OUTPUT 10
Breeding for insect and other arthropods resistance and development of alternative methods for their control

Activity 10.1. Evaluation of cassava germplasm for resistance to whiteflies (Aleurotrachelus socialis) during 2003-2004.

Rationale

Stable HPR (Host Plant Resistance) in cassava offers a practical, low-cost, long-term solution for reducing whitefly populations below economic damage levels. Whiteflies are major pests of many cassava growing regions of the Neotropics where 11 species are reported. As direct-feeding pests or as virus vectors, whiteflies can reduce cassava yield considerably. In the Americas two species cause yield losses; *Aleurotrachelus socialis* predominates in northern South America (Colombia, Venezuela and Ecuador), reducing cassava yields by 5 to 79%, depending on the duration of the attack. In Brazil, especially Northeast Brazil, *Aleurothrixus aepim* is found in high populations and reducing cassava yields by ≈ 40%. Cassava farmers will apply pesticides for whitefly control, often needing 6 to 8 applications to achieve acceptable results. Studies by CIAT with cassava producers in the Cauca Department show that pesticide application is un-economical for the small farmers (1-3 ha) and barely economical for the larger cassava grows.

Host plant resistance to whiteflies is rare in cultivated crops. The large-scale screening or evaluation of an extensive collection of genotypes or selected wild or cultivated species for whitefly resistance has been limited. In recent years, due to the importance and economic damage caused by *Bemisia tabaci* across a wide range of crop species, HPR activities with whiteflies has increased. However, in general a narrow range of germplasm has been evaluated and there are few deliberate germplasm improvement program designed to identify and select resistant parental genotypes to combine with genotypes of high agronomic value and produce quality cultivars with resistance to whiteflies.

The CIAT Cassava Improvement Project (IP-3) in collaboration with the IPDM project (PE-1) is paying special attention to the need to develop high yielding cassava cultivars that contain resistance to whiteflies. The CIAT Cassava Germplasm Bank contains nearly 6000 accessions; 93% of these are landraces (locally selected cultivars), many collected from farmers' fields and national program germplasm collections, in the tropical regions of the world, especially the Neotropics. We have now systematically screened more than 5400 landrace cultivars from this germplasm collection for resistance to *A. socialis*. We have identified numerous resistant genotypes and combined with a comprehensive breeding scheme, whitefly resistance has been incorporated into a commercial hybrid (Figure 10.1).

During March of 2003, CORPOICA (Colombia MADR) officially released the whitefly (*A. socialis*) resistant cassava cultivar, Nataima-31 (CG 489-31; CIAT Breeding Code). Nataima-31 was originally developed to meet the farmers demand in the Tolima Valley region of Colombia, where whitefly populations are consistently high, causing yield reduction (± 35% in local farmers cultivars, and often obligating farmers to apply costly insecticides. Cassava producers in other regions of Colombia have requested Nataima-31; the cultivar has now been distributed to farmers in the Cauca regions as well as the Tolima Valley. Preliminary response from farmers growing Nataima-31, indicate that it has good whitefly resistance and

is out yielding the local cultivars. A CIAT developed rapid propagation scheme to increase vegetative planting material will contribute to a more rapid adaptation and wider distribution of the cultivar.

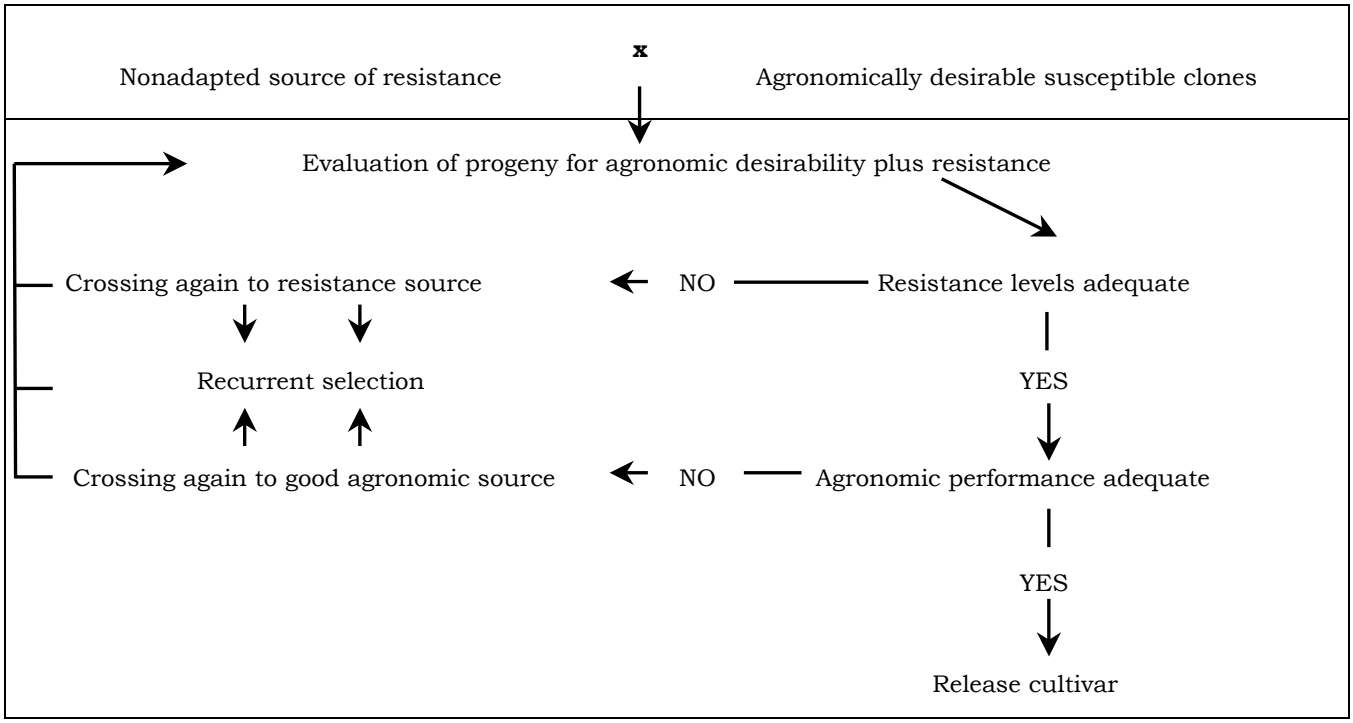

Figure 10.1. Scheme for incorporating whitefly resistance from nonadapted cassava sources into commercial hybrids.

Additional whitefly resistant hybrids are being developed and will be available to farmers in future years. These newer cultivars will be both high yielding with increased whitefly resistance than Nataima-31. Requests for whitefly resistant germplasm, including the commercial hybrid Nataima-31, have come from Ecuador, Brazil and Cuba. In addition, through a collaborative research project with the Natural Resources Institute (NRI) in the UK, whitefly resistant genotypes will be made available to East African countries. Studies in the UK as part of a whitefly IPM research project funded by DFID, indicate that whitefly (*A. socialis*) resistant genotypes originating from neotropical germplasm, also has varying levels of resistance to the whitefly species *Bemisia tabaci*, the vector of African Cassava Mosaic Disease (ACMD).

In recent years, it has been shown that *B. tabaci* also causes direct feeding damage, reducing yields in cassava plantations in Uganda and other East African countries. Whitefly resistant germplasm could be an important component in a pest management program in this region. As part of the strategy during the third phase of the ´Tropical Whitefly IPM Project," the introduction of whitefly resistant germplasm from CIAT, through NRI in the UK, into Uganda is planned.

Field screening of cassava accessions from the CIAT germplasm bank as well as evaluations of progeny from the cassava germplasm improvement project, is part of the long-term strategy of Cassava Project IP-3. In addition we are evaluating the Wild Manihot species as a

source of resistance genes to whiteflies as well as other major cassava pests. We have also developed a mapping population of genotypes suitable for identifying molecular markers for whitefly resistant traits, using MEcu 72 as the resistant parent and MCol 2246 as the susceptible parent (Fam: CM 8996). This NZAID (New Zealand Agency for International Development) funded project has resulted in a preliminary cassava framework map of MEcu 72 for resistance to *A. socialis*. During 2004, a research project has been initiated to determine the biochemical factors involved in whitefly resistance. This USAID (United States Agency for International Development) funded project includes a close collaboration with the USDA Laboratories in Ft Pierce, Florida, USA. Data and observations from these projects are included in the activities of this report.

Specific Objectives

A. Evaluation of the family CM 8996 for a genetic study for whitefly (A. socialis) resistance at CORPOICA, Nataima, Tolima (2003-04).

The screening of cassava germplasm for whitefly (*A. socialis)* resistance is carried out primarily at two sites, the CORPOICA field station "Nataima," at El Espinal, Tolima, and at the CIAT farm in Santander de Quilichao. Both sites are characterized by usually having high whitefly field populations, facilitating germplasm evaluations.

The family CM 8996 was developed from a cross of the *A. socialis* resistant cultivar MEcu 72 and the susceptible MCol 2246; the progeny from this cross (approximately 700 genotypes) are being evaluated for whitefly feeding damage as part of a study to determine the genetics and inheritance of resistance. These progeny have also been evaluated since 2002 for yield, dry matter content, plant type and other agronomic characteristics. Selections are made each year for whitefly resistance combined with the abovementioned qualities. The ultimate goal of this phase of the project is to identify progeny that may eventually be released as a whitefly resistant commercial cultivar. Results from the release for Nataima-31, indicate that hybrids developed for the Tolima Valley agroecosystems, will also perform well in additional regions such as Cauca and the Atlantic Coast of Colombia. In 2002, 718 progeny from the MEcu 72 x MCol 2246 cross were planted and evaluated; 332 genotypes were selected for whitefly resistance and yield and sown in 2003 and 263 were harvested (some genotypes were lost due to poor quality planting material or poor adaptation.

Methodology

Methodologies employed for planting cassava genotypes and whitefly resistance evaluations are similar at the Nataima, Tolima and the Santander de Quilichao, Cauca sites. The Tolima site is 420 m.a.s.l., with an average temperature of 27°C; soils are sandy and annual average rainfall is 1000 to 1300 mm. The 273 genotypes were planted in one replication, seven plants to the row; a control clone, CMC 40 (MCol 1468) is sown every 15 to 20 rows, as an indicator for whitefly population levels and distribution. The five control plants of each row are harvested and data recorded on variables such as total number of roots, number of commercial roots, total and commercial root weight (t/ha), aerial plant weight and harvest index.

Three whitefly (*A. socialis*) population and damage evaluations were performed during the crop cycle. A 1 to 6-whitefly damage and population scale was employed (Table 10.1) where 1 indicates the absence of whiteflies and damage, and 6 indicates severe damage and maximum populations. Populations of adults, nymphs, pupae and eggs are recorded at

different plant levels (top, mid and bottom third). Leaf damage symptoms can show chloratic mottling, leaf curling, reduction in leaf area and the presence of sooty mold on the mid and lower leaves of the plant. A damage rating of 4.0 or above indicates as susceptible clone and usually eliminates it from further screening. A damage rating of 1 or 2 may indicate resistance and clones will be replanted for further evaluation, while a rating of 3.0 signals a low to moderate level of resistance and, depending upon agronomic qualities, such as yield, may warrant continued evaluation.

Table 10.1. 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

Whitefly populations and subsequent damage were low throughout the crop cycle at Nataima, Tolima. All clones had whitefly populations feeding on them but plant damage levels remained low (Figure 10.2). One hundred thirty-two of the 273 (48.3%) clones had a whitefly population rating of 1.1 to 2.0 and 138 (50.5%) clones had a population level of 2.1 to 3.0 (intermediate level). Few cultivars showed leaf damage symptoms; 230 of the 273 clones, (84.2%) had a damage rating of 1.0, that is no damage symptoms; 40 clones had a very low rating of 1.1 to 2.0, expressed by a slight flaccidity of the young leaves. Only 3 clones had a significant damage rating of 3.0, twisting and curling of young leaves (a detailed list of all recorded field evaluations is available).

As mentioned in the methodology section, the susceptible clone, CMC 40, is planted as a whitefly population "indicator" clone and spaced every 15 to 20 rows throughout the field. *A. socialis* populations and plant damage was considerably higher on this susceptible clone. Of the 66 rows planted to CMC 40, only 2 rows (3%) had a low population/damage rating (1.1-2.0) (Figure 10.3). Twenty-three rows (34.8%) had a population level of 2.1-3.0 and 41 rows, 62.1% had populations above the 3.2-5.0 range. Corresponding whitefly damage levels were moderate to high, 92% of the CMC 40 rows had a damage level above 4.0. In addition it can be noted that the whitefly population was well distributed throughout the field.

These observations and results indicate that the levels of *A. socialis* resistance in the 273 progeny from the MEcu 72 x MCol 2246 cross that have been selected after 3 years of field screening, contain moderate to high levels of whitefly resistance. Low whitefly populations

and damage observed on the Fam. CM 8996, were mostly due to the inherent whitefly resistance and not to overall low whitefly field populations. These results are supported by previous year's evaluations, and indicate the value of planting susceptible control clones throughout an evaluation field.

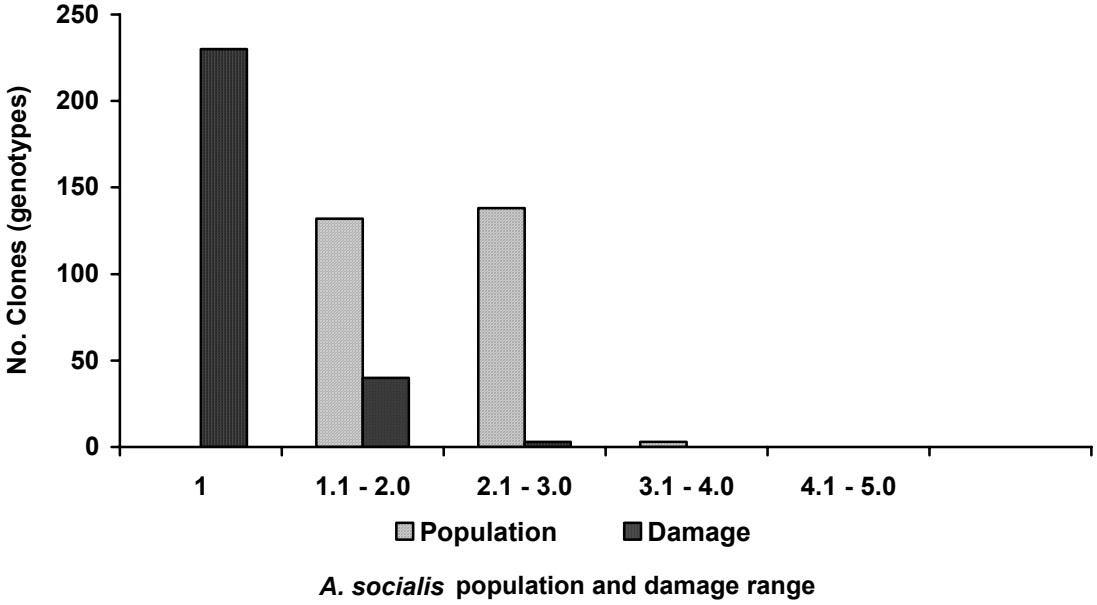

Figure 10.2. Whitefly (*A. socialis*) population and damage rating of genotypes from the cassava family CM 8996, evaluated at CORPOICSA, Nataima (Tolima) during the 2003-04 crop cycle.

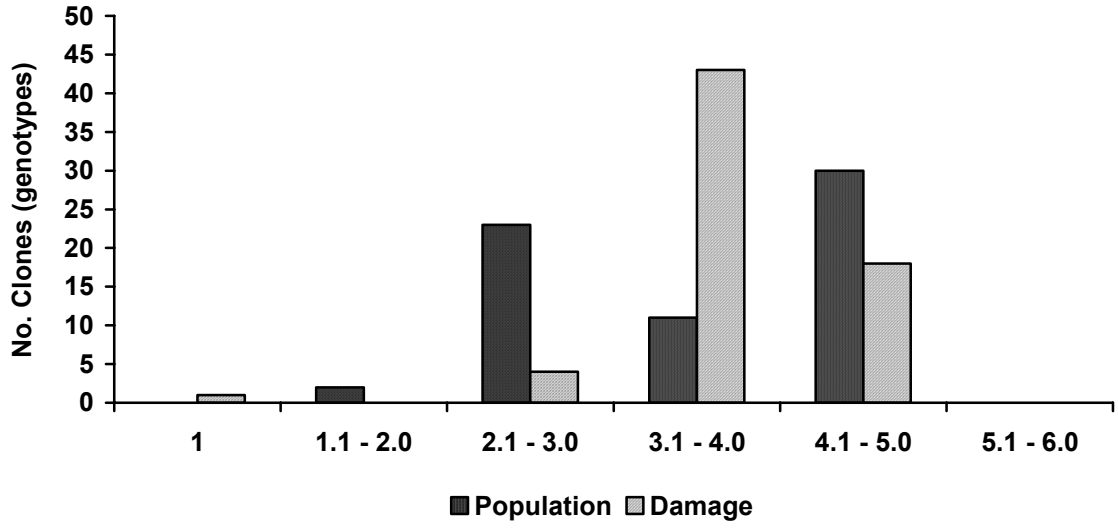

Figure 10.3. Whitefly (*A. socialis*) populations and damage ratings on the susceptible cassava cultivar CMC 40 (MCol 1468) planted with cassava family CM 8996 at CORPOICA, Nataima (Tolima) during 2003-04.

Root yields during this crop cycle were considerably lower than in previous years (Figure 10.4). This reduction in yield is primarily due to poor plant stand; numerous plants were lost due to inadequate field preparation and growing conditions. Planting was done immediately following the previous crop harvest in the same field due to inaccessibility of sufficient land. This contributed to the poor plant stand. Sixty-nine cultivars of the 332 sown were lost during this cropping cycle. One hundred sixty five of the remaining 263 harvested (62.7%) had root yields of 0.0 to 15 t/ha and 35 clones (13.3%) resulted in root yields of 15.1 to 20 t/ha. Fifty-two clones (20.0%) produced yields of 20 to 30 t/ha and 8 (3.0%) had yields of 30-40 t/ha. There were some notable exceptions, 3 cultivars produced yields above 40 t/ha and one had a yield equivalent to 85.7 t/ha (Figure 10.4).

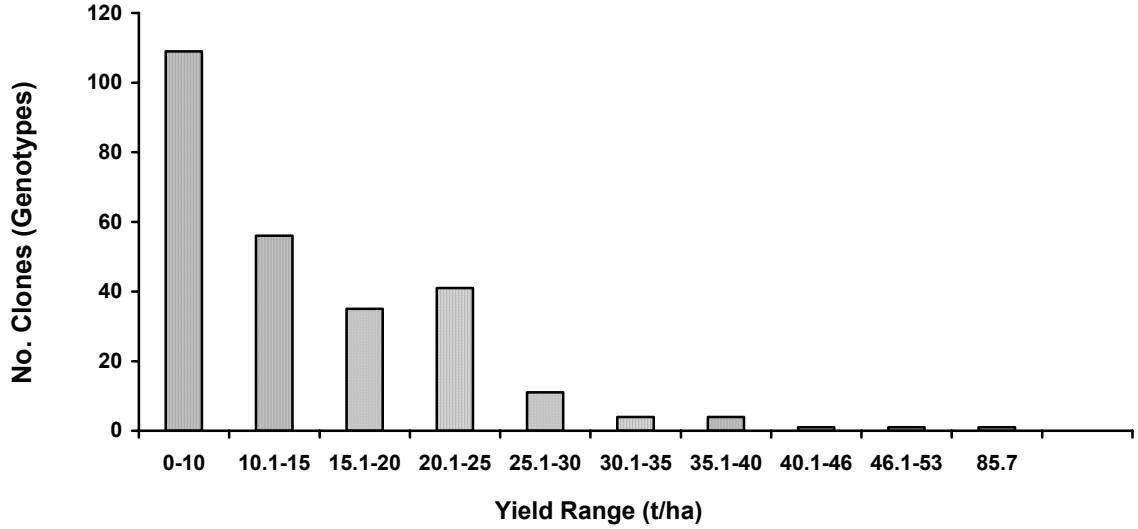

Figure 10.4. Yield distribution for cassava family CM 8996, under whitefly (*A. socialis*) population pressure at CORPOICA, Nataima (Tolima) during the 2003-04 crop cycle.

In general, root yields for this family have been moderate to high. This observation is supported with the results of the planting of the same cultivars at Santander de Quilichao (see following Activity). Data on yield, harvest index and root dry matter is presented in Table 10.2. It should be noted that farmers' yields with local cultivars in this region are generally low (an average of about 9-10 t/ha). Many of the clones evaluated have a higher yield potential than the local traditional varieties. The progeny in Fam. CM 8996 are all low HCN or "sweet" varieties and thereby acceptable in both the fresh consumption or the industrial (e.g. animal feed) market.

Table 10.2. Yield parameters (harvest index and % dry matter) of 263 clones from Family CM 8996 subject to whitefly (*A. socialis*) feeding pressure at CORPOICA, Nataima (Tolima) during 2003-04 crop cycle.

Yield t/ha			Harvest Index			% Dry Matter		
Range	No. Clones	%	Range	No. Clones	%	Range	No. Clones	%
0.0-15.0	165	62.7	0.3-0.4	36	13.7	0.0 - 20	11	4.1
15.1-20.0	35	13.3	0.41-0.49	90	34.2	20.1 - 29	124	47.1
20.1-30.0	52	20.0	0.5-0.6	92	35.0	29.1 - 32	100	38.0
30.1-40.0	8	3.0	0.61-0.70	31	11.8	32.1-35.4	27	10.3
40.1-85.7	3	1.0	0.71-0.86	14	5.3	35.5-43.6	1	0.3

B. Evaluation of the cassava family CM 8996 for a genetic study of whitefly (A. socialis) resistance and genotype yield at CIAT, Santander de Quilichao (Cauca) (2003-04).

Since 2001 the CIAT farm at Santander de Quilichao has been used as a site to evaluate the progeny from the MEcu 72 x MCol 2246 cross (Fam. CM 8996). This collaborative project involving three CIAT projects, Biotechnology (SB2), Cassava Improvement (IP-3) and IPDM (PE-1) proposes to determine the genetic inheritance of whitefly (*A. socialis*) resistance in cassava. The results and progress in this study is reported as a separate Activity (see Annual Report SB-2, 2004). Selected progeny from Family CM 8996 are planted separately and evaluated for additional agronomic characteristics, especially yield, dry matter content and culinary qualities, as well as whitefly resistance. Whitefly populations at Santander de Quilichao are usually high enough to exert sufficient selection pressure for adequate resistance screening. Cassava plantings at Santander de Quilichao, however, are sometimes complicated by the occurrence of Cassava Frogskin Disease (CFSD). CFSD damages cassava roots, reducing yield and quality.

