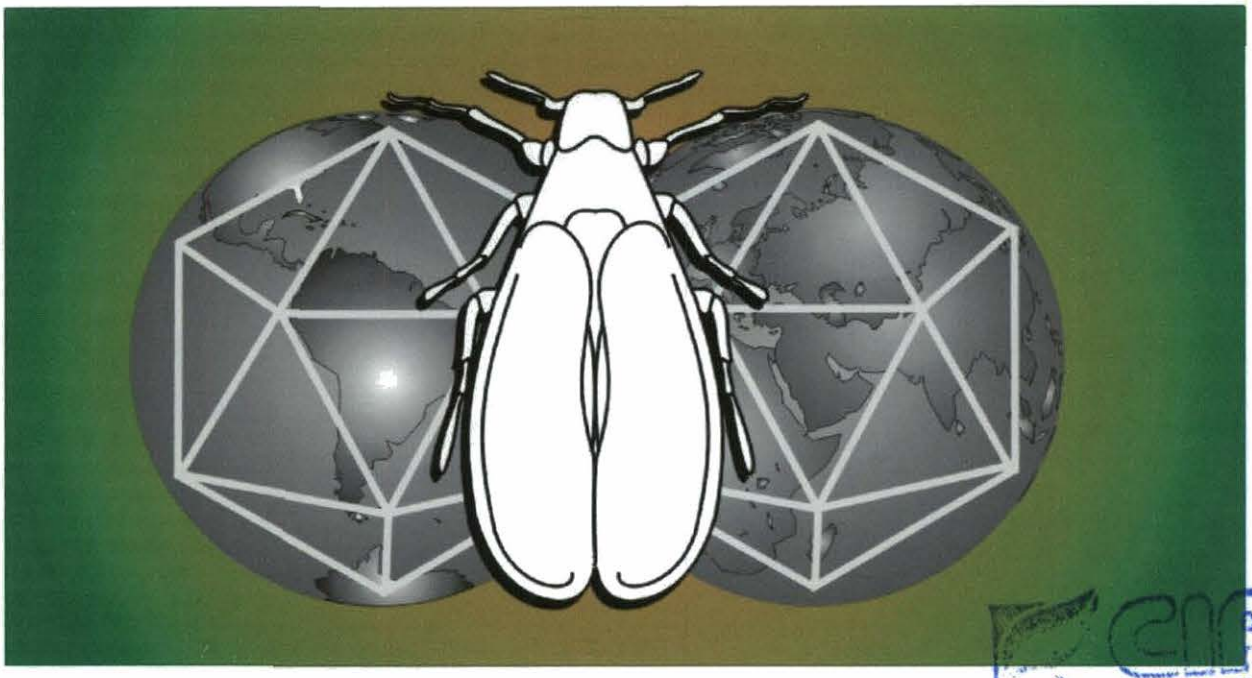
PROGRESS REPORT

Sustainable Integrated Management of Whiteflies through Host Plant Resistance



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Funding Agency: MFAT, New Zealand

1999 - 2000

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

Contact Persons: A.C. Bellotti, J. Tohme
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Project Purpose:

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

Project Objectives:

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

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

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

Project Summary:

As direct-feeding pests and vectors of plant viruses, whiteflies constitute a major problem in cassava production in Africa, the Americas and Asia. Whitefly feeding affects cassava in there ways. Direct damage is caused by feeding on the phloem of the leaves, inducing chlorosis and leaf fall, which results in considerable reduction in root yield if prolonged feeding occurs. Yield losses of this type are common in the neotropics. Whitefly feeding also produces a honeydew, which provides a medium for sooty mold growth that also reduces yield. More importantly, whiteflies are major vectors of cassava viruses such as African Cassava Mosaic Disease, Common Mosaic and Frogskin Disease. Although

numerous natural enemies of cassava whiteflies have been identified, especially in the neotropics, their effectiveness in controlling whitefly populations, is still being studied.

Host Plant Resistance (HPR) offers a low cost, sustainable solution to losses from cassava whitefly damage. HPR research at CIAT was initiated nearly 15 years ago and, at present, more than 5000 varieties from the CIAT cassava germplasm bank have been evaluated for whitefly resistance. In recent years the Systemwide Whitefly Project has achieved some very important advances with support from New Zealand's MFAT. In addition to the continual evaluation of landrace varieties, several thousand hybrids and breeding populations have also been evaluated.

Whitefly resistance in agricultural crops is rare and a review of the literature indicates that the levels of resistance that we have identified in cassava germplasm is unique. Several sources of resistance in cassava to the whitefly *Aleurotrachelus socialis* have now been selected. Prior to 1999, the varieties MEcu 72, MPer 335, MEcu 64, MPer 415 and the hybrids CG 489-4, CG 489-34, CG 489-31 and CG 489-23 were selected as moderately to highly resistant. Research during 1999, added several additional varieties, including MPer 317, MPer 215, MPer 221, MPer 265, MPer 266 and MPer 365. In additional several hybrids, including CM8424-6, CM 8424-33 and CM8424-4 were also selected as resistant after several years of field evaluations. Based on these results, *A. socialis* resistance appears to be concentrated in germplasm originating from Ecuador and Peru, presenting a phenomenon that needs to be investigated further.

Research is also being done at CIAT to identify molecular markers linked to genes conferring resistance to *A. socialis* in order to evaluate further and understand the genetics of whitefly resistance in cassava. Different breeding populations have been obtained from viruses between resistant and susceptible genotypes. We are using Amplified Fragment Length Polymorphism (AFLP's) and Simple Sequence Repeat (SSR) combination with the Bulk Segregant Analysis (BSA) method to find markers associated to resistance for mapping and ultimately cloning the resistance genes. Using AFLP's, we found bands cosegregating with resistance to the whitefly. These bands are being sequenced to generate SCAR markers. The new SCAR's markers will be used for the identification of resistant materials in breeding programs. A linkage map for resistance to whiteflies is being constructed using the AFLP's and SCAR's markers.

Cross resistance studies with additional whitefly species have also been initiated. During 1999, it was found, through laboratory studies at CIAT, that the new B biotype of *Bemisia tabaci* will feed on cassava. This is supported by field observations in several countries in Latin America, and by reports from taxonomists. Once a colony of this biotype is established on cassava, studies in HPR will be initiated. It is felt that the employment of whitefly resistance combined with virus resistance will reduce or deter the development of cassava virus epidemics.

Present research being funded by MFAT/HPR Project consists of four major areas of activity:

1. Cassava germplasm evaluation to identify sources of whitefly resistance in landrace varieties from CIAT's germplasm bank.

2. Identification of genomic regions responsible for the expression of whitefly resistance in cassava.

3. Identification of whitefly resistance mechanisms in cassava.

4. Construction of a linkage map for resistance to whiteflies.

Present Research: 1999 Activities and Results.

Preliminary studies: Whitefly species diversity on cassava at CIAT, Palmira

Cassava whitefly populations at CIAT have increased dramatically in recent years, causing crop damage. Concurrently, the incidence of cassava frogskin virus disease, which is whitefly transmitted has also increased. Traditionally, whitefly infestations at CIAT have consisted of three species; *Aleurotrachelus socialis* has always been the predominant species while *Bemisia tuberculata* and *Trialeurodes variabilis* are found in much lower populations. This follows the pattern observed in most commercial cassava plantations in Colombia (Castillo et al. 1999). Since frogskin disease is transmitted by *B. tuberculata*, and the incidence of the disease has increased in recent years, it was decided to monitour the populations of the whitefly species population on cassava at CIAT.

Leaf samples were taken from cassava plants at 98 randomly selected sites on the CIAT farm. Leaves were removed from the lower portion of the plant, and brought to the laboratory for stereoscope observation and individuals were identified to species.

A total of 13, 194 whitefly individuals were collected from the leaf samples; 12, 987, or 98.5% corresponded to the species *A. socialis*. *B. tuberculata* and *T. variabilis* were represented by 90 (0.68%) and 114 (0.86%) individuals respectively. These results indicate that there has probably been no shift in the relationship of whitefly species populations in recent years. Although populations of *B. tuberculata* are very low, they may be sufficient to cause the increase in the incidence of frogskin disease, especially if there exist a high source of disease inoculum. Whitefly populations will continue to be monitored in the future.

Development of a colony of *Bemisia tabaci* on cassava.

B. tabaci is the vector of African Cassava Mosaic Disease (ACMD) in Africa. Until recently, the *B. tabaci* biotypes found in the Americas did not feed on cassava. It has been speculated that the absence of ACMD in the Americas may be related to the inability of its vector, *B. tabaci*, to colonize cassava. Since the early 1990's a new biotype (B) of *B. tabaci*, considered by some to be a separate species (*B. argentifolii*) has

been found feeding on cassava in the neotropics. It is considered that ACMD now possess a more serious threat to cassava production, as most traditional varieties in the neotropics are highly susceptible to the disease. In addition the B biotype of *B. tabaci*, as a virus vector, causes heavy crop losses on numerous other crops in the neotropics, and these are often grown in association with cassava, or in the same area. The possibility of virus diseases moving between crops, or the appearance of previously unrecorded viruses has become a potential threat.

