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Project 7: Integrated Cassava Crop Management in Major Agroecosystems of Latin America and Asia

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PROJECT 7: INTEGRATED CASSAVA CROP MANAGEMENT IN MAJOR AGROECOSYSTEMS OF LATIN AMERICA AND ASIA

1. Introduction

The purpose of an integrated pest and crop management project in cassava is to promote sustainable cassava production in major agroecosystems of Latin America and Asia by developing principals of and component technologies for integrated crop management. Many of these technologies and methodologies are also being employed in Africa through close collaboration with researchers at IITA. The origin of *Manihot esculenta* is the neotropics; CIAT, therefore has the global responsibility to identify, collect, evaluate, characterize and assess the biotic biodiversity associated with the crop and make it available to our collaborator around the world.

The cassava growth cycle varies from 7 to 24 months, depending on the ecosystem. Agrochemicals are seldom used for controlling pests and diseases or for maintaining soil fertility. To realize cassava's true production potential, adapted germplasm must be used, but integrated with cultural and biological management practices that enhance the crop's biotic and abiotic environment, thus reducing erosion, maintaining soil fertility, and avoiding use of pesticides.

Crop management projects in collaboration with selected national program in Latin America and Asia are showing that small-scale farmers will obtain higher productivity, lower costs, and a safer working environment. National programs are benefiting from research conducted at representative sites in several ecosystems; from close collaboration with regional, national, and international partners; from new research techniques of participatory diagnosis, validation adaptation, and on-farm testing of generated technologies to ensure adoption and thus more sustainable production; and from feedback to ensure orientation of research toward users' needs.

2. Summary of main achievements of whole project

RESEARCH HIGHLIGHTS

Integrated Pest Management in Major Agroecosystems of Latin America.

Entomology

1. Cassava mulched with lemon grass and vetives grass reduced the level of damage caused by the cassava burrowing bug (*C. bergi*), without reducing cassava root yield.

2. Soil moisture was found to have an effect on the behavior of *C. bergi*; higher soil moisture is preferred and sought, and drier condition will induce emigration.

3. Based on soil moisture, air humidity and temperature studies, and in collaboration with the CIAT GIS section, a model for *C. bergi* climates was calculated and mapped to predict potential areas of pest problems.

4. Research on entomopathogens identified three strains (9206, 9236, and 9501) of the fungus *Metarhizium anisopliae* as highly pathogenic to *C. bergi*.

5. The release of the cassava mealybug parasitoids, *Apoanagyrus diversicornis, Acerophagus coccois* and *Aenasius vexans* into mealybug infested cassava areas of Brazil and Colombia provides excellent control of this pest.

6. The parasitoid species *A. vexans* and *A. diversicornis* respond positively to mealybug damaged cassava plants indicating a chemically mediated searching behavior.

7. Surveys in 3 ecological regions of Colombia have identified 10 parasitoid species of cassava whiteflies; 7 are newly recorded species for Colombia, and 4 are undescribed species.

Pathology

1. A disease survey in 34 farms of four Colombian departments revealed that root and stem rots caused by *Phytophthora* spp. are a major problem in cassava. The impact of root rots on cassava yield of cultivar MBRA 12 was analyzed. Average root yield reduction by root rots was calculated 7.1 T/ha per growing cycle for the period 1981-1995 of 60 field experiments performed at CIAT- Palmira.

2. Isolation of *Phytophthora* spp. from cassava roots and stem was accomplished by new techniques (baiting and direct plating on selective media). For the first time a collection of 75 strains was obtained from cassava fields at Colombia.

3. A new methodology to screen germplasm for resistance to *Phytophthora nicotianae*, *P. drechsleri* and *P. erythroseptica* was identified. Tolerant cultivars to root and stem rot diseases were selected.

4. A novel polymerase chain reaction (PCR) method was developed and standardized to characterize cassava rot pathogens. This unique method has been adopted by CNPMF and EMBRAPA scientists in Brazil.

5. An effective thermotherapy method was designed to eradicate *Phytophthora* species in cassava stem cuttings. In collaboration with the National University Palmira a study was initiated to induce enzyme production by which stakes resist relatively high temperatures.

6. Extracellular production of antifungal compounds by selected biocontrol strains of *Trichoderma* spp. inhibited *Diplodia manihotis* and *Fusarium oxysporum*. Positive correlation was determined between in vitro and greenhouse results. The incubation time of *Trichoderma* spp. in liquid media was successfully reduced from one week to two days obtaining an inoculum concentration of 10^6 conidia/ml.

Virology

1. A Ugandan geminivirus that cause of African cassava mosaic disease was found to be distinct from either African cassava mosaic virus (ACMV) or East Africa cassava mosaic virus (EACMV), and it may actually be a hybrid of these viruses. The Ugandan geminivirus is causing an extremely severe disease and is a major factor in the epidemic causing very large losses of production.

2. The isolates of cassava vein mosaic virus were collected from six states in Brazil. All the isolates were able to be detected using polymerase chain reaction (PCR) amplification. The detection method functions even when visible symptoms are not present.

3. One region at the terminus of the coat protein was found to be more variable than other regions and is the focus of the biodiversity of CVMV.

4. An microscopic analysis of the roots of frogskin disease affected cassava revealed very different structural feature that are being evaluated as method to select FSD tolerant cassava.

5. PCR markers that are differentially amplified in health versus disease plants are being tested as diagnostic probes for the rapid detection of FSD.

(Acarology)

1. The predatory mite, *Typhlodromalus aripo*, is established and spreading at an average rate of 100 km per year in West Africa covering over 150,000 km^2. It is reducing cassava green mite populations dramatically and increasing root yields by at least 30%, which translates to an estimated yearly benefit of US $60 million for just 4 countries in West Africa.

2. Predatory mites collected by CIAT are continuing to be released by CNPMF/EMBRAPA in Northeast Brazil, and two species appear to be established.

3. Over 10,990 tetranychid mite specimens were reviewed to determine the range of host species and geographic distribution of the mite pathogen *Neozygites*.

4. Eleven major documents (2,800 pages) on cassava research were scanned and formatted for publication in a CD-ROM in collaboration with IITA and the University of Florida.

5. A computer model was developed to optimize mass rearing of predatory mites and has undergone initial validation with experimental data.

6. A taxonomic key to 30 species of phytoseiid mites was developed in collaboration with Gilberto de Moraes, CNPMA/EMBRAPA.

7. Explorations for natural enemies of cassava green mite were conducted in Ecuador and Colombia, and strains of phytoseiids and *Neozygites* were sent to CNPMF in Brazil, IITA in Benin, University of Amsterdam in the Netherlands, the Boyce Thompson Institute in New York, and the Swiss Federal Res. Station for Agronomy in Zurich.

8. Suitability of a wide range of prey and foods were evaluated for 8 species of phytoseiids.

Integrated Crop Management in Major Agroecosystems in Latin America and Asia.

Physiology

I. Germplasm characterization for nutrient use efficiency in different plant types, tolerance to low-K soils and tolerance to acidity:

1. Medium and short cassava genotypes were found to be more efficient (14 to 40% increase) in nutrient use as compared to tall cassava genotypes - These genotypes are advantageous for sustaining productivity in poor soils.

2. Several elite cassava lines were screened for tolerance to low-K soils and to acidity. The most tolerant lines to low-K were CM 4365-3, M Col 1505 and CM 5460-5. The most tolerant lines to acidity were CM 6740-7, CG 333-4 and CG 1141-1. The CG 1141-1 was recently released in northern Colombia under the name "Costeña".

II. Crop/soil management in infertile acid soils and in sandy soils in Colombia.

1. Long-term (13-Y) NPK trial confirmed that the most limiting nutrient in acid Inceptisols to cassava productivity is potassium (> 200% response to K), followed by nitrogen (≈ 35% response to N) and phosphorus (≈ 20% response to P).

2. Long-term (8-Y) responses to surface plant mulch and NPK in poor sandy soils in northern Colombia, confirmed that sustained cassava yield could be achieved by either application of moderate levels of NPK or by application of plant mulch.

3. Leaf photosynthesis of cassava is not affected by the quality of planting material, but moderately reduced by prolonged water stress.

Crop Management

1. A comprehensive database was compiled on the productivity and labour requirements of important barrier grasses under farmer field conditions.

2. Maize cropping systems were tested on erosion/runoff plots in long term management treatments in its 8th year. An excellent balance between

conservation and productivity was obtained with minimum tillage and ley-farming practices.

3. Interinstitutional collaboration for further development testing and diffusion of conservation technology with NGO's was consolidated in 1996.

Crop/Soil Management: Asia

1. On-farm trials in several Asian countries showed that erosion losses were significantly reduced and yields increased when cassava was planted in the early dry season; in addition planting cassava at closer spacing, on contour ridges, and with adequate fertilization, was the best package of practices to reduce erosion and obtain higher yields.

2. Long term NPR trials in three countries show the importance of potassium fertilization for long-term soil fertility maintenance.

3. Farmer participatory research projects to develop locally adapted soil conservation practices and enhance their adoption by farmer are being conducted in Thailand, Indonesia, Vietnam, China, and the Philippines.

3. Subproject 1. Purpose: ***To develop improved pest and disease management components and implementations strategy for cassava production system in Latin America and Africa.***

3.1 Output 1.1 Identification and quantification of major arthropod complexes in selected agroecosystems; development of control measures.

3.1.1 Mealybug (*Phenacoccus herreni*)

The cassava mealybug *P. herreni* causes severe crop losses, especially in N.E. Brazil where in certain areas farmers have abandoned cassava production due to mealybug attacks. As part of a UNDP funded project; in collaboration with EMBRAPA, three parasitoids from Colombia and Venezuela have been introduced and releases into the Brazilian States of Bahia, Pernambuco and Ceara. All three parasitoids, *Aenasius vexans, Apoanagyrus diversicornis* and *Acerophagus coccois* have become established and two of the species *A. coccois* and *A. diversicornis* are dispersing rapidly. Mealybug control has been so effective due to these parasitoids that cassava production has returned to some of the areas where it had been abandoned. The actual data an impact assessment on these studies will be included in the final project document report to UNDP. In addition journal publications are being prepared.

In the Llanos of Colombia and Venezuela cassava is attacked by *P. herreni*, causing crop losses. Studies to evaluate the effectiveness of biological control were conducted in Villavicencio in collaboration with CORPOICA/La Libertad. This research formed part of a MS thesis requirement for a Colombian student from Georg-August University, Gottingen, Germany.

Field experiments were initiated by artificially infesting cassava plants with *P. herreni* to insure adequate mealybug populations. Three parasitoid species, *A. vexans, A. diversicornis* and *A. coccois* were subsequently released into 3 cassava fields; one parasitoid species per field; in a fourth field all 3 species were released. In control plots cassava growing points were protected from parasitoids with a gauze sleeve. The percentage of parasitism was obtained by dissecting 1887 2nd, 3rd and 4th instar mealybugs.

The study resulted in parasitism rate of 15.5% and a level of encapsulation of 37.6%. In this experiment low CM densities produced higher percentages of encapsulation, while a higher CM population caused lower percentages. The parasitoid *A. vexans* had the highest percentage of parasitism. In addition parasitism rates were higher in the field where all three species were released.

Chemical Mediated Searching Behavior of Parasitoids

Plants when attacked by insects often release volatiles that can attract natural enemies. Previous results from this on-going research have indicated that two parasitoid species *A. vexans* and *A. diversicornis* respond significantly to infested plants while *A.. coccois* was not attracted to infested plants over healthy ones, though it was attracted by cassava plants. More recent research using Y-tube experiments have tried to determine the cause of the insect attraction, plant synomones released by infested cassava plants, or odours emitted by the mealybug and its by-products. The following choices were offered to *A. vexans* and *A. diversicornis*; infested vs. washed infested leaves (Cl); Blank vs. Cl; and healthy vs. Cl. To obtain clean leaves (Cl), mealybugs and exuviae were removed from two infested cassava leaves and leaves were thoroughly washed with wet cotton to remove honey dew and fungi. For the mb treatment, all products originated by the mealybug presence were removed from two infested cassava leaves. Mealybugs and exuviae were deposited on wet cotton, and honeydew and fungi were collected.

Initial results indicate that both parasitoid species are attracted to the infested leaves (Cl) over the mealybug (Mb). A significantly higher proportion of female parasitoid of both species chose the infested but cleaned leaves over the blank (no leaf) or the mealybug and its exudates. There was no significant differences in the Cl vs. healthy leaves or the Cl vs. infested leaves, indicating that plant volatiles from mealybug damaged leaves attract the parasitoids.

Field experiments, to determine if a bean intercrop will have an influence on the capacity of two parasitoids *A. vexans* and *A. diversicornis*, to parasitize *P. herreni* on cassava, were initiated during 1996. Three treatments were tested, plots with only cassava plants, plots with mixed cassava and beans, and plots with only bean plants. Eight potted cassava plants infested with second and third instar mealybugs, were placed in each plot and 20 parasitoid females of one species were released. After five days plants were brought into the greenhouse and parasitism determined.

Initial results show that the parasitism rate of *A. vexans* is higher than *A. diversicornis*, but the difference may not be significant. In addition *A. vexans* appears to have a better searching capacity in pure cassava stands, while *A. diversicornis* appears to prefer the bean culture, but more data is needed to verify these results.

3.1.2 Whiteflies

Aleurotrachellus socialis, *Bemisia tuberculata* and *Trialeourodes variabilis* are the most common species of whiteflies attacking cassava in Colombia. Previous surveys show that *A. socialis* is the predominant species, and can cause severe cassava yield reductions when high populations occur. For the past two years, as part of the UNDP funded cassava IPM project and as a prelude to a major DANIDA funded whitefly IPM project, considerable survey work has been undertaken to more accurately define the pest distribution and its associated natural enemy complex.

Results from these surveys (the 3 zones extensively surveyed, the Andean zone, the Llanos, and the North Coast are describe in the 1995 Annual Report) show that *A. socialis* and *B. tuberculata* have the widest distribution and are found in all areas surveyed. They are usually found in lower altitudes, sharing the same climatic conditions and altitudes most favorable for the cassava crop. *T. variabilis* was not observed in the Savannahs. High altitudes above 1000 meters such as the Andean Region favored this species. Neither temperature nor relative humidity were factors related to populations of the three species. *A. socialis* was found mostly in those sites with temperatures above 35°C.

Numerous microhymenopteran parasitoid natural enemies found associated with each species of whitefly were collected and identified; several were new species recorded from Colombia and others were unrecorded species.

The highest level of parasitism was found on *A. socialis* in the Llanos at 15.3% of the pupae parasitized. The highest levels of parasitism for *T. variabilis* and *B. tuberculata* were in the Andean Region with 12.1% and 13.9% respectively.

Ten species of parasitoids were recovered from the three whitefly species (Table 1). Seven of these were recorded for the first time in Colombia, and four of these are new, undescribed species, recorded for the first time to science. *Encarsia hispida* (Aphelinidae) was the predominant parasite with 64.2% of the total parasites collected. Three species of the genus *Eretmocerus* are new, undescribed species (referred to as *Eretmocerus* sp "a", "b" and "c"). *Eretmocerus* sp. "b" was the second most numerous parasite collected with 16.9% of the individuals.

Two species *E. hispida* and *Eretmocerus* sp "b" were the most widely distributed throughout the three regions surveyed. They were the most abundant species in the Llanos with 79.3% and 69.1% respectively; in the Andean Zone they represented 20% and 24% respectively.

Some whitefly vs. parasitoid specificity was also noted. *A. spiniferus* was only obtained from *A. socialis*; *E. hispida* and Eretmocerus sp "b" were only collected from *A. socialis* in the Atlantic Coast, and the Llanos and highly preferred *A. socialis* in the Andean Zone. Parasitoid species obtained from *B. tuberculata* were rare in all three regions; *Eretmocerus* sp "c" was the most frequent species found on *B. tuberculata* in the Andean Zone. The other new species *E. bellotti* was found parasitizing *A. socialis* and *T. variabilis* in the Andean region. Research on the behavior of these parasitoids has been initiated.

These results further indicate the great potential for arthropod biodiversity that exists in agroecosystems, that is yet undiscovered and need to be further explored.

Table 1. Natural enemies associated with whiteflies on cassava from surveys of three ecological zones (Andean, Savannahs and Atlantic Coast) of Colombia.

Parasite Species	N	%
Aphelinidae (Fam.)		
Encarsia hispida	1845	64.2
Encarsia pergandiella	26	0.9
Encarsia bellottii	13	0.5
Encarsia sp.	48	1.7
Eretmocerus sp. "a"	30	1.0
Eretmocerus sp. "b"	485	16.9
Eretmocerus sp. "c"	12	0.4
Eretmocerus sp.	178	6.2
Platygasteridae (Fam.)		
Amitus spiniferus	159	5.5
Amitus sp.	79	2.7
Total	2875	
Depredators		
Coccinellidae (Fam.)		
Delphastus sp.	143	

3.1.3 The Burrowing Bug: *Cyrtomenus bergi*

The burrowing bug, *C. bergi* is a multihost pest that can cause severe cassava losses in affected areas. Additional host include maize, onion, commercial peanut (*Arachis hypogea*), wild peanut (*A. pintoi*) and sorghum. More recently considerable damage has been reported on asparagus, especially by growers in the Dept. of Cauca, Colombia. Laboratory colonies are efficiently maintained on germinating maize kernels. Present research concentrates on the relationship between soil moisture, relative humidity and pest population dynamics. Research on control of has emphasized cultural practices and biological control, especially the use of entomopathogens and entomopathogenic nematodes. In addition actual and potential distribution maps based on edafic and climatic relationships are being developed.

Mulch

The effect of mulch on damage level on cassava caused by the burrower bug *Cyrtomenus bergi* was repeated this year, as no evident conclusion could be drawn from the results of the previous year. This year, the experiment improved as the more susceptible variety MCol 2066 was used, which created at higher damage level in general. This year's data also showed an improved symmetry compared to the previous year (Univariate Procedure, SAS).