Methodology

The methodologies used in germplasm evaluation at Santander de Quilichao are similar to those described for CORPOICA, Nataima. This site differs from Nataima, Tolima in that it is at a higher altitude, 1100 m.a.s.l. and soils are acid (calcium is applied at a rate of 30 grams per plant). In April 2003, 324 clones from the CM 8996 family were sown in one replication with 8 plants in each row. The susceptible control or "indicator" cultivar (CMC 40) was planted every 15 to 20 rows throughout the experimental plot. At harvest the middle 6 plants of the 8-plant row was harvested and data on root yield, dry matter content, harvest index, etc. as described in the previous activity was recorded.

During the crop cycle, three whitefly population and damage evaluations were carried out; one in each of the months of July, August and September, 2003, when whitefly populations are high. The damage and populations scale described in Table 10.3 of the previous activity was employed. Evaluations are done on the six central plants of each clone (row). Data was analyzed by combining the recording from the three evaluations. The highest damage rating is considered the most important and most indicative of resistance or susceptibility levels.

Results

Whitefly (*A. socialis*), at varying population levels was observed on all of the cultivars evaluated (Figure 10.5). However, 127 (37.5%) of the 339 clones evaluated presented no damage symptoms. Whitefly populations were low, between 1.1 and 2.5 (1 to 6 population scale), on 255 or 75.2% of the cultivars. One hundred cultivars (29.5%) showed some apical leaf curling damage symptoms and 74 (21.8%) cultivars had a damage rating of 3 to 3.5. Forty cultivars resulted in severe damage symptoms, a rating of 4.0 to 6.0 on the damage scale. This latter group can be classified as very susceptible to *A. socialis*.

Whitefly (*A. socialis*) populations on the susceptible control CMC 40 were high and damage symptoms severe (Table 10.3). Damage ratings on the upper, middle and lower leaves ranged from a low of 3.0 to a high of 4.5 on the damage scale. This data also indicates that the high whitefly populations were relatively uniform throughout the experimental plot. These results also lead us to conclude that the lower *A. socialis* populations and damage recorded on the cultivars in family CM 8996, are due to the higher levels of whitefly resistance contained in many of these cultivars, as can be observed in Photo 1.

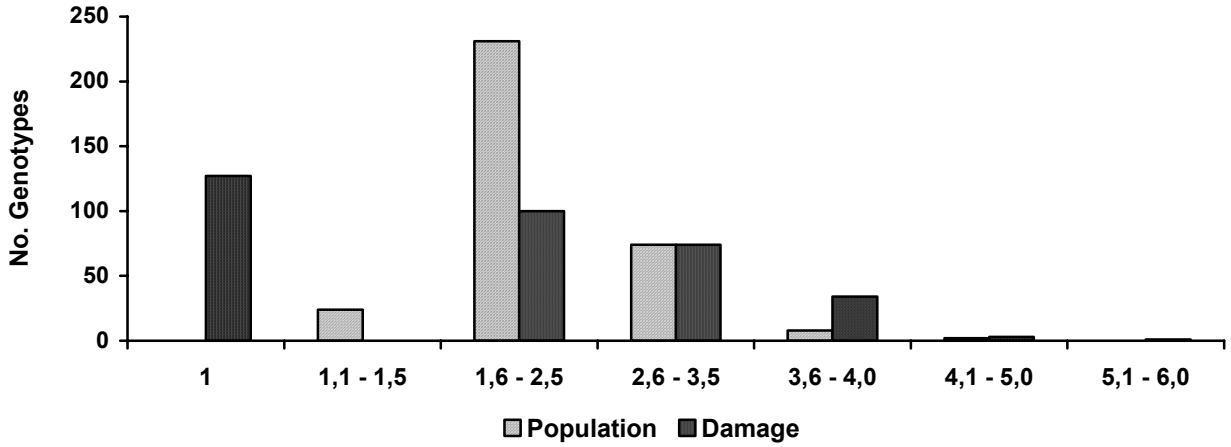

Figure 10.5. Whitefly (*A. socialis*) population and damage rating of genotypes from the cassava family CM 8896, evaluated at Santander de Quilichao (Cauca) during the 2003-04 crop cycle.

Table 10.3. Whitefly (*A. socialis*) populations and damage rating on the susceptible cassava cultivar CMC 40 (MCol 1468) planted with cassava family CM 8996 and other resistant and susceptible genotypes (included in the table) at the CIAT farm in Santander de Quilichao (Cauca) during 2003-04 crop cycle.

	Population Rating									
	Apical Leaves			Middle Leaves		Lower Leaves		Damage Rating		
Genotype	Adult	Egg	Nymph	Pupae	Nymph	Pupae	Pupae	Superior	Middle	Lower
CMC 40	2.0	4.0	4.0	1.0	3.5	2.5	3.0	4.0	4.0	4.0
CMC 40	2.0	4.0	4.0	1.0	3.5	3.5	3.0	4.0	4.0	4.0
CMC 40	2.0	4.0	4.5	1.0	3.5	3.5	3.0	3.5	4.0	4.0
CMC 40	3.5	4.0	4.0	1.0	4.0	4.5	4.5	4.0	3.0	3.0
CMC 40	2.0	4.0	4.0	1.0	4.5	4.5	3.5	3.0	4.0	3.5
CMC 40	3.5	4.0	4.0	1.0	5.0	4.5	4.0	4.0	4.0	3.0
CMC 40	4.0	5.0	4.5	1.0	4.5	4.5	4.0	4.0	4.0	4.0
CMC 40	3.0	4.5	5.0	1.0	4.0	4.0	3.5	4.0	4.5	4.0
CMC 40	3.0	4.0	5.0	1.0	4.5	4.5	3.0	3.5	4.5	3.5
CMC 40	3.0	4.5	4.5	1.0	4.5	4.5	4.0	4.0	4.5	4.0
CMC 40	4.0	5.0	5.0	2.0	4.0	4.0	3.0	4.5	4.0	4.0
CG 489-31	1.0	1.0	1.5	2.0	1.0	3.0	1.0	1.0	2.0	1.0
CG 489-34	2.0	4.5	5.0	1.0	5.0	5.0	2.0	3.5	4.5	2.5
MEcu 72	1.5	1.5	1.0	1.0	4.0	4.0	2.0	1.0	3.5	1.0
MPer 334	1.5	1.5	3.0	1.0	1.0	4.0	1.0	1.0	2.0	1.0
MBra 304	3.0	5.0	6.0	5.0	3.0	6.0	4.0	5.0	5.5	5.0
MCol 2246	2.0	3.5	3.0	1.0	4.0	4.0	4.5	2.0	4.0	4.0
MCol 2643	3.0	4.0	5.0	4.0	1.0	5.0	2.5	3.0	3.5	2.0
MCol 2025	4.0	5.0	5.0	4.0	1.0	5.0	2.5	3.5	5.5	3.0

Additional cassava clones were planted for observation in this trial. CG 489-31 (Nataima-31) continues to show low whitefly population and damage levels (Table 10.3). However CG 489-34, a sister hybrid to CG 489-31 had higher population and damage rating than have previously been recorded. MEcu 72, the resistant female parent in these crosses, also had population and damage ratings slightly higher than normally observed. MPer 334, also a resistant cultivar, maintained low damage ratings while MBra 304, MCol 2643 and MCol 2025 had high *A. socialis* populations and damage ratings. The cultivar MCol 2246, the male parent in the CM 8996 family, as expected supported moderately high populations and damage ratings.

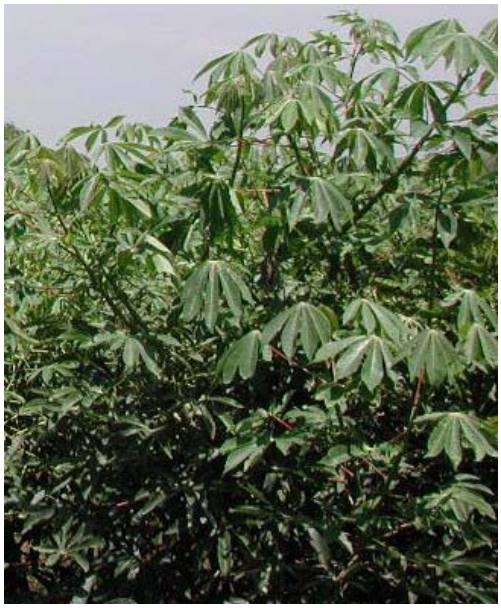

Photo 1. Plants representing the cassava family CM 8996 at Santander de Quilichao (Cauca) during 2003-04 crop cycle: note absence of whitefly (*A. socialis*) damage.

Yields of the 314 cultivars ranged from the very low (0-10 t/ha) to the very high (> 60t/ha) (Figure 10.6). In general yields were good (Photo 2), in spite of the fact that yields were probably adversely affected by the presence of super elongation disease (*Sphaceloma manihoticola*) (Photo 3). Periodic applications of a fungicide, Score, (14cc per 20 l of water) were made in an attempt to try and control the disease. Sixty-five of the 314 cultivars (20.7%) produced root yields between 0.0 and 15 t/ha. Fifty-five cultivars (17.5%) produced root yields between 15.1 and 20 t/ha and 146 (46.5%) yielded between 20.1 and 35.0 t/ha. Forty cultivars produced yields of 35 to 50 t/ha, while 8 cultivars (2.5%) yielded above 50.0 t/ha. The average yield for all cultivars in this trial was 24.2 t/ha and 155 cultivars (48.1%) yielded above this average.

Additional data on root yield, harvest index, dry matter content and culinary quality is presented in Table 10.4. 26.3% (51) of the 194 cultivars evaluated resulted in a dry matter above 30% and of these only 12 cultivars (6.2%) resulted in dry matter content above 32%. This level of dry matter is considered very low. Testing for culinary quality shows that only 15 cultivars (7.7%) presented a grade of 1.0 (very good palatability and boiling quality after 25 minutes). Forty-tree cultivars (22.2%) were graded at level 2.0 (good palatability) while 136 cultivars (70.1%) were classified between 3.0 and 4.0 (poor quality). Root quality may

have been affected by the pressure of super-elongation disease. In previous trials with these same cultivars, culinary quality of the roots, on the average was rated much higher (see Annual Report Project IP-3, 2003).

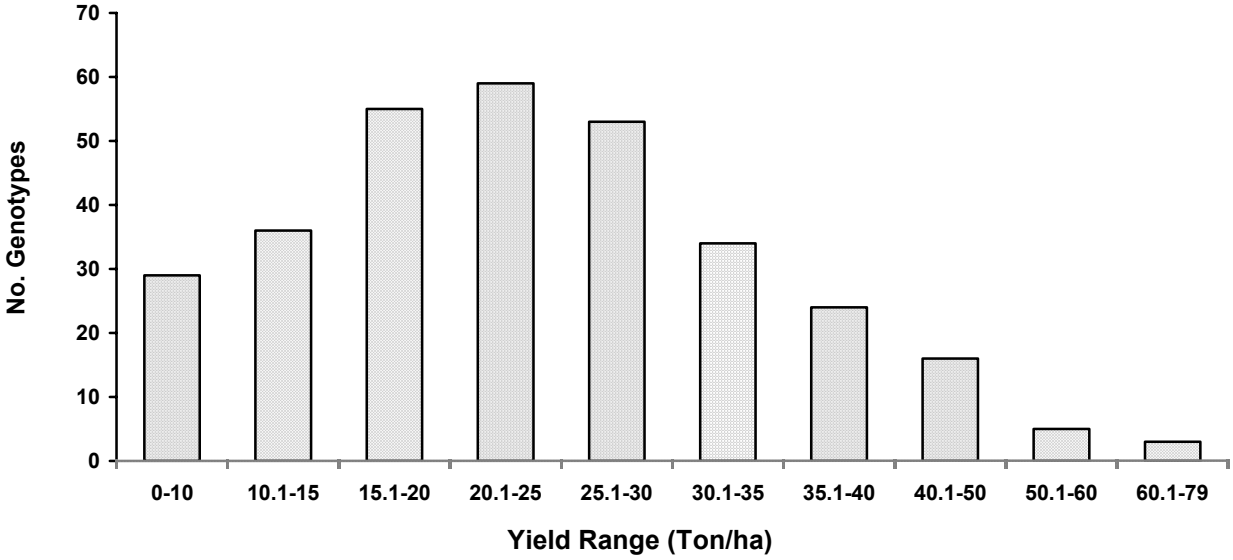

Figure 10.6. Yield distribution for cassava family CM 8996, under whitefly (*A. socialis*) population pressure at CIAT farm, Santander de Quilichao (Cauca) during 2003-04 crop cycle.

Photo 2. Root yield of genotype from cassava family (CM 8996 at Santander de Quilichao (Cauca) during 2003-04 crop cycle: These cassava plants yield well in spite of whitefly (*A socialis*) attack.

Photo 3. Cassava plants from family CM 8995 showing symptoms of super elongation disease (*Sphaceloma manihoticola*) at Santander de Quilichao (Cauca) during 2003-04 crop cycle.

Table 10.4. Yield parameters (t/ha, harvest index, % dry matter and culinary quality) of genotypes from the cassava family CM 8996 under whitefly (*A. socialis*) feeding pressure at Santander de Quilichao (Cauca) during 2003-04 crop cycle.

Yield Ton/ha		Harvest Index		% Dry Matter		Culinary Quality	
Range	No. Clones	Range	No. Clones	Range	No. Clones	Grade	No. Clones
0.0 – 15.0	65	0.0 - 0.39	16	21.0 - 25.0	22	1.0	15
15.1 – 20.0	55	0.4 - 0.49	29	25.1 - 30.0	121	2.0 - 2.5	43
20.1 – 35.0	146	0.5 - 0.59	94	30.1 - 32.0	39	3.0 - 3.5	66
35.1 – 50.0	40	0.6 - 0.69	125	32.1 - 33.4	12	4.0	70
>50.1	8	0.7- 0.8	58	-	-	5.0	0

Contributors: Bernardo Arias, Anthony C. Bellotti.

Collaborators: Gustavo Trujillo, Gerardino Pérez, Carlos Ñañes.

Activity 10.2 Evaluation of whitefly (*Aleurotrachelus socialis*) populations and damage on cassava genotypes from GM and CM families (developed for genome mapping studies for root dry matter) at Santander de Quilichao, 2004.

Rationale
The entomology section of the cassava project operates in close collaboration with the cassava breeders and geneticists and participates in the evaluation of genetic and breeding materials. These trials may be planted at various sites around Colombia and therefore subject to attack from different cassava pests. These evaluations take advantage of the naturally occurring pest attacks in cassava fields in different agro-ecosystems and provide important data and information related to pest susceptibility or resistance available in these genetic materials. At present, the major pest attack in cassava fields in various agro ecosystems is the whitefly, *Aleurotrachelus socialis*.

Objective
Evaluate 455 cassava genotypes from CG and CM families, developed as part of a genomic study for mapping root dry matter inheritance.

Methodology
The CIAT farm at Santander de Quilichao is located 1100 m.a.s.l., with an average temperature of 26-27°C and characterized by low fertility, acid soils. Four hundred fifty five cassava genotypes were evaluated for whitefly (*A. socialis*) populations and damage during the 2003-04 crop cycle. The genotypes from CG and CM families were planted in three main plots; each plot consisted of several blocks of plants. The whitefly evaluations were carried out in two of these plots. The first plot consisted of 18 blocks and the second plot of 10 blocks; each block consisted of 10 to 40 rows for a total of 807 rows of five plants each. Whitefly evaluations were carried out between, July 21 to 29 2004, when *A. socialis* populations are very high and plant damage is easily discernable. Evaluations were done using the 1 to 6 populations and damage rating scale described in Table 10.1 of Activity 10.1 in this report.

Data from the evaluations was organized and analyzed using an Excel program. Repetitions of each of the 455 genotypes were grouped and the highest values were recorded. Genotypes were listed by the lowest to the highest damage ratings (list of all genotypes and damage and population ratings is available), facilitating the selection of the best (most resistant) genotypes available. Genotypes receiving a damage rating between, 1 to 2.5 are considered most "promising" (a "resistant" rating would require several field evaluations). Evaluations of 4.0 to 6.0 indicate susceptible genotypes.

Plant damage due to thrips (*Frankliniella williamsi*) and mites (*Mononychellus tanajoa*) was observed and recorded on several of the genotypes.

Results
Whitefly *(A. socialis*) populations were high permitting good selection pressure on the genotypes. 94.7% (431 of 455) of the genotypes had a rating between 4.0 to 6.0 on the population scale, indicating a population of 500 to 4000 per leaf (Figure 10.7). Damage ratings were similarly high; 91.9% (418 of 455) of the genotypes had a damage evaluation between 4.0 and 6.0 (Figure 10.7). This indicates severe curling and twisting of apical leaves, leaf yellowing, the presence of "sooty mold," leaf necrosis and defoliation. Only seven clones

(1.5%) had a damage rating between 1.1 to 2.5. These genotypes should be further evaluated in subsequent crop cycles to verify if this is actual resistance and not escapes. These clones are GM 315-57, GM 312-10, GM 311-14, GM 312-65, GM 306-1, GM 312-9 and GM 313-15. If these genotypes are also high yielding and with high dry matter, they could should be further evaluated and may be of value in a germplasm development program for the Cauca and Valle del Cauca departments.

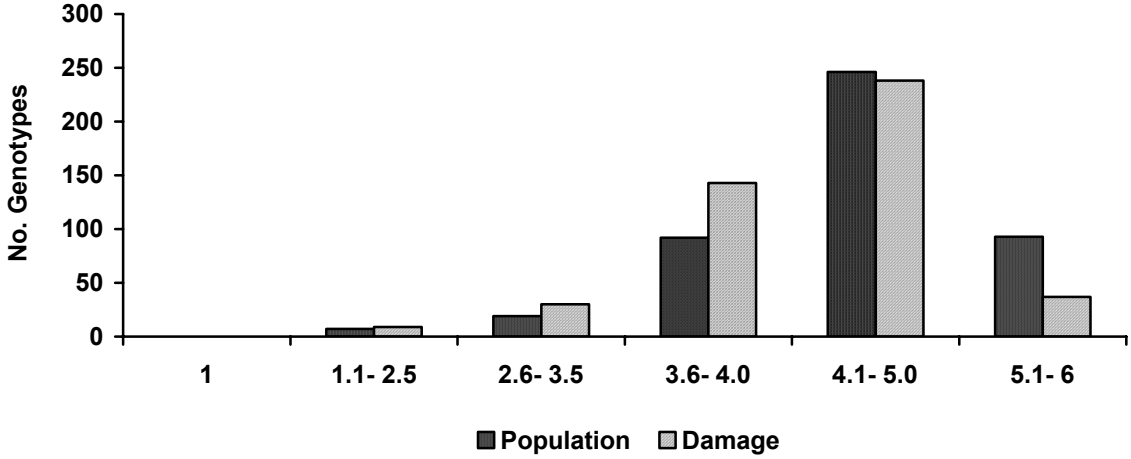

Figure 10.7. Whitefly (*Aleurotrachelus socialis*) population ratings on genotypes of cassava families GM and CM, developed for genetic mapping studies on root dry matter content at Santander de Quilichao (Cauca, Colombia), CIAT, 2003-04.

Contributors: Bernardo Arias, Anthony C. Bellotti.

Collaborators: Gerardino Pérez.

Activity 10.3 Intrinsic rate of increase of Biotype "B"* Bemisia tabaci *on two African cassava genotypes MNg 2 and MNg 11.

Bemisia tabaci (Homoptera: Aleyrodidae) as the vector of Africa Cassava Mosaic Disease (CMD), caused by a geminivirus (CMGs) (Legg et al, 2002), causes yield loss reported as ranging from 12-25% of the cassava crop in Africa (Thresh et al, 1997). It has been speculated that the absence of CMD in the Americas is related to the inability of *B. tabaci* to colonize cassava in the Neotropics (Costa and Russell, 1975). However, in the early 1990's a new biotype "B" of *B. tabaci* was collected feeding on cassava in the Americas. Biotype "B" is considered by some authors and taxonomists to be a separate species, *Bemisia argentifolii* (Bellows and Perring) (Bellows et al, 1994). *B. tabaci* "B" is now viewed as a possible threat to vector CMD (or other geminiviruses) in the Americas if the disease were inadvertently introduced; traditional landrace cassava varieties cultivated in the Americas are considered highly susceptible to CMD (Bellotti and Arias, 2001). In addition, cassava damage evaluation caused by the increase in a *B. tabaci* population in East and Central Africa indicate yield losses above 50% due to direct feeding by whiteflies, even on varieties known to be resistant to CMD (CIAT, 2003-2004). Those reports thereby indicate that cassava varieties that contain resistance only to CMD may not be adequate to resist yield losses due to the direct feeding damage caused by *B. tabaci.*

The search for resistance (HPR) to the whitefly, *B. tabaci*, in cassava genotypes offers an alternative and additional low cost and stable option for maintaining lower populations of the whitefly and reducing crop losses. Research experiments were designed to measure and compare the development of "B" biotype of *B. tabaci* populations found in Colombia, on two African cassava genotypes, TMS 30572 (MNg 2) and TMS 60444 (MNg 11). These genotypes were developed during the 1950 as part of a project to identify germplasm resistant to CMD (CIAT, 2004).

Objective: Determine the intrinsic rate of increase of populations of Biotype "B" of *B. tabaci* on two African cassava genotypes, MNg 2 and MNg 11.

