OUTPUT 6
Breeding for insect and other arthropods resistance and development of alternative methods for their control

Activity 6.1. Evaluation of cassava germplasm for resistance to whiteflies (Aleurotrachelus socialis) during 2002.

Rationale

As direct feeding pests and virus vectors, whiteflies cause major damage in cassava based agroecosystems in the Americas, Africa and to a lesser degree in Asia. Eleven species have been identified feeding on cassava in the Neotropics but two predominate, *Aleurotrachelus socialis* in the Northern region of South America (Colombia, Venezuela and Ecuador), while *Aleurothrixus aepim* is the major species causing crop damage in Brazil. In Africa *Bemisia tabaci* is the vector of African Cassava Mosaic Disease (ACMD) while in recent years *Bemisia afer,* originally from East Africa, has now been found in the Americas (Perú) and may pose as a future threat to the cassava crop if rapid adaptation and dissemination occurs.

Both *A. socialis* and *A. aepim* cause direct damage to cassava by feeding on the phloem of leaves, causing chlorosis and leaf fall, which can result in considerable crop loss. Neither species is known to transmit virus diseases. Research at CIAT to control whiteflies has concentrated on a combination of host plant resistance (HPR) and biological control (BC), with initial emphasis being given to HPR (See PE-1 Annual Report for research activities with BC). Although whitefly resistance in agricultural crops in general is rare, several good sources of resistance have been identified in cassava and high-yielding, whitefly resistant cassava hybrids are being developed. One of these CG 489-31 (CIAT Breeding Code) is being released officially by CORPOICA (Colombia, MADR) in November of 2002.

A continued high incidence of frog skin disease at CIAT during the 2001-2002 growing season, deterred much of our efforts to carry out pest research on the CIAT station. There was a constant shortage of disease free germplasm that is needed to carry out many of the pest resistance mechanism studies that confirms and explains much of our field results and observations. Several of the planned studies had to be postponed due to lack of planting material of several of the resistant genotypes. Even some of the off station plantings, that had previously supplied germplasm, became infested with frog skin, limiting access to planting materials.

Materials and methods

Whitefly evaluations of cassava germplasm were done at principally two sites, at the CIAT farm in Santander de Quilichao and at CORPOICA, Nataima, Tolima. At CORPOICA Nataima, 722 hybrids of the family CM 8996 were evaluated. This family is from a cross of MEcu 72 x MCol 2246 (whitefly resistant x whitefly susceptible). The objective of this on-going research is to study the genetics of resistance of whitefly (*A. socialis*) in cassava. The same cultivars were planted in March 2002 at Santander de Quilichao, and although some whitefly damage and population data has been taken, yield data will be known when the trial is harvested in March 2003. The CORPOICA, Nataima planting was done in May 2001, and harvested in March of 2002 so that yield data is available.

