PROGRESS REPORT
2000 - 2001

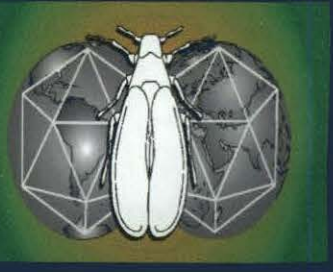
Sustainable Integrated Management of Whiteflies through Host Plant Resistance

CIAT - Cali, Colombia
Anthony C. Bellotti, Joe Tohme

Crop and Food Research, Levin, New Zealand
M. Dunbier, G. Timmerman

Funding Agency: MFAT, New Zealand

System-wide Programme on
Integrated Pest Management



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PROGRESS REPORT

Title: Sustainable Integrated Management of Whiteflies through Host Plant Resistance

Collaborating Institutions: CIAT – Cali, Colombia
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Project Purpose:

To reduce crop losses due to whitefly feeding damage and whitefly-transmitted viruses, and prevent further environmental degradation and food contamination due to excessive pesticide use, leading to a more productive and sustainable agricultural system.

Project Objectives:

1. To identify and access exotic or novel genes and gene combinations which can contribute to germplasm enhancement for whitefly resistance in cassava.

2. To study the genetics of resistance and to map genes for whitefly resistance in cassava and develop molecular markers for their incorporation into improved African, Latin American and Asian germplasm.

3. To develop crop management options for reducing whitefly populations, and the transmission of whitefly transmitted viruses.

Project Summary:

Whiteflies are considered one of the world's major agricultural pests, attacking a wide range of crop hosts and causing considerable crop loss. Whiteflies are one of the most difficult pests to control, especially when trying to use chemical pesticides. They rapidly acquire resistance to pesticides and their short life cycles (30 to 35 days) make chemical control economically difficult for resource scarce small farmers. Whiteflies are particularly damaging to crops in the tropical and subtropical regions of the world.

Eleven whitefly species are reported attacking cassava (*Manihot esculenta* Crantz) from Latin America, Africa and Asia. They cause damage to cassava as direct-feeding pests and vectors of plant viruses. There are three major species attacking cassava: *Aleurotrachelus socialis, Aleurothrixus aepim* and *Bemisia tabaci* (Bellotti et al, 1999) *A. socialis* and *A. aepim* cause considerable direct-damage yield losses in the northern part of South America and Brazil. *A. socialis* is predominant in Colombia, Venezuela and Ecuador, while *A. aepim* is found in high populations, causing yield losses in northeast Brazil. These areas are dominated by small cassava farmers with limited resources. *Bemisia tabaci*, the vector of African Cassava Mosaic Disease (ACMD), has a pantropical distribution, feeding on cassava throughout Africa, several countries in Asia, and more recently in the neotropics.

Host Plant Resistance (HPR) offers a sustainable solution for reducing whitely damage; however, whitefly resistance in agricultural crops is rare. A recent review of the literature indicates that whitefly resistance has been evaluated across a wide range of crops including vegetables, fruits, legumes, cotton, melons, tabacco, potato, squashes and alfalfa. Results show that only low levels of resistance, often expressed as crop or varietal "preference vs. non-preference," have been observed.

The high levels of resistance that we are finding in cassava germplasm are unique. The research on HPR at CIAT, with support from New Zealand's MFAT as part of the CGIAR Systemwide Whitefly IPM Project, is an invaluable contribution to achieving and understanding the mechanism of whitefly resistance in agricultural crops. This unique research on the genetics of this resistance could lead to important advances in achieving HPR, not only in cassava, but also other important agricultural crops.

Over the last 4 years, CORPOICA, the Colombian Institute of Agronomy, has been evaluating high-yielding, whitefly-resistant hybrids developed by CIAT at CORPOICA field research stations. The hybrid CG 489-31 is a progeny of a MEcu 72 (Resistant) x MBra 12 (Tolerant) cross (See 1999 Progress Report; MFAT), which combines high yield, whitefly resistance, and excellent eating quality. It will be released to farmers in Colombia during 2001. The hybrid is presently being multiplied so that sufficient planting material (stakes or cuttings) will be available to farmers.

The CIAT cassava germplasm bank contains 6000 landrace varieties collected mostly from farmers' fields across the neotropics, the origin of *Manihot esculenta*. More than 5000 varieties have been evaluated for whitefly resistance at several sites in Colombia. Approximately 13 varieties have been selected with moderate to high levels of resistance, and form the basis of our whitefly resistance breeding program (See Table 1). MEcu 72 continues to be our most resistant variety.

An outbreak of the frog-skin virus disease at CIAT and other regions of Colombia rendered evaluations of the remaining varieties (approximately 1000 varieties) difficult during 2000. Frog-skin infected varieties cannot be moved off of the CIAT station and must be destroyed. Nonetheless, during 2000 approximately 450 cassava cultivars were field evaluated for whitefly resistance and the results are included in this report. Many of the cultivars are hybrids from the cassava germplasm development program. In spite of high whitefly populations and heavy selection pressure, approximately 7% (30 cultivars) had very low damage ratings and more than

20% (91 cultivars) displayed low to moderate levels of resistance. These results indicate that whitefly resistance is being successfully introduced into cassava germplasm, and eventually will be available to small farmers in developing countries where cassava is a major staple.

Table 1. Cassava germplasm[1] evaluated for whiteflies resistance from 1992 to 2000 at different localities in Colombia.

Locality	No. Clones Evaluated[2]	No. Promissory Clones	No. Selected Clones	No. Advanced Clones
CIAT-Palmira	6872	1511	109	7
Espinal-Tolima	3030	359	111	6
Villavicencio-Meta	167	61	3	---
Pivijay-Magdalena	124	12	2	---
Total Germ. Eval.	>5000	845	225	13

[1] = CIAT germplasm bank about 6000 clones.
[2] = Some clones evaluated more than once.

In addition, evaluation of previously selected resistant germplasm was carried out in the laboratory under controlled conditions, to determine whitefly resistance mechanisms. Several varieties, including MEcu 64, MEcu 72, MPer 335 and MPer 415 provoked high mortality of whitefly nymphs, indicating an antibiosis type of resistance mechanism. Studies are now underway to determine the compound or compounds in cassava leaves responsible for nymphal mortality.

Of the 5363 genotypes that have been field evaluated for whitefly resistance, 1466 genotypes (27%) have received damage ratings below 3.5 (on a 1-6 damage scale) and 479 (8.9%) are below 3.0. Future resistance screening will also concentrate on this group to eliminate escapes and identify additional sources of resistance.

At present our most resistant varieties are MEcu 72, MPer 335, MEcu 64, MPer 415, MPer 317, MPer 215, MPer 221, MPer 265, MPer 266 and MPer 365. The most resistant hybrids include CG 489-4, CG 489-34, CG 489-31, CG 489-23, CM 8424-6, CM 8424-33, and CM8424-4.

Due to the importance of the whitefly as a pest and virus vector, it is necessary to know about the nature of genes that confer resistance to whiteflies. Therefore, the F1 segregation of crosses made with MEcu 72, the resistant genotype, using molecular markers, would help isolate and identify resistant genes. Two hundred and eighty two seeds from a MEcu 72 and a MCol 2246 (susceptible) cross were planted in pots and evaluated in the greenhouse. Simple sequence repeats (SSR's) are being used to find markers associated with resistance for mapping and ultimately cloning of resistant genes. The SSR's are random repeat sequences across all eukaryotic genome. SSR's show high polymorphism, are locus-specific and multiallelic; they exhibit a Mendelian inheritance and are also codominant. A high percentage (>60%) of polymorphism was found between parents MEcu 72 and MCol 2246 and 130 polymorphic SSR's have been obtained from the parents. Segregation from the SSRs and greenhouse evaluation will contribute to the construction of a linkage map for whitefly resistance in cassava.

This research is critical to understanding the mechanisms of whitefly resistance, and combined with a knowledge of the genetics of resistance, will provide the basis for incorporating HPR for whitefly into agricultural crops.

Efforts are now underway to combine the resistance to the viral disease, such as ACMD, with resistance to the whitefly in cassava. ACMD free, virus resistant, germplasm has been brought to CIAT from IITA. Crosses are being made with whitefly resistant germplasm at CIAT. Progeny can be evaluated for whitefly resistance at CIAT, however materials will have to be sent to Africa for evaluation of virus resistance.

In addition, IITA scientists have now requested whitefly resistant germplasm from CIAT to introduce into African varieties. Resistant germplasm is presently being prepared in tissue culture form for introduction into Africa.

