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/MADR 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 MEcu 72 has consistently express high levels of resistance. The variety Nataima-31 is the progeny of a cross between MEcu 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 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

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).

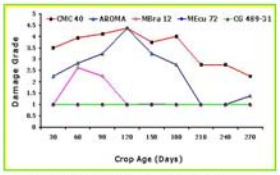

Fig. 4. Whitefly *A. socialis* populations on five cassava genotypes

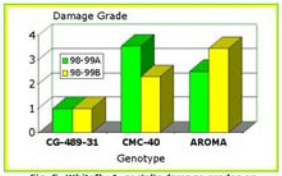

Fig. 5. Whitefly *A. socialis* damage grades on Nataima-31 (R), CMC-40 (S) and Aroma (Farmers Variety)

Additional studies have shown that Nataima-31, as well as its resistant female parent, MEcu 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	291
CMC 40	57	75.2	A	104
CG489-23	54	69.7	AB	119
MCol 1505	70	59.3	B	148
MEcu 72	75	40.4	B	141
CG 489-34	102	20.5	C	134
CG 489-31	77	19.0	C	149

[1] Values followed by the same letter are not significantly different (Duncan alfa = 0.005).

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 1000 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 008, 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 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.

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RECENT ADVANCES IN HOST PLANT RESISTANCE TO WHITEFLIES IN CASSAVA

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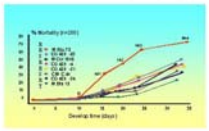
INTRODUCTION

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 (R), 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.

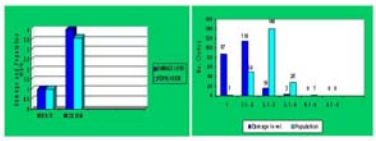


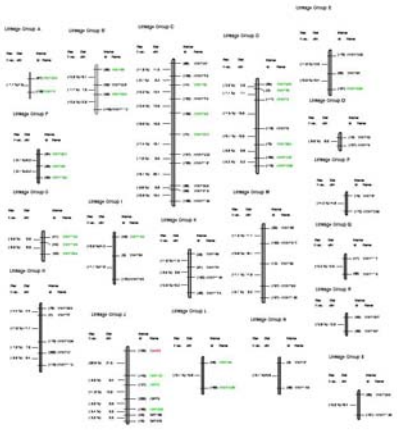
Fig. 3. Cassava damage and whitefly population ratings due to *A. socialis* feeding on parental genotypes MEcu-72, MCol-2246 and clones from the family CM 8996 at CORPOICA, Nataima (Tolima, Colombia).

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.

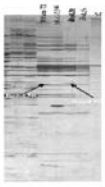
Fig. 4: Preliminary Cassava framework Map of MEcu-72 for Resistance to White Fly, consisting of SSRs and a RGA (Contig39) (Lod = 25 and theta = 25)



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, show the polymorphic band # 50 unique in the resistants.



ASSOCIATION BETWEEN MOLECULAR MARKERS AND RESISTANCE

The molecular data are being analyzed using QTL packages (QTL cartographer Qgene) 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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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 afected 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 reach cassava cultivar MCol 2063 "Secundina," well known by its susceptibility to the whiteflies *Aleurotrachelus socialis* and *B. tuberculata* was selected as the final host.

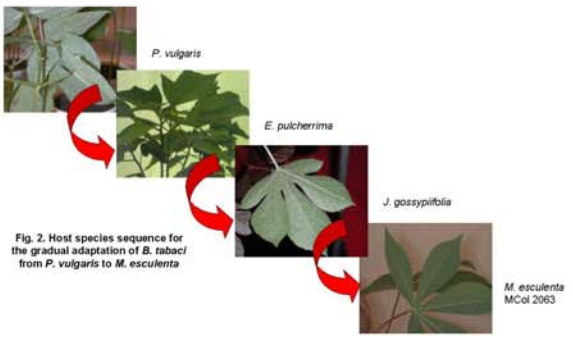

Fig. 2. Host species sequence for the gradual adaptation of *B. tabaci* from *P. vulgaris* to *M. esculenta*

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, forty 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).

Average Parameter	Host of Origin		
	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< 0.0001, 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).

Parameter	Host of Origin		
	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.[a] χ^2, 2 df, 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 (individual/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 8.3% 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).

Parameter	Host of Origin		
	J. gossypiifolia	*E. pulcherrima*	*P. vulgaris*
Ro	8.63	11.6	1.82
T	44.76	56.03	51.3
r_m	0.048	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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Continued