Methodology

1. **Genotypes of Manihot esculenta:** In vitro plantlets (20) of the *M. esculenta* genotypes MNg 2 (TMS 30572) and MNg 11 (TMS 60444) were obtained from the CIAT Biotechnology Project (Agrobiodiversity and Biotechnology SB-2). Plantlets were subsequently planted in plastic bags and pots. Eight, 40 day old plants of each genotype were placed in nylon mesh, wooden framed cages (1m x 1m x 1m).
2. ***Bemisia tabaci:*** The source of *B. tabaci* was obtained from a CIAT colony established on *Jatropha gossypiifolia* (Euphorbiacea). The colony had been established for 15 generations on *J. gossypiifolia* in the previously described cages under growth chamber conditions (25±2°C, 70±5% RH and 12:12 photoperiod). The colony is periodically checked for species purity by RAPD-OCR of adult specimens (CIAT, 1999).
3. **Biological and demographic parameters of *B. tabaci* on MNg 2 and MNg 11.**

Longevity and fecundity: Forty pairs (40 males: 40 females) of recently emerged *B. tabaci* adults were collected from *J. gossypiifolia* using a technique described by Eichelkraut and Cardona (1989). One pair was placed in clip-cages (2.5 mm diameter x 2.0 mm depth) and attached to cassava leaves of MNg 2 and MNg 11 so that whiteflies fed on the leaf undersurface. Every 48 hours, the whiteflies were moved to a different area of the leaf. This procedure was repeated throughout the study until the natural death of the females; males were replaced whenever they perished before their mate. Fecundity was estimated by recording the number of eggs oviposited by each female during the 48 hour periods, while longevity was calculated as the time (days) that the female survived.

Development time, rate of survival and proportion of females: Fifty two day old *B. tabaci* adults (male and females) were removed from *J. gossypiifolia* plants with the aid of a buccal aspirator (constructed with a Pasteur pipette). Adults are then placed in small clip cages (2.5 cm diameter x 3.0 cm depth) and attached to the undersides of MNg 2 and MNg 11 leaves. Adults are allowed to oviposit for six hours before being removed and 300 eggs are selected at random. The development time from egg to adult is obtained and survival rate of the immature stages and proportion of females is determined.

Demographic parameters: Data on development time is combined with experimental data on reproduction '1_x-m_x,' generating life tables which are used to calculate the demographic parameters as defined by Price (1975): 1) Net reproduction rate (R_o), the average number of females descendents produced by one female per generation; 2) generational time (T),

equivalent to the time contained between parental birth and progeny birth and 3) the intrinsic rate of population increase (r_m) estimated using the equation (Carey, 1993).

$$\sum \exp(-r_m x)\ 1_x m_x = 1$$

Where x is the female age (days); l_x is specific survival age and m_x, the proportion of females from a female progeny at age x. To calculate the values of r_m, the corrected age X+0.5 and the equation $1n\ 2/r_m$ were used to estimate the days required to double the population (Carey, 1993).

Statistical analysis: Statistical analysis was carried out by utilizing the program Stat View, version 5.0.1 (SAS Institute, 1999). The values for longevity, fecundity, oviposition rate and development time were analyzed using Mann-Whitney test; this permits comparing the means of two distributions without needing to determine the supposition that the error is normally distributed. Rate of survival values were compared using chi-square (x^2).

Results and Discussion

1. Biology and demographic parameters of *B. tabaci* feeding on MNg 2 (TMS 30572) and MNg 11 (TMS 60444).

Longevity and fecundity: the most extensive longevity range, 2 to 10 days, was achieved by *B. tabaci* females feeding on MNg 2, exceeding by approximately 4 days those females feeding on MNg 11. After six days, mortality reached 60% and 100% on MNg 11 and MNg4 respectively (Figure 10.8) and their respective longevities were significantly different (Mann-Whitney $P < 0.05$) Table 10.5.

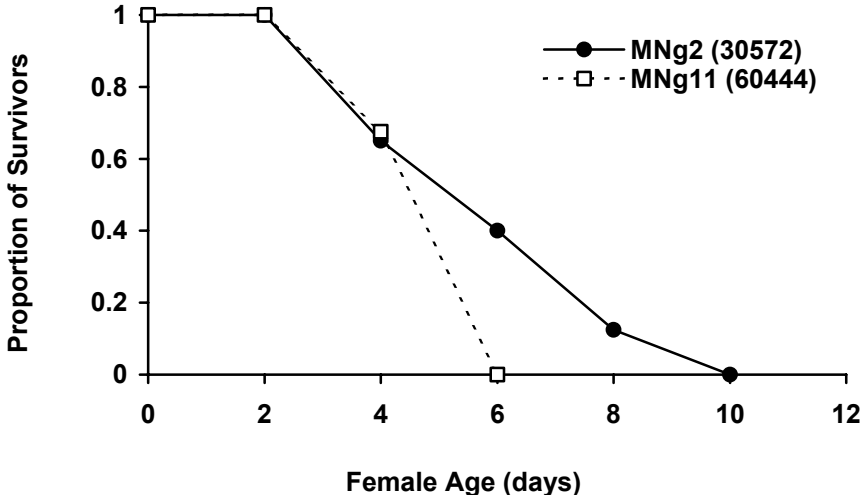

Figure 10.8. Female survivors of *B. tabaci*, B Biotype, feeding on the African Cassava genotypes MNg 2 (TMS 30572) and MNg 11 (TMS 60444) in the growth chamber (CIAT, 2004).

Table 10.5. Average longevity (days), average fecundity (eggs/female) and oviposition rate (eggs/female/2 days) of *B. tabaci,* B biotype, feeding on the African cassava genotypes MNg 2 and MNg 11 (CIAT-2004).

Parameter	MNg 2	MNg 11
Average longevity	4.5 a	3.3 b
Range	2-8	2-4
Average fecundity	8.1 a	3.7 b
Range	1-25	2-16
Average oviposition rate	1.8 a	1.1 b
Range	0.5-11.5	0.5-4

Averages followed by different letters across the columns are significantly different (Mann-Whitney P< 0.05).

Initial oviposition on both genotypes was similar in that *B. tabaci* females oviposited 66% of their total oviposition within the first 48 hours. The difference in average ovipositional rate for each genotype permits predicting that, in a limited way, either of the two hosts would the adequate for development of nymphal stages. The highest ovipositional rate (1.8 eggs/2 days/female) was achieved on MNg 2 with a significantly higher value than achieved on MNg 11 (Mann-Whitney P < 0.05). Maximum oviposition on both genotypes occurred during the first two days. These differences reveal a certain preference for *B. tabaci* to oviposit on MNg 2.

The average fecundity was significantly higher on MNg 2 compared with that on MNg 11 (Mann-Whitney P < 0.05) (Figure 10.9, Table 10.5).

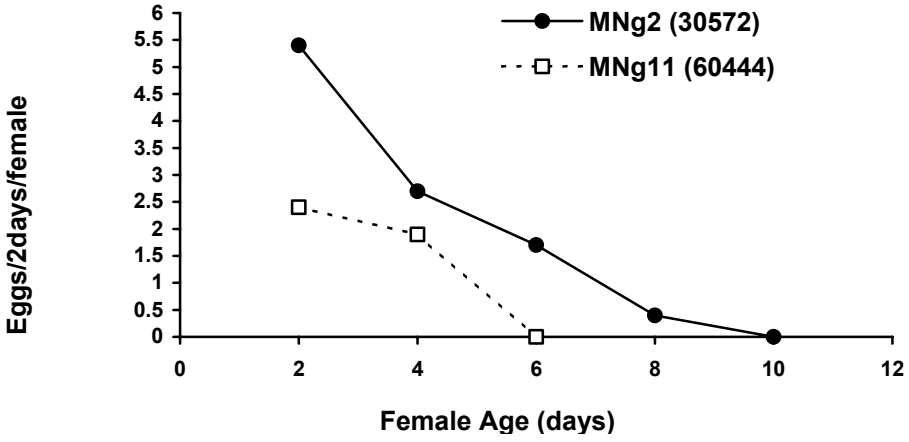

Figure 10.9. *B. tabaci* reproduction curves when feeding on African cassava genotypes MNg 2 and MNg 11 in the growth chamber (CIAT, 2004).

2. Development time, rate of survival of immature stages and proportion of females.

The development time of *B. tabaci* feeding on MNg 2 was significantly shorter by 30 days than those feeding on MNg 11 (Table 10.6). Development time for *B. tabaci* feeding on MNg 2 was

37.9 days, and 68.0 days while feeding on MNg 11. The highest levels of nymphal mortality occurred in the first instar on both genotypes. Mortality also occurred during the second and third instars feeding on MNg 2, but only occurred during the second instar for those feeding on MNg 11. In each case, nymphs entered into a latent state without reaching the adult stage. These results suggest *B. tabaci* biologically adapts more readily on the genotype MNg 2. *B. tabaci* survival rates for immature stages were significantly different on the two genotypes (Chi-Square = 44.58, 1d.f., P < 0.0001) Table 10.6). Results show that of the 200 *B. tabaci* eggs, 45 individuals survived to adult stage when feeding on MNg 2; compared to only 2 adults surviving on MNg 11. This parameter is a good indication of the potential capacity of *B. tabaci* to develop higher populations on MNg 2, compared to that on MNg 11. In general, the proportion of females and males was not affected by genotype.

Table 10.6. Development time, survival and proportions of female *B. tabaci* feeding on two African genotypes, MNg 2 and MNg 11 (CIAT, 2004).

Parameter	MNg 2	MNg 11
Development time (days)*	37.9 b	68 a
No. Insects	45	2
Survival rate (%)♣	22.5 a	1 b
No. Insects	200	200
Proportion of females (%)	60	50
No. Insects	45	2

* Averages followed by different letters across columns are significantly different Mann-Whitney P<0.05.

♣ Chi-Square = 44.58, 1d.f., P<0.0001 (CIAT, 2004).

2. Demographic parameters.

The net rate of reproduction (R_o) allows us to estimate that, on average, at the end of a generation, *B. tabaci* populations could multiply 8.1 times (individual/individual) on MNg 2 (Table 10.7), this being 1.9 times greater than on MNg 11. One generation of *B. tabaci* would be completed in 39.6 and 68.8 days on MNg 2 and MNg 11 respectively (Table 10.7). These results allow us to predict that *B. tabaci* would complete nine generations per year on MNg 2, while only five generations on MNg 11.

The results are equally consistent when comparing the intrinsic rate of increase (r_m). This analysis shows a greater population build up on MNg 2, 62% greater than on MNg 11. Likewise, the value of r_m reflects the time of population doubling. On MNg 2, *B. tabaci* requires 21 days less to duplicate its population compared to MNg 11 (Table 10.7).

Table 10.7. Demographic parameters of biotype B of *Bemisia tabaci* feeding on MNg 2 (TMS 30572) and MNg 11 (TMS 60444) in the growth chamber (CIAT, 2004).

Parameter	MNg 2	MNg 11
Net reproduction rate (R_o) $\Sigma l_x m_x$	8.1	4.2
Generation time (T)	39.6	68.8
Intrinsic rate of increase (r_m)	0.053	0.02
Days to duplicate population (TD) $\ln 2/r_m$	13	34.5

Results on longevity, fecundity, development time, survival rate and demographic parameters, suggest that the genotype MNg 11 (TMS 60444) is not a suitable host for biotype

B of *B. tabaci* in Colombia. These results, however, do differ than those reported by Costa and Russell (1975), where none of the *M. esculenta* genotypes tested permitted survival or reproduction of *B. tabaci*. Bird (1957) also reported that he was not able to rear *B. tabaci* on *M. esculenta*, with whiteflies previously reared on *J. gossypiifolia*. In addition, the results from this study suggest that the African genotypes of *M. esculenta* are potential hosts of the B biotype of *B. tabaci* found in Colombia.

In recent experiments with the genotype TMS 60444 (MNg 11), resistance to the cassava hornworm, *Erinnyis ello*, was observed on this genotype (Chavarriaga et al, unpublished data) (Activity 10.8). *E. ello* is an important cassava pest in the Neotropics (Bellotti, 1981). The TMS 60444 genotype was developed in Nigeria in the 1950's by using the third backcross derived from an interspecific cross between *M. esculenta* and *M. glaziovii*, as a source of resistance to CMD (CIAT, 2003). The other progeny TMS 30572, also derived from the backcross with *M. glaziovii* was used to construct the genetic map of cassava (Fregene et al, 1997) and shows genomic regions that are probably inherited from *M. glaziovii*. One of their regions is found in ligament D, which shows QTLs for resistance associated with CMD and CBB (CIAT, 2003). These findings, together with the results of this study permit speculating about a possible resistance in TMS 60444 (MNg 11) to biotype B of *B. tabaci* found in Colombia. This could be related to that region on the genome for the QTL's previously mentioned.

References

Bellotti, A.C.; Arias, B. 2001. Host plant resistance to whiteflies with emphasis on cassava as a case study. Crop Prot. 20:813-823.

Bellotti, A.C. 1981. Cassava hornworm. *In:* Lozano, J.C., Bellotti, A.C., Reyes, J.A., Howeler R., Leihner, D., and Doll J. (eds) Field Problems in Cassava. CIAT, Cali Colombia. pp. 84-85.

Bellows, T.S. Jr.; Perring, T.M.; Gill, R.J.; Headrick, D.H. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 87:195-206.

Bird, J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypiifolia*. Univ. Puerto Rico, Agric., Exp. Stn., 22-35.

Carey, J.R. 1993. Applied demography for biologist. Nueva York. Oxford University Press. 206 p.

CIAT (Centro Internacional de Agricultura Tropical) 1999. Integrated pest and disease management in mayor agroecosystems: Annual Report, Project PE-1. Cali, CO. 386 pp.

CIAT (Centro Internacional de Agricultura Tropical) 2004. Sustainable Integrated Management of Whiteflies Through Host plant Resistance: Progress Report, 2003-2004. Cali, CO. 77 p.

Costa, A.S.; Russell, L.M. 1975. Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera: Aleyrodidae). Cienc. Cult. Sao Paulo 27:388-390.

Development and use of biotechnology tools for cassava improvement. Output 8. 2003. *In:* CIAT (Centro Internacional de Agricultura Tropical) 2003. Improved Cassava for the developing World: Summary Annual Report, Project IP-3. Cali, CO. p 1-104.

Eichelkraut, K; Cardona, C. 1989. Biología, cría masal y aspectos ecológicos de la mosca blanca *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), como plaga del frijol común. Turrialba. 39:55-62.

Fregene, M.A.; Angel, F.; Gómez, R.; Rodríguez, F.; Roca, W.; Tohme, J.; M. Bonierbale. 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). Theor. And Appl. Genet. TAG 95(3):431-441.

Legg, J.P.; French, R.; Rogan, D.; Okao-Okuja, G.; Brown, K. 2002. A distinct *Bemisia tabaci* (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae) genotype cluster is associated with the epidemic of severe cassava mosaic virus disease in Uganda. Molecular Ecology 11:1219-1229.

Price, P. 1975. Insect Ecology. John Wiley & Sons, New York. 514 p.

SAS Institute, 1999. SAS Institute, Cary, N.C. USA.

Thresh, J.M.; Otim-Nape, G.W.; Legg, J.P.; Fargette, D. 1997. African cassava mosaic virus disease: the magnitude of the problem. African Journal of Root and Tuber Crops 2:13-19.

Contributors: Arturo Carabalí, Adriano Muñoz, Anthony C. Bellotti.

Activity 10.4 Studies on the biology and behavior of biotype "B" of Bemisia tabaci *on a wild* Manihot *sp,* M. flabellifolia.

Whiteflies are a major agricultural pest group, attacking a wide range of crop species. As direct feeding pests or virus vectors, whiteflies cause yield losses in cassava based agroecosystems in the Americas, Africa and Asia. The origin of cassava (*Manihot esculenta*) is in the neotropics and two whitefly species cause considerable crop damage in the region; *Aleurotrachelus socialis* predominates in northern South America (Colombia, Venezuela and Ecuador), while *Aleurothrixus aepim* is the major species in Brazil. *Bemisia tabaci* is a pantropical species that is the vector of Africa Cassava Mosaic Disease (CMD) in Africa and parts of Asia. Biotype "B" of *Bemisia tabaci* has been collected feeding on cassava in the Americas but has not been reported, nor observed, transmitting virus diseases on cassava in the neotropics. Host plant resistance in cassava to whiteflies is seen as a practical, low cost, long-term solution for reducing whitefly populations and damage.

The wild species within the genus *Manihot* are seen as potential source of genes for resistance in the control of major cassava pests (see 2003 Annual Report; Project IP-3). There is a precedence for this in that resistance to CMD resulted from an interspecific cross between *M. esculenta* and *M. glaziovii*. However, apart from this one successful case, wild Manihot species have not been exploited as a source of resistance to cassava pests and diseases (also see Activity 10.8).

The development of pest and diseases resistant varieties resulting from interspecific crosses involving wild *Manihot* species is difficult and time consuming and no continued effort has been attempted to take advantage of this potential source of resistance genes. However recent advances in the development of the molecular genetic map of cassava facilitates gene transfer and transformation. It is presently considered that with the modern tools of genetic engineering now available, access to resistance genes in the wild species will be more efficient, providing quicker manipulation at the molecular level.

Objective

The objective of this present study is to evaluate biological, populational and demographic aspects of Biotype "B" of *B. tabaci* found in Colombia, on *Manihot flabellifolia*.

Methodology

a) Source of *M. flabellifolia* and *B. tabaci*.

Plantlets of *M. flabellifolia* were obtained from the CIAT Biotechnology Unit (Agrobiodiversity and Biotechnology Project, SB-2) where they were propagated in-vitro. These were transplanted to plastic bags or pots. Eight 40-day old plants were selected and placed in nylon mesh wooden frame cages (1m x 1m x 1m).

The source of *B. tabaci* whiteflies was a CIAT established colony being reared on *Jatropha gossypiifolia* (Euphorbiacea). These had been reared for 15 generations on *J. gossypiifolia* in nylon meshed wooden cages (1m x 1m x 1m) in the growth chamber (25±2°C, 70±5% RH, 12:12 photoperiod). The species quality (uncontaminated) of the *B. tabaci* colony is periodically verified through RAPD-PCR testing of adults (CIAT, 1999).

b) Biology of *B. tabaci* on *M. flabellifolia*.

Longevity and fecundity were evaluated by placing 40 recently emerged adult pairs (40 males + 40 females) of *B. tabaci* from the *J. gossypiifolia* colony, in small clip cages (1.5 cm diameter + 2.0 cm depth) (one pair per cage), on the underside of *M. flabellifolia* leaves. Adults were removed every 48 hours to a different site on the leaf; this procedure was repeated until the natural death of the females. Fecundity was estimated by counting the number of eggs oviposited every 48 hours by each female, while longevity was estimated based on the number of days that females survived.

Development time, survival and female/male ratio was estimated by placing 50 two day old adults (males and females) removed from the *J. gossypiifolia* colony, in round clip cages (2.5 x 2.0 cm) on the underside of *M. flabellifolia* leaves. After six hours, adults were removed and 200 eggs were randomly selected. Egg to adult development time, survival of immature stages and proportion of females was observed and recorded.

Demographic parameters were calculated by combining data on development time and reproduction (1_x-m_x), generating life tables (Price, 1975): 1) net reproduction rate (R_o), the average number of females that one female produces in one generation; 2) generational time (T), equal to that period between birth of the parents and of the progeny and 3) intrinsic rate of increase of the population (r_m), estimated using Carey's formula (1993),

$$\sum \exp(-r_m x)\, l_x m_x = 1$$

where x is the age of the female in days, l_x, the age of species survival, and m_x, the proportion of female progeny of one female at age x.

Results

Longevity and Fecundity: Results show a range of *B. tabaci* female survival of 2 to 8 days when feeding on *M. flabellifolia*, with an average of 3.5 days (Figure 10.10A and Table 10.8). An average of 3.3 eggs (range 1-16 eggs) were oviposited per female. Ninety percent of female initiated oviposition during the first 48 hours and by the 4th day, 87% of oviposition had occurred (Figure 10.10B).

Table 10.8. Average longevity, average fecundity and rate of oviposition (eggs/female/2 days) of biotype "B" of *Bemisia tabaci* feeding on *Manihot flabellifolia* in the growth chamber.

Parameter	*M. flabellifolia*
Average longevity Range No. Insects	3.5 2-8 40
Average fecundity Range	3.3 1-16
Average Oviposition rate Range	0.98 0.25-4

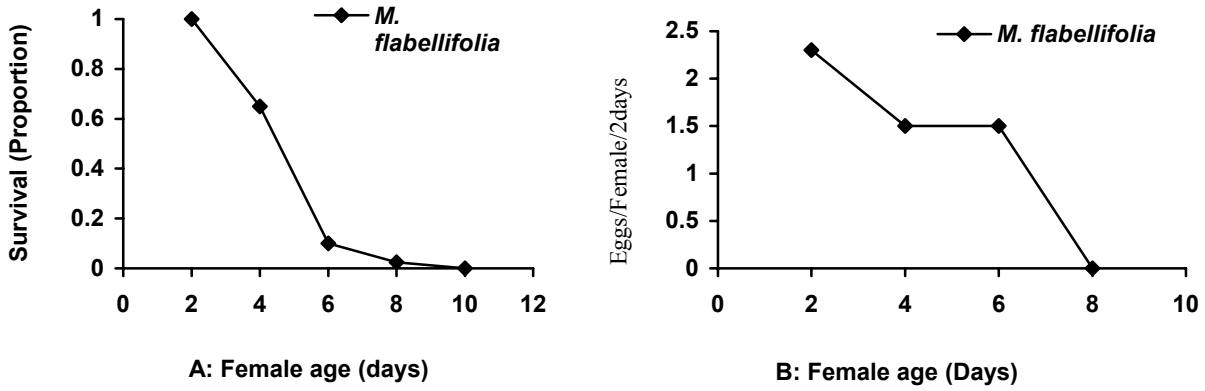

Figure 10.10. *Bemisia tabaci* (biotype B) reproduction (A) and survival (B) curves when feeding on *Manihot flabellifolia* in the growth chamber.

Development Time, Survival, Proportion of Females: Development time of B. tabaci (biotype B) individuals feeding on M. flabellifolia was 47.2 days (Table 10.9). The proportion of females was 50% and survival 8%.

Table 10.9. Development time, survival and proportion of females of Biotype "B" of *Bemisia tabaci* feeding on *M. Flabellifolia* (n=200) in the growth chamber.

Parameter	Values
Development time (days)	47.2
Rate of survival (%)	8
Proportion of females (%)	56

The net reproduction rate (Ro) estimates that *B. tabaci* population will increase three fold during one generation (Table 10.10). *B. tabaci* will complete one generation in 48 days feeding on *M. flabellifolia*, resulting in seven generations in one year. In addition the r_m value indicates a 77% population decrease when compared to the reproductive rate of *B. tabaci* on its original host, *J. gossypiifolia*. Feeding on *M. flabellifolia*, *B. tabaci* requires 31 days to duplicate its population, compared to only 25 days on *J. gossypiifolia* (Carabalí, 2004).