During February and March of 2000, both Joe Tohme and Anthony Bellotti had the opportunity to visit MFAT in Wellington and the Crop and Food Research in Levin, New Zealand. At MFAT/Wellington persons contacted included Dr. Keneti Faulalo, Dr. Steve Thompson and Dr. Helen Anderson. A brief presentation was made to each on the goals and activities of the MFAT-funded Whitefly HPR project. Emphasis was also given to the unique role of the project in the overall objectives of the Systemwide project.

At Crop and Food Research meetings were realized with the Chief Executive Dr. Mike Dumbier and numerous scientists including Drs. John McCollum, Ross Bicknell, David Teulon, John Marshall and Gail Timmerman. Future project collaboration was discussed and a seminar on Whitefly Resistance in Cassava was presented.

Present research being funded by the MFAT Cassava Host Plant Resistance Project consists of four major areas of activity:

1. Cassava germplasm evaluation to identify sources of whitefly resistance in landrace varieties from the CIAT cassava germplasm bank.
2. Identification of genomic regions responsible for the expression of whitefly resistance in cassava.
3. Identification of whitefly resistance mechanisms in cassava.
4. Construction of a linkage map for resistance to whiteflies.

PROJECT REPORT: 2000

Introduction

Whiteflies, considered one of the world's major agricultural pests, are particularly damaging to crops in the tropical regions of the world. Eleven species are reported on cassava: *Aleurotrachelus socialis, Trialeurodes variabilis, Bemisia tuberculata, Aleurothrixus aepim, Bemisia tabaci, Bemisia argentifolii, Trialeurodes abutiloneus, Aleurodicus dispersus, Paraleyrodes* sp., *Aleuronudus* sp. *and Tetraleurodes* sp. The whitefly complex reported from other crops, such as vegetables, fruits, cotton and legumes, is too extensive to list. However, important species collected from the Andean region of South America include *Bemisia tabaci* and *Trialeurodes vaporariorum.*

B. tabaci has a pantropical distribution, feeding on numerous crops throughout the tropical regions of the world. If feeds on cassava throughout Africa where it is the vector of ACMD (Africa Cassava Mosaic Disease, caused by several geminiviruses), and is also reported from India and Malaysia. Since the early 1990's a new biotype (B) of *B. tabaci*, considered by some a separate species (*B. argentifolii*) has been found feeding on cassava in the neotropics. Recent reports, and personal observations, indicate that *B. tabaci* is feeding on cassava in several areas of Brazil, Northern South America, Central America and the Caribbean. Although ACMD has not been reported from the Americas it is considered that ACMD now poses a more serious threat to cassava production, as most traditional cultivars in the regions are highly susceptible to the disease. In addition, the *B. tabaci* biotype complex is the vector of several virus of crops, especially vegetables and legumes, that are often grown in association with cassava, posing a potential threat for these viruses to move to cassava.

Whiteflies cause direct damage to cassava and other crops by feeding on the phloem of leaves (Photo 1), inducing chlorosis and leaf fall, which can result in crop loss. Yield losses of this type are common owing to *A. socialis* and *A. aepim*, feeding on cassava in Colombia, and Brazil respectively. There is a correlation between duration of whitefly attack and root loss; losses over 70% have been reported from Colombia, and over 40% from Brazil.

Two cassava viruses are known to be transmitted by whiteflies. ACMD is caused by several geminiviruses transmitted by *B. tabaci* ACMD is reported causing crop losses of 28-40% (159, 160) from all African cassava producing countries. Resistance to ACMD has been introduced into cassava germplasm; however outbreaks of the disease still occur in regions of Africa. Severe crop losses due to ACMD have occurred in recent years in East Africa, especially in Uganda. *Bemisia tuberculata* is the reported vector of cassava frog-skin disease in the neotropics. This disease is causing considerable yield loss in northern South America, especially in Colombia. The combination of direct whitefly feeding damage and its threat as a vector of virus diseases, both resulting in considerable crop losses, justify a continued strong research effort on whiteflies of cassava.

Complementary to the research in cassava to determine and employ resistance to whiteflies, a project was designed to determine the complex of indigenous South American parasitoids and other natural enemies. After nearly three years of surveys (in several countries such as Colombia,

Ecuador and Venezuela) numerous parasitoid species (more than 10) have been identified. Several of these are new or unrecorded species and are being identified by taxonomists. In addition studies are in progress to determine the efficacy of these parasitoids and their interaction with whitefly resistant germplasm. The results of these surveys for natural enemies are not included in this report but they are available upon request.

Photo 1. Whitefly (*A. socialis*) adults feeding on the under surface of young, apical cassava leaves.

PRESENT RESEARCH: 2000

Activities and Results

Whitefly populations on cassava during the 1999-2000 growing season continued to remain high at CIAT. As studies during 1999 (Photo 2) (See IP3 Annual Report, 1999) indicated, the predominant species is *Aleurotrachelus socialis*, which accounts for 98.5% of the population. *Bemisia tuberculata* and *Trialeurodes variabilis* account for the remaining 1.5%. Whitefly populations were so high and extensive (all cassava plots/fields were heavily infested) throughout the CIAT farm that it was impossible to carry out experiments on other pests or almost any other type of cassava experiments (i.e. agronomic or physiological). In addition, the species *B. tuberculata* is reported as a vector of cassava frog skin virus disease. This disease is also endemic at CIAT, with high incidence and most varieties/fields being infested.

The combination of high whitefly populations and frog skin disease has rendered cassava field research at CIAT impractical. Land outside of CIAT, in areas where there is scarce cassava plantings and low whitefly populations, has been obtained to produce pest and disease free cassava for entomological experimentation. Clean cassava plants are required for host-plant resistance mechanism studies with *A. socialis* and *B. tabaci* and for the maintenance of pest and parasitoid studies with mealybugs and whiteflies and predator research with mites.

Whitefly population eruptions and epidemiology is not well documented and understood. *A. socialis* has been observed and collected on the CIAT farm for more than 25 years. However it is only in the past 5 years that populations have reached epidemic proportions. This could be due to several factors, some not well understood:

- Most cassava germplasm planted at CIAT is susceptible.
- Cassava has been continually grown at CIAT for more than 30 years.
- Environmental conditions, especially adequate to high rainfall, are favorable for whitefly population increases.
- The staggered planting pattern followed at CIAT, where cassava plantings are programmed almost continually throughout the year, provides a continuum of young, vigorous foliage that is preferred for oviposition and feeding.
- Pesticides use in the Germplasm Bank and on other experimental fields may have caused an unbalance in the pest-natural enemy relationship.
- A new "biotype" of *A. socialis*, that is particularly aggressive and with a high reproductive capacity may have been inadvertently introduced into the region.

The third factor; the high rainfall pattern of recent years is considered to be the major factor for increased populations. Number 6, the introduction of a new biotype is least favored of the reasons indicated.

Photo 2. The pupal stage of the whitefly species *Aleurotrachelus socialis* and *Trialeurodes variabilis* on cassava leaves.

Activity I. Biology of *Aleurotrachelus socialis*

There is conflicting information in the literature on some aspects of *A. socialis* biology. This is especially true for female ovipositional rates which are reported at 116 eggs per female throughout its 12 day adult duration.

A. socialis eruptions and extremely high populations, observed in recent years at CIAT and other localities, indicate that ovipositional rates may be higher than reported.

A small experiment was designed to try to more accurately measure female *A. socialis* oviposition. A 9x 1.5cm (diameter x height) plastic petri dish, with a perforated lid (to prevent moisture build up, which can cause adult whitefly mortality) was filled with agar to within 2mm of the top. Upon cooling, a cassava leaf portion (Var. MCol 1468) was placed on the agar. A pair of recently emerged whitefly adults was introduced into each petri dish, in the laboratory (25±2°C & 70±5%RH). Three hundred replicates of each treatment were evaluated for oviposition for 30 days.

Females oviposited (Photo 3) for a maximum of 18 days. Previous report indicated a 12-day adult duration. Maximum and minimum ovipositional rates varied considerably indicated by the high standard deviation. Accumulated oviposition reached a high of 244 eggs, an average of 181 and a minimum of 155 (Table 2) (Figure 1). These results show that *A. socialis* oviposition is higher than previously recorded (116 eggs per female) and partially accounts for the rapid population builds-up we observed in the field.

Photo 3. Whitefly adult ovipositing on the undersurface of cassava leaves.

Table 2. Accumulated ovipositional potential of *A. socialis* females on cassava (Var. MCol 1468) under laboratory conditions.

