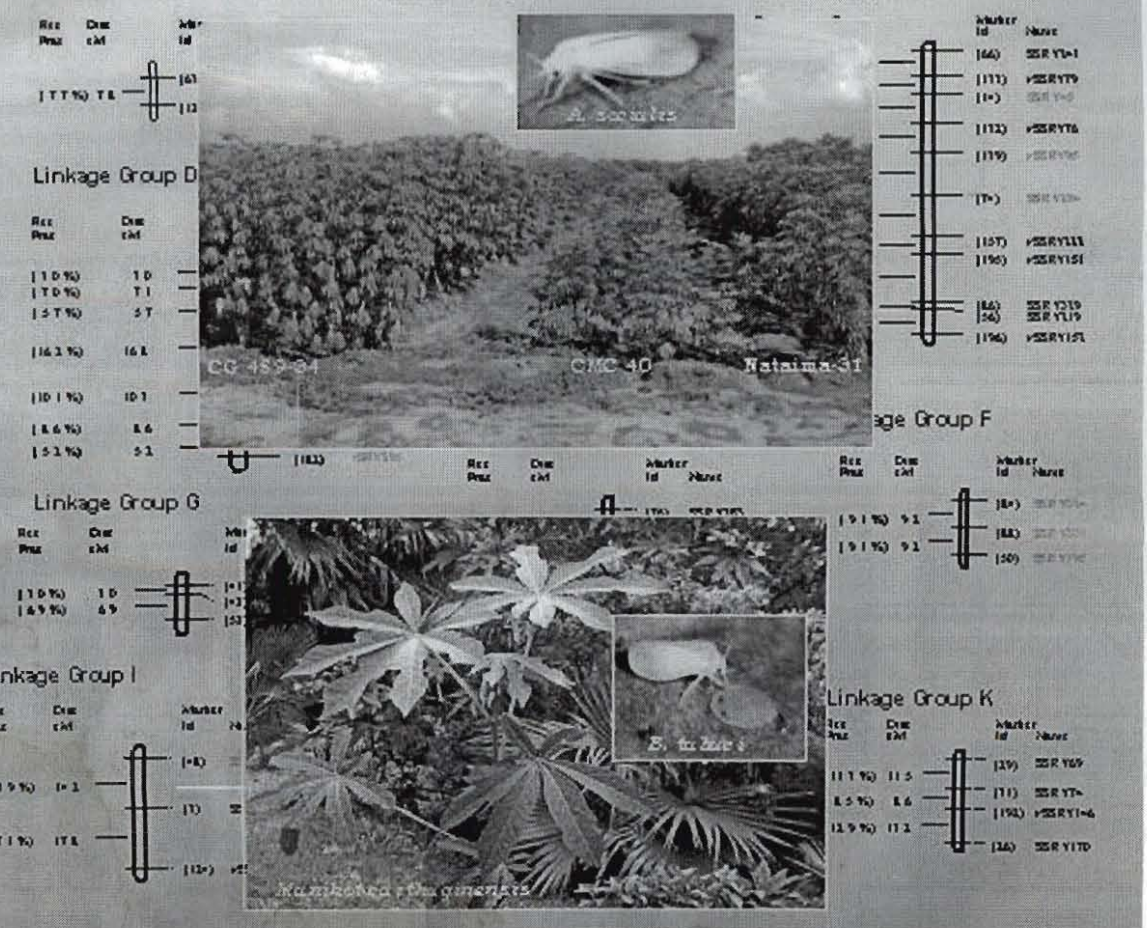
Sustainable Integrated Management of Whiteflies Through Host Plant Resistance



Progress Report 2003-2004

CIAT

nzaid

System-wide Programme on
Integrated Pest Management

COVER

Nataima-31 (CG 489-31 and CG 489-34) are two progeny from the MEcu 72 x MBra 12 cross. Nataima-31 has been released as a commercial variety by the Colombia MADR. CG 489-34 is presently being considered for commercial released.

Wild Manihot Species: A new source of whitefly resistance discovered for incorporating into cultivated cassava.

Background: Part of molecular linkage map for whitefly resistance.

CGIAR Systemwide program on IPM: The NZAID contribution is part of the SPIPM project on Tropical Whitefly IPM.

Sustainable Integrated Management of Whiteflies Through Host Plant Resistance

Collaborating Institutions: CIAT, Cali, Colombia
Crop and Food Research, Levin, New Zealand

Contact Persons: Anthony C. Bellotti, Joe Tohme
Paul Tocker, Gaigl M. Timmerman-Vanghan

Funding Agency:
NZAID, New Zealand

April 2004

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PROGRESS REPORT
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Funding Agency: MFAT, New Zealand

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 constitute a major pest problem in agricultural systems in the tropical and subtropical regions of the world. Whiteflies have been described in the popular press as the pest of the century, due to the extensive crop losses caused by their direct feeding and their ability to vector virus diseases. Yield losses due to whiteflies feeding on a wide range of hosts is estimated to total hundreds of millions of dollars. In addition, an impact study in California concluded that for every million dollars of crop loss, there is an estimated $1.2 million loss in personnel income and the elimination of 42 jobs. The current heavy use of chemical pesticides for whitefly control is not economically sound or sustainable.

As direct feeding, pests and virus vectors, whiteflies cause severe damage in cassava (Euphorbiaceae: *Manihot esculenta* Crantz) based agroecosystems in the Americas Africa and in Asia. The largest complex is found in the neotropics where 11 species are reported. The most important species causing direct feeding damage are *Aleurotrachelus socialis* (in Colombia, Venezuela and Ecuador) and *Aleurothrixus aepim* (in Brazil). *Bemisia tabaci*, the vector of Cassava Mosaic Disease (CMD); (previously referred to as Africa Cassava Mosaic Disease) has a pantropical distribution, feeding on cassava throughout Africa, several countries in Asia, and more recently in the neotropics. CMD is caused by several geminiviruses and it is often speculated that the absence of CMD in the Americas may be related to the inability of its vector, *B. tabaci*, to effectively colonize cassava.

Since the early 1990's, a new biotype (B) of *B. tabaci*, regarded by some as a separate species, (*B. argentifolii*) has been found feeding on cassava in the neotropics. It is now considered that CMD poses a more serious threat to cassava production given that most traditional varieties in the neotropics are highly susceptible to the disease. In addition, recent studies in Africa indicate that CMD is a complex of eight distinct cassava-infecting geminivirus species, with possibly more being discovered in the coming years.

Until recently, *B. tabaci* damage to cassava was limited primarily to its ability to vector CMD. However, in addition to their vectoring ability, *B. tabaci* populations have so dramatically increased in parts of East and Central Africa (especially Uganda, Tanzania, Rwanda and western Democratic Republic of Congo) that they are causing direct physical damage to the cassava crop. Yield losses have been estimated as high as 50%, even in those varieties with known resistance to CMD. These results indicate that cassava cultivars containing only resistance to CMD will not be adequate to avoid or resist yield losses due to the direct feeding of high populations of *B. tabaci*. Additional control measures will be required to reduce *B. tabaci* populations below economic injury levels. Past experience has indicated that farmers will turn to the indiscriminate use of costly and toxic pesticides to reduce whitefly populations.

Host plant resistance (HPR) offers a low-cost, practical, long-term, environmentally sound, farmer friendly solution for maintaining lower whitefly populations and reducing crop losses. This is especially important for cassava, which has a long growing cycle (1 year or more) and is often grown by resource limited, small farmers who cannot afford costly inputs. Host plant resistance to whiteflies in cultivated crops is rare. The large-scale screening or evaluation of a

wide collection of genotypes or selected wild or cultivated species is limited. A major exception to this trend is the case of cassava. A systematic evaluation of more than 5000 cassava accessions has now resulted in identifying several resistant genotypes. This moderate to high levels of resistance in cassava germplasm is unique among major food crops. This resistance is highly heritable and has been incorporated into high-yielding, good quality hybrids that are being released to farmers and are being commercially grown in Colombia. In addition, requests from several other countries, including Ecuador, Venezuela, Brazil, India and several countries in Africa, indicate the high demand for whitefly resistant cassava germplasm.

The contribution of NZAID has been invaluable in providing this technology to NARS and cassava growers, potentially across three continents.

NZAID's support for the research on host plant resistance to whiteflies is a sub-project within the CIAT coordinated "Systemwide Tropical Whitefly IPM Program (SP-IPM-TWFP). The cassava HPR subproject is therefore linked to the other subprojects based in sub Saharan Africa, Southeast Asia and the Americas. These subprojects are funded by separate donors, namely DFID (The United Kingdom Department for International Development), the United States USAID, and ACIAR (Australian Center for International Agricultural Research). A DFID supported project involving NRI (Natural Resources Institute) in the UK is evaluating the whitefly resistant germplasm that we identify from the Cassava Germplasm Bank (CIAT) for resistance to *B. tabaci* the major whitefly species in Africa and the vector of Cassava Mosaic Disease (CMD). The *B. tabaci* biotype found in Africa is obviously distinct from the biotype in the neotropics, as the Africa biotype readily feed and reproduces on cassava while the neotropics biotype is not successful on cassava.

In order to evaluate neotropical cassava genotypes against the Africa *B. tabaci* biotype a neutral site was required where neotropical genotypes could be evaluated in the absence of CMD but with the Africa *B. tabaci* biotype. This combination of events can take place at the NRI facilities in the UK.

Nearly three years of collaboration between CIAT, NRI and Uganda, have resulted in identifying the cassava genotype MEcu 72 as resistant to the *B. tabaci* biotype from Africa. MEcu 72 was selected at CIAT, Colombia as being the most resistant genotype to the whitefly species. *Aleurotrachelus socialis*, the major species found in Northern South America. This important finding indicates that resistance to the African *B. tabaci* can be obtained from neotropical germplasm. Since several additional whitefly resistant genotypes have been identified at CIAT, the possibility for introducing whitefly resistance becomes a potential reality. The procedure to develop whitefly resistant cassava varieties adapted to the African and Asian agroecosystems is now being developed. Links between CIAT, NRI, IITA and African National Research Institutes, primarily NARO in Uganda have been established. This will be of primary benefit to the African cassava farmers. In addition, links with cassava breeders/genetists in India have also been established and it is envisioned that whitefly resistant genotypes will be introduce into India in the near future.

Highlights

1. Mapping and association between molecular markers and resistance.

An AFLP analysis was made of 128 combinations of primers with both parentals (MEcu 72 and MCol 2246) and both bulks of 10 whitefly-resistant DNA and 10 susceptible DNA. We obtained 53 polymorphic combinations, in which there were 425 polymorphic bands between the resistant and the susceptible. All combinations were amplified in the F1. Approximately 155 of the SSRs evaluated were polymorphics in the parentals and were evaluated in the F1 (286 individuals). For the construction of linkage map 103 SSRs, 1 RGA and 57 AFLPs were analyzed of which 89 were anchored. A genetic linkage map of cassava was constructed with 89 markers segregating from the heterozygous female parent (MEcu-72) of an intraspecific cross (see Figure 2A-2B/Activity 1). The map consists of 19 linkage groups, which represent the haploid genome of cassava. These linkage groups span is 550,2 cM and the average marker density is 1 per 7,9 cM. The position of 75 SSRs markers, shown in the figure, on the framework (LOD = 25 and tetha (?) = 25) molecular genetic map of cassava. Map distances are sho wn in Kosambi map units and analyzed by Q gene. So far, 25 SSRs markers were mapped on the cassava framework map, the other 64 markers are new. The molecular data are being analyzed using QTL packages (QTL cartographer Q gene) to determine linkages between the SSR, RGA and AFLPs markers and the phenotypic characterization. Preliminary analysis (X^2 and Simple Linear Regression at the 5% level) was done using SAS. Subroutine associations were found between (32 markers SSRs, RGA and AFLPs, shown by * in the Figure 2A-2B). And the field phenotypic characterization. We observed that all markers anchored in the linkage group B are associated with the resistance.

2. Nataima-31, a whitefly resistant cassava variety (see photos page 8).

The development of Nataima-31, a whitefly resistant cassava hybrid developed by CAT and CORPOICA in Colombia was described in detail in the 2002-2003 Progress Report prepared for NZAID. This hybrid developed during the NZAID funded project, and released by the Colombian Ministry of Agriculture in 2003, has now been distributed to hundreds of cassava farmers. The variety is being multiplied in the field at the CORPOICA station in Tolima, Colombia, for continued distribution to demanding cassava producers. In addition, the variety is going through a "rapid multiplication" at CIAT; this is a process for quickly producing massive numbers of plantlets for rapid distribution. This process, developed at CIAT, is ideally suited for cassava, a vegetatively propagated crop, in that it speeds up a normally slow reproductive cycle and facilitates the rapid introduction of new varieties. It is estimated the Nataima-31 will increase cassava yields by 25% per hectare (see following highlight).

3. Economic; production and social importance of Nataima-31.

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

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

4. Additional cassava varieties identified as resistant to whiteflies (*A. socialis*).

Three additional cassava varieties were selected for resistance to the whitefly, *Aleurotrachelus socialis*, MEcu 64, MPer 334 and MPer 273. Mortality levels on the three varieties were higher in the first generation than in the second generation. In field trials at CORPOICA, Nataima, Tolima, the cassava varieties MPer 317, MPer 334 and MPer 273, and the hybrids Nataima-31 and CG 489-34 had very low damage ratings (1.0 to 1.7 on the 1.0 to 6.0 damage scale).

5. Evolution of biotype B of *B. tabaci* to cassava (see diagram page 9).

Biotype B of the whitefly species *Bemisia tabaci*, the vector of CMD in Africa, has been observed feeding on cassava in the neotropics, but rarely in high populations. The potential of *B. tabaci* to vector a wide range of plant damaging geminiviruses, especially in cassava, is of constant concern. Most farmer grown cassava cultivars in the neotropics are considered highly susceptible to Cassava Mosaic Disease and related geminiviruses. Therefore, the ability for *B. tabaci* to adapt to cassava poses a potential threat to cassava production in the neotropics. Recent laboratory studies indicate a possible pathway for the Biotype B of *B. tabaci* to evolve from its traditional hosts, such as beans (*Phaseolus vulgaris*), to cassava. A successful transfer from beans to cassava occurred only after *B. tabaci* completed several generations on other Euphorbiaceae species such as *Euphorbia pulcherrima* (Poinsettia) and *Jatropha gossypiifolia* (Jatropha).

Results show that if *B. tabaci* is transferred directly from beans to cassava, there is only a 2% survival and little or no reproduction; a transfer from poinsettia to cassava results in a 3% survival and, again, little or no reproduction. However, when *B. tabaci* is transferred from Jatropha to cassava there is a 27.5% survival and considerable reproduction. *B. tabaci* also

readily transfers from Jatropha to *Manihot carthaginensis* a closely related wild relative to *M. esculenta* (cultivated cassava); *B. tabaci* survival in this case is 60% and reproduction is more successful than when feeding on *M. esculenta.* *B. tabaci* populations when feeding on *M. carthaginensis* has a shorter development time, a higher rate of survival and a shorter generation time than when feeding on cassava.

These results provide evidence that biotype B of *B. tabaci* can adapt to Wild Manihot species and successfully reproduce high populations. The adaptation from Wild Manihot relatives to cassava could easily facilitate an even more rapid adaptation of *B. tabaci* on cassava. This represents a serious potential threat to cassava in the neotropics and this transfer and adaptation process is being further evaluated.

6. Resistance to biotype B of *B. tabaci* in *M. esculenta.*

A collaborative research project between CIAT and the Natural Resources Institute (NRI) in the UK is evaluating whitefly resistant cassava genotypes from the neotropics against the *B. tabaci* biotype from Africa (the vector of CMD). Four cassava genotypes, MCol 1468 (Susceptible), MCol 2063 (S), MEcu 72 (Resistant), and CG489-34 (Tolerant) were sent from CIAT to NRI via tissue culture in 2001. These were evaluated against three whitefly colonies established on cassava at NRI; *B. tabaci* from Namulonge (Uganda), *B. afer* from Entebbe (Uganda), and *B. tabaci* from Trivandrum (India). Although the three whitefly populations successfully oviposited on the four cassava varieties, oviposition of the African *B. tabaci* was lowest on the resistant genotype MEcu 72. Of the four cassava genotypes, MEcu 72 also had the lowest number of nymphs and was clearly the most resistant of the genotypes. *B. afer*, similarly, had low number of eggs and nymphs present on MEcu 72, CG 489-34 and MCol 1468. The results of the studies being carried out at NRI clearly indicates that MEcu 72 shows the most consistent pattern of resistance to *B. tabaci* from Africa and India. Future research to exploit the potential of evaluating cassava genotypes from the neotropics for whitefly resistance is being planned. NZAID's support for introducing whitefly resistant germplasm into Africa is an important contribution to this work.

7. Interspecific crosses as a source of whitefly resistance.

Accessions from the wild *Manihot* species, *M. flabellifolia* and *M. peruviana* were evaluated for resistance to mealybugs (*Phenacoccus herreni*), mites (*Mononychellus tanajoa*), and whiteflies (*Aleurotrachelus socialis*). Both wild species were moderately resistant to mites and mealybugs and highly resistant to whiteflies. These results show that the possibility of using the wild *Manihot* species as a source of resistance to cassava pests has considerable potential for the future.

Interspecific crosses between cassava varieties (MCol 2215, CG 501-16, CMC 2766-5) and the wild species *M. flabellifolia*, were evaluated against the cassava green mite (*M. tanajoa*). Most progeny had mite ovipositional rates 37 to 62% below that of the susceptible control, CMC 40. Progenies of MEcu 72, the source of resistance to whiteflies also showed excellent levels of resistance to mites.

8. The Cassava Biotechnology Network (CBN).

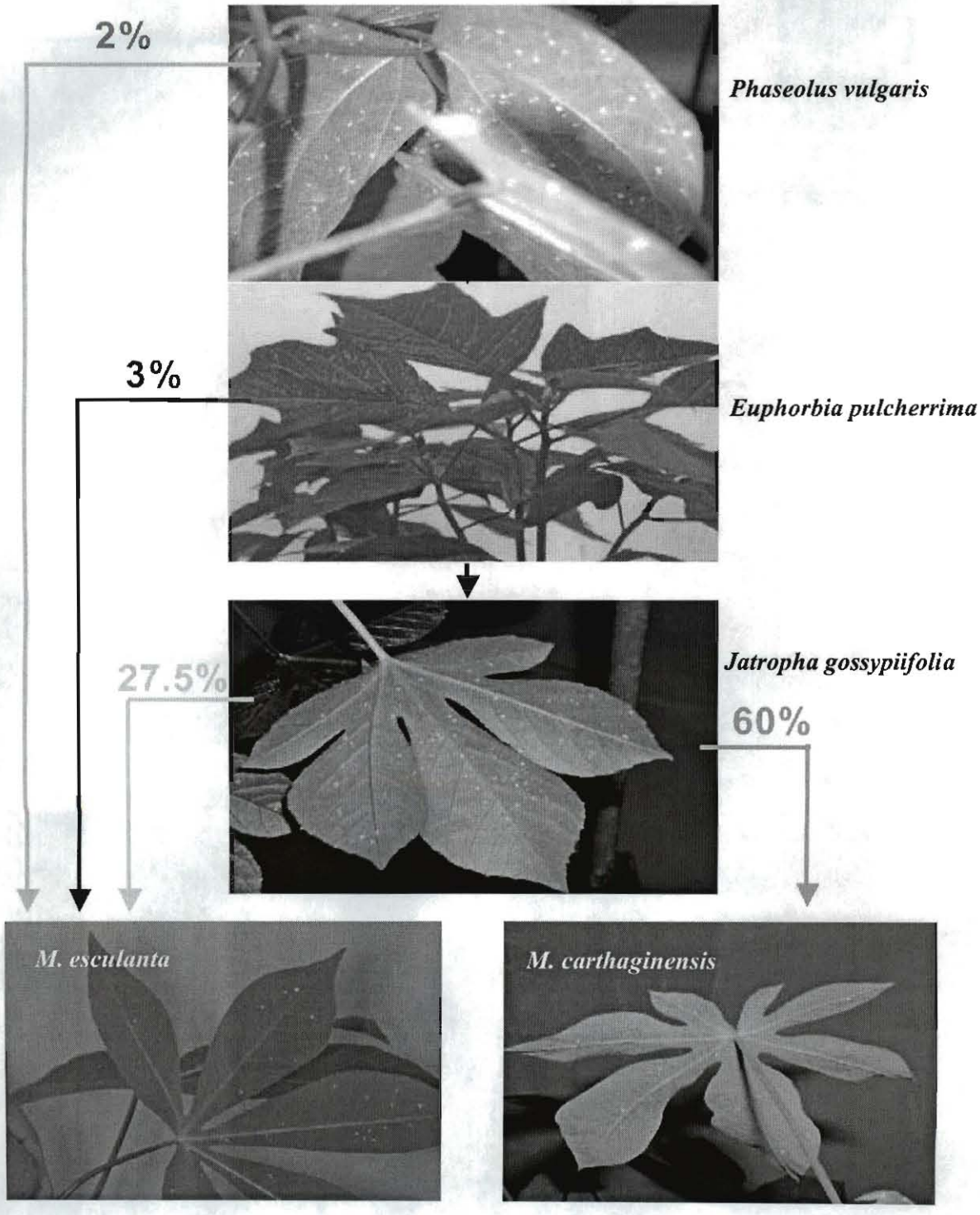
Colombia and CIAT hosted the CBN's Sixth International Meeting on March 8-14, 2004. The goal of CBN is "to strengthen and speed effort to maximize the contribution of modern biotechnology tools to the agronomic improvement of cassava and thereby contribute to improved food security in the tropics." CBN sponsors both worldwide and regional activities dedicated to cassava improvement. The 2004 meeting had more than 150 participants representing 30 countries and five continents. Approximately 160 papers were presented on a range of subjects, from product development, biodiversity, genomics, biotic and abiotic stresses and post harvest. More than 25 presentations and posters dealt with the theme of whiteflies and related virus diseases, indicating the importance of whiteflies as direct feeders and virus vectors on cassava production throughout the tropical regions of the world. Three posters and one oral presentation were funded through the NZAID support project (see posters, pages 10-12).

Nataima-31, the whitefly resistant variety, seen on the left, is a progeny from the cross of MEcu 72 (seen on the right) the resistant female parent and MBra 12.

Nataima-31, a whitefly resistant variety developed with NZAID funding and officially released by the Colombia Ministry of Agriculture, is being multiplied at several sites (including CIAT) for distribution to cassava farmers. Note upright plant type, vigorous growth and high leaf retention.

Evolution of Biotype B of *Bemisia tabaci* to Cassava

Bemisia tabaci **(Biotype B) % Survival on** ***Manihot esculenta*** **(Cassava) and** ***Manihot carthaginensis*** **Wild Species when Populations Originate from Three Alternate Hosts**



Poster 1.

RECENT ADVANCES IN HOST PLANT RESISTANCE TO WHITEFLIES IN CASSAVA

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INTRODUCTION

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

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

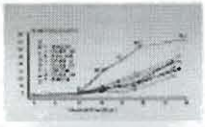


Fig. 2. Whitefly (*A. socialis*) nymphal mortality on resistant (Rg, tolerant (T) and susceptible (S) cassava clones.

MATERIALS AND METHODS

PLANT MATERIAL

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

MOLECULAR ANALYSIS

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

RESULTS AND DISCUSSION

FIELD EVALUATION

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

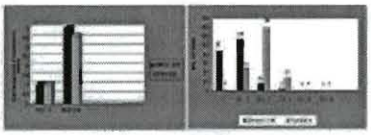
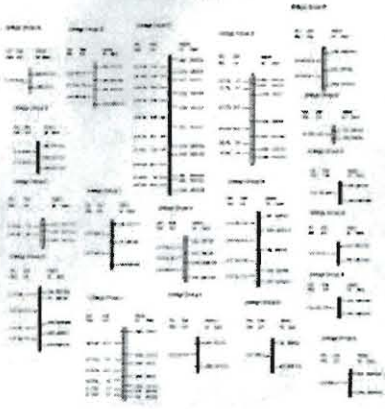


Fig. 3. Cassava damage and whitefly population ratings due to *A. socialis* feeding on parental genotypes MEcu-72, MCol-2246 and clones from the family CM 3966 at CORPOICA, Nataima (Tolima, Colombia).

MOLECULAR ANALYSIS

Both parents, MEcu-72 and MCol-2246, were evaluated with 343 cassava SSR markers (Mba et al, 2001), including 156 cDNA SSRs developed by Mba et al (submitted).
Approximately 155 of the SSRs were polymorphic in the parentals and were evaluated in the F1 (286 individuals). For the construction of the linkage map, 103 SSRs were analyzed, of which 71 were anchored and segregating from the heterozygous female parent (MEcu-72) of an interspecific cross. The map consists of 19 linkage groups, which represent the haploid genome of cassava (Fig. 4). These linkage groups span 550,2 cM and an average marker density of 1 per 7,9 cM. The position of the 71 SSRs markers is shown in figure 5 of the cassava molecular genetic map (LOD = 25 and tetha (θ) = 25). Map distances are shown in Kosambi map units. So far, 26 SSRs markers (shown in green, Fig. 4) have been previously placed on the cassava framework map (Fregene et al, 1997), the other 45 SSRs are new. Thirty one of the 71 SSRs were cDNA sequences (Mba, in preparation) and the others were genomic DNA.

Fig. 4. Preliminary Cassava Framework Map of MEcu-72 for Resistance to White Fly, consisting of SSRs and a RGA (Contigs) (Lod ≥ 3 and theta ≤ 25)



AFLPs Analysis

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

Fig. 5. Silver stained polyacrylamide gel showing combination ACA-CTT of AFLP of both parents (R resistant, S susceptible) and Bulks resistants and susceptibles, where the polymorphic band 4.10 (maybe in the resistants).