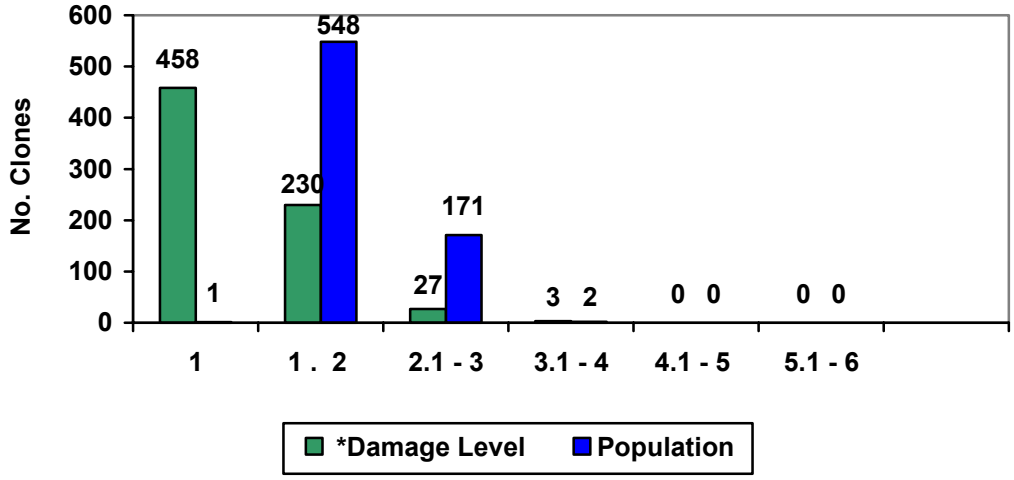
Results

CORPOICA, Nataima: Field evaluations of *A. socialis* damage and populations were carried out three times during the crop cycle, using a 1 to 6 damage and whitefly population scale (Table 6.1). Whitefly (*A. socialis*) populations were low throughout the crop cycle and this resulted in low damage ratings for the genotypes evaluated (Figure 6.1). Neither whitefly populations nor damage reached the 4.0 level on the 1-6 scale and only on a few cultivars did the levels reach 3.0. The low whitefly incidence makes it nearly impossible to separate the resistant from susceptible cultivars, and this trial will need to be repeated. Interestingly, the local farmers cultivar, Aroma, which is highly susceptible to whiteflies, was planted throughout the field as a local control. Whitefly populations on Aroma were relatively higher than on the CM 8996 family (Figure 6.2), reaching the 3.0 to 4.0 level on a majority of the sites where planted in the fields. The difference in whitefly populations and damage between the CM 8996 progeny and the control may, at least in part be due to the resistance conferred by MEcu 72.

Table 6.1. 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.

At harvest, the exceptional vigor of the genotypes from the CM 8996 family was notable (Photo 6.1). Both parents of this family are vigorous varieties and combined with the whitefly resistance conferred by MEcu 72, these results are not unexpected. Harvest data shows a range in plant yield from 4.5 to 86.5 T/ha, and numerous cultivars with high harvest index, high dry matter, good root color and cooking quality. 75 sister cultivars were selected for high yield, between 20 and 86.5 T/ha, such as cultivars CM 8996-342 and Cm 8996-54 respectively (Figure 6.3). Dry matter content was as high as 42% in genotype CM8999-314 (Table 6.2). These 75 genotypes will be evaluated in more detail, with increased replications in future trials.



* Four cultivars not evaluated due to extreme stunting of plants.

Figure 6.1. Cassava damage and whitefly population ratings due to whitefly (*Aleurotrachelus socialis*) feeding on clones from the family CM 8996 (MEcu 72 x MCol 2246) at CORPOICA, Nataima (Tolima, 2002).

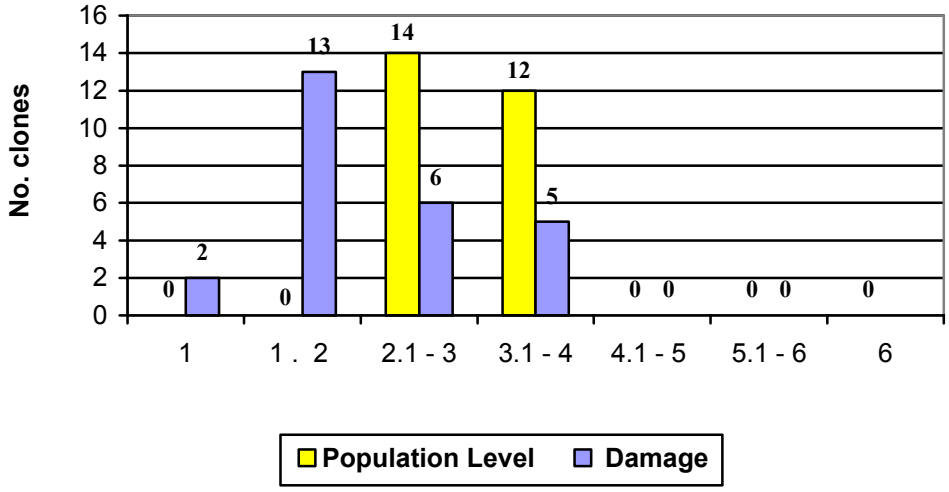


Figure 6.2. Whitefly (*Aleurotrachelus socialis*) populations and damage ratings on the variety Aroma (local farmers variety) at CORPOICA, Nataima (Tolima, 2002).