Day	1	2	3	4	5	7	8	9	10	11	12	13	14	15	16	17	18
Average Oviposition	0.2	11.8	18.9	31.6	67.4	64.5	71.4	121	130	90.33	110.9	100.4	126.3	126.9	124	106.3	181
Maximum Oviposition	1	18	39	66	91	112	125	144	128	145	177	182	184	203	212	147	244
Minimum Oviposition	0	8	9	11	40	29	38	99	72	47	64	74	117	75	96	65	155
S.D	0.4	3.7	10.6	19.9	14.9	34.4	25.9	15.6	21.6	42.2	34.7	31.7	32.2	44	37	33.6	46.3
N	10	9	9	8	9	8	8	10	9	9	11	9	9	10	8	4	4

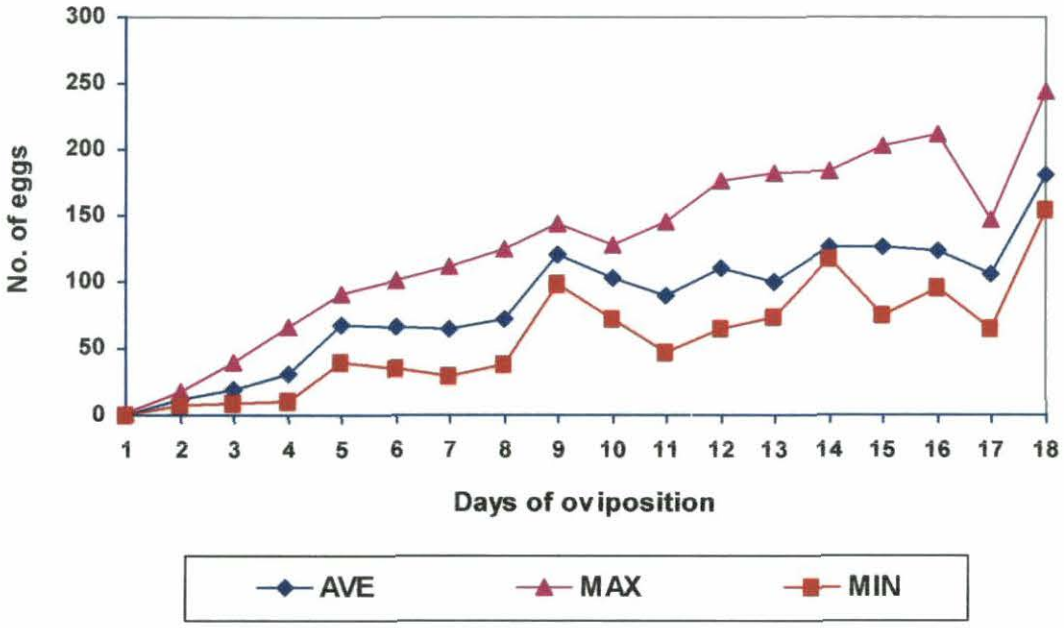

Figure 1. Accumulated oviposition of *Aleurotrachelus socialis* females on cassava varieties in the laboratory (26±2°C & 70±5%RH).

Activity II. Evaluation of cassava germplasm for resistance to *Bemisia tabaci*

In recent years, *B. tabaci*, the vector of African Cassava Mosaic Disease (ACMD), has been collected feeding on cassava in the neotropics. ACMD has not been observed in the Americas, and it has been speculated that its absence may have been related to the inability of its vector, *B. tabaci* to feed on cassava. Since the early 1990's a new biotype (B) of *B. tabaci* (considered by some investigators to be a separate species, *B. argentifolii*) has been found feeding on cassava in several areas of the neotropics, including Brazil, Ecuador, Colombia and several countries in the Caribbean region. ACMD, therefore, now poses a more serious threat, as most traditional varieties in the neotropics are susceptible to the disease.

During 1999 (See Previous Report) we were able to confirm, with greenhouse studies that the B biotype of *B. tabaci* will feed and reproduce on cassava. However it was difficult to establish the colony since *B. tabaci* was being collected from beans (*Phaseolus vulgaris*), a species very distant from cassava. It was therefore decided to establish a colony on Poinsettia (*Euphorbia palcherrima*). Poinsettia was chosen since, in addition to being a reported host of *B. tabaci*, it is of the Euphorbiaceae Family and shares commonalties with cassava.

A working colony was established on poinsettia by placing pupae from the bean colony onto poinsettia leaves in 1m x 1m nylon mesh cages in the greenhouse. Using this technique it was possible to establish a now flourishing colony on poinsettia. In collaboration with the virology unit, and using the RAPD's-PCR technique, it has been possible to generate molecular markers for whitefly biotype identification. The amplified products indicate a polymorphism between biotypes A & B of *B. tabaci*. Fragments of amplified DNA observed in biotype B were absent in A. It was therefore possible to confirm the establishment of biotype B on both beans and poinsettia.

The next step in the process was to see if *B. tabaci* could be established on a wild *Manihot* species, especially one closely related to cassava. Previous observations have indicated that whiteflies will readily colonize certain Manihot species under field conditions. In caged experiments in the growth chamber, 2 month plants of *Jatropha gossypiifolia* were exposed to *B. tabaci* adults from the poinsettia colony. Leaf cages, which had been employed in previous attempts to establish *B. tabaci* colonies were not needed.

The *B. tabaci* colony from poinsettia readily took to *J. gossypiifolia* plants and a colony was quickly established. This colony will be allowed to complete at least two cycles on *J. gossypiifolia* before transferring it to cassava. Preliminary observations indicate that this *J. gossypiifolia* colony will more easily establish on cassava.

As part of the MFAT whitefly resistance project a student from a local Colombia University has been contracted to do on MS degree thesis to evaluate *B. tabaci* feeding, oviposition and development on whitefly resistant (to the species *Aleurotrachelus socialis*) and susceptible cassava cultivars.

Activity III. Evaluation of cassava cultivars for resistance to *Aleurotrachelus socialis*

Cassava germplasm from several sources was evaluated for whitefly resistance during the 1999-2000 crop cycle at CIAT. Due to the heavy infestation of frog skin disease in CIAT germplasm, it was not possible to introduce this germplasm into the CORPOICA, Nataima station for evaluation at that site. Germplasm evaluated included core collection, crossing blocks, multiplication and yield trial materials. All were planted in collaboration with the cassava germplasm improvement project, sown at CIAT and subjected to high field populations of *A. socialis*. Emphasis was given to those varieties/cultivars that had not been previously evaluated, had few evaluations or had received very low damage ratings in previous evaluations. Approximately 450 materials were evaluated.

A considerable portion of the germplasm evaluated were hybrids (CM & SM) while others were from the core collection, mostly MBra (Brazil), MCol (Colombia), MEcu (Ecuador), MGua (Guatemala), and MMex (Mexico). The hybrids evaluated were developed for several ecosystems, such as the lowland tropics, acid-soil savannas and inter-andean valleys and also include materials for genetic mapping, whitefly resistance mapping, post harvest root deterioration and phytophthora and bacteriosis mapping.

Periodic evaluations for plant damage and whitefly populations were made throughout the crop cycle. A 1 to 6 damage scale (1 = no damage, 6 = severe damage) (Photo 4) is used to measure whitefly damage, and a 1 to 6 scale is also used to measure whitefly populations (Table 3).

Photo 4. Cassava leaf damage symptoms caused by whitefly (*Aleurotrachelus socialis*) feeding.

Table 3. Population and damage scales for evaluating cassava germplasm for resistance to whiteflies.

Population scale (nymphs and pupae)
1 = no whitefly stages present
2 = 1-200 individuals per cassava leaf
3 = 201-500 per leaf
4 = 501-2000 per leaf
5 = 2001-4000 per leaf
6 = > 4000 per leaf
Damage scale
1 = no leaf damage
2 = young leaves still green but slightly flaccid
3 = some twisting of young leaves, slight leaf curling
4 = apical leaves curled and twisted; yellow-green mottled appearance
5 = same as 4, but with "sooty mold" and yellowing of leaves
6 = considerable leaf necrosis and defoliation, sooty mold on mid and lower leaves and young stems.

Results show that whitefly populations at CIAT were extremely high and caused considerable selection pressure on the cassava clones. Of the 449 clones evaluated, more than 50% had a damage rating above 4.0, and 86% above 3.0 (Figure 2). However, in spite of the high selection pressure, 30 cultivars (6.6%) presented no damage symptoms, and 35 cultivars had low damage ratings (between 2.0 and 3.0) on the 1 to 6 damage scale. Ninety-one cultivars had a damage rating below 3.5, indicating that they will be re-evaluated in subsequent planting cycles.