The treatments consisted of mulch of Lemon grass [*Cymbopogon nardus* (DC. Ex Ness) Stapf], Vetiver grass [*Vetiveria zizanioides* (L.) Nash] and an unmulched control. Each of three replicates consisted of five by five cassava plants of which the nine centre plants were assessed. Damage level of each roots was assessed using a 0-6 scale and data on yield were collected. A Kruskal-Wallis test was applied for the analysis.

Mulch had a significant decreasing effect on damage ($P<0.0001$) and no difference was found between the two types of mulch. No difference was found in yield either, however, this might be due to a very high variation, see table 1 below.

Table 2. Damage level, percent root damage and yield of cassava (var. MCol 2066) in treatments of mulch Lemon grass (*Cymbopogon nardus*), Vetiver grass (*Vetiveria zizanioides*) and an unmulched control.

Mulch	Damage Level* (0-6 scale)	% Root Damage (≥3 on the Damage Scale)	Yield* (t/ha)
Control	3.45 ± 0.15	51.50%	17.2 ± 8.9
Lemon grass	2.47 ± 0.14	30.39%	24.1 ± 13.8
Vetiver grass	2.19 ± 0.14	26.05%	20.8 ± 3.8

*Values are mean ± standard errors

As seen in Table 2, the effect of mulch on the decrease in damage is evident, but low. Hence, the effect of mulch in the reduction of damage caused by *C. bergi* is limited and its use should only be recommend if other benefits from mulching are to be gained as well.

Most probably mulch decimates reduces the population of *C. bergi*, but its function as such is to be considered. Mulch was previously thought to favour *C. bergi* as mulch is known to decrease maximum soil temperature and increase soil moisture content. Now we speculate that mulch might act as a physical barrier which resist *C. bergi* from landing and descend into the soil. Another speculation is that mulch might favour the environment for entomopathogens controlling *C. bergi*.

Soil moisture and relative air humidity

C. bergi thrives under moist condition and drought can cause the population to go extinct. The development rate of juvenile instars was found at its highest at soil moisture levels between field capacity and water saturation, (see Figure 1). Soil water content lower than wilting point impedes the development and in dusty soils the population decreases dramatically.

Reproduction is even more significantly influenced by soil moisture levels, (Figure 2). The maximum reproduction occurred while insects were kept at soil moisture levels between field capacity and water saturation. Water saturation impedes the reproduction significantly, but any soil water content lower than wilting point killed off the imago within 3-5 weeks without oviposition.

C. bergi has shown attraction to moist soil when placed in a horizontal soil water gradient. The movement behaviour of the insect was therefor observed in vertical soil moisture gradients simulating dry season (dry soil on top and moist soil at the bottom), rain season (moist soil on top and dry soil at the bottom) and a control of intermediate soil water content throughout the vertical soil tube. The insects were placed at three different release points in the tubes with three replicates of each and their position was registered after 20-24 hours. The set up was repeated 14 times, seven times in the morning (8:00-9:30 am) and seven times in the afternoon (4:40-6:00 pm).

The different release points did not significantly influence the final position of the insects. *C. bergi* showed a hereditary characteristic of geotaxy, (Figure 3). When soil conditions are moist at the soil surface and few centimeters below, *C. bergi* will descend until unfavourable conditions are met, e.g. unfavourably low soil moisture, compact soil texture and others. Nevertheless, under dry conditions at the soil surface, *C. bergi* changes behaviour despite the fact that soil water conditions are favourable in deeper soil layers. The threatening conditions of drought at soil surface, which are unfavourable to host plants as well induce emigration. Half of the population was found to ascend under such conditions in the search for more favourable conditions. If the emigrating insect fail to find a retreat of more moist conditions, they will die within a short time frame of approximately 48 hours.

As adults fly at night we found the insects, as expected, closer to the soil surface in the morning hours than in the afternoon hours and many more insects walking above ground in the morning compared to the afternoon, (Figure 3). As insect ascended in the dry season simulating vertical moisture gradients the different vertical distribution of insects in the morning compared to the afternoon was more distinctive in the dry season simulation than in the rain season simulation. While conducting a field collection of *C. bergi* in June 1993 (La Bella, Risaralda, Colombia) we observed the insects as more shallow during the morning compared to the afternoon. On this basis, it was decided to conduct a systematic assessment of insects during early morning hours and late afternoon hours during the rainseason while high population density was expected (December 1993 at the same locality). However, this exercise did not confirmed our first observation. On the basis of the results from the season simulating vertical moisture gradients we can now explain our first observation of a evident diurnal vertical movement of *C. bergi* as this was carried out during the dry season. We did not succeed in the systematic assessment of vertical distribution as this was conducted during the rainseason during which the diurnal vertical movement is less distinctive between morning and afternoon hours.

High relative air humidity was found to induce flight at night. The frequency of individuals flying over night was found as a function of the relative air humidity over night, measured as the mean of relative humidity at 7 pm and at the following 7 am. A double reciprocal model gave the best fit, r=0.85 ($P<0.0009$), (Figure 4). Significant increase in fly frequency was observed above 89%. As we have no data selected at relative humidities above 97% we can not predict the continuation of the curve illustrated in figure 5, but rather describe what we observed within the humidity range from which we have selected data. We assume that high relative air humidity stimulates any migrational activity above ground level.

C. bergi Climate Distribution (GIS)

The damage caused by *C. bergi* does not affect the vigour of the crop and is left undetectable until the peeling of the roots, hence, farmers are most often taken by surprise at the time of harvest. Therefore, a mapping of the potential geographic distribution and risk zones of this pest is of great benefit. *C. bergi* has been reported from various sites only in the neotropics. The pest has a temperature range from 18 -28 °C and enhanced survivorship and reproduction when exposed to moist soil. The amount of rainfall, wet day frequency and temperature are suggested as important climatic factors for the vigorous development of the pest populations. Reports on collections sites of *C. bergi* from the CIAT insect collection, agronomists and the literature were gathered in a data base of 66 accessions, (see map in Figure 5).

Dr. Peter Jones from the CIAT GIS section extracted the passport data for each of the accessions and used the latitude, longitude and elevation of the collection points to find climate data for each site. Monthly rainfall, temperature and daily temperature range data were used to calibrate a model for the *C. bergi* climates and calculated the probability that a climate belongs to the *C. bergi* climate. The climate probability model uses a principal component analysis to concentrate the information in a small number of variables. When plotting the accessions against the variable scores it appears that there are groups of climate types. A cluster analysis was made and resolved the accessions into five independent climate clusters. The climates of each cluster were mapped to see where similar climates exists in the neotropics.

Cluster 1 (Figure 6).

Tropical highlands along the sides of the Andes mountain range at 1000-1700 meters above sea level. Locations in Honduras (Copán, Cortés, Olancho), Nicaragua (Jinotega, Chontales y Region Autónoma Atlántico Norte), Costa Rica (Limón), Dominican Republic (San Cristóbal, Santiago Rodríguez y Barahona), Puerto Rico (Utuado, Comerio), north cost of Venezuela (Sucre), the Guyana Highlands and East Brazil (Bahía). Mean daily temperature is 20-21°C and there is a high precipitation all year round without any real dry season.

Cluster 2 (Figure 6).

Low Andes region at 350-1000 meters above sea level. Locations in the Dominican Republic (María Trinidad Sánchez), Trinidad, northern Venezuela and Guyana, North East Brazil and Amazonas of Brazil. Mean daily temperature is 25°C and there are two one-month dry seasons yearly.

Cluster 3 (Figure 7).

Jungle areas throughout tropical Latin America. Mean daily temperature is 25-26°C and there is a very high precipitation all year round with no monthly precipitation less than 100 mm.

Cluster 4 (Figure 7).

Very hot areas in Panama next to the Golf of Panama, the north cost of Colombia and the Bolivian lowlands in particular. Locations on the west cost of Nicaragua, savannas of Colombia and Venezuela, Peruvian lowlands, Amazonas of Brazil and North East Brazil. Mean daily temperature of 27°C and there is a four-month dry season yearly.

Cluster 5 (Figure 8).

Sites in Panama (Chiriqui, Veraguas, Coclé, Herrera, Namá and Darién), Costa Rica (San Ramón, Pérez Zeledón) and Perú (Pasco). Mean daily temperature is 22°C and the is a three- month dry season yearly.

Cluster 1 includes the majority of 62% of the accessions, but this might reflect the fact that most collections have taken place within Colombia. A dendrogram of the accessions shows that cluster 2 and 3 are the most similar which again are related to cluster 1 and cluster 5 is the most distinct from the others. The clusters are described as follows:

The grading of the probability of pest presence within each map together with knowledge on potential host plants will serve as a guide to farmers, extensionists and advisors. The areas of high probability within each map indicate a risk zones of pest outbreak due to its favourable climate to *C. bergi*, but it does not necessarily confirm that he insect is there. There may be other host or geographic reasons for the absence of the insect. However, the new potential areas which emerged from the map and from which the pest has not yet been reported should be scouted for this pest. Nevertheless, the mapping indicates a high risk of pest outbreak in case of an accidental introduction of the pest into these new areas.

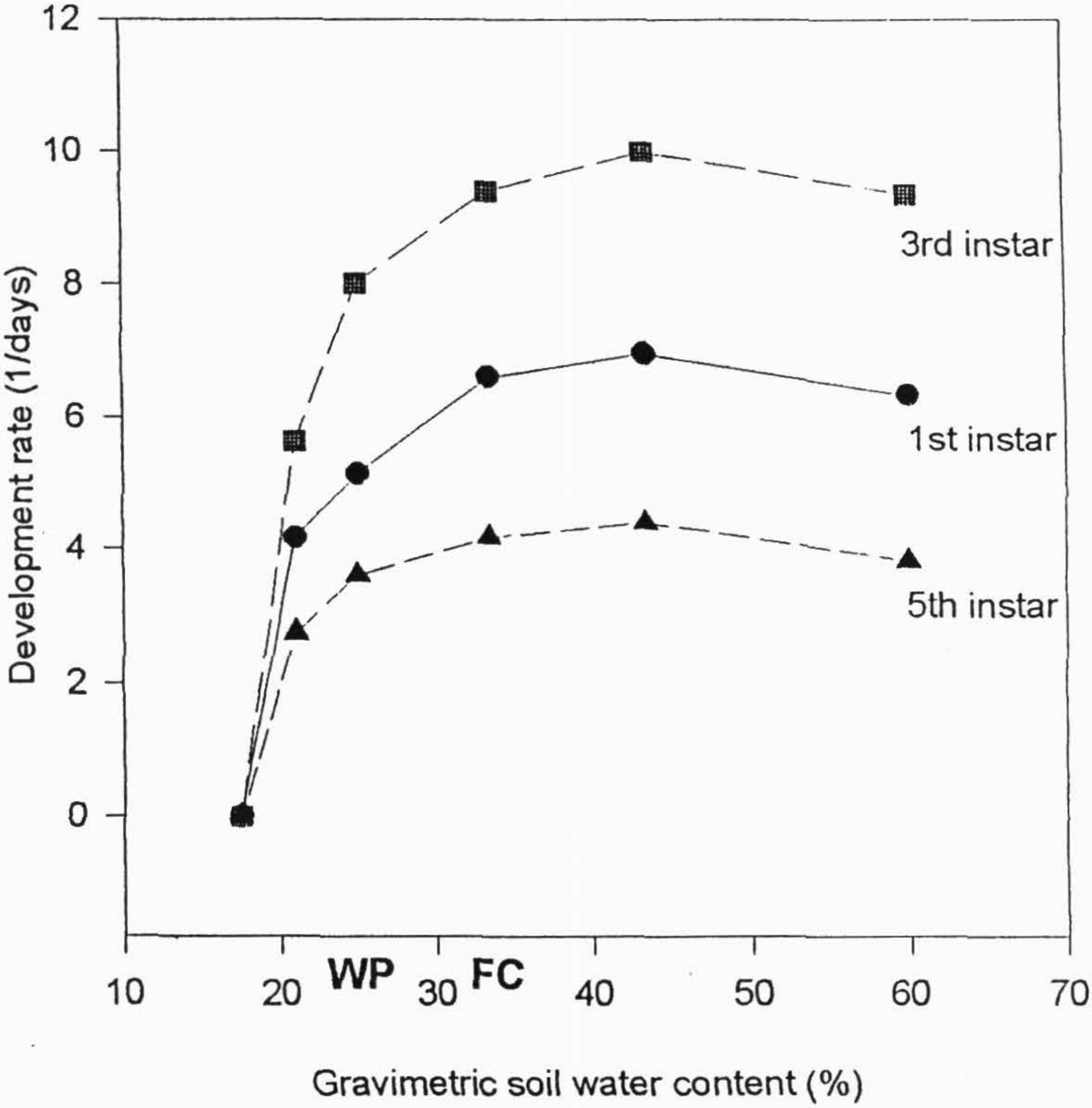

Figure 1. Development rate of 1st, 3rd and 5th juvenile instar of *Cyrtomenus bergi* at increasing soil water contents and a constant temperature of 25°C. WP=wilting point, FC=field capacity.

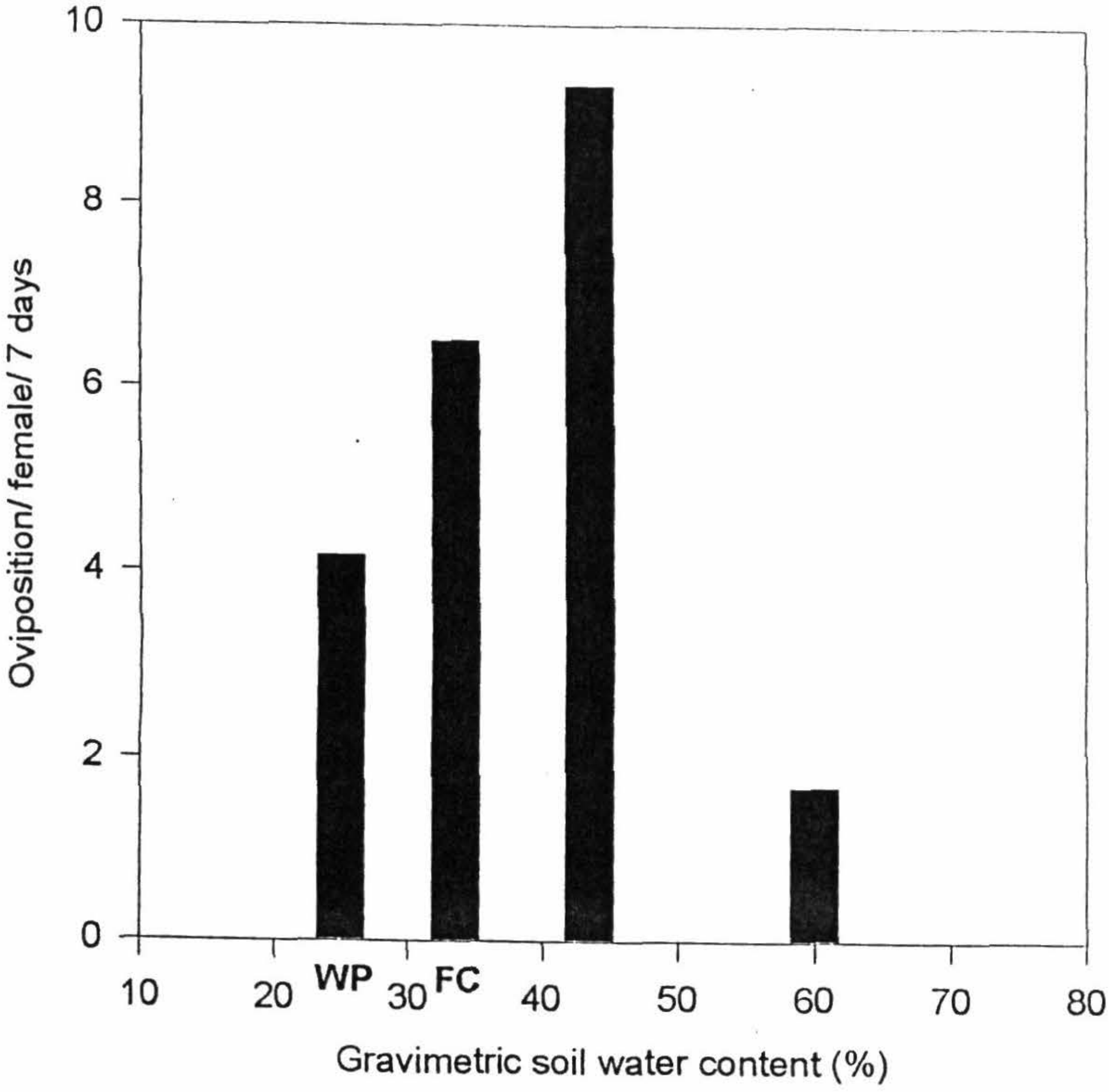

Figure 2. Reproduction of *Cyrtomenus bergi* at increasing soil water content and a constant temperature of 25°C. WP=wilting point, FC=field capacity.