Photo 6.1. Genotypes of family CM 8996 (MEcu 72 x MCol 2246) harvested at CORPOICA, Nataima; Note exceptional vigor of plants.

This family in its entirety has also been replanted in CORPOICA, Nataima for a second cycle of evaluations. Whitefly damage and population data will be taken throughout the year and the trial will be harvested during early 2003.

Results

Santander de Quilichao. At this site, as at the Tolima site, initial whitefly populations were lower than expected. However populations' levels did reach 4.0 on about 30 cultivars (Figure 6.4). *A. socialis* populations started early in the crop cycle but did not increase greatly over time and other pests, notably mites and thrips, occurred and their populations quickly increased. 326 of 756 clones (43%) presented moderate level of damage to thrips and 302 (40%) had high or severe levels of damage (Figure 6.5). Only 128 (17%) had low levels of damage (1-2 on a 1 to 6 damage scale). Since MEcu 72, the female parent, is highly resistant to thrips, and MCol 2246 is susceptible this kind of segregation in the progeny is not unexpected. Of the 128 clones displaying resistance to thrips, 38 belong to the group of selected cultivars from Tolima, as previously described (Figure 6.6). Of the 75 clones selected in Tolima (Figure 6.3), 33 (44%) are susceptible to thrips and 38 or about 50% are resistant. The cultivar CMC-40 was planted at Santander de Quilichao as the control, and is highly susceptible to thrips and gave thrips damage ratings above 4.0, on 100% of the plants evaluated, often reaching 5 or 6 on the damage scale, again indicating the high level of thrips population.