ASSOCIATION BETWEEN MOLECULAR MARKERS AND RESISTANCE

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

CONCLUSIONS

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

ON GOING WORK

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

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March 8-14, 2004 – CIMMYT

Poster 2.

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

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INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

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

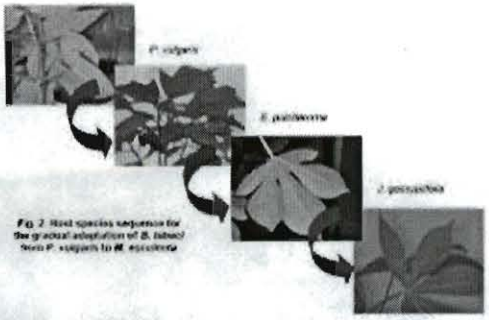

Fig. 2. Host species sequence for the gradual adaptation of *B. tabaci* from *P. vulgaris* to *M. esculenta*

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

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

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

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

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

RESULTS AND DISCUSSION

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

The average longevity of the females of biotype B was significantly higher in females coming from *E. pulcherrima* (5.6 days). The highest oviposition rate (2.64 eggs/female/2 days) was found in females coming from *J. gossypiifolia*, being significantly higher than that of females coming from the other two hosts (Table 1).

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

	Host of Origin		
Average Parameter	*J. gossypiifolia*	*E. pulcherrima*	*P. vulgaris*
Longevity	3.25 b	5.6 a	3.1 b
Fecundity	8.6 a	7.65 a	1.82 b
Oviposition rate	2.64 a	1.36 b	0.58 c

Averages followed by different letters in the columns differ significantly (Kruskal-Wallis P< 0.001, followed by Student-Newman-Keuls P< 0.05).

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

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

	Host of Origin		
Parameter	*J. gossypiifolia*	*E. pulcherrima*	*P. vulgaris*
Development time (days)*	44.4 b	50.6 a	49.5 a
Survival rate (%)	27.5 a	3.0 b	2.0 b
Proportion of females (%)	50.9	50	50

Averages followed by different letters in the columns differ significantly * (Kruskal-Wallis P< 0.0001, followed by Student-Newman-Keuls P< 0.05; ** χ^2, 2 d.f., P< 0.0002, followed by Student-Newman-Keuls P< 0.05).

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

Intrinsic growth rates (r_m) revealed a higher population growth on *M. esculenta* when coming from *J. gossypiifolia*, exceeding those from *E. pulcherrima* by 8.9% and up to 58.3% for those from *P. vulgaris*.

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

	Host of Origin		
Parameter	*J. gossypiifolia*	*E. pulcherrima*	*P. vulgaris*
Ro	5.53	11.6	1.82
T	44.76	36.53	51.3
r_m	0.042	0.044	0.02

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

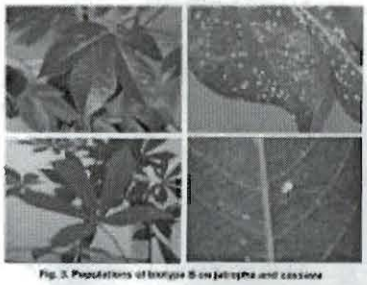

Fig. 3. Populations of biotype B on jatropha and cassava

CONCLUSIONS

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

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March 8-14, 2004 – CIEN-M

Poster 3.

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

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INTRODUCTION

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

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

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

ORIGIN OF Nataima-31

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

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

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

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

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

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

1 = no leaf damage
2 = young leaves still green but slightly leaves
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

Fig. 2 Nataima-31 plant type

Fig. 3 Root yield, Nataima-31

VARIETAL REACTION TO WHITEFLY ATTACK

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

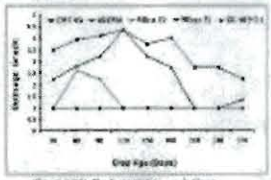

Fig. 4. Whitefly *A. socialis* populations on five cassava genotypes

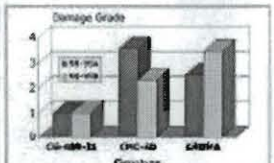

Fig. 5. Whitefly *A. socialis* damage grades on Nataima-31 (R), CMC-40 (S) and Aroma (Farmers Variety)

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

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

Genotype	No. Leaves	Eggs/Leaf[1] Average	C.V
MBra 12	92	82.1 A	295
CMC 40	97	75.2 A	104
CG489-23	94	69.7 AB	119
MCol 1505	70	59.3 B	142
HEcu 72	75	40.4 B	141
CG 489-34	102	20.5 C	134
CG 489-31	77	19.0 C	140

[1] Values followed by the same letter are not significantly different (Duncan alfa = 0.05).

It is therefore estimated that Nataima-31 will not require chemical pesticide applications for effective whitefly control in area such as Tolima, where *A. socialis* is endemic and, farmers are presently applying up to 6 insecticide applications per crop cycle. These pesticide applications increase production costs and adversely effect the environment. The ideal range of adaptation for Nataima-31 is between 400 to 1600 m.a.s.l., and yields more than the regional variety Aroma (23.0 T/ha vs. 16.2 T/ha).

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

THE RELEASE OF Nataima-31

On March 28, 2003, Nataima-31 was officially released by the CORPOICA Research Station in Espinal, Tolima, with ICA (Instituto Colombiano Agropecuario) register number 808, July 10, 2002.

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

Newspaper Coverage of the Release of Nataima-31

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

ECONOMIC, PRODUCTION AND SOCIAL IMPORTANCE OF Nataima-31

It is estimated that the Colombian aviculture industry will require 280,000 tons of cassava for poultry feed. It is planned that the Departments of Tolima, Huila and Cundinamarca will plant 14,500 hectares of cassava toward this goal and by 2007; approximately 3,000 hectares may be planted to Nataima-31 in the high, warm Rio Magdalena valley. This could have the following effects on the region:

- An increase of 3,000 hectares with Nataima-31 above the already 8,900 hectares presently being grown using regional varieties.
- A generation of about 177,000 new jobs in the production phase, 48,000 jobs in the post harvest phase and 24,000 indirect jobs.
- A 25% yield increase from the present average of 10 T/ha to 20 T/ha with Nataima-31 for a regional average production of 12.5 T/ha.
- The 3,000 ha sown to Nataima-31 will produce 60,000 tons, and overall production will increase from 89,000 tons annually to 149,000 tons in 2007, an increase of 67.4%.
- A production cost reduction of 6.7% per hectare due to the reduced pesticide applications (a minimum of 2) presently being applied for whitefly control.
- At present between 3,600 to 7,200 kg of active ingredient of pesticides is being applied. This represents an expenditure of 324 to 628 million pesos. Planting Nataima-31 will reduce this cost.
- Nataima 31 maintains the high dry matter and quality of the regional cultivars and is superior in being less susceptible to physiological deterioration; this is an advantage in time of transport to markets.
- Nataima-31 will bring direct benefits to approximately 1,500 rural families and an indirect benefic to 4,500 families in the Rio Magdalena Valley region.

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March 8-14, 2004 - CBN-VI

Activity 1. Identification of gene (s) responsible for conferring resistance to whitefly (*Aleurotrachelus socialis)* in cassava.

Rationale

The whitefly (*Aleurotrachelus socialis*) is one of the most serious pests that affect agricultural production in the neotropics. In cassava (*Manihot esculenta* Crantz), the whitefly causes from 70 to 80 percent economic losses. The most important source of resistance genes is the genotype MEcu 72. Due to the whitefly's importance as a pest, it is necessary to understand the nature of genes that confer resistance to the whitefly in genotypes such as MEcu 72. A linkage map was constructed of MEcu 72 (resistant genotype), using Single Sequences Repeats (SSRs) and a Resistance Genes Analogs (RGA). This will help tag resistance genes for whitefly, as well as initiate the fine mapping for resistance genes (R genes). It I hypothesized that these resistant genes may also be effective against other whitefly species, especially *Bemisia tabaci*, the species that is a vector of ACMD, a virus that causes severe crop losses in Africa and Asia. Whitefly resistant genotypes (such as MEcu 72) from the neotropics are displaying resistance to *B. tabaci* in greenhouse trials being carried out by NRI in the UK.

The application of molecular genetic analysis for cassava breeding has been limited compared to others crops. Recently progress has been made in the development of genomic and bioinformatics tools to increase our knowledge of cassava genome structure and cassava gene function. Expressed Séquense Tag (EST) provides an immediate and productive method of gene discovery. In cassava a total of 14168 ESTs were obtained in CIAT and Perpignan Universite (Lopez, et al, submitted), of these 105 have SSRs, for which we designed primers.

An additional step toward a better understanding of the attack response of the whitefly to cassava was the establishment of a cDNA library, which was developed with a new, highly effective method known as differential subtraction chain (DSC). Using this approach, two mRNA populations, extracted from both resistant and susceptible genotypes, were examined to elucidate the differential gene expression between them. Functional genomic tools such as the cassava microarray gives a first comprehensive overview of the molecular basis of the cassava defense response to the whitefly attack and will help to understanding the defense mechanisms to other important pests and diseases. Microarray-expression profiling will be used to identify putative early response regulatory and/or signaling genes and to test the function of selected candidate genes using reverse genetics.

Materials and Methods

For this work an F1 cross (family CM 8996, 276 individuals) between MEcu 72 (as the resistant parent) and MCol 2246 (as the susceptible parent), elite cassava cultivars from Ecuador and Colombia, respectively, was used. The parents and their offspring were evaluated in the field at two sites: Nataima (Tolima) and Santander de Quilichao (Cauca) in Colombia. The purpose of this evaluation was to identify gene segregation in the offspring and select the resistant and susceptible materials and identified associations between molecular markers and the resistance materials. Cassava SSRs and AFLPs (Vos et al., 1995) were be used to find markers associated to resistance for mapping and ultimately cloning the resistant genes. Silver staining is being used to visualize the allelic segregation of the markers.

For the isolation of expressed sequences, 21 forty-day-old plants were used, seven of each genotype (MEcu 72 and MPer 334 resistant and MCol 2246 susceptible). These plants were taken to the greenhouse, where they were infested with 300 whitefly adults per plant, for a population of 2100 whiteflies per cage. Leaves were collected at six different times for RNA extraction. For the differential subtraction chain (DSC), the follow strategy was used: Genotype MEcu 72 was infested for use as the tester, while genotype MCol 2246 was used as the driver. At present, the DSC technology is being performed according to Luo et al (1999). We designed primers SSRs from ESTs sequences using the software Primer 3 and these SSR were amplified in the parentals and the polymorphics were mapping in the F1.

Results

Field evaluation

The field evaluation showed high pressure being exerted by the pest in Nataima, and Santander de Quilichao where test materials had high damage ratings; however, some materials had lower levels of damage in the evaluations. We can conclude that these genotypes show a resistance level similar to parental MEcu 72.

Mapping of SSRs from ESTs

We designed 51 pairs of SSRs primers (Table 1) which 29 were polymorphics for cross (Figure 1)

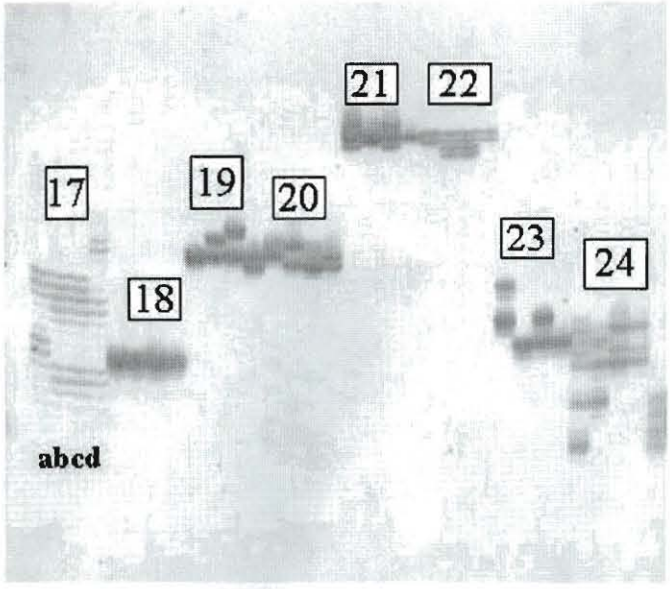

Figure 1. SSRs from ESTs in parentals, a is MNig2, b is CM21772 (both parentals used in ESTs), c is MEcu 72 and d is MCol 2246.

Mapping and association between molecular markers and resistance.

An AFLP analysis was made of 128 combinations of primers with both parentals (MEcu 72 and MCol 2246) and both bulks of 10 whitefly-resistant DNA and 10 susceptible DNA. We obtained 53 polymorphic combinations, in which there were 425 polymorphic bands between the resistant and the susceptible. All combinations were amplified in the F1. Approximately 155 of the SSRs

evaluated were polymorphics in the parentals and were evaluated in the F1 (286 individuals). For the construction of linkage map 103 SSRs, 1 RGA and 57 AFLPs were analyzed of which 89 were anchored. A genetic linkage map of cassava was constructed with 89 markers segregating from the heterozygous female parent (MEcu-72) of an intraspecific cross (see Figure 2A-2B/Activity 1). The map consists of 19 linkage groups, which represent the haploid genome of cassava. These linkage groups span is 550,2 cM and the average marker density is 1 per 7,9 cM. The position of 75 SSRs markers, shown in the figure, on the framework (LOD = 25 and tetha (?) = 25) molecular genetic map of cassava. Map distances are shown in Kosambi map units and analyzed by Q gene. So far, 25 SSRs markers were mapped on the cassava framework map, the other 64 markers are new. The molecular data are being analyzed using QTL packages (QTL cartographer Q gene) to determine linkages between the SSR, RGA and AFLPs markers and the phenotypic characterization. Preliminary analysis (X^2 and Simple Linear Regression at the 5% level) was done using SAS. Subroutine associations were found between (32 markers SSRs, RGA and AFLPs, shown by * in the Figure 2A-2B). And the field phenotypic characterization. We observed that all markers anchored in the linkage group B are associated with the resistance.

Table 1. SSRs from ESTs primers designed.

No.	EST Name	Motif	No. Repeat	No.	EST Name	Motif	No. Repeat
1	cn1375-1	atgg	5	27	cn1304-1	atg	9
2	cn1004-1	tatt	6	28	cn1351-1	aga	10
3	cn1098-1	aga	6	29	si.03.G1.5-1	cca	10
4	cn1388-1	tct	6	30	gi17923193gbBM260153.	taa	11
5	cn1457-1	agc	6	31	rni.06.I21.5-1	tct	11
6	cn255-1	tcc	6	32	si.02.O10.5-1	aat	11
7	cn416-2	gat	6	33	cn1635-1	aag	12
8	cn44-1	tta	6	34	m.01.H14.5-1	tc	12
9	cn700-3	ttc	6	35	si.01.E12.5-1	tc	12
10	c.04.C18.5-3	atg	7	36	cn1460-1	ag	13
11	c.05.I1.5-1	tct	7	37	cn1498-1	at	13
12	cn1186-1	ttc	7	38	cn1587-1	ata	13
13	cn2269-1	tgg	7	39	cn2418-1	ag	13
14	cn393-1	cat	7	40	m.04.K18.5-1	ct	13
15	cn732-1	aag	7	41	aflp_28-2	ga	15
16	cn764-2	tca	7	42	m.06.H4.5-1	ct	15
17	gi17922797gbBM25976 5.1BM259765-2	aag	7	43	rni.06.N9.5-1	at	15
18	m.04.K21.5-1	ctt	7	44	cn1009-1	ct	16
19	m.05.I2.5-1	ttc	7	45	cn1722-1	tct	17
20	rni.06.N10.5-1	tta	7	46	m.05.L3.5-1	ag	17
21	rni.09.D10.5-1	ctg	7	47	cn47-1	ct	18
22	si.03.B22.5-1	tct	7	48	m.09.N13.5-1	ct	18
23	cn1131-1	tcc	8	49	gi17922797gbBM259765.1BM259 765-1	agc	19
24	m.10.J19.5-1	gat	8	50	m.08.G23.5-1	at	20
25	m.11.K5.5-1	gat	8	51	cn1880-1	at	29
26	rni.05.L17.5-1	tga	8				

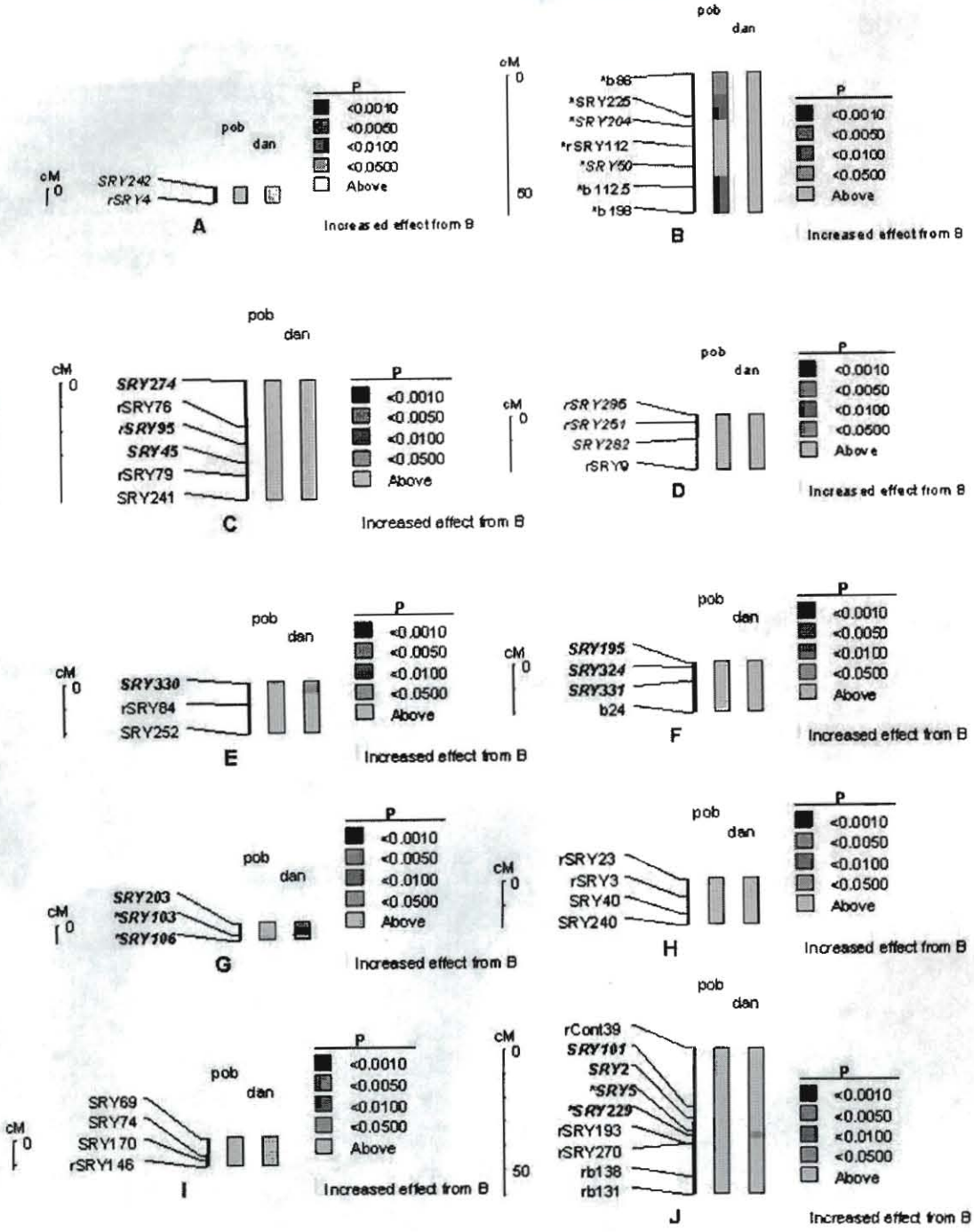

Figure 2A. Preliminary linkage map of MEcu 72 analyzed by Qgene. Associated markers with the resistance showed with*.

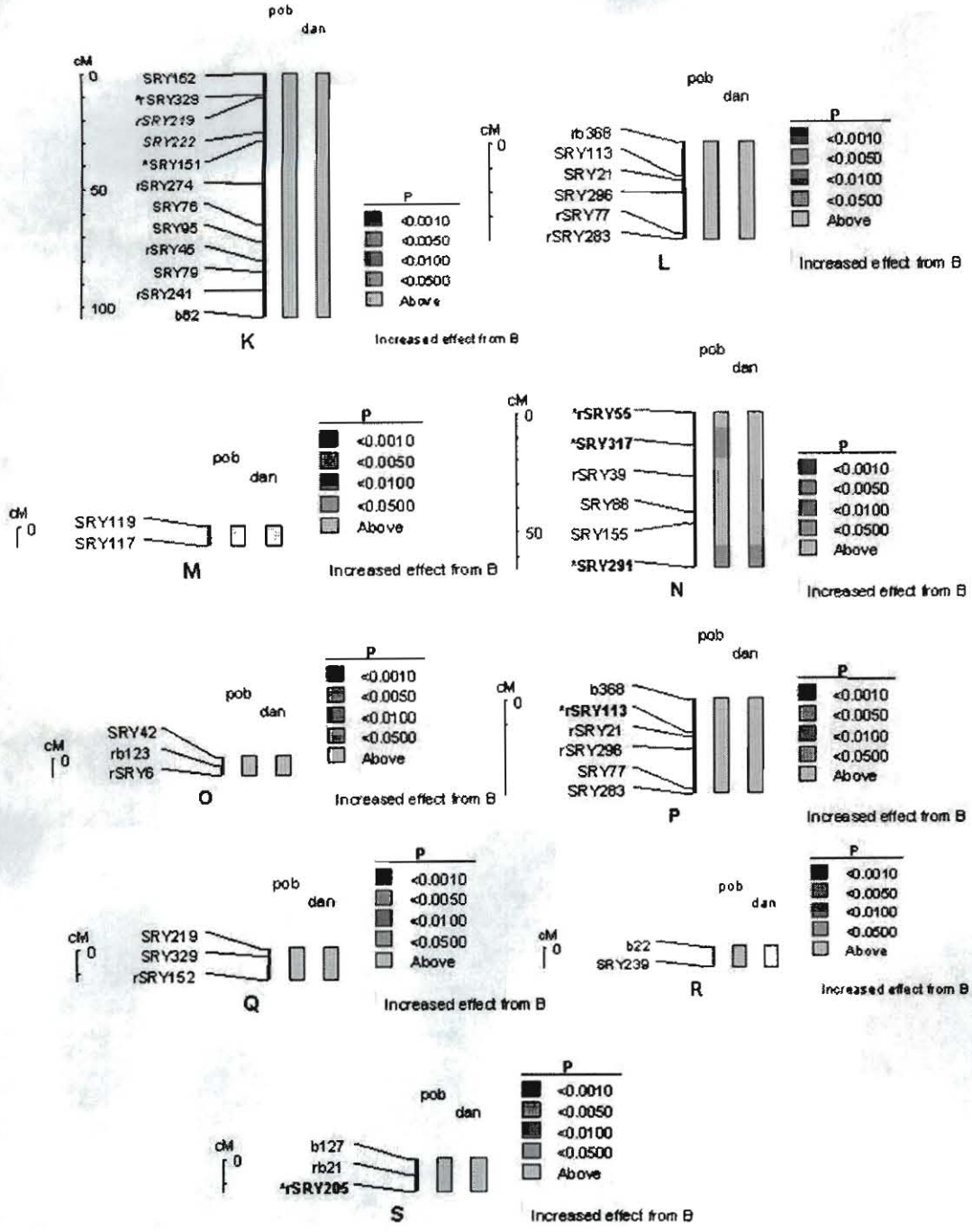

Figure 2B. Preliminary linkage map of MEcu 72 analyzed by Qgene. Associated markers with the resistance showed with*.

Differential subtraction: RNA extraction and amplicon generations for hybridizations.
RNA was isolated from young leaves of MEcu 72 (E), M Per 334 (P) and MCol 2246 (M), colleted in the greenhouse. To isolate total RNA, the Rneasy Plant Mini Kit QIAGEN? was used. Genomic DNA was removed prior to isolation of poly $(A)^+$ RNA with DNAse I. The SV Total Isolation System of Promega? was used. The generation of cDNA was done using poly A+ mRNA as the substrate, which was isolated using the protocol Oligotex mRNA Spin Column of QIAGEN? . First-strand cDNA synthesis and cDNA amplification were done using SMART PCR cDNA Synthesis kit" de Clontech? .

The PCR products from the amplification of cDNA were purified using QIAquick PCR Purification kit QIAGEN? . Then digestion ligation was done, where the cDNA was digested with DpnII, and then adapters (BamI and BamII) were ligated. Finally the generation of a PCR amplicon was done, which is representative of the original mRNA from MEcu 72 and MCol 2246. For "tester" MEcu 72 (E), 150 ng was obtained; and for "driver" MCol 2246 (C), 15 ?g (Figure 3).