For many years we have maintained an active program to identify cassava germplasm resistant to whiteflies and develop resistant hybrids. The species most frequently used in our evaluations has been, and continues to be, *A. socialis*. However, in light of the fact that there is a biotype of *B. tabaci* now capable of feeding on cassava, and its capacity to transmit geminiviruses, research has been initiated to study the potential of *B. tabaci* as a pest or vector on cassava. Therefore an attempt was initiated to establish a colony of *B. tabaci* biotype found in Colombia, on cassava.

Adults of *B. tabaci* were collected from several hosts, including lettuce, beans, cotton, squash, and cabbage (supplied by the bean entomology project).

Potted, five week old, cassava (Var. CMC 40) plants were infested by placing 20 to 25 adult whiteflies in small leaf cages, and they were allowed to oviposit 48 and 96 hours (two separate experiments).

Preliminary results indicate that the local *B. tabaci* biotype "B" does not easily survive on cassava. Whiteflies collected from all of the aforementioned hosts were able to oviposit on cassava, and nymphs emerged and initiated feeding on cassava. A total of 898 nymphs were produced in the two experiments; however only 10 adults (1.1%) emerged. In the first experiment, 257 nymphs were produced and resulted in 10 adults. In the second experiment of the 641 nymphs produced, there was 100% mortality during the nymphal stage. Nymphal mortality occurred during both early or late stages.

Although these results indicate a poor adaptation of *B. tabaci* on cassava, the fact that some oviposition and nymphal development occurred, indicates that populations of *B. tabaci* could move onto cassava. This is already occurring in certain areas (i.e. Brazil), indicating that the local biotype may not yet have had sufficient opportunity to adapt to cassava. We will continue to monitour this situation.

Whiteflies: Germplasm evaluations at CIAT, Palmira

Cassava is often considered more tolerant to pests than most crops because it does not have critical periods that affect yield forming organs. Nevertheless, research, and field observations, have shown that several pests can reduce yield significantly when pest populations are high and/or environmental conditions are unfavorable. An estimated 200 species of arthropods feed on cassava in the neotropics and many of these are specific to cassava and have adapted in varying degrees to the array of natural biochemical defenses

that include laticifers and HCN content. A successful integrated pest management (IPM) program in cassava will depend on having effective, environmentally sound, low-cost pest management technologies available to cassava farmers in developing countries. Stable host plant resistance (HPR) offers a practical long term solution for maintaining reduced pests populations. Sources of resistance have been identified for mites, lacebugs, whiteflies, thrips and burrower bug. The CIAT cassava germplasm bank of approximately 6000 accessions is continually being screened for resistance to arthropod pests. Current emphasis is being given to whiteflies, mites and stemborers.

Whitefly populations at CIAT, especially those of the species *A. socialis*, continued to remain high (see previous section) during 1999. Whitefly resistance in agricultural crops is rare, however after evaluating nearly 5000 cultivars, several good sources of resistance have been identified and high yielding, whitefly resistant hybrids have been developed. Three of these hybrids are presently being evaluated by CORPOICA and may soon be released to producers.

The clone MEcu 72 has consistently expressed the highest level of resistance. Three additional clones MEcu 64, MPer 335 and MPer 415 have also been selected for high levels of resistance. The progeny from MEcu 72 x Bra 12 cross, CG 489-34, CG 489-4, CG 489-31 an CG 489-23 have consistently displayed moderate levels of whitefly resistance. The families evaluated were as follows:

Hybrid family		**Female Parent**		**Male Parent**
CM 8984	=	MCol 1505	x	CG 489-34
CM 8990	=	MCol 2026	x	CG 489-34
CM 8991	=	MCol 2026	x	MBra 12
CM 8995	=	MEcu 72	x	MCol 1468
CM 8996	=	MEcu 72	x	MCol 2246
CM 3317	=	MBra 12	x	MCol 1468
CM 5438	=	MBra 12	x	MCol 1505
CM 7559	=	MNGA 2	x	MBra 12
CM 8891	=	MCol 1468	x	CG 489-4
CM 8884	=	CG 489-4	X	MCol 1468

The clones MCol 1505, MCol 2026, MCol 1468, MCol 2246 and MNGUA are susceptible to whiteflies and MBra 12 is tolerant. These families were planted in fields at CIAT and at CORPOICA, Nataima, Tolima. Plantings were done at both sites in early November 1998 and whitefly damage on populations were done during April - May 1999. A 1 to 6 damage scale (1 = no damage, 6 severe damage) is used to measure whitefly damage and A 1 to 6 scale is also used to measure whitefly populations **(Table 1.2.3.1).**

Table 1.2.3.1 Population and damage scales for evaluating cassava germplasm for resistance to whiteflies.

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

Results show that whitefly (*A. socialis*) populations at CIAT were extremely high and probably caused excessive selection pressure on the cassava clones. Of the 671 progeny and parents evaluated 637 or 95% had damage ratings above 4.1 and 47% between 5.1 and 6.0 **(Figure 1.2.3.1).** Only 7 had ratings below 3.5 and two of these were parents. MEcu 72, our most resistant variety held up fairly well under this heavy selection pressure with a damage rating of 2.0, 3.0 and 3.5 in the three repetitions.

That whitefly populations were extremely high can be seen in **figure 1.2.3.2**; 658 of the 671 progeny, or 98%, had whitefly populations ratings above 4.1, and none had populations below 2.5 on the population scale. If we look at just one family, CM 8990 (MCol 2026 x CG 489-34) 170 of the 179 progeny (95%) had damage ratings above 4.1 and only 1 clone below 3.6 **(Figure 1.2.3.3).** This family is being evaluated in the greenhouse using potted plants and a controlled whitefly population.

Whiteflies: Germplasm evaluations with CORPOICA, Nataima, Tolima

The cassava cultivars from the crosses between resistant and susceptible cultivars (as described in the previous section) were also planted at CORPOICA, Nataima, Tolima. We have been evaluating germplasm for whitefly resistance at this experiment station for about 15 years. Whitefly populations have traditionally been high, making this an excellent site for HPR evaluations. However in recent years whitefly population pressure has decreased while, as previously noted, populations have increased dramatically at CIAT. The reasons for this phenomena are not fully understood.

Evaluations at CORPOICA, Nataima, contrast with those taken at CIAT, on the same cultivars, in that damage levels were considerably lower. Of the 554 cultivars tested at Nataima, 290 or 52% had damage ratings of 2.5 or lower **(Figure 1.2.4.1)**, while at CIAT, no cultivars had damage ratings below 2.5. At Nataima, only 53 cultivars (9.6%)

had damage ratings above 4.0; at CIAT, 637 cultivars, or 95% had damage ratings above 4.0.

Data recorded on whitefly (*A. socialis)* populations show a higher population level at CIAT than at Nataima **(Figures 1.2.4.2 and 1.2.3.1)**.

At CIAT, 658 cultivars, or 98%, had whitefly populations rating above 4.0 and 173 (25.8%) of these were between 5.5 and 6.0 **(Figure 1.2.3.1)**. At Nataima, only 82 cultivars (14.8%) had population rating above 4.0 and none above 5.5 on the 0 to 6 scale **(Figure 1.2.4.2)**.

A comparison of the progeny from the family CM 8990 between the two evaluation sites presents similar results **(Figures 1.2.3.3 and 1.2.4.3)**. At Nataima the cultivars were well distributed in their rating from 1.0 to 5.5, and no cultivars in the 5.5 to 6.0 range **(Figure 1.2.4.3)**. Of the 104 cultivars, 49, nearly one half (47%), were rated in the 1.0 to 2.5 range; while at CIAT no cultivars fell within this range **(Figure 1.2.3.3)**. In addition, at CIAT, 74 cultivars (41.3%) received a rating of 5.1 to 5; at Nataima only 13 cultivars (12.5%) received a damage rating above 4.0.

The susceptible check in these trials is CMC-40 (MCol 1468) and at Nataima this cultivar was planted at systematic intervals through the trial area. This facilitates "measuring" the levels, intensity and distribution of whitefly populations and damage levels. CMC-40 received a moderate to high damage (3.0 to 5.0) and population (3.0 to 4.5) throughout the trial. At CIAT the high CMC-40 damage rating and whitefly population rating was 5.5 and 4.5 respectively; at Nataima it was 5.0 and 4.5 **(Table 1.2.4.1)**, slightly lower than at CIAT but also indicating that there were adequate whitefly populations.

Whitefly damage and population ratings were consistently higher at CIAT. Table 1.2.4.1 contains a representative sample of the ratings at the two sites for selected cultivars. The cultivars MCol 1505, MCol 2026 and CMC-40 are susceptible (data from previous trial) and the three had high damage ratings at both sites; MCol 2246, also susceptible, did not follow this pattern. The clones CG 489-23, CG 489-31, CG 489-34, and CG 489-4 (selected resistant progeny from a MEcu 72 x MBra 12 cross), and the resistant cultivar MEcu 72, had low damage ratings, as expected, at Nataima. These results are consistent with several previous trials done over numerous years at this site.

The results from these two trials, one at CIAT, the second at Nataima probably express the difficulty in obtaining an ideal situation, with the optimal amount of pest selection pressure needed to do this type of genetic study. Whitefly populations and consequently cassava plant damage was so great at CIAT that small or moderate differences could not be detected in the cultivars. Whitefly populations at Nataima appears to be too low, resulting in insufficient damage and too many cultivars with low ratings. Controlling whitefly populations in the field will always be difficult; perhaps the greenhouse studies will provide more optimal selection pressure in order to detect differences in resistance from these crosses.