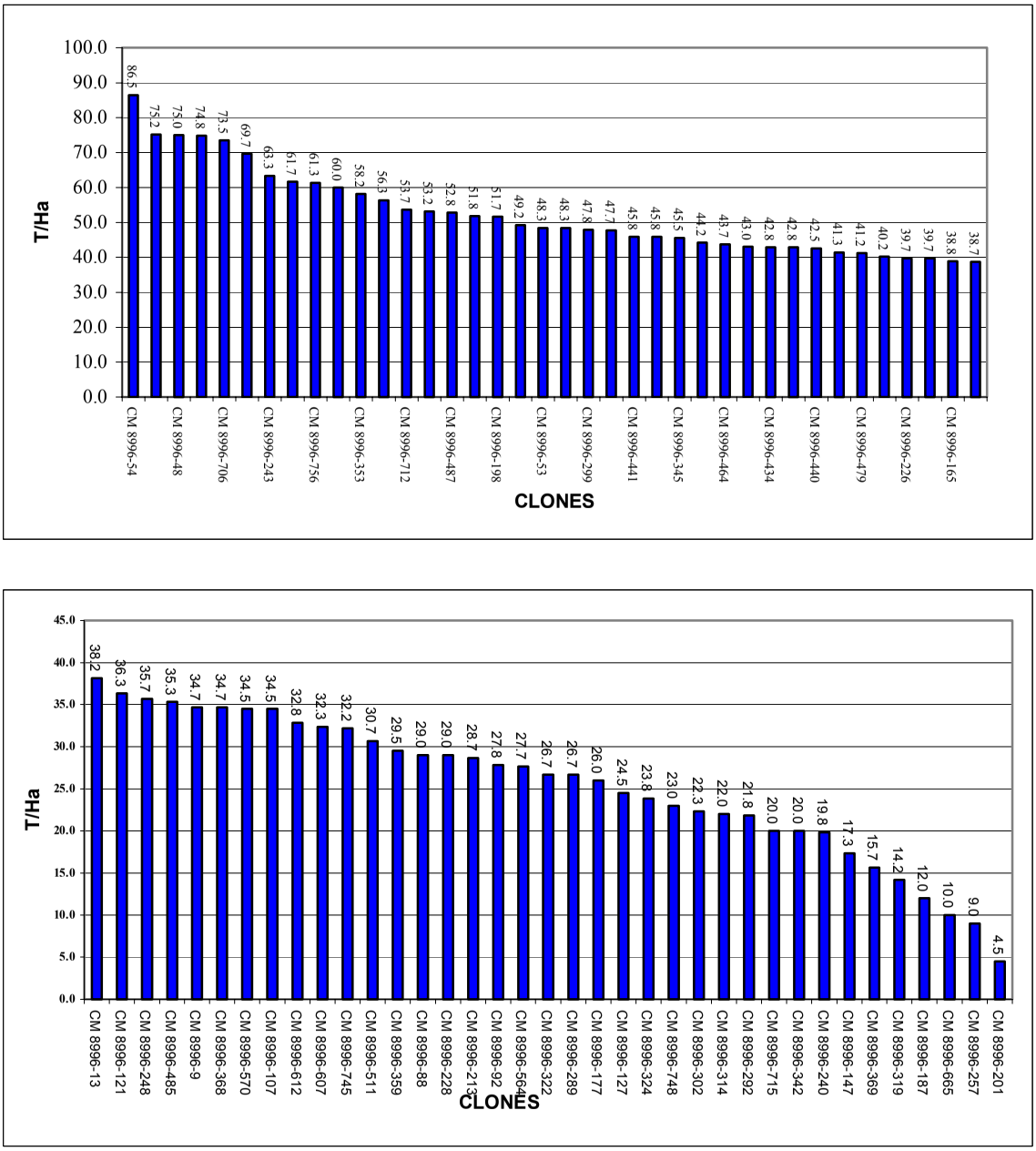


Figure 6.3. Range in yield of 75 cassava clones selected for whitefly resistance at CORPOICA, Nataima, 2002.

Table 6.2. Harvest index, total yield and % dry matter content of 75 selected cassava progeny from a MEcu 72 x MCol 2246 cross and evaluated for resistance to the whitefly, *Aleurotrachelus socialis* at CORPOICA, Nataima.

Clone	Harvest Index	Total Yield	Dry Matter
CM 8996-314	0.59	22.0	42.0
CM 8996-257	0.45	9.0	40.2
CM 8996-665	0.62	10.0	38.8
CM 8996-511	0.50	30.7	38.7
CM 8996-201	0.52	4.5	38.5
CM 8996-189	0.56	41.3	37.5
CM 8996-302	0.50	22.3	37.3
CM 8996-88	0.55	29.0	36.8
CM 8996-319	0.49	14.2	36.8
CM 8996-261	0.61	40.2	36.6
CM 8996-324	0.58	23.8	36.6
CM 8996-369	0.38	15.7	36.5
CM 8996-292	0.53	21.8	36.4
CM 8996-147	0.40	17.3	36.3
CM 8996-368	0.57	34.7	36.3
CM 8996-213	0.44	28.7	36.2
CM 8996-208	0.60	49.2	36.2
CM 8996-177	0.59	26.0	36.2
CM 8996-210	0.68	45.8	36.2
CM 8996-748	0.71	23.0	36.0
CM 8996-92	0.55	27.8	35.9
CM 8996-358	0.57	38.7	35.9
CM 8996-342	0.51	20.0	35.8
CM 8996-187	0.46	12.0	35.7
CM 8996-228	0.48	29.0	35.5
CM 8996-248	0.54	35.7	35.5
CM 8996-289	0.54	26.7	35.5
CM 8996-243	0.66	63.3	35.4
CM 8996-127	0.67	24.5	35.4
CM 8996-479	0.65	41.2	35.4
CM 8996-715	0.62	20.0	35.4
CM 8996-322	0.49	26.7	35.3
CM 8996-745	0.44	32.2	35.2
CM 8996-485	0.56	35.3	35.2
CM 8996-353	0.56	58.2	35.1
CM 8996-107	0.65	34.5	35.0
CM 8996-226	0.61	39.7	35.0
CM 8996-607	0.53	32.3	34.8
CM 8996-199	0.61	39.7	34.8
CM 8996-570	0.50	34.5	34.8
CM 8996-564	0.51	27.7	34.7
CM 8996-165	0.58	38.8	34.6
CM 8996-53	0.57	48.3	34.5
CM 8996-9	0.41	34.7	34.5
CM 8996-359	0.51	29.5	34.3