Table 10.10. Demographic parameters of individuals of biotype "B" of *Bemisia tabaci* feeding on *Manihot flabellifolia* (n=200) in the greenhouse.

Parameter	Values
Net reproduction rate (Ro) ∑lxmx	3.0
Generation time (T)	48.3
Intrinsic rate of increase (r_m)	0.0222
Days to duplicate population Ln2/rm	31.2

In recent studies, *M. flabellifolia* was evaluated for resistance to the cassava mealybug (*Phenacoccus herreni*), the cassava green mite (*Mononychellus tanajoa*), and the whitefly (*Aleurotrachelus socialis*). *M. flabellifolia* showed moderate levels of resistance to the mealybug and mite, and high levels to the Whitefly (Burbano, 2003). Present results further indicate that the wild *Manihot* species are a potential source of whitefly resistance genes and in particular a resistance source to biotype B of *B. tabaci*.

References

Burbano M., M. 2003. Multiplicación de material de especies silvestres y domesticadas del género Manihot y estudio de su resistencia natural a tres plagas de cultivo (*Mononychellus tanajoa, Aleurotrachelus socialis,* y *Phenacoccus herreni*) en condiciones controladas de temperatura y humedad relativa. Thesis (Biologist). Universidad del Valle, *Facultad* de Ciencias, Cali, Colombia. 107 p.

Carabalí, A. 2004. Potencial de resistencia de diferentes genotipos de yuca *Manihot esculenta* Crantz al biotipo "B" de *Bemisia tabaci* (Gennadius). Thesis MS, Univ. del Valle, Cali, Colombia, 85pp.

Carey, J.R. 1993. Applied demography for biologist. Nueva York. Oxford University Press. 206p.

CIAT (Centro Internacional de Agricultura Tropical), 1999. Integrated pest and disease management in mayor agroecosystems: Annual Report, Project PE-1. Cali, CO. 386pp.

Price, P., 1975. Insect Ecology. John Wiley & Sons, New York. 514p.

Contributors: Arturo Carabalí, Adriano Muñoz, Anthony C. Bellotti.

Activity 10.5 Evaluation of cassava germplasm in several breeding and genetic trials for insect and mite pest damage at several localities on the Colombia Atlantic Coast.

Rationale

The north coast of Colombia denotes an important cassava agro ecosystem, representing the lowland, seasonally dry tropics. It is also indicative of small farmers in traditional cassava production systems. Pesticide use in traditional cassava agroecosystems is usually minimal due to their prohibitive costs and the long cassava crop cycle. However this seasonally dry agroecosystem is also representative of numerous arthropod pests of cassava. In general arthropod pest are most damaging to cassava during the dry season and do not appear to cause significant damage in areas of considerable and consistent rainfall. Dry season feeding by pests, tends to cause the greatest yield loss in cassava.

It is therefore important that cassava germplasm developed for the seasonally dry, lowland, tropical agroecosystems, have the capacity to produce good yields under arthropod pest pressure. The entomology section collaborates with the cassava breeders by evaluating arthropod pest damage on germplasm being tested and developed for this agroecosystems. This germplasm is attacked by a range of pests, including whiteflies, mites (several species), thrips, stemborers, hornworm and others. These evaluations can determine the levels of susceptibility or resistance available in the genotypes being evaluated. These results afford knowledge on possible pest behavior or potential crop damage prior to varietal release and assist in designing a pest management system that will aid in maximizing cassava yields while minimizing production costs.

Objective

Determine arthropod pest damage levels on cassava genotypes in collaboration with the Cassava Germplasm Development Project, being grown in numerous field trials on the north (Atlantic) coast of Colombia.

Methodology

Germplasm development (breeding) field trials were evaluated at several sites in the departments of Atlántico and Magdalena. These sites and trials are described in Table 10.11. Soils in all these localities are sandy to sandy loams, temperatures average around 30°C and rainfall is from 1000 to 1200 mm. All actual data from these evaluations is available in the cassava entomology and breeding databases. Field evaluations were made using standard 1 (absence of pest and damage) to 6 (high pest populations and severe plant damage) rating scales.

In general, whitefly (*A. socialis*) populations were present in all the fields evaluated; however, populations were usually low, between 1 and 3 on the 1 to 6 population scale. The mites, *Oligonychus peruvianus, Tetranychus* sp. and *Mononychellus tanajoa* were observed at low to high levels, depending on the species present. *O. peruvianus* appears to be the predominate species in the region and is found at higher populations than the other species. The lepidopteran stemborer, *Chilomima clarkei*, was observed in numerous fields.

Table 10.11. Localities on the Colombian Atlantic Coast (departments of Atlántico and Magdalena for evaluation of breeding materials from the Cassava Germplasm Improvement Project.

Department/Municipality	Locality/Site	Trial
Atlántico		
Santo Tomás	Finca "El Esfuerzo" (Alvaro Barros)	3 trials
	Finca "El Desquite" (Alvaro Barros)	4 trials
	Finca Absalón Charris	2 trials
	Finca Tomás Fontalvo	4 experiments (4, 5, 6, 7)
Pitalito	Finca Teobaldo La Rosa	3 trials (Exp. 3, yield trial, and 8 Corpoica released clones
Baranoa	Finca Palapa (Industrias del Maíz)	Yield trial; and 8 clones from 2nd year Paulina selection
Caracolí	Finca Germán Jaramillo	8 clones
Magdalena		
Ciénaga		2 yield trials (ER and PR)
Tamalameque		2 yield trials (ER and PR)

1. Observation fields 1, 2, and 3 at Santo Tomás (Atlántico).

These plantings consisted of CM, GM and SM hybrids; including CM 8379, 9106, 9832, 9904, 9906, 9907, 9910, 9913, 9914, 9924, 9926, 9945, 9946, 9955, 9957, 9958; GM 248, 259, 262, 266, 288, 383, 385, 389, 406, 408, 409, 410, 413, 428, 436, 439, 443, 451, 456, 462, 465, 466, 468, 521, 546, 549, 578, 579. SM 2621, 2750, 3052, 3054, 3058, 3061, 3062, 3063, 3067. These genotypes were sown in three groups and no replications. The small plots (1157) were grown in single rows of seven plants; observations were made on the central five plants using the afore-mentioned 1 to 6 damage and population scales. Stemborer damage was recorded by counting the number of adult exit holes in the stem. Data was tabulated using Excel Program and genotypes were listed according to damage and population ranges. Harvest of genotypes is carried out by the breeding program and selection for continued evaluation in subsequent crop cycles is determined. Data on yield, % dry matter, harvest index and other morphological or agronomic characteristics is recorded.

Results

Three arthropod pests were evaluated, the mite *O. peruvianus*, whiteflies (*A. socialis*) and the stemborer *C. clarkei*. *O. peruvianus* populations were high; 469 of the 1156 genotypes (40.6%) had a population rating of 3.1 to 6.0 (Figure 10.11). This indicates that 50 to 100% of the leaf was infested with the mite. This is also reflected in the damage ratings where 374 (32%) of the genotypes had more than 50% of the leaf area covered with necrotic lesions due to mite feeding (Figure 10.12). *O. peruvianus* usually attacks and damages lower or mid third leaves, seldom damaging upper or bud leaves (in contrast to *M. tanajoa* where bud leaves are severely attacked) and therefore is considered of lesser importance, in terms of the effect on root yield, than *M. tanajoa*. However *O. peruvianus* feeding can cause defoliation on the mid to lower levels of the plant. Lower leaf retention has been shown to be important in root yield, especially in this agroecosystems, indicating that the population and damage levels observed may be affecting root yield.

Whitefly (*A. socialis*) populations were low and 570 genotypes (49.3%) showed no presence of the pest (Figure 10.11); 566 (49%) had a low (1.2–2.0 on the 1 to 6 scale) population level and 20 genotypes had a population rating above 3.0. None of the genotypes displayed leaf damage. *M. tanajoa* mite occurred on 28 genotypes (2.4%), of which 17 had a damage level between 3 to 5 (grade 5.0 = SM 3054-6; grade 4.0 = SM 3063-29, GM 451-5; grade 3.5 = GM 451-19, GM 428-6).

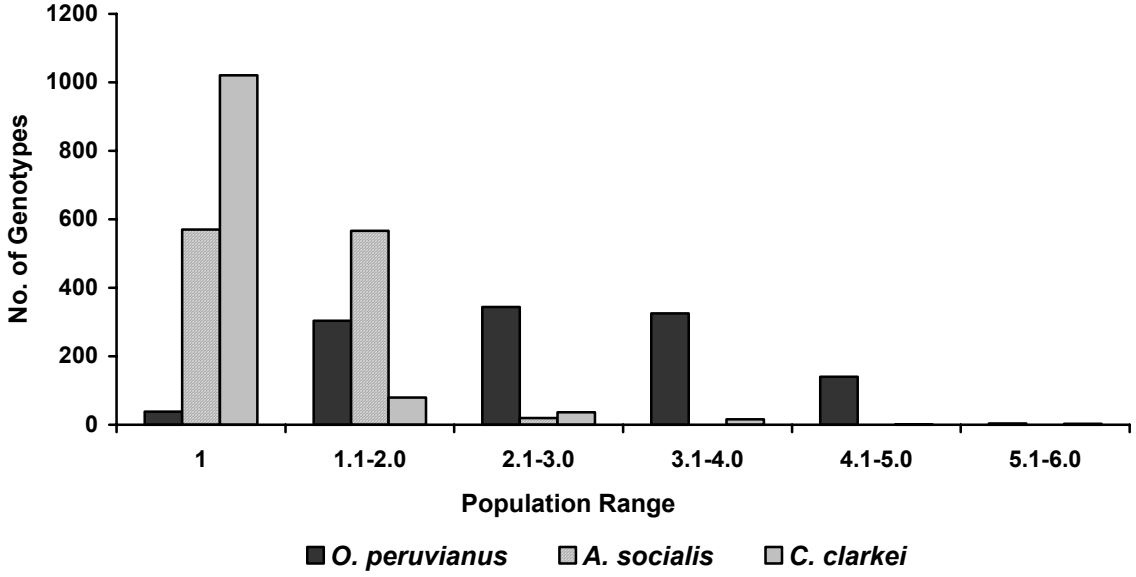

Figure 10.11. Arthropod pest populations (*Oligonychus peruvianus, Aleurotrachelus socialis* and *Chilomima clarkei*) on 1156 cassava genotypes in three Observation Field Trials at Santo Tomás (Atlántico, Colombia), CIAT 2003-04.

The stemborer, *C. clarkei* is a major pest on the Atlantic Coast. Population and damage incidence on these genotypes was low during this crop cycle (Figure 10.12); 1026 of the 1156 genotypes (89%) had no exit perforation for 6 plants, 36 (3.1%) presented 2 perforations per 6 plants, 16 (1.4%) had 3, 2 (0.2%) had 4 and 3 (2.6%) had 5 perforations per 6 plants (the latter 3 were CM 9832-1, CM 9944-1 and GM 406-21).

In conclusion, pest populations were probably too low to determine accurate levels of resistance/susceptibility in the genotypes. Known susceptible checks to these pests were not planted with the genotypes evaluated. It is therefore difficult to determine if the low pest populations were a natural phenomena or due to resistance available in the genotypes. These results do indicate that *O. peruvianus* was the major pest present during this crop cycle and that *C. clarkei* damage was not severe enough to cause stem breakage (stem breakage can cause yield reduction).

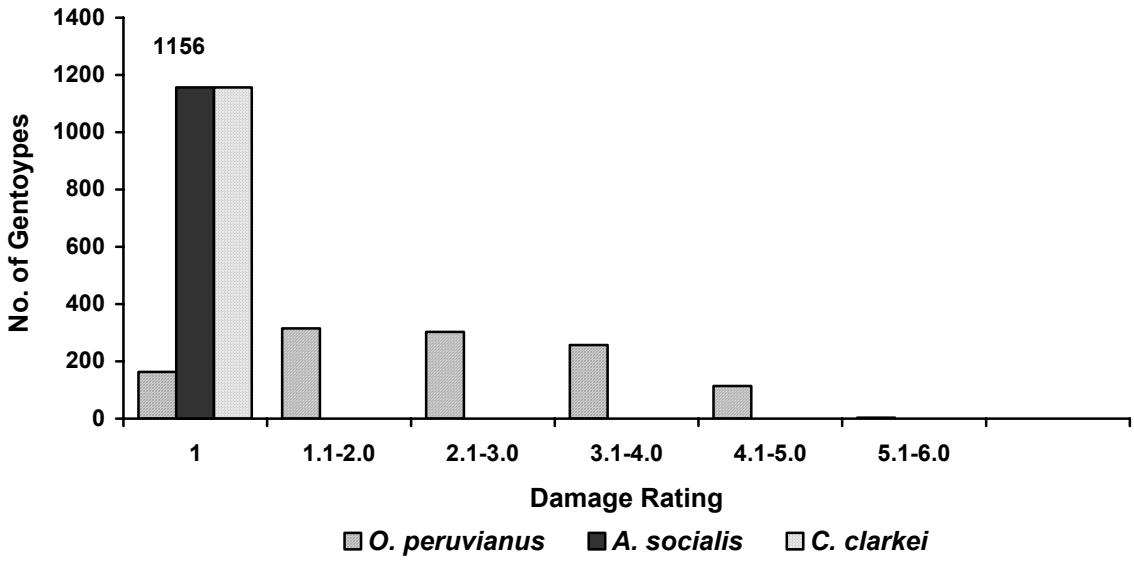

Figure 10.12. Arthropod pest (*Oligonychus peruvianus, Aleurotrachelus socialis* and *Chilomima clarkei*) damage ratings on 1156 cassava genotypes in three Observation Field Trials at Santo Tomás (Atlántico, Colombia), CIAT 2003-04.

2. Santo Tomás (Atlántico), Experiment 2. Low vs. high leaf retention trial. Finca "El Desquite" Alvaro Barros.

This experiment studied the genetics of leaf retention in cassava germplasm. It consisted of 180 small plots of two families SM 2783 and SM 2615 with 29 entries and 3 replications of each. The control was they hybrid CG 1141-1. The pest populations observed in this trail were the mite's *O. peruvianus* and *Tetranychus* sp., and the whitefly, *A. socialis*. *O. peruvianus* had the highest pest populations; 34 of 60 genotypes (57.0%) had a population grade between 4.0 and 5.0 (indicating a 60 to 75% leaf infestation with necrotic lesions) (Figure 10.13). Twenty-four genotypes (40%) showed an intermediate population (3.0) and two genotypes a low population rating (2.0). High *O. peruvianus* populations will accelerate leaf necrosis and defoliation on the lower 2/3 of the plant, greatly affecting the leaf retention qualities being sought in these genotypes. *O. peruvianus* is primarily a dry season pest, that period when leaf retention is of most importance. The genotypes with the lowest population and damage ratings were SM 2783-46, SM 2783-30 (grade of 2.0). SM 2615-53, SM 2783-29, SM 2783-56, SM 2783-59, SM 2615-54 had a rating of 2.5 in population and 2.0 damage (indicating less than 10% of the leaf damaged). The control CG 1141-1 had a damage grade of 3.0 (40% of the leaf damaged).

The whitefly, *A. socialis* and *Tetranychus* mite were observed in low populations.

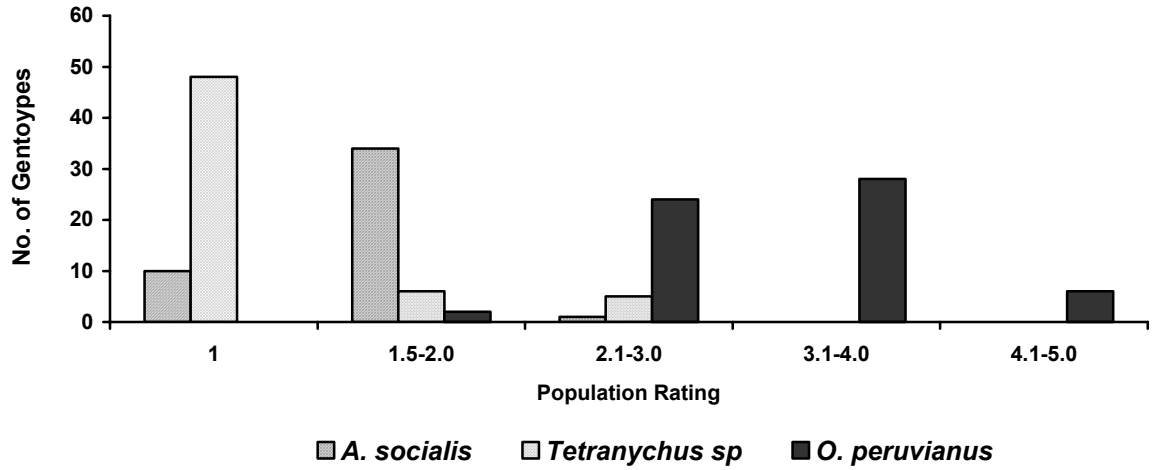

Figure 10.13. Arthropod pest (*Oligonychus peruvianus, Aleurotrachelus socialis*, and *Tetranychus* sp.) populations on cassava genotypes in Leaf Retention Trials at Santo Tomás (Atlántico, Colombia) (Experiment 2: CIAT 2003-04).

3. Santo Tomás (Atlántico), Experiment 1. Leaf retention; multiplication. Finca "El Desquite."

This trial consisted of 159 plots of the Families CM 9775 (9 entries), CM 9791 (18 entries), CM 9794 (18 entries), CM 9797 (8 entries), with three replications.

Results show that four pest species were present during the crop cycle; three mite species, *M. tanajoa*, *Tetranychus* sp., and *O peruvianus*, and the whitefly, *A. socialis*. Of the four, only *O. peruvianus* was present in relatively high populations. 82.4% of the genotypes had an *O. peruvianus* population and damage rating between 4.0 and 5.0 (with 60 to 75% of the leaf surface covered with necrotic lesions) (Figure 10.14). One genotype, CM 9794-51 had a population rating of 2.0 and 8 had a rating of 3.0 (CM 9775-12, CM 9775-5, CM 9794-19, CM 9794-8, CM 9797-11, CM 9797-25, CM 9794-21 and CM 9797-31).

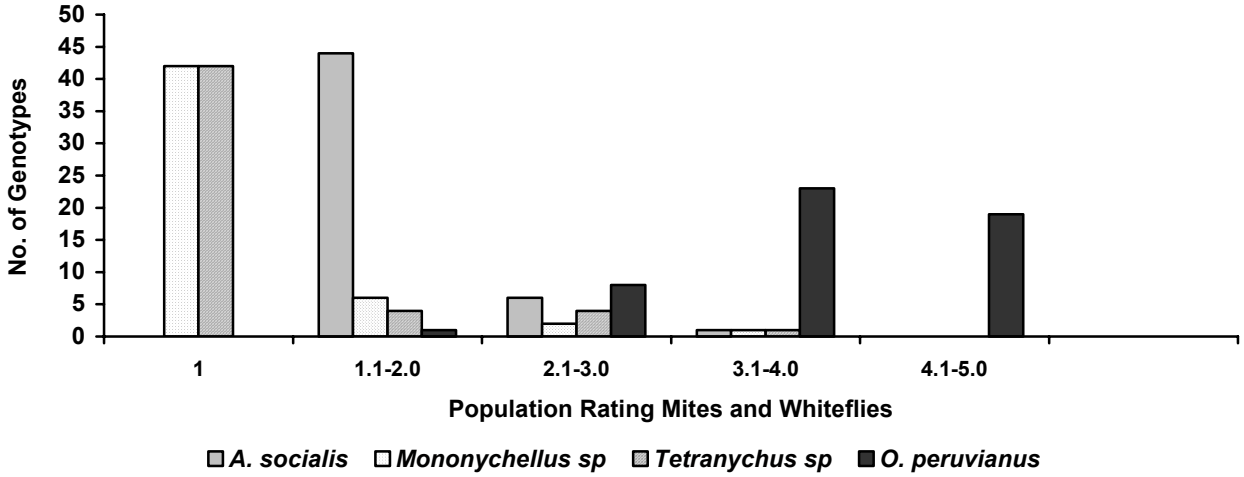

Figure 10.14. Arthropod pest (*Aleurotrachelus socialis, Mononychellus sp., Tetranychus* sp., and *Oligonychus peruvianus*) population ratings on cassava genotypes in Leaf Retention Trials at Santo Tomás (Atlántico, Colombia), CIAT 2003-04.

4. Santo Tomás (Atlántico), Yield trial, New Experiment 1. Observation field. Finca "El Desquite."

This trial consisted of 78 hybrid genotypes of CM and GM families, planted in three replications, totaling 243 plots. These genotypes were selected from the field Observation Trial at Santo Tomás during 2003. Primarily two pest species were present during the crop cycle, *A. socialis* and the mite, *O. peruvianus*. Whitefly populations were very low on all of the genotypes in all of the replications (Figure 10.15). Seventy-seven of the 78 genotypes had *A. socialis* populations below 3.0.

O. peruvianus population and damage was high. Forty-five of the 78 genotypes (57.7%) resulted in a population rating between 4.0 and 5.0 and 66% (52 genotypes) had a damage rating between 4.0 and 5.0. Four genotypes, CM 9923-56, CM 8209-64, GM 490-36 and CM 9958-37 had a low population rating of 2.0, and three of these also had a low damage rating of 2.0.
The cassava green mite, *M. tanajoa* showed up at low populations on a few genotypes, CM 9923-59 (population and damage rating of 2.0), CM 9952-58 and CM 9907-63 (population and damage rating of 3.0), CM 9958-53 and CM 9949-42 (population and damage rating 4.0). The *Tetranychus* mite was observed on CM 9958-37 (population 2.0), CM 9958-40 (population 2.0), CM 9952-32 (population 3.0) and GM 290=61 (population 2.0).

The control cultivar MTai 8 had high populations and damage due to *O. peruvianus*, ranging from 2.0 to 5.0 for both ratings. MTai 8 had low populations and damage due to *A. socialis* and the feeding of the other mite species. SM 1438-2 had similar results as MTai 8.