In general the hybrid clones presented very low damage ratings (Table 4). This table includes those clones and cultivars that received a damage rating below 3.5 and will be re-evaluated. Approximately 64% of these are represented by hybrids (SM = 47, 51.6% and CM = 11, 12%) of the germplasm accessions, MCol represents 15% and MEcu and MPer 6% each. These results indicate that there is a good basis for resistance to whiteflies in Andean germplasm, a phenomenon that has been noted in previous evaluations. These results also reinforce previous observations that the cultivar MECU 72 continues to display high levels of resistance, even under high levels of whitefly selection pressure (Photo 5).

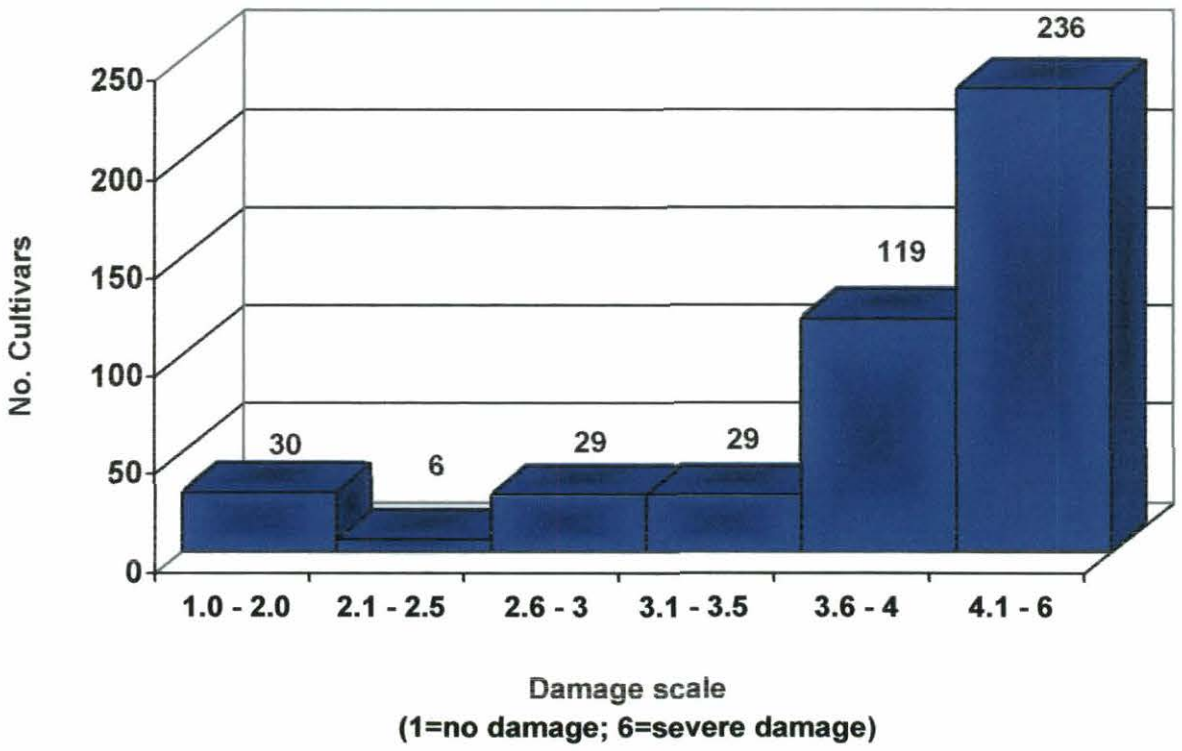

Figure 2. Evaluation of 449 cassava cultivars (hybrids and core collection varieties) for whitefly (*Aleurotrachelus socialis)* damage at CIAT/Palmira during 1999-2000 crop cycle.

Table 4. Cassava cultivars evaluated for whitefly (*Aleurotrachelus socialis*) damage during 1999-2000 crop cycle, at CIAT, with damage ratings below 3.5 (1=no damage, 6=severe damage).

Clone	Code	Damage Grade	Population Levels/Leaf				
			Adult	Eggs	Nymph	Pupae[1]	Pupae[2]
CM 6320 - 2	MULTN3	1.0	2.0	2.0	1.5	2.0	1.0
CM 8024 - 2	MULTN3	1.0	2.0	2.0	2.0	2.5	2.0
SM 1143 - 21	MULTN3	1.0	2.0	2.0	2.0	2.0	2.0
SM 1517 - 9	MULTN3	1.0	2.0	2.0	1.0	1.0	1.0
SM 1519 - 2	MULTN3	1.0	1.0	1.0	2.0	2.0	2.0
SM 1624 - 2	MULTN3	1.0	1.0	1.0	2.0	1.0	1.0
SM 1657 - 7	MULTN3	1.0	1.0	1.0	1.0	2.0	2.5
SM 1673 - 11	MULTN3	1.0	1.5	1.0	1.0	2.0	2.0
SM 1673 - 5	MULTN3	1.0	1.0	1.0	1.0	1.0	1.0
SM 1684 - 13	MULTN3	1.0	1.5	1.5	1.0	1.5	2.0
SM 1694 - 2	MULTN3	1.0	2.0	1.0	1.0	2.0	2.0
SM 1778 - 53	MULTN3	1.0	1.0	1.0	1.0	2.0	2.0
SM 1896 - 3	MULTN3	1.0	2.0	2.0	1.5	1.5	2.0
SM 2065 - 8	MULTN3	1.0	2.0	2.0	1.5	1.5	1.0
SM 985 - 9	MULTN3	1.0	1.5	1.0	2.0	2.0	1.0
MPer 385		1.5	2.0	2.0	2.5	2.5	2.0
SM 1788 - 16	MULTN3	1.5	2.0	2.0	1.0	1.0	1.5
SM 1955 - 6	MULTN3	1.5	2.0	2.0	2.0	2.5	1.5
SM 2069 - 2	MULTN3	1.5	2.0	2.0	2.0	2.0	2.0
CM 7951 - 5	MULTN3	2.0	2.0	2.0	2.5	2.5	2.0
CM 909 - 25	MULTN3	2.0	2.0	2.0	2.5	2.5	2.5
MBra 489	MULTN3	2.0	1.0	1.0	1.0	2.0	2.0
MCol 2019		2.0	2.0	2.0	4.0	4.0	3.0
MCol 297		2.0	1.0	1.0	2.0	2.0	2.0
MEcu 82		2.0	2.0	2.0	3.0	3.0	2.0
MEcu 72	CruzMap Whitefly	2.0	1.0	1.0	0.0	0.0	1.0
SM 1778 - 44	MULTN3	2.0	2.0	1.5	1.5	2.0	1.5
SM 1794 - 18	MULTN3	2.0	1.0	1.0	1.0	2.0	3.0
SM 1828 - 11	MULTN3	2.0	1.0	1.0	1.0	2.0	2.0
SM 1673 - 10		2.0	2.0	2.0	3.0	4.0	3.0
MCol 317		2.5	3.0	3.5	4.0	4.0	2.0
MPer 368		2.5	2.5	2.5	4.0	4.0	2.5
MVen 67 B		2.5	3.0	3.0	3.0	3.0	2.0
SM 1682 - 2	MULTN3	2.5	1.5	1.5	1.5	2.5	3.0
SM 1948 - 29	MULTN3	2.5	2.0	2.0	3.0	2.5	3.0
SM 2141 - 1		2.5	2.0	2.0	2.0	2.0	3.0
CM 1111 - 8	ERCIAT00	3.0	2.0	2.0	3.5	3.5	2.5
CM 5438 - 12	MULTN3	3.0	2.0	2.0	2.5	2.5	2.0
CM 7593 - 15	ERCIAT00	3.0	2.0	2.0	3.0	3.0	3.5
CM 8378 - 3	MULTN3	3.0	2.0	2.0	2.5	3.0	2.0

Clone	Code	Damage Grade	Population Levels/Leaf				
			Adult	Eggs	Nymph	Pupae[1]	Pupae[2]
MCol 113	Sterile male	3.0	3.0	4.0	3.0	3.0	4.0
MCol 1522		3.0	4.0	4.0	4.0	4.0	2.0
MCol 1795		3.0	3.5	5.0	5.0	5.0	2.5
MCol 2131		3.0	4.0	4.0	3.5	3.5	2.0
MCol 346		3.0	2.0	2.0	3.0	2.5	2.0
MCol 725		3.0	4.0	4.0	4.0	4.0	3.5
MEcu 151		3.0	3.0	3.0	4.5	4.5	2.5
MEcu 41		3.0	4.0	3.5	5.0	5.0	2.5
MEcu 43		3.0	3.0	3.5	3.0	3.0	3.5
MEX 71		3.0	3.0	3.0	3.5	3.0	3.0
MMex 108	MULTN3	3.0	2.0	2.0	2.5	3.0	2.5
MPer 183		3.0	2.0	2.0	2.0	3.0	3.5
MPHI 3		3.0	2.0	2.0	2.0	3.0	3.0
SM 1688 - 20	ERCIAT00	3.0	2.0	2.0	2.0	3.0	3.0
SM 1689 - 18	MULTN3	3.0	2.0	2.0	2.0	2.0	3.0
SM 1799 - 18	MULTN3	3.0	2.0	2.0	1.0	1.0	3.0
SM 1812 - 55	MULTN3	3.0	2.0	2.0	3.5	3.5	3.0
SM 1861 - 18		3.0	3.0	3.0	3.0	3.0	3.0
SM 1862 - 25		3.0	2.0	2.0	2.0	2.0	3.0
SM 1870 - 31		3.0	2.0	2.0	3.0	3.0	3.0
SM 1920 - 1	MULTN3	3.0	2.0	2.0	2.0	3.0	3.0
SM 1927 - 9	MULTN3	3.0	1.0	1.0	1.0	2.0	3.0
SM 1953 - 30	MULTN3	3.0	1.5	1.5	2.0	2.5	2.5
SM 1965 - 1	ERCIAT00	3.0	2.0	2.0	2.0	2.0	3.0
SM 909 - 25	MULTN3	3.0	2.0	2.0	3.0	3.5	2.0
CM 5655 - 4	Z4	3.5	2.0	2.0	3.0	3.0	3.5
CM 6370 - 2	MULTN3	3.5	2.0	2.0	2.5	2.5	3.0
CM 6787 - 4	MULTN3	3.5	2.0	2.0	3.5	3.5	2.5
*Brasilera		3.5	2.0	3.0	3.0	3.5	3.5
MCol 1722		3.5	4.0	5.0	4.5	4.5	3.5
MCol 1780		3.5	4.0	5.0	5.0	5.0	3.0
MCol 191		3.5	3.0	3.0	3.5	4.5	3.0
MCol 1964		3.5	4.5	5.0	5.0	5.5	3.0
MCol 2493		3.5	3.5	4.0	4.5	5.5	2.0
MCub 42		3.5	3.0	3.5	4.0	5.0	4.0
MDom 5		3.5	3.0	3.5	5.0	5.0	2.0
MEcu 171		3.5	2.5	3.0	4.0	4.0	3.0
MEcu 47		3.5	4.5	5.0	5.0	4.5	4.0
MFJI 6		3.5	6.0	4.5	5.0	5.0	2.5
MPer 209		3.5	4.0	4.0	3.5	3.5	3.0
MPer 372		3.5	2.0	2.0	2.5	4.0	3.5
MPer 488		3.5	4.0	3.5	4.0	3.5	3.5
SM 1468 - 9	MULTN3	3.5	2.0	2.0	2.5	3.0	2.5

Clone	Code	Damage Grade	Population Levels/Leaf				
			Adult	Eggs	Nymph	Pupae[1]	Pupae[2]
SM 1543 -16		3.5	2.0	3.0	4.0	3.0	3.0
SM 1602 - 13	MULTN3	3.5	2.0	2.0	3.0	3.5	3.0
SM 1642 - 20	MULTN3	3.5	2.0	2.0	3.0	3.0	3.0
SM 1754 - 21		3.5	2.0	3.5	2.5	3.5	3.0
SM 1754 - 46	MULTN3	3.5	2.0	2.0	3.0	3.0	3.0
SM 1779 - 8		3.5	3.0	3.0	3.0	4.0	3.0
SM 1780 - 27	MULTN3	3.5	2.0	2.0	3.5	3.0	3.0
SM 1868 - 29	MULTN3	3.5	1.0	1.0	2.0	2.0	2.0
SM 2160 - 2		3.5	2.0	3.0	3.5	3.0	3.5
SM 2216 - 12	ERCIAT00	3.5	2.0	2.0	2.0	2.0	2.0
SM 653 - 14	MULTN3	3.5	2.5	2.0	3.0	3.5	2.0

[1] Pupae on leaves of mid 1/3 of plant.
[2] Pupae on leaves of lower ½ of plant.

Photo 5. Whitefly (*A. socialis*) populations on two cassava varieties; MEcu 72 is the resistant variety with few whitefly adults and immatures is compared with a susceptible variety.

Activity IV. Whitefly resistance in cassava germplasm collection: A comprehensive evaluation

The research for resistance to cassava whiteflies (especially the species *Aleurotrachelus socialis)* has been an important segment of the cassava research program for several years. This research has been successful in identifying numerous cultivars with resistance to whiteflies (whitefly resistance in agricultural crops is rare) and in developing commercial hybrids with resistance.
From 1992 to present, 5363 genotypes have been field evaluated for whitefly damage/resistance at four different sites in Colombia (Figure 3). The bulk of these screening have been at two sites, CIAT and the CORPOICA station in Nataima, Tolima. The results of these evaluations, which include both plant damage and whitefly population ratings, are contained in a database and available to researchers. In the case of numerous (most) clones, more than one evaluation has been made; the highest score received is considered the most important and first that appears in the data bank.

Of the 5363 genotypes screened, 3897 (73%) have received a damage rating above 3.5 (1 = no damage, 6 = severe damage) and are considered susceptible (Figure 3). No further evaluations are planned for these materials. The remaining 1466 genotypes (27%) with damage ratings below 3.5 are considered "promising" and will continue to be evaluated. Emphasis will be given first to those materials with damage rating below 2.0 (479 or 8.9%). Most of these are probably escapes, i.e. selection pressure was not sufficiently high enough to get an accurate evaluation.

Approximately 44% of the materials evaluated are hybrids (Figure 4). This figure has increases considerably in recent years as more crosses and subsequent hybrids are being produced by the germplasm development project and many of these are screened for arthropod resistance, especially whiteflies, mites and thrips.

Germplasm (landrace varieties) from several other countries have also been screened and can be appreciated in Figures 4 and 5. Based on present results, since much of the whitefly resistant germplasm has originated in the Andean zone, increased emphasis will be given to accessions from Ecuador and Peru.

Landrace varieties have been collected from numerous countries, especially in the neotropics and these have been included in the CIAT germplasm bank. These accessions have also been systematically screened. The highest number are from Colombia 1030 (33.6%) of the 3038 accessions screened (Figure 4). Colombia is followed by Brazil (167 or 5.5%), Venezuela (118 or 3.9%) and, finally, Ecuador (115 or 3.8%).

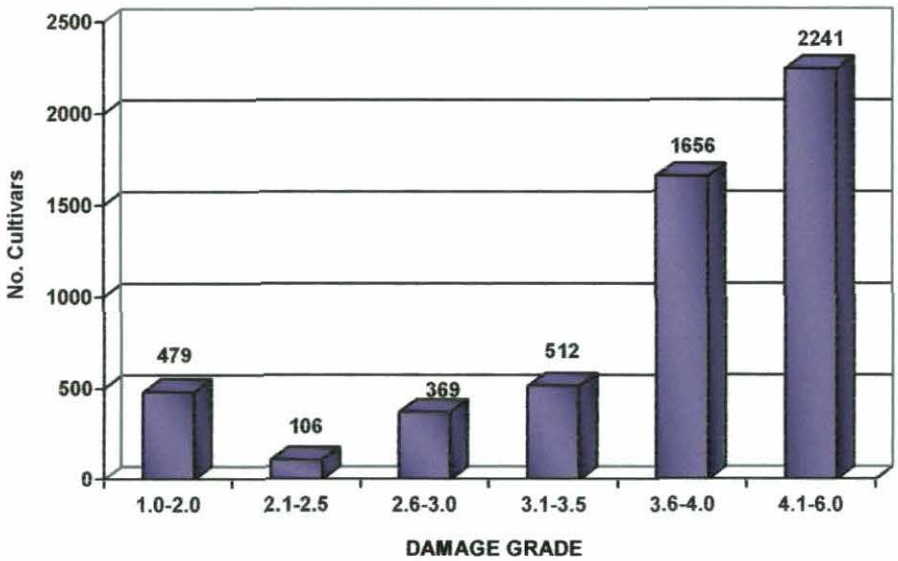

Figure 3. CIAT cassava germplasm evaluated in Colombia for resistance to whiteflies (*A. socialis*) from 1992 to 2000; damage scores are based on a 1 (no damage) to 6 (severe damage) rating scale.