Clone	Harvest Index	Total Yield	Dry Matter
CM 8996-612	0.71	32.8	34.3
CM 8996-434	0.56	42.8	34.2
CM 8996-299	0.64	47.8	34.2
CM 8996-13	0.56	38.2	34.2
CM 8996-121	0.54	36.3	34.1
CM 8996-42	0.54	56.3	34.1
CM 8996-101	0.52	44.2	34.0
CM 8996-712	0.65	53.7	34.0
CM 8996-217	0.59	51.8	34.0
CM 8996-441	0.55	45.8	33.9
CM 8996-214	0.62	53.2	33.6
CM 8996-178	0.65	42.8	33.6
CM 8996-345	0.50	45.5	33.5
CM 8996-467	0.61	48.3	33.5
CM 8996-306	0.53	43.0	33.4
CM 8996-198	0.53	51.7	33.3
CM 8996-240	0.58	19.8	33.3
CM 8996-440	0.71	42.5	33.2
CM 8996-714	0.72	47.7	33.2
CM 8996-616	0.59	69.7	32.9
CM 8996-487	0.66	52.8	32.7
CM 8996-756	0.60	61.3	32.4
CM 8996-706	0.66	73.5	32.3
CM 8996-464	0.51	43.7	32.3
CM 8996-643	0.61	61.7	32.3
CM 8996-298	0.55	60.0	31.9
CM 8996-216	0.54	74.8	31.3
CM 8996-54	0.63	86.5	29.2
CM 8996-323	0.65	75.2	29.0
CM 8996-48	0.61	75.0	28.6

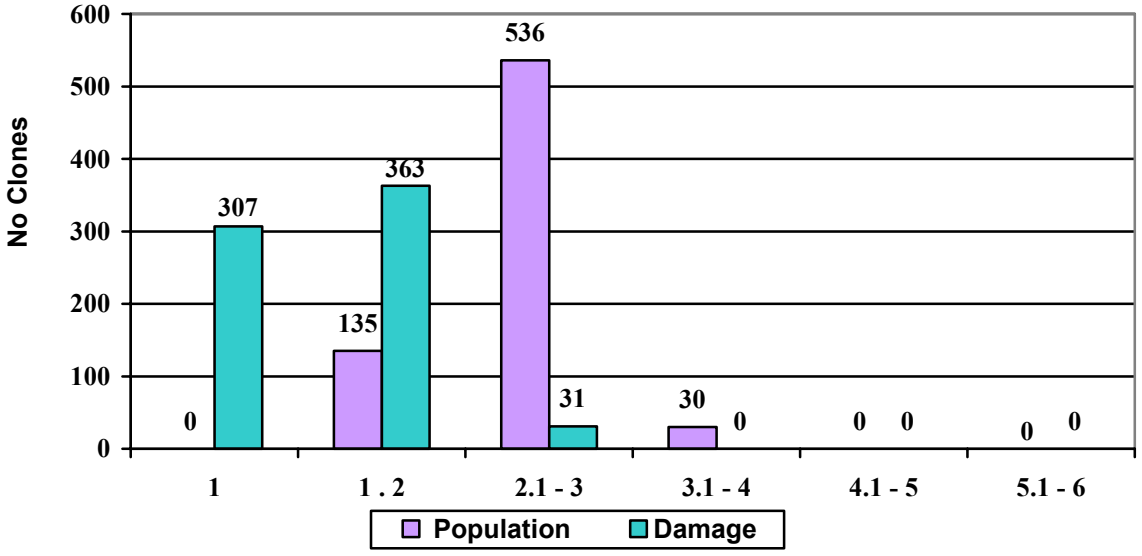


Figure 6.4. Whitefly (*Aleurotrachelus socialis*) populations and damage ratings on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) (originating from CORPOICA, Nataima) at Santander de Quilichao (Cauca, 2002).