The high whitefly populations/severe damage syndrome at CIAT during early 1999, is an indication of the potential severity of whiteflies as a cassava pest, and reinforces the need to develop highly resistant cultivars.

Table 1.2.4.1. Whitefly (*Aleurotrachelus socialis*) populations and damage on selected cassava cultivars at two evaluation sties, CIAT, Palmira, Valle and CORPOICA, Natima, Tolima.

	CIAT		Nataima	
Cultivars	**Damage[1]/Populations**		**Damage/Populations**	
CM 5438-194	6.0	5.9	1.0	2.75
CM 8991-129	5.5	5.7	1.0	1.5
MEcu	3.0	3.5	1.0	1.1
CG 489-4	4.0	4.6	1.5	2.1
CG 489-23	4.5	4.3	1.5	1.6
CG 489-31	3.0	3.4	2.0	2.0
CG 489-34	5.5	4.6	1.0	1.1
CM 8984-60	4.5	4.5	1.0	1.5
MCol 2246	5.0	4.8	1.5	3.0
CM 8995-4	5.0	4.7	1.5	1.8
CM 8991-37	5.5	4.3	4.0	3.6
CM 5438-74	3.0	4.9	1.0	1.8
MCol 2026	6.0	5.6	5.5	5.2
CMC 40 (MCol 1468)	5.5	4.9	5.0	4.5
MBra 12	5.5	5.5	2.5	3.7
MCol 1505	6.0	5.7	3.5	3.8

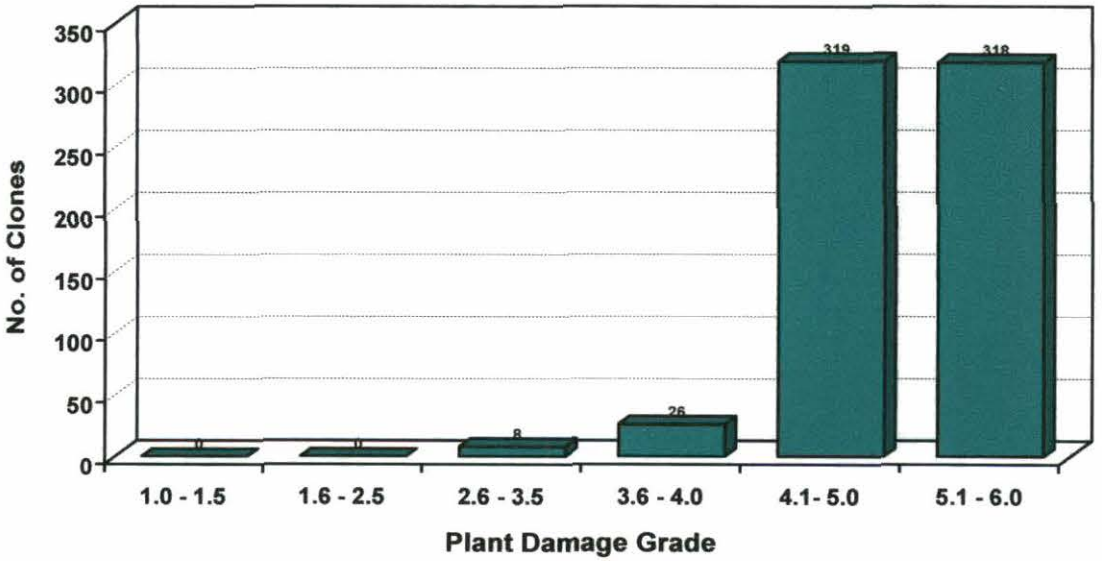


Figure 1.2.3.1 Evaluations of cassava clones from crosses for whitefly damage (*Aleurotrachelus socialis*) on resistant and susceptible cultivars at CIAT (1998-1999).

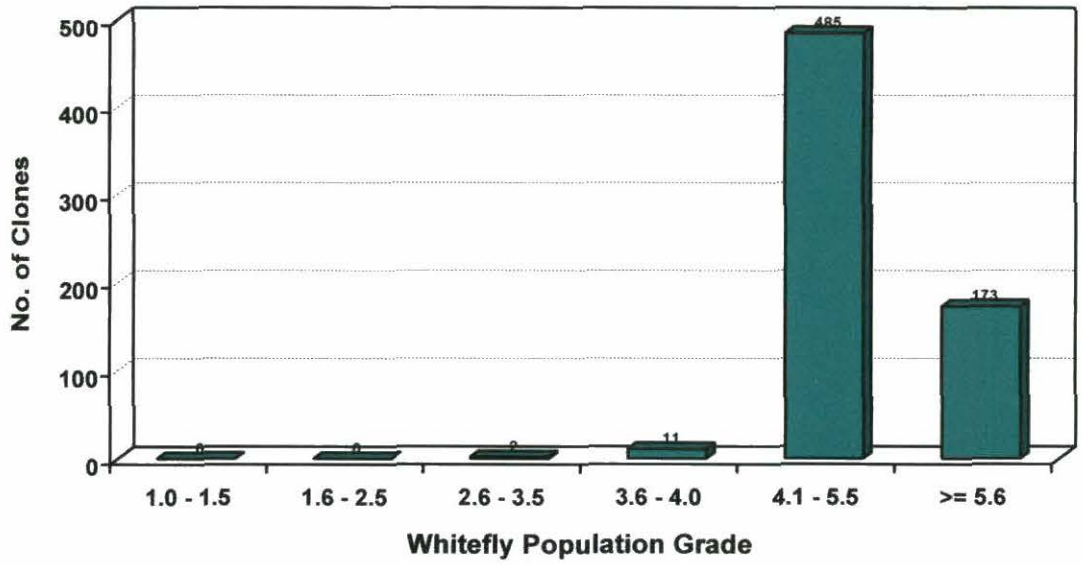


Figure 1.2.3.2 **Evaluations of whitefly (*Aleurotrachelus socialis*) populations on cassava clones derived from crosses between whitefly resistant and susceptible cultivars at CIAT (1998-1999).**

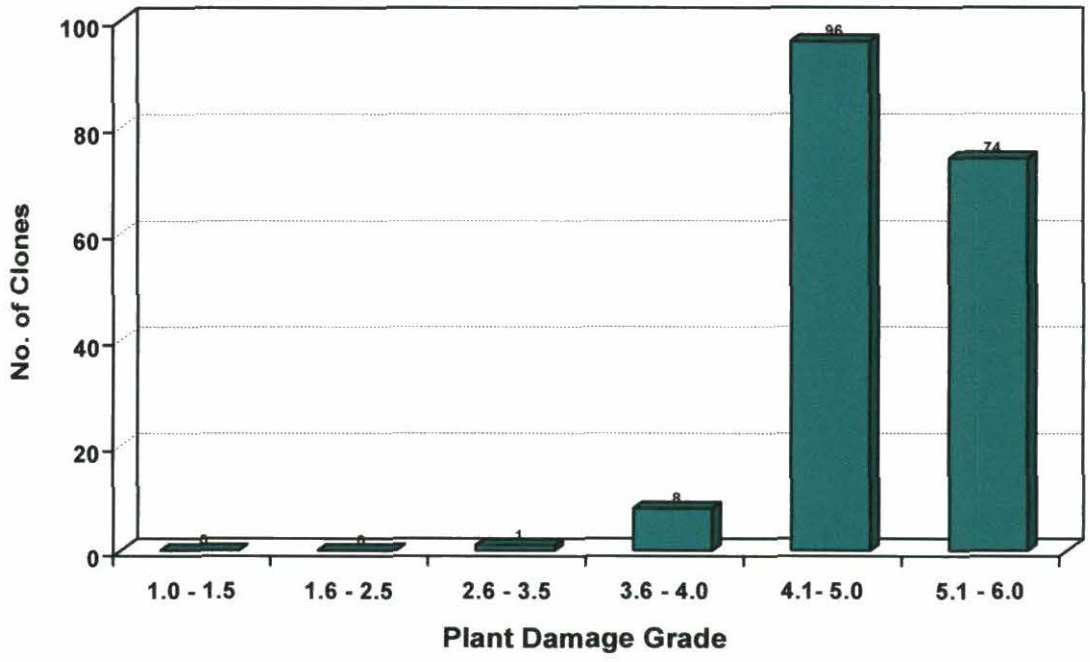


Figure 1.2.3.3. **Evaluations of whitefly (*Aleurotrachelus socialis*) damage on cassava clones resulting from a MCol 2026(S) x CG 489-34(R) cross (Fam. CM 8990) at CIAT, Palmira (1998-1999).**

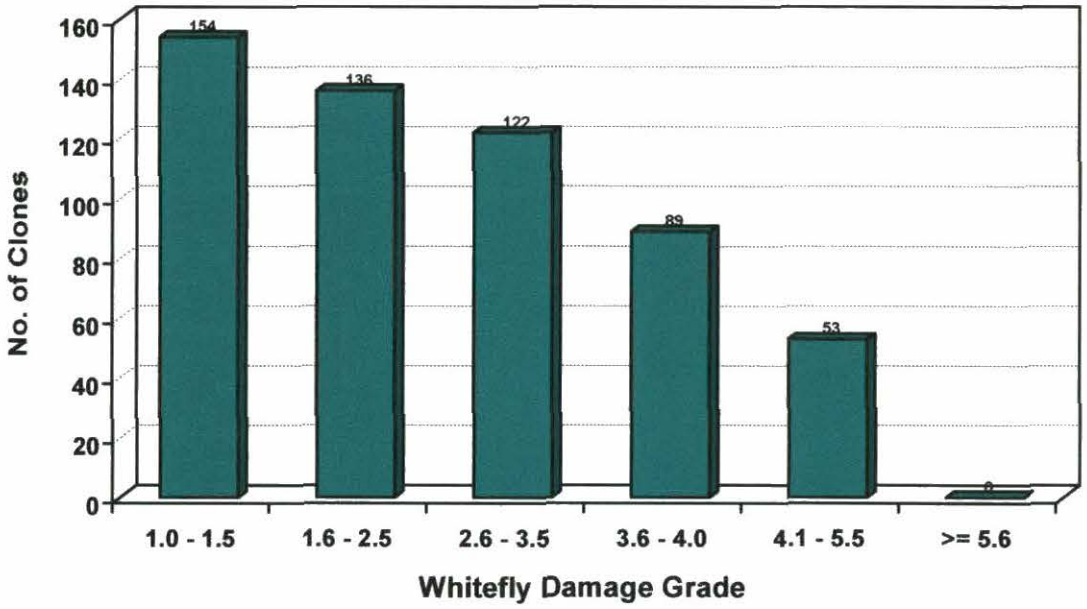
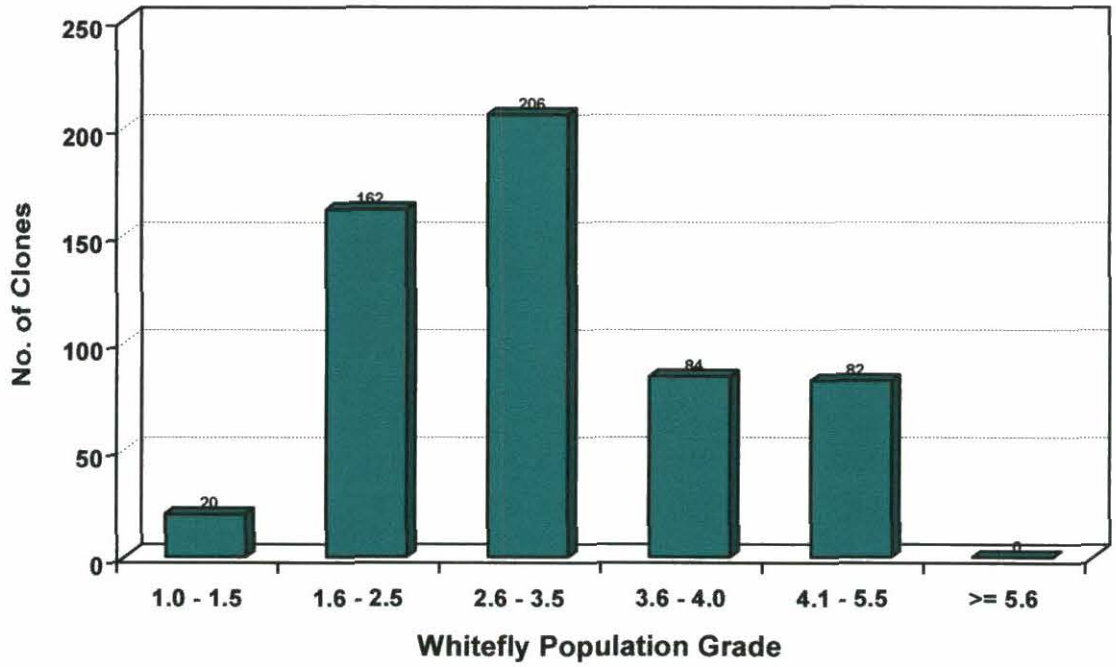


Figure 1.2.4.1 **Evaluations of whitefly (*Aleurotrachelus socialis*) damage on cassava clones derived from crosses of whitefly resistant x susceptible cultivars at CORPOICA, Nataima (1998-1999).**



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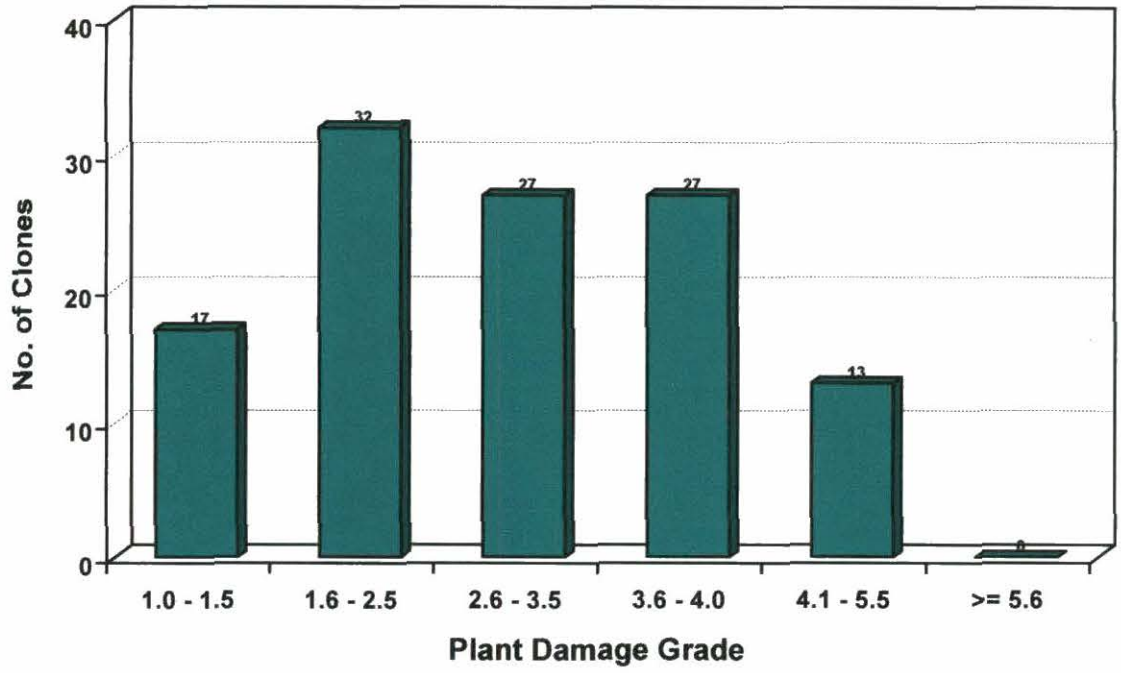


Figure 1.2.4.3 Evaluations of whitefly (*Aleurotrachelus socialis*) damage on cassava clones resulting from a MCol 2026(S) x CG 489-34 (R) cross (Fam. CM 8990) at CORPOICA, Nataima (1998-1999).

Tritrophic interactions: Studies to determine the effect of HPR on whitefly parasitism

Biological control and host plant resistance can offer a low-cost sustainable solution to cassava losses from whitefly damage. Host plant resistance studies at CIAT, especially with the whitefly species *Aleurotrachelus socialis* are well advanced and several resistance sources have been identified.

In recent years we have increased our activities in biological control, surveying for natural enemies in several regions of Colombia and Venezuela. Numerous parasitoids have been collected from cassava whiteflies; these will be identified, studied and evaluated. The most frequently observed parasitoid species of *A. socialis* is *Encarsia hispida*. The genus *Encarsia* is recognized as possessing good searching ability, dispersion and adaptation.

Present research is investigating the compatibility between host plant resistance in biological control, the two most important components in an integrated pest management system. Experiments were designed to determine the compatibility of the parasitoid *E. hispida* on the three cassava genotypes. This phase of the study had two major objectives:

1. Determine the preferred instar of *A. socialis* for *E. hispida* parasitism.

2. Determine the effect of four cassava varieties, MEcu 72, CG489-4, MBra-12 and CMC-40, on the emergence and survival of *E. hispida* parasitizing *A. socialis.*

Materials and Methods

These four varieties were selected because of their resistance or susceptibility to *A. socialis*. MEcu 72 has consistently expressed a high level of resistance to *A. socialis;* CMC-40 is a highly susceptible variety (the cassava whitefly colony is maintained on CMC-40); MBra-12 is a tolerant (low levels of resistance) variety with good agronomic qualities; and CG489-34 is moderately resistant to A. *socialis* and the progeny of a MEcu 72 x MBra 12 cross.

Potted cassava plants of the above mentioned varieties are maintained in the screen house until 4 to 5 weeks of age. They are transferred to a whitefly infestation chamber in the greenhouse and subjected to *A. socialis* oviposition for approximately 36 hours. Infested varieties are maintained in a growth room for exposure to the parasitoid *E. hispida.*

The *E. hispida* colony was developed by collecting cassava leaves with whitefly parasitized pupae from the field. These leaves were placed in plastic boxes with a paper towel on the bottom and darkened with black cheesecloth. Clear glass gars were connected to an opening in the lid of the box, where parasitoids were drawn to the light and collected.

Studies on *A. socialis* instar preference by *E. hispida* were done on the variety CMC-40. Adult whiteflies were placed in small leaf cages on cassava leaves and allowed to oviposit for 8 hours. This was done periodically so that the cassava leaves eventually contained patches of immatures of instars I, II, III and IV of *A. socialis.* Infested leaves were isolated by placing a nylon mesh "bag" over each leaf. Twenty five (25) females collected from the field were released into each bag.

Studies on the biology of *E. hispida* were done on six week plants, and *A. socialis* infestations were done every second day with small leaf cages. When nymphal "patches" reached the third instar, one *E. hispida* parasitoid was introduced to each patch; there were 30 repetitions. The parasitoid was transferred to patches of the same instar three times each week until parasitoid death. The patches with parasitized nymphs remained on the plants until parasitoid emergence.

Results

Results of this experiment show that *E. hispida* prefers to parasitize third and forth instar nymphs (**Fig. 1.2.5.1**). There was no significant difference between the two instars. Parasitism of the first instar was negligible, and very low in the second instar. A high number of nymphs had no parasitoid emergence. This phenomena occurred for all instars but was significantly higher in the forth instar (**Fig. 1.2.5.2**). These non emerged nymphs were either "non viable nymphs" or, as reported in the literature within the genus

Encarsia, it is characteristic for the adult parasitoids to feed on its host. This host feeding characteristic can cause considerable nymphal mortality, especially in the early instars. The fact that the highest number of non-viable nymphs were in the forth instar indicates a possible preference for parasitoid feeding on this instar.

The survival of *E. hispida* does not appear to be adversely affected by any of the four genotypes used in this experiment (**Fig. 1.2.5.3**). Female adult longevity was 27, 28, 32 and 35 days respectively on the varieties MEcu 27, CMC-40, MBra-12 and CG 489-34 respectively. MEcu 72 and CMC-40, the highly resistant and susceptible verities respectively gave very similar results related to longevity. Why longevity is about 25% longer on CG 489-34 is not known unless there are chemical factors in the leaf that are conducive to parasitoid longevity. MEcu-72 is a highly pubescent variety and CMC-40 is a non pubescent variety; MBra-12 and CG 489-34 are intermediate. It has been suggested that pubescence might play a role, perhaps a detrimental effect, to parasitoid longevity. These results do not support that hypothesis.

The emergence of *E. hispida* parasitoids from *A. socialis* pupae on the variety CMC-40, indicate that peak oviposition occurs on about the third days after parasitoid emergence, and tapers off rapidly with continued oviposition for about 23 days (**Fig. 1.2.5.4**). Results also dramatically indicate that genotype can have an effect on parasitoid development or emergence. Parasitoid emergence was considerably lower from *A. socialis* pupae feeding on MEcu-72 and CG 489-34 (**Fig. 1.2.5.4**). It was not possible to get results from MBra-12 as plant leaves dried up and dropped during the experiment. These results strongly indicate that whitefly resistant genotypes could have a detrimental effect on biological control agents, especially parasitoids.

It can generally be concluded, from these experiments, that *E. hispida* will parasitize all instars of *A. socialis* but is most successful on 3rd and 4th instars. There was no genotype effect on survival and longevity of *E. hispida* and that leaf tricomes do not alter these factors. However the low parasitoid emergence rate on the resistant cultivars MEcu-72 and CG489-34 indicate a possible negative effect of whitefly varietal resistance on biological control.

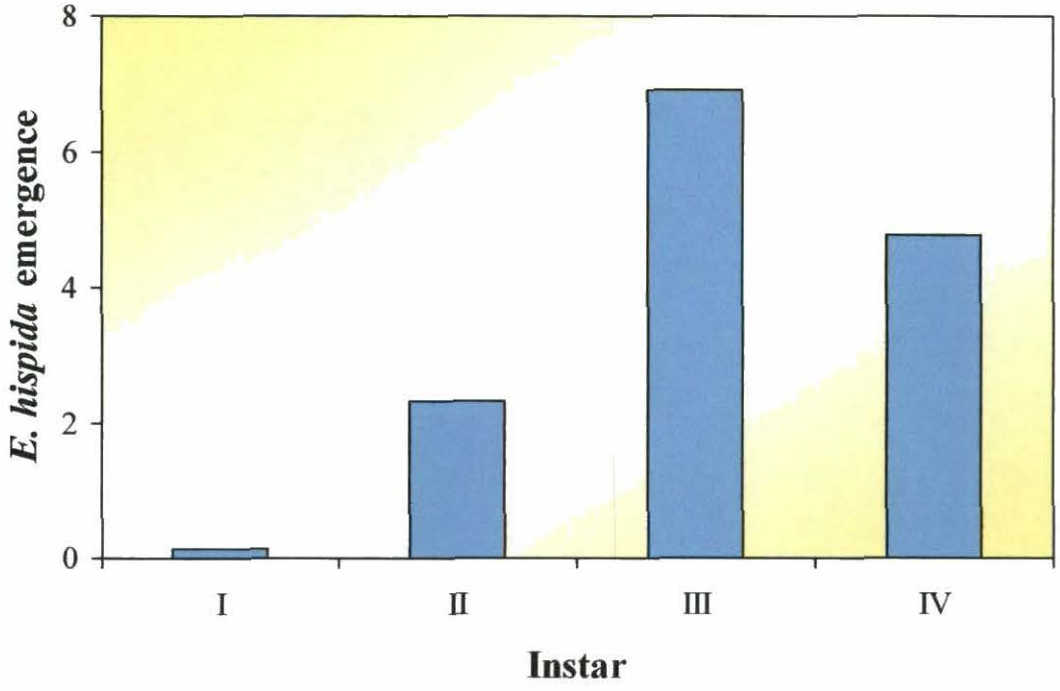


Fig. 1.2.5.1. Emergence of the parasitoid *Encarsia hispida* from four instars of the cassava whitefly *Aleurotrachelus socialis.*

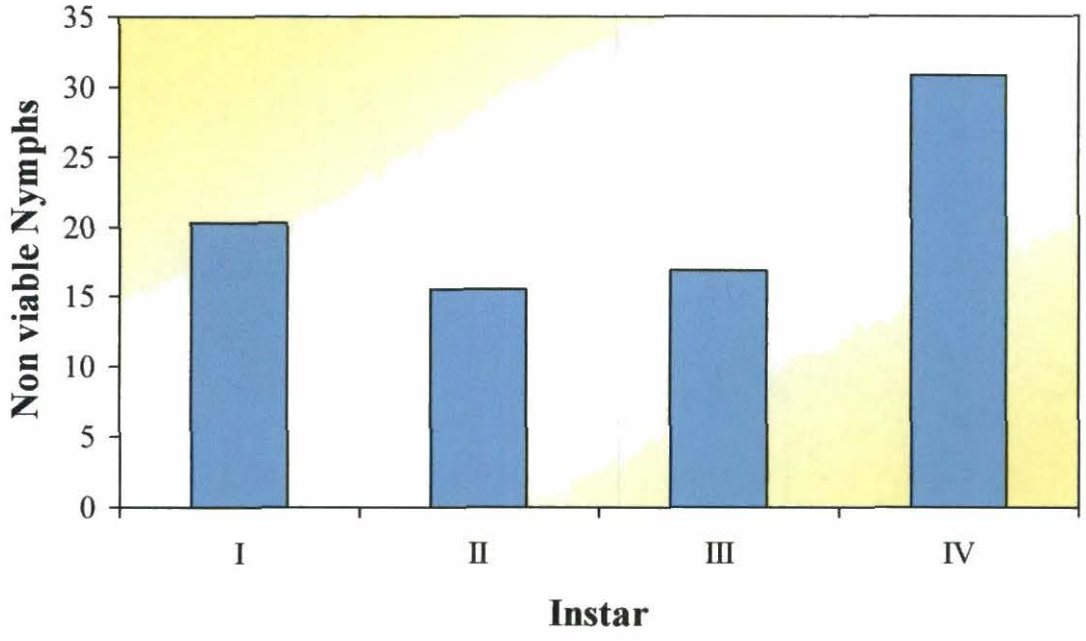


Fig. 1.2.5.2. *Aleurotrachelus socialis* nymphs that were "non viable" in that no *Encarsia hispida* emergal.

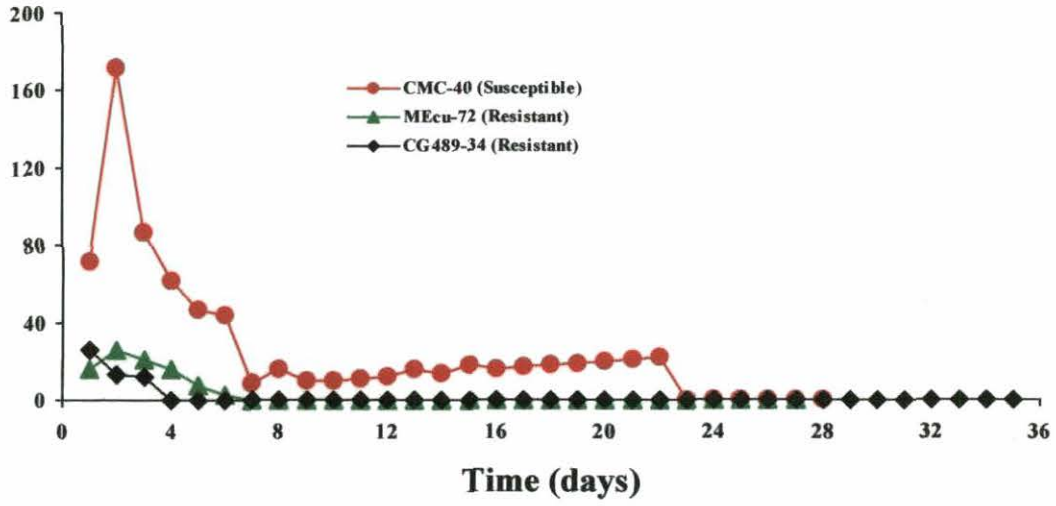


Fig. 1.2.5.3. The effect of four cassava varieties on the survival of the parasitoid *Encarsia hispida* on the cassava whitefly *Aleurotrachelus socialis.*

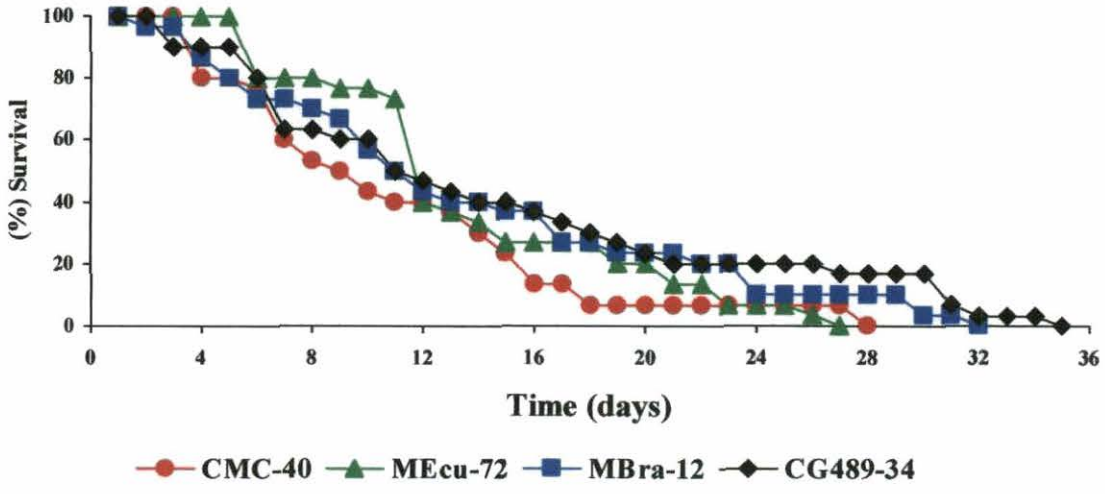


Fig. 1.2.5.4. The effect of three cassava varieties on the emergence of whitefly parasitoid *Encarsia hispida* from parasitized *Aleurotrachelus socialis* pupae.

Identification of molecular markers liked to genes conferring to whitefly in cassava

Introduction

Whitefly (*Aleurotrachelus socialis*) is one of the most serious pests and disease vectors that affect agricultural production in the world. There are almost 1200 species with a wide range of hosts like legumes, fruit trees and ornamentals where this insect causes major economic losses. In cassava (*Manihot esculenta* Crantz), whitefly causes between 70 to 80 percent of economical losses. The principal symptoms in the plant are: total chlorosis of the leaves, curling of the apical leaves; yellowing and drying of the basal leaves; and stoppage of the plant's development.

The adult insects are found preferentially in the apical zones of the plant where they extract large quantities of the sap from the conductive vessels, causing a considerable damage by loss of vigour. This of course leads to reduced yield. The honeydew which they excrete as a result of the copious sap intake serves as a substrate for sooty mold fungi, which can also damage hosts by preventing photosynthesis.

The main objective of the mapping component of the project is to assist cassava breeders, entomologists and virologists in their work on one of the most important constraint to cassava production. A primary target for the work is the tagging of gene(s) conferring resistance to whiteflies species. The markers linked to resistance gene (s) will serve as tools for an efficient, and cost-effective scheme to screen cassava segregating populations and to initiate a marker assisted selection scheme. In the long term, outputs of the project will provide the basis to initiate the positional cloning of the resistance gene (s). Two related projects being conducted will allow us to proceed quickly toward both goals; 1) CIAT has developed a molecular map for cassava consisting of some 120 RFLP markers and more recently of 112 microsatellites and 2) The group of Dr. Rod Wing at the University of Clemson is developing a BAC library form Ecu- 72 the most resistant genotype found in the CIAT cassava germplasm bank. We are using molecular markers Amplified Fragment Length Ploymorphisms (AFLPs) and Simple Sequences Repeat (SSR) to find markers associated to resistance.

Materials and Methods

Different sources of resistance to Whitefly has been reported (CIAT, 1995). The most important sources of the resistance genes are: MBra-12 and Ecu-72. These have been used as parentals in the generation of new genotypes. One of the offsprings, CG489-34 has shown the highest resistance to this pest. Some of the very susceptible genotypes were MCol 2026 and MCol 2246. Different breeding populations have been obtained from crosses between the resistant and susceptible genotypes. The cross CG489-34 X MCol-2026 produced 131 individuals

Field and greenhouse screening.

The mapping population together with the genotypes MBra-12, Ecu-72 CG489-34 and were screened under field and greenhouse conditions. The evaluation under greenhouse condition consisted of three replications. Plants at 40 days with only the first five leaves were used. The genotypes were infested with a whitefly adult population for 72 hours. After that the plants were removed to a space free of white flies and the number of eggs on the first two leaves were counted using the method developed at CIAT (Arias, B 1995). The counting of live nymph on the first two leaves was conducted at ten days after infestation. The number of pupas was counted on the same first two leaves at twenty days after the initial infestation (**Fig. 1.2.6.1**).

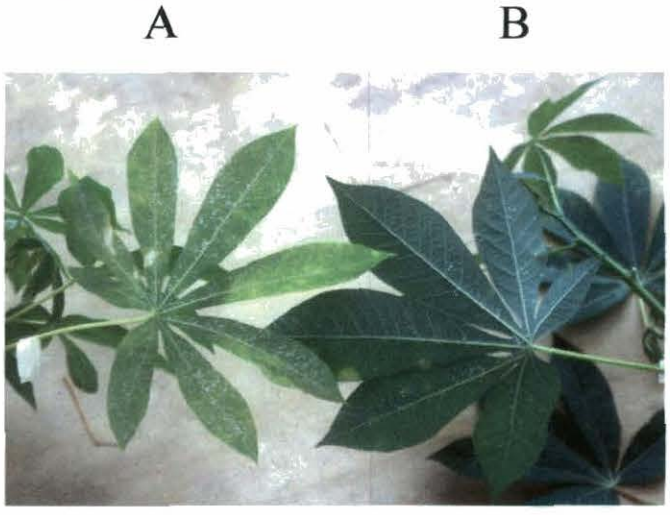


Fig. 1.2.6.1. Cassava leaves infected with second whitefly instar nymphs. A) Susceptible genotype var. Mcol 2026; B) Resistant genotype CG 489-34.

DNA extraction, AFLP and SSR analysis.

Extraction of DNA from all the accessions was according to Dellaporta (1983). AFLP kits from Life Technology was used. Sixty-four different combinations of AFLPs were evaluated using Bulk Segregant Analysis on bulks using from 6-10 resistant and susceptible based on field evaluation. The resistant parentals Ecu-72 and CG489-34 and susceptible parentals MCol 2026 and MCol 2246 were evaluated with all 186 cassava SSR markers developed at CIAT (Mba et al, In preparation).

Results and Discussion

Fifteen combinations resulted polymorphic and provided 22 polymorphic bands present only in the resistant bulk. The resulted were confirmed when the AFLP analysis was conducted one each of the genotype of the bulk (**Fig.1.2.6.2 and 1.2.6.3**). These bands were eluted for sequencing later and will be used to generate Scar markers. The polymorphic combination was then evaluated on the whole progeny.

Out the 186 SSR screened approximately 90 SSRs were polymorphic between the two parental genotypes. So far 40 SSR's have been evaluated with F1 (**Fig. 1.2.6.6**) population. (**Fig. 1.2.6.4, 1.2.6.5, Table 1.2.6.1**).

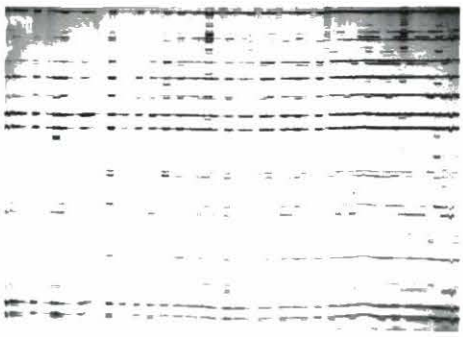


Fig. 1.2.6.2 AFLP AGG-CTC in the F1

Fig. 1.2.6.3 AFLP AAC-CTA in bulks resistant and susceptible

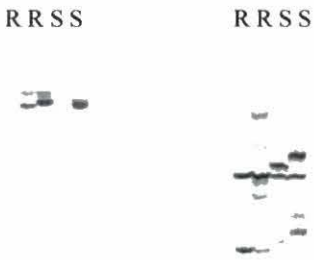


Fig. 1.2.6.4 SSR 215 and 300 in parentals

Fig. 1.2.6.5 SSR 338 in parentals

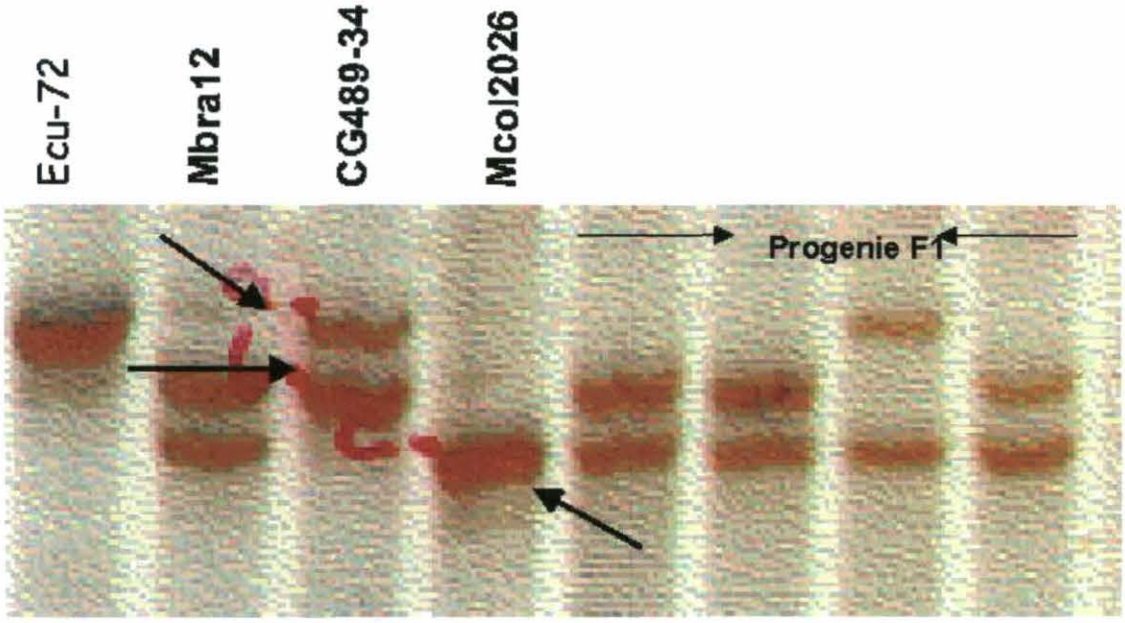


Fig. 1.2.6.6 Silver stained polyacrylamide gel showing unique alleles in both parents of the progeny. Several F1 progenies show the inheritance of these alleles.

Statistical analysis

We have started the preliminary analysis with the 40 SSR screened on the mapping. An analysis of variance was conducted on the data (log transformed) from the counting of eggs, live nymphs and emerged pupas (figs. 1.2.6.7, 1.2.6.8 and 1.2.6.9) So far we have not we have not detected any significance association between the SSR. Field and greenhouse data.

Table 1.2.6.1. SSRs polymorphics to offspring CG489-34 x Mcol 2026.

SSR #	SSR Primer	Size of band	Alignment Temp. °C		SSR #	SSR Primer	Size of band	Alignment Temp. °C
SSRY1	11'	197	45		SSRY89	416	120	55
SSRY2	22'	225	55		SSRY93	473	289	55
SSRY3	1	247	45		SSRY94	S478	268	55
SSRY4	2	287	45		SSRY96	486	149	55
SSRY5	S10	173	55		SSRY97	S490	194	55
SSRY7	13	250	45		SSRY98	491	209	55
SSRY11	24	265	55		SSRY99	494	192	55
SSRY16	43	218	55		SSRY100	498	210	55
SSRY20	67	143	55		SSRY101	500	213	55
SSRY23	82	247	45		SSRY105	506	225	55
SSRY26	103	121	55		SSRY107	508	120	45
SSRY27	108	277	50		SSRY108	511	203	55
SSRY28	118	180	55		SSRY109	521	125	55
SSRY30	120	220	50		SSRY112	542	117	55
SSRY34	132	279	55		SSRY113	2(F)	187	45
SSRY38	153	122	55		SSRY117	7(F)	142	55
SSRY39	155	293	50		SSRY119	f11	155	55
SSRY44	169	194	50		SSRY120	12(F)	139	55
SSRY45	170	228	50		SSRY122	f14	273	45
SSRY48	181	178	50		SSRY123	16(R)	136	55
SSRY49	184	300	50		SSRY130	30(R)	223	55
SSRY51	198	298	50		SSRY135	f34(C)	253	55
SSRY52	200	266	55		SSRY143	62(F)	153	55
SSRY54	203	151	55		SSRY145	GA-12	143	45
SSRY57	215	293	55		SSRY146	GA-13	139	45
SSRY60	254	137	55		SSRY148	GA-21	114	55
SSRY64	275	194	55		SSRY149	GA-57	500	45
SSRY65	286	299	55		SSRY151	GA-126	182	55
SSRY68	300	287	55		SSRY152	GA-127	233	45
SSRY69	313	239	55		SSRY153	GA-131	117	45
SSRY70	324	249	55		SSRY161	68	220	55
SSRY71	332	217	55		SSRY164	127	187	55
SSRY72	338	141	55		SSRY168	268	277	55
SSRY74	352	114	55		SSRY169	270	100	55
SSRY79	373	210	55		SSRY170	277	299	55
SSRY80	377	299	55		SSRY171	283	291	55
SSRY82	381	211	55		SSRY175	S410	136	55
SSRY84	397	203	55		SSRY177	436	268	55
SSRY87	406	102	55		SSRY179	449	226	55
					SSRY185	64(F)	243	50

Fig. 1.2.6.7 Counting of whitefly's eggs
Offspring CG489-34XMCOL2026

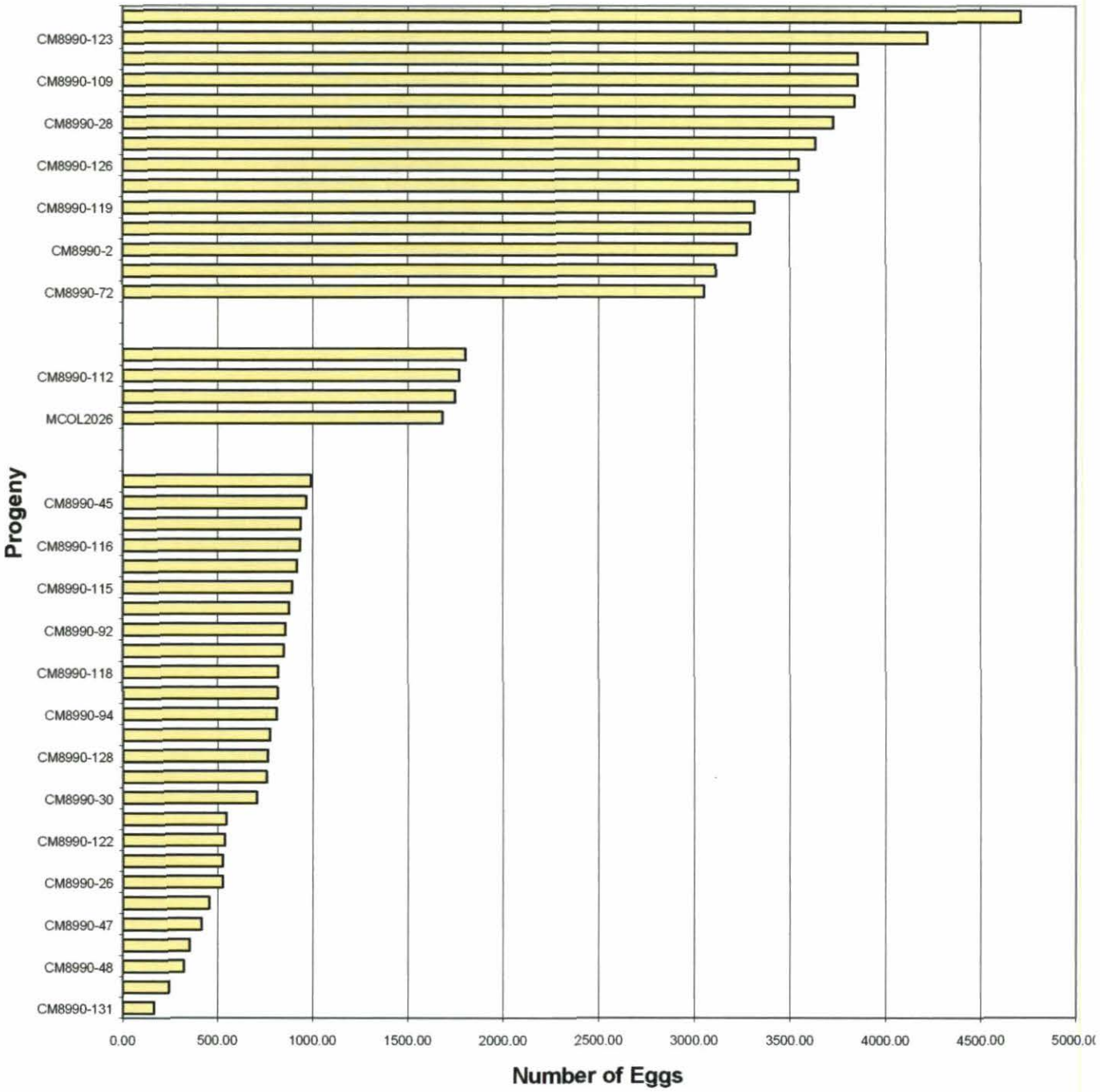


Fig. 1.2.6.8 Counting of whitefly's nymphs offspring CG489-34XMCOL2026

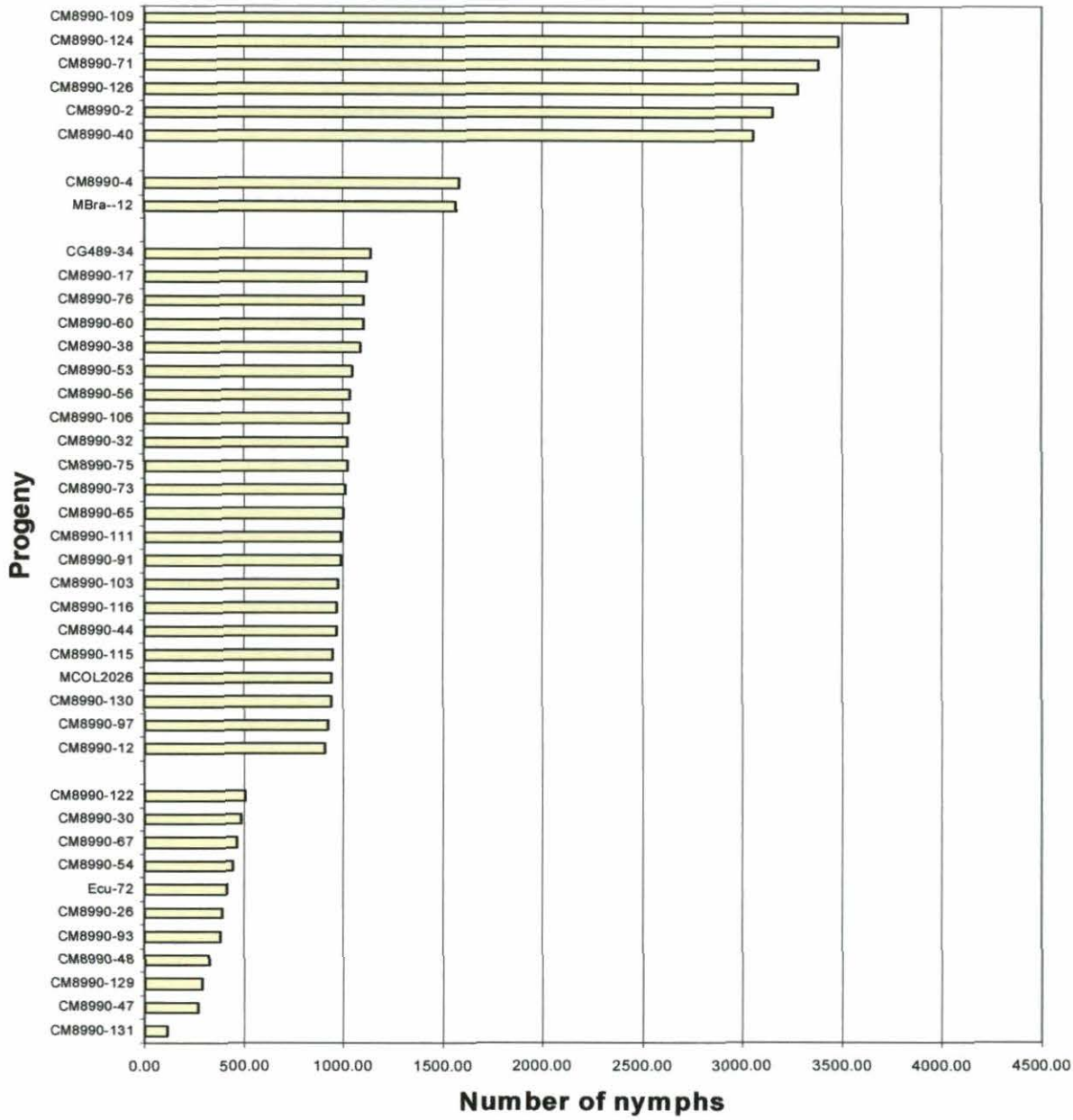
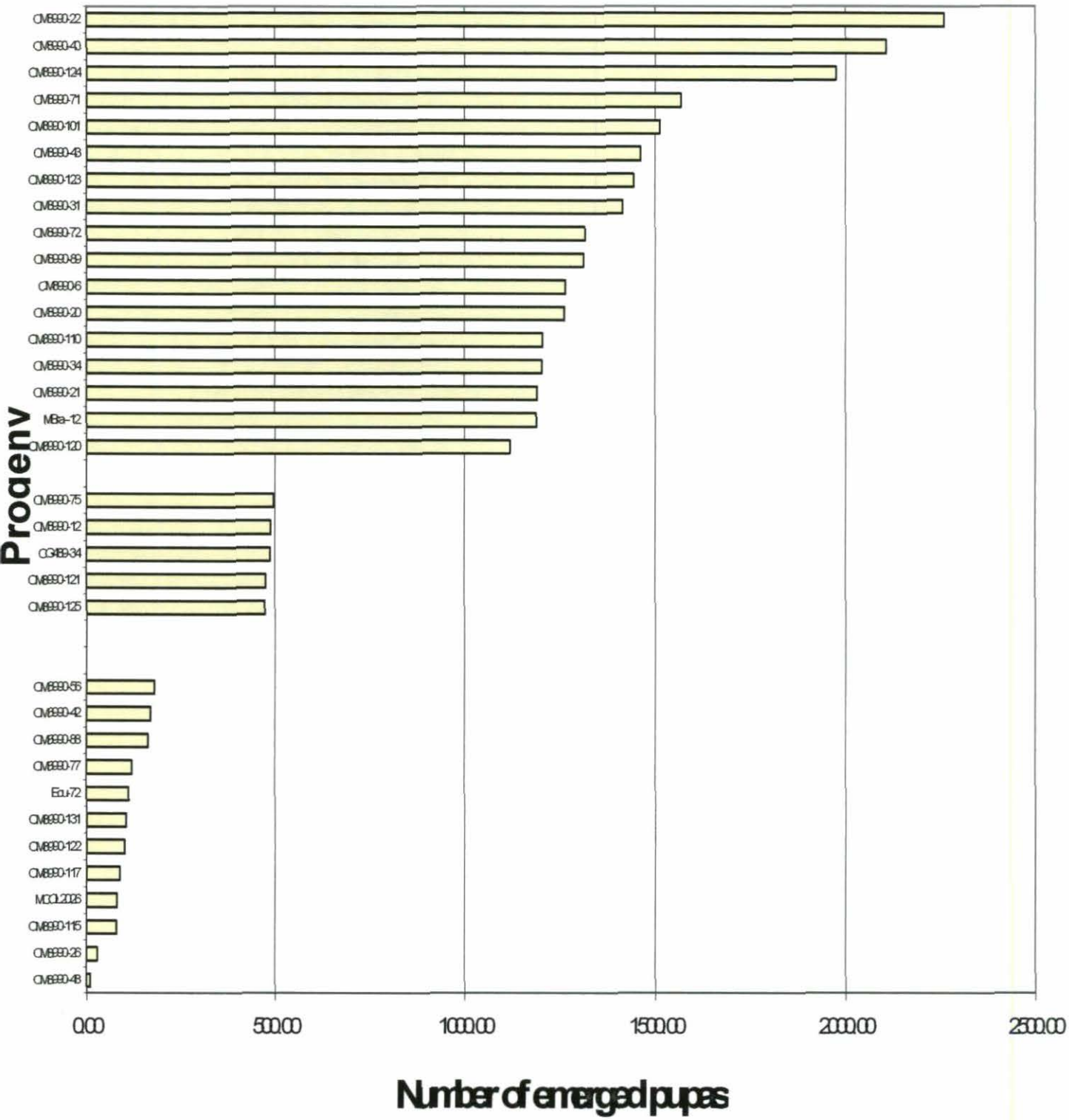


Fig 1.26.9 Counting of whitefly's emerged pupas offspring
CG489-34xMcd2026



On going activities

Using AFLPs, we found bands present only the resistant bulk. These bands will be sequenced to generate a Scar for screening the whole population.

We are pursuing the screening of the remaining 60% of the polymorphic SSR. We have also initiated another cross using Ecu-72 as the resistant parent and MCol 2246 as the susceptible parent will be made. MCol 2246 has such good attributes as tolerance to other pests like mites and thrips, its flowering is good but is very susceptible to white fly. We expect that by using Ecu-72 directly as a parental donor we will be able to identify more marked difference in the reaction to whitefly damage.

References

Arias B. 1995. Estudio sobre el comportamiento de la "Mosca Blanca" *Aleurotrachelus socialis* Bondar (homoptera: Aleyrodidae) en diferentes genotipos de Yuca, *Manihot esculenta* Crantz. Tesis.

Brown, J. K., D. R. Frolich, and R. C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex. Annu. Rev. Entomol. 40: 511-534.

Butler, G. D., Jr. and T. J. Henneberry. 1994. *Bemisia* and *Trialeurodes* (Hemiptera: *aleyrodidae*), pp.325-352. In G.A. Matthews and J. P. Turmstall, Insect pests of cotton. CAB, Wallingford, U.K.

CIAT, 1995. Cassava program annual report. 54p.

Fregene, M., F. Angel, R. Gomez, F. Rodriguez, P. Chavarriaga, W. Roca, J. Tohme, M.

Bonierbale. 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). Thor. Appl. Genet. 95: 431-441.

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