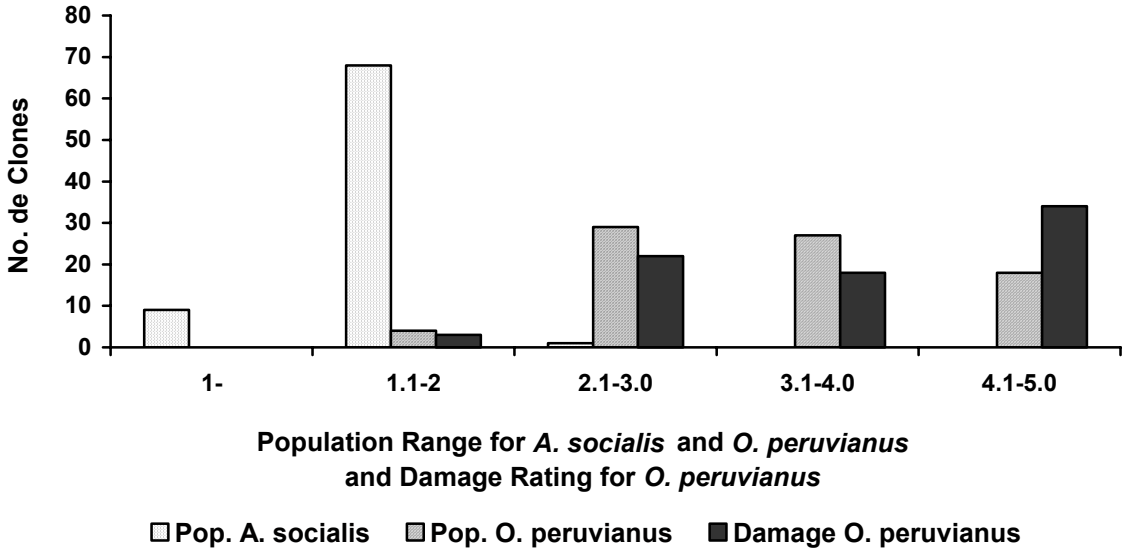

Figure 10.15. Arthropod pest (*Aleurotrachelus socialis* and *Oligonychus peruvianus*) populations on cassava genotypes in New Experiment #1, Finca "El Desquite," Observation Field Trial, Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

5. Santo Tomas (Atlántico), Yield Trial, New Experiment 2. Selections from Observation Fields.

This trial consisted of 192 plots with genotypes selected from Observation Field Trails during 2003 in Santo Tomás. Sixty-four genotypes were planted in three replications (58 genotypes were evaluated). Control plots consisted of MCol 2215, MTai 8 and CG 1141-1. The genotypes evaluated were primarily GM and some CM families. They were planted in small plots of 10 plants.

Again in this trial, the mite, *O., peruvianus* resulted in the primary pest attack. Forty-nine genotypes (84.5%) had a populations rating between 3.0 and 5.0 (Figure 10.16) (this indicates a 50 to 75% of the leaf with necrotic lesions or spots). Damage levels were similar. The genotype GM 211-20 had no *O peruvianus* present and several genotypes had a population and damage rating of 3.0 (CM 4919-1, CM 9921-44, CM 9957-61, CM 9958-81, GM 238-47, GM 214-78, GM 213-56 and GM 247-43). The control cultivars, MCol 2215 and MTai 8, had *O. peruvianus* populations between 4.0 and 5.0, and CG 1141-1 had a 2.0 and 1.5 population and damage rating respectively. However the latter cultivar had a *M. Tanajoa* population and damage rating between 3.0 and 4.0.

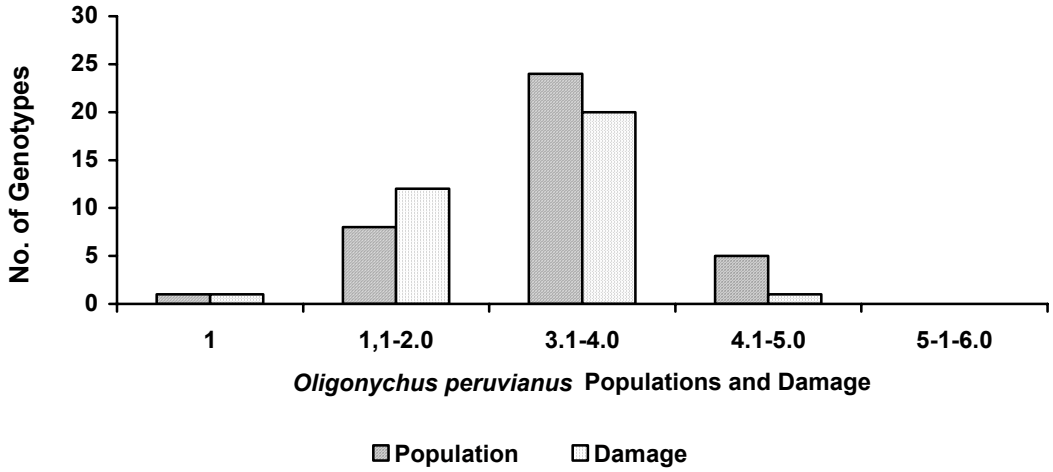

Figure 10.16. *Oligonychus peruvianus* mite populations and damage on cassava genotypes in Yield Trials, New Experiment #2; selections from Observation Field Trials, Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

M. tanajoa populations were low on 94.8 % (55 of 58) genotypes with 1.0 to 2.0 population rating. Three genotypes had an intermediate (3.0) rating; while damage evaluations resulted in 6 genotypes (10.3%) with a 3.0 rating and 4 genotypes had a 4.0 rating (GM 213-2, CM 9958-84, CM 9966-50, GM 302-25). The *Tetranychus* mite was also observed in low populations; four genotypes, GM 2313-2, GM 247-32, GM 214-48 and CM 9958-84, had ratings between 3.0 and 4.0.

6. Santo Tomás (Atlántico), Yield Trial, New Experiment 3. Selections from Observation Fields (Pitalito, Atlántico).

This trial consisted of 216 plots with mostly GM and some CM hybrids. A total of 69 hybrids were planted in 10 plants per plot in three replications. Four pests were present during the crop cycle, the three mite species, *M. tanajoa*, *Tetranychus* sp., and *O. peruvianus*, and the whitefly, *A. socialis*. *O peruvianus* was present in high populations and damage while the other three pest species were represented in comparatively low populations and damage.

O. peruvianus populations were high (4.0 to 5.0) in 60 of the 69 (88.4%) of the genotypes (Figure 10.17), indicating in a 75 to 100% of the leaf surface damaged. This was the trial with the highest *O. peruvianus* damage on the Atlantic Coast during this crop cycle (2003-04). No genotypes had a populations or damage rating below 3.0.

Whitefly (*A. socialis*) populations were low; 56 genotypes (81.2%) had a population rating of only 2 and 2.5, with no significant accompanying leaf damage. *Tetranychus* sp. was very low or non-existent, while *M. tanajoa* was observed only on GM 302-48 (grade 3.0 population and damage), CM 262-40 (3.0 damage), CM 9923-34 (4.5 damage) CM 255-53 (4.0 damage) and GM 217-31 (3.0 population and damage).

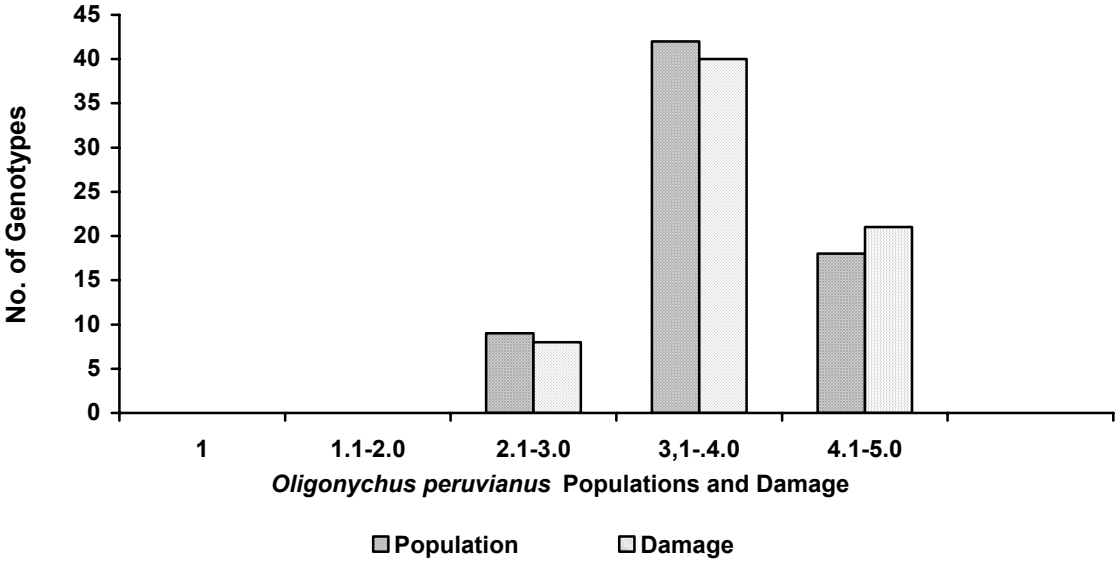

Figure 10.17. *Oligonychus peruvianus* populations and damage on cassava genotypes in Yield Trials, New Experiment #3, from Observation Field Trial selections. Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

7. Santo Tomás (Atlántico), Observation Field Trial; Finca Tomás Fontalvo.

This trial consisted of 110 cultivars of 3 replications, totaling 330 plots. The cassava germplasm in this trial consisted of SM, CM, CT and GM hybrids, and the controls MTai 8 and CG 1141-1. Again *O. peruvianus* resulted in the highest pest populations. The other two mite species, *M. tanajoa* and *Tetranychus* sp. as well as the whitefly, *A. socialis* were also present but remained at low populations throughout the crop cycle.

High populations of *O. peruvianus* were observed on 79.1% (87 of 110) of the hybrids with a population rating between 4.0 and 5.0 (Figure 10.18). A similar situation was evident in the damage ratings; only four hybrids CT 59-17, CM 9912-15, SM 2779-30 and SM 2546-80 had a damage rating as low as 2.0 (SM 2546-80 had a 3.0 population and a 2.0 damage evaluation).

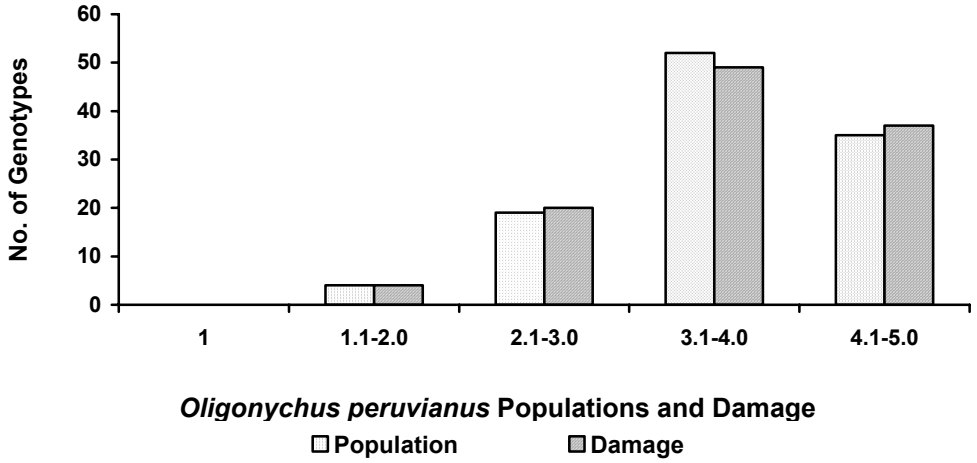

Figure 10.18. *Oligonychus peruvianus* populations and damage on cassava genotypes in Observation Field Trials, Experiment #4. Santo Tomás (Atlántico, Colombia), CIAT 2003-04.

8. Santo Tomás (Atlántico), Observation Field Trial 2; 2003-04.

This trial was planted with 10 hybrid cultivars principally of SM and CT families and to a lesser number of GM, CM and the controls CG 1141-1 and MTai 8. These were planted in three replications for a total of 330 field plots of 10 plants each. The two pest species that predominated during this trial were the mite *O. peruvianus* and whitefly, *A. socialis*.

A. socialis populations were observed feeding on almost all of the genotypes although mostly in low populations (Figure 10.19). 83.6% (92 of 110) of the genotypes had a population rating of 1.5 to 2.0. A small group of genotypes (12 = 10.9%) had an intermediate rating of 3.0. These low populations did not result in noticeable leaf damage symptoms.]

O. peruvianus populations and damage were much higher. Seventy-eight genotypes (71%) supported a population level between 4.0 and 5.0 and expressed a similar level of damage (Figure 10.19). Six genotypes produced a low population and damage rating of 3.0 (GM 281-93, SM 2954-20, SM 2957-13, SM 2773-90) of these 6, two presented a population level of 3.0 and genotype CM 9912-27 have a population level of 4.5 but a damage rating of only 2.0.

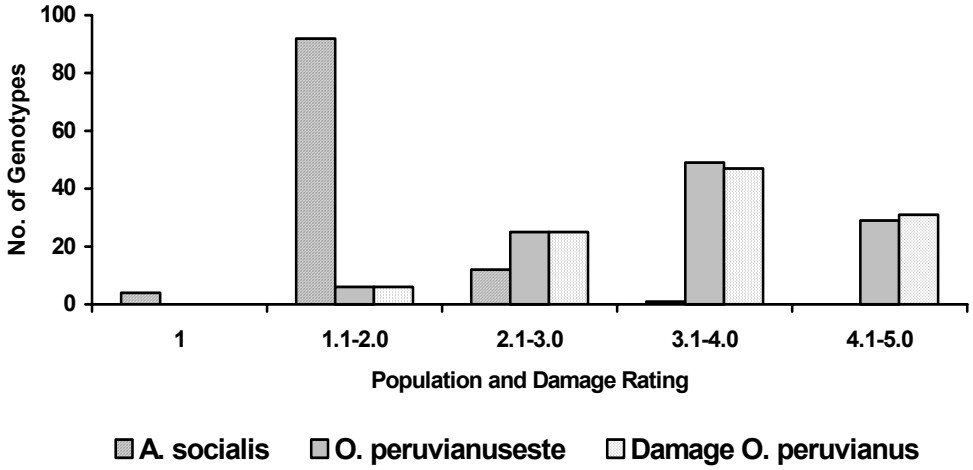

Figure 10.19. *Oligonychus peruvianus* and *Aleurotrachelus socialis* populations and damage rating for cassava genotypes in Observation Field Trial 2, Experiment #5 (from OFT 2002-03) at Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

9. Santo Tomás (Atlántico), Observation Field Trial, Experiment 6. Finca Tomás Fontalvo.

This trial was composed of 110 hybrid cultivars planted in three replications and consisting primarily of SM and CT families with some CM and GM plus the controls CG 1141-1 and MTai 8. Again two pest species predominated, the mite, *O. peruvianus* had the highest populations (Figure 10.20). Ninety-six genotypes (87.3%) had a mite population rating between 4.0 and 5.0. This indicates that 75 to 90% of the leaf is covered with necrotic spots. Only two genotypes (SM 2547-117 and SM 2546-116) had population and damage ratings around 3.0. Several genotype resulted in being highly susceptible with a damage level of 6.0, 100% of the leaf infested and damaged; these include SM 2547-124, SM 2547-114, SM 2834-41, SM 2954-40, CT 59-60 and CT 57-28). The control MTai 8 had population and damage levels of 5.0, as did the hybrid CG 1141-1.

Whitefly populations were observed on all of the genotypes but at low to moderate levels in 91 of 110 genotypes (82.7%): 14 genotypes (12.7%) had a population rating of 3.5 to 4.0. *M. tanajoa* appeared on six genotypes, SM 2882-49, CM 9794-58, GM 288-68, SM 2773-114, SM 2618-52 and CT 54-27, at a damage level of 3.5 to 5.0. The *Tetranychus* mite was also present at the same levels on SM 2620-93, SM 2621-63, GM 288-68, CT 54-30, SM 2773-113 and SM 2834-40. Neither of these latter two mite species was observed on the MTai 8 and CG 1141-1 controls.

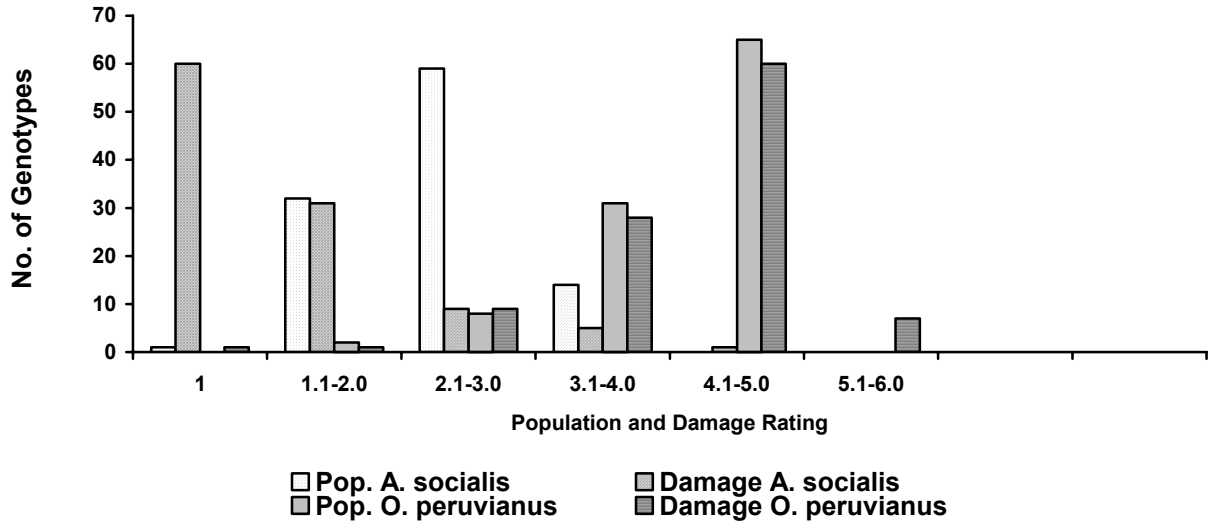

Figure 10.20. *Aleurotrachelus socialis* and *Oligonychus peruvianus* populations and damage on cassava genotypes in Observation Field Trial 3, Experiment #6 at Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

10. Santo Tomás (Atlántico), Multiplication and Observation Fields, Dialelic Selections, 2003; Finca "El Esfuerzo."

Sixty-six (66) GM and CM cultivars were planted in three replications. These materials were selected from the dialelic crosses evaluated during 2002-03. Sixty-two cultivars survived and were evaluated, primarily for *O. peruvianus* mite populations and damage. Fifty-two of the 62 genotypes (84.0%) had a population and damage rating between 4.0 and 5.0; two genotypes, CM 258-2 and GM 237-13 resulted in a damage level of 2.0 (Figure 10.21). *A. socialis* populations appeared on 84% (52 of 62) of the genotypes at a low 2.0 level.

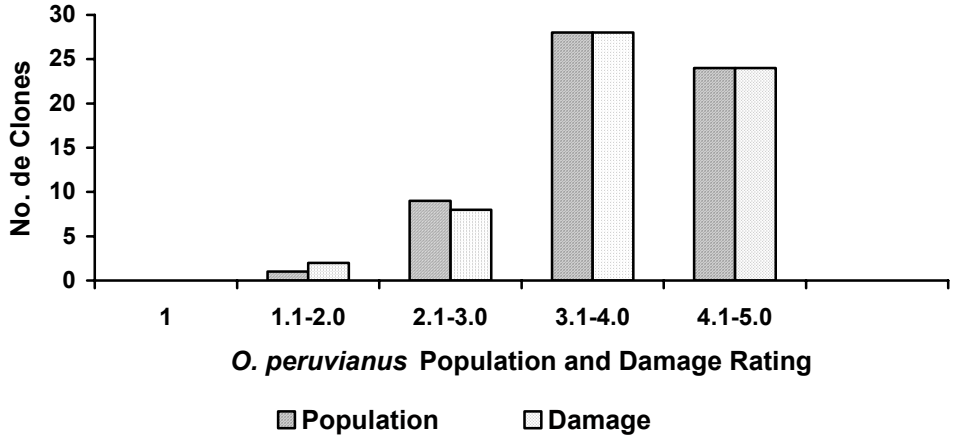

Figure 10.21. *Oligonychus peruvianus* populations and damage on cassava genotypes in Observation Field Trials, Dialelic Selections. Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

11. Baranoa (Atlántico), Advanced Yield Trial, selections from Paulina 2002 Yield Trial. Finca "Palapa."

In this trial, 105 plots were planted, consisting of 34 genotypes in three replications. Most of the genotypes were of an SM family with a few CM. The controls were MTai 8 and CG 1141-1. The two principal pests that appeared at these plantings were the mite *O. peruvianus* and the stemborer *C. clarkei*. *O. peruvianus* populations reached the level of a 4.0 to 5.0 population and damage rating on 73.5% (25 of 34) of the genotypes (Figure 10.22). Two genotypes, SM 2621-21 and SM 2775-2 had a population level of 2.0 and 2.0 and damage level of 2.0 and 3.0 respectively.

C. clarkei damage was noted in 20 of the 34 clones (58.8%) with two perforations in the stem per 6 plants; 4 clones (11.7%) with 3 perforations, 3 clones (8.8%) with 4 holes and two clones with seven holes. Five cultivars, CM 9456-12, SM 2949-40, SM 2620-7, SM 3773-32 and SM 25=621-21 had no stem perforations. The control MTai 8 had *O. peruvianus* damage and population ratings between 3.0 and 5.0, and 3 *C. clarkei* perforations in 6 plants.