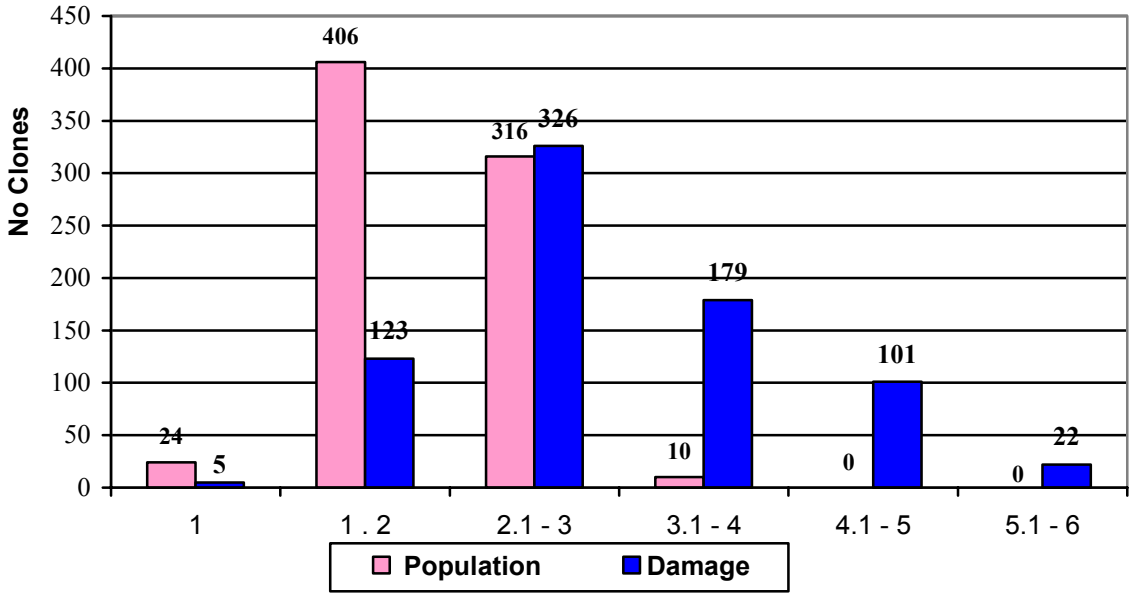


Figure 6.5. Thrips (*Frankliniella williamsi)* population and damage ratings on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Cauca, 2002).

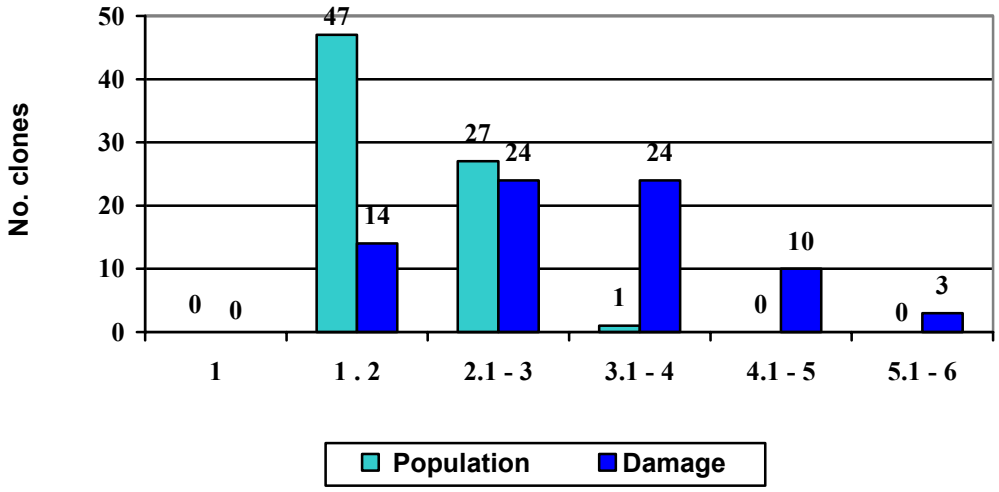


Figure 6.6. Thrips (*Frankliniella williamsi*) population and damage in selected cultivars (from CORPOICA, Nataima) of family CM 8996, at Santander de Quilichao (Cauca, 2002).

Mite (*Mononychellus tanajoa*) attacks also occurred during the dry season in this trial and high populations were evident. 705 of the 706 (99.8%) clones evaluated resulted in high damage levels, above 3.1 on the 1 to 6 damage scale. Only 1 clone (0.14%), CM8996-641, presented low or no damage (Figure 6.7). The mite damage on the susceptible control, CMC-40 was above 4.0 on 72.5% of the plants and where low damage was observed, thrips attack was so severe that it masked mite populations and damage (Figure 6.8). Of the 75 selected clones from the Tolima planting, none showed any resistance to mites, all having damage ratings above 3.0 (Figure 6.9).