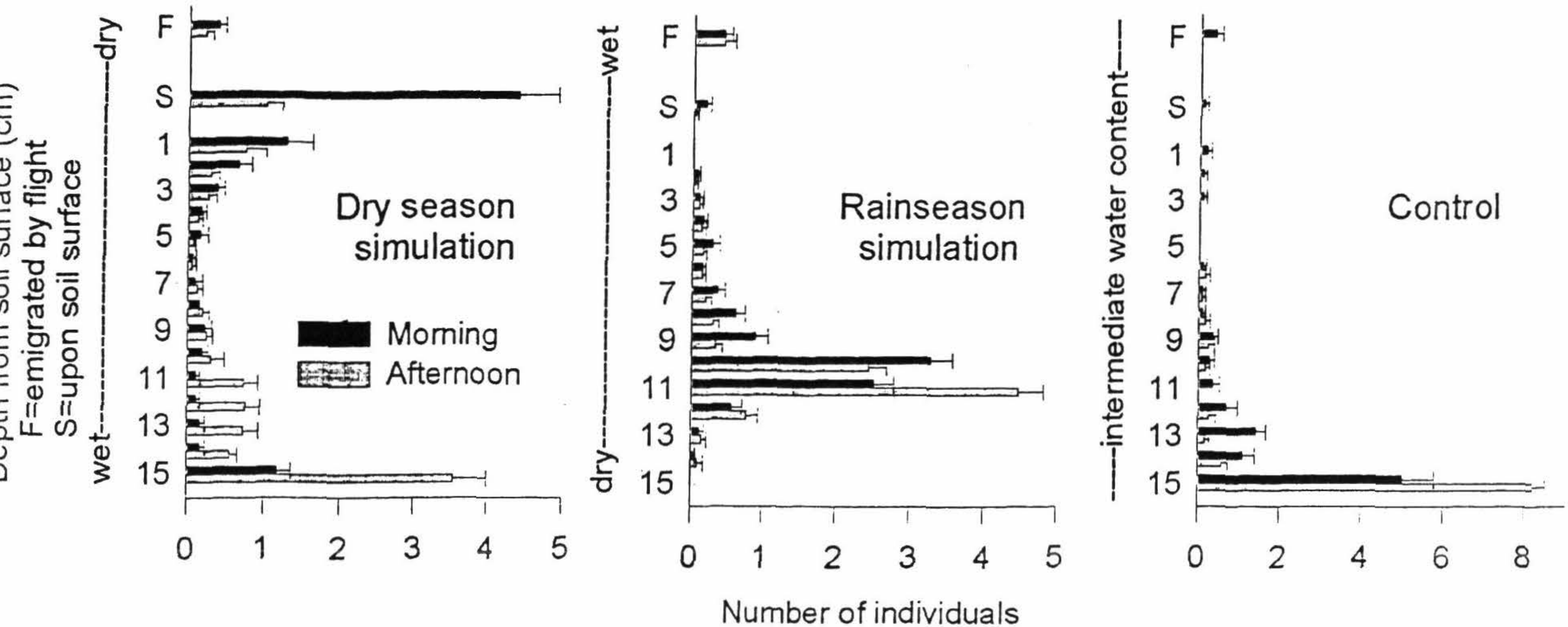

Figure 3. Aggregation of females of *C.bergi* at each centimeter depth in vertical soil water gradient tubes, on soil surface (S) and individuals escaped by flight (F) from tubes simulating dry season (increasing soil water content from top down), early rainseason (decreasing soil water content from top down) and a control with an intermediate soil water content.

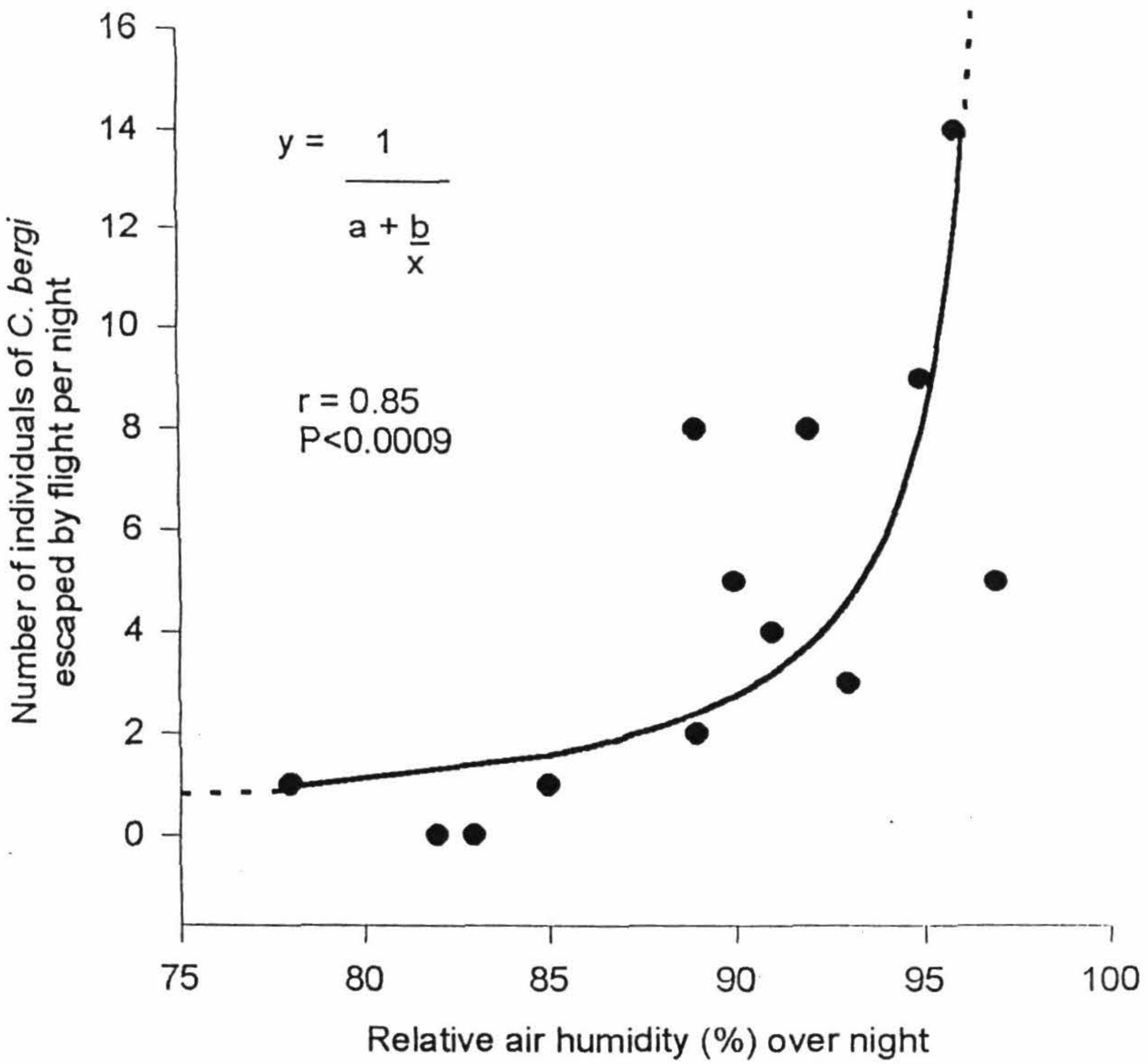

Figure 4. Nightly escape of individuals of *Cyrtomenus bergi* from vertical tubes as a funcion of the relative humidity over night (mean of relative humidity at 7 pm and at the following 7 am).

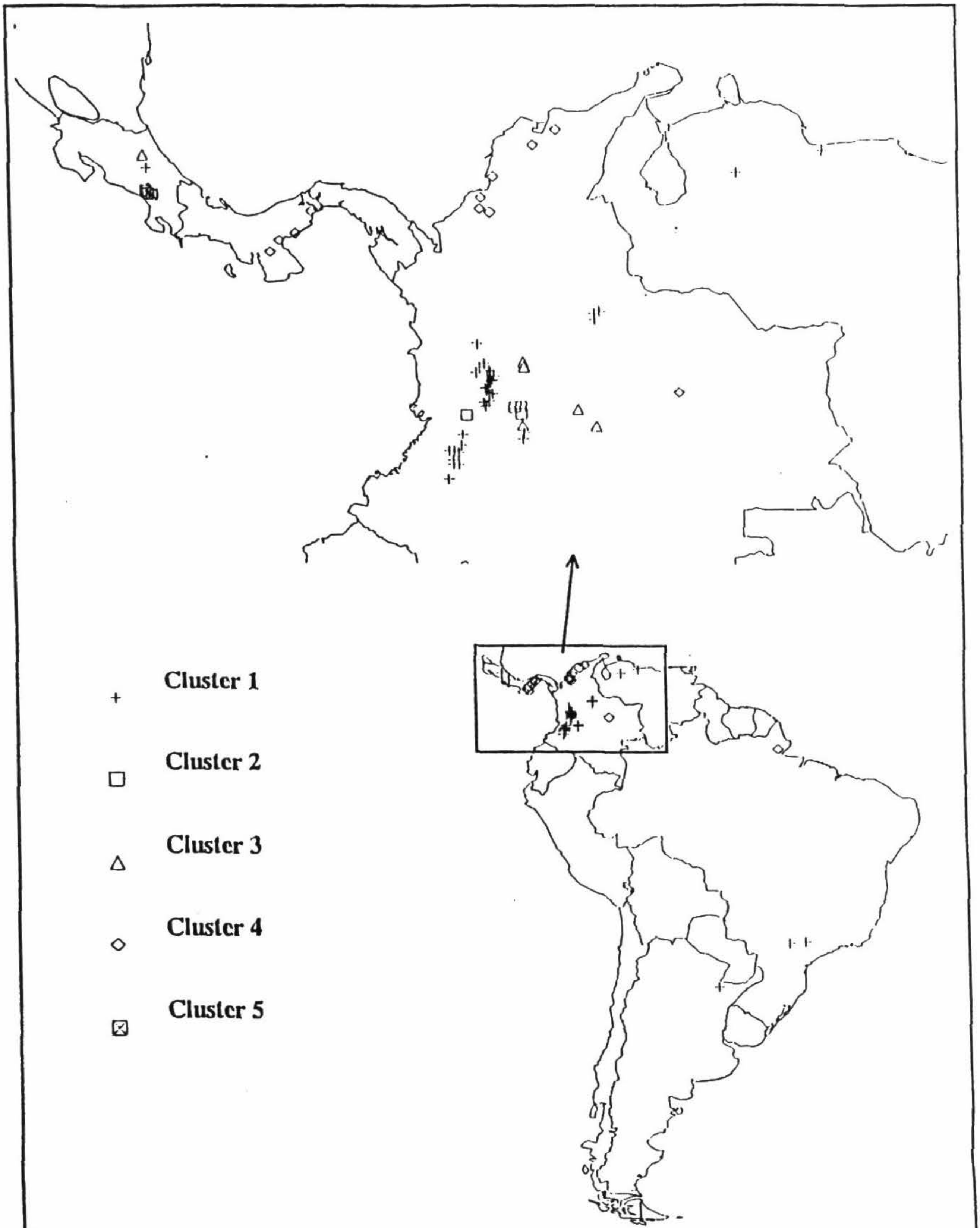

Figure 5. Collection sites of *Cyrtomenus bergi*. Each site belongs to one of five climate clusters.

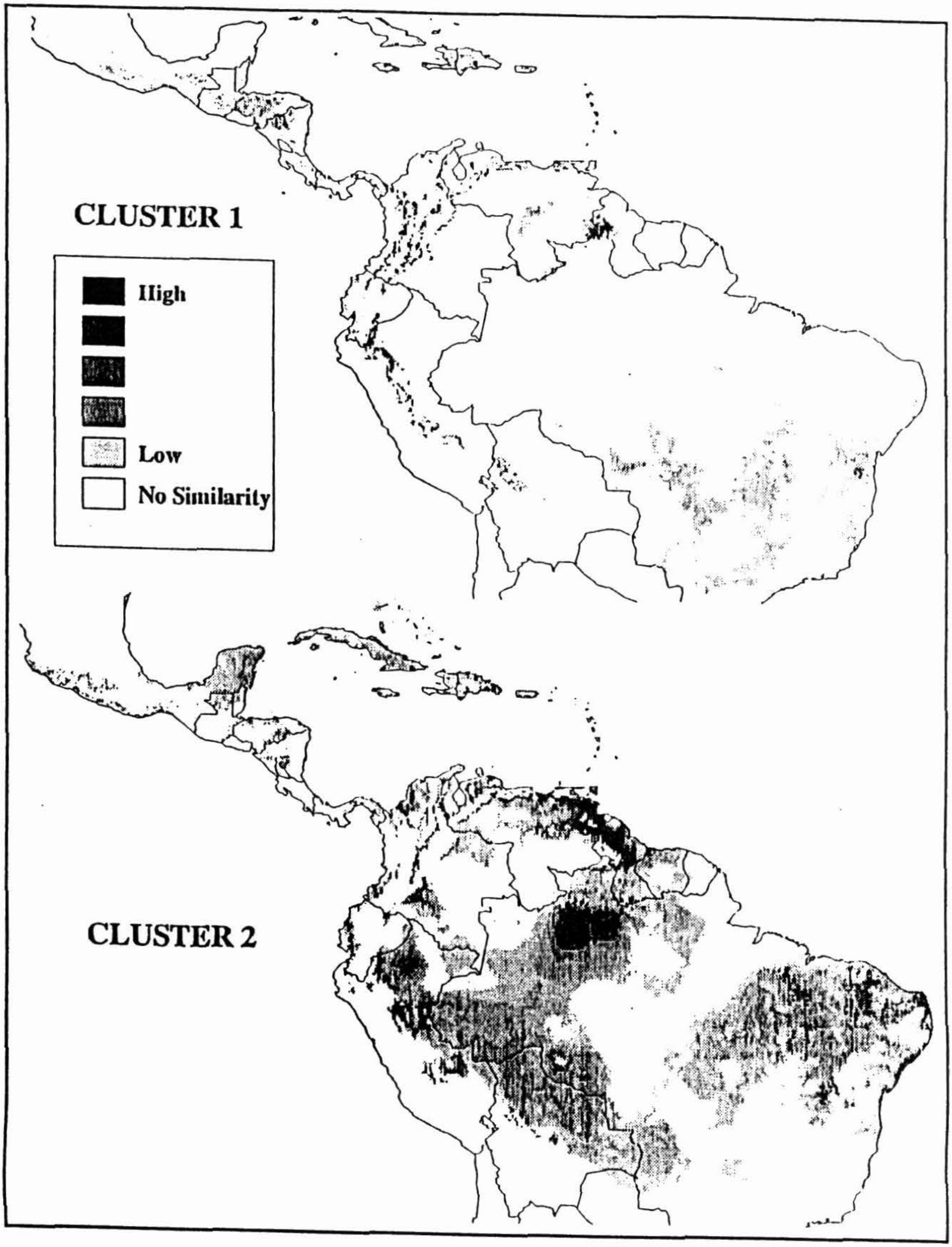

Figure 6. Climates of *Cyrtomenus bergi*; climate clusters 1 and 2.

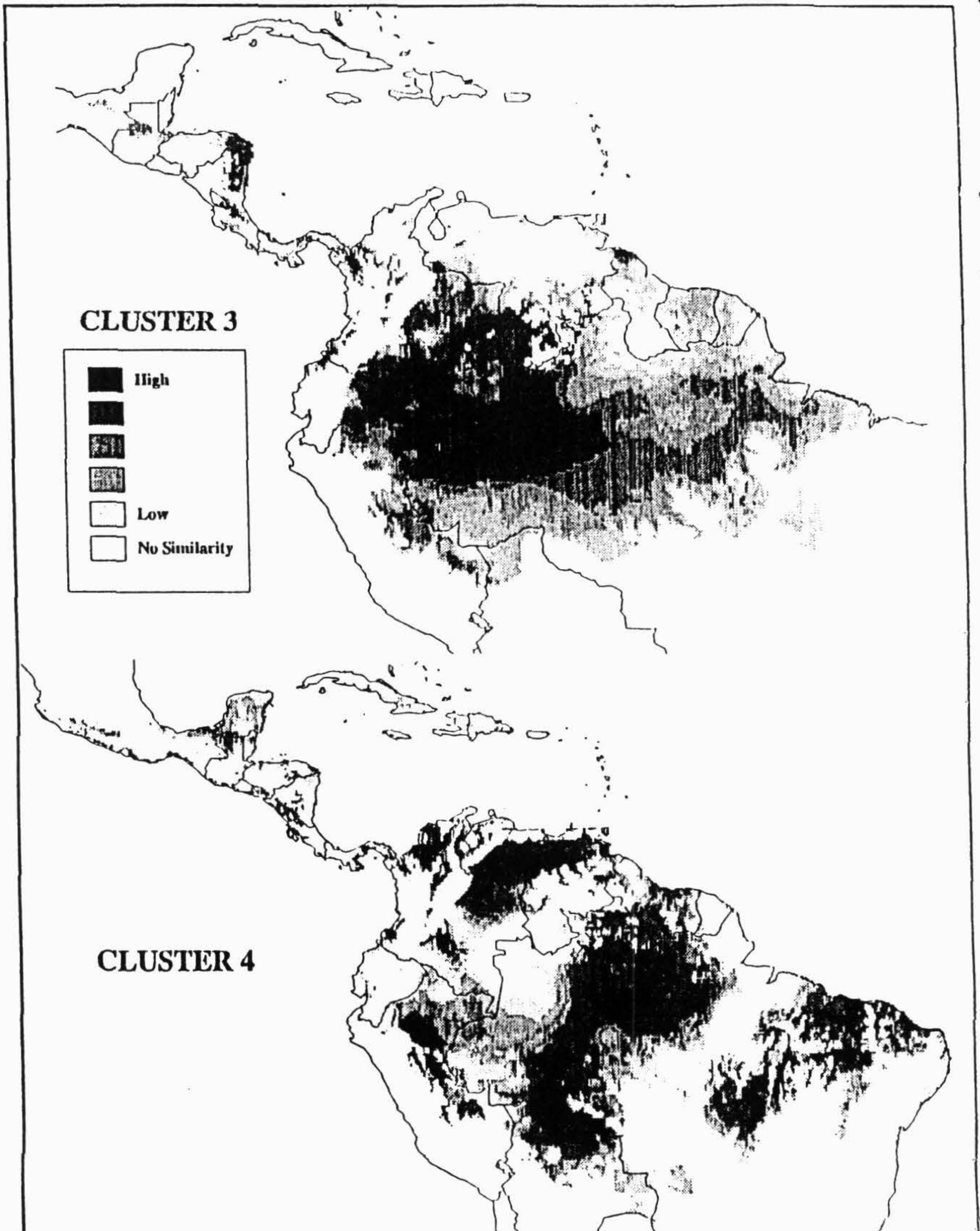

Figure 7. Climates of *Cyrtomenus bergi*; climate clusters 3 and 4.