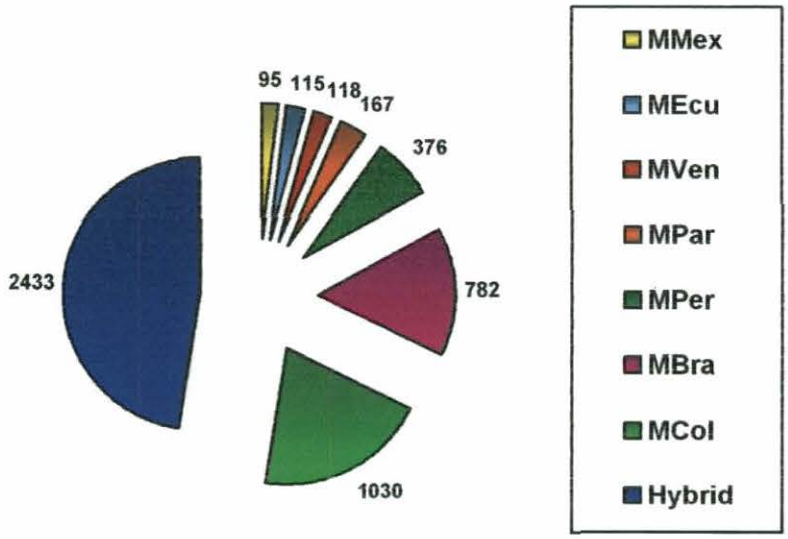

Figure 4. Cassava landrace cultivars collected from numerous countries, evaluated for whitefly (*A. socialis*) resistance from 1999 to 2000.

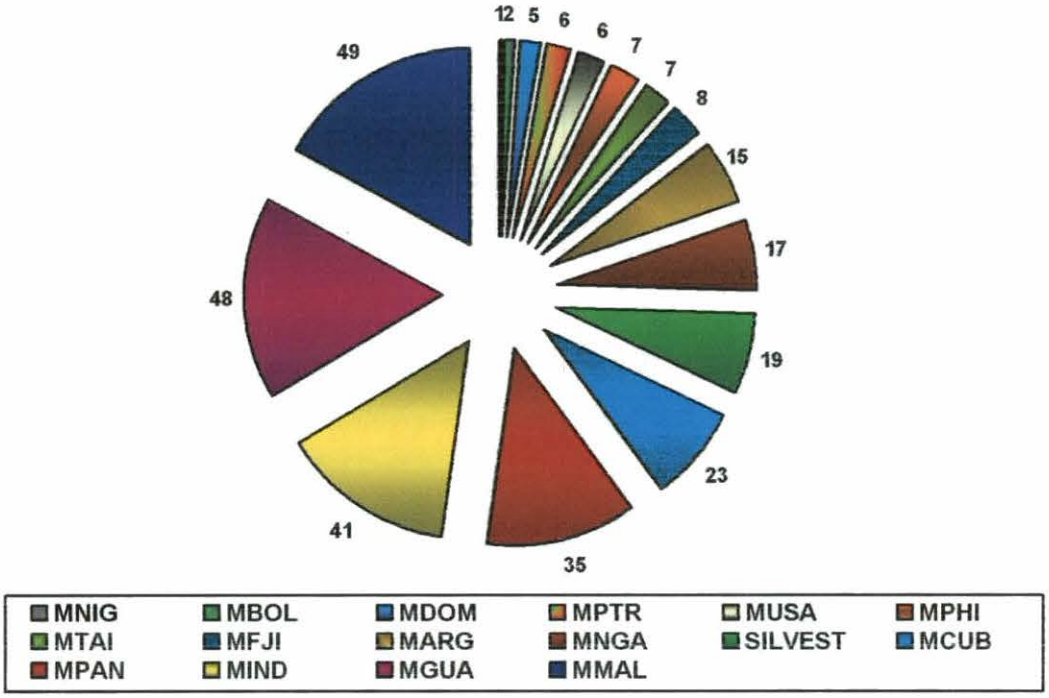

Figure 5. Cassava landrace cultivars collected from several countries, and CIAT-produced hybrids, evaluated for whitefly (*A. socialis*) resistance from 1999 to 2000.

Activity V. Whitefly resistance mechanisms studies in cassava

Field evaluations of cassava germplasm over the past several years have identified several varieties expressing moderate to high levels of resistance. During 2000, whitefly (*A. socialis*) biology and behavior was studied on six cassava varieties selected as resistant from field evaluations. The major objectives of this study were:

1. To determine the life cycle duration of *A. socialis* on 7 cassava genotypes.
2. To evaluate whitefly behavior, genotype interaction.
3. To measure whitefly survival (mortality) on resistant vs. susceptible cassava genotypes.

These studies were done at CIAT in growth (environmental) chambers where temperature (Average 27°C), humidity (68% RH) and photo period (12:12, daylight/night), were controlled throughout the experiment.

The cassava varieties selected for the evaluation were MEcu 72, MEcu 64, MPer 317, MPer 611, MPer 415, MPer 335, all resistant to *A. socialis* and the susceptible control variety CMC 40. Four potted plants of each variety were infested with whiteflies from the CIAT colony. Infestation was accomplished by attaching small (2.5 cm diameter) clip-cages (five per plant) to cassava leaves. Ten whitefly (*A. socialis*) females were introduced into each cage and allowed to oviposit for 24

hours after which cages and adults were removed. The whitefly infested plants were maintained in the growth chamber and watered regularly.

To study the biological cycle, 50 whitefly eggs per plant were selected and an "infestation map" was designed so that daily evaluations of eggs, nymphal instars and pupae could be easily accomplished. A total of 1400 whitefly individuals on 7 cassava varieties were constantly observed and evaluated throughout the experiment. Daily evaluations were made by observing the leaf undersurface with the aid of a stereo-microscope. In order to minimalize leaf damage and not disturb nor injure whitefly immatures, a method was devised to least disturb developing nymphs. The potted plants are inverted on an iron support ring attached to an iron rod that allows upward/downward movement for optimal positioning for observance with the stereo-microscope. A rubber plate inserted at the base of the plant stem at the soil line, prevents soil loss or plant movement and injury when the potted plant is inverted.

Daily observation noted changes in instars (molting) at each stage of whitefly development as well as individual mortality and the possible cause of mortality.

Results and Discussion

The total life cycle of egg to pupae of *A. socialis* on the 7 cassava varieties ranged from 33.9 to 37.4 days (Table 5). The duration of the four feeding stages, the first through forth nymphal stage ranged from 22.2 days (MEcu 64) to 27.1 (MPer 415). MEcu 64, MEcu 72 and MPer 317 showed the shortest nymphal cycle of 22.2, 22.4 and 22.2 days respectfully. The average duration including the egg stage was 34.6 days with an average range of 31 to 44 days (Table 6). The susceptible control CMC40 and the resistant variety, MPer 611 both had biological cycles above the average. In general, resistant varieties had a shorter biological cycle than the susceptible control. These data indicate that the biological cycle duration may not be a reliable measure to distinguish resistant varieties. In previous studies the duration of the biological stages was longer on the resistant clones and shortest on susceptible clones.

A. socialis nymphal mortality was highest on MEcu 64 and lowest on CMC-40. Only 18% of the nymphs feeding on CMC-40 died while 71% nymphal mortality occurred on MEcu 64. Nymphal mortality was also high on MEcu 72 (47.5%) and MPer 415 (43%) and intermediate on MPer 317 (31.8%), MPer 335 (39%) and MPer 611 (34.5%) (Table 7). Mortality was most severe during the nymphal stages, but also occurred during the egg stage and pupae (IV instar). In most cases, the highest mortality occurred during the first instar and lowest during the egg stage. The primary feeding stages are 1st through 3rd instar and all of the resistant clones, with the exception of MPer 611, highest mortality occurred during these three instars. There was relatively high pupal mortality observed on MEcu 64 and MPer 611 (Table 7). On varieties such as MEcu 64, MEcu 72 and MPer 415, first instar nymphs express difficulty in "fixing" themselves to the leaf undersurface to initiate feeding. These nymphs quickly dry up and fall from the leaf surface.

These trials will be repeated on some of the aforementioned varieties as well as other varieties. In addition, trials will be designed to measure whitefly feeding and survival over several generations on the same clone. The fact that mortality was considerably higher on the resistant clones than on

the susceptible clone (CMC-40) further indicates that good levels of resistance to whiteflies (*A. socialis*) is present in cassava germplasm.

Table 5. Life cycle duration of the whitefly *Aleurotrachelus socialis* on seven cassava varieties in the growth chamber.

Variety	Egg	I Instar	II Instar	III Instar	IV Instar (Pupae)	Total Duration	Nymphal Duration
CMC-40	11.4	6.2	3.4	4.2	10.5	35.7	24.3
MEcu 64	11.7	4.4	4.3	4.1	9.4	33.9	22.2
MEcu 72	11.5	4.8	3.7	4.2	9.7	33.9	22.4
MPer 317	11.8	5.6	3.6	4.1	8.9	34.0	22.2
MPer 335	10.3	5.5	4.3	4.7	9.6	34.4	24.1
MPer 415	10.3	5.8	4.7	4.9	11.7	37.4	27.1
MPer 611	9.9	5.4	4.4	4.3	10.6	34.4	24.5
Average	11.0	5.4	4.1	4.4	10.1	35.1	24.1

Table 6. The life cycle duration of *Aleurotrachelus socialis* on seven cassava varieties.

Variety	No. Observations	Duration/Days	Average Duration (Days)	S.D.
CMC-40	164	30 – 43	35.7	1.41
MEcu 64	43	28 – 41	33.9	2.27
MEcu 72	105	31 – 40	33.9	1.93
MPer 317	102	31 – 40	34.0	1.72
MPer 335	61	30 – 43	34.4	1.88
MPer 415	57	34 – 45	37.4	2.04
MPer 611	131	31 – 44	34.6	1.74

Table 7. Percent mortality of *Aleurotrachelus socialis* feeding on seven cassava varieties.

Variety	Egg	I Instar	II Instar	III Instar	IV Instar (Pupae)	% Mortality
CMC-40	0.0	5.0	3.5	3.0	6.5	18.0
MEcu 64	4.6	39.3	6.6	4.6	16.0	71.1
MEcu 72	3.5	24.0	6.0	5.0	9.0	47.5
MPer 317	1.3	13.3	4.6	2.6	10.0	31.8
MPer 335	0.0	23.0	2.0	4.0	10.0	39.0
MPer 415	0.0	23.0	6.0	7.0	7.0	43.0
MPer 611	0.5	13.0	1.0	2.0	18.0	34.5

Activity VI. Identification of genomic regions responsible for conferring resistance to whitefly in cassava

Different sources of resistance to white fly have been reported (CIAT, 1995). The most important source of resistance genes was a genotype MEcu 72. Due to the importance of the whitefly as a pest and virus vector, we have initiated genetic studies to understand the inheritance of the resistance to the whitefly in genotypes like MEcu 72 and to tag the resistance gene (s). For this purpose we are analyzing the F1 segregation of cross the MEcu 72 (resistant genotype) x any very susceptible genotype, using molecular markers. This would help to accelerate selection of resistant materials to whiteflies and also to isolate resistant genes.

Materials and Methods

An F_1 population of 282 individuals from the cross MEcu72 X Mcol2246 were used for this study with the former being the identified source of resistance to white fly and the latter the susceptible parent. In addition, Mcol2246 though being susceptible to whitefly infestation is resistant to other pests like mite and thrips and also flowers quite copiously.

Genomic DNA from these 282 F_1 individuals was isolated from fresh young leaves using a slightly modified method adapted from Dellaporta *et al.* (1983). Tissue from these young leaves were macerated in liquid nitrogen and 0.3g of this put in a 1.5ml eppendorf tube containing 1ml of 100mM Tris-HCl, 50mM EDTA, 500mM NaCl, 1.25% SDS and 0.38g/ml Sodium Bisulphite. This mixture was incubated for 45 minutes at 60°C followed by the addition of 0.4ml of 5M Potassium Acetate and subsequently placed on ice for 30 minutes. This was followed by centrifugation in a Sorvall Table top Centrifuge maintained at 4°C at 4000rpm for 10 minutes. The supernatant was decanted and the nucleic acids then precipitated by the addition of one volume of isopropanol and 1/10 volume of Sodium Acetate (pH 5.2). An incubation period of 15 minutes at –80°C followed. After this low temperature incubation, the mixture was again centrifuged at a temperature of 4°C in a Sorvall Table Top Centrifuge at 12000rpm for 5 minutes and the supernatant decanted. The pellets were then washed in 70% ethanol, which was also decanted. The pellets were re-suspended in 100µl TE buffer. The integrity of the DNA was confirmed by agarose gel electrophoresis of the aliquots.

Seeds from these 282 F_1 individuals were planted in plastic dishes filled with sterile soil and left to grow for 6 to 8 weeks under greenhouse conditions with the temperature maintained at approximately 30°C. The seedlings were then transferred to the field for multiplication.

For the greenhouse evaluations, the planting materials were rapidly multiplied *in vitro* in order to generate enough propagules in a short time, approximately 3 months. This is a significant reduction from the 6 months it normally takes to obtain stakes for planting from cassava stems when grown in the field. Also, this helped to obtain relatively cleaner planting materials.

In vitro propagation methodology developed by Escobar (1991) will be used in the present work. This methodology is based in cutting plant tips, which are transferred to the lab, disinfected first by washing them with sterile deionized water, ethanol 70%, hypochlorite 0,25% and finally washed three times with sterile deionized water. The tips are cultured in 4E medium (Roca, 1984) in 16 ml

assay tubes. The calculated growing period will be from 60 to 80 days. Following this period a second in vitro propagation in 4E medium in 100 ml small flasks will be performed for increasing the material amount per clone. After this we will cut the tips of each clone for culturing in 17N rooting medium (Roca, 1984), during 30-40 days. Finally the plants will be transferred to the greenhouse.

Photo 6. Greenhouse screening of potted cassava plants to evaluate whitefly (*A. socialis*) feeding damage and oviposition.

This methodology will allow the conservation of material under optimal health conditions, and it will supply sufficient material in a reduced space.

The parents MEcu 72, MCol 2246 and their offspring will be evaluated in the greenhouse with the "clip cage" methodology which consist in two polyethylene cylinders of different height joined by forceps. Both cylinder bases are covered by muslin, and the highest cylinder has a small hole through which flies are introduced. With this evaluation we pretend to identify the gene segregation in the offspring and select the resistant and susceptible materials.

We are using Simple Sequences Repeat SSR, to find markers associated to resistance for mapping and ultimately cloning the resistant genes. The SSR are random repeat sequences across all eukaryotic genome. These simple repeats can range from two to six base pairs (bp). SSRs show high polymorphism, are locus specific and multiallelic, they have a mendelian inheritance and also are codominant. We are using silver staining to visualize the allelic segregation of the markers.

Results

Genomic DNA of 282 individuals offspring was isolated from fresh, young tissue of cassava leaves powdered with liquid nitrogen, according to Dellaporta et al. (1983) method modified (Figure 6).

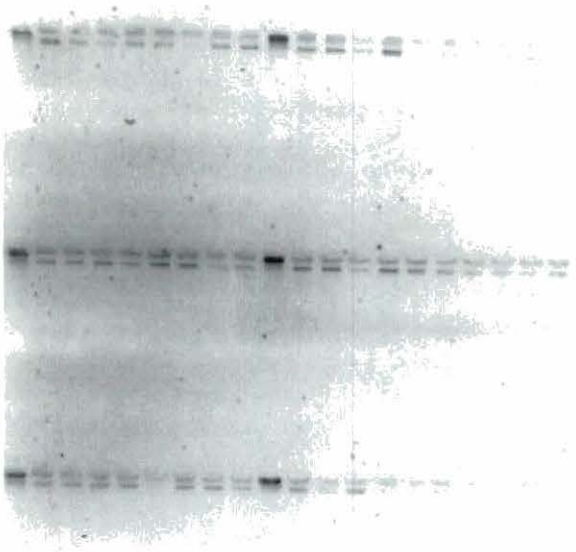

Figure 6. Agarose gel ethidium bromide staining showing the white fly DNA's isolated with the Dellaporta method modified.

Both parents Ecu-72 and MCol 2246 were evaluated with 343 cassava SSRs including 157 cDNA SSRs recently developed (Mba et al, submitted). Approximate 60% of the SSRs were polymorphic. (Figure 7, Table 8).

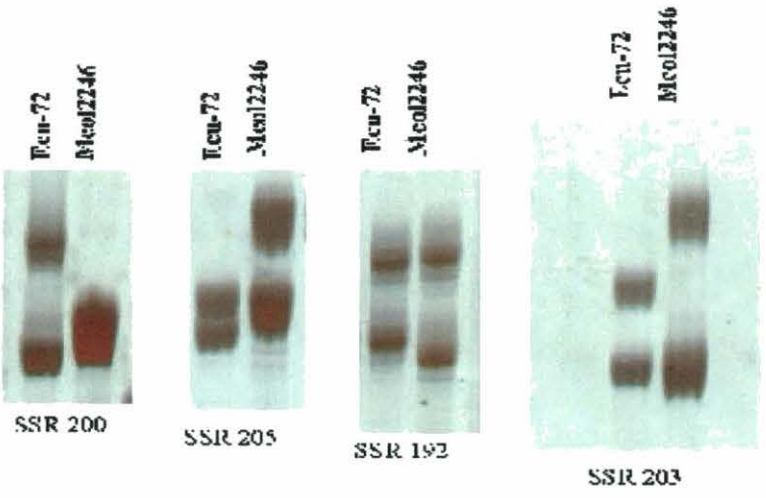

Figure 7. Silver stained polyacrylamide gel showing SSRs of cDNA in both parents Ecu-72 (female) and Mcol-2246 (male).

Table 8. SSRs in parents Ecu-72 x MCol2246.

SSR #	Size (bp)	T. Anneal°C	Polymorphics	SSR #	Size (bp)	T. Anneal°C	Polymorphics
SSRY1	197	45	X	SSRY51	298	50	X
SSRY2	225	55	X	SSRY52	266	55	X
SSRY3	247	45	X	SSRY53	138	55	Monomorphic
SSRY4	287	45	X	SSRY54	151	55	X
SSRY5	173	55	X	SSRY55	145	50	X
SSRY6	298	45	X	SSRY56	137	50	Monomorphic
SSRY7	250	45	X	SSRY57	293	55	X
SSRY8	288	45	X	SSRY58	217	55	X
SSRY9	278	55	Monomorphic	SSRY59	158	55	X
SSRY10	153	55	X	SSRY60	137	55	X
SSRY11	265	55	X	SSRY61	233	55	Monomorphic
SSRY12	266	55	Monomorphic	SSRY62	250	55	Monomorphic
SSRY13	234	50	X	SSRY63	290	55	Monomorphic
SSRY14	300	55	Monomorphic	SSRY64	194	55	X
SSRY15	215	50	Monomorphic	SSRY65	299	55	X
SSRY16	218	55	X	SSRY66	261	55	Monomorphic
SSRY17	277	50	X	SSRY67	278	55	Monomorphic
SSRY18	198	44	Monomorphic	SSRY68	287	55	X
SSRY19	214	50	X	SSRY69	239	55	X
SSRY20	143	55	X	SSRY70	249	55	X
SSRY21	192	55	X	SSRY71	217	55	X
SSRY22	299	43	Monomorphic	SSRY72	141	55	X
SSRY23	247	45	X	SSRY73	265	50	Monomorphic
SSRY24	100	45	Monomorphic	SSRY74	114	55	X
SSRY25	296	45	Monomorphic	SSRY75	284	55	X
SSRY26	121	55	X	SSRY76	273	55	X
SSRY27	277	50	X	SSRY77	275	55	X
SSRY28	180	55	Monomorphic	SSRY78	248	55	X
SSRY29	281	55	Monomorphic	SSRY79	210	55	X
SSRY30	220	50	X	SSRY80	299	55	X
SSRY31	188	50	X	SSRY81	204	55	Monomorphic
SSRY32	298	50	Monomorphic	SSRY82	211	55	X
SSRY33	273	50	Monomorphic	SSRY83	239	55	Monomorphic
SSRY34	279	55	X	SSRY84	203	55	X
SSRY35	282	55	Monomorphic	SSRY85	292	50	X
SSRY36	134	55	X	SSRY86	296	50	X
SSRY37	187	50	Monomorphic	SSRY87	102	55	X
SSRY38	122	55	X	SSRY88	243	55	X
SSRY39	293	50	X	SSRY89	120	55	X
SSRY40	231	50	X	SSRY90	193	55	Monomorphic
SSRY41	271		X	SSRY91	300	55	Monomorphic
SSRY42	221	50	X	SSRY92	171	55	Monomorphic
SSRY43	255	43	Monomorphic	SSRY93	289	55	X
SSRY44	194	50	Monomorphic	SSRY94	268	55	X
SSRY45	228	50	X	SSRY95	282	55	X
SSRY46	268	50	Monomorphic	SSRY96	149	55	X
SSRY47	244	55	X	SSRY97	194	55	X
SSRY48	178	50	Monomorphic	SSRY98	209	55	Monomorphic
SSRY49	300	50	Monomorphic	SSRY99	192	55	X
SSRY50	271	50	X	SSRY100	210	55	X

SSR #	Size (bp)	T. Anneal°C	Polymorphics	SSR #	Size (bp)	T. Anneal°C	Polymorphics
SSRY101	213	55	X	SSRY153	117	45	X
SSRY102	179	55	Monomorphic	SSRY154	318	55	X
SSRY103	272	55	X	SSRY155	158	55	X
SSRY104	258	52	Monomorphic	SSRY156	160	44	Monomorphic
SSRY105	225	55	Monomorphic	SSRY157	500	45	Monomorphic
SSRY106	270	55	X	SSRY158	224	45	Monomorphic
SSRY107	120	45	X	SSRY159	159	45	Monomorphic
SSRY108	203	55	X	SSRY160	151	50	X
SSRY109	125	55	X	SSRY161	220	55	X
SSRY110	247	55	Monomorphic	SSRY162	126	43	X
SSRY111	235	55	Monomorphic	SSRY163	231	44	Monomorphic
SSRY112	117	55	X	SSRY164	187	55	X
SSRY113	187	45	X	SSRY165	243	55	X
SSRY114	167	55	X	SSRY166	244	55	X
SSRY115	296	.	No amplified	SSRY167	183	45	X
SSRY116	167		No amplified	SSRY168	277	55	Monomorphic
SSRY117	142	55	X	SSRY169	100	55	X
SSRY118	169	55	Monomorphic	SSRY170	299	55	X
SSRY119	155	55	X	SSRY171	291	55	X
SSRY120	139	55	X	SSRY172	201	55	X
SSRY121	168	43	X	SSRY173	281		NO
SSRY122	273	45	X	SSRY174	136	43	X
SSRY123	136	55	X	SSRY175	136	55	X
SSRY124	146	55	Monomorphic	SSRY176	112	45	Monomorphic
SSRY125	247	55	Monomorphic	SSRY177	268	55	X
SSRY126	245	55	Monomorphic	SSRY178	104	55	Monomorphic
SSRY127	130	44	Monomorphic	SSRY179	226	55	X
SSRY128	243	45	X	SSRY180	163	55	X
SSRY129	205	55	Monomorphic	SSRY181	199	55	X
SSRY130	223	55	X	SSRY182	253	50	Monomorphic
SSRY131	111	45	Monomorphic	SSRY183	221	50	X
SSRY132	196	45	Monomorphic	SSRY184	163	50	X
SSRY133	295	55	Monomorphic	SSRY185	243	50	X
SSRY134	213	55	Monomorphic	SSRY186	297	55	
SSRY135	253	55	X	SSRY187	160	55	
SSRY136	296	55	Monomorphic	SSRY188	198	55	Monomorphic
SSRY137	157	55	Monomorphic	SSRY189	185	55	X
SSRY138	129	50	Monomorphic	SSRY190	164	55	
SSRY139	129	44	Monomorphic	SSRY191	186	55	Monomorphic
SSRY140	212	43	Monomorphic	SSRY192	183	55	X
SSRY141	262	55	X	SSRY193	218	55	X
SSRY142	206	55	X	SSRY194	196	55	
SSRY143	153	55	Monomorphic	SSRY195	186	55	X
SSRY144	117	55	X	SSRY196	188	55	
SSRY145	143	45	X	SSRY197	209	55	X
SSRY146	139	45	X	SSRY198	219	55	
SSRY147	113	45	Monomorphic	SSRY199	205	55	
SSRY148	114	55	Monomorphic	SSRY200	205	55	X
SSRY149	500	45	X	SSRY201	197	55	X
SSRY150	175	45	Monomorphic	SSRY202	191	55	
SSRY151	182	55	X	SSRY203	246	55	X

SSR #	Size (bp)	T. Anneal°C	Polymorphics	SSR #	Size (bp)	T. Anneal°C	Polymorphics
SSRY152	233	45	X	SSRY204	182	55	X
SSRY205	201	55	X	SSRY212	238	55	
SSRY206	219	55		SSRY213	199	55	
SSRY207	199	55		SSRY214	234	55	
SSRY208	198	55		SSRY215	204	55	X
SSRY209	195	55		SSRY216	210	55	
SSRY210	219	55	Monomorphic	SSRY217	181	55	X
SSRY211	202	55	Monomorphic	SSRY218	203	55	X

We are using 130 polymorphic microsatellites to screen the 282 individuals and we are evaluated the 125 new SSRs of cDNA in both parents Ecu-72 and Mcol-2246.

Conclusions and On Going Work

- We found a high polymorphism percentage (more than 60%) between parents Ecu-72 and MCol-2246.
- We will also screen the population with a new set of SSRs generated in the CIAT lab from cDNA.
- Segregation data from the SSR evaluations and greenhouse evaluation, from the 282 F1 individuals, will be used for the construction of a linkage map and for QTL analysis of the resistance to white fly.

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