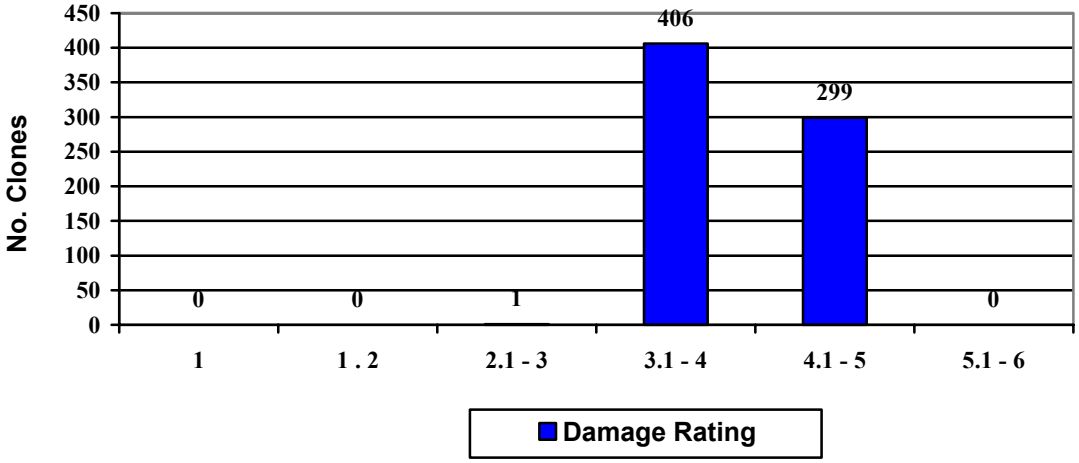


Figure 6.7. Mite (*Mononychellus tanajoa*) population and damage rating on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Cauca, 2002).

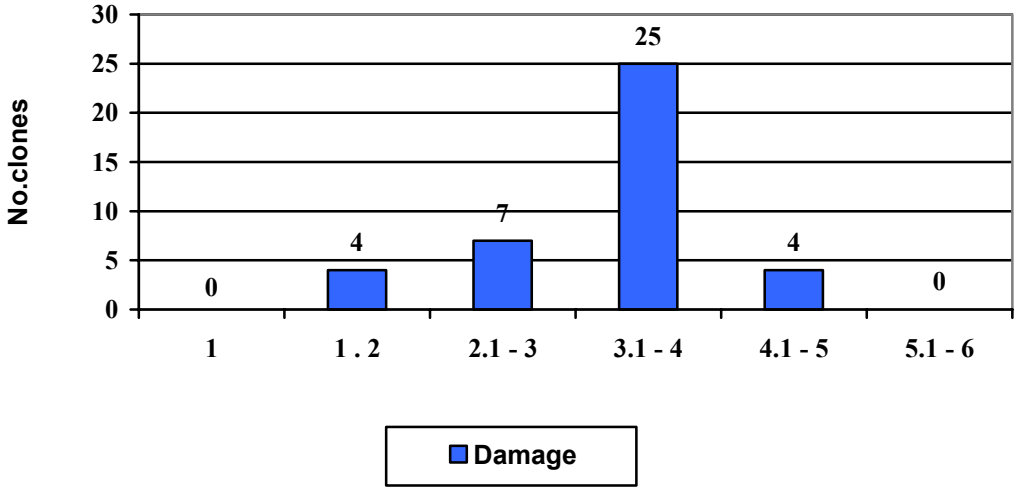


Figure 6.8. Mite (*Mononychellus tanajoa*) damage ratings on CMC-40 (control plots) at Santander de Quilichao (Cauca, 2002).

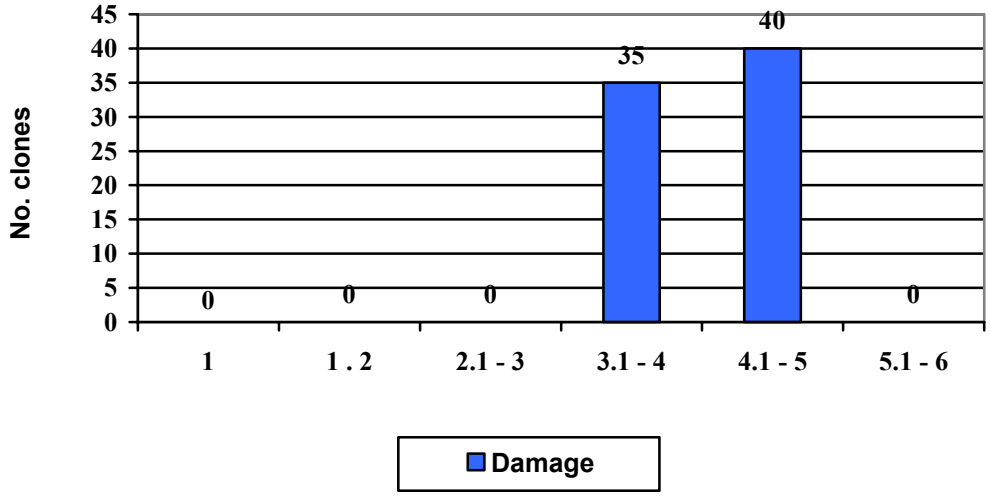


Figure 6.9. Mite (*Mononychellus tanajoa*) damage ratings in selected cultivars (from CORPOICA, Nataima) from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Cauca, 2002).

Of the 75 clones selected in Tolima for yield and low whitefly damage, all responded well at Santander de Quilichao with low whitefly populations (Figure 6.10). Both damage ratings and whitefly populations were low.

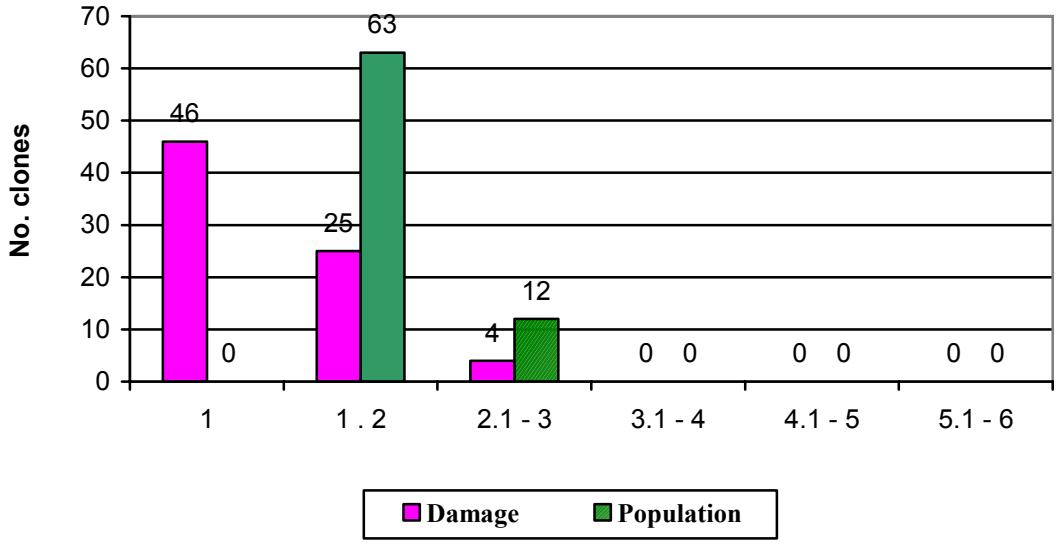


Figure 6.10. Whitefly (*Aleurotrachelus socialis*) population and damage rating on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Valle del Cauca), previously selected at CORPOICA, Nataima (2002).

In a breeding or germplasm development program it is always advantageous to identify cultivars that exhibit multi resistance, that is resistance to several pests in a single cultivar. Table 6.3. Lists the reaction of several cultivars to thrips and whitefly attack, where whitefly is the key pest.

Table 6.3. Cultivars from family CM 8996 (MEcu 72 x MCol 2246) showing low whitefly and thrips damage ratings at CORPOