The TWFP has two pilot sites in Middle America, one in El Salvador, Central America (Zapotitan Valley), and a second one in Yucatan, Mexico. The research activities in El Salvador are primarily conducted in collaboration with the national agricultural research program (CENTA); and with the Mexican national program (INIFAP) in Merida, Mexico. The valley of Zapotitan used to be the 'pantry' of the capital city San Salvador, but the emergence of the whitefly problem in this valley put an end to the cultivation of basic food staples, such as beans, and horticultural crops, such as tomato, pepper and various cucurbits. Thus, the objective of the TWFP was to recover the production of these crops in the valley, particularly during the dry months of the year (November-April), when most of the land in the valley is left in fallow even though there is irrigation. The strategy with common bean was to promote the evaluation and release of a new line developed by the Pan American School (Dr. Juan Carlos Rosas) in Honduras. This line (EAP-9510-77) has excellent agronomic characteristics, a high level of geminivirus resistance **(Figure 1)** and, moreover, the commercial color and cooking quality that farmers require. This line has been already registered as a new variety (CENTA-San Andres) and is currently being multiplied for general distribution **(Figure 2)**. The use of this cultivar is expected to reduce pesticide use in more than 70%.

Figure 1. Virus-resistant (background) and golden yellow mosaic-susceptible bean cultivars in El Salvador.

PROMUEVEN VARIEDAD

FRIJOL CENTA San Andrés

"Esta variedad de frijol es buena. Es más tolerante a los virus. El grano y la vaina es gruesa. La vaina carga demasiado... a una mata hasta se le han contado unas 50 vainas o un poquito más. Estamos contentos con esta variedad que nos ha dado el CENTA", testimonió el señor Julio Acevedo, productor de frijol de la zona de Zapotitán.

Un grupo de agricultores escuchan charla sobre el manejo agronómico del cultivo del Frijol CENTA San Andrés, en la parcela del señor José Magaña, Distrito 3, Zapotitán.

El frijol CENTA San Andrés, por sus excelentes características agronómicas se convertirá este año, en el **"caballito de batalla"** de los productores de frijol, ya que la variedad es tolerante a la mosca blanca y ofrece una producción de 33 quintales por manzana.

El frijol CENTA San Andrés, fue introducido al país en 1998, como línea EAP 9510-77, con el apoyo de PROFRIJOL, y está a disposición de los productores desde noviembre del año pasado. La variedad es resistente al virus del mosaico dorado amarillo del frijol, presenta buena tolerancia a altas temperaturas alto potencial de rendimiento, amplia adaptación y excelente calidad comercial de grano.

A través del Convenio entre el CENTA y el Centro Internacional de Agricultura Tropical (CIAT) - Proyecto MIP Mosca Blanca, se inició un Programa que contempla investigación, estudios socioeconómicos y transferencia de tecnología a través de Giras de Observación, a parcelas de productores innovadores, quienes se convierten posteriormente en productores irradiadores de la tecnología.

El convenio considera otros cultivos, como son: el chile dulce, el tomate y el loroco, con el objetivo de que los productores puedan sembrar y cosechar sobre todo en época seca que es la de mayor incidencia de virosis, manifestó el ingeniero José Eduardo Vides, coordinador del Proyecto MIP Mosca Blanca-CIAT.

La semilla de la nueva variedad de frijol CENTA San Andrés, ya está disponible para los productores en la Unidad de Semilla Básica (las 200) en el CENTA, pudiendo comprar desde una bolsa de 50 libras. O pueden obtenerla a través de los productores propietarios de las parcelas visitadas, quienes dejarán la cosecha para semilla.

NotiCENTA

Figure 2. Promotion of the new geminivirus-resistant bean cultivar 'CENTA-San Andres' in El Salvador.

The second most important activity has been the evaluation of physical barriers against insect (virus) vectors, specifically 'micro-tunnels'. These structures are made with simple materials (a synthetic mesh and wire) to protect crops (mainly tomato and peppers) during the first three weeks of vegetative growth, when plants are most susceptible to viruses. The adoption of this simple technology has been outstanding **(Figure 3)** and the economic returns are significant (over $ 8,000 USD/ha in tomato). This technology was first observed in the Yucatan Peninsula, where small-scale farmers adopted it with good results. Unfortunately, the advent of new insecticides effective against whiteflies, and the aggressive marketing strategies of agrochemical companies that have sufficient personnel to reach farmers, resulted in the abandonment of physical barriers in Yucatan. However, the new insecticides are very expensive and do not protect against aphid-borne viruses, which are as abundant as whitefly-transmitted viruses. Farmers have now suffered significant yield losses and, thus, are willing to accept IPM methods, including the use of micro-tunnels and new ferti-irrigation (drip irrigation to apply agrochemicals as well) techniques that facilitate cultural practices for crops grown under cover. The success obtained with these IPM strategies has fostered their adoption by other projects that conduct research in other Central American countries, such as Guatemala, Honduras and Nicaragua.

Figure 3. Micro-tunnels used by small-scale farmers in Central America to control whitefly and aphid-transmitted viruses affecting horticultural crops.

The third activity has been the search for sources of resistance to whitefly-transmitted viruses in horticultural crops, primarily in tomato and pepper. Currently, most of the seed is imported from developed countries, and these varieties do not have resistance against most of the plant viruses found in the Americas. Collaborative research between the Middle American and Southeastern Whitefly subprojects has lead to close collaboration between CIAT and AVRDC to develop tomato varieties possessing resistance to the whitefly-borne viruses present in Central America, Mexico and the Caribbean Region. This year, a total of ten selected tomato lines were evaluated both in Yucatan and the valley of Zapotitan for their reaction to the viruses endemic to these regions. In both localities we were able to select the same tomato genotypes that did not show symptoms of virus infection in the presence of 100% virus-infected controls **(Figure 4)**. These outstanding sources of resistance are now being used as parental materials for crosses with commercial Mexican and Salvadorian tomato genotypes.

The Middle American subproject also includes training and extension activities for national program scientists **(Figure 5)** and farmers **(Figure 5)** under laboratory and field conditions. We expect to enter a third Phase in the TWFP, when the best IPM strategies identified and validated, would be transferred to farmers not only in the Americas, but in other regions of the world.

Figure 4. **Geminivirus-resistant tomato lines (foreground) selected in Middle America to control whitefly-borne viruses.**

Figure 5. **Transfer of IPM technology to agricultural scientists in Central America.**

Publications

Book Chapter

Morales, F.J., Palma, E., Paiz, C., Carrillo, E., Esquivel, I., Guillespie, V., Ordoñez, J.L., and Viana, A. 2003. Socioeconomic and agricultural factors associated with mixed cropping systems in small farms of southwestern Guatemala. Cropping Systems:Trends and Advances. The Haworth Press, Inc., N.Y. 650 p.

Book Chapters-In Press

Morales, F.J. Detection, Diagnosis and Identification of Plant Viruses Based on Physical, Biochemical and Biological Properties. In: Handbook of Plant Virology. The Howarth Press, Inc. N.Y.

Morales, F.J. 2003. Bean Viruses. In: Compendia of Bean Diseases. American Phytopathological Society, St. Paul. MN.

Morales, F.J. 2003-4. Common Bean Viruses. In: Plant Virus Diseases of Major Food Crops in Developing Countries. Kluwer Academic Publishers, The Netherlands.

Refereed Journals

Morales, F.J., Martinez, A.K., y Velasco, A.C. 2003. Nuevos brotes de begomovirus en Colombia. Fitopatologia Colombiana. 26: 75-79.

Morales, F.J., Palma, E., Paiz, C., Carrillo, E., Esquivel, I., Guillespie, V., Ordoñez, J.L., and Viana, A. 2003. Socioeconomic and agricultural factors associated with mixed cropping systems in small farms of southwestern Guatemala. Journal of Crop Production. Vol 9: 649-659.

Morales, F.J., Lozano, I., Arroyave, J.A., Castao, M., Sedano, R., and Velasco, A.C. 2002. Enfermedades virales de la palma de aceite en el suroccidente colombiano y sus agents causales. Fitopatología Colombiana 26: 81-86.

Murcia, N., Osorio, J.A., Caicedo, A., Calvert, L., and Morales, F. 2002. Distribución y caracterización serológica de aislamientos del virus de la tristeza de los cítricos en Colombia. Fitopatologia Colombiana 26: 21-26.

Reichel, H., Perez, R., Martínez, A.K., Sedano, R., Cuervo, M., Arroyave, J.A., and Morales, F.J. 2002. Caracterización parcial de un potexvirus aislado de pitahaya amarilla (*Selenicereus megalanthus*) afectada por mosaico en Colombia. Fitopatología Colombiana 26: 73-74.

In Press

Morales, F.J., and Jones, P. 2003. The ecology and epidemiology of whitefly-transmitted viruses in Latin America. Virus Research.