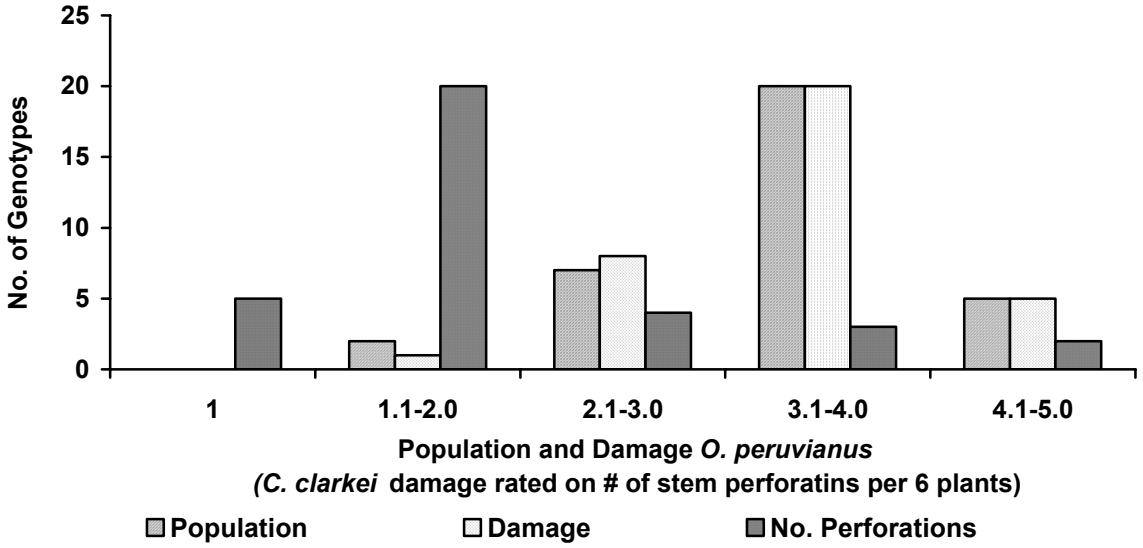

Figure 10.22. *Oligonychus peruvianus* and *Chilomima clarkei* population and damage on cassava genotypes in Advance Yield Trial at Baranoa (Atlántico, Colombia), CIAT, 2003-04.

12. Santo Tomás (Atlántico), Advance Yield Trial, Multiplication Field; selections from Paulino 2002; Absalón Charria.

This trial includes 36 cultivars, mostly SM and a few CM families, planted in three replications in 105 field plots. Three pests, *O. peruvianus, A. socialis* and the stemborer *C. clarkei* appeared during the crop cycle. The highest populations were of the mite, *O. peruvianus.*

Results show that 78% (28 of 36) of the cultivars had an *O. peruvianus* population rating between 4.0 and 5.0 (Figure 10.23); 7 clones (19.4%) had an intermediate population rating (3.0), while one clone, SM 2773-21 had a population and damage rating of 1.0. Nineteen

clones (52.8%) had a 1 to 2 stemborer (*C. clarkei*) perforations per 6 plants evaluated. Eleven (30.6%) clones resulted in 3 perforations, 2 clones with 4, 1 clone with 5 and 2 clones with 6 to 7 adult exit holes per 6 plants.

Whitefly (*A. socialis*) presence was recorded on 31 (86.1%) clones at a population rating of 2.0; there was no leaf damage detected.

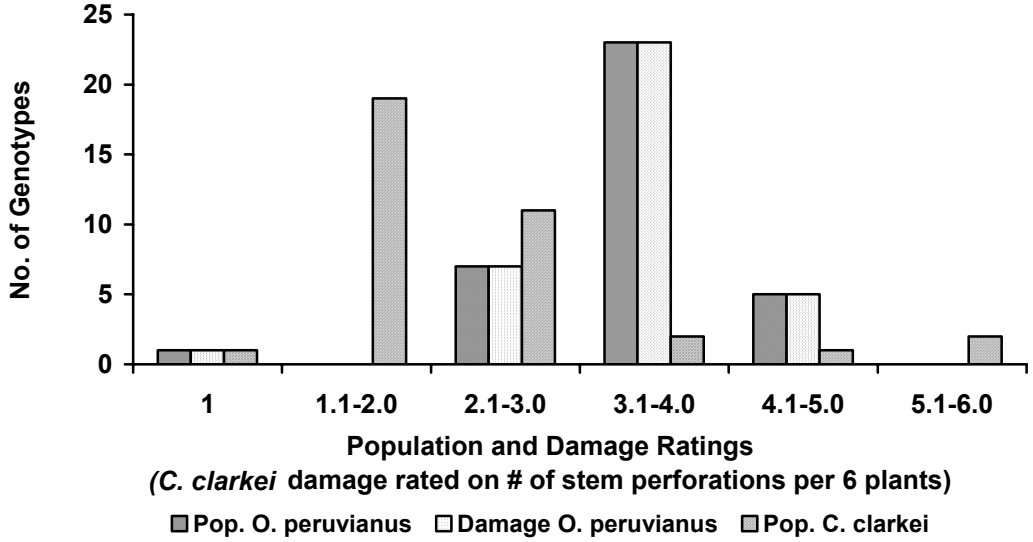

Figure 10.23. *Oligonychus peruvianus* and *Chilomima clarkei* populations and damage on cassava genotypes in Advanced Yield Trials (selected from Paulina Yield Trials) at Absalón Charris, Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

13. Santo Tomás (Atlántico), Regional Trial CORSUCRE, Lote Teobaldo, La Rosa.

This trial consisted of 29 clones, planted in three replications, totaling 90 field plots and including SGB, SM, CM families plus MVen and MPan germplasm bank accessions and the control MTai 8. Several pests occurred during the crop cycle, including two mite species, *O. peruvianus, Mononychellus* sp, whiteflies and the stemborer, *C. clarkei*. The *Mononychellus mite* was present only in a few cultivars.

O. peruvianus populations reached the 4.0 to 5.0 levels in 21 of the 29 clones (72.4%); 7 clones were at the intermediate (3.0) level and one clone; SM 2450-5 had a 2.0 population level (Figure 10.24). The control MTai 8 had population ratings between 3.0 and 4.0.

All 39 cultivars supported whitefly (*A. socialis*) populations but at low levels; 28 (96.6%) were recorded at intermediate (2.0to 3.0) levels, and with no apparent leaf damage.

Stemborer, *C. clarkei*, levels were low to intermediate; 12 cultivars (41.4%) had no adult exit perforations in the cassava stems. Twelve cultivars had 1 to 2 perforations per six plants, 3 had 3 perforations, 1 with 4 perforations and 1 with 5 (these included SM 2450-5, SM 1521-10, SM 1759-29, SM 1973-25 and SM 1669-5).

Mononychellus mite damage occurred on the clones CM 6119-5 (levels 3.0 and 4.0 for population and damage respectively), SM 2450-5 (damage rating 3.5), SM 1759-29 (3.0 damage) and SM 1669-5 (3.0 damage).

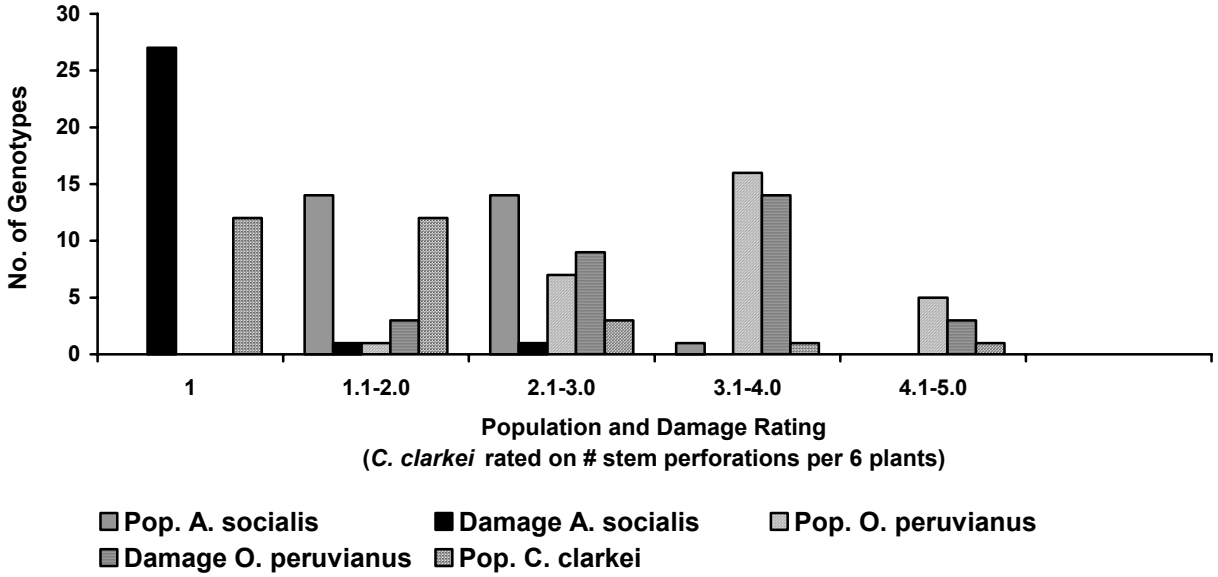

Figure 10.24. Pest populations (*Oligonychus peruvianus, Chilomima clarkei* and *Aleurotrachelus socialis*) and damage on cassava genotypes at Corsucre Regional Trial, Teobaldo La Rosa Block, Pitalito, Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

14. Santo Tomás (Atlántico), Field Selections/Observation Trial of Dialelics. Finca Tomás Fontalvo.

In this trial 60 cultivars were planted in three replications (180 field plots) and included GM and some CM families plus two controls, MTai 8 and SM 1438-2. Two pests predominated, the mite *O. peruvianus* and the whitefly, *A. socialis*.

Results show that whitefly populations were present on 651 of the 58 cultivars (88%); however few damage symptoms were observed (Figure 10.25), as population levels were around 2.0. Only one cultivar, CM 266-3 reached population level 4.0 and CM 9966-24 had no whiteflies present.

Highest pest populations were of *O. peruvianus*; 38 of the 58 cultivars (65.5%) presented a population and damage rating between 4.0 and 5.0 (Figure 10.25). Six cultivars (CM 9907-3, GM 238-4, GM 258-25, GM 289-13, GM 258-2 and GM 272-9) resulted in a 2.0 damage rating.

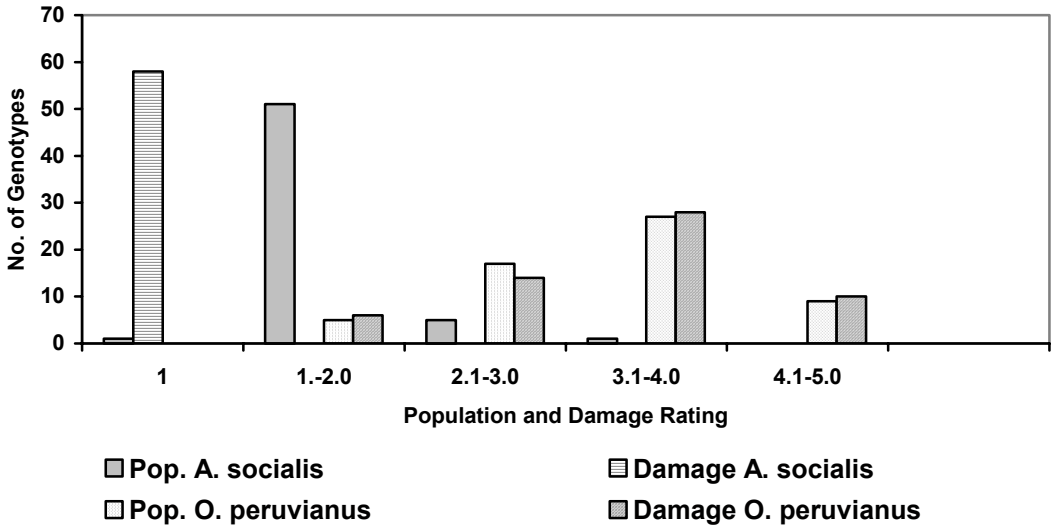

Figure 10.25. *Oligonychus peruvianus* and *Aleurotrachelus socialis* population and damage ratings on cassava genotypes in Observation Field Trials, Dialelic Crosses, Experiment # 7. Santo Tomás (Atlántico, Colombia), CIAT, 2003-04.

15. Regional Trials (8 cultivars).

This trial consists of advanced cultivars that have been evaluated numerous times, and also includes varieties that have been released by CORPOICA as commercial varieties. The 8 cultivars in this trial were planted at three different sites (Caracolí, Pitalito and Santo Tomás) 3 replications of 24 plots at each locality.

Three pest species appeared in the trial across the three sites, the mite, *O. peruvianus*, stemborers (*C. clarkei*) and whiteflies (*A. socialis*). *O. peruvianus* maintained the highest population, and levels were consistent throughout the three trial sites (population was slightly higher at the Caracolí site) (Table 10.13 Activity 10.6). Whiteflies and stemborer (*C. clarkei*) populations were higher at the Pitalito site and lowest at the Finca Teobaldo La Rosa, in Santo Tomás.

These results indicate that the cultivars comprising this trial are susceptible to the mite *O. peruvianus*. Observations on *C. clarkei* presence in these trials show that the cultivars CM 4843-1 and CM 491-1 had the lowest number of adult exit perforations pr 6 plants (1 hole per 6 plants) while the highest was CM 3306-19 with an average of 3.3 holes per 6 plants.

Whitefly (*A. socialis*) was low at all three sites.

Contributors: Bernardo Arias, Anthony C. Bellotti.

Collaborators: José María Guerrero, Gustavo Trujillo, Gerardino Pérez, Jorge Iván Lenis.

Activity 10.6 Observations on the incidence, damage and behavior of whitefly (A. socialis), mites (O. peruvianus) and other arthropod pests in germplasm (breeding) trials on the Atlantic Coast of Colombia.

This activity is a composite of the results presented in the numerous trials and experiments described in the previous Activity 10.5. These trials are part of the "Cassava Germplasm Improvement Project" to develop commercial varieties for the lowland tropical, seasonally dry agroecosystems. Several arthropod pests were observed and recorded during the year cropping cycle in these trials. However two pests, the mite, *Oligonychus peruvianus*, and the whitefly *Aleurotrachelus socialis* predominated and occurred in most of the fields where the trials were located. Other pests that occurred sporadically or at very low populations include the *Tetranychus* and *Mononychellus* mites, the stemborer, *Chilomima clarkei* and thrips (*Franklinella williamsi*).

A. socialis populations were present in nearly all trials in all fields (Table 10.12) although usually in low populations. Whitefly incidence seldom went above 2.0 on the population scale (1 = no whitefly present; 6 = high populations > 4000 per leaf); this rating represents evidence of whitefly presence, but usually leaves are void of any damage symptoms. High whitefly (*A. socialis*) populations have not been recorded nor observed on the North Cost of Colombia. This may be due to several factors, including rainfall patterns, temperature, humidity or other abiotic constraints. As can be seen in Table 10.12, whitefly populations were above 3.0 rating on few clones and at few sites.

Table 10.12. Summary of whitefly (*A. socialis*) incidence and populations levels on numerous germplasm (breeding) trials on the Atlantic Coast of Colombia (2003-04).

Trial	Population Rating of *Aleurotrachelus socialis*					
	1	1.1-2	2.1-3	3.1-4	4.1-5	5.1-6
O.F.T 1, 2, 3 (1155)	570	566	20	0	0	0
Exp. 2. Leaf retention (60)	10	34	1	0	0	0
Exp. 1. Leaf retention (51)	0	44	6	1	0	0
New Exp. 1. Sel.. O.F.T Santo Tomás (78)	9	68	1	0	0	0
New Exp. 2. Sel. O.F.T. Santo Tomás (58)	19	39	0	0	0	0
New Exp. 3. Sel. O.F.T. Santo Tomás (69)	0	49	20	0	0	0
Exp. 4. O.F.T. 2002-03 (110)	7	95	8	0	0	0
Exp. 5. O.F.T. 2. 2002-03 (109)	4	92	12	1	0	0
Exp. 6. O.F.T. 3. 2002-03 (106)	1	32	59	14	0	0
Exp. 7. Sel. O.F.T Dialelic (58)	1	51	5	1	0	0
Multipl. O.F.T Sel. Dialelic 2003 (62)	10	52	0	0	0	0
A.Y.T. Sel. in Yield Trials Paulina. Palapa (34)	18	16	0	0	0	0
A.Y.T. Sel. in Yield Trials Paulina (36)	0	5	31	0	0	0
A.Y.T. Sel. in Yield Trials Paulina (29)	0	0	14	14	1	0
R.T. Pitalito (8)	0	4	3	1	0	0
R.T. Santo Tomás (8)	0	8	0	0	0	0
R.T. Caracolí (8)	0	8	0	0	0	0

A.Y.T. = Advanced Yield Trials, O.F.T. = Observation Field Trails, R.T. = Regional Trials

O. peruvianus populations were present in all of the sites and fields, and often at very high populations (Table 10.13). In fact few sites had populations at the lower level of the scale (1.0 to 2.0). In all sites and fields, populations reached the plant damage level (3.0 to 6.0) (Table 10.13). High populations at the 3.0 to 5.0 level occurred frequently. This data indicates that the mite *O. peruvianus* may be a more important economic pest in this

agroecosystem than we originally considered. Plant physiology studies have shown that leaf retention is an important plant characteristic to achieve and maintain high cassava root yields in this agroecosystems. *O. peruvianus* primarily attacks the lower to mid plant leaves causing chlorotic spotting, necrosis and eventually defoliation. Leaf retention, therefore, could be greatly hindered by *O. peruvianus* attack and adversely effect root yields. This needs to be investigated.

Table 10.13. Summary of *Oligonychus peruvianus* mite incidence and population levels on numerous cassava germplasm trials on the Atlantic Coast of Colombia (2003-04).

Trial	Population Rating *Oligonychus peruvianus*					
	1	1.1-2	2.1-3	3.1-4	4.1-5	5.1-6
O.F.T. 1, 2, 3 (1155)	38	304	344	325	140	4
Exp. 2, Leaf retention (60)	0	2	24	28	6	0
Exp. 1, Leaf retention (51)	0	1	8	23	19	0
New Exp. 1. Sel. O.F.T. Santo Tomás (78)	0	4	29	27	18	0
New Exp. 2. Sel. O.F.T. Santo Tomás (58)	1	8	20	24	5	0
New Exp. 3. Sel. C.O. Santo Tomás (69)	0	0	9	42	18	0
Exp. 4. O.F.T. 2002-03 (110)	0	4	19	52	35	0
Exp. 5. O.F.T. 2.2002-03 (109)	0	6	25	49	29	0
Exp. 6. O.F.T. 3.2002-03 (106)	0	2	8	31	65	0
Exp. 7. Sel. O.F.T. Dialelic (58)	0	5	17	27	9	0
Multipl. O.F.T. Sel. Dialelic 2003 (62)	0	1	9	28	24	0
A.Y.T. Sel. in Yield Trials Paulina. Palapa (34)	0	2	7	20	5	0
A.Y.T. Sel. in Yield Trials Paulina (36)	1	0	7	23	5	0
A.Y.T. Sel. in Yield Trials Paulina (29)	0	1	7	16	5	0
R.T. Pitalito (8)	0	0	0	3	5	0
R.T. Santo Tomás (8)	0	0	2	5	1	0
R.T. Caracolí (8)	0	0	0	3	5	0

A.Y.T. = Advanced Yield Trials, O.F.T. = Observation Field Trails, R.T. = Regional Trials

Contributors: Bernardo Arias, Anthony C. Bellotti.

Collaborators: José María Guerrero, Gerardino Pérez, Gustavo Trujillo.

Activity 10.7 Cassava germplasm evaluations to identify resistance to the cassava green mite, Mononychellus tanajoa.

More than 40 species of mites have been reported as feeding on cassava from the numerous cassava growing regions of the world. Mites are a universal pest of cassava and can cause severe yield reductions, especially in seasonally dry agroecosystems with a three-month or longer dry period. The cassava green mite (CGM), *M. tanajoa*, is considered the major species causing cassava crop damage on two continents, the Americas and Africa,

In Africa yield losses are reported ranging from 13 to 80% (Yaninek and Herren, 1988), while in the Americas yield loss of 15 to 73% are reported, depending on duration of attack and varietal susceptibility (Byrne, et al, 1982, 1983). HPR research at CIAT has concentrated on identifying cassava genotypes resistant to *M. tanajoa*. More than 5000 landrace cultivars in the CIAT Cassava Germplasm Bank have now been evaluated for CGM resistance. Approximately 6% (about 300 cultivars) have been identified as having low-to-moderate levels of resistance. After considerable effort over numerous years, some cultivars with moderate levels of resistance have been developed and released to farmers.

A considerable effort has also been invested in the biological control of the CGM and numerous natural enemies, primarily the mite predators from the family Phytoseiidae, have been identified and evaluated. The role of these predators as biological control agents is well documented (Bellotti et al, 1999). It is considered that a CGM management strategy based on the combination of low to moderate levels of resistance and adequate biological control is critical for sustaining high cassava yields in seasonally dry agroecosystems where frequent mite outbreaks occur.

The cassava germplasm development project (IP-3) develops hybrids with varying levels of resistance to mites as well as other important cassava pests. A considerable number of progeny are produced each year from controlled crosses. Many of these progeny are evaluated for CGM resistance/susceptibility, in close collaboration with the cassava plant breeders.

Objective
The overall objective of this activity is to identify cassava landrace cultivars, and genotypes (progeny) from controlled crosses for resistance to CGM (*M. tanajoa*).

Methodology
The genotypes evaluated during the course of the year were mostly planted during August of 2003. Most mite (CGM) damage evaluations were done during January 2004, at the height of the dry season, when mite populations are at their highest. Cassava plants are about 5 months old at this time.

A total of 1286 genotypes were evaluated; these were divided in seven separate experiments, all planted at CIAT, Palmira (zone 4). One experiment is an observation field of 878 genotypes (progeny) from controlled crosses. Three experiments totaling 300 genotypes being evaluated for adaptation to zone 4, the mid altitude tropics, represented by the CIAT agroecosystems. The third group of these experiments, totaling 108 genotypes, are selected and evaluated for adaptation to the Tolima-Huila cassava agroecosystems. Evaluations are accomplished using a 1 to 6 mite damage scale, where 1 = no damage and 6 = growing point completely reduce, no apical leaves and severe yellowing of lower leaves.

Results
In general, mite populations were high. Figure 10.26 is a composite of all the genotypes evaluated in the seven experiments. Of the 1286 genotypes evaluated, 152, nearly 12%, had a damage rating of 2.0 or lower and 519 or 40%, had a rating of 3.0 or lower. Sixty percent (767 genotypes) had a damage rating of 4.0 or above, indicating considered CGM selection pressure in the field. This latter group is considered susceptible to the CGM and not included for continued evaluations. Those genotypes with 1-3 ratings, indicating low to moderate levels of resistance, are further evaluated for agronomic qualities as determined by the cassava germplasm improvement sections. This includes fresh root yield, dry matter content, plant type, and harvest index (see Annual Report, Project IP-3, 2003).

Figure 10.26. Damage ratings (1=no damage; 6=severe damage) caused by the cassava green mite, *Mononychellus tanajoa* feeding on 1286 cassava genotypes evaluated at CIAT, Palmira, during the 2003-04 growing cycle.

In experiment 1, the Observation Field, Zone 4, 878 progeny from 49 families were evaluated (Table 10.14). Forty eight percent (425) of the genotypes resulted in a damage rating between 2.0 and 3.0 (Figure 10.27). Of these, only 112 genotypes were selected to be included in the next evaluation cycle; of these, 41 genotypes had a damage rating of 2.0 (Table 10.15).

Table 10.14. Observation Field Trial; cassava families/genotypes evaluated for *Mononychellus tanajoa* (CGM) feeding damage at CIAT, Palmira, during the 2003-04 crop cycle.

Family	Parents		No. Progenies Evaluated	No. Selected < 3.0 Damage Rating	No. Selected for Continued Cycle
	Female	Male			
CM 9901	SM 1219-9	CM 6740-7	27	18	7
CM 9903	CM 6740-7	SM 1741-1	24	18	7
CM 9919	CM 7951-5	SM 1565-17	6	3	1
CM 9920	CM 7951-5	SM 1741-1	3	2	1
CM 9953	SM 1741-1	SM 1219-9	21	13	8
GM 228	CM 6740-7	SM 1278-2	18	9	1
GM 230	CM 6740-7	SM 1636-24	15	9	2
GM 254	SM 1219-9	SM 1278-2	10	4	2
GM 260	SM 1673-10	SM 1219-9	4	1	1
GM 266	MTAI 8	SM 1219-9	11	1	0
GM 268	SM 1278-2	SM 1673-10	15	5	2
GM 269	SM 1741-1	SM 1278-2	9	1	1
GM 284	SM 1741-1	SM 1636-24	20	9	5
GM 291	SM 1665-2	MTAI 8	18	4	3
GM 292	SM 1741-1	SM 1673-10	16	5	2
GM 295	SM 1741-1	SM 2219-11	20	4	1
GM 297	SM 1741-1	MPER 183	36	14	4
GM 306	MECU 72	MPER 183	26	21	0
GM 308	MECU 72	CM 6740-7	35	33	8
GM 309	MECU 72	SM 1219-9	17	13	6
GM 314	MECU 72	HMC 1	16	12	2

Family	Parents		No. Progenies Evaluated	No. Selected < 3.0 Damage Rating	No. Selected for Continued Cycle
	Female	Male			
GM 370	CM 2772-3	SM 1210-4	4	2	1
GM 372	SM 1460-1	CM 2772-3	17	9	2
GM 373	SM 1557-17	CM 2772-3	26	10	1
GM 374	CM 2772-3	SM 1660-4	34	8	4
GM 375	SM 1689-18	CM 2772-3	21	6	0
GM 473	SM 8151-1	CM 8370-11	13	5	1
GM 501	SM 1219-9	SM 1210-4	11	6	2
GM 502	SM 1210-4	SM 1460-1	14	9	0
GM 503	SM 1557-17	SM 1210-4	23	11	1
GM 509	SM 1557-17	SM 1219-9	22	8	2
GM 555	SM 1660-4	CM 8370-11	7	3	1
GM 556	SM 1660-4	SM 1210-4	12	5	0
SM 2802	SM 1219-9		20	6	2
SM 2859	CM 6740-7		3	2	0
SM 2860	CM 7951-5		12	4	0
SM 2982	CM 2772-3		13	6	2
SM 2983	CM 6740-7		7	4	1
SM 2985	SM 1219-9		21	9	1
SM 3085	SM 2772-3		34	15	3
SM 3087	CM 6740-7		28	13	3
SM 3090	SM 1210-4		11	6	1
SM 3091	SM 1219-9		29	10	5
SM 3092	SM 1460-1		26	14	2
SM 3094	SM 1660-4		14	9	0
SM 3096	SM 1741-1		23	14	6
SM 3097	MECU 72		16	15	4
SM 3098	MPER 183		28	10	3
SM 3099	MTAI 8		22	7	0

Three groups of genotypes (100 genotypes per experiment) with adaptation for zone 4, the mid altitude Andean ecosystem, were evaluated for mite (CGM) damage at CIAT. The 300 genotypes comprised 52 families (Table 10.16). Mite populations again were high and only 52 (17.3%) genotypes had a damage rating of 3.0 or lower and only 2 genotypes had a damage rating of 2.0 (Figure 10.28). Seventeen genotypes, or 5.7% were selected for continued evaluation by the breeding section (Table 10.17).

Figure 10.27. Damage rating (1 = no damage; 6 = severe damage) for *Mononychellus tanajoa* feeding on 878 genotypes from Field Observational Trials for zone 4 (mid- altitude tropics) at CIAT, Palmira, during 2003-04 crop cycle.

Table 10.15. Field Observation Trials: Cassava genotypes showing moderate levels of resistance to *Mononychellus tanajoa* and selected for further field evaluations at CIAT (2003-04).

Genotype	Genotype
CM 9901-167	GM 314-64
CM 9901-172	GM 372-4
CM 9903-201	GM 373-5
CM 9953-163	GM 374-22
CM 9953-173	GM 374-23
CM 9953-177	GM 503-22
CM 9953-178	GM 509-3
GM 230-74	SM 2802-104
GM 254-91	SM 2982-77
GM 260-90	SM 2982-79
GM 284-89	SM 2983-48
GM 292-48	SM 3085-8
GM 297-113	SM 3087-5
GM 308-52	SM 3087-18
GM 308-61	SM 3092-6
GM 308-78	SM 3092-10
GM 308-79	SM 3097-8
GM 308-84	SM 3097-9
GM 308-85	SM 3097-13
GM 308-86	SM 3098-13
GM 309-84	

The three groups of genotypes (experiments) selected for the Tolima-Huila agroecosystems were evaluated for *M. tanajoa* resistance at CIAT, Palmira. Twenty-seven families consisting of 108 genotypes were evaluated (Table 10.18). Forty-two genotypes resulted in a 1-3 damage rating (Figure 10.29) and of these 18 genotypes were selected for further planting and evaluation (Table 10.19).

Table 10.16. Zone 4, Mid-altitude Andean adaptation: cassava families evaluated for *Mononychellus tanajoa* feeding damage (1=no damage, 6=severe damage) at CIAT, Palmira, during 2003-04 crop cycle.

Family	Parents		No. Progeny Evaluated	Damage < 3.0	Selected Further Cycle
	Female	Male			
CM 6979	CM 523-7	HMC 1	3	0	0
CM 8884	CG 489-4	MCOL 1468	1	0	0
CM 8885	CG 489-4	MCOL 1505	3	0	0
CM 9642	MPER 183	CM 6740-7	3	0	0
CM 9901	CM 6740-7	SM 1219-9	3	1	1
CM 9903	SM 1741-1	CM 6740-7	15	5	1
CM 9953	SM 1219-9	SM 1741-1	9	0	0
GM 128	SM 2075-4	MTAI 8	6	0	0
GM 228	CM 6740-7	SM 1278-2	1	0	0
GM 234	HMC 1	CM 6740-7	6	2	1
GM 254	SM 1219-9	SM 1278-2	4	0	0
GM 260	SM 1219-9	SM 1673-10	3	0	0
GM 264	SM 1219-9	HMC 1	7	0	0
GM 265	SM 1219-9	MPER 183	5	1	0
GM 269	SM 1741-1	SM 1278-2	6	1	0
GM 270	SM 1278-2	HMC 1	1	0	0
GM 295	SM 1741-1	SM 2219-11	15	4	1
GM 297	SM 1741-1	MPER 183	17	3	3
SM 2651	CM 7951-5		7	1	0
SM 2652	SM 643-17		6	1	0
SM 2656	SM 1210-4		1	0	0
SM 2657	SM 1406-1		1	0	0
SM 2658	SM 1460-1		3	1	0
SM 2661	SM 1565-15		2	1	0
SM 2663	SM 1677-4		1	0	0
SM 2799	SM 643-17		5	5	0
SM 2801	SM 1210-4		4	0	0
SM 2802	SM 1219-9		6	0	0
SM 2803	SM 1460-1		4	0	0
SM 2804	SM 1565-17		10	4	2
SM 2858	CM 5655-4		22	7	4
SM 2860	CM 7951-5		9	1	0
SM 2861	SM 643-17		7	1	0
SM 2862	SM 653-14		14	2	1
SM 2863	SM 909-25		8	1	1
SM 2864	SM 1210-4		4	1	0
SM 2865	SM 1219-9		12	0	0
SM 2866	SM 1460-1		7	1	1
SM 2867	SM 1479-8		2	1	0
SM 2868	SM 1557-17		3	0	0
SM 2869	SM 1565-17		6	1	0
SM 2870	SM 1741-1		4	0	0
SM 2871	MBRA 12		3	2	0
SM 2913	CM 8602-12		9	1	0

Family	Parents		No. Progeny Evaluated	Damage < 3.0	Selected Further Cycle
	Female	Male			
SM 2982	CM 2772-3		2	0	0
SM 2983	CM 6740-7		11	1	1
SM 2988	SM 1543-16		7	0	0
SM 2992	SM 1673-10		2	0	0
SM 2998	MECU 72		3	2	0
SM 2999	MPER 183		1	0	0
SM 3045	HMC 1		4	0	0
SM 3047	MCOL 1505		4	0	0

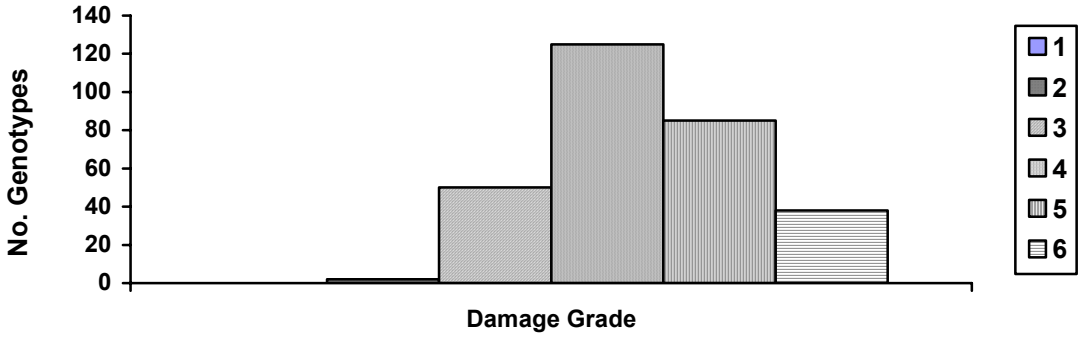

Figure 10.28. Zone 4, Mid-altitude Andean adaptation; damage ratings (1=no damage, 6=severe damage) on 300 genotypes of *Mononychellus tanajoa* feeding at CIAT, Palmira, during 2003-04 crop cycle.

Table 10.17. Zone 4, Mid-altitude Andean adaptation: Selected cassava genotypes for resistance to *Mononychellus tanajoa* (CGM) at CIAT, Palmira, during 2003-04 crop cycle.

Genotype	Damage/Reps. (1=no damage, 6=severe damage)		
	I	II	III
CM 9901-137	3	3	2
CM 9903-137	3	3	3
GM 234-132	2	3	3
GM 295-18	3	3	3
GM 297-47	2	3	3
GM 297-68	3	3	3
GM 297-69	3	3	3
SM 2804-33	3	3	3
SM 2804-45	2	3	3
SM 2858-4	2	2	3
SM 2858-14	3	3	3
SM 2858-31	3	3	3
SM 2858-47	3	3	3
SM 2862-15	2	2	3
SM 2863-9	2	2	3
SM 2866-10	2	3	2
SM 2983-13	3	2	3

Table 10.18. Tolima-Huila Agroecosystem Adaptation: Cassava families evaluated for *Mononychellus tanajoa* feeding damage (1=no damage, 6=severe damage) at CIAT, Palmira, during 2003-04 crop cycle.

Family	Parents		No. Progeny Evaluated	Damage < 3.0	Selected for Planting
	Female	Male			
CM 8035	MTAI 8	HMC 1	4	1	0
CM 9642	CM 6740-7	MPER 183	1	1	0
CM 9733	HMC-1	MPER 183	1	0	0
CM 9765	CM 6754-8	SM 653-16	1	0	0
CM 9791	SM 1433-4	MNGA 19	3	3	1
CM 9912	SM 1433-4	CM 7514-8	6	5	1
CM 9914	CM 7514-8	SM 1565-17	10	2	0
CM 9926	SM 1565-17	CM 8027-3	2	2	0
CM 9961	SM 1433-4	MTAI 8	2	1	0
CM 9962	SM 1438-2	SM 1565-17	5	2	1
CM 9966	SM 1565-17	MTAI 8	1	0	0
GM 234	CM 6740-7	HMC 1	7	2	1
GM 265	SM 1219-9	MPER 183	7	1	1
SM 1521	CM 3299-4		2	1	1
SM 2802	SM 1219-9		6	3	3
SM 2804	SM 1565-17		3	1	1
SM 2805	SM 1741-1		4	1	1
SM 2826	CM 4365-3		7	1	0
SM 2829	CM 7395-5		2	1	0
SM 2834	SM 1411-5		8	3	3
SM 2836	SM 1433-4		1	1	1
SM 2839	SM 1565-17		6	2	0
SM 2865	SM 1219-9		9	5	3
SM 2866	SM 1460-1		4	2	0
SM 2870	SM 1741-1		2	0	0
SM 2871	MBRA 12		1	0	0
SM 2882	CM 3372-4		3	1	0

The genotype CM 9912-92 displayed the highest resistance to CGM with a damage rating of 1.0 (no damage) in the three repetitions.

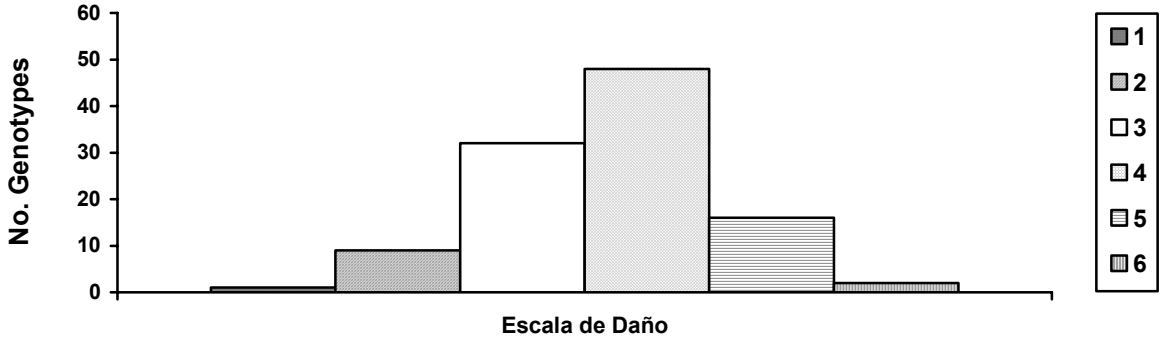

Figure 10.29. Tolima-Huila Agroecosystem Adaptation: Damage ratings (1=no damage, 6=severe damage) for *Mononychellus tanajoa* feeding on 108 genotypes at CIAT, Palmira, during 2003-04 crop cycle.

Table 10.19. Tolima-Huila Agroecosystem Adaptation: Selected cassava genotypes for resistance to *Mononychellus tanajoa* (CGM) at CIAT, Palmira, during 2003-04 crop cycle.

Genotypes	Damage/Reps.		
	I	II	III
CM 9791-64	2	2	2
CM 9912-92	1	1	1
CM 9962-1	2	2	2
GM 234-171	3	3	3
GM 265-173	3	3	3
SM 1521-27	1	2	2
SM 2802-76	3	3	3
SM 2802-78	2	3	3
SM 2802-84	2	2	2
SM 2804-63	2	2	3
SM 2805-21	2	2	3
SM 2834-43	2	3	3
SM 2834-55	3	3	3
SM 2834-60	3	3	3
SM 2836-59	3	3	3
SM 2865-64	3	3	2
SM 2865-97	2	2	2
SM 2865-99	3	3	2

It should be noted that mite populations in the field seldom display an even or uniform distribution. Therefore, genotypes that might appear as resistant in one crop cycle evaluation might result in a high (susceptible) damage rating in a subsequent evaluation. For this reason, damage ratings from numerous field evaluations are required before confirming CGM resistance in a particular cassava genotype. Laboratory or growth chamber studies can be used to support field observations.

References

Bellotti, A.C.; Smith, L.; Lapointe, S.L. 1999. Recent advances in cassava pest management. Annu. Rev. Entomol. 44:343-70.

Byrne, D.H.; Guerrero, J.M.; Bellotti, A.C.; Gracen, V.E. 1982. Yield and plant growth responses of *Mononychellus*_mite resistant and susceptible cassava cultivars under protected vs. Infected conditions. Crop Science 22(5-6):486-550.

Byrne, D.H.; Bellotti, A.C.; Guerrero, J.M. 1983. The cassava mites. Tropical Pest Management 29(4):378-394.

Yaninek, J.S.; Heren, H.R. 1988. Introduction and spread of the cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), an exotic pest in Africa, and the search for appropriate control methods: A review. Bulletin of Entomological Research 78:1-13.

Contributors: José María Guerrero, Fernando Calle, Anthony C. Bellotti.

Activity 10.8 Testing of transgenic cassava (Africa genotype TMS 60444) plants displaying indications of resistance to the cassava hornworm, Erinnyis ello.

Erinnyis ello, the cassava hornworm, is one of the most serious cassava pests in the neotropics (Bellotti et al, 1992). It has a broad geographic range, extending from the southern cone (Brazil, Argentina and Paraguay) of South America to the Caribbean Basin and southern USA. Hornworm larval feeding will defoliate cassava plants causing considerable yield reductions, especially if repeated attacks occur. Based on extensive research of this pest by CIAT and NAR's scientists an IPM program for hornworm control has been developed. The basis of this program is centered around biological control, especially the use of a baculovirus that has recently been developed as a commercial biopesticide (CIAT Annual Report, Project PE-1, 2002 and 2003).

The CIAT cassava germplasm bank consists of nearly 6,000 genotypes. Most accessions are traditional land race cultivars collected from farmers' fields and this material offers entomologists and breeders a potential pool of pest resistant genes. A high (60 to 70%) percentage of genotypes in this germplasm bank are consistently being grown in the field and subject to pest attack. Periodic evaluations of these genotypes when hornworm attacks have occurred have indicated that genetic resistance to *E. ello* is not available in cultivated cassava, *Manihot esculenta.*

Several years ago CIAT initiated research based on introducing insect resistant *Bacillus thuringiensis* (Bt) genes (Cry 1Ab) through *Agrobacterium*-mediated transformation into cassava embryonic tissue to develop lepidopteran resistant cultivars. Transgenic plants of the model variety of African origin, TMS 60444 (MNg 11) have been developed. This genotype is the progeny of an interspecific cross of the wild species *Manihot glaziovii* and *M. esculenta*. *M. glaziovii* is also the source of resistance to ACMD (African Cassava Mosaic Disease), and in preliminary evaluations at CIAT has displayed resistance to other pests such us whiteflies. TMS 60444 was selected because of its high transformation capacity and relatively rapid regeneration (Bellotti et al, 2002).

Objective

1. Determine the leaf consumption rate of the cassava hornworm, *E. ello*, on different genetically modified lines the variety TMS 60444.
2. Quantify the effect of the *Gen* Cry 1Ab in transgenic lines on the behavior and feeding of the cassava hornworm.

Methodology

Hornworm larvae were obtained from the laboratory/field colony maintained at CIAT. The cassava variety CMC-40, a susceptible genotype was grown out in farmers and CIAT fields. TMS 60444, non-modified genetically and resistant to the hornworm was grown at CIAT. The genetically modified lines L27, L80 and L92, originally from TMS 60444 were produced at CIAT.

The cassava hornworm, *E. ello*, colony is maintained by placing adults (male and females) in large field cages (2m x 2m x 2m) where females can readily oviposit on growing cassava plants. Eggs are removed to the laboratory where larval instars (5) develop in cages while feeding on cassava leaves. Recently emerged first instar larvae were used in all experiments; the first leaves fed-upon were those of each respective treatment.

The experiment had six treatments and twenty replications per treatment. The experimental arena was a plastic petri dish (15mm x 2.5mm) that contained excised cassava leaves. One first instar *E. ello* larvae was introduced into each petri dish and allowed to feed on the cassava leaf. All larvae were weighed on an analytical balance prior to being placed in the petri dish. It was therefore possible to record any weight gain or loss during the larval feeding period. Larvae were weighed every 24 hours and cassava leaves were replaced on a daily basis, until pupation or larval mortality occurred. Chi square analysis was used to evaluate mortality vs. variety (treatment).

Results

Hornworm (*E. ello*) mortality reached 100% on the transgenic lines L80, L92, and 85% mortality on L27 (Figure 10.30). On the latter 15% of the larvae reached the prepupal stage. Mortality on the non-modified control variety, CMC-40 was 25%. Mortality on the non-modified variety, TMS 60444, was 100%. The Chi square test showed that the mortality was independent of the genotype.

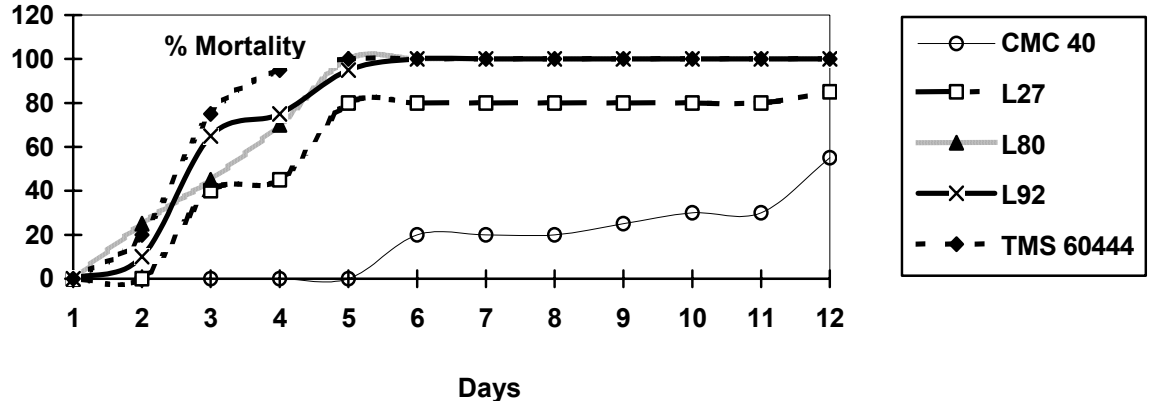

Figure 10.30. Percent mortality of cassava hornworm (*Erinnyis ello*) larvae consuming leaves from Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) cassava genotypes (CIAT, 2004).

Peak mortality on the transgenic lines and non-modified TMS 60444 occurred during the first 3 to 5 days of larval development. Larval mortality on the susceptible control, CMC-40, first occurred at 6.6 days (Table 10.20). There were no statistical differences between the transgenic genotypes and TMS 60444, but all four genotypes were statistically different from CMC 40 (Table 10.20).

These results show that the TMS 60444 genotypes, have a "natural" resistance to *E. ello* and that this resistance masks the effect of the Bt gene inserted into the transgenic lines. The rapid mortality of *E. ello* larvae feeding on the modified or non-modified TMS 60444 genotypes, when compared to the susceptible control (CMC 40) is additional evidence of the effectiveness of the natural resistance in TMS 60444.

Table 10.20. Average number of days when initial hornworm (*Erinnyis ello*) larval mortality occurs on transformed (BT, Cry 1Ab) (L27, L92, L80) and non-transformed (CMC 40, TMS 60444) cassava genotypes (CIAT, 2004).

Genotypes	Days Mortality Initiated (Average)
CMC-40	6.6 A
TMS 40666	3.1 B
L-27	4.4 B
L-92	3.5 B
L-80	3.6 B

E. ello larvae feeding on TMS 60444 and the transgenic lines show a significant reduction in daily weight gain when compared to the susceptible control, CMC 40 (Figure 10.31). Daily weight gain on TMS 60444, L80 and L92 was significantly lower than on L27, which was significantly lower than on CMC 40 (Table 10.21).

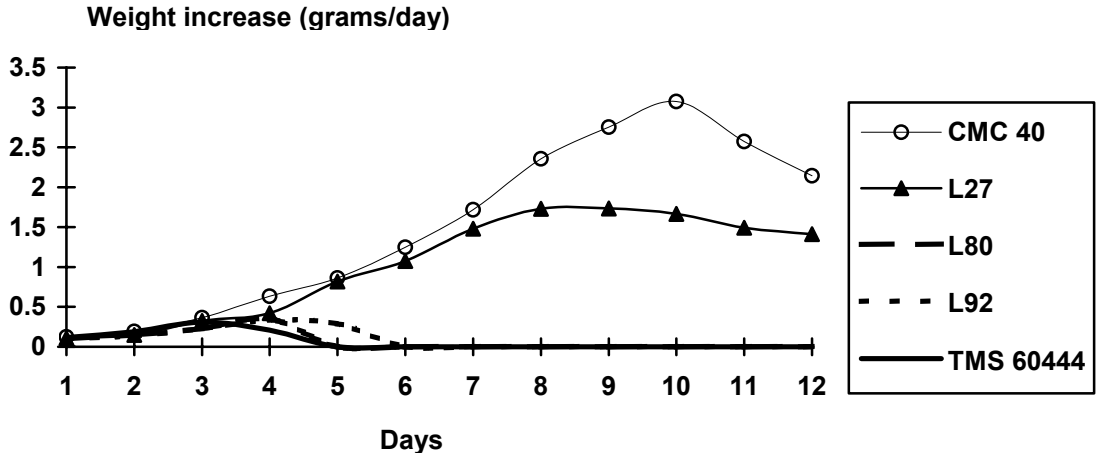

Figure 10.31. Daily weight increase of cassava hornworm (*Erinnyis ello*) larvae feeding on leaves from Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) on cassava genotypes (CIAT, 2004).

Table 10.21. The area below the growth curve as a function of the weight and mortality of cassava hornworm (*Erinnyis ello*) larval feeding on Bt transformed (L27, L80, L92) and non-transformed (CMC 40, TMS 60444) cassava genotypes (CIAT, 2004).

Cassava Genotypes	Area Below Curve
CMC 40	9.1176 A
L27	6.7492 B
L80	5.8309 C
L92	5.8156 C
TMS 60444	5.6588 C

The area below the growth curve is a function of larval weight increase and mortality on the Bt transformed and non-transformed genotypes.
These results show no significant difference between the transgenic lines L80, L92 containing the Cry 1Ab gene from *Bacillus thuringienses* and the non-modified TMS 60444. This

indicates that the TMS 60444 genotype has genes independent of Cry 1Ab that expresses resistance to the cassava hornworm, *E. ello*. As stated earlier, numerous years of observation (at least 30) of the CIAT *M. esculenta* germplasm bank did not detect any resistance to *E. ello*. This leads to the speculation that the source of the "natural" resistance found in TMS 60444 originated from the interspecific cross with *M. glaziovii*, a parent in its development in Africa nearly 70 years ago.

A genetic study is needed to identify the gene or gene sequence responsible for the *E. ello* resistance detected in the TMS 60444 lines.

References

CIAT (Centro Internacional de Agricultura Tropical). 2002. Integrated pest and disease management in major agroecosystems. Annual report, Project PE-1, Cali, CO. 264 p.

CIAT (Centro Internacional de Agricultura Tropical). 2003. Integrated pest and disease management in major agroecosystems. Annual report, Project PE-1, Cali, CO. 258 p.

Bellotti, A.C.; Arias, B. Guzmán, O.L. 1992. Biological Control of the Cassava Hornworm, *Erinnyis ello* (Lepidoptera: Sphingidae). Florida Entomology. 75:506-515.

Bellotti, A.C.; Roca, W.; Tohme, J.; Chavarriaga, P.; Escobar, R.; Herrera, C.J. 2002. Biotecnología para el manejo de plagas en la producción de semilla limpia. *In:* La yuca en el tercer milenio; sistema moderno de producción, procesamiento, utilización y comercialización. Centro Internacional de Agricultura Tropical (CIAT), Clayuca, Cali, CO. p. 255-268.

Collaborators: Carlos Julio Herrera, Anthony C. Bellotti, Paul Chavarriaga, Danilo López.

Activity 10.9 Determining the plant metabolites involved in whitefly (*Aleurotrachelus socialis*) resistant cassava varieties, MEcu 64, MEcu 72 and MPer 334.

The whitefly, *Aleurotrachelus socialis*, is a major pest of cassava, reducing root yield and the formation of cassava planting material (cuttings or stakes). Field evaluations during a 1, 6- and 11-month attack resulted in yield losses of 5, 42 and 79% respectively (Bellotti and Vargas, 1986). Whiteflies cause direct damage to cassava by feeding on the phloem of leaves, inducing leaf chlorosis and abscission, which results in reduction in root yield if feeding is prolonged (Bellotti, 2002). Additional yield reduction can be caused by the growth of a "sooty-mold" that grows on whitefly exudates deposited on cassava leaves and deters photosynthesis (Bellotti and Vargas, 1986).

The CIAT cassava germplasm bank contains nearly 6000 accessions, of which 93% are landraces (locally selected cultivars), collected from tropical and subtropical regions of the world, but mainly from the Neotropics. This germplasm collection has been extensively screened in the field for whitefly (*A. socialis*) resistance, more than 5400 landrace cultivars have been evaluated. Sources of resistance to *A. socialis* have now been identified. The clone "MEcu 72" has consistently expressed high level of resistance. Several additional cultivars, including "MEcu 64; MPer 334, MPer 415, MPer 317, MPer216, MPer 221, MPer 266 and

MPer 365, have expressed moderate to high levels of resistance. These results also indicate that *A. socialis* resistance may be concentrated in Peruvian and Ecuadorian germplasm. In greenhouse and field studies show that *A. socialis* feeding on resistant clones had less oviposition, longer development period reduced size and higher mortality than those feeding on susceptible one (Arias, 1995). *A. socialis* nymphal instars feeding on MEcu 72 suffered a 72.5% mortality, mostly in the early instars (Arias, 1995, Bellotti and Arias, 2001).

Recent studies under controlled conditions in the growth chamber, *A. socialis* had a longer development cycle when feeding on MEcu 64, MEcu 72 and MPer 344 when compared to the susceptible control, CMC 40. Nymphal mortality was highest on MPer 334 (77.5%), followed by MEcu 64 and MEcu 72 with 68.5% and 68.0% respectively. In addition genomic sequences possibly involved in *A. socialis* resistance have been detected in MEcu 72 using AFLP and microsatelite markers (Bellotti, et al, 2003).

Plant strategies for resisting insect attack often involved biochemical factors or activities. Studies were therefore initiated to determine what plant metabolites might be involved in the development of *A. socialis* resistance found in the resistant genotypes. MEcu 64, MEcu 72 and MPer 334.

Materials and Methods

Electrophoresis, employing polyacrylamide gels (PAGE) has proven to be a very useful technique for the analysis and characterization of complex protein mixtures. Nevertheless, since access into the interior of protein matrixes is limited, information generated about the individual components is usually restricted to molecular weight and isoelectric dots. The transfer of proteins by PAGE to an unfixed membrane, permits the utilization of diverse tests for an improved characterization. One of the more precise applications for the transfer of proteins to membranes, is through inmunodetection which consists of the identification and characterization of a fixed antigen by means of antibody tests (Timmons and Dunbar, 1990); Garfin, 1990; Anderson, 1988; Hames and Richwood, 1988; Dunbar, 1987),

Inmune-detection permits estimating by semiquantitative means, the mass or abundance of a specific protein in a determinate tissue. This technique is regularly employed in experimental studies in which the objective is to detect a specified protein or to observe its variation under diverse conditions.

It was decided that the first stage of this study would be carried out to determine if a relationship exists between leaf proteins in the resistant genotypes, MEcu 64, MEcu 72 and MPer 334, and the resistant characteristics they display to *A. socialis*; the susceptible genotype CMC 40 was used as the control. The plan includes obtaining polyclonal antibodies from the immunization of rabbits against protein extracts for each of the materials, and later to determine by means of immunodetection, and the combination of Western Blot and 2D SDS-PAGE techniques, the differences between each of the protein extracts. This process will be carried out using healthy plants (non-infested), and plants infested with *A. socialis*, for each of the genotypes, to see if a proteic response occurs in infested plants. In addition, *A. socialis* feeding on resistant plants will be examined for the presence of a plant protein.

Total Protein Extraction

To extract the total protein, cassava leaves (without petioles) were macerated in liquid nitrogen, obtaining a very fine powder that was subsequently homogenized for five hours at

4°C with the buffer Tris HCL, pH 8.0, and containing 1mM of EDTA (metalloprotease inhibitor), 5 mM of DTT (reduction agent), 1% PVP (antiphenolic), and 5 mM of PMSF (serine protease inhibitor) at a proportion of 1g macerated leaf to 3ml of buffer. The following step consisted of filtering this mixture and centrifuging it at 15000 rpm for 30 minutes at 4°C, to clarify the extract and eliminate vegetative tissue. The supernadant is dialyzed with a dialysis membrane of W.M. Co. 3.5 Kd and finally lyophilized to obtain an extract in powder form, in order to manipulate the concentration by weight units.

Immunization and Production of Polyclonal Antibodies against Cassava Proteins

Polyclonal antibodies were used as they contain different sub-classes of antibodies, including IgG, IGM, IGE, IgA and IgD. Each antibody represents the product of only one stimulated lymphocyte and its clonal progeny. An antigen complex such as a protein can contain several distinct or epitopes or determinant antigens, each of which is specifically recognized by antibodies from only one clonal lymphocyte (Dunbar and Schwoebel, 1990).

To produce polyclonal antibodies the following steps were developed:

- Two milligrams of each protein was dissolved in 1 ml of the buffer Tris-Glicina pH 6.8 and later emulsified with one ml of Freund's complete adjuvant.
- Four New Zealand breed rabbits were employed. Each of them was subcutaneously injected four times with 0.5 ml of each of the prepared proteins. The injections were applied to the animal's loin.
- After three weeks, the four applications were repeated on each rabbit, but at this time the proteins were emulsified with 1ml of Freund's incomplete adjuvant. Two of the injections were intermuscular.
- Ten days after the last injections, the animals were bled, obtaining 15-20 ml of blood from each.
- The collected blood was left at room temperature for 24 hours, then centrifuged and the serum was stored coagulated in aliquots for later analysis.

Test for Antibody Recognition using the Dot Blot Technique

A test for antibody recognition using the Dot Blot technique was carried out to verify that the antibodies produced were in good condition. The following steps were developed:

- One milligram of each of the proteins was dissolved with 200 µl of Tris Glycine (pH 6.8) buffer. On each nitrocellulose membrane 5 µl of the stock solution was applied to each of the proteins.
- Blockage of the nitrocellulose membrane with the sample in TBS containing 1% gelatine.
- Exposure of the membrane to 30 µl of the first antibody dissolved in 30 ml of blockage solution.
- Four washings of the membrane of 15-minutes each. The first three with TTBS (TBS containing 1% tween 20) and the last with TBS.
- Exposure of the membrane in 30 µl of the second antibody (Bound to PER) dissolved in 30 ml of the blockage solution.
- Four washings of the membrane of 15-minutes each. The first three with TTBS (TBS containing 1% tween 20) and the last with TBS.
- Addition of 5 ml of revealed solution (40 ml of TBS, 3 µl of hydrogen peroxide and 30 mg of 4 Chloro-1-Naphtol dissolved in 10 ml of methanol). This solution is preheated at 35°C.

SDS-PAGE Electrophoresis

Using electrophoresis trials with polyacrilamide gels in disnatured conditions (SDS-PAGE) it was determined:

- Protein sample concentrations (mg/ml) carried on gel pools for a visualization of the bands. To do this, concentrations of 200 mg/ml, 100 mg/ml, 75 mg/ml, 50 mg/ml, 25mg/ml, 10 mg/ml and 2mg/ml were tested.
- Adequate concentrations of the resolving phase of the gel were achieved for a good view of the protein bands. To do this, concentrations of 10%, 14%, and 17% were tested. It should be noted that the phase stacking concentration was 4% at all times.
- Polymorphism by molecular weight for each of the proteins for each genotype evaluated. To do this a marker of the Prestained SDS-PAGE from Biorad Laboratories (with a arrange of 106 to 20.8 Kd) molecular weight was utilized.

These tests were carried out in a Biorad Mini Protean electrophoresis chamber and followed the protocol established by the manufacturer for both the electrophoresis as well as the staining of the gels.

Results

Tests for antibody recognition using Dot Blot. By sing the afore-described methodology a clear recognition of the antibodies for each of the genotype extracts was achieved and evaluated. In addition a good staining (concentration) of the polyclonal antibodies originating from each genotype was observed, owing to the high intensity of each marker (Figure 10.32).

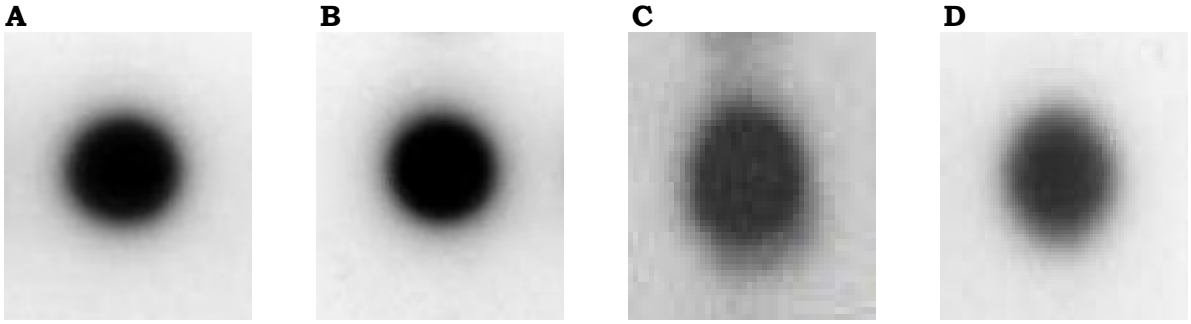

Figure 10.32. Test for antibody recognition using the Dot Blot technique. A: antibodies against MEcu 72, B: antibodies against MEcu 64, C: antibodies against MPer 334, D: antibodies against CMC 40.

These results indicate that the process for immunization and production of the antibodies using the described procedures was successful; therefore it is possible to continue with the cross-tests for immunodetection of proteins for both the varieties being evaluated, as well as for *A. socialis*.

SDS-PAGE Electrophoresis

It was determined that the protein sample concentration that best provides a good visualization of the bands is 2mg/ml. This concentration provided for well defined bands without vertical streaking of protein, as occurred with the other concentration evaluated (Figure 10.33).

The protein concentration that gave adequate results for the resolving phase by providing good visualization of the protein bands was 14% (Figure 10.33). With the other concentrations the distribution of the bands along the gel were not uniform and very congested on the lower part of the get at the 10% concentration, while they were congested at the top of the gel at the 17% concentration.

In Figure 10.33, polymorphic bands can be observed between the resistant and susceptible genotypes, with molecular weights between 47.5 and 35 Kd. A common polymorphic band is clearly noted in the resistant genotypes (black arrow), although it is less intense for MEcu 64. The genotype MPer 334 shows a high polymorphism as well as an additional band that is absent in the other genotypes (yellow arrow). The yellow circle on Figure 10.33, indicates the absence of these aforementioned protein bands on the susceptible genotype, CMC 40. These results are a good indication that these protein immunodetection tests should be continued on these genotypes; the differences shown between the resistant and susceptible genotypes is a good indication that a relationship exists between these proteins and the presence of resistance to *A. socialis*.

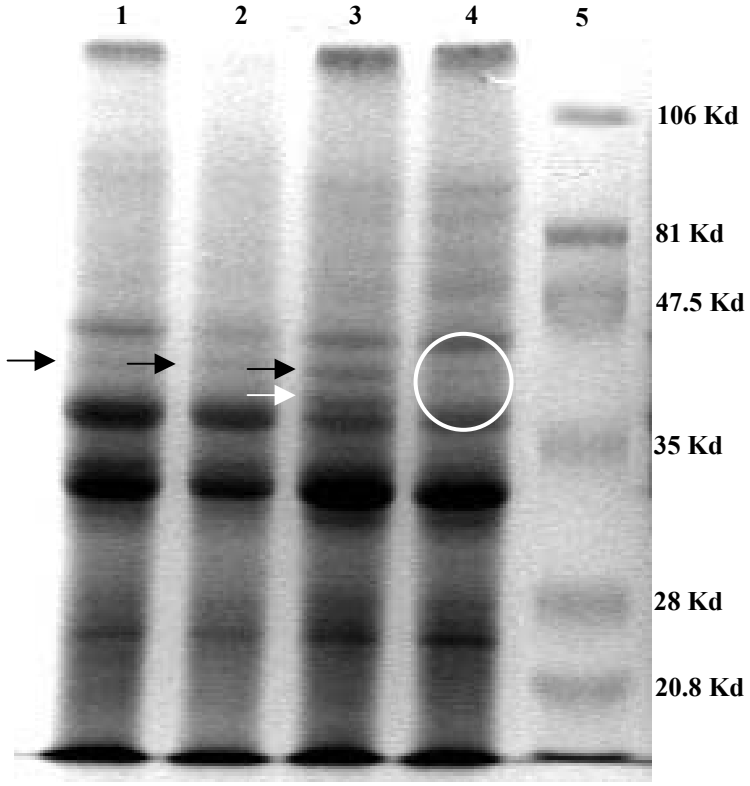

Figure 10.33. SDS-Page. Phase resolving concentration of 14%, Sample concentration of 2 mg/ml. 1: MEcu 72, 2: MEcu 64, 3: MPer 334, 4: CMC 40; 5: Molecular weight marker (Kd). The black arrow indicates the polymorphic band commonly present in the resistant genotypes and absent in the susceptible, CMC 40, indicated by the yellow circle. The yellow arrows show an additional polymorphic band that is only evident in the resistant genotype MPer 334.

Projections

With the polycloned antibodies tested and the standardization of conditions for the SDS-PAGE achieved, we can proceed to develop cross-immunodetection tests of the genotypes and *A. socialis* utilizing the Western Blot and 2D SDS-PAGE techniques.

References

Anderson, L. 1988. Two-dimensional electrophoresis: Operation of the ISO-DALT System. Large scale Biol. Press, Washington, D.C.

Arias, B. 1995. Estudio sobre el comportamiento de la mosca blanca *Aleurotrachelus socialis* Bondar (Homóptera: Aleyrodidae) en diferentes genotipos de yuca *Manihot esculenta* Crantz. Tesis (Maestría en fitomejoramineto). Universidad Nacional de Colombia, Palmira, Colombia. 164 p.

Bellotti, A.C. 2002. Arthropod pests. *In:* Eds. R.J. Hillocks, J.M. Thresh and A.C. Bellotti. CASSAVA: Biology, Production and Utilization. CABI Publishing, UK. p. 209-235.

Bellotti, A.C.; Arias, B. 2001. Host plant resístanse to whiteflies with emphasis on cassava as a case study. Crop Protection 20:813-823.

Bellotti, A.C.; Bohórquez, A.; Arias, B.; Vargas, J.; Vargas, H.L.; Mba, Ch.; Duque, M.C.; Tohme, J. 2003. Avances recientes en la identificación de genes de resistencia a mosca blanca, *Aleurotrachelus socialis* Bondar (Homoptera: Aleyrodidae) en yuca (*Manihot esculenta* Crantz). Memorias XXX Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 17-19, Cali, Colombia.

Bellotti, A.C.; Vargas, O. 1986. Mosca blanca del cultivo de la yuca: Biología y control [Conjunto audiotutorial]. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia.

Dunbar, B.S. 1987. Two-dimensional electrophoresis and imnmunological techniques. Planum, New York. 372 p.

Dunbar, B.S.; Schwoebel E. D. 1990. Preparation of polyclonal antibodies. En: Methods in enzymology Vol. 182. Guide to protein purification. Academic press, INC. San Diego, CA, USA. p 663-670.

Garfin, D.E. 1990. One-dimensional gel electrophoresis. En: Methods in enzymology Vol. 182. Guide to protein purification. Academic press, INC. San Diego, CA, USA. p 459-477.

Hames, B.D; Rickwood**,** D. 1988. Gel electrophoresis of proteins: A practical approach. IRL. Press, Washington, D.C. 383 p.

Timmons T.M.; Dunbar, B.S. 1990. Protein blotting and inmunodetection. En: Methods in enzymology Vol. 182. Guide to protein purification. Academic press, INC. San Diego, CA, USA. p 679-688.

Collaborators: Diego Fernando Múnera S., Anthony C. Bellotti, Arnubio Valencia. Universidad de Caldas, Manizales, Colombia, Stephen L. Lapointe. United States Department of Agriculture (USDA), Paul-André Calatayud, IRD, France.