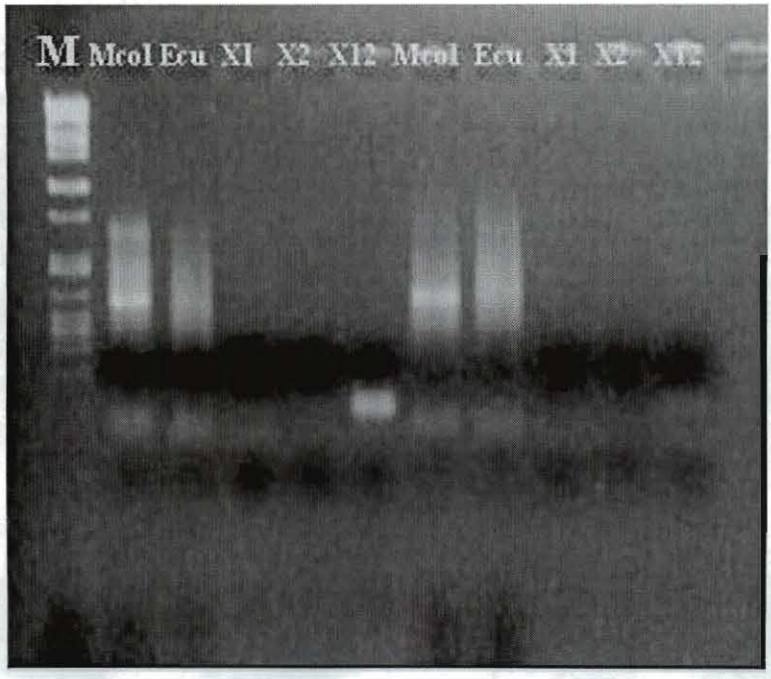

Figure 3. **Amplicons of MEcu 72 and MCol 2246. M: ? PstI.**
MCol: MCol 2246 with primer Bam II.
MEcu 72: MEcu 72 with primer Bam I.
X1: blank (without DNA) with primer Bam I.
X2: blank (without DNA) with primer Bam II.
X12: blank (without DNA) with primers Bam I and Bam II.

Ongoing Activities:

- Saturation of linkage map of MEcu 72, using AFLPs.
- Isolation, cloning, sequencing and mapping of AFLPs polymorphic bands between resistant and susceptible genotypes.
- Design of SCARs for marker-assisted selection.
- QTL analysis for whitefly resistance.
- Mapping of cassava SSRs from ESTs in F1 (276 genotypes).
- Subtractive hybridization of the amplicon MEcu 72 (tester) and MCol 2246 (driver), during which amplified portions of differentially expressed genes are enriched and common sequences are depleted.
- Cloning and screening of the resulting products of expressed sequences during the defense response of MEcu 72 to whitefly attack.
- Microarray of clones in order to identify differentially expressed sequences.

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Activity 2. Evaluation of cassava germplasm for resistance to whiteflies (*Aleurotrachelus socialis*) during 2002-2003.

Rationale

Research in host plant resistance (HPR) to whiteflies has increased in recent years, primarily because of the extensive damage caused by the *Bemisia tabaci* species complex on a wide range of agricultural crops. In general, a narrow range of germplasm has been tested and there are few deliberate breeding programs designed to develop higher levels of resistance in cultivars. In some cases, related wild species have been evaluated for a source of whitefly resistance in breeding programs, but examples are limited. Consequently, HPR to whiteflies in cultivated crops is rare.

The CIAT cassava and IPDM project (IP-3 and PE-1) have placed a special emphasis on our ongoing efforts to develop whitefly resistant cultivars in cassava. This project is unique because we are systematically screening a large germplasm bank (?6000 clones), and more recently, wild *Manihot* species. We continue to identify resistant genotypes and through a comprehensive breeding scheme, develop commercial hybrids containing whitefly resistance. We have also developed a mapping population of genotypes suitable for identifying molecular markers for a given trait, using MEcu 72 as the resistant parent and MCol 2246 as the susceptible parent (Fam: CM 8996).

Whiteflies, especially in the Neotropics, cause direct damage to cassava by feeding on the phloem of the leaves. This causes leaf curling, chlorosis, and leaf fall, which results in considerable reduction in root yield if prolonged feeding occurs. There are two major species causing direct feeding damage in the Neotropics, *Aleurotrachelus socialis* in the Northern region of South America (Colombia, Ecuador and Venezuela) and *Aleurothrixus aepim* in Brazil. Yield losses resulting from *A. socialis* and *A. aepim* feeding are common in both regions. In Colombia cassava field losses as high as 79% are reported caused by *A. socialis* and about 40% due to *A. aepim* in Brazil.

HPR offers a low-cost practical, long-term solution for maintaining lower whitefly populations and reducing crop losses. This is especially important for cassava, as it has a long growing cycle and is often grown by resource limited smallholder farmers who cannot afford costly inputs. During March of 2003, CORPOICA (Colombia, MADR) officially released a whitefly (*A. socialis*) resistant cassava cultivar, Nataima-31 (CG 489-31; CIAT Breeding Code). This cultivar, a progeny of a MEcu 72 x MBra 12 cross, was developed over a 15 year period in a collaborative CIAT-CORPOICA (Nataima, El Espinal, Tolima) effort. A field day to commemorate the varietal released attracted about 200 cassava producers from the El Espinal/Tolima region.

Specific Objectives:

A. Evaluation of the family CM 8996 for a genetic study for whitefly (*A. socialis*) resistance at CORPOICA, Nataima, Tolima (2002-03).

Materials and Methods

Systematic and continuing whitefly (*A. socialis*) evaluations on cassava germplasm are carried out at primarily two sites, the CIAT farm in Santander de Quilichao and at CORPOICA, Nataima, Tolima.

The family CM 8996 was developed from a cross of the whitefly resistant cultivar, MEcu 72 and the susceptible cultivar MCol 2246. The resulting progeny, approximately 700 genotypes, are being systematically evaluated at the two aforementioned sites with the objective being to study the genetics and inheritance of whitefly (*A. socialis*) resistance in cassava. The Santander de Quilichao plantings were done in April 2002 and harvested in March 2003. The CORPOICA, El Espinal plantings were also planted in April 2002 and harvested during May 2003. Both sites traditionally have moderate to high whitefly populations although in some years *A. socialis* populations have been too low for reliable screening for resistance. At CORPOICA, El Espinal; 654 genotypes (progeny) of family CM 8996 were planted, while at CIAT/Santander de Quilichao 700 genotypes were planted. Each genotype was planted in rows of five plants and every 20 rows, the susceptible cultivar CMC 40 (MCol 1468) was planted (this "indicator" cultivar provides a record of whitefly population levels and distribution). Three evaluations for whitefly populations and damage levels were carried out during the course of the crop cycle at Santander and 4 at Nataima. A 1 to 6 damage and population scale was employed (Table 2). Root yield data was recorded at harvest by harvesting the central three plants of each genotype/row.

Table 2. 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

CORPOICA, Nataima. This is the second cycle of evaluating this family CM 8996 at Nataima. Last year (Cycle 1, 2001-2002) whitefly (*A. socialis*) populations were low throughout the crop cycle, resulting in low damage ratings. Whitefly populations during the second cycle *(2002-03)*

were higher and present on all 633 genotypes evaluated (Figure 4). The highest number, 338 (53.4%) genotypes occurred in the 2.0 to 3.0 population range, indicating up to 500 *A. socialis* individuals per leaf, while 127 (20.1%), genotypes fell into the 1.1 to 2.0 range, or 1-200 per leaf. CMC 40, the susceptible check had an average damage rating of 4.5, indicating about 1000 to 2000 whitefly individuals per leaf and signifying a moderate to high *A. socialis* population. This population was fairly evenly distributed throughout the evaluation field. CMC 40 also had high damage ratings, consistently between 4.0 and 5.0 throughout the field (Figure 5). This level of population and damage results in sufficient selection pressure to do adequate evaluations of germplasm.

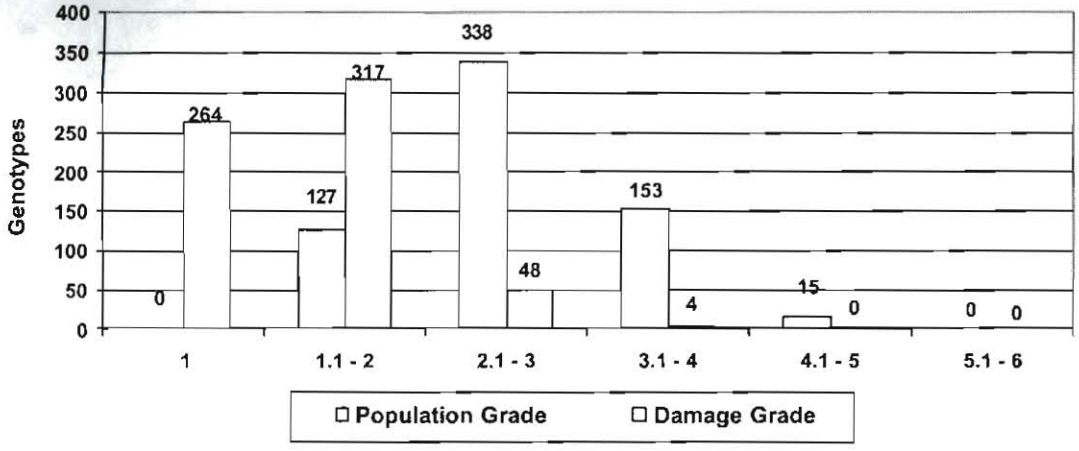


Figure 4. Whitefly (*A. socialis*) population and damage ratings of genotypes from the cassava family CM 8996 evaluated at CORPOICA, Nataima (Tolima) during the 2002-03 crop cycle.

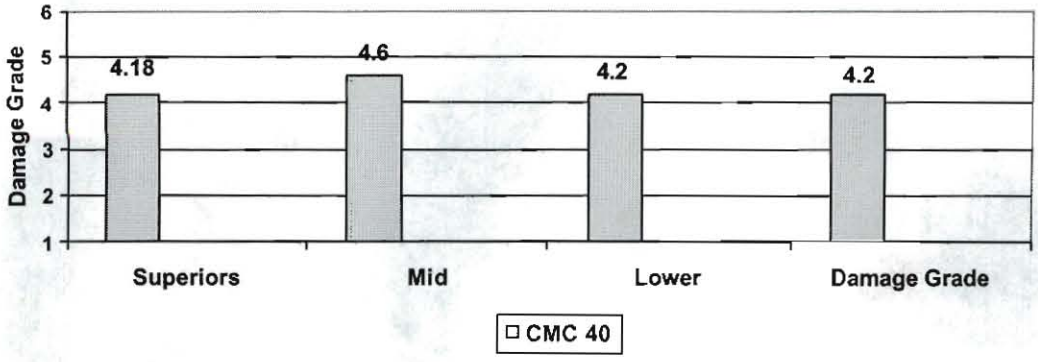


Figure 5. Average whitefly (*A. socialis*) damage and population ratings at three plant levels (superior, middle and lower leaves) of the susceptible control clone, CMC 40 at CORPOICA, Nataima (Tolima) during 2002-03.

The majority of the genotypes, however, had very low damage scores: 264 (41.7%) genotypes had a damage rating of 1.0, indicating no visible damage. Three hundred seventeen (50.1%) had a damage rating of 2.0 (young leaves slightly flaccid) while 48 (7.6%) had a 3.0 rating (some twisting of young leaves, slight leaf curling). Only four (0.6%) genotypes had, what can be

considered, a high damage rating (4.0). Both *A. socialis* populations and damage were higher during the 2002-03 cycle than the 2001-02 cycle, as indicated by the > 4.0 damage rating (curling and twisting of apical leaves, mosaic-like appearance to the leaves and sooty mold) for the susceptible control CMC-40 (average of 19 rows evaluate) (Table 3).

Table 3. Whitefly (*A. socialis*) populations and damage ratings on the susceptible cassava cultivar CMC 40 planted with the cassava Family CM 8996 at CORPOICA, Nataima (Tolima) during 2002-03.

	Population Grade									
	Upper Bud				Medium		Lower	Damage Grade		
Clone	Adult	Egg	Nymph	Pupa	Nymph	Pupa	Pupa	Upper	Medium	Lower
CMC 40	3.0	4.0	4.0	5.0	4.0	4.0	4.0	4.5	4.0	4.0
CMC 40	3.0	4.0	4.0	4.0	4.0	4.0	4.0	4.5	4.0	4.0
CMC 40	3.0	4.0	5.0	5.0	5.0	4.0	4.0	4.5	4.5	4.5
CMC 40	4.0	3.0	5.0	5.0	5.0	4.0	5.0	4.5	4.5	4.0
CMC 40	4.0	3.0	4.0	4.0	5.0	5.0	4.0	4.0	4.0	4.0
CMC 40	3.5	4.0	4.0	4.5	4.0	5.0	4.0	4.5	4.0	3.5
CMC 40	4.0	3.5	4.5	5.5	5.0	6.0	4.0	4.5	5.5	5.0
CMC 40	4.0	3.0	4.0	4.0	5.0	4.0	4.0	4.0	4.0	4.0
CMC 40	3.0	4.0	4.0	4.0	4.0	4.0	4.0	4.5	4.0	4.0
CMC 40	4.0	4.0	4.5	4.0	5.0	5.0	4.5	4.0	4.5	4.0
CMC 40	3.0	3.0	4.5	4.0	3.0	3.0	3.0	4.0	4.0	4.0
CMC 40	4.0	4.0	6.0	6.0	6.0	4.0	4.0	4.0	4.0	4.5
CMC 40	4.0	4.0	6.0	6.0	5.5	5.5	5.0	4.0	4.0	4.5
CMC 40	4.0	4.0	5.5	5.5	5.5	4.0	4.0	4.0	4.0	4.0
CMC 40	3.0	3.0	6.0	6.0	6.0	5.0	4.0	4.0	4.5	4.0
CMC 40	4.0	4.0	5.0	5.0	5.0	4.0	4.0	4.0	4.0	4.0
CMC 40	3.0	4.0	4.0	4.0	4.0	4.0	4.0	4.5	4.0	4.0
CMC 40	4.5	4.0	4.5	4.5	5.0	4.5	4.5	4.0	4.5	5.0
CMC 40	4.0	4.5	4.0	3.0	4.5	5.0	5.0	4.0	4.5	5.0

These results may be indicating that there exists at least a moderate level of *A. socialis* resistance in nearly all of the CM 8996 genotypes. Since the female parent, MEcu 72, is highly resistant to *A. socialis*, these results are not completely unexpected. Both parents of this family are vigorous varieties and the resulting vigor in the progeny should be expected.

At harvest, the range of segregation in the F1 can be observed in the yield data. Based on only the three central plants of each row of each genotype, yields ranged 0.0 T/ha (no roots produced) for CM 8996-170 to 98.7 T/ha for genotype CM 8996-679 (Table 4).

About 80 (12.7) genotypes yielded less than 10 T/ha, or below the national average, while 115(18.3%) yielded between 11 and 19.8 T/ha or somewhere in the range of the national average. This means that 68.9% (433 genotypes) yielded above 20 T/ha; 65 of these (10.4%)

yielded above 50 T/ha (Table 4). Since these yield estimates are based on only three plants, this data is presented to indicate the range of yield segregation in the F1. It is interesting to note that we do not see this type of segregation in whitefly resistance, where most of the F1 genotypes are in the moderate to high level of resistance.

Table 4. Yield parameters (harvest index, % dry matter and cooking quality) of 628 cassava genotypes from the family CM 8996 at CORPOICA, Nataima (Tolima) during 2002-03 crop.

Yield T/ha		Harvest Index		% Dry Matter		Cooking Quality	
Range	No. Genotypes	Range	No. Genotypes	Range	No. Genotypes	Grade	No. Genotypes
0–10	80	0-0.39	75	17–29.96	107	1	128
11–19.8	115	0.4–0.49	102	30–32.96	228	2	261
20–29.9	162	0.5–0.59	187	33–35.93	231	3	125
30–49.6	206	0.6–0.69	213	36–40.34	57	4	97
50–69.2	53	0.7–0.86	51	41–48.16	2	5	10
70–98.7	12	1.0	0	-	-	-	-

This data however does indicate the possible yield potential of some of the genotypes. Harvest index show a similar range; 451 genotypes (71.8%) had a harvest index equal to or above 0.5 and 264 genotypes (42.0%) were above 0.6 (Table 4). This data further supports the production capacity of this family (CM 8996). Root dry matter also displays a range of segregation; more than half (235=53.3%) of the genotypes had dry matter below 33%, which is unacceptable for a commercial variety. Nearly 37% (231 genotypes) of the F1's had a root dry matter between 33 and 36%, this is acceptable but higher is preferred. Fifty-nine genotypes (9.4%) had dry matter content above 36% and two of these were above 41% (Table 4).

More than half of the genotypes (389 or 62.6%) had a cooking quality rating between 1 and 2, indicating roots that soften or are made edible in 20 to 25 minutes, a high portion of starch and excellent eating texture (Table 4). These results further indicate that it is possible to combine good whitefly resistance with high yield and excellent commercial qualities.

B. Evaluation of the Family CM 8996 for a genetic mapping study for whitefly (*A. socialis*) resistance at Santander de Quilichao (2002-03).

Results

Santander de Quilichao. The methodology for the trial at Santander is basically the same as that for CORPOICA, Tolima, described in the previous section. In May 2003, 673 genotypes of family CM 8996 were harvested. Three whitefly damage and population ratings were made during the growing cycle. Growing conditions at Santander differ from those at Tolima in that soils at Santander are acid and clay loam in texture while at Nataima soils are not acid and more a sandy leam. Planting material for this trial originated from the 2001-02 trial at Nataima, Tolima. In addition, at Santander several other pests are present and affect cassava growth, especially mites and thrips. The presence of these additional pests may suppress whitefly populations and damage symptoms and also affect yield.

Root yield of the CM 8996 genotypes at Santander ranged from 0.0 to 42.2 T/ha (Figure 6) while at Nataima yields ranged from 0.0 to 98.7 T/ha (a difference of about 57.2%). At Santander 432 of the 673 genotypes (64.2%) had yields of 10 T/ha or lower, while at Nataima only 80 genotypes (12.7%) fell into this range (Figure 6). At Santander only 50 genotypes 7.4%) had yields above 20 T/ha (Table 5) with a maximum production of 42.2 T/ha, while at Nataima, 68.9% (433) of the genotypes had yields above 20 T/ha (Figure 6) and a maximum of 98.7 T/ha.

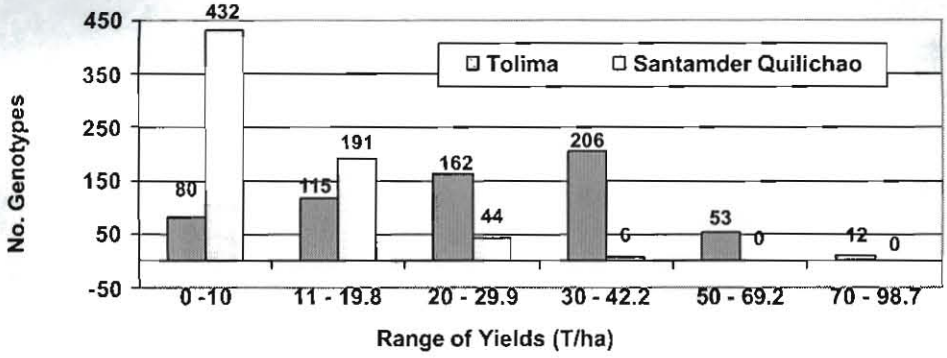


Figure 6. Yields for the cassava family CM 8996 (MEcu 72 x MCol 2246) under whitefly (*Aleurotrachelus socialis*) pressure at two evaluation sites, Santander de Quilichao (Cauca) and CORPOICA, El Espinal (Tolima) during 2002-03 crop cycle.

Harvest index of Santander was between 0.39 and 0.89 (Table 5). The accuracy of this data is questionable due to the damage caused by mite and thrips attack as well as other variables such as the presence super-elongation disease (*Sphaceloma* sp) toward the end of the crop cycle. Mite, thrips and super elongation attack did not occur at Nataima.

Results on dry matter content did not differ greatly between the two sites. At Santander, as in Nataima, more than half the genotypes had a dry mater content below 33.0% (Tables 4 and 5). At Santander 55% (374) genotypes (53.3% and 335 genotypes at Nataima) had a dry mater content below 33.0%; 32.4% (218 genotypes) fall in the range of 33 to 36% (231 and 37.0% at Nataima) and 10.5% (71 genotypes above 36% (59 and 9.4% at Nataima). Cooking quality of the roots harvested at Santander ranged from very good to very poor. About 32.4% (210) of the genotypes had excellent cooking quality, ranging between 1 to 2 (Table 5), while nearly 29% (194) were intermediate at grade 3. A higher percentage of the genotypes had a poorer cooking quality at Santander than at Nataima (36.4% vs. 17.0%) (Table 4 and 5). All genotypes are sweet (i.e. low HCN).

Table 5. Yield parameters (harvest index, % dry matter and cooking quality) of 673 genotypes from the family CM 8996 at Santander de Quilichao (Cauca) during the 2002-03 crop cycle.

Yield T/ha		Harvest Index		% Dry Matter		Cooking Quality	
Range	No. Genotypes	Range	No. Genotypes	Range	No. Genotypes	Grade	No. Genotypes
0-10.9	432	0-0.39	88	20.2–29.9	123	1	42
11-19.8	191	0.4-0.49	112	30–32.9	251	2	168
20-29.9	44	0.5-0.59	212	33–35.9	218	3	194
30-38.4	5	0.6–0.69	200	36–40.4	62	4	196
40-42.2	1	0.7–0.89	61	41-55.8	9	5	49

A comparison of genotypes with the highest yields at Nataima, Tolima and Santander de Quilichao can be found in Table 6. It can be observed that those genotypes having the highest yield in Tolima had very low yields at Santander. For example, CM 8996-79 yielded the equivalent of 98.7 T/ha at Tolima and yielded nothing at Santander. CM 8996-250 yielded 71.3 T/ha at Tolima and only 4.3 T/ha at Santander (Table 6). However, we can observe that those genotypes that yielded highest at Santander also yielded well at Tolima, in several cases yielding higher. These results suggest that clones that yield well in a harsher environment, in most cases will do even better when sown in a more favorable environment.

Table 6. Highest yielding cultivars of the cassava family CM 8996 evaluated at two sties for whitefly (*A. socialis*), Santander de Quilichao (Cauca) and CORPOICA, Nataima (Tolima) during 2002-03.

Clone	Yield Tolima	Yield S. de Quilichao	Clone	Yield S. de Quilichao	Yield Tolima
CM 8996-79	98.7	-	CM 8996-318	42.3	48.4
CM 8996-14	95.5	13.5	CM 8996-596	38.4	52.8
CM 8996-126	84.5	7.5	CM 8996-410	37.5	34.5
CM 8996-290	82.9	20.7	CM 8996-282	37.3	22.3
CM 8996-536	80.3	9.9	CM 8996-404	35.9	42.5
CM 8996-244	78.7	3.7	CM 8996-280	30.68	34.73
CM 8996-401	78.5	14.9	CM 8996-88	29.9	42.8
CM 8996-482	76.5	4.8	CM 8996-81	29.9	35.8
CM 8996-660	75.5	6.9	CM 8996-305	29.7	12.93
CM 8996-250	71.3	4.3	CM 8996-65	29.5	16.4

C. Evaluation of selected cassava varieties for whitefly (*A. socialis*) resistance at CORPOICA, Nataima, Tolima (2002-03).

Rationale

The systematic evaluation of the cassava germplasm bank (>5000 landrace varieties) has resulted in the identification of numerous cultivars as potential sources of whitefly resistance. These selected varieties will go through numerous field cycles. The methodology used for evaluation is similar to that described in the previous activity.

Results

Whitefly populations and pressure was uniformly high for this trial indicating good selection pressure (Table 7. Eleven of the 16 varieties evaluated had population levels of 4.0 or higher (Figure 7). The trial was composed primarily of Peruvian, Colombian and Brazilian varieties and included Nataima-31 (CG 489-31) and CG 489-34, both whitefly resistant progeny from the MEcu 72 x MBra 12 cross. The five varieties with the lowest whitefly population levels and consequently the lowest damage ratings were Nataima 31 (2.5 popl. Vs 1.0 damage), CG 489-34 (3.2 and 1.3), MPer 317 (1.8 and 1.3), MPer 334 (2.4 and 1.3), MPer 273 (2.0 and 1.7) (Table 7. All other varieties had populations' levels above 4.0 and damage rating ranging from 2.7 to 4.3. The majority of the varieties were of Brazilian origin (9) and all appeared more susceptible than the three Peruvian varieties. This again supports the observation that most whitefly (*A. socialis*) resistance is concentrated in Ecuadorian and Peruvian varieties. MPer 317, MPer 334 and MPer 273 have consistently shown low populations and damage levels through several field evaluations and growth chamber trials. The two hybrids from the MEcu 72 x MBra 12 cross, Nataima 31 (CG 489-31) and CG 489-34 continue to express good field resistance with low whitefly populations and damage levels.

Table 7 Selected Peruvian, Brazilian and Colombian cassava cultivars and hybrids evaluated for whitefly (*A. socialis*) resistance at CORPOICA, Nataima (Tolima) during 2002-03 crop cycle.