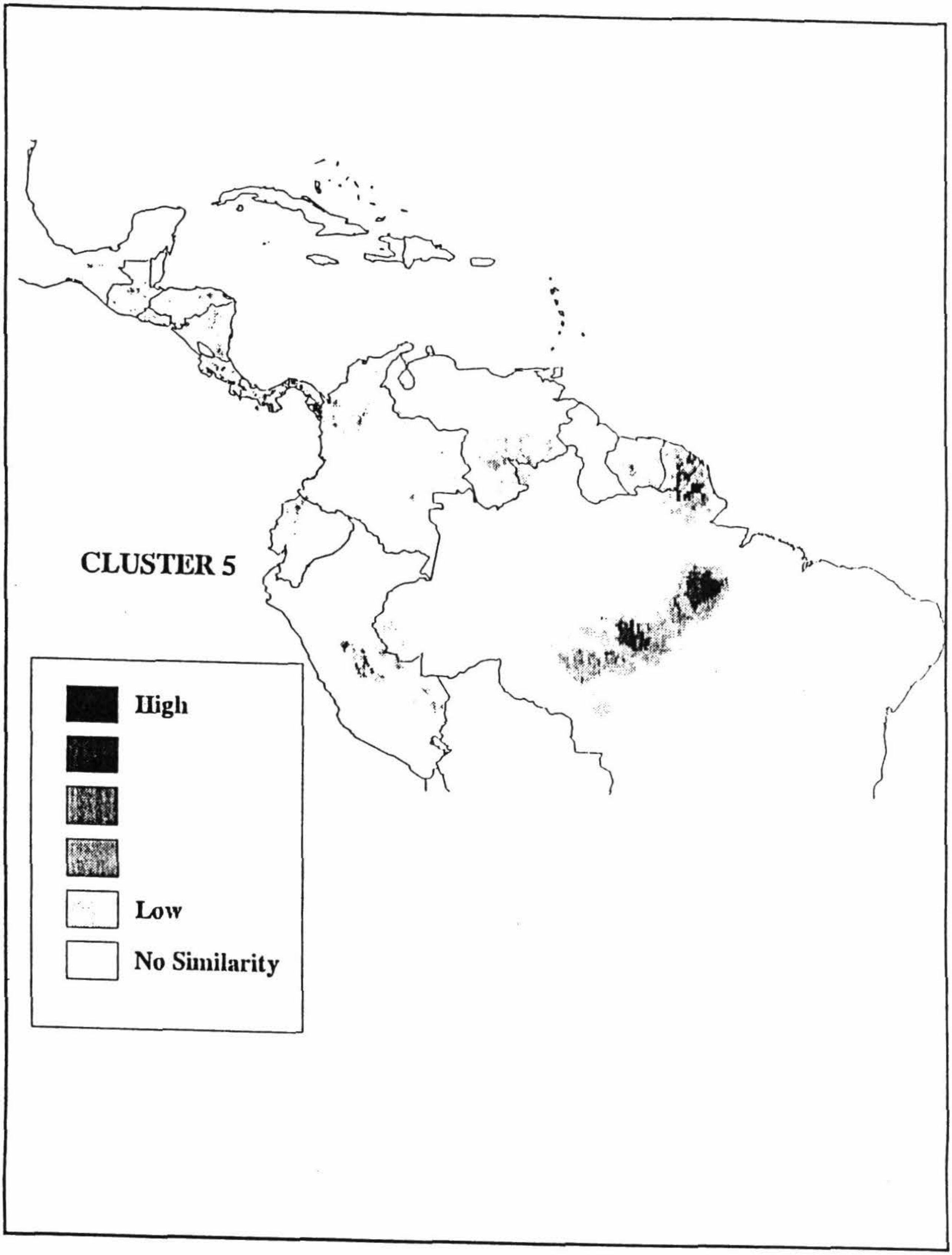

Figure 8. Climates of *Cyrtomenus bergi*; climate cluster 5.

Biological Control of *C. bergi*

During 1996 biological control research concentrated on the entomopathogen fungus *Metarhizium anisopliae* in the laboratory . These studies had four major objectives:

1. Develop a methodology to evaluate the pathogenicity of entomopathogenic fungi on *C. bergi*.

2. Evaluate the pathogenicity of different strains of *M. anisopliae* on *C. bergi* and determine the most susceptible life stage.

3. Determine the most pathogenic strain of *M. anisopliae*.

4. Determine the LD.50 of the most pathogenic isolates.

C. bergi individuals were produced in a laboratory colony reared on peanut in sterilized soil at 20% humidity in plastic boxes. Twelve isolates of *M. anisopliae* and one of *Beauveria bassiana* from different zones of Colombia and provided by CENICAFE, were evaluated. Isolates were maintained on a Sabourand dextrose Agar + yeast extract at 0.1% SADY. Healthy test *C. bergi* were selected and disinfected with a 0.5% sodium hypochlorite solution for two minutes. Suspensions of conidia from the fungus *M. anisopliae* Sorok, *B. bassiana* (Bals.) Vuill, and *Paecilomyces lilacinus* (Deuteromycotina: Hyphomycetes) were evaluated in experiments under laboratory conditions on adults of *C. bergi*. Three media were evaluated corresponding to sterilized soil, unsterilized soil and moist filter paper. Two methods of inoculation by a conidia suspension: one consisted in inoculating the medium and the second inoculating the insect directly by contact with a suspension. The insects of the control treatment were inoculated with sterilized distilled water.

Significant differences in mortality were found between the species of fungus used and the inoculation medium of X^2=11.9; p=0.01 and X^2=6.39; $p \leq 0.05$ respectively. No significant differences were found between the evaluated media; therefore the moisture filter paper was chosen as the media for all experiments.

Significant differences in mortality were detected between the three different entomopathogens evaluated; *M. anisopliae*, 47.5%, *B. bassiana*, 35%; *P. lilacinus* 12.5%; and the control 5% (X^2=11.9, gl=3, $p \leq 0.01$).. *M. anisopliae* was therefore chosen as the most effective control agent for future experiments. The most effective inoculation method corresponded to direct inoculation with the suspension of conidia.

Using this methodology of moist filter paper and a conidia suspension, the six life stages (5 nymph plus adult) of *C. bergi* were evaluated to determine the most susceptible life stage.

Significant differences in mortality were found between all five nymphal instars and the adult stage ($p \leq 0.05$) (Fig. 9). There were no significant differences detected between the nymphal instars with a high of 98.75% in the first instar and a low of 86.25% in the second instar. The 3rd, 4th and 5th instars registered mortalities of 95, 97.5, and 95% respectively. At all stages there was a highly significant difference between mortality of the treated insects and the non-treated control. Highest mortality of the control occurred during the 1st and 2nd instars.

Nymphs were more susceptible than the adult stage of *M. anisopliae*, and because of the low mortality in the control, the fifth instar nymphs were chosen for future experiments. Thirteen isolates of Colombian *M. anisopliae* were therefore evaluated on fifth instar nymphs.

Results show that the most promising isolates were 9206 (originating from *Aenolamia reducta*), 9236 (unknown origin) and 9501 (originating from *C. bergi*) with control levels of 62, 84 and 70% respectively (Fig. 10). Using isolate 9236, which gave the highest mortality the LD_{50} was determined at 1.19×10^8 viable conidia per ml^{-1} with confidence limits of 5.91×10^3 and 3.36×10^9 and an LT_{50} between 4.7 and 8 days.

Utilizing a distinct methodology, in which sterilized soil was used as a medium and the three best isolates applied in a granulated formulation, it was determined that the isolate 9206 was superior to isolates 9236 and 9501 with mortality levels of 63.9, 57.2 and 54.5% respectively (Fig. 11). Isolates 9206 and 9236 gave significantly higher mortality than isolate 9501.

Evaluations under field condition using *M. anisopliae* and *entomopathogenic* nematodes (see Annual Report 1995) are being planned.

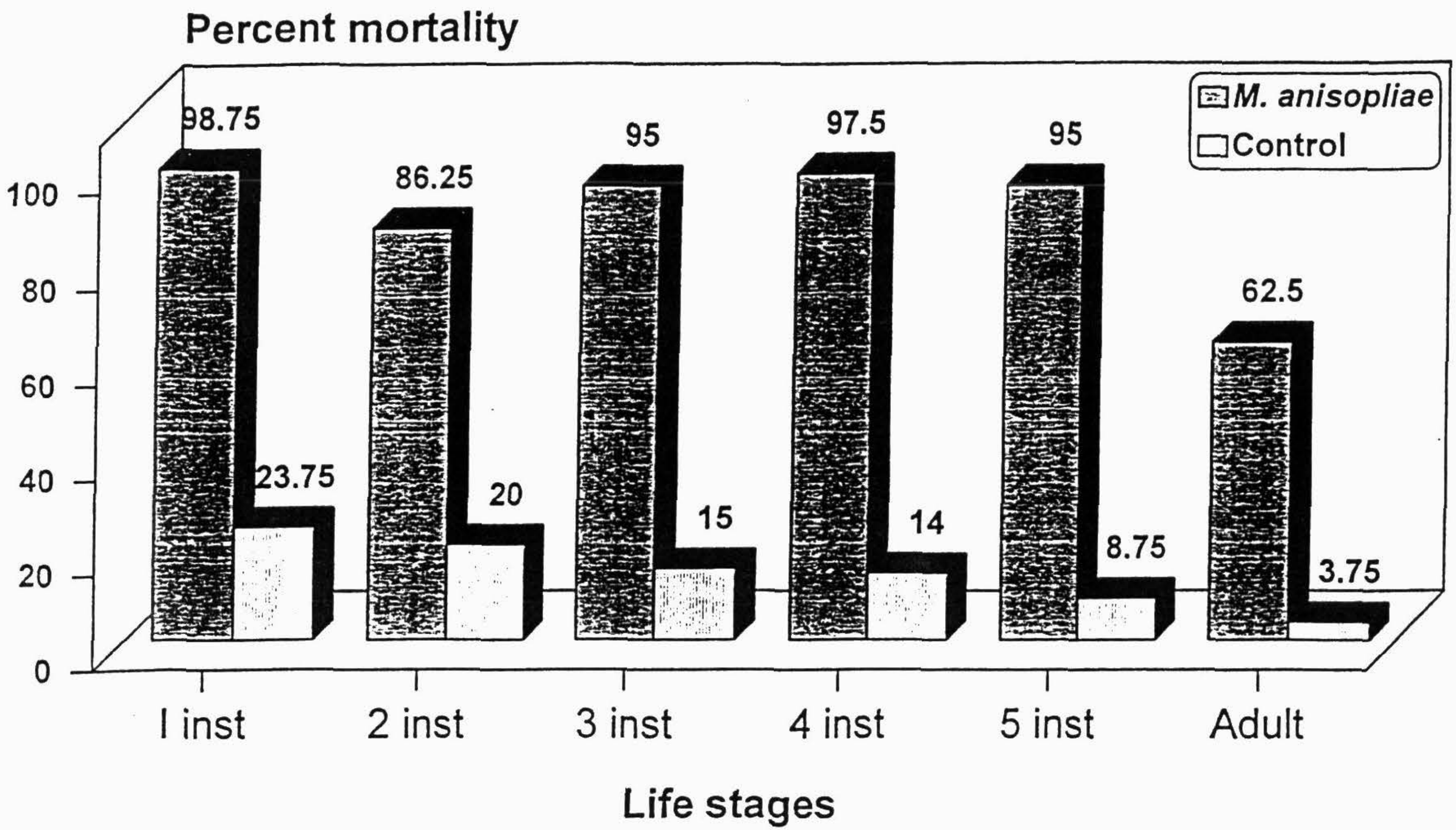

Figure 9. Percent mortality of the different life stages of *Cyrtomenus bergi* caused by *Metarhizium anisopliae* 9501 at a concentration of 1×10^{10} conidias per ml.

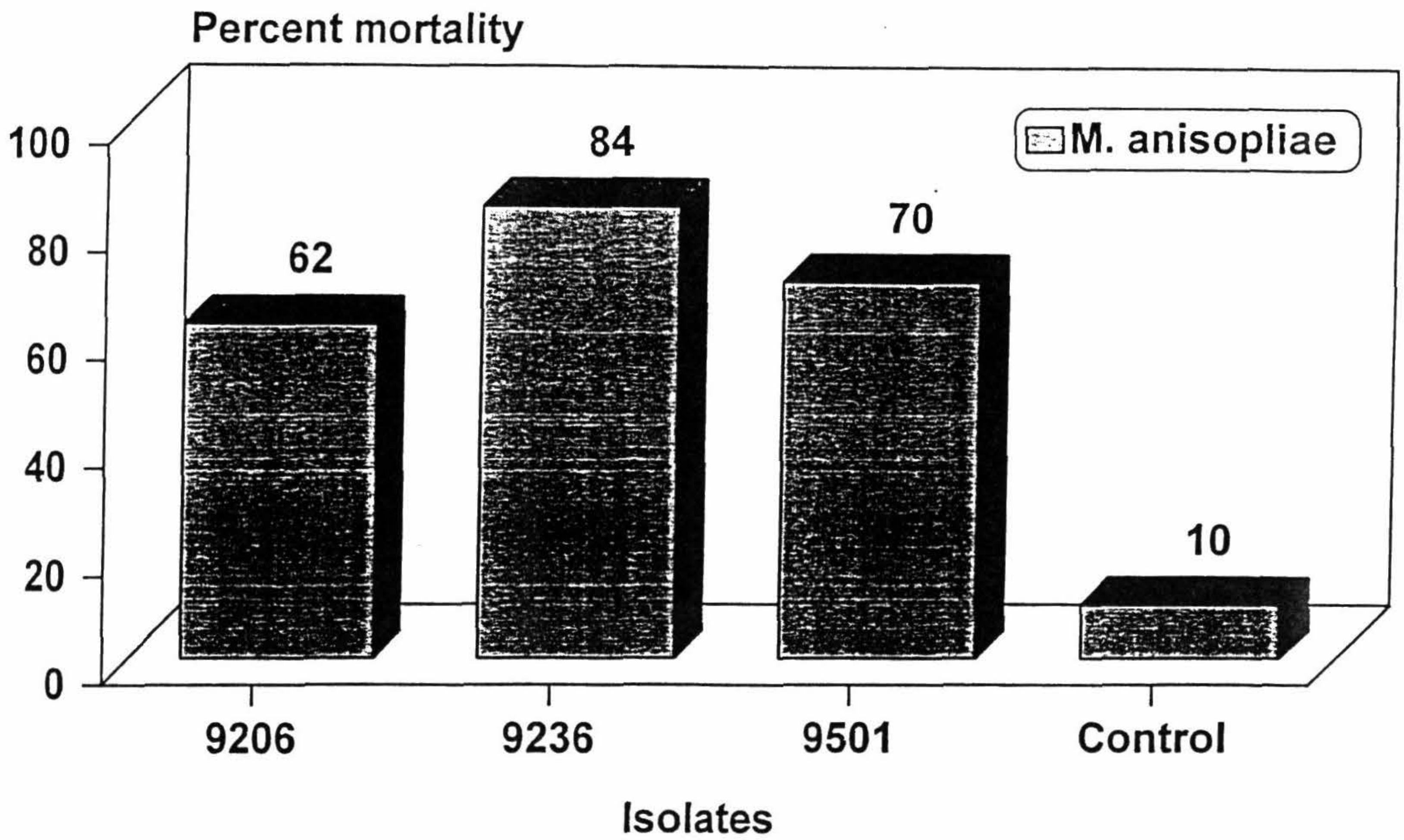

Figure 10. Percent mortality caused by three isolates of *M. anisopliae* in *C. bergi* on moist filter paper in the laboratory.

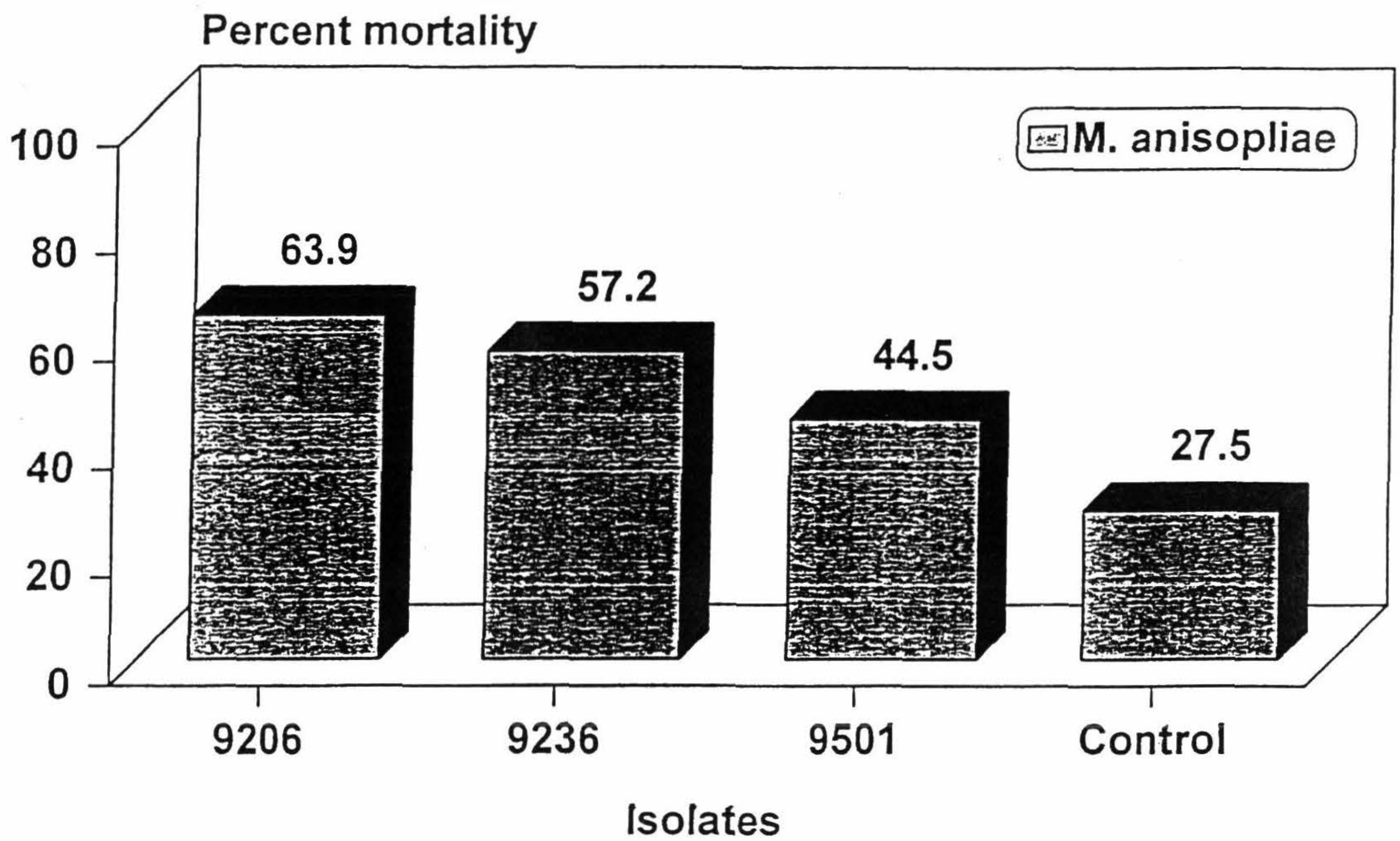

Figure 11. Percent mortality of *C. bergi* inoculated with three isolates of *M. anisopliae* using a granular formulation in the soil

3.2 Output 1.2 Identification of races of CBB, root rot pathogens; development of methodologies for rapid detection; development of control measures.

3.2.1 Phytophthora Root Rot and Leaf Blight Diseases

In collaboration with PROFISMA, CNPMF/EMBRAPA, EBDA, CORPOICA, Brazilian and Colombian Universities a working plan is designed to investigate cassava root and leaf blight. The objective is to develop simple integrated strategies to control soilborne pathogens, embedded in a farming- and agro-ecological management approach. In 1996 main CIAT activities were isolation of root and stem rot pathogens and development of isolation, inoculation methods and molecular technology. Biocontrol experiments were realized to select antagonistic *Trichoderma* spp. and *Pseudomonas* spp. strains.

3.2.1.1 Disease survey and isolation of the pathogens

The major limiting factor of cassava root and blight research is understanding of rot causing pathogens, therefore the following methodology is developed to isolate *Phytophthora* spp.

In 1996 34 farms of the Colombian departments Valle, Quindío, Cauca and Bolivar were visited. The survey revealed that root and stem rots caused by *Phytophthora* spp. are a major problem. In these regions root rots have been known to cause losses in excess of 80% of potential production, while 26% of the farms visited, cassava was badly affected by root and stem rots. For the first time the impact of root rots on cassava yield was analyzed (**Fig. 1.**). Despiteful chemical stem cutting treatment, cultivation on ridges and the relative long dry periods and crop rotation at CIAT cassava is, severely, affected by root rots. Average root yield reduction by root rots was calculated 7.1 T/ha per growing cycle for the period 1981-1995 by analysis of 60 field experiments.

At the visited farms random samples were taken from affected plants. *Phytophthora* spp. was isolate by direct plating of diseased plant tissue on selective media and by baiting a selective host. For direct plating small fragments were placed on V8 juice agar (V8A) and Potato-Dextrose Agar (PDA) amended with thiabendazole (0.5 μ/ml), benomyl (20 μg/ml), ampicillin (250 μg/ml), penicillin (400 μg/ml) and rifampicin (10 μg/ml). The baiting technique was as follows: soil or macerated plant tissue was suspended in sterile deionized water, and cassava sprout fragments of approx. 2 cm were placed in the suspension. Approx. 1 wk after incubation fragments of the sprouts were placed on the described selective media. **Table 1** presents a summary of successful isolation.

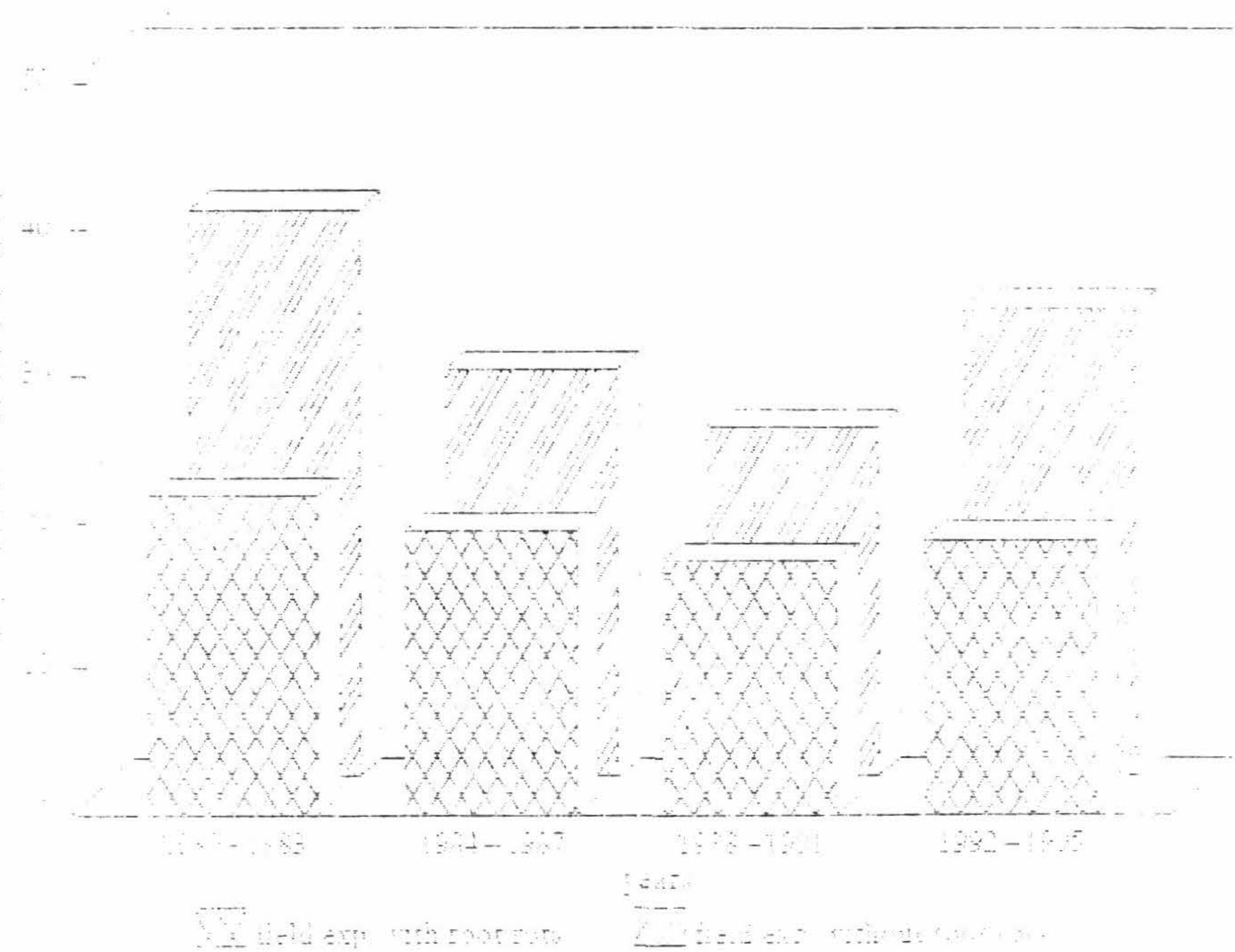

Figure 1. Root rot development of cultivar MBRA 12 at CIAT.

Table 1. Phytophthora root and stem rot diseases observed at 4 Colombian departments and their isolation.

Collection Site	Number of Farms Visited	Positive Recovery[1]			
		Root	Sprout	Rhizosphere	Number of Isolates
Cauca	10	1[1]	1	4	7
Quindio	15	3	2	12	41
Valle	10	3	0	14	25
Bolivar	1	2	0	0	2

[1] number of farms from where *Phytophthora* spp. were isolated

Isolation of *Phytophthora* spp. was effective using the described techniques, and 75 isolates were obtained. Preliminary inoculations of some of the isolates confirmed their virulence to cassava. A rapid and accurate method to detect these pathogens in plant tissue, soil and water samples is required. Research is ongoing to develop a polymerase chain reaction (PCR) method.

3.2.1.2 Culture of cassava *Phytophthora* species

It is necessary to have information about culture of *Phytophthora* species to determine the identity of species, to study their responses to ecological factors, to develop inoculum for use in pathogenicity tests and others.

Phytophthora spp. isolated from cassava grow and sporulate best on V8A compared with PDA, at 20-30°C with or without light.

Phytophthora cultures are always contaminated with bacteria. Therefore the following techniques were tested to obtain bacteria free cultures: 1, placing a small piece of fungal growth on the inside of a glass Van Wiegham cell that has been placed on a selective medium; 2, inverting the entire agar culture in a petri plate; 3, selective weak medium (medium diluted with water). Most efficient method is transferring fungal discs to water agar that contains appropriate antibiotics.

To check if a *Phytophthora* isolate is free from bacterial contamination a fungal plug was transferred to a test tube with Φ liquid medium (g/lt: peptone, 5; casamino acids, 0.5; malt extract, 0.5; pH 7.2) if the culture is contaminated with bacteria, the liquid becomes cloudy within 48 h of incubation at 30°C, but if bacteria-free, the liquid remains clear. *Phytophthora* spp. were successfully transferred from Φ medium to V8A.

Single-zoosporic isolation was readily accomplished by isolation of a single germinated cyst or not cysted zoospore after a highly concentrated *fungal suspension* was been spread thinly on the surface of 3% water agar on a microscope slide. After 12-24 h, germinated spores were detected on the surface of the agar by use of a microscope with a magnification of 400. With a bent Pasteur pipet a germinated spore was marked, and by use of a needle a plug was placed on V8A. Activities to identify and to conserve strains are started.

3.2.1.3 Development of bioassays to study pathogen variability and to test cassava germplasm for resistance

Known methods are not tested on correlation with field resistance of cultivars. Therefore 5 new *in vitro* and greenhouse methods of inoculation, described in **Table 2**, were designed to inoculate cassava plants with pathogen strains. To compare the efficiency of the methods 22 cultivars were inoculated with 4 *Phytophthora* spp. isolates. Extent of root rot and stem necrosis caused by *Phytophthora* spp. was recorded.

Table 2. Tested bioassays for root and sprout inoculations.

Parameter			Root & Sprout		Sprout
Plant material	*slice*[a] *of* swollen root	*cylinder of* swollen root[b]	pot plant of 3 wk[c]	pot plant of 4 wk[c]	excised shoot of 2½ wk[c]
Technique	disc in center of each slice	cylinder placed vertical in suspension[d]	application of suspension[d] to soil; flooding *of soil and* removing foliage	fragment on 5 mm wound of stem	cut sprout in suspensi on[d]
Incubation conditions	cellophane in incubator	plastic box in lab.	screenhouse	greenhous e	glass tubes in lab.
Evaluation period[e]	14	14	14	28	28
Efficiency[f]	24	24	15	15	60

[a] 1.5 cm X approx. 5 cm; [b]1.5 cm X 3 cm; [c]propagated by stem cutting; [d]10^5 spores/ml; [e] d after *inoculation*; [f]*avg. number of inoculations per field* plant

All tested *Phytophthora* strains infected roots and stems severely in the proven systems, results are specified in **Table 3**. Inoculated roots were characterized by a yellow discolorization, tissue decompose, is soft and smells pungent. Between cultivars there were only small differences in amount of rot obtained.

On young cassava plants, infection by *Phytophthora* spp. produces necrotic lesions on the stems, and the infected stem shrunken. Inoculated plants presented light brown or almost black discolorization of stems. The first observable symptoms are brown patches on the petioles and older leaves turn yellow and loss turgidity and a general dieback of the whole plant occurs. Leaves eventually turn brown and do not fall down. Significant differences in lesion expression caused by *Phytophthora* spp. was detected by inoculating cassava stems. Tolerant cultivars exhibited shorter or less intense lesions than the susceptible cultivars. The plants of some cultivars plants were killed, or recovered by new sprouts.

Table 3. Results of laboratory and greenhouse bioassays of *P. drechsleri* and *P. nicotianae* (*P. parasitica*) inoculated on cassava cultivars.

Pathogen & Cultivar	Root				Sprout			Field Reaction[1]
	Whole	Slice	Cylinder	Soil	Soil	Wound	Excised Sprout	
P. drechsleri								
CG 1355-2	R[2]	S	S	S	R	R	R/S	
MCOL 2280	R	S	S	R	R	R/S	R	
MCOL 1505	S	S	[3]	R	S	S	R	
MBRA 1045		S	S	R	R	R		R
MBRA 532[4]	S		S			R/S	R/S	R
P. nicotianae								
MCR 54	S	S	R	S	R/S	R/S	R/S	
MCOL 2280	R/S	R	S	S	R/S	S	S	
CG 1355-2		S	S	S	R/S	R/S	S	
MCOL 1505		S	S	S	S	S	S	
MBRA 532[4]		S	R			S	R/S	R

[1] According to farmers; [2]S, susceptible (high pathogenicity); S/R, intermediate pathogenicity; R, resistant (low pathogenicity); [3] Not sufficient plant material available; [4] Ossoduro

Resistance to root rot not necessary implies resistance to leaf blight and vice versa. To be sure of an adequate root inoculation test other field resistant and susceptible cultivars have to be identified and inoculated.

3.2.1.4 Cassava infected by *Phytophthora* spp. at CIAT

Phytophthora root rot and leaf blight were not considered to be important limiting factors for cassava stake production at CIAT. By use of advanced isolation methods it was found out that the 2 diseases are present at CIAT, and severely reduced stake quality. In 1996 the following observations were made about stem cutting and seedling infections by *Phytophthora* spp.

Greenhouse plants propagated from a CIAT cassava field showed disease expression, varying from stem necrosis to death. From 200 cultivars, all core collection, 61 expressed Phytophthora leaf blight disease.

In plants a general blight - yellow leaves and/or necrotic lesions form on sprouts - was observed 3-5 wk after planting stem cuttings. Cassava plantlets in the greenhouse can be predisposed to the severe expression of stem rot if the soil in the pots is allowed to dry to near the wilting point before excessive watering.

About 3 ha of commercial cassava production was severely affected by root and stem rots, the field was infested with *Phytophthora* spp. which was isolated from irrigation water, soil and cassava sprout tissue. In plants a general dieback was observed 3 months after planting stem cuttings, 7 months later the swollen roots were rotten. Under less favorable conditions plants recuperated. Most plants at all stages of growth from germination to maturity showed symptoms. It is concluded that under severe conditions or severe infection plants may be killed, but in plants that survive the stakes are infected:

The disease was observed in seedlings propagated by botanical seeds in seedling containers. It affected seedlings severely, 90 % of the plants died. The infected stem shrivels, cause wilt and death of the seedling within 10 d.

Phytophthora spp. can be considered to be the main cassava root rot causing factor. From the above can be concluded the systemic transmission of these pathogens by stake material. To obtain high yields of cassava under marginal conditions, it is important to start with good sanitary quality of propagation material. Severe outbreaks can be expected in the nearby future because cassava production continues to become more intensive, at CIAT as well as outside.

In course of 1996 various control methods, e.g. thermotherapy of stem cuttings and chemical control, have been evaluated to reduce infection at CIAT. From 3 heat treatments (dry heat, vapor and hot water bath), immersion of stakes in water of 50°

during 20 min was the most efficient. Stake germination and plant vigor were similar to control plants. Development of rapid diagnosis, like PCR, of the 2 diseases is started. A more detailed study to know if Phytophthora diseases are transmitted by cassava stem cuttings have to be programmed.

3.2.1.5 Molecular variation in strains of *Phytophthora* species affecting cassava stake quality and root yield

Phytophthora strains were obtained from 34 cassava farms of the Colombian departments Valle, Quindio, Cauca and Bolivar. 10 strains were compared in virulence tests. Virulence was determined by inoculating cassava plants by stem wounding. The effectiveness of this procedure in distinguishing the severity of disease reactions produced by different strains was evaluated. Considerable variation in lesion size was observed. The symptoms caused by individual strains of *Phytophthora* species were very similar although symptom severity differed among strains and species. The virulence test of 76 strains is ongoing.

Amplification of the ITS (Internal Transcribed Spacer) region of the rDNA was obtained with template DNA from *P. drechsleri*, *P. nicotianae* and *P. erythroseptica* using extracted DNA or by scraping mycelium. The amplified product for the ITS region of all species was the same. Restriction Digest with *Alu*I of the product amplified for the ITS region showed 3 different restriction patterns, which corresponded to the 3 species tested. DNA from 76 *Phytophthora* strains will be digested with *Cfo*I, *Msp*I, *Mse*I restriction enzymes to differentiate the species. In addition RAPD analysis will be conducted to determine genetic variation among strains.

3.2.2 Biocontrol of Root and Stem Diseases

Antagonism of *Trichoderma* spp. and *Pseudomonas* spp. - potential biocontrol agents - to *Phytophthora* spp., *Diplodia manihotis* and *Fusarium oxysporum* the causal agents of cassava root and stem rot diseases in America and Africa, was investigated.

3.2.2.1 Extracellular production of antifungal compounds by *Trichoderma* spp.

Trichoderma spp., isolated from the rhizosphere of cassava plants were screened *in vitro* for antifungal activity against a range of fungal pathogens: 2 isolates of *P. drechsleri* and *P. nicotianae*, *D. manihotis* and 1 of *F. oxysporum*. A control treatment with *Thielaviopsis* spp., a causal agent of bud rot of oilpalm, was included.

The activity of 14 one wk cultures differed among the pathogens. Filtrates from liquid cultures of isolate 11TSM-4 caused marked reduction in growth of the pathogen *D. manihotis* after 24 h of incubation. Filtrates of 14PDA-4 were most effective in reducing the growth of *F. oxysporum*. The culture filtrates of this *Trichoderma* spp. isolate caused 40% growth inhibition of the pathogen. They were toxic in each replication and until the end of the experimentation period of 1 wk. Experiments are ongoing to study the effect on spore germination and germ tube elongation of efficient isolates; and application of pure and mixed extracts of these isolates to infected cassava plants.

3.2.2.2 Evaluation of *Pseudomonas* spp. by excised shoot bioassay

Biocontrol strains from the genera *Pseudomonas* were tested for antifungal activity in bioassays *in vitro*. This study is undertaken to select strains to control *Phytophthora* spp. Shoots of cassava cultivar CG 1-37 in an excised shoot bioassay were bacterized, and 3 d later inoculated by a *Phytophthora* spp. suspension. Preliminary observations indicate that from 9 isolates 1 antagonist inhibited lesion development of the cassava sprouts.

3.2.2.3 Production *Trichoderma* spp. in liquid medium

Production of *Trichoderma* spp. using a liquid culture system on media based on molasses, V8 juice and mineral solutions was studied. These media were incubated at 30° C for 1 wk under darkness of shake culture. Of the media evaluated, a mineral salt medium supported max. conidia production of 27 *Trichoderma* spp. strains. High antifungal activity was observed by culture filtrates prepared with the medium. Field plots and cassava stem cuttings, infested by *Phytophthora* spp., were inoculated by strains produced in the identified medium.

3.2.3 Study on Stem Rot Disease Caused by *Fusarium* Species

Fusarium solani and *F. oxysporum* strains were obtained from 86 cassava samples including stem cuttings, swollen and secondary roots, and soil obtained from different zones in Colombia (Maria La Baja, Carimagua, Media Luna, Pivijay, Calarcá, Palmira, Villavicencio, Alcalá, Pescador, Granada and Quilichao).

In addition 4 methods of classification were tested for differentiation of the strains, including morphology, growth, pathogenicity and site directed (rDNA) PCR. These species were compared in pathogenicity tests, in which the symptoms caused by individual strains were very similar, although symptom severity differed. Infected seedlings exhibited necrotic stem lesions compared with healthy controls. The majority of strains were not clearly differentiated on PDA by colony type, growth or pigment production.

Amplification of the ITS region of the rDNA was obtained with template DNA from 10 strains using extracted DNA. Restriction digest with *Alu*I, of the product amplified for the ITS region showed 2 different restriction patterns, which corresponded to the 2 species tested. The PCR analysis was more reliable in differentiating the strain than the other methods. In addition rDNA analysis offered a distinct advantage by requiring less time for distinguishing among the species. The simplicity of the PCR analysis presented in the study, and the accuracy of their predictions make them acceptable for identification of *Fusarium* species on cassava. The most pathogenic of the *F. solani* and *F. oxysporum* strains will be distinguished from the other *Fusarium* strains by RAPD markers.

Furthermore a screening of 51 cassava cultivars has been conducted by inoculating 28 *Fusarium* strains to evaluate resistance.

3.2.4 Study on Stem Rot Caused by *Diplodia manihotis*

A survey in cassava growing areas in Colombia was conducted in 1995-1996 and samples including cassava stem cuttings and roots were collected. 55 strains of *D. manihotis* were isolated and monosporic cultures were obtained.

Three inoculation methods were used for pathogenicity studies, they included stem injection, stem wounding and root cylinder inoculation. Clear symptom development was observed by stem wounding. From a total of 55 strains, 8 were highly pathogenic on cassava plants. A pathogenic specialization study has been initiated, at the moment 20 cassava cultivars have been inoculated with 40 strains selected from the *D. manihotis* collection.

3.2.5 Characterization of *Sphaceloma manihoticola*

3.2.5.1 Characterization of strains by colony morphology, growth and pathogenicity

A collection of 30 *S. manihoticola* strains were selected from cassava samples including petioles, stems and leaves. Strains were originally isolated from natural PDA and monosporic cultures were obtained. Cultures were tested for growth, morphology and pathogenicity.

Colony morphology was assessed, after incubation at 26°C for 7 to 21 d. Color production and conidial morphology were also studied after 21-28 d growth. Great variability in pigmentation was observed among strains making grouping by colony morphology alone difficult. Spore measurements showed no differences between strains.

Growth. Fungal plugs (5 mm diameter) were transferred to petri plates containing natural PDA and the extent of linear growth was determined after incubation at 26°C for 21 d. 3 groups were differentiated. No correlation was found between colony growth rate and pathogenicity.

Pathogenicity. Strains were inoculated into 20 d old greenhouse-grown cassava stem cuttings by spraying the stem, petioles and leaves with an aqueous spore suspension

(3 X 10^6 spores/ml). The plants were incubated at 95% relative air humidity and 26-28°C for 48 h with a photoperiod of 12 h. Symptoms were evaluated 10-20 d after inoculation. The results of the pathogenicity tests showed high variability among strains when inoculating MCOL 22 and MVEN 77 (a susceptible and a resistant cultivar respectively) with 30 *S. manihoticola* strains. Of the 31 strains of *S. manihoticola* that were obtained from cassava samples 9 were highly pathogenic showing severe stem infection. Intermedium reactions were observed with 11 strains and symptoms were observed only on leaves and petioles. 10 strains were low pathogenic.

3.2.5.2 Molecular characterization

Template DNA was extracted from 10 strains of *S. manihoticola* using the method of Lee and Taylor in PCR Protocols[a].

PCR analysis of rDNA the ITS region of the rDNA was amplified using the primers ITS_4 - ITS_5. Amplification of the ITS region was obtained with all strains using the extracted DNA. The PCR products generated with the primer pair ITS_4 - ITS_5 will be digested with each of 11 restriction enzymes to detect variation among strains.

A pathogenic specialization study was initiated. 20 cassava cultivars have been inoculated with 20 strains selected from the *S. manihoticola* collection.

[a] Innis, M.A., Gelfland, D.H., Sninsky, J.J. and White, T.J. 1990. PCR Protocols. San Diego, California

3.2.6 Effectiveness of Potassium Fertilization on Reduction of Anthracnose disease

Five cassava cultivars were evaluated under 2 different levels of potassium fertilization by field trials at Santander de Quilichao. The results are presented in **Table 4**.

The results showed clearly a reduction of anthracnose disease after potassium fertilization. The result of a new experiment at the same plot will be compared with the results obtained in the previous year.

Table 4. Performance of cassava cultivars to Anthracnose disease under 2 levels of potassium fertilization.

Cultivar	Reaction[a]	Potassium Level[b]
CM 3299-4	S	0
CM 3299-4	S	100
CM 3311-3	S	0
CM 3311-3	S	100
CM 2766-5	S	0
CM 4779-4	S	0
CM 4770-2	R	0
CM 4770-2	R	100

[a] Resistant (R), and Susceptible (S) reaction; [b] gr potassium chloride per plant

3.3 Output 1.3 Characterization and identification of cassava viruses; development of rapid detection methods; development of control measures.

3.3.1 Characterization of Cassava vein mosaic virus (CVMV)

The molecular characterization of the genome of one isolate of CVMV was completed and an article was published during 1995. It was determined that CVMV is a member of the family of plant pararetroviruses but was not as closely related to the Cauliflower mosaic virus group as previously thought. Because this virus is so unique, this study is being continued to determine the diversity of isolates from the different states in the northeast of Brazil, to confirm that all isolates could be identified using the diagnostic methods developed during the molecular characterization and to confirm the genomic structure.

3.3.2 Development of rapid detection methods for CVMV

Samples were taken from plants collected from several states in Brazil that showed typical CVMV symptoms. Nine isolates of CVMV were amplified using PCR. The isolates were from Fortaleza, Crato and Tiangua in Cerea, Araripina and Petrolina in Pernambuco, Bento Fernandes in Rio Grande do Norte, Piretiba and Iteberra in Bahia, and Seripe in Alogoas. The diagnostic test used specific PCR primers for two regions of the genome. All of the samples were positive for CVMV using both sets of primers. The virus complex at this level appears to be conserved and the PCR detection can be considered a reliable detection method over the range of CVMV. Fresh material is more reliable for detection but if the samples are not for immediate analysis, it is important to desiccate the leaves rapidly. This method of detection is currently being used in Brazil and the seed health laboratory of CIAT.

Survey of virus isolate diversity

The analysis of CVMV isolate diversity was done by molecular analysis of some isolates used for the confirmation of the PCR detection method. The CVMV PCR products were cloned into bacterial plasmids, sequenced, and analyzed for homology among the CVMV isolates. For the majority of the isolates, two distinct regions were analyzed. These were the part of the genome that encodes the replicase gene and the region containing the putative coat protein and movement protein. A comparison of the region that encodes the replicase gene reveals that the percentage of homology was always more than 95%. This area appears to be highly conserved with little variation between the isolates.

The region encoding the coat protein and movement proteins has a higher level of variation. While the homology levels were generally more than 90% over the entire region, the junction between the two proteins showed a high degree of diversity. One of the key differences is that the coat protein does not read through to the movement protein as a polyprotein. Because these changes are important in understanding the pathogen diversity and the genomic structure, additional isolates are being characterized. It can be concluded that over the range of CVMV that there is a significant degree of molecular diversity and that care should be taken not to spread the isolates through infected germplasm.

3.3.3 Molecular marker for the identification of CFSV

To develop a PCR method for the rapid detection of CFSD, seventy five combinations of oligonucleotide primers were used to determine if they produce differential amplified products when comparing CFSD affected versus non-affected cassava. Of these, four sets of primers are undergoing intensive evaluation as a rapid detection marker for CFSV.

The amplified product from one set of primers was cloned and sequenced and has a moderated degree of similarity with rice ragged stunt reovirus (RRSV). Although this product has a fair degree of similarity with RRSV, there are still doubt that this is a virus product since similar products are sometimes amplified from the apparently healthy controls. Other differentially PCR products are in the process of further analysis and these studies will continue until a rapid detection method and the etiology of FSD is clearly identified.

3.3.4 Analysis of the signs of CFSD

Cytological studies of the roots of normal and CFSD affected roots were made using both light and electron microscope. There were dramatic differences in the root periderm and cortex in the accumulation of starch. In CFSD affected roots, the starch granules were either absent or apparently empty. The cell layers were much more densely pack in the CFSD affected roots and this is due to the absence of starch granules and the hyperplasia.

The other characteristic of CFSD is the enlarge lip shaped fissures on the surface of the roots. These studies suggest that these fissure on the outside portion are caused by a hyperplasia of the cells in the root cortex. The root cortex is much thicker than normal yet there is no starch granules in these cells. The hyperplasia is not equal all along the cambium meristem causing the lip shaped fissures.

These studies were made to identify characteristics that can be used in screening for resistance to cassava frogskin disease (CFSD). The problem with using just root weight is that there is a high degree of variability with the core collection because some of the materials are more adapted than others to the growing conditions in Cauca. It is important to be able to distinguish between poor root growth due to poor adaptation to the growing conditions as compared to the affects caused by CFSD. This assay using the light microscope is relatively rapid and inexpensive and can determine if the starch accumulation and cytology of the cells is abnormal. This assay will be used in the screening of the core collection for tolerance toCFSD.

3.3.5 Screening the core collection for resistance or tolerance to cassava frogskin virus.

During 1995, a field experiment was begun to analyze the 630 accession of the cassava core collection for tolerance or resistance to cassava frogskin virus (CFSV). The methodology is to inoculate the core collection using a single source of CFSV. Characteristics including yield and starch accumulation will be analyzed and compared with CFSD free accessions. Over 500 of these accessions are currently in the field in either the first or second cycle of evaluation. Most of the remaining accessions have been inoculated and will be planted during this season. The number of infected plants per accession will be increased and screened over several years. The most promising clones will be tested in multilocational trials. This is a continuing activity that will take at least three to five years to get reliable results. Any resistant or tolerant accessions will be used as parents in development of resistant germplasm pools.

3.3.6 Identification of geminiviruses infecting cassava

African cassava mosaic virus (ACMV) serotype A is endemic throughout Uganda and causes substantial albeit manageable yield losses. Since 1990, there has been a severe epidemic of African cassava mosaic disease (ACMD) that has moved from the northern regions towards the south at the rate of 10-20 kilometers per year. The symptoms in the plants after the epidemic are very severe with the leaves becoming extreme small and deformed. When infected stem cuttings are used in the following planting cycle, the plants produce roots of no value causing yield losses of nearly 100%. This epidemic has caused nearly a 50% decrease in total production throughout Uganda and total loss of production in many regions.

In collaboration with IITA, SCRI and NARO (Uganda NAR) and molecular analysis of the A component of the severe Ugandan isolate was made. This represents 50% of the genome of a geminivirus and the result is that the Uganda isolate appears to be a hybrid between ACMV (serotype A) and East Africa cassava mosaic virus (serotype B). The coat protein of this isolate is almost identical to ACMV and cannot be distinguished from ACMV using the currently available monoclonal antibodies specific to serotype A. The common region and the replicase gene are more similar to EACMV.

Since there is such deformity to the leaves, the whiteflies move from severely infected plants to cassava planting not yet affected by the severe ACMD in a epidemic front. When this front moved through the NARO experiment station near Kampala, NARO/IITA materials showed a fair degree of resistance or tolerance to the Ugandan geminivirus. Many of these varieties are high in cyanide and while are good for making gari are not suitable for the Ugandan market where fresh cassava is eaten. There are breeding efforts by IITA and NARO to address this problem and produce resistant varieties that are low in cyanide. The result of a distinct geminivirus and not simply higher levels of whiteflies confirms the policy of NARO to insist that only resistant varieties are replanted in the areas behind the epidemic front.

3.3.7 Monitor "B" biotype of *Bemisia tabaci* spread throughout Americas

The "B" biotype of *B. tabaci* (also known as *B. argentinfolia*) is expanding its range and is causing problems on a wide range of hosts. This whitefly is a vector of geminiviruses and as the range of the "B"biotype expands, there are increasing epidemics of geminiviruses in beans, tomatoes and many other crops. As part of the IPM whitefly project a systematic survey of the range of this and other whitefly species and biotypes will be made. This biotype is of concern on cassava because it has the potential to transmit geminiviruses to this host. There is already some limited evidence that a new world geminivirus has been transmitted to cassava. The other biotypes of *B. tabaci* in the Americas did not have cassava as a host an therefore by exclusion was not a threat to cassava.

The most common criteria for stating that the "B" biotype is present is the number of hosts that are being attacked in a region. Unfortunately this can easily be confused with natural outbreaks of endemic whiteflies due to favorable climatic conditions, introduction of new crops that are good host all whiteflies, or other factors such as changes in insecticide usage. It is proposed to develop markers based on regions of the genome that have been used extensively for evolutionary studies for other species. This work will be part of the IPM whitefly grant and results should be available during 1997. The mapping of biotypes is an important element in the understanding of whitefly outbreaks and geminivirus epidemics. To a crop like cassava, it is important to anticipate the new threat and be prepared to introduce resistance varieties if geminiviruses become a problem in cassava grown in tropical America.

3.4 Output 1.4 Components of sustainable cassava plant protection in South America and Africa developed (UNDP-Sponsored).

3.4.1

The principal objectives of the acarology unit are to find natural enemies of the cassava green mite (CGM, *Mononychellus tanajoa*), evaluate them for safety and suitability as biological control agents, and send them to EMBRAPA in Brazil and IITA in Benin for release.

Explorations were conducted to find phytoseiid predatory mites in two target ecological zones: 1) hot semi-arid (corresponding to NE Brazil) and 2) high altitude/subtropical (corresponding to the East African plateau. For the first target, we collected 5 species of phytoseiids in Manabí, Ecuador from 11 sites with 425-1500 mm annual precipitation, 6-9 dry months (<60 mm rain), 24-26●C average temperature, and 16-120 m elevation. For the second target, we collected 6 species of phytoseiids from 20 sites in Antioquia, Boyacá, Caldas, Cundinamarca, Quindío, Risaralda, Santander, and Tolima, Colombia from sites with 425-1500 mm annual precipitation, 0-4 dry months, 14-26●C average temperature, and 495-2216 m elevation. We established 7 new colonies of phytoseiids from high elevations for evaluation. A culture of *Typhlodromalus aripo* from Palmira was established for biological investigations.

Laboratory experiments were conducted to determine the suitability of a range of different prey and food sources for the development, survivorship and oviposition of 9 species of phytoseiids. The cassava green mite and its close relative, *M. caribbeanae* were the most suitable prey for predator development, survivorship and fecundity. The two-spotted spider mite was also suitable for several *Neoseiulus* and *Galendromus* species, as was the mite *Oligonychus peruvianus* for two *Neoseiulus* species. Other types of prey such as thrips, whitefly, mealybug, *Oidium* leaf fungus, and cassava exudate were not very suitable, though pollen could sustain development of *Neoseiulus*, *Typhlodromalus* and *Euseius* species.

Investigations were conducted to develop a computer model to help optimize and facilitate the management of the mass-production of phytoseiids. Data on life history, rate of stage-specific prey consumption, and intraspecific competition were collected using *T. tenuiscutus* to provide inputs for the model. Data were collected from mass-rearing cultures to "validate" the model. It was determined that additional information is needed on the rate of cannibalism.

Tritrophic host plant resistance investigations were conducted in the laboratory on the effect of three varieties of cassava (previously classified as susceptible, tolerant and resistant to CGM) on populations of CGM and the phytoseiid predator *N. californicus*. The three varieties affected development time, immature survival, adult longevity, fecundity and sex ratio of progeny. Intrinsic rate of increase was highest on the "susceptible" variety CM 3306-4, intermediate on the "tolerant" variety Ecu-72, and lowest on Bra-12. Data on the phytoseiid are still being collected.

A taxonomic key to 30 phytoseiid species commonly found on cassava in northern South America was developed in collaboration with Gilberto de Moraes (CNPMA/EMBRAPA, São Paulo, Brazil). Phytoseiids collected in the field during explorations and experiments were identified and curated. The taxonomic collection is being reviewed to verify old identifications and correct the computer database. AFLP analysis of genetic variation showed that geographic strains of *N. idaeus* were very homogeneous whereas those of *T. manihoti* differed greatly. Genetic crosses are being made between field strains of *T. manihoti* to determine if there was any evidence of reproductive incompatibility which may reveal the presence of subspecies.

We scanned and formatted 11 major documents (2,800 pages) including recent Cassava Program annual reports, CIAT Working Documents, scientific meeting proceedings and Cassava Newsletters to be included in a CD-ROM being published by IITA and the University of Florida, Gainesville.

A grant proposal co-submitted with the University of Amsterdam to WOTRO to fund a Ph.D. student to study multitrophic interactions of insects and mites in cassava was rejected at the beginning of 1996. This proposal was modified and re-submitted.

3.4.4

Neoseiulus californicus from Portoviejo, Ecuador was sent to Brazil, through quarantine at CNPMA/EMBRAPA in São Paulo, for mass-rearing and release by CNPMF/EMBRAPA in Bahía. Strains of *Typhlodromalus manihoti* previously collected from Barbosa, Cajibío and Copacabana, Colombia were sent to IITA, Benin via quarantine at the University of Amsterdam. To date, three species of phytoseiids have been established in Africa. Of these, *T. aripo* and *T. manihoti* are spreading rapidly and are reducing cassava green mite populations significantly. Estimated benefits for 4 countries in West Africa are on the order of US $60 million per year. Two species released in northeast Brazil (*T. tenuiscutus* & *N. californicus*) have been recovered in small numbers.

Cultures of the fungal pathogen *Neozygites* sp. were collected from the cassava green mite and two-spotted spider mite (*Tetranychus urticae*) at CIAT and from CGM at Media Luna, Colombia. Other cultures obtained from Brazil and Africa were included in a laboratory evaluation of host specificity on *M. tanajoa*, *M. caribbeanae* and *T. urticae*. None of the 3 strains from *M. tanajoa* infected *T. urticae*, and one of the strains from *T. urticae* failed to infect *M. tanajoa*. All 5 strains infected *M. caribbeanae*. Specimens were sent to Siegfried Keller (Swiss Federal Res. Station for Agronomy, Zurich, Switzerland) for taxonomic identification. The five strains were grown in axenic *in vitro* cultures for DNA extraction. New methods for *in vitro* culturing developed by the Brazilian Luis Leite working in Don Roberts' laboratory (Boyce Thompson Institute, Ithaca, New York) are being adopted. Genetic characterization of the strains was attempted using AFLP, but the initial trials failed, apparently due to insufficient quantities of DNA. Consequently we have changed our strategy and are now attempting to use RAPD primers.

We reviewed 10,782 specimens of tetranychid mites on microscope slides in our collection, representing 907 sample sites, to determine the host range and geographic range of this pathogen. *Neozygites* infection was observed in 14 species of tetranychid mites from 11 neotropical, 2 African and 2 Asian countries, indicating that this pathogen may be much more important than was previously thought.

A strain of *Neozygites* sp. from *T. urticae* was released to Biocontrol, SA and Coinbiol, SA., Palmira, Colombia for development as a commercial biological control agent.

4. Subproject 2. Purpose: ***To develop improved crop/soil management components relevant to cassava production systems in Latin America and Asia.***

4.1 Output 2.1 Crop/soil management in poor acid and sandy soils in Colombia.

4.1.1 Long-term response of cassava to NPK levels in acid infertile soils at Santander de Quilichao.

Table 4.1 contains data on the long-term response (13th year) to NPK fertilizer in infertile acid soils at Santander de Quilichao. In absence of NPK fertilizer application, dry root yield and top biomass were significantly reduced in both clones (M Col 1684 and CM 91-3). After 13 consecutive cropping cycles in this soils, dry root yield was about 4 t ha^{-1}. With application of 100 kg ha^{-1} each of NPK, root yields were dramatically increased (about 10 t dry root ha^{-1}). The largest observed response was for K as indicated by much lower yields in absence of K application and with 100 kg ha^{-1} of N and P. Lesser responses were observed for N and P. These findings again demonstrate that K is the most limiting nutrient in these soils with high organic matter content (≈ 7%). To sustain reasonable productivity, K fertilizer must be applied annually since most K uptake (> 60%) is removed in the harvested roots.

4.1.2 Long-term effects of surface mulch, fertilizer and tillage on cassava productivity in poor sandy soil in northern Colombia.

Table 4.2 presents data on root yield and top biomass as affected by plant surface mulch, NPK fertilizer and tillage on sandy soils at Media Luna, northern Colombia. Both dry root yield and top biomass were significantly increased by the application of either mulch or NPK fertilizer at a moderate level. On the other hand, conventional tillage increased yield only with NPK application in absence of mulch. The combination of surface mulch and zero tillage gave the highest root yield with and without fertilizer. These findings indicate that consecutive cultivation of cassava in these poor soils would lead to very low yields unless appropriate measures are followed to maintain soil fertility. The choice between application of NPK fertilizer or surface plant mulch would depend on various factors related to the socio-economic conditions of the local farmers. However, the potential economic return due to fertilization is high in this case. Moreover, the application of fertilizer would ensure enough quantity of planting material with high quality.

Table 4.3 contains data on the response of cassava to both plant mulch and NPK fertilizer in the soils of Santander de Quilichao. Although these soils contain greater organic matter than the sandy soils of northern Colombia, mulch application increased root yield even in the first year of the trial. Again, these findings indicate the beneficial effect of plant residues as mulch. Fertilizer application also increased root yield in these soils which have been under cassava cultivation for several years. Both top biomass and root dry matter content were also enhanced by applications of surface mulch and NPK fertilizer. When native weeds were repeatedly cut, without uprooting, and used for mulch, yields were depressed in absence of NPK fertilizer. This finding suggests that weeds significantly compete for nutrients in this case. Previous research (Cassava Program Annual Reports, 1987-1992) indicated that using post-emergence herbicides at an advanced stage of weed growth enhanced cassava productivity as compared with no weed mulch.

4.1.3 Effects of quality of planting material and water stress on cassava productivity (1996/1997).

Figures 4.1, 4.2, 4.3, presents data on leaf photosynthesis, stomatal conductance and midday leaf water potential as affected by stake quality and water stress. In this trial, planting material of M Col 1684 were obtained from the long-term NPK trial at Quilichao on the 12th cropping cycle. Mother plants with the following NPK levels were used: 0,0,0 (T_1), 50-50-50 (T_2), 100, 100, 100 (T_3), and 100, 100, 0 (T_4) kg ha^{-1} N, P, K. The trial was conducted in the field lysimeter at Quilichao, where half of the experimental area was deprived of water at 65 days of planting by applying plastic sheets for 3 months. Leaf net photosynthetic rate (Fig. 4.1) was similar in both the control and the water-stressed crops irrespective of the origin of the planting material. Apparently, the stake quality did not affect leaf photosynthesis. Stomatal conductance was significantly reduced by water stress in all stake origins. However, this effect was observed only at 60 days after stress was initiated (Fig. 4.2). The same trend was observed in midday leaf water potential (Fig. 4.3). These data indicate that cassava tolerates prolonged water stress and its leaves maintain high level of photosynthesis during water stress period. Moreover, cassava conserves water by partially closing its stomata during prolonged water stress.

4.1.4 Evaluation of crop/soil management practices in farmers fields in northern Colombia.

In collaboration with CORPOICA, several field trials were established in April-May 1996 in various sites to evaluate the effects of the application of mulch and fertilizer on productivity of cassava/maize system in farmers fields. These trials are located in Los Palmitos, Dept. of Sucre, in Plato, Dept. of Magdalena, Pueblo Nueva, Dept. of Cordoba, Pivijay, Dept. of Magdalena, Chiriguana, Department of Cesar, and in El Carmen de Bolívar, Dept. of Bolívar. These trials will be harvested in Feb/March 1977.

In addition to the research mentioned above, the Associate, Luis F. Cadavid, have participated in two training workshops for specialists of CORPOICA in regions 2 and 3. He prepared and dictated two courses on the integrated crop/soil management. The first course was in Barranquilla, Atlántico in May 1996, and the second was in Turipana, Montería, Córdoba in August 1996.

Table 4.1 Effect of NPK rates on dry root yield (t ha^{-1}) and dry top (t ha^{-1}). 1995-1996 season (13th cycle). Santander de Quilichao, Cauca, Colombia.

Fertilizer treatment (kg ha^{-1})			M Col 1684		CM 91-3	
N	P	K	Dry root (t ha^{-1})	Dry top (t ha^{-1})	Dry root (t ha^{-1})	Dry top (t ha^{-1})
0	0	0	4.4	2.0	3.9	2.3
50	50	50	9.1	3.5	7.8	3.9
0	100	100	10.4	3.3	5.2	2.9
50	100	100	10.1	4.4	7.8	3.8
100	100	100	10.5	4.3	9.2	3.9
100	0	100	9.8	4.0	8.9	4.4
100	50	100	13.0	4.2	8.4	3.8
100	100	0	7.5	3.0	5.2	3.1
100	100	50	10.3	4.0	7.6	4.1

LSD 5% (Tukey) for comparison between NPK treatments for dry root 3.75
LSD 5% (Tukey) for comparison between NPK treatments for dry top 1.57

Table 4.2 Dry root yield and dry top (t ha^{-1}) in cassava (cv. M Col 1505) at Media Luna, Magdalena, Colombia. Season (1995-1996), 8th cropping cycle.

	Fertilized [1]				Unfertilized			
	DRY (t ha^{-1})				DRY (t ha^{-1})			
Treatment	Root		Top		Root		Top	
Conventional tillage	4.3	bc[3]	2.4	b	1.2	b	1.4	b
Conventional tillage + mulch [2]	5.9	ab	3.6	ba	4.6	a	2.7	a
Zero tillage	3.3	c	2.3	b	1.4	b	1.2	b
Zero tillage + mulch	6.9	a	4.2	a	4.9	a	2.8	a

1 Fertilized with 330 kg ha^{-1} 15-15-15 NPK (50 N; 21.6 P; 41.7 K kg ha^{-1})

2 Mulch, 12 t ha^{-1} dry grasses applied annually

3 Values followed by the same letters are not significantly different at 5%.

Table 4.3 Dry root yield and dry top (t ha^{-1}) and root dry matter (%) (cv. CM 523-7 and CM 507-37) at Santander de Quilichao, Cauca, Colombia. First season (1995-1996).

	CM 523-7						CM 507-37					
	Unfertilized			Fertilized [1]			Unfertilized			Fertilized		
	DRY (t ha^{-1})			DRY (t ha^{-1})			DRY (t ha^{-1})			DRY (t ha^{-1})		
Treatment	Root	Top	Root dry matter (%)	Root	Top	Root dry matter (%)	Root	Top	Root dry matter (%)	Root	Top	Root dry matter (%)
Without mulch	9.7	3.9	40.9	12.2	5.3	40.8	10.3	3.9	34.8	13.9	5.7	36.9
Mulch Brachiaria [2]	11.0	5.7	41.3	13.8	5.5	41.1	12.6	4.4	36.8	16.3	7.5	38.2
Weed mulch [3]	7.1	2.8	40.7	13.4	5.1	41.3	8.3	2.8	33.4	13.3	5.1	36.3
Avg.	9.3	4.1	41.0	13.1	5.3	41.1	10.4	3.7	35.0	14.5	6.1	37.1
LSD 5% (Tukey)	2.65	1.06	2.91									

1 Fertilized with 500 kg ha^{-1} 10-20-20 NPK (50 N; 44 P; 83 K kg ha^{-1})

2 Mulch of *Brachiaria decumbens*, 12 t ha^{-1} dry applied annually

3 Mulch of dry weed, 4.44 t ha^{-1}; top biomass of in situ weeds were used through repeated cuttings.

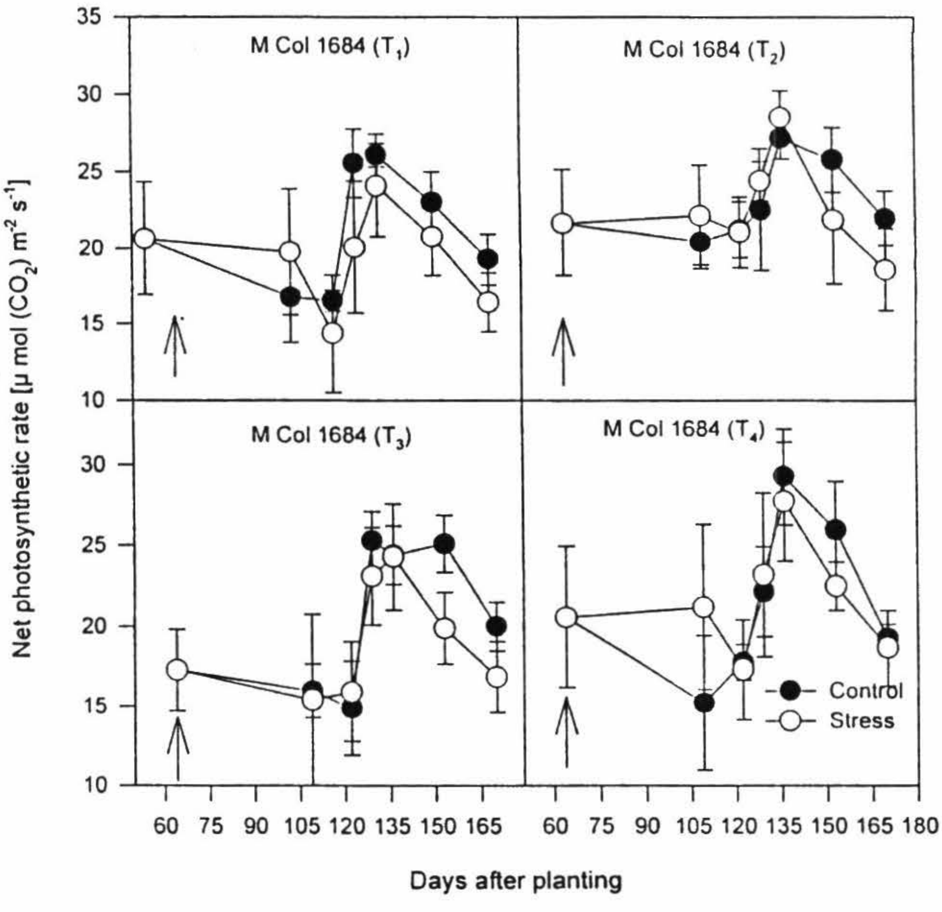

Fig. 4.1 Effects of the quality of planting material and water stress on leaf photosynthesis. ↑ Initiation of stress. Stakes were obtained from mother plants on the 13th cycle of a long-term NPK trial that received annually the following treatments: T_1 = 0,0,0 NPK; T_2 = 50,50,50 kg ha^{-1} NPK; T_3 = 100,100,100 kg ha^{-1} NPK; T_4 = 100,100,0 kg ha^{-1} NPK. Bars ± sd.

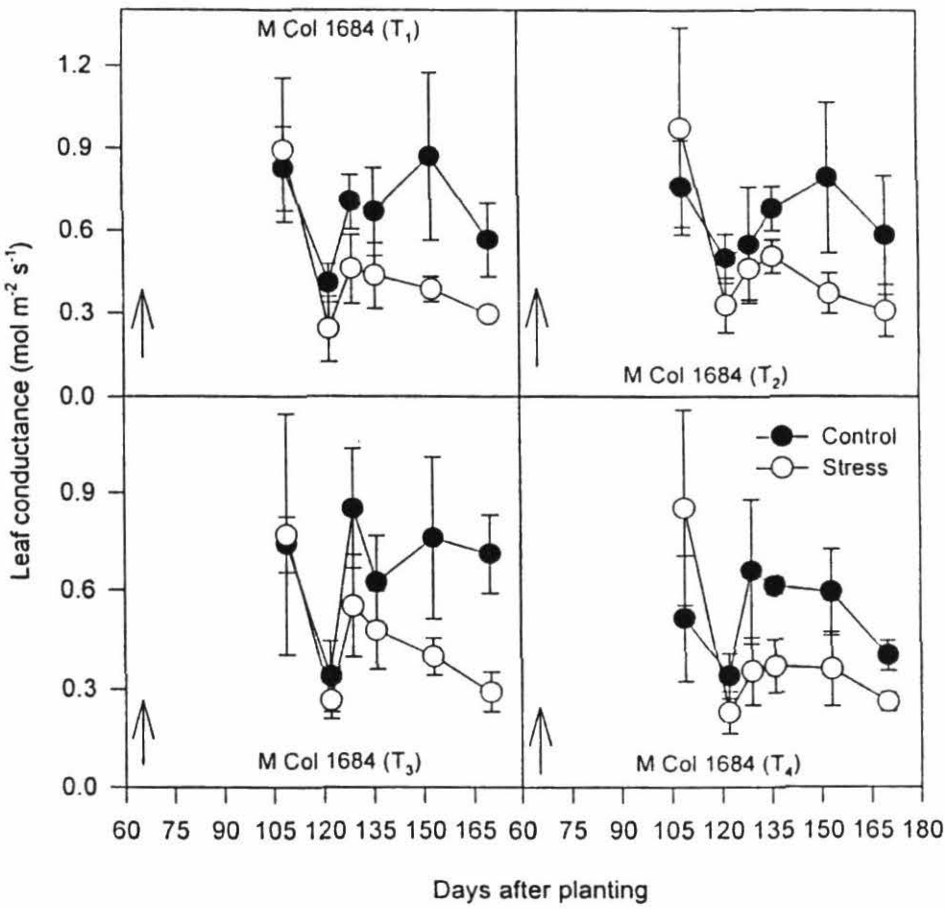

Fig. 4.2 Effects of the quality of planting material and water stress on leaf conductance. ↑ Initiation of stress. Stakes were obtained from mother plants on the 13th cycle of a long-term NPK trial that received annually the following treatments: T_1 = 0,0,0 NPK; T_2 = 50,50,50 kg ha^{-1} NPK; T_3 = 100,100,100 kg ha^{-1} NPK; T_4 = 100,100,0 kg ha^{-1} NPK. Bars ± sd.

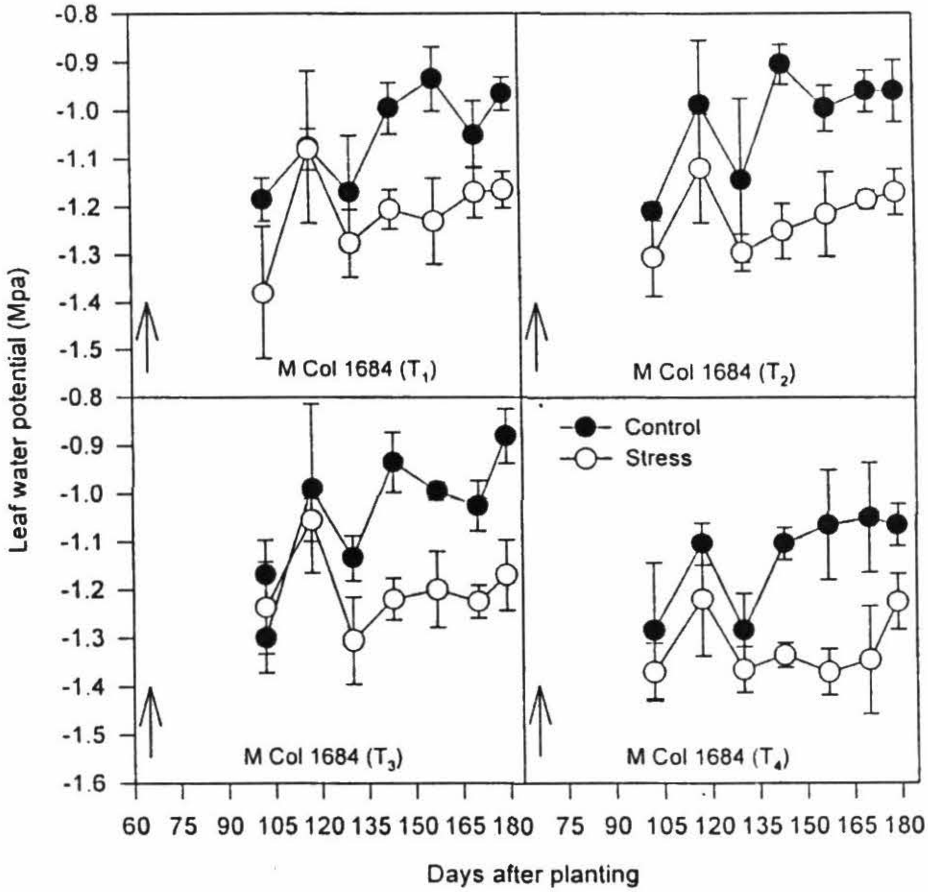

Fig. 4.3 Effects of the quality of planting material and water stress on midday leaf water potential. ↑ Initiation of stress. Stakes were obtained from mother plants on the 13th cycle of a long-term NPK trial that received annually the following treatments: T_1 = 0,0,0 NPK; T_2 = 50,50,50 kg ha^{-1} NPK; T_3 = 100,100,100 kg ha^{-1} NPK; T_4 = 100,100,0 kg ha^{-1} NPK. Bars ± sd.

4.2 Output 2.2 Quantification of soil degradation in cassava based systems in Andean hillsides and development of improved and productive conservation measures

Activities developed in this area of research were continued on the bases of an ongoing research effort in the Cassava Program that started in the late seventies. Since 1990, research was developed within the framework of a special project.

In 1996 the Special Project on Soil Degradation and Crop Productivity Research obtained funding for a two year prolongation phase to conclude a ten years period of research activities.

In this final phase of the project, major emphasis is put on further characterizing rainfall and soil water balance, on management related indicators of erodibility, on socioeconomic implications and on possibilities to improve adoption levels. Sustained support to collaborating institutions and joint development of training and dissemination activities also form part of the agenda. Access to data and results will be facilitated by the creation of a single Mega-database and a practical manual.

Due to the partitioning of project outputs in several CIAT projects, research results on soil health indicators, erosion-productivity relationships and on economic aspects are reported in separate project reports.

4.2.1 Measurement of rainfall characteristics for R-factor calculations and intensity-energy relationships

Erosivity, the capacity of rainfall to cause erosion, is best estimated by direct measurements of a rainstorms energy load. The data base for these measurements however, is limited to a few regions and no data on the energy-intensity relationships exist for the Andean Zone of northern South America.

Aggressiveness or erosivity of rainfalls can, however only be calculated reliably, if intensity-energy equations are available for the region.

First rainfall measurements with an electronic raindrop sensor computer system (Distrometer) were done in 1994 and suggested no major differences between the typical Andean site and relationships found in the temperate zone of North America. The dataset so far analyzed are however, too small a sample to allow conclusions.

Additional measurements were therefore initiated August'96 in Santander de Quilichao and will be carried out throughout 1996 and 1997 to examine the energy-intensity relationships of rainfall in the Andean zone and to base erosivity (R-factor) calculations on a solid and sufficiently representative set of data. Parallel to this measurements splash, is measured and related to rainfall energy, intensity and drop size distribution.

4.2.2 Relating soil water balance and-status to cropping technology

Monitoring of soil conservation and management practices with respect to runoff, infiltration and subsequent effects on water conservation, water retention, water distribution and availability in the soil profile is required to give a complete picture of conservation practices.

On one hand, knowledge on relationships between soil water-dynamics and conservation technology are important for making better use of rainfall for crop production. On the other hand, differences in soil water dynamics, related to cropping systems and conservation technologies in hillsides, affect the supply of drinking water from wells and creeks and hence merit a deeper understanding.

In order to get the respective informations from soil profiles, TDR-probes (Time Domaine Reflectometry) are installed in erosion plots to monitor soil water together with runoff, erosion and crop performance in long term cropping and management treatments.

Due to persistent difficulties to properly install the probes (0-120 cm) to the adequate depth in this clay soils, preliminary data are not expected before March 1997.

4.2.3 Relating erodibility indicators to soil/crop management

Besides rainfall, slope, cover and management, the physical, chemical and biological properties of soils are important factors determining the risk of soil loss. The Universal Soil Loss Equation, one of the most widespread models to predict soil losses, considers mainly soil texture, infiltration capacity and soil organic matter (SOM) to express a soils susceptibility to erosion. Nevertheless changes in soil erodibility due to management practices often occur before significant changes in those parameters can be measured.

Based on this observation research activities were initiated to detect early indicators for soils changes in erodibility (eg. fractions of SOM) and which are sensitive enough to reflect the trends induced by specific soil/crop management practices.

First experiences, with hot water extractable carbohydrates (HWCH) revealed, that correlation coefficients of HWCH content with stability indicators like Mean Weight Diameter and proportion of water stable aggregates > 2 mm were not superior to those obtained with total soil organic matter. On the other hand cropping treatments could not be differentiated by soil organic matter content but were well reflected by values of the relationship of HWCH to SOM. Further in detail studies on soils with similar SOM content are required.

4.2.4 Cooperation with national institutions in conservation technology development and transfer

Collaborative work with institutions engaged in soil and water conservation, working in close cooperation with farmers, rural schools and farmers groups in the last year concentrated on four main areas:

a) Characterization and development of barrier components.
b) Joint maintenance and establishment of demonstration trials in the northern Cauca Department.
c) Identification and development of local pilot activities linking opportunities for income generation to improved soil and water management.
d) Participation in training courses, seminars and in the development of training materials.

With regard to barriers, comprehensive data on the performance of grasses under rainfed field conditions on acid, low P-soils in the tropical, subhumid mid altitudes (1100-1600 m.a.s.l.) in now available for *Vetiveria zizanioides*, *Cymbopogon nardus, Pennisetum purpureum "Mott", Axonopus scoparius, Tripsacum sp.* and *Leptocoryphium lanatum*. Data from "El Pital", municipality of Caldono, Cauca are presented in **Table 1.**

Table 1. Results from long term evaluation of barrier hedgerows on a farmers field in "El Pital", 1450 m.a.s.l. [1)]

Grass species	Annual yield Mg DM.ha^{-1} [2)]	Seasonal fluctuations	Labour requirements [3)] per cut in Mandays.ha^{-1}
Citronella grass (Cymbopogon nardus)	5,074	3,4 - 7,0	2,54
Vetiver grass (Vetiveria zizanioides)	2,636	2,0 - 3,1	2,36
Dwarf Elephant grass (Pennisetum purpureum "Mott")	8,813	6 - 11,4	2,61

1) Mean of 4 rep., 9 m rows, sampled in 3-4 month intervals between 12/94 and 5/96.
2) One ha refers to 1000 lineal meters/ha or a barrier every 10 m across the slope.
3) Vetiver cut with sickle, others with machete.

Erosion-productivity trials carried out in the Pescador area in collaboration with FIDAR (Fundación para la Investigación y el Desarrollo de la Agroindustria Rural) on soils developed from volcanic material (> 14% Soil Organic Matter) produced surprisingly low levels of soil loss on slopes of 26-30% inclination. Two bean crops fertilized with 3 tons of commercial chicken manure produced 4,36 and 1,83 Mg of dry soil loss per hectare with conventional tillage, less than 1 Mg with grass barriers every 10 m and less than 0,5 Mg per ha with mulch and minimum tillage. Yield levels were not affected by minimum tillage and slightly higher incidence of diseases was controlled by spraying fungicides.

As a result of the cooperation with CETEC (Corporación para Estudios Interdisciplinarios y Asesoría Técnica) and a farmers community in the municipality of Buenos Aires, Cauca, two minor projects to induce more sustainable land management have been developed, building on results from previous experimentation with farmers in the area. Both projects focus on new opportunities for income generation, developed from components for improved soil management on hillsides. In the first case, small processing units for broom manufacturing will be financed through a rotating fund to add value to barriers of Partiña brooms-grass. In the second case, small scale egg production, based on local feed resources, derived from diversification of cassava cropping systems with introduced multipurpose cover crops like Vigna sp. and rotation or hedgerow crops is envisaged.

Collaboration with CVC, a semiofficial, regional corporation for natural resources management, concentrated on the development of concepts for low cost revegetationing of small gullies and technical and logistic support to a research oriented pilot activity.

4.2.5 Conservation technology development and measurement of the impact on crop production

Development of soil conserving and at the same time productive conservation technology is required to increase levels of adoption and to reduce the gap between farmer's economic logic and ecological considerations.

Study of trade offs between conservation effectiveness and productivity are therefore an integral part of technology development carried out on runoff-erosion plots.

Preliminary results from two cropping cycles with maize (Sikuani - V110) following two years of contrasting crop rotations and a cassava crop are shown in the next table.

Not considering the bare fallow treatment, soil losses were highest in cassava and with sole maize in a conventional rotation scheme; intermediate losses occurred in maize with chicken manure and low to very low losses with undersown *Chamaecrista rotundifolia* forage legume, minimum tillage and Vetiver grass barriers planted at 8 m distance. Surprisingly low values were also produced with maize in treatment 8. Grass/legume ley elements after 1-2 years still exerted an effect on structural stability.

Cost of erosion control in terms of maize yield were relatively high for Vetiver grass barriers (-38%) and for undersown Chamaecrista forage cover (-27%) and very low or non existent with minimum tillage/mulch and the residual effects of a biannual grass/legume forage crop (treatment 8).

Results in Mondomo (not shown) followed the same tendencies, however *Ch. rotundifolia* did not well adapt to higher altitudes and continued chicken manure applications had a pronounced effect on reducing soil losses in these soils.

Table 2. Preliminary results on dry soil losses and cumulative maize grain yields for the first and second 1995 cropping season with different production systems and rotations in Santander de Quilichao (5/95 - 3/96).[1]

Features of long term treatments	Cropping treatment 1st and 2nd season	Soil loss in Mg x ha^{-1}	se	Maize grain yield Mg ha ha^{-1}	se
1. Bare fallow	Bare fallow	**212,14**	(24,5)	-	-
2. Crop rotation with chicken manure; 2 Mg ha^{-1} and crop.	Maize, manure	**4,48**	(1,22)	**6,91**	(0,98)
3. Continuous cassava [2]	Cassava	**11,97**	(6,86)	**22,58** [3]	(0,55)
4. Crop rotation, minimum tillage, grass/legume ley in 92-94.	Maize, mulch Min. tillage	**0.85**	(0,12)	**6,24**	(0,37)
5. Crop rotation (weed fallow 92-94)	Maize	**12,88**	(3,2)	**6,28**	(0,59)
6. Crop rotation, chicken manure, Vetiver barriers.	Maize, manure Vetiver barriers	**0,34**	(0,01)	**3,88**	(0,40)
7. Crop rotation including a legume component.	Maize, undersown Ch. rotundifolia forage	**1,73**	(0,18)	**4,56**	(1,13)
8. Crop rotation, grass/legume ley in 92-94.	Maize	**1,42**	(0,24)	**6,91**	(0,89)

1) Mean slope 10%.
2) In 1994/95 all treatments, except "bare fallow" were planted to cassava.
3) Cassava fresh roots.

4.2.6 Documentation of results

Past and recent results of project work were summarized and published in technical journals, conference proceedings and with poster presentations at an international conference.

4.3 Output 2.3 Integrated crop/soil management for sustainable cassava-based production systems in Asia developed and tested through FPR (Sasakaw-sponsored).

4.3.1 Collaborative research with national programs on technology components concerning soil fertility maintenance and erosion control as well as other cultural practices.

Through research contracts of $2000-4000 per year, national programs in Thailand, Indonesia, Vietnam, China and the Philippines are conducting in collaboration with CIAT cassava agronomy experiments mainly in the area of soil fertility maintenance and erosion control.

In 1996 long-term NPK trials were continued in eight locations in three countries, most having completed 4-8 consecutive cropping cycles. A significant response to N was obtained in six, to K in seven and to P in three locations, indicating the importance of K fertilization for long-term soil fertility maintenance. In most soils, N application to cassava is also essential to obtain high yields, while P application is seldom necessary in the cassava growing areas of Asia. Maintaining soil fertility by biological means, i.e. through green manuring, mulching, intercropping or alley cropping, was studied in Thailand and Vietnam. Green manuring or intercropping (and mulching after 2 months) with *Crotalaria juncia* or *Canavalia ensiformis* appeared promising in Thailand, while intercropping and mulching of cowpea or *Tephrosia candida* were most effective in Vietnam.

Various types of erosion control experiments were conducted in six on-station trials and five on-farm trials in four countries. In Thailand it was found that time of planting had a significant effect on erosion, with highest soil losses occurring when the crop is planted in the early wet season, as is done by most cassava farmers. Erosion losses were significantly reduced and yields increased when cassava was planted in the early dry season (Nov-Dec). On-farm trials showed that planting cassava at closer spacing, on contour ridges, and with adequate fertilization, was the best package of practices to reduce erosion and obtain high yields. Various intercropping practices were also found to reduce erosion, but intercropping with peanut was usually most effective, while also producing the highest net income. Live barriers of various grasses and legume species, planted as contour hedgerows, were also investigated. Most effective were barriers of vetiver grass and elephant grass, or double row barriers of *Flemingia* *congesta, Gliricidia sepium* and *Tephrosia candida*; these legume species also help to maintain or improve soil fertility if tops are cut off and mulched among cassava plants.

4.3.2 Farmer participatory research (FPR) to develop locally adapted soil conservation practices and enhance their adoption by farmers.

As part of this project the following activities were conducted in Thailand, Indonesia, Vietnam and China. A similar project is being initiated in the Philippines.

A. Rapid Rural Appraisal (RRA). These were conducted in various cassava growing areas in each country in order to better understand the bio-physical conditions, the farmers' cultural practices, their socio-economic situation as well as their needs and limitations. From the results of these RRAs 2-3 suitable pilot sites were selected in each country. A more formal socio-agronomic survey in the selected pilot sites is still pending.

B. Demonstrations. A large number of demonstration plots were planted by researchers on-station or at the pilot sites, in order to show farmers a wide range of technological options and their effect on yield (or income) and on erosion. Soil loss was measured by weighing the eroded sediments that had washed into plastic-covered channels below each plot. A field day was organized and farmers from the pilot sites were invited to visit these plots, to evaluate and discuss the advantages and disadvantages of each treatment, and to select a few options that they considered most useful for their own conditions.

C. Farmer Participatory Research. Participants farmers in each pilot site, with the help of researchers and extension agents involved in the project, set out simple erosion control trials to test some of the selected practices on their own fields. These erosion control trials also had plastic-covered channels below each plot, so farmers could see the effect of each treatment on erosion. The sediments that collected in these channels were weighed periodically to have a quantitative measure of erosion. During the growing season, the FPR team members visited and evaluated these trials together with the farmers. Since improved varieties and cultural practices are essential components in increasing yields and farmers' income, while often contributing to more rapid canopy closure and thus better soil protection against erosion, other trials, usually testing new varieties, intercropping systems, or fertilization practices, were also conducted by farmers in the pilot sites. At the end of the cropping cycle all trials were harvested and the results were discussed with the farmers. From the data on yield, production costs, net income and erosion, farmers were asked again to select best varieties and the most suitable practices, which would then be retested in the following year.

After the first year of FPR trials, farmers in the two pilot sites in both Thailand and Vietnam selected the planting of vetiver grass contour hedgerows (combined with intercropping cassava with peanut in Vietnam), as the most suitable practice to reduce erosion while maintaining high yields or income. However, since vetiver grass planting material is not always available, and the planting is costly and time-consuming, other potions are still being investigated. In Thailand, farmers are also testing the planting of cassava on contour ridges, the application of grass mulch, or intercropping with mungbean, sweet corn or squash. In Vietnam farmers are also testing double contour hedgerows of *Tephrosia candida* or *Tephrosia* and vetiver combinations, as well as intercropping with peanut, black bean, soybean or sesame.

In China and Indonesia the results of the first year's trials were difficult to interpret as most farmers had tested only 1-3 options which generally differed among farms. In China, the intercropping with peanut was quite effective in reducing erosion and produced the highest income. In the two Indonesian pilot sites cassava is always intercropped with maize. An additional intercrop of cowpea followed by peanut resulted in the highest net income, while contour barriers of *Calliandra calothyrsus, Flemingia congesta* or elephant grass tended to reduce erosion. These options are being retested in 1996/97.

In the various other FPR trials, farmers selected the most adapted new varieties, usually bases on root yield and starch content, but in Hainan island of China also considering typhoon tolerance, and in north Vietnam stake storability during the winter. In Vietnam they found that intercropping with peanut was more productive than intercropping with black bean or planting in monoculture, and that applications of N and K were more effective than those of P or large amounts of pig manure in increasing cassava yields.

4.3.3 Fifth Regional Cassava Workshop

This workshop will be held in Hainan island of China in Nov. 1996, with the objective of bringing together the cassava researchers in Asia to present and discuss the progress made in breeding and varietal dissemination, in agronomy research, and in the FPR projects. The activities and the results so far obtained in these projects will be critically evaluated in an attempt to improve the effectiveness of the project and to enhance the wider adoption by farmers of those practices that were found to be most effective.

5. Subproject 3. Purpose: ***To enhance adoption of technology, strengthen NARS and link with advanced institutions.***