	Population Grade											
	Upper Leaves				Middle Leaves		Lower	Damage Grade			Average	
Clone	Adult	Egg	Nymph	Pupa	Nymph	Pupa	Pupa	Upper	Mid	Lower	Pop.	Damage
Nataima-31	1.5	1	2	3	3	2.5	2	1	1	1	2.5	1
CG 489-34	4	4	4	4	3	3	2	1	1	2	3.2	1.3
MPer 317	2	2	3	2	1.5	1.5	1	1	1	2	1.8	1.3
MPer 334	2	2	2	3	3	2	2	1	1	2	2.4	1.3
MPer 273	2	2	1	1	2.5	3	2.5	1	1.5	2.5	2	1.7
MBra 292	3	3	5	4	4	4	4.5	1	3	4	4.3	2.7
MBra 81	3	4	5	5	5	5	4	3	3	2	4.8	2.7
MCol 2025	4	4	4	5	4	4	4	2.5	2.5	3	4.2	2.7
MBra 29	3	4	5	5	4.5	4	4.5	1	3	5	4.6	3
MBra 442	3.5	3.5	5	5	5	5	4	3	3	4	4.8	3.3
0MBra 532	4	3	4.5	4.5	4	4	4	4	3.5	4	4.2	3.8
CG 936-7	3	4	5	5	5	4	4	4	4	4	4.6	4
MBra 303	3	4	4	4	5	5	5	4	4	4	4.6	4
MCol 2246	2	3	5	5	4	3	3	4	4	4	4	4
MBra222	5	4	4	5	5	5	5	4	4.5	4	4.8	4.2
MBra370	4	4	5	5	5	4	5	4	4.5	4.5	4.8	4.3

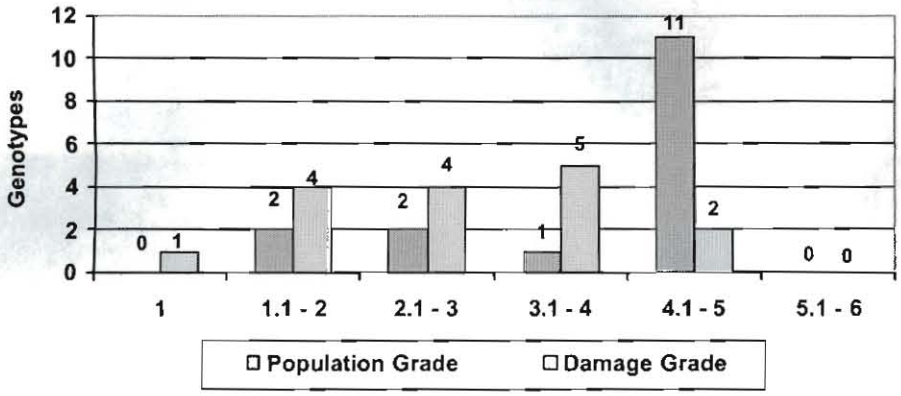


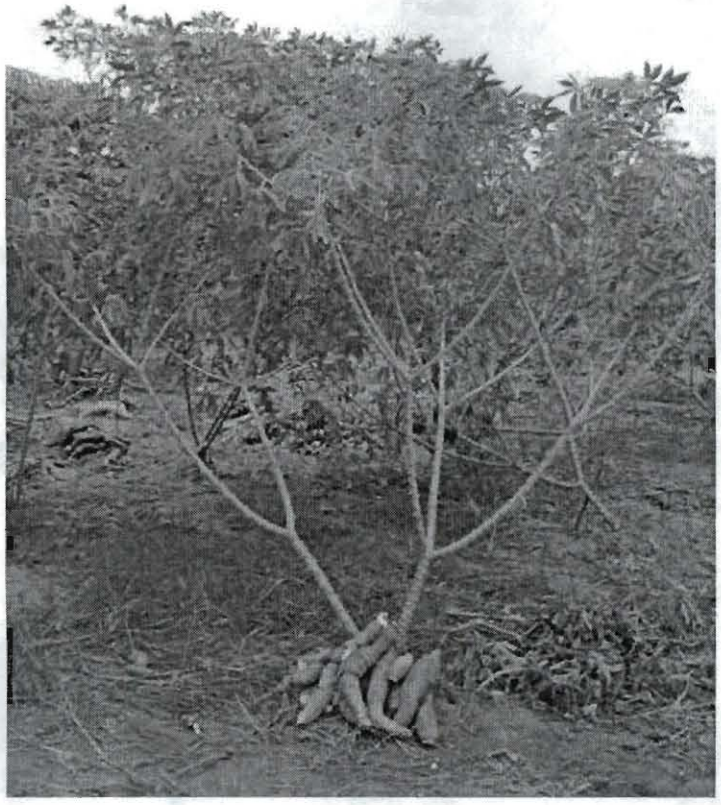
Figure 7. Whitefly (*A. socialis*) population and damage ratings on Peruvian, Brazilian and Colombian cultivars and hybrids evaluated for resistance at CORPOICA, Nataima (Tolima) during 2002-03.

Harvesting, Evaluating and Planting Cassava

In order to determine the genetics of whitefly resistance a cross was made between the highly resistant MEcu 72 x the very susceptible MCol 2246. A mapping population of 276 individual genotypes (family CM 8996) is planted each year at two sites (CIAT/Santander de Quilichao and CORPOICA/Nataima, Tolima) in Colombia and evaluated for whitefly damage and populations, yield, dry matter and culinary qualities. The major objective of the evaluations is to determine whitefly resistance/susceptibility as an indication of gene segregation in the offspring and identify associations between molecular markers and the resistant materials. The following series of photos is a pictorial description of the field trails of this mapping population.

Contributors: Bernardo Arias, Anthony C. Bellotti.

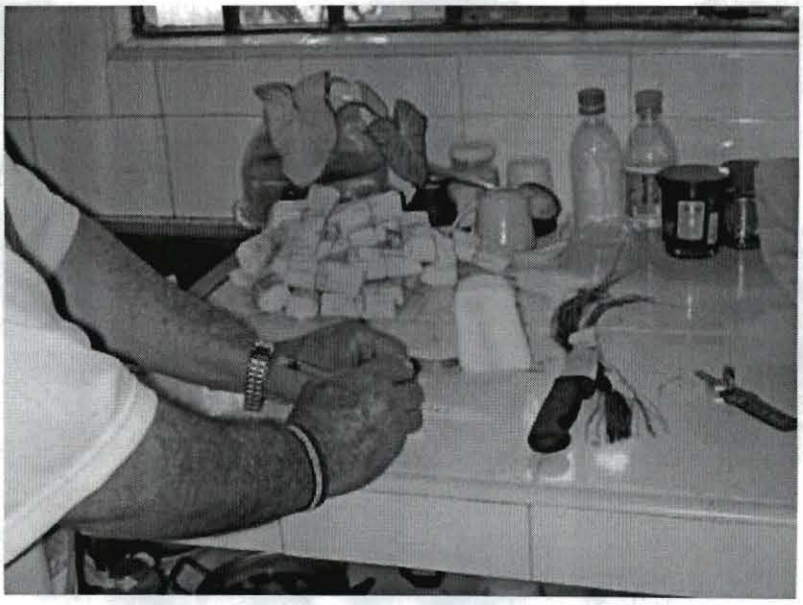
Collaborators: Gustavo Trujillo, Gerardino Pérez.



The per-plant harvest of one genotype of the MEcu 72 x MCol 2246 cross. The harvest is usually done 11 to 12 months after planting.

The weighting of plant roots is done in the field.

The harvesting process consists of first removing plant stems/leaves, and then pulling up the roots. Root weight is determined on a per-plant basis and on total plant population. Plant biomass, including stems and leaves may also be included.



The culinary or cooking quality of selected genotypes is evaluated in the laboratory. The sections of cassava roots seen in the photo are boiled and tasted.

Cassava stems for each genotype are maintained separately. Note the stem color variation between the different genotypes (family CM 8996).

Cassava is vegetatively propagated using 20 cm stem cuttings. A selection of stem cuttings is made prior to planting with the objective being the use of uniform and healthy planting material.

Field planting of the selected genotypes. Approximately 300 genotypes will be planted in three replications and evaluated for whitefly populations and damage on a periodic basis. Note that cassava cuttings (stakes) are planted vertically with the upper portion above ground.

Activity 3. Manihot wild species and hybrids evaluated for whitefly (*A. socialis*) populations damage at Santander de Quilichao during 2002-03 crop cycle.

Rationale

Wild *Manihot* species have provided a potential source of resistance genes for arthropod pests **(see Activity 9, this reports).** In collaboration with the cassava genetics and plant breeding sections, field plantings of hybrids from crosses between *M. esculenta* x *M. flabellifolia* and *M. peruviana* wild *Manihot* species are evaluated when arthropod attacks occur. During 2002-03 about 549 genotypes, consisting of wild cultivars (CW), GM and CM hybrids were evaluated for whitefly (*A. socialis*) damage at Santander de Quilichao.

Results

Whitefly damage ratings, in general, were moderate and of the 549 genotypes, 150 (27.3%) resulted in no damage symptoms (Figure 8). Two hundred fourteen (39.0%) showed light damage symptoms (1.1 to 2.5 on the 1-6 damage scale, Activity 2, Table 2, while 44 (8.0) had intermediate damage symptoms (2.6-3.0) and 141 (25.7%) genotypes showed damage symptoms above 3.0. Of these 37 genotypes (6.7%) had severe damage (4.1 to 5.0).

All genotypes were infested with whiteflies with populations ranging from one (<200 individuals per leaf) to four (500 to 2000 per leaf). Of the a 549 genotypes, 371 (67.6%) had low whitefly populations (below 2.5 on the 1 to 6 scale), while 178 (32.4%) resulted in an intermediate damage rating (2.6 to 4.1) (Figure 8).

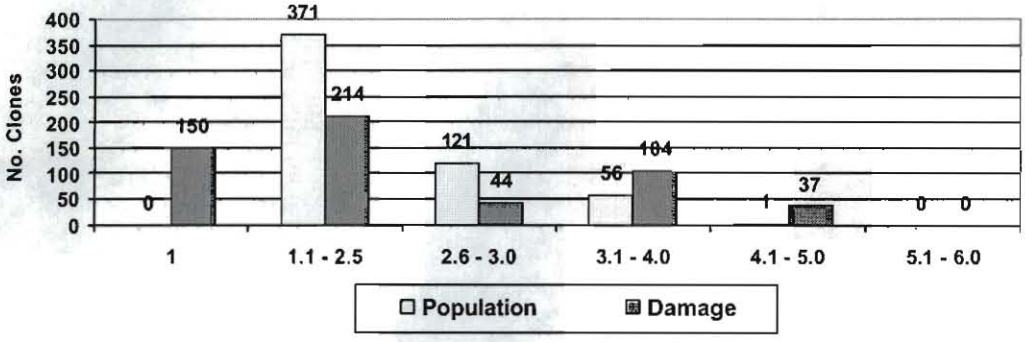


Figure 8. Whitefly (*Aleurotrachelus socialis*) populations and damage on 549 wild *Manihot* hybrids and other hybrid at Santander de Quilichao (Cauca) during the 2002-03 crop cycle.

Of the 296 *Manihot* hybrids (CW) evaluated, nearly half, 48.6%, had no whitefly damage symptoms (Figure 9). Only 4.7% (13 genotypes) had a damage rating of 3.0 or higher. Whitefly populations were present on all genotypes but populations were in the low range (1.0 to 2.5) on nearly all (95.2%) of the genotypes. Whitefly populations and damage were higher on the GM genotypes (Figures 10 and 11). In the GM and CM hybrid plantings (Figure 10), whitefly damage ranged from 1.1 to 5.0 on the damage scale. Of the 224 genotypes, 65.6% had damage ratings above 2.5 and about 55% had whitefly populations above 2.5 (Figure 10). In the CM planting of 29 hybrids genotypes, nearly 83% had damage ratings above 2.5 (Figure 11) and no genotypes were without damage. Whitefly populations were moderate.

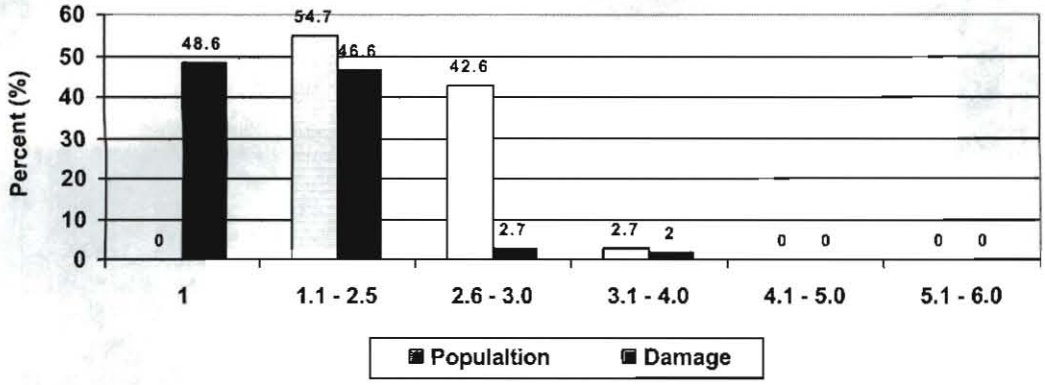


Figure 9. **Percent whitefly (*A. socialis*) populations and damage recorded on 296 wild *Manihot* hybrids at Santander de Quilichao (Cauca) during the 2002-2003 crop cycle.**

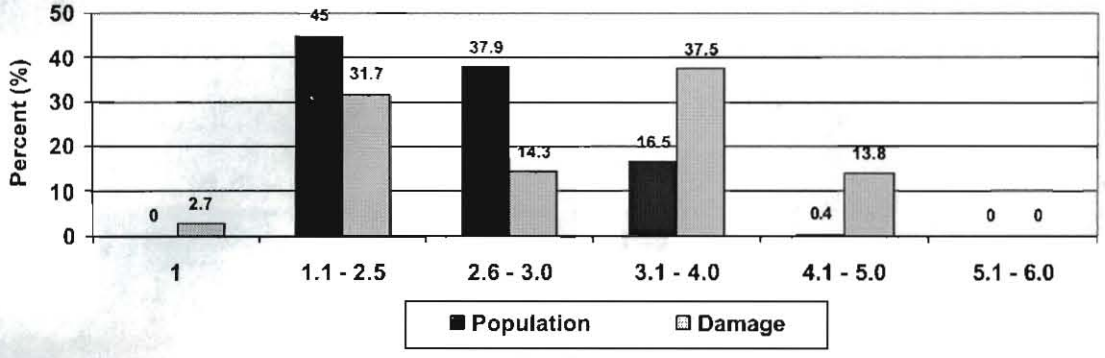


Figure 10. **Percent whitefly (*A. socialis*) populations and damage recorded on 224 GM hybrids planted at Santander de Quilichao (Cauca) during the 2002-03 cycle.**

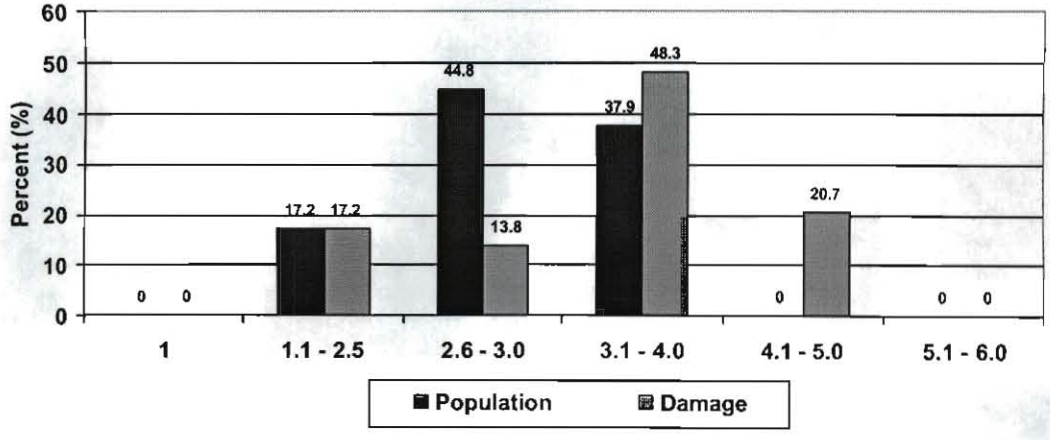


Figure 11. **Percent whitefly (*A. socialis*) populations and damage recorded on 29 hybrids (CM) planted at Santander de Quilichao (Cauca) during the 2002-03 cycle.**

The fact that the Wild *Manihot* hybrids had both low whitefly populations and damage is not completely unexpected. Separate research and observations over the years have indicated that the wild *Manihot* species may contain arthropod resistant genes that can provide a potential source for resistance to the cultivated *M. esculenta*. Modern biotechnological tools provide the means to achieve a more effective success in the development of pest resistant germplasm. The possible whitefly resistance observed in the CW wild *Manihot* hybrids is further evidence of this potential and needs to be pursued.

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Activity 4. Evaluation of cassava dialelic crosses for whitefly (*A. socialis*) resistance, using MEcu 72 as the resistant female parent (at Santander de Quilichao).

MEcu 72 has consistently displayed high levels of resistance to the whitefly, *A. socialis*. This variety was used as the resistant female parent in a dialelic cross with several other genotypes, including MPer 183. CM 6740-7, SM 1219-9, SM 1278-2, SM 1636-24, SM 1673-10, SM 1741-1 and HMC-1. The resulting progeny were planted out at Santander de Quilichao and evaluated for whitefly resistance and damage.

Results

The dialelic cross resulted in 225 progeny. All progeny had whitefly populations but in general, both damage and populations were low (Figure 12). Nearly all of the progeny had whitefly populations between 1.1 to 2.5 (99.1%). One hundred four genotypes (46.2%) resulted in no damage symptoms, while 111 progeny (49.3%) had a damage rating between 1.1 and 2.5 (Figure 12). Ten genotypes had a damage level of 3.0, considered to be intermediate but susceptible. The genotype GM 310-21 had a high damage rating; the male parent SM 1278-2, also had a high damage (4.0).

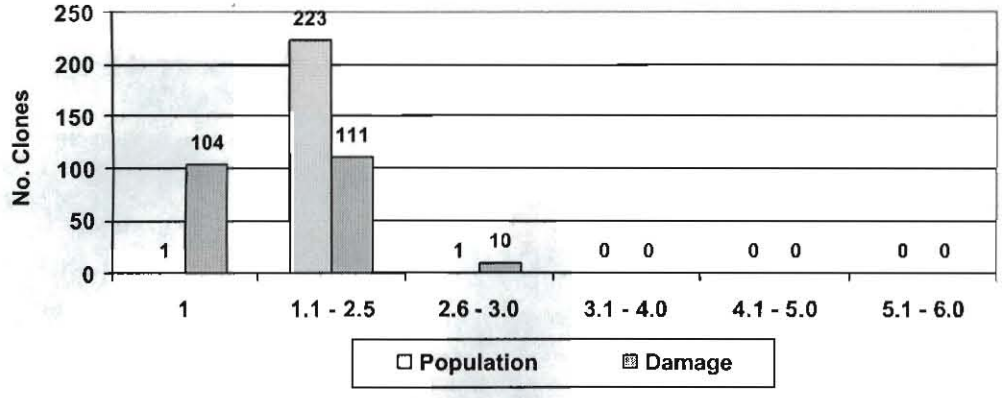

Figure 12. Whitefly (*A. socialis*) populations and damage on 225 cassava hybrid progeny from a dialelic cross using MEcu 72 as the resistant female parent (Santander de Quilichao, Cauca, 2002-03).

These results, low whitefly populations and corresponding low damage, of the progeny from this cross, are not unexpected. MEcu 72, the female parent, is highly resistant and it appears that this resistance is readily passed on to the progeny.

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Activity 5. Evaluation of cassava germplasm in several breeding and genetic trials for whitefly (*A. socialis*) damage at Santander de Quilichao (Cauca).

The cassava entomology section participates in the screening of genetic and breeding materials in close collaboration with breeders and geneticists. The trials evaluated include 1) Yield trials, 2) Regional trials, 3) Beta carotene varietal selection, 4) Observation trial, 5) Multiplication trials. A brief description of results from these evaluations follows. Actual data for all evaluations of each clone is available in the database.

1. Yield trial: Whitefly (*A. socialis*) populations were generally high; high adult and egg populations were found on the upper leaves while the middle and lower leaves had high nymph and pupae populations. Of the 75 clones evaluated, only one, SM 2583-3 displayed no damage symptoms (Figure 13), while 45 clones (60%) had a damage rating above 3.0 and 26 (34.7%) had a rating between 4.0 and 5.0 (leaf curling, severe clorosis and sooty mold). Nearly two-thirds of the clones (65.3%) had a population rating of 3.0 or lower and 30 clones (40%) were rated 2.5 or lower.

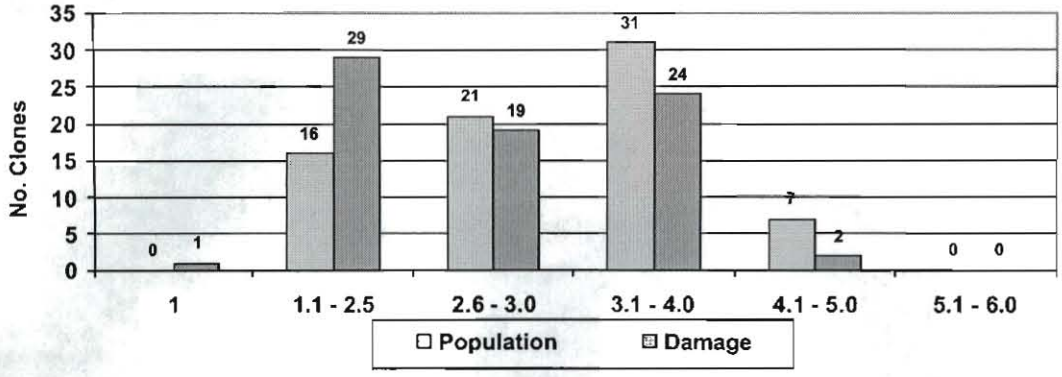

Figure 13. Average whitefly (*A. socialis*) population and damage ratings for 82 clones in a cassava yield trial at Santander de Quilichao (Cauca) during the 2002-03 crop cycle.

2. The regional trial consisted of 29 clones evaluated at Santander de Quilichao for whitefly (*A. socialis*) damage. These 29 clones were planted in three replicates. All clones had average whitefly populations that ranged from 2.5 to 5.0, indicating a moderate to high selection pressure (Figure 14). Two of the clones that consistently presented very low damage ratings across the three replicates were SM 1871-33 (1.0, 2.0 and 1.7) and SM 2085-7 (1.3, 1.7 and 1.7). Populations on some clones, especially on the upper and mid leaves were very high, reaching 5.0 to 6.0 on the damage rating scale. Few clones show low damage ratings; five of the 29 clones (17.2%) had damage ratings below 2.5 (although populations on some of these reached 4.0 to 5.0). In general, many of these are vigorous clones and may "outgrow" some of the damage symptoms or display some tolerance to whitefly attack. Twenty-seven of the 29 clones (93.1%) had whitefly population ratings above 3.0, again indicating high selection pressure (Figure 14).

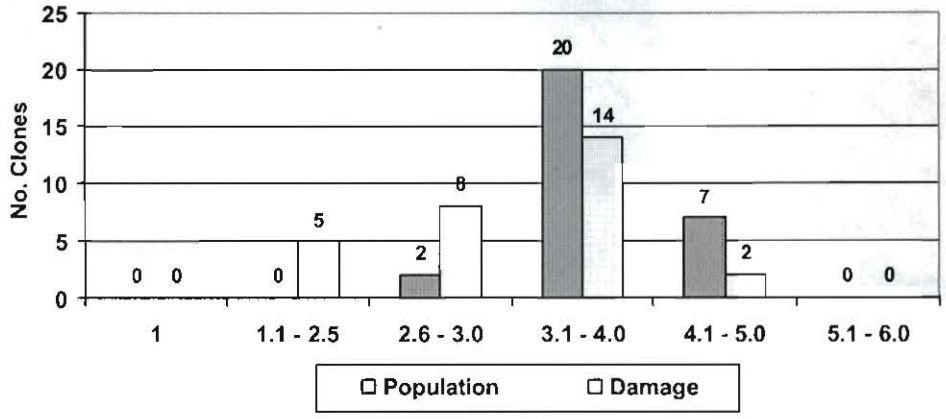

Figure 14. Average whitefly (*A. socialis*) population and damage rating of 29 clones in a cassava regional trial at Santander de Quilichao (Cauca) during the 2002-03 crop cycle.

3. Yellow pulp (Beta carotene) varieties. In this trial of 27 clones average whitefly populations ranged from 2.6 to 4.9 indicating moderate to high selection pressure. Whitefly (*A. socialis*) damage levels ranged from 2.0 to 4.0 (Figure 15). Twenty-two clones (81%) had whitefly population levels of 3.0 or higher but only 12 of these had damage levels of 3.0 or higher. The clones CM 9731-2, CM 9731-11 and CM 9712-7 combined low damage levels (2.0) and low whitefly populations (2.6). These clones, or this group of clones, should be re-evaluated in another crop cycle.

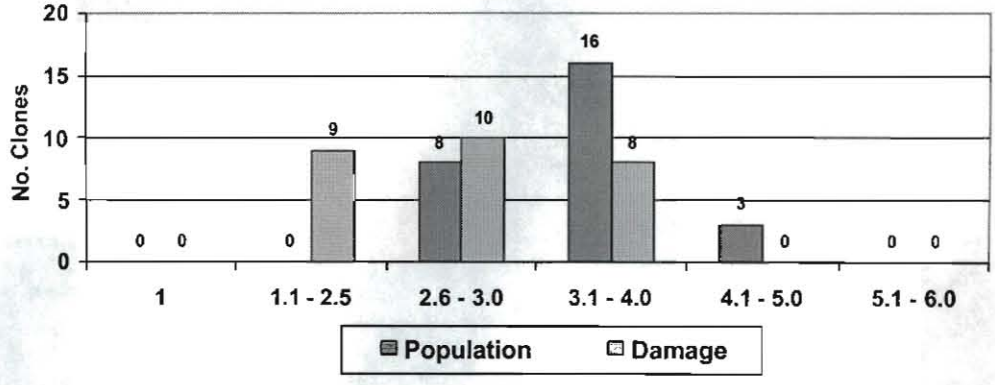

Figure 15. Whitefly (*A. socialis*) population and damage ratings for 27 yellow pulp cassava clones planted at Santander de Quilichao during the 2002-03 crop cycle.

4. Observational trial: This observational trial of the zone 2 (Colombia, Llanos Orientales) only 11 clones was planted and evaluated in Santander de Quilichao (Cauca). Whitefly (*A. socialis*) populations were low to moderate, not higher than 3.0 on any of the clones. Consequently, damage ratings were also low to moderate, ranging from 1.7 to 3.0 (Figure 16). Five clones, SM 2730-51, SM 2741-26, SM 2731-9, SM 2740-18 and SM 2743-18 had damage ratings of 1.7 and whitefly population ratings of 2.3 to 2.4.

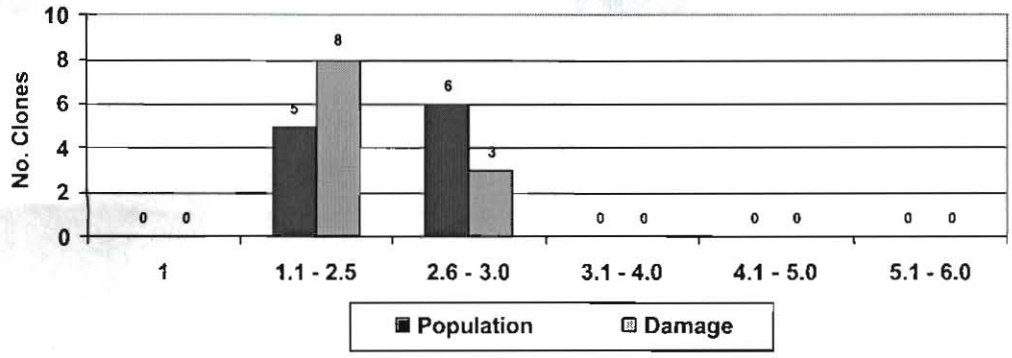

Figure 16. Whitefly (*A. socialis*) population and damage ratings for 11 clones of zone 2 (Colombian Llanos Orientales) in a cassava observational trial in Santander de Quilichao (Cauca) during the 2202-03 crop cycle.

5. Regional trial, Atlantic Coast: This regional trial of the zone 1 (Colombian Atlantic Coast), only evaluated six cassava clones in Santander de Quilichao (Cauca). Whitefly (*A. socialis*) population ratings ranged from 3.0 to 4.0, indicating a moderate 2.3 to 3.3, also a moderate level (Figure 17). This trial has not yet been harvested.

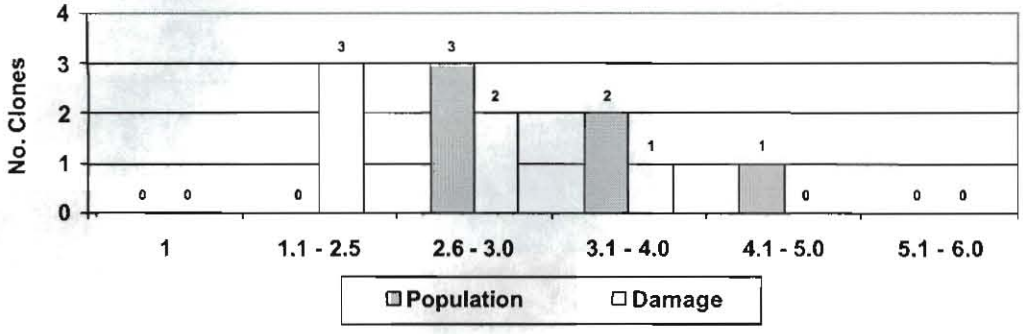

Figure 17. Whitefly (*A. socialis*) population and damage ratings of six cassava clones of zone 1 (Colombian Atlantic Coast), regional multiplication trial in Santander de Quilichao during the 2002-03 crop cycle.

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Activity 6. Studies on whitefly (*Aleurotrachelus socialis*) resistance mechanisms in selected cassava genotypes.

Rationale

As direct feeding pests and virus vectors, whiteflies cause major damage in cassava based agroecosystems in the Americas, Africa and, to a lesser extent Asia. The largest complex of whitefly pests on cassava is in the Neotropics, where 11 species are reported. Eight whitefly species are reported feeding on cassava in Colombia. *Aleurotrachelus socialis* is the major species on cassava in Northern South America (Colombia, Ecuador, Venezuela) while *Aleurotrachelus aepim* predominates in Brazil and *Bemisia tabaci* in Africa and parts of Asia. In whitefly surveys on cassava in Colombia, approximately 92% of the species population is *A. socialis*. For this reason, *A. socialis* receives most of the research effort, especially in the identification of whitefly resistant cassava genotypes and the development of resistant varieties.

The first symptoms of whitefly damage are manifested by curling of the apical leaves and yellowing, necrosis and abscission of lower leaves. This results in plant retardation and considerable reduction in root yield if feeding is prolonged. Damage and yield losses of this type are common with *A. socialis* and *A. aepim*. There is a correlation between duration of whitefly attack and yield loss, which has been recorded as high as 79% in prolonged (11 months) attacks and on susceptible cultivars. Cassava farmers will respond to whitefly attack with frequent applications of toxic chemical pesticides. Pesticide use is costly, often causes environmental contamination, a hazard to human health and may not provide effective control.

Stable host plant resistance (HPR) offers a practical long-term low cost solution for maintaining reduced whitefly populations. Although whitefly resistance in agricultural crops is rare, several good sources of resistance have been identified in cassava and high-yielding, whitefly resistant cassava hybrids are being developed. At CIAT we are systematically evaluating the cassava germplasm bank of more than 6000 accessions. The clone MEcu 72 has consistently expressed the highest level of resistance and is being employed in a breeding scheme to develop whitefly resistant hybrids (see CIAT 2002, IP-3 Annual Report). Additional cultivars expressing moderate to high levels of resistance in field trials include MEcu 64, MPer 335, MPer 415, MPer 317, MPer 216, MPer 221, MPer 265, MPer 266 and MPer 365.

The objective of these present studies is to evaluate several selected genotypes for mechanisms of resistance to *A. socialis* under controlled growth chamber conditions.

Materials and Methods

The genotypes selected for evaluation were MEcu 64, MPer 273 and MPer 334; CMC 40 was the susceptible control and MEcu 72 the resistant control. All genotypes have been field evaluated during numerous trials at CORPOICA, Nataima (Tolima). As previously mentioned MEcu 72 has consistently shown resistance to *A. socialis* and in laboratory controlled resistance mechanisms evaluations, resulted in a 72% mortality, a lower oviposition rate, longer development time and reduced size. CMC 40 supports high *A. socialis* populations and low mortality. In field trials MPer 273, MPer 334 and MEcu 64 genotypes showed low to moderate *A. socialis* populations and few damage symptoms. Four *A. socialis* development parameters were evaluated, mortality/survival, duration of the life cycle, nymphal development size,

(Antibiosis), and ovipositional preference (Antixenosis). This was done in two separate experimental designs.

1. Antibiosis Experiments: This was done in two parts; in the first, test plants were infested and evaluated by using *A. socialis* adults harvested directly from the greenhouse maintained colony being reared on the susceptible CMC 40 (Figure 18A). The second evaluations were done by first preconditioning *A. socialis* on the selected test genotypes for two generations. These individual colonies on the five aforementioned genotypes were reared in wooden, nylon mesh lined cages (1m x 1m x 1m) in the greenhouse (Figure 18B). All antibiosis experiments were carried out in the growth chamber (28?1?C, 60-70% RH, 12 hrs. light) by measuring the life cycle development of *A. socialis* as the aforementioned resistant and susceptible genotypes. Cassava plants were grown in plastic pots and were 4 to 5 weeks of age at infestation. Plant infestation was accomplished by introducing 20 whitefly adults into small leaf cages, supported by plastic straws (Figure 19). Each leaf cage has a small lateral opening and with the aid of a pasteur aspirator, *A. socialis* adults are encouraged to enter the leaf cages. Five leaf lobes were infested on each plant (total 180) during a 4-hour period with 3600 adults (Figure 20A). *A. socialis* adults were allowed to oviposit for 24 hours, thereby assuring a uniform population. Leaf cages and adults were then removed and egg infested plants were placed in the growth chamber (Figure 20B). Each leaf lobe was sequentially numbered to assure accurate data collection on each of the tested genotypes.

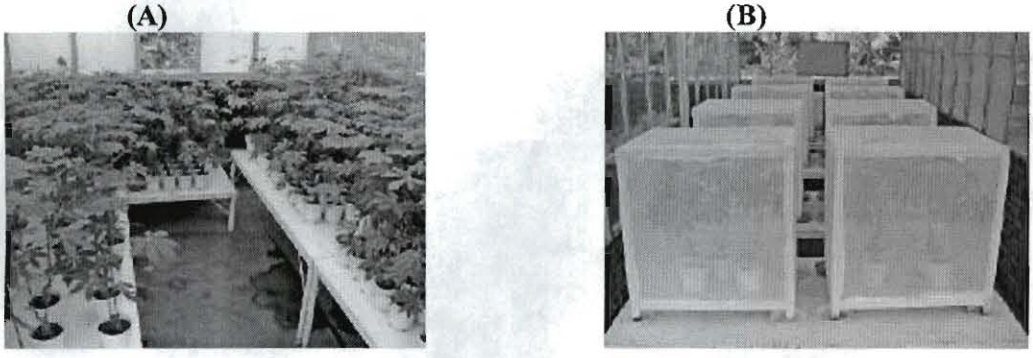

Figure 18. Antibiosis experiments. (A) Greenhouse colony of *Aleurotrachelus socialis* on CMC 40 (not-preconditioned); (B) *A. socialis* pre-conditioned and reared in nylon-meshed cages on resistant and susceptible genotypes.

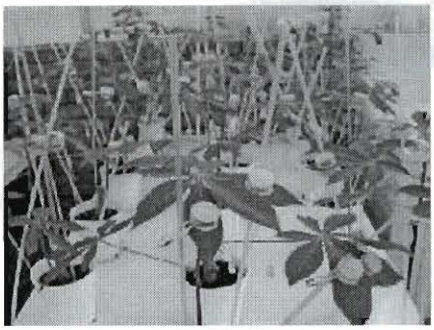

Figure 19. Thirty-day cassava plants grown in plastic pots and with attached leaf cages conditioned for *Aleurotrachelus socialis* infestation.

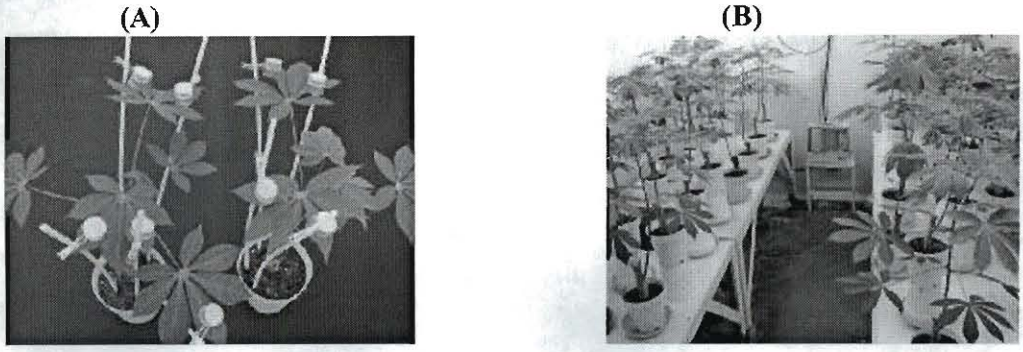

Figure 20. (A) Cassava plants conditioned for *A. socialis* infestation; (B) cassava plants infested with *A. socialis* eggs in the growth chamber (28? 1?C, 60-70% RH, 12 hrs light).

To determine the biological cycle of *A. socialis* on resistant and susceptible genotypes, 200 eggs are selected per plant, and an "infestation map" was designed so that daily evaluations of immature development can be recorded for instar changes, growth characteristics and survival/mortality. Daily evaluations were done with the aid of a stereomicroscope on the leaf undersurface. The potted plants, fastened to an iron support rod that allows upward-downward movement for optimal positioning, are inverted for easy observance. A rubber disk inserted at the base of the plant stem at the soil line prevents soil loss or plant movement and injury when the potted plants are invested (Figure 21).

Figure 21. Inverted cassava plants fastened to an iron support rod allowing easy observance of *Aleurotrachelus socialis* development stages with the use of a stereomicroscope.

The differences in duration of biological stages, time of development, morphological measurements of immature stages and adult dry weight were analyzed using the Ryan-Einot-Gabriel-Welsch Multiple range test (REGW). The rate of survival and relationship between sexes was analyzed with the Chi-Square (X^2) test.

Morphological measurements were done by removing 10 individuals per leaf lobe (40 individuals total per genotype) and taking measurements of the 2nd and 3rd instar nymphs and the pupal stage. A stereomicroscope with a digital dispositive for micro measurement (Wild MMS 225/MMS 2535) (Figure 22).

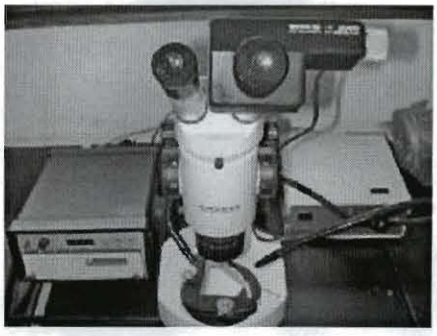

Figure 22. Digital micrometric measuring devise to determine morphological size of *Aleurotrachelus socialis* immatures.

Dry weight of adult whiteflies was done by placing well-developed pupae in the small leaf cages to prevent adult escape upon hatching. Sexing was done under the stereomicroscope using adult anal morphological characteristics to separate male and females. Captured adults from each of the tested genotypes were placed in plastic vials with cotton stoppers and dried in a Blue-M stove at 37?C for 72 hours. These were weighted on a CAHN C-30 microbalance, sensitive to 1 ? g.

2. Antixenosis Experiments. These experiments compared and determined the ovipositional and feeding preferences of *A. socialis* on the five genotypes. One potted plant of each genotype was randomly placed in a 1m x 1m x 1m wooden, nylon meshed lined cage. Each 30-day-old plant contained only three leaves, numbered in descending order from the top, middle and lower portions of the plant. This design allowed measurement of both total and vertical plant preference of oviposition. All plants were of equal height and distributed in a circular fashion to provide each genotype with an equal chance for oviposition (Figure 23). Five hundred *A. socialis* adults of the same age and randomly selected from the whitefly colony being reared on CMC 40, were introduced into the center of each cage. Recorded data was logarithmically transformed (Log H+1) and significant differences were determined using the Ryan-Einot-Gabriel-Welsch multiple F test. The variables were, 1) the number of whiteflies perched on each genotype at 24 and 48 hours after infestation, and 2) the number of eggs oviposited on each genotype after 48 hours. A visual count of perched adults was accomplished by carefully opening each cage without disturbing plants and whitefly adults. Egg counts were made under the stereomicroscope. The evaluation was done 3 times with four repetitions using a randomized block design in the growth room (28? 1?C, 60-70% RH and 12 hr. light).

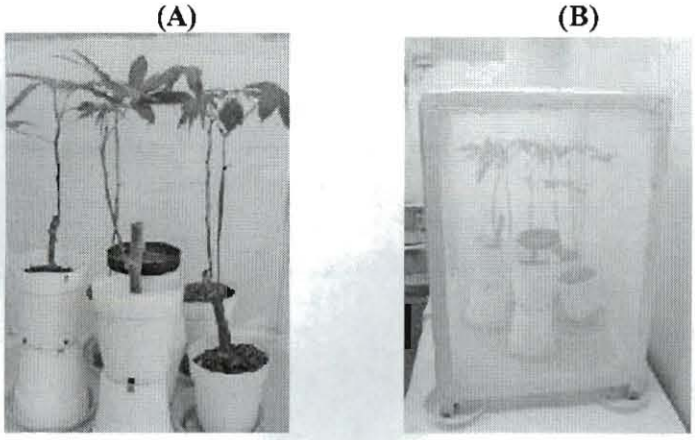

Figure 23. Cassava genotypes (Resistant and Susceptible) placed in nylon meshed cages and infested with 500 *A. socialis* adults for free choice ovipositional preference evaluations in the growth chamber.

Results

1. Antibiosis: No preconditioning.

A. socialis individuals (adult infestation directly from the greenhouse colony = un-preconditioned) feeding on MEcu 64 and MPer 334 had a significantly longer development period than on the other genotypes (Table 8 with 36.8 and 36.4 days respectively. MEcu 72 and MPer 273 resulted in a duration of 35.2 and 33.6 days, while CMC 40 the susceptible control had a significantly more rapid development of 32.7 days. The duration of the egg stage ranged from 10.1 (CMC 40) to 11.1 (MEcu 64) and difference between genotypes were significant (Table 9. The greatest differences occurred in the first nymphal instar. Most rapid development occurred on CMC 40 (4.9 days) and longest on MEcu 64 (6.4 days); MPer 334, MEcu 72 and MPer 273 had first instar duration of 6.1, 6.1 and 5.6 days respectively (Table 9. Significant differences between genotypes also occurred in the 2nd and 3rd nymphal instars but they were not as dramatic

as in the first instar. The duration of the pupal stage ranged from 9.6 days (CMC 40) to 10.5 days (MPer 334). The relationship between sexes was approximately 1:1 in all of the genotypes evaluated (Table 8.

Table 8. Average development time of *Aleurotrachelus socialis* (non-preconditioned) feeding on five cassava genotypes (resistant and susceptible) in the growth chamber.

Genotypes	n.	Average ? SD	Sex Relation
MEcu 64	63	36.8 ± 2.09 a1	1.0 : 1.0
MPer 334	45	36.4 ± 2.21 a	1.1 : 1.0
MPer 273	94	33.6 ± 1.55 c	0.7 : 1.0
MEcu 72	62	35.2 ± 2.56 b	1.2 : 1.0
CMC 40	152	32.7 ± 1.65 d	1.3 : 1.0
			X^2 : NS^2

1. Ryan-Einot-Gabriel-Welsch Multiple Range test. Columns with the same letter are not significantly different at the 5% level.
2. Independent Test. Female/Male sex relation is 1:1 in all genotypes.

Table 9. Duration of *Aleurotrachelus socialis* developmental stages on whitefly resistant and susceptible genotypes (non-preconditioned) (n=200).

Genotypes	Egg	Nymph 1	Nymph 2	Nymph 3	Pupae
MEcu 64	11.1 ± 0.57 a[1]	6.4 ± 1.08 a	4.3 ± 0.71 a	5.1 ± 0.66 a	10.4 ± 0.93 a
MPer 334	10.7 ± 0.47 b	6.1 ± 1.08 b	4.3 ± 0.95 a	5.0 ± 0.94 a	10.5 ± 1.07 a
MPer 273	10.4 ± 0.49 c	5.6 ± 1.01 c	3.7 ± 0.64 b	4.4 ± 0.63 b	10.0 ± 0.84 b
MEcu 72	10.6 ± 0.58 b	6.1 ± 1.05 b	4.4 ± 0.63 a	4.6 ± 0.96 b	9.9 ± 0.87 b
CMC 40	10.1 ± 0.50 d	4.9 ± 0.85 d	3.8 ± 0.59 b	4.4 ± 0.54 b	9.6 ± 0.83 b

1. Ryan-Einot-Gabriel-Welsch Multiple Range (F) test. Columns with the same letter are not significantly different at the 5% level.

2. Antibiosis: With preconditioned *A. socialis.*

Development time for *A. socialis* in this experiment was significantly longer when reared on MEcu 64 (34.5 days) compared to the other genotypes. It was shortest on CMC 40 (31.8 days) and intermediate for the remaining three genotypes (Table 10). Nymphal duration was longest during the first instar; MEcu 64 was longest (6.3 days) and CMC 40 was shortest duration (5.0 days). The remaining three genotypes, MPer 334 (5.6 days), MPer 273 (5.5 days) and MEcu 72 (5.7 days) were significantly different from the susceptible genotype CMC 40 (Table 11). Differences in development duration in the second and third instars were not as dramatic as in the first instar. Duration of the pupal stage ranged from MEcu 64 (10.5 days), the longest, to CMC 40 (9.5 days) the shortest and the remaining genotypes, intermediate (Table 11). Results in this experiment were similar to those in the un-preconditioned experiment, however, the values were lower or of shorter duration, indicating that preconditioning *A. socialis* effects development time.

Table 10. ***Aleurotrachelus socialis*** **development time on cassava genotypes (resistant and susceptible) during preconditioning phase.**

Genotypes	N	Average ? SD	Sex Relation
MEcu 64	96	34.5 ± 1.94 a[1]	1.0 : 1.0
MPer 334	124	33.0 ± 1.76 bc	0.9 : 1.0
MPer 273	127	32.8 ± 2.22 c	1.3 : 1.0
MEcu 72	127	33.5 ± 1.82 b	0.8 : 1.0
CMC 40	140	31.8 ± 1.61 d	1.5 : 1.0
			X^2 : NS[2]

1. Ryan-Einot-Gabriel-Welsch Multiple Range test. Columns with the same letter are not significantly different at the 5% level.

Table 11. **Duration of** ***Aleurotrachelus socialis*** **development stages on whitefly resistant and susceptible genotypes (*n*=200) (preconditioning phase) in the growth room.**

Genotypes	Egg	Nymph 1	Nymph 2	Nymph 3	Pupae
MEcu 64	9.7 ± 0.54 b[1]	6.3 ± 1.34 a	4.2 ± 0.86 a	4.2 ± 0.70 a	10.5 ± 1.05 a
MPer 334	9.4 ± 0.54 c	5.6 ± 0.92 b	3.8 ± 0.83 b	4.0 ± 0.52 a	10.4 ± 1.04 a
MPer 273	10.0 ± 0.72 a	5.5 ± 0.99 b	3.8 ± 0.77 b	4.0 ± 0.61 a	9.7 ± 1.21 bc
MEcu 72	9.7 ± 0.62 b	5.7 ± 1.11 b	4.2 ± 1.01 a	4.1 ± 0.58 a	10.0 ± 1.02 b
CMC 40	9.6 ± 0.71 b	5.0 ± 0.87 c	3.8 ± 1.02 b	4.1 ± 0.614 a	9.5 ± 0.95 c

1. Ryan-Einot-Gabriel-Welsch Multiple Range test. Columns with the same letter are not significantly different at the 5% level.

A. socialis survival on resistant genotypes (MEcu 64, MEcu 72, MPer 334 and MPer 273) is significantly lower than on the susceptible check, CMC 40 (Figures 24 and 25). First instar nymphs are the most effected; they have difficulty adhering to the leaf undersurface and initiating feeding on resistant genotypes. This is not a problem on the susceptible genotype CMC 40, where establishment and feeding readily occur (Figure 24A). In the two experiments *A. socialis* survival remained the same (76 and 75% survival) (Figure 25). Without precondition *A. socialis* survival on MPer 344, MEcu 72, MEcu 64 and MPer 273 were 22.5, 31.0, 31.5 and 47.0% respectively. For preconditioned *A. socialis*, the results were similar but the rate of survival was higher for all of the resistant genotypes (Figure 25). For example, in the first experiment MEcu 64 survival was 31.5%, wile in the second it was 48.0%. In both experiments, the resistant genotypes had a significantly lower survival rate than the susceptible genotypes (P=0.05). These results indicate that constant rearing of *A. socialis* on resistant genotypes may reduce the effectiveness of the resistant factors. This will play a role in the deployment of resistant cultivars in field plantings.

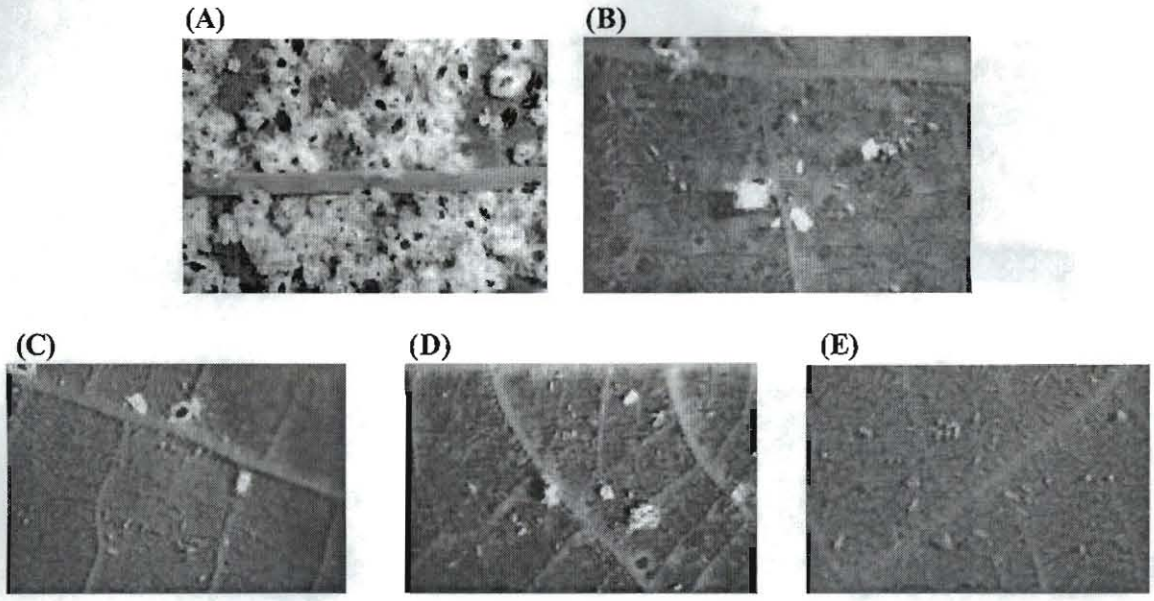

Figure 24. ***Aleurotrachelus socialis*** **nymphal survival on cassava (A) Susceptible control CMC 40; (B) Resistant control, MEcu 72; (C) MPer 273; (D) MPer 334; (E) MEcu 64.**

Morphological measurements of *A. socialis* feeding on resistant and susceptible genotypes show that 2nd and 3rd instar nymphs and pupae were significantly longer on CMC 40 than on the resistant genotypes (Figure 26) (P=0.05). The results for width were similar although differences were not always significant. *A. socialis* adult dry weight was significantly lower when feeding on MEcu 64, followed by MPer 334, MPer 273 and MEcu 74 (P=0.05) (Figure 27). All resistant genotypes were significantly lower than the susceptible check, CMC 40, for both the non-preconditioned and preconditioned *A. socialis*.

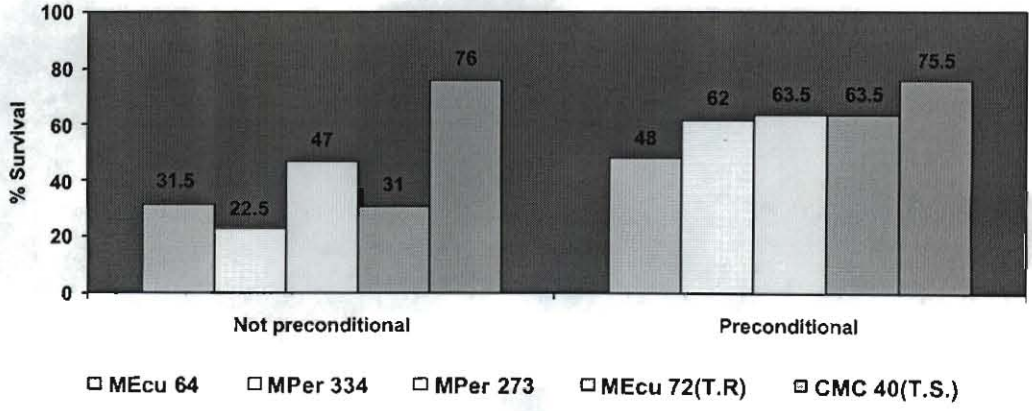

Figure 25. Percent survival of ***Aleurotrachelus socialis*** **feeding on five cassava genotypes (resistant and susceptible) in the growth chamber (28? 1?C, 60-70% RH, 12 hrs. light).**

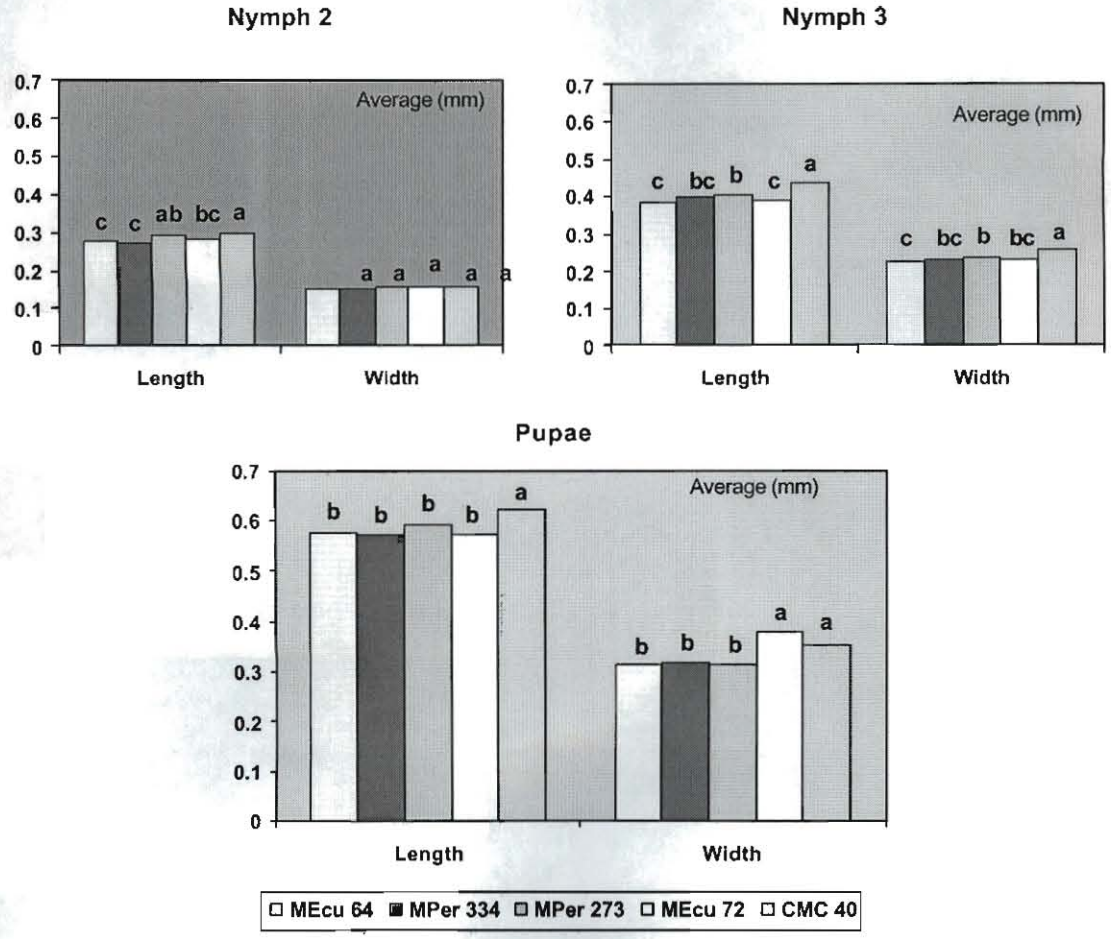

Figure 26. Morphological measurements of *Aleurotrachelus socialis* 2nd and 3rd instar nymphs and pupal stage on five cassava genotypes in the growth chamber.

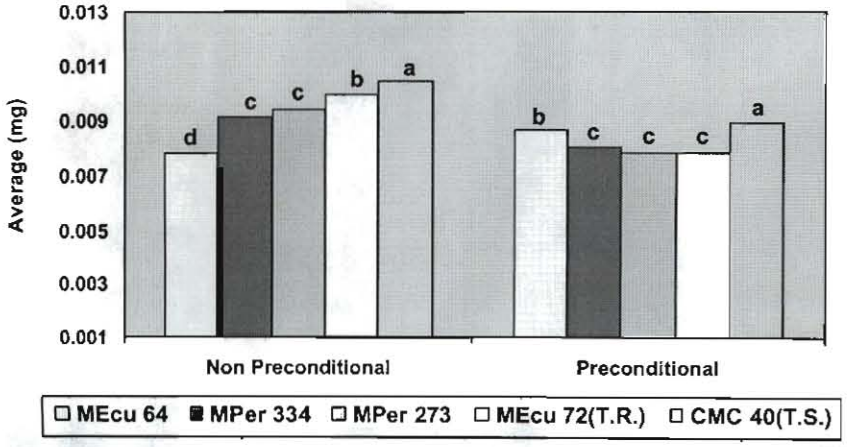

Figure 27. Dry weight of *Aleurotrachelus socialis* adults reared on five cassava genotypes (2 experiments) in the growth chamber.

3. Antixenosis: Free choice feeding preference.
Under a free choice evaluation where *A. socialis* adults were offered five randomly placed genotypes, a significantly higher feeding preference occurred on CMC 40 (Figure 28) (P=0.05). There was no significant difference among the remaining resistant genotype, although feeding was lowest on MEcu 64 (the data was logarithmically transformed (X+1)). An interaction was noted between experiment, time (hour) and leaf, where the time of evaluation influenced results on the first leaf, where preference for *A. socialis* feeding was the same at 24 and 48 hours. Leaf one, or the upper most leaf, was the most preferred for feeding (Figure 29) in all three experiments, for all genotypes. There was no significant difference in feeding preference on leaves 2 and 3, but in general, feeding activity was higher during the initial 24-hour period (Figure 29).

Oviposition was effected by genotype. Oviposition on MEcu 64 was significantly lower (P=0.05) than on the susceptible check (CMC 40) in all three experiments (Figure 30). In experimental 1, all resistant genotypes were significantly lower than CMC 40; however in experiment 2, only MEcu 64 was significantly lower, and in experiment 3, both MEcu 64 and MEcu 72 were lower (Figure 30). Total oviposition was significantly higher on the upper leaf in all three experiments (Table 12); 75% of the eggs were oviposited on the upper leaf, 15% on the second and 10% on the third leaf.

The combined results for feeding and ovipositional preference and those for mortality and nymphal development indicate that MEcu 64 along with MEcu 72 are the most *A. socialis* resistant genotypes.

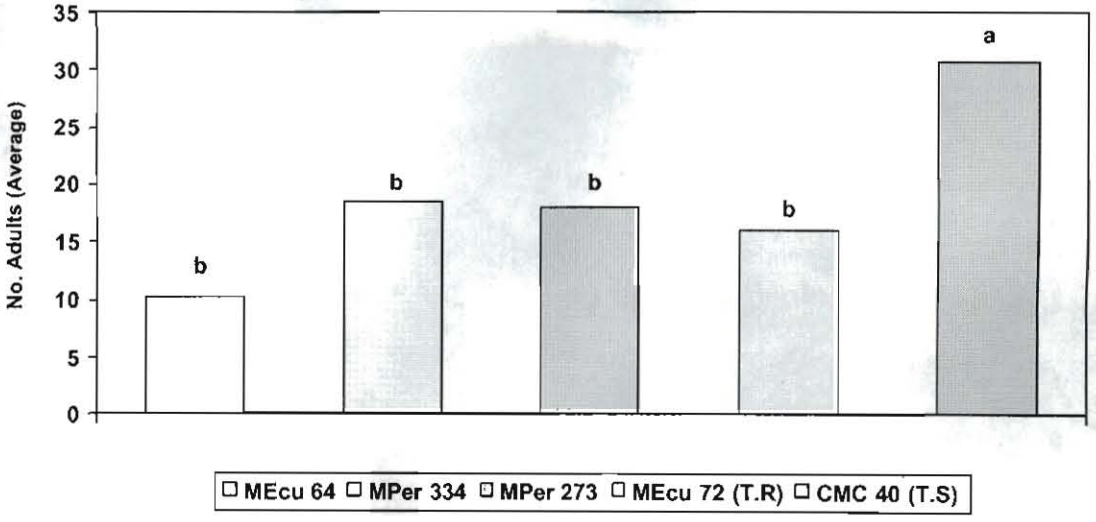

Figure 28. Free choice *Aleurotrachelus socialis* feeding trials on five cassava genotypes (3 leaves per plant and 3 repetitions over a 48hr. period.

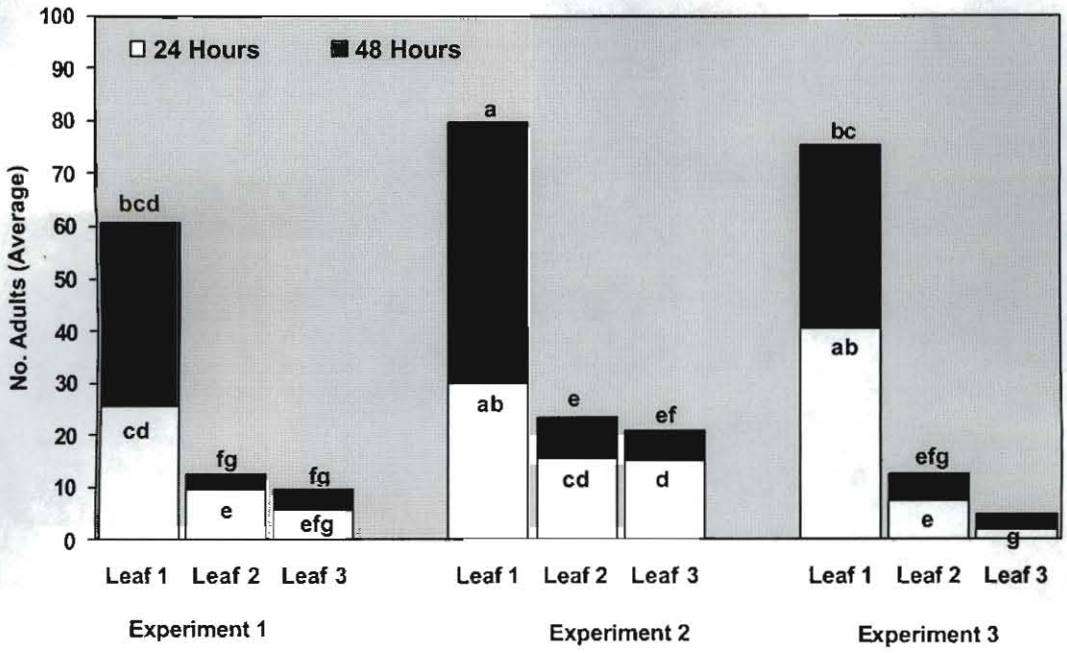

Figure 29. **Free choice *Aleurotrachelus socialis* feeding preferred trials on five cassava genotypes on three leaves per plant during a 48-hour period.**

Table 12. ***Aleurotrachelus socialis* ovipositional distribution on three cassava leaves of five genotypes in free choice trials.**

Leaf Position	Hour	Experimental 1	Experimental 2	Experimental 3
1	48	335.3 a[1]	418.8 a	661.0 a
2	48	50.0 b	135.7 b	93.2 b
3	48	46.6 b	111.7 b	32.9 c

1. Ryan-Einot-Gabriel-Welsch Multiple Range (F) test. Columns with the same letter are not significantly different at the 5% level. Analysis with transformed data. Log (x=1).

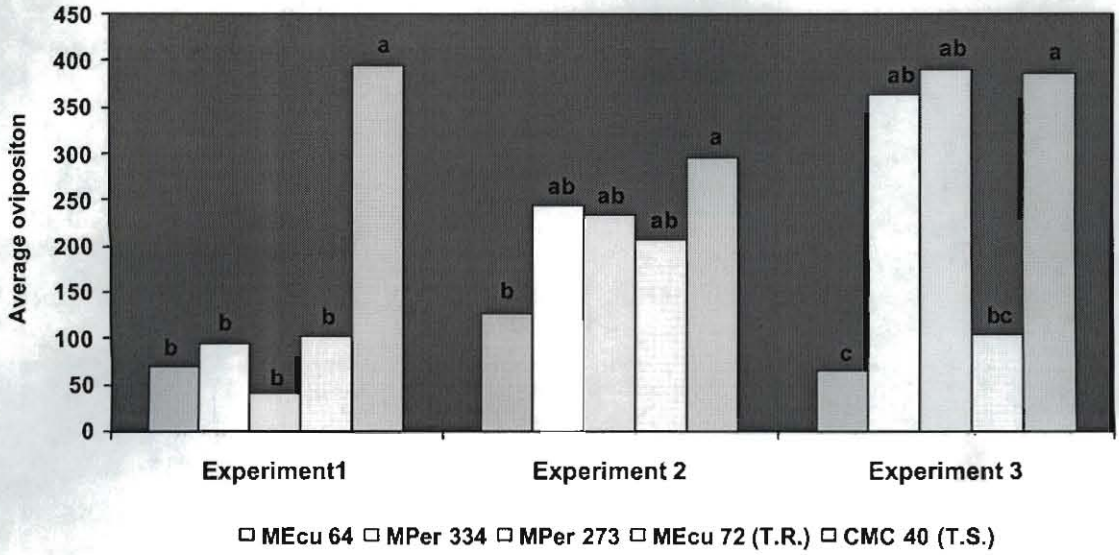

Figure 30. Free choice ovipositional preference of *Aleurotrachelus socialis* on five cassava genotypes (three experiments).

Contributors: Miller J. Gómez, A.C. Bellotti

Collaborators: Myriam C. Duque, Claudia M. Holguín, Bernardo Arias, Diego F. Múnera

Activity 7. Studies on the biology and development of biotype B of *Bemisia tabaci* on cassava, *Manihot esculenta* and the wild species, *Manihot carthaginensis*.

Rationale

Whiteflies are major pests of cassava in the Americas, Africa and Asia. Several species are involved; *Aleurotrachelus socialis* predominates in Northern South America (Colombia, Venezuela and Ecuador), while *Aleurothrixus aepim* is the major species in Brazil. *Bemisia tabaci*, a pantropical species prevails in Africa and parts of Asia (i.e. India) where it is the vector of Africa Cassava Mosaic virus (ACMV) and related viruses. Until the early 1990's, *B. tabaci* biotypes found in the neotropics did not feed on cassava, and it has been speculated that the absence of ACMD in the Neotropics may be related to the inability of *B. tabaci* to colonize cassava. Biotype B of *B. tabaci*, has been collected feeding on cassava in the neotropics. However, recent research at CIAT indicates that cassava is not a very successful host (see CIAT Pest and Disease Management Annual Report, 2002, pp. 25-35). *B. tabaci* feeding on beans (*Phaseolus vulgaris*) was successfully transferred to cassava only after completing several generations on other Euphorbiaceae species such as *Euphorbia pulcherrima* (Poinsettia) and *Jatropha gossypiifolia* (Jatropha). However mean longevity, female fecundity, oviposition and adult survival were low when compared to other whitefly species feeding on cassava.

This present study evaluates the potential of *B. tabaci* to adapt to wild *Manihot* species, such as *M. carthaginensis* and compares this to the development of *B. tabaci* on cultivated cassava *Manihot esculenta*, variety MCol 2063.

Materials and Methods

Life table parameters of *B. tabaci* were evaluated in the growth chamber on potted plants of *M. carthaginensis* and the cassava variety MCol 2063. *B. tabaci* longevity, fecundity, development time, survival and demography were calculated. *B. tabaci* populations originated from a colony maintained on *Jatropha gossypiifolia* (Euphorbiaceae), in screened cages (1m x 1m x 1m) for 9 generations (25±5?C, 70±5% RH and 12/12hr. photoperiod). Longevity and fecundity were evaluated by placing 40 pairs (1m x 1f) of recently emerged Biotype B of *B. tabaci* adults in small leaf cages (2.5cm diameter x 2.0cm deep) on test plants. Every 48 hours adults were moved to another leaf area and this was repeated until all (40) females died. When males died, they were replaced until female mortality occurred. Fecundity was estimated by counting eggs oviposited by each female during the 48 hour period; longevity was estimated by the maximal survival of each female.

Development time and survival were studied by placing 50 adults (25 males + 25 females) in the small leaf cages and allowed to feed on the leaf undersurface for 6 hours. Adults were then removed and 200 eggs were selected to evaluate development time from egg to adult and record nymphal survival and sex ratio. Life tables for *B. tabaci* were calculated (Price, 1975) using net reproduction rate (R_o), generation time (T), intrinsic growth rate (r_m) of the population, and employing the formula:

? exp(-r_mx)1xmx=1

where: x = age

lx = age specific survival

mx = proportion of female progeny from female x

For the calculated values of r_m the corrected age of x + 0.5 were used (Carey, 1993).

Results

The longevity of *B. tabaci* on *M. carthaginensis* and *M. esculenta* (MCol 2063) were similar. It was two days longer on *M. carthaginensis* (12 days) than on *M. esculenta* (10 days) (Figure 31). By the end of 6 days 65% of the females on *M. carthaginensis* and 82.5% of the females on *M. esculenta* had died. The average longevity on the two genotypes differed significantly (Student-Newman-Keuls P<0.05, after K-Wallis P<0.0001).

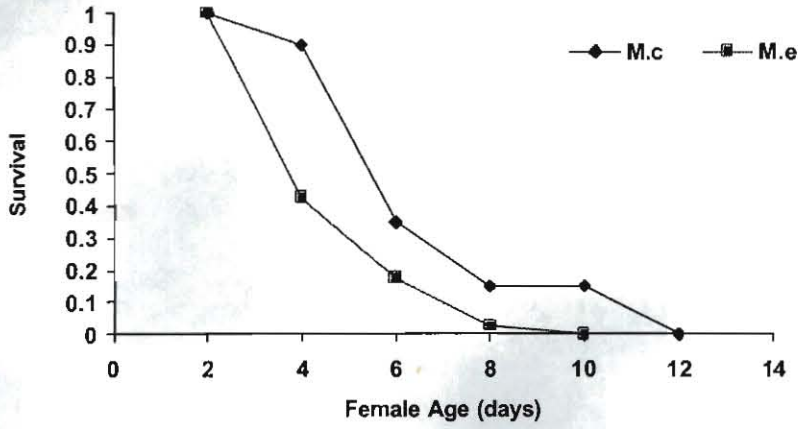

Figure 31. Survival curves of Biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Oviposition occurred readily on both genotypes but the range was greater on *M. esculenta,* although the difference was not significant (K-Wallis P<0.0001, followed by Student-Newman-Keuls P <0.0.5) (Figure 32, Table 13). The mean ovipositional rate was significantly higher on *M. esculenta* (eggs per female/2 days). All females of *B. tabaci* initiated oviposition within 48 hours of eclosion on both genotypes. On *M. esculenta* 72% of the total oviposition occurred during this 48-hour period while only 35.5% occurred on *M. carthaginensis*. These results indicate a preference of *B. tabaci* to oviposit on *M. esculenta.* Highest oviposition on *M. esculenta* occurred on day 2, while on *M. carthaginensis* it was on days 4 to 6.

B. tabaci development time was significantly lower or faster on *M. carthaginensis* than on *M. esculenta* (Table 14). The development time or life cycle on *M. esculenta* was 11 days (44.4 days) longer than on *M. carthaginensis* (33.3), indicating a more rapid adaptation of the immatures when feeding on *M. carthaginensis*. Taking into consideration that fecundity was higher on *M. esculenta* (8.6 eggs vs. 5.3) (Table 13) and combines this with the faster development time on *M. carthaginensis*, it results in the intrinsic growth rate (r_m) to be the same

for both genotypes (Table 14). These results indicate that populations of Biotype B of *B. tabaci*, in spite of a higher fecundity on *M. esculenta*, will be of equal growth rates on both genotypes.

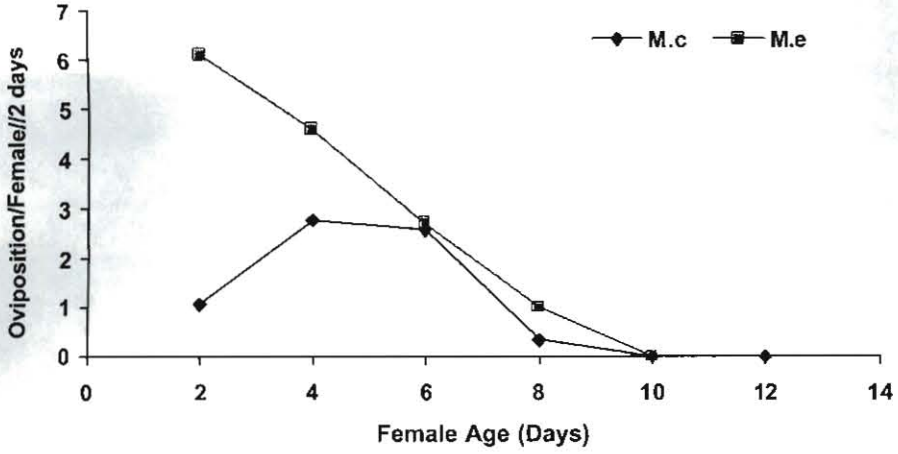

Figure 32. Reproduction curves of Biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Table 13. Average longevity, fecundity and ovipositional rate (eggs/female/2 days) of Biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Parameters	*M. carthaginensis*	*M. esculenta*
Average Longevity	5.1 a	3.25 b
Range	2-12	2-10
No insects	30	40
Average Fecundity	5.35 a	8.6 a
Range	1-35	1-41
Average Oviposition Rate	1.05 a	2.64 b
Range	0.25-3.6	0.5-8

Figures followed by different letters across columns indicate significant differences (Kruskal-Wallis) $P<0.001$, followed by Student-Newman-Keuls $P<0.05$).

Survival rates were significantly higher on *M. carthaginensis* (Table 14). Results show that of 200 eggs oviposited on *M. carthaginensis,* 120 or 60% survived to the adult stage, while only 55 eggs (36%) survived to adulthood on *M. esculenta* (Figure 33). Immature survival is a good indication of the eventual ability of a biotype to develop on a genotype. These results indicate that *M esculenta* (MCol 2063) is not an optimal host for Biotype B of *B. tabaci* (Figure 33).

Significant differences in the net reproductive rate were obtained between the two genotypes. It was estimated that at the end of a generation, populations of Biotype B of *B. tabaci* would multiply 8.6 times on *M. esculenta* (MCol 2063), three times greater than on *M. carthaginensis* (Table 14). This can be explained by total reproduction was less on *M. carthaginensis.* One generation of *B. tabaci* on *M. carthaginensis* is 35.6 days vs. 44.8 on *M. esculenta.* These results indicate that *B. tabaci* can complete 10 generations per year on *M. carthaginensis* and eight on

M. esculenta. Population growth of *B. tabaci* was the same on both genotypes (Table 14). The difference in development time was a more important criterion for the population increase of *B. tabaci* on *M. carthaginensis*, than were the differences in ovipositional rate. Population increases of *B. tabaci* on *M. esculenta* were more influenced by changes in reproduction rate. It should be noted that the high rate of oviposition of *B. tabaci* on *M. esculenta* can be independent of subsequent development of the immature stages.

Table 14. Demographic parameters of biotype of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

Parameter	*M. carthaginensis*	*M. esculenta*
Development time (d)	33.3 a	44.41 b
Rate of survival (%)	60 a	27.5 b
Proportion of females (%)	50.6	50.9
Intrinsic rate of increase (r_m)	0.048	0.048
Net reproductive rate (Ro) ? lxmx	5.35	8.63
Generation time (T)	35.6	44.76
Days to duplicate population Ln2/ r_m	14.4	14.4

Development time: different letters across columns indicate significant differences (K-Wallis P?0.0001, followed by Student-Newman-Keuls P? 0.05). Rate of survival: ($?^2$=29.9, 1df, P? 0.0001).

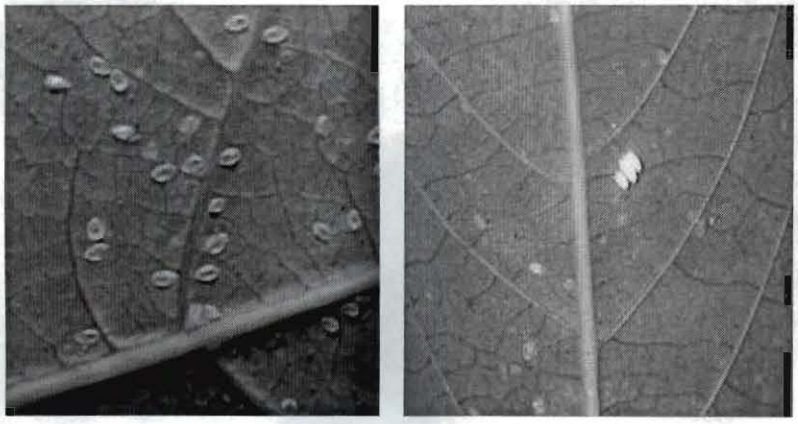

Figure 33. Pupal capsules, pupae and adults of biotype B of *B. tabaci* feeding on *M. carthaginensis* and *M. esculenta* (MCol 2063).

It can be concluded that Biotype B of *B. tabaci* can successfully develop on both *M. esculenta* (MCol 2063) and *M. carthaginensis.* In this case, however, it should be noted that these populations of *B. tabaci* had already adapted to related Euphorbiaceae, Jatropha, prior to being evaluated on the two aforementioned genotypes. Previous research has shown that when the *B. tabaci* populations originate on an unrelated genotype, such as beans (*P. vulgaris)*, they do not readily adapt to *M. esculenta.* These results, however, do provide evidence that biotype B of *B. tabaci* can adapt to Wild *Manihot* species as well as the cultivated species, *M. esculenta* and represents a potential threat to cassava production in the Neotropics.

Contributor: Arturo Carabali.

Activity 8. Wild Manihot species as a source of resistance to cassava arthropod pests.

Several cassava arthropod pests will significantly reduce root yield. Emphasis is given to two complementary systems, host plant resistance and biological control, for the effective environmentally sound and low cost methods of controlling cassava pests. Different levels of resistance to cassava pests have been identified within *M. esculenta.* For example resistance to mealybugs, lace bugs, stemborer and burrower bugs (within low HCN varieties) is very low; resistance levels to mites is low to moderate; resistance to thrips and whiteflies is moderate to high, while no resistance to hornworms and white grubs have been identified.

The wild species of *Manihot* offer a potential "source" of resistance genes for the control of major cassava pests. This "source" of resistance has already been exploited for the control of Africa Cassava Mosaic Disease (ACMD) in Africa. ACMD resistance was obtained by intercrossing cassava varieties with *Manihot glaziouii* and other species of *Manihot.* Interspecies hybrids were backcrossed to cassava and this resulted in varieties highly resistant to ACMD. This research was initiated in the 1930's and 1940's, when modern biotechnology tools and information were not available.

The development of pest and disease resistant varieties using interspecific crosses with wild *Manihot* species was difficult and slow. Therefore, the use of wild *Manihot* species as a source of pest and disease resistant genes was not effectively pursued. Recent advances in genetic mapping, gene transfer, transformation and genetic engineering allow for a more efficient production of new cassava varieties resistant to pests and diseases (Fregene, 2002).

The objectives of the present research is to evaluate species of wild *Manihot* to determine their potential as a source of resistance to three major cassava pests, mites (*Mononychellus tanajoa*) whiteflies (*Aleurotrachelus socialis*) and mealybugs *(Phenacoccus herreni).* Mites, whiteflies and mealybugs cause significant yield losses in the Americas, Africa and Asia.

This research was divided into two parts; the first consists of the acquisition and establishment of vegetative material of the *Manihot* species. Different methodologies were used, including rooting techniques, soil sand mixtures and soil source (site or location). The second part consists of infesting genotypes of the different wild species, as well as control genotypes with the aforementioned arthropod pets and evaluating population dynamics, behavior, survival and damage.

Multiplication of Wild Manihot Species

Developing a methodology for the multiplication of wild *Manihot* species was both difficult and time consuming (see PE-1 Annual Report, 2002, pp 67-69) (Figure 34). Originally, attempts were made to establish genotypes of four wild *Manihot* species, *M. flabellifolia, M. carthaginensis, M. peruviana* and *M. tristis.* Several methods and rooting media were tested for achieving the establishment of the wild species. The rooting media that gave the best results was a mixture of three parts construction sand (this is a coarse grain of sand that permits good drainage) and one-part rice husks. However, some genotypes rooted and established more successfully than others. The genotype that resulted in the highest percentage of germination were *M. carthaginensis* (MCTH 37-8); a genotype of *M. peruviana* (203-3) and of *M.*

flabelliflora (MFLA 444-033). Although stem rooting was achieved in many of the genotypes of the wild species, plant establishment was not always successful. Since vigorous growing potted plants with adequate foliage was needed for pest infestation, establishment and evaluation, many of the wild genotypes were discarded from this final phase of the project.

Genotypes from the wild species, *M. flabellifolia* and *M. peruviana* were selected for the pest infestation and evaluation phase. These were compared to several *M. esculenta* cassava varieties, including several genotypes from Vaupés Department, that are being cultivated by indigenous peoples in that region (Tables 15a and 15b).

Table 15a. *Manihot* genotypes evaluated for resistance to mites (*Mononychellus tanajoa*) and mealybugs (*Phenacoccus herreni*).

Species	Genotype
Manihot esculenta	CMC 40 CM 7395 MEcu 72
Manihot esculenta (Vaupés)	Ibacaba Cassava de Mico (Roja) Abeja Cassava de Garza Cassava de Piña Abiyú
M. flabellifolia	MFla 444-002
M. peruviana	MPer 417-003 MPer 417-005

Table 15b. *Manihot* genotypes evaluated for resistance to whiteflies (*Aleurotrachelus socialis*).

Species	Genotype
Manihot esculenta	CMC 40
Manihot esculenta (Vaupés)	CM 7395 MEcu 72 Nupará Flores
M. flabellifolia	MFla 444-002
M. peruviana	MPer 417-003 MPer 417-005

Mite (*M. tanajoa*), mealybug (*P. herreni*) and whitefly (*A. socialis*) colonies were established in the greenhouse or screen house on *M. esculenta* (usually variety CMC 40). Mite infestation of the test genotypes were made by placing a cassava leaf lobe from the colony on the test plant; this resulted in an infestation of 150 to 200 individuals. Whitefly infestations were made with 200 adults (100 males and 100 females), while mealybug infestations were done by placing ovisacs on plant stems at the leaf axial. All test plants were place in four or six chambered wooden cages enclosed with a fine nylon mesh to prevent arthropod movement into or out of the cages (Figure 35). Each chamber was .50 L x .50 W x 1.0 H in meters.

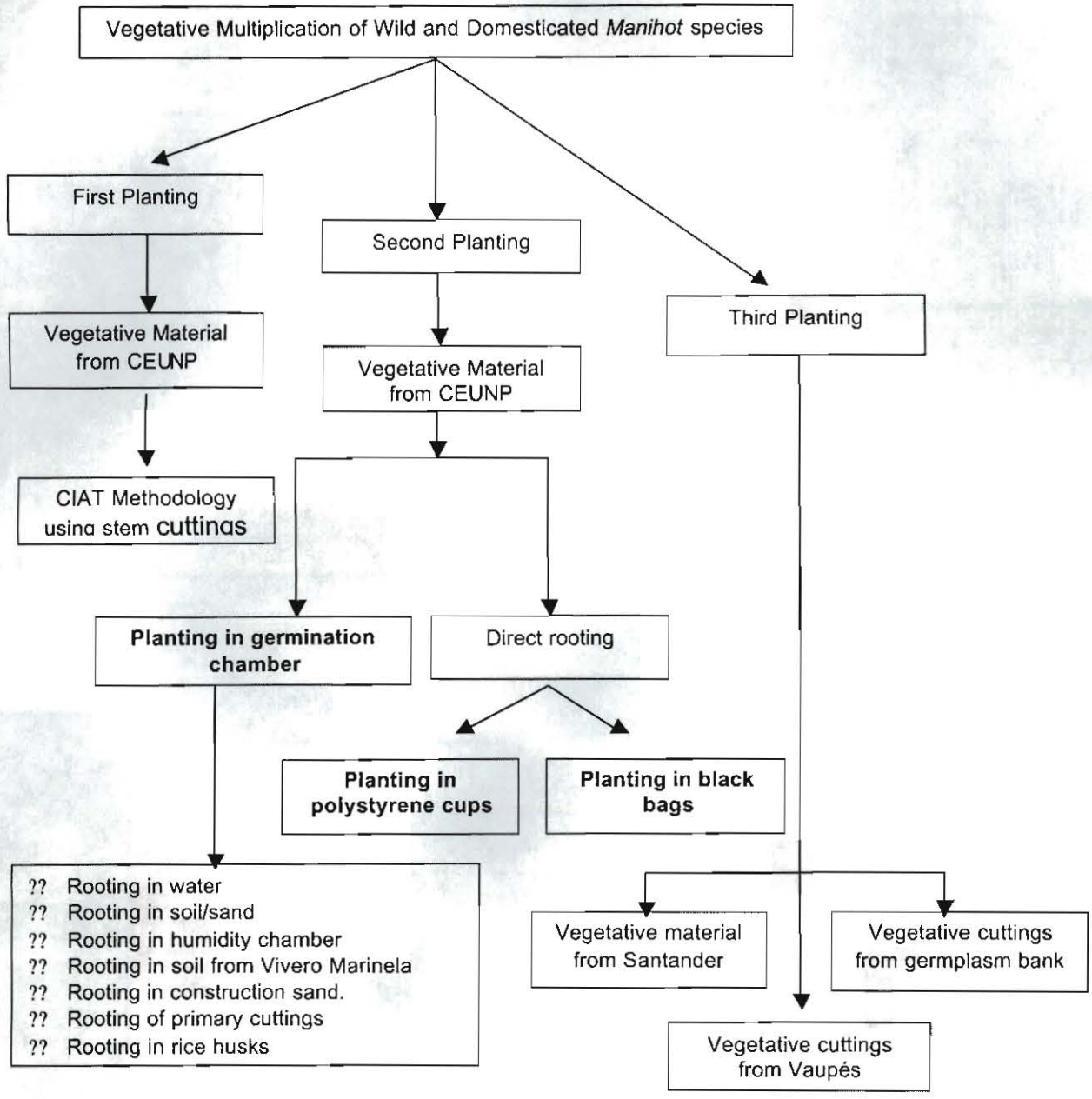

Figure 34. Flowchart of methodology for the vegetative multiplication of *Manihot* species.

Evaluations were carried out periodically for both pest populations and plant damage using a 1 (low) to 6 (high) population and damage scales. Mite evaluations were made every 5 days during a 4-week period (4 evaluations). Mealybug and whitefly evaluations were carried out every 10 days and a total of 6 evaluations were done for each pest species (Figure 35).

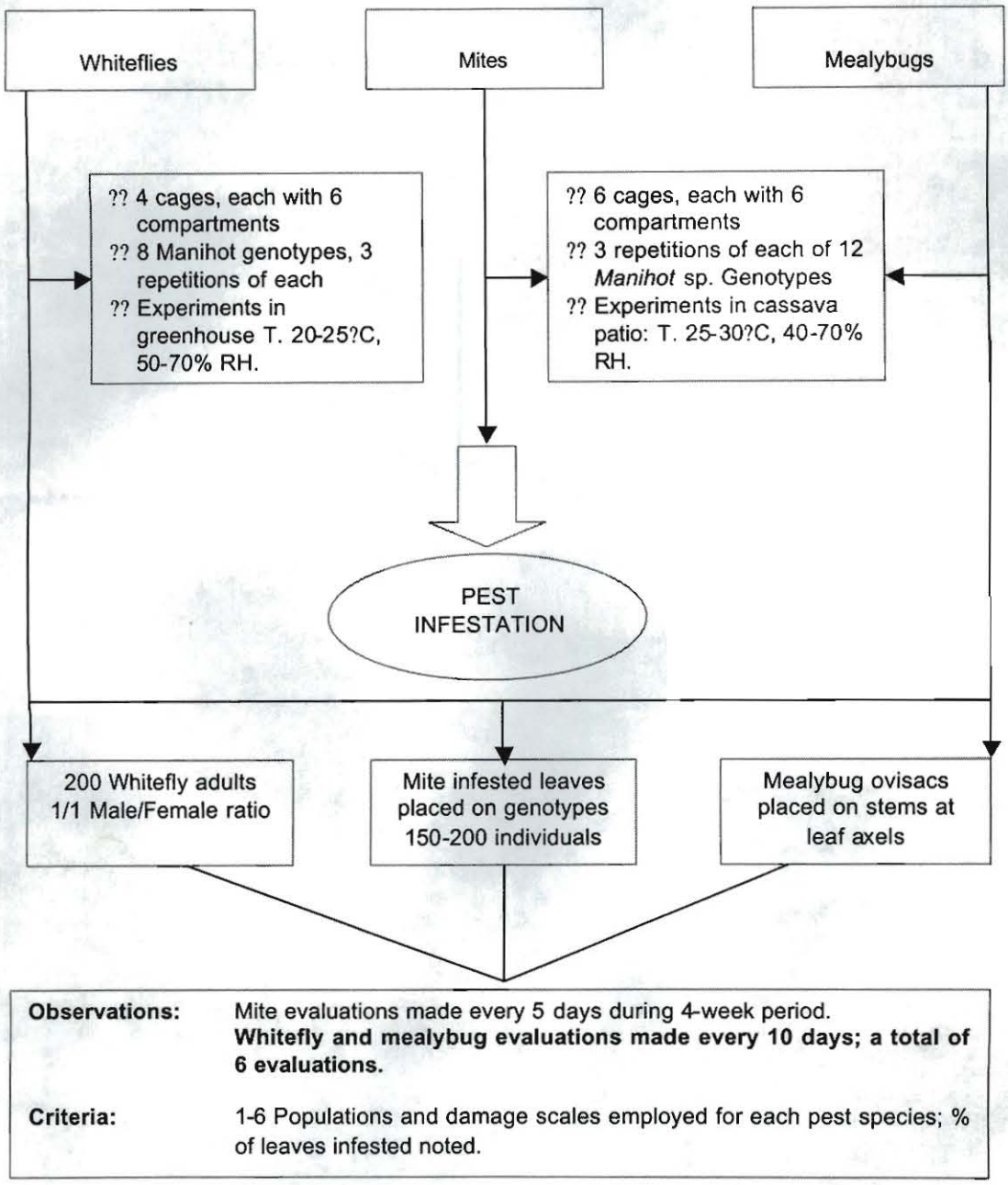

Figure 35. Bioassay methodologies for the evaluation of Wild *Manihot* species and cassava varieties and three cassava pests, mites, whiteflies and mealybugs.

Results

Mite damage, infestation and percentage of infested leaves were significantly different for the genotypes evaluated (AOV; $F_{(11.132)} = 66.72$, $F_{(11.132)} = 29.86$ and $F_{(11.132)} = 5.61$ respectively with a $P<0.005$, for the three variables).

Significant differences in damage were observed between three wild *Manihot* genotypes, MFla 444-002, MPer 417-003 and MPer 417-005 (Turkey and Newman-Kewls Multiple Comparison) and all other genotypes (Figure 36). On the 1 to 6 damage scale, these three genotypes were between 2.0 and 3.0 rating, with MFla 444-002 being the lowest. All the *M. esculenta* genotypes had a damage rating of 5.3 to 5.7 after 25 days of infestation. Results for infestation levels were similar in that the genotypes evaluated separated into two groups. The three wild *Manihot* genotypes, MFla 444-002, MPer 417-003 and MPer 417-005 were significantly lower (Turkey and Newman Kewls Multiple Comparison) than all of the *M. esculenta* genotypes (Figure 37). The number of leaves infested was similar for all the genotypes tested and there was no significant difference between the wild *Manihot* genotypes and the *M. esculenta* genotypes. These results indicate that the wild *Manihot* genotypes may possess low to moderate levels of resistance to *M. tanajoa*.

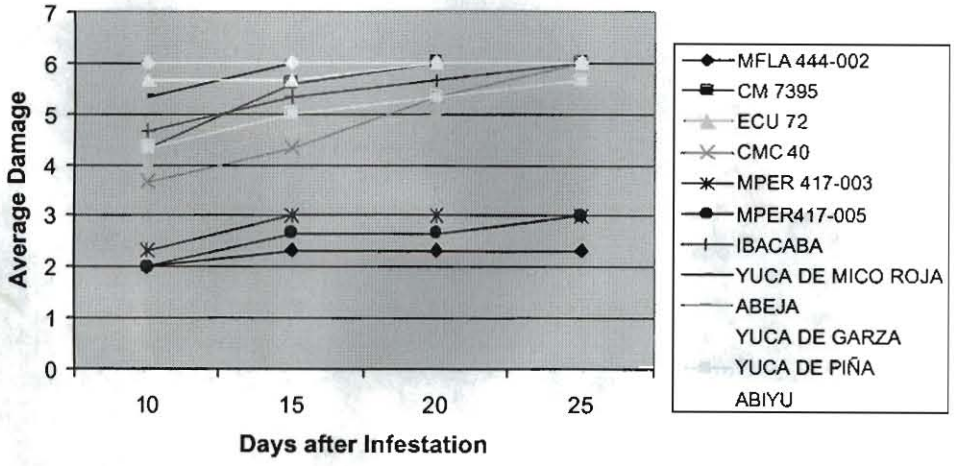

Figure 36. Average damage ratings (using a 1 to 6 damage scale) of *Manihot* species after 25 days of mite (*Mononychellus tanajoa*) infestation.

Mealybug (*P. herreni*) damage, infestation level and percentage of infested leaves were significantly different for the genotypes evaluated (AOV $F_{(11.204)} = 2.83$, $F_{(11.204)} = 8.24$, and $F_{(11.204)} = 4.41$ respectively, with a $P<0.005$ for the three variables).

MPer 417-003 had a significantly lower damage level than the other genotypes tested (Figure 38). The other two wild *Manihot* genotypes (MPer 417-005 and MFla 444-002) also had lower damage ratings than the *M. esculenta* genotypes but the differences were not always significant. MPer 417-003 had a damage rating of 2.3 60 days after infestation, while MPer 417-005 and MFla 444-002 had damage ratings of 3.6 and 4.6 respectively. All *M. esculenta* genotypes had damage ratings of 5.0 or higher (Figure 38). Infestation levels were lower for MPer 417-003,

MFla 444-002 and CM 7395 and were significantly lower than all the remaining genotypes (Figure 39). The three previously mentioned genotypes all had infestation levels below 3.0 while all the remaining genotypes had ratings of 5.0 or higher. MPer 417-003 and MFla 444-002 can be seen as having low to moderate levels of resistance to *P. herreni* since both also had low damage levels. However, CM 7395, although it has a low infestation level had a high damage level of 5.0. MPer 417-003 and MFla 444-002 also had low percentage of leaves infested.

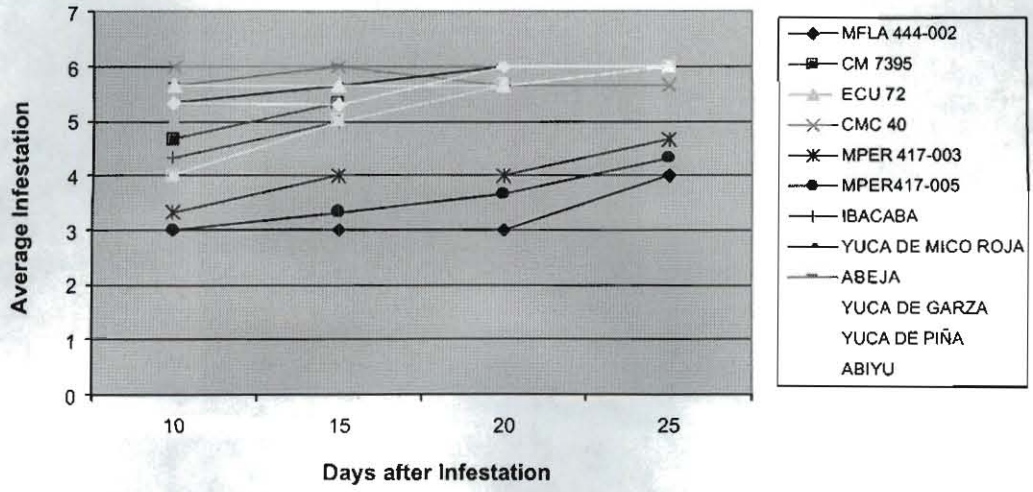

Figure 37. Average infestation level ratings (1 to 6 scale) of *Manihot* species after 25 days of mite (*Mononychellus tanajoa*) infestation.

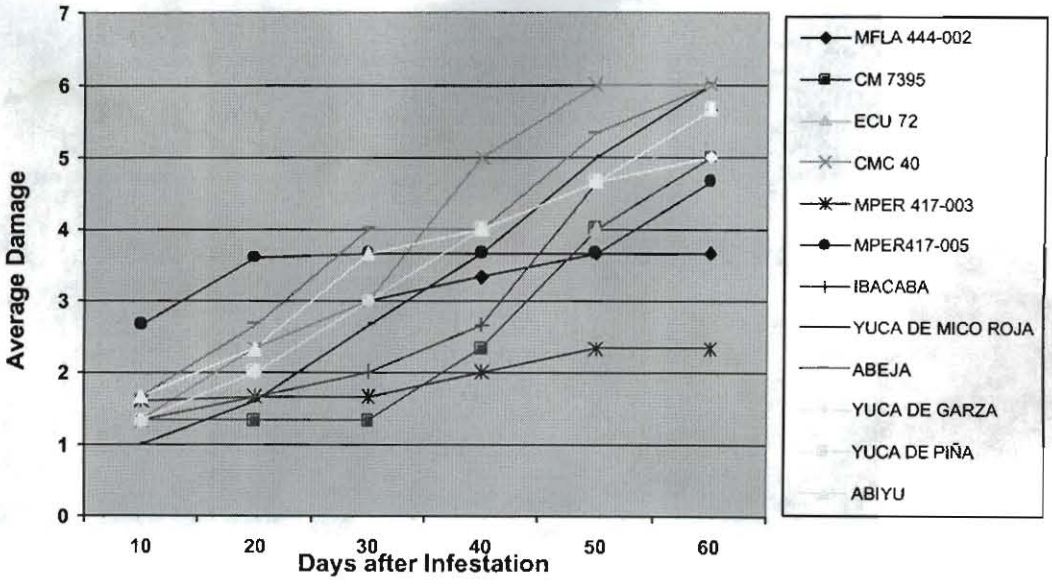

Figure 38. Average damage ratings (1 to 6 damage scale) of *Manihot* species after 60 days of mealybug (*Phenacoccus herreni*) infestation.

Whitefly damage, infestation and percentage of infested leaves were significantly different for the genotypes evaluated (AOV; $F_{(7.136)} = 19.54$, $F_{(7.126)} = 12.3$ and $F_{(7.126)} = 17.12$ respectively, with a $P<0.005$ for the three variables).

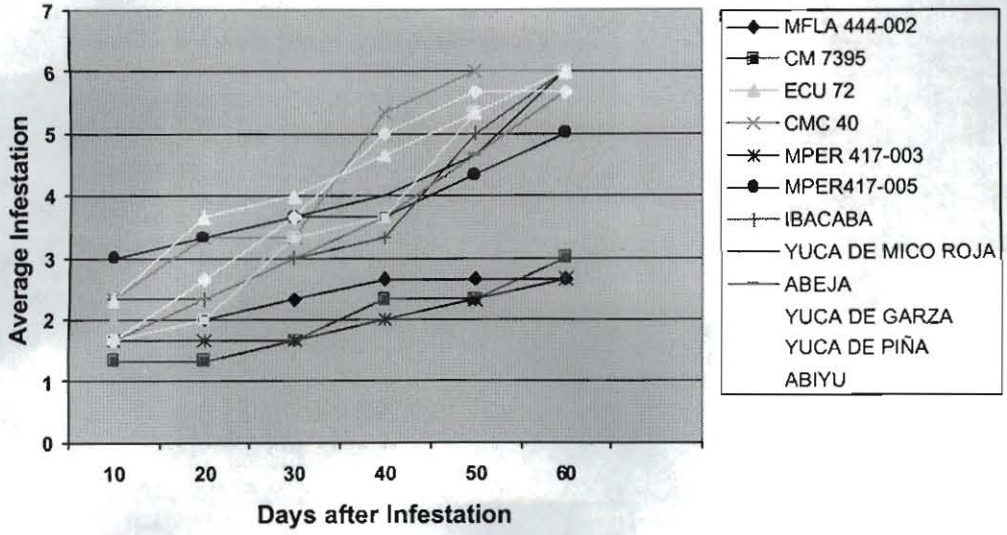

Figure 39. Average infestation level ratings (1 to 6 scale) of *Manihot* species after 60 days of mealybug (*Phenacoccus herreni*) infestation.

Results in damage with whiteflies differed from the other two pest species, mites and mealybugs. The three wild *Manihot* genotypes, MEcu 72 and NUPARA all had significantly lower damage ratings than the remaining *M. esculenta* genotypes (Figure 40). The three wild *Manihot* genotypes and MEcu 72 had a 1.0 damage rating, and NUPARA had a 2.0 rating while the three remaining *M. esculenta* genotypes (CM 7395, CMC 40 and FLORES) all had ratings of 5.3 or higher. MEcu 72 had been selected as the most resistant *M. esculenta* genotype from previous evaluations of the cassava germplasm bank.

The three wild *Manihot* genotypes (MFla 444-002, MPer 417-003 and MPer 417-005) all had very low infestation levels (1.3, 1.3 and 1.6 respectively) while MEcu 72 and NUPARA both had infestation levels of 3.0 (Figure 41). The percentage of leaves infested was lowest for the three wild *Manihot* species, intermediate for MEcu 72 and NUPARA and highest (nearly 100%) for CM 7395, CMC 40 and FLORES. These results indicate that there exist high levels of resistance to whiteflies (*A. socialis*) in the wild *Manihot* species. Higher levels of resistance are indicated for whiteflies than for the other two pests evaluated, mites and mealybugs.

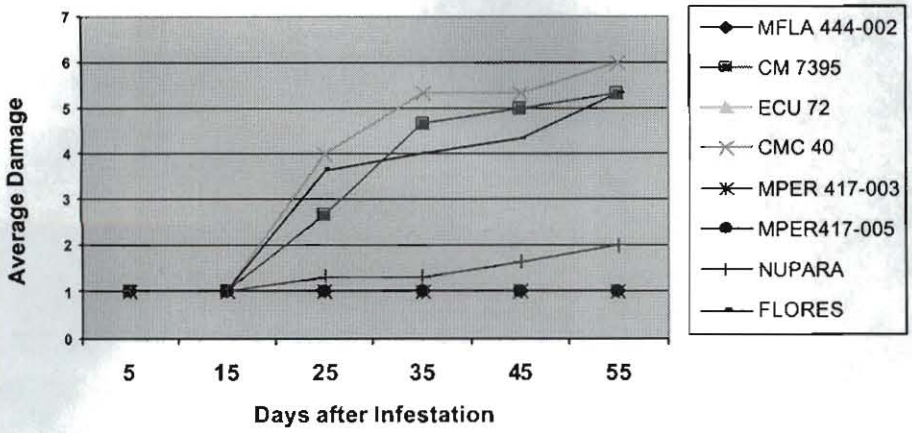

Figure 40. Average damage ratings (1 to damage scale) of *Manihot* species after 55 days of whitefly (*Aleurotrachelus socialis*) infestation.

Overall results show that the possibility of using the wild Manihot species as a source of resistance to cassava pests has considerable potential for the future. This line of research needs to be continued and expanded.

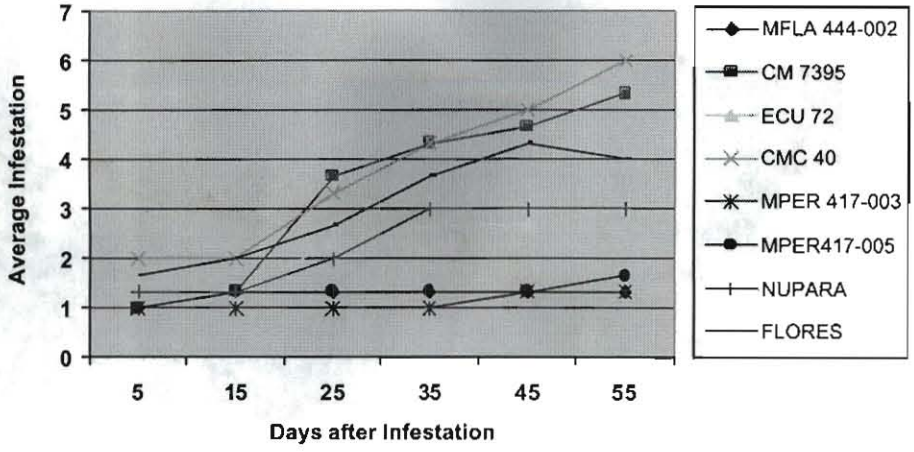

Figure 41. Average infestation level ratings (1 to 6 scale) of *Manihot* species after 55 days of whitefly (*Aleurotrachelus socialis*) infestation.

Contributor: Maritza Burbano.

Activity 9. Biological control of cassava whiteflies.

Rationale

Whiteflies are major pest of cassava throughout most of the tropical and subtropical regions of the world, as direct feeding pests and virus disease vectors. Eleven species of whiteflies have been identified feeding on cassava, eight of these are present in Colombia. *Aleurotrachelus socialis* is the predominant species in Northern South America (Colombia, Venezuela and Ecuador) where it can cause considerable crop loss due to its direct feeding on young cassava plants. *Aleurothrixus aepim* is the major species in Brazil, especially the Northeast (States of Bahia, Pernambuco and Ceara), where yield losses are reported. Additional species of importance are *Bemisia tuberculata* and *Trialeurodes variabilis*, both found throughout several regions of the Neotropics. *Bemisia afer* was recently introduced from East Africa into Peru and warrants considerable attention as it is an important pest in cassava in Africa.

Although *B. tabaci* has pan-tropical distribution, indications are that its populations are not uniform and may actually be a complex of species and biotypes. In Africa, moderate to high populations of *B. tabaci* are observed on cassava and it is the known vector of Africa Cassava Mosaic Disease (ACMD). In the Americas, however, high populations of *B. tabaci* on cassava are seldom, if ever, observed, and it is not presently known to transmit any virus diseases in the neotropics. However, its presence on cassava in the Americas, even if only in low populations is cause for concern as it has the potential to vector virus diseases, including ACMD, were it to be inadvertently introduced into the Americas. In surveys that we have performed over the past several years throughout numerous cassava growing regions of Colombia, Venezuela and Ecuador, we have not collected *B. tabaci* feeding on cassava from any site.

Whitefly populations may fluctuate considerably from one year or growing season to another; the direct cause for these population eruptions is not fully understood, although the overuse or misuse of pesticides may play an important role. Twenty to twenty five years ago high populations of *A. socialis* in Colombia were limited primarily to the Tolima Valley. In more recent years, we have observed even higher populations in the Cauca Valley as well as other regions of Colombia. For example, as plantations size of cassava has increased in the Cauca Valley, *A. socialis* populations have also increased and in some cases it has been necessary to suspend cassava production in certain regions. It has also been observed that a change in planting pattern, where cassava is sown on a "staggered" basis, every 2 to 3 months, *A. socialis* populations may increase considerably, probably due to the continued presence of young cassava leaves, the preferred ovipositional sites for whitefly adults.

Control efforts for *A. socialis* on cassava at CIAT have traditionally concentrated on host plant resistance. In recent years, added emphasis has also been given to biological control. The Colombia Ministry of Agriculture (MADR) through CORPOICA will release a cassava whitefly resistant variety. This variety, Nataima-31, was developed through a joint CIAT/CORPOICA collaboration over a 15 year period. The adoption of a new variety of cassava is a slow process and the variety may not be adapted to all cassava ecosystems. It is therefore necessary to develop alternative control methods, including, biological control and the use of selective pesticides. In recent Annual Reports (1999 to 2001) we have discussed the results of surveys to

detect natural enemies, especially parasitoids. We are also evaluating the role of fungal entomopathogens in whitefly IPM.

Objective: Determine the pathogenicity of several fungal hyphomycetes for control of the cassava whitefly *Aleurotrachelus socialis* under laboratory conditions.

Materials and Methods

Six entomopathogenic fungal isolates were selected from the CIAT "Ceparío;" these isolates had been previously collected from *A. socialis* in different cassava zones of Colombia (Table 16). These isolates had been stored at -20°C in the CIAT collection, using the dry filter paper technique. They were reactivated by placing 10 to 20 adult *A. socialis* (from CIAT colony) in petri dishes with wet filter paper (distilled water) and the fungal entomopathogen. After 5 days, those adults with fungal mycelium present were isolated and placed on PDA.

Table 16. Isolates of native fungal entomopathogens collected in Colombia and evaluated for the whitefly, *Aleurotrachelus socialis* control in cassava.

Isolate	Fungal Entomopathogen	Host
CIAT 210	*Paecilomyces fumosoroseus*	*Trialeurodes vaporariorum*
CIAT 211	*Paecilomyces fumosoroseus*	*Trialeurodes vaporariorum*
CIAT 212	*Paecilomyces fumosoroseus*	*Trialeurodes vaporariorum*
CIAT 215	*Verticillium lecanii*	*Aleurotrachelus socialis*
CIAT 216	*Paecilomyces fumosoroseus*	*Aleurotrachelus socialis*
CIAT 217	*Beauveria bassiana*	*Aleurotrachelus socialis*

Once purified, the fungus was placed on a 0.5 % "insect agar" which consists of *A. socialis* adults that were collected from the field and the CIAT colony, and added to the PDA, previously sterilized (Figure 42). 0.5 grams of macerated *A socialis* was added to 100 ml of agar, and autoclaved at 10 psi and 110°C for 10 minutes. The sterilized agar was poured into petri dishes and was sown with the six fungal isolates. A similar procedure was also carried out with *A. socialis* eggs and nymphs.

Evaluations of the isolates were accomplished on potted cassava plants (Var. CMC-40) infested with *A. socialis* adults, placed in nylon mesh cages in the greenhouse (30 ± 2°C and 50-60 % RH). Each plant was infested with 20-30 adults selected from the CIAT colony, and placed in small leaf cages located on cassava leaves, for a 24hr. period. After this period, the adults were removed. This procedure was also carried out at intervals of 4, 7, 14 and 23 days so that all development stages of the whitefly would be present upon fungal application.

The fungal pathogens were applied with a micro aspirator at 10 PSI. Spray coverage was evaluated using a hydrosensitive sheet of paper. The applied volume per treatment was 4.0 ± 0.5 ml per nymph. After application, plants were placed in a growth room (28 ± 2°C and 80-90 % RH). Evaluations were made when adults emerged by counting nymphal skins, live and dead nymphs and dead nymphs with or without micosis. Leaves with nymphs and fungal application were removed and placed on moist filter paper for 4 to 5 days.

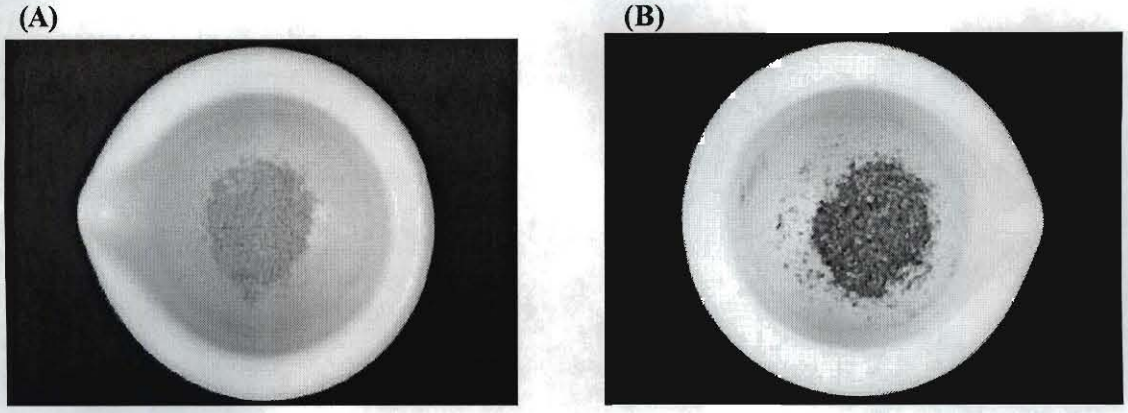

Figure 42. A. Adults of *Aleurotrachelus socialis* – B. Eggs and nymphs of *A. socialis*.

The experimental design utilized was completely randomized, with 10 replications per treatment, and each leaf cage was considered as an experimental unit. Controls consisted of sterile distilled water, and sterile distilled water plus tween 80 at 0.05%. Once the most susceptible whitefly stage to the fungus was determined, the most promising isolate was evaluated at concentrations of 1×10^3, 1×10^4, 1×10^5, 1×10^6, 5.0×10^6, 1×10^7 and 5.0×10^7 conc./ml.

Additionally six commercial products were evaluated at the recommended doses by each commercial source. Initially a quality control procedure was carried out for each product to determine product purity. Products were then tested for pathogenicity by applying them to whitefly eggs about to hatch, as this is the most susceptible stage.

Results

Evaluation of the six native isolates on the different development stages of *A. socialis* resulted in isolated CIAT 215 (*Verticillium lecanii*) being selected as the most promising (Figure 43). Whitefly mortality was highest with this isolate, reaching 65.4%; isolate CIAT 217 (*Beauveria basssiana*) was next highest at 47.1% mortality. Mortality in the control treatments averaged 16%, which was lower than all of the isolates evaluated. This also indicates that the methodology utilized was adequate for evaluating the fungal entomopathogens on the different whitefly development stages.

Consequently, the isolate CIAT 215, having caused the highest mortality, was used to determine the whitefly stage most susceptible to fungal entomopathogens. It was observed that this isolate, *V. lecanii*, resulted in fungal mycelium growing on egg and nymphal stages of *A. socialis* (Figure 45). When *V. lecanii* (CIAT 215) was applied to the egg and nymphal stages of *A. socialis*, mortality was above 50 % for all stages (egg and 4 nymphal stages) (Figure 44). Mortality was highest when applied to the egg stage at 74 %, followed by 72 % for 2nd instar nymphs. Although these two differences were not significant, it was decided to use the egg stage to evaluate concentrations of the isolate CIAT 215. The commercial products were also evaluated using the egg stage.

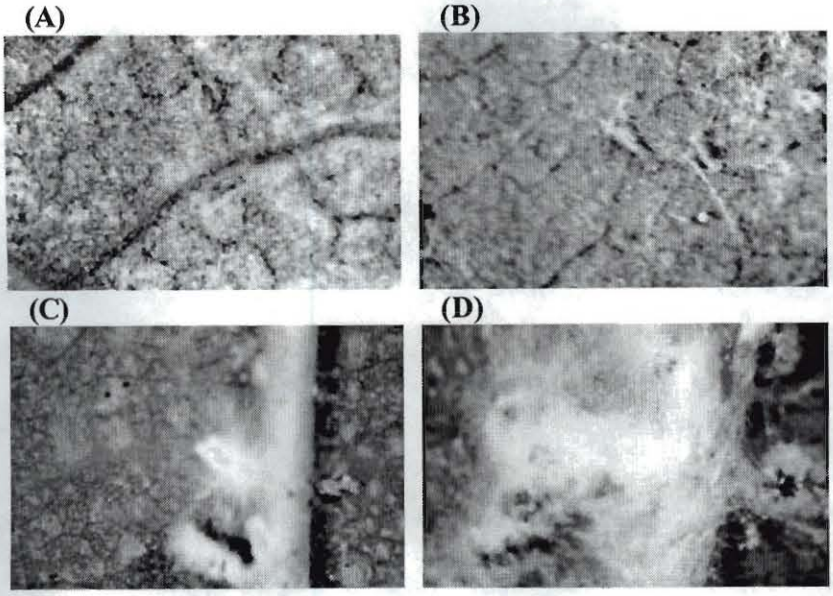

Figure 45. ***Verticillium lecanii*** **mycelium, present on life stages of** ***Aleurotrachelus socialis*** **(A) eggs; (B) 1st instar nymphs; (C) 2nd instar nymphs; (D) 3rd and 4th instar nymphs.**

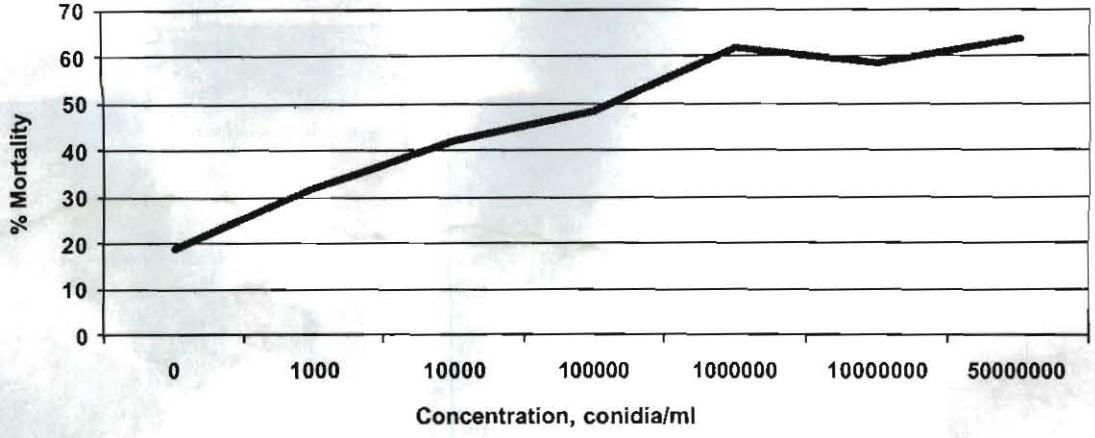

Figure 46. Egg (near hatch) mortality of ***Aleurotrachelus socialis*** **infested with several concentrations of the fungal entomopathogen isolate CIAT 215 (*Verticillium lecanii*).**

Table 17. Determination of LC_{50} and LC_{90} of the fungal entomopathogen isolate CIAT 215 (*Verticillium lecanii*).

N	CL_{50} (LC)*	CL_{90} (LC)	B ± EEM	X^2	$P > X^2$
2146	1.4×10^{7} (3.6×10^{5}-1.5×10^{9})	2.3×10^{12} (9.3×10^{9}-4.1×10^{21})	0.24±0.05	12.6	0.01

* Confidence limit at 95%

The evaluations of quality control for commercial formulated products show that these products did not contain the quantity of spores claimed on the label, rather at a lower concentration (Table 18). Viability tests show that only one product, *Paecilomyces fumosoroseus*, had a viability rating above 85%, the minimum percent that a formulated product should have, for a quick knock-down or kill in field applications (Table 19). The purity test established the proportion of the biological agent in the formulated product, and also identified any of the contaminants that might be present. In two products evaluated, there was no growth of the active ingredient, the fungal entomopathogen (Table 19).

Table 18. Spore counts of several commercially formulated fungal entomopathogen products that were evaluated for *Aleurotrachelus socialis* control on cassava.

Product	Spore Counts Conidia/ml (Actual)	Spore Counts Conidia/ml (Ticketed)
Paecilomyces fumosoroseus	$6.6x10^8$	$2x10^{10}$
Verticillium lecanii	$8.1x10^6$	$2x10^7$
Beauveria bassiana	$1.9x10^8$	$2x10^9$
Verticillium lecanii	$1.3x10^8$	$2x10^{10}$
Verticillium lecanii	$9.0x10^7$	$2x10^{10}$
Verticillium lecanii	$2.0x10^7$	$2x10^9$

The pH can also influence fungal germination, with optimal range between 5.5 and 7.0. Only two products were in this range, both were *V. lecanii.* "Suspendibility" determines the time that a wettable powder requires to become suspended; some product suspension occurs rapidly, while others do not, or require more time, which could lead to occasional clogging of nozzle opening when applying the product (Table 19).

Finally, each formulated commercial product was evaluated by applying it to *A. socialis* eggs that were near hatching. All commercial products evaluated resulted in whitefly mortalities below 50% (Figure 47). However, the resulted mortalities on all products were significantly different than the control. Two products, *Beauveria bassiana* and *V. lecanii*, achieved 49.9% mortality while the control was 19.0%, indicating, at best, mediocre *A. socialis* control. It should be noted that these formulated products have not been recommended for *A. socialis* species on cassava, but rather are recommended for other species on other crops. This could be the reason for the lower mortality, results. These products could be tested on other development stages of *A. socialis* to determine if a higher mortality if feasible.

Results from these experiments indicate that the CIAT 215 isolate of *V. lecanii* has the potential to be commercially formulated and promoted or recommended for *A. socialis* control in cassava.

Table 19. Quality control of formulated commercial fungal entomopathogen products that were evaluated for *Aleurotrachelus socialis* control on cassava.

Product	Viability 24 h	Purity	PH	Suspensionability
Paecilomyces fumosoroseus	95%	98%	5.35	2 min.
Verticillium lecanii	35%	100%	5.54	4 min.
Beauveria bassiana	40%	100%	5.14	50 min.
Verticillium lecanii	22%	-	4.80	1.30 min.
Verticillium lecanii	13%	-	4.87	-
Verticillium lecanii	40%	40%	5.59	None

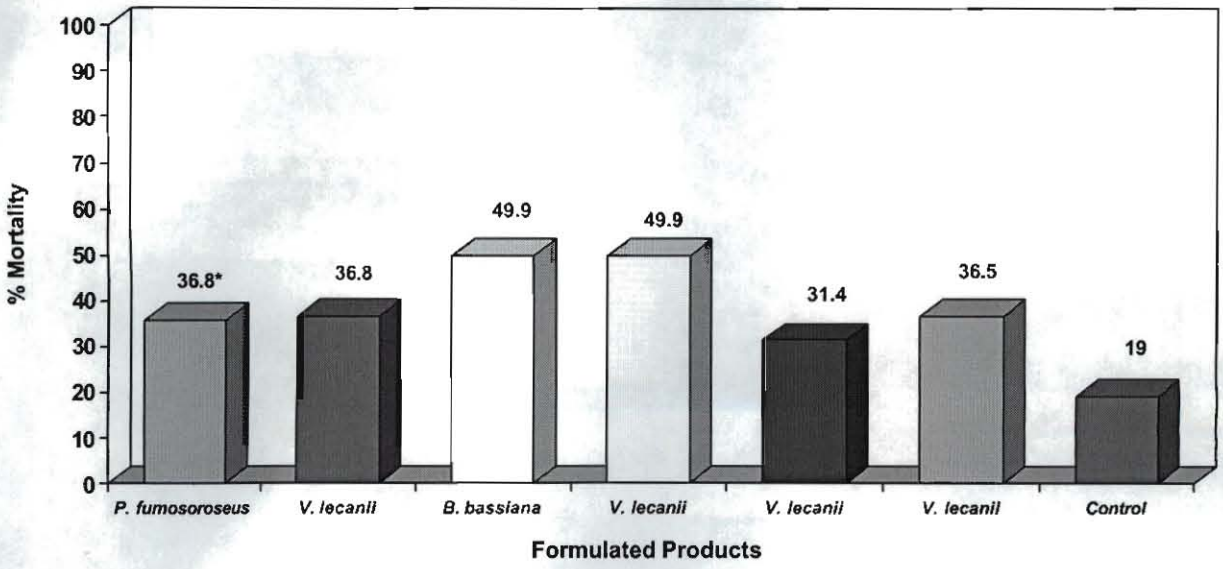

Figure 47. ***Aleurotrachelus socialis*** **egg (near hatch) mortality with applications of formulated commercial fungal entomopathogen products.**

Contributors: I. Aleán, A. Morales, C.M. Holguín, A.C. Bellotti.

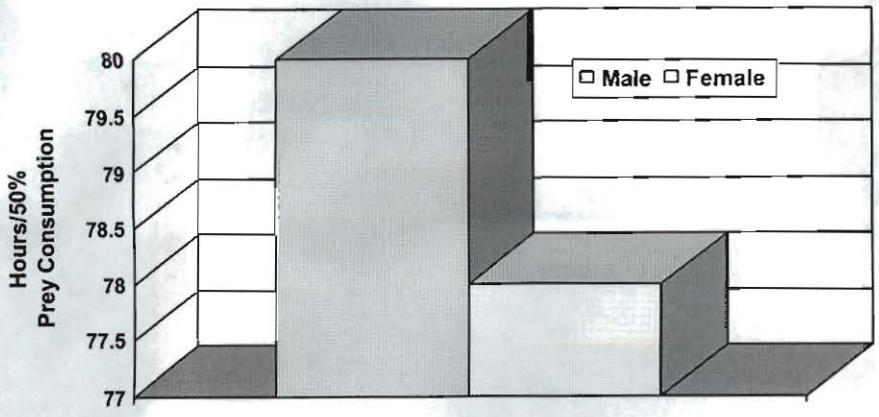

Figure 49. Consumption of *A. socialis* immatures by male and female *C. carnea* adults in laboratory studies.

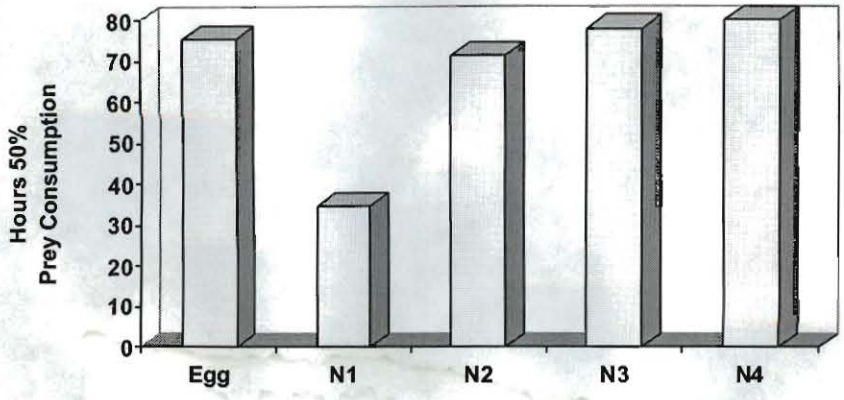

Figure 50. Consumption of *A. socialis* immatures by *C. carnea* larvae in laboratory studies (Duncan Multiple Comparison test at 0.05%).

It was observed that oviposition was initiated on the 4th day that adult *C. carnea* were introduced into the experimental units. Females lived on the average of 27 days but oviposition occurred primarily between the 4th to 12th day (Figure 51). Between the 6th and 7th day oviposition peaked at a 19.5 average, while the overall average was 14.0 eggs per day during the 8 day period. Each *C. carnea* female oviposited an average of 112 eggs during its ovipositional period. This is considered low and may have been negatively influenced by the artificial diet that was offered.

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

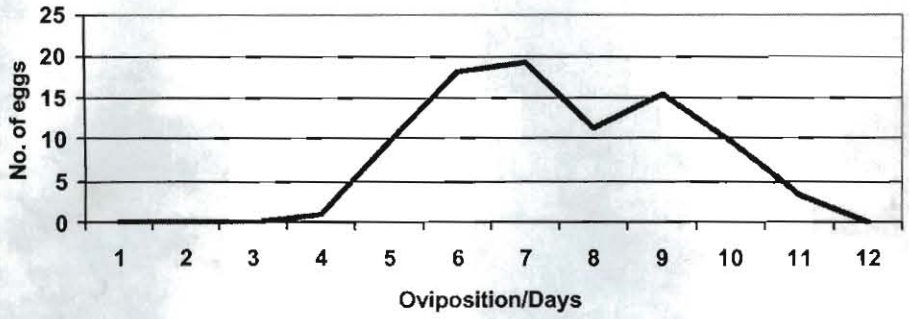

Figure 51. Oviposition of *C. carnea* adult females feeding on an artificial diet in laboratory studies.

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

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Submitted

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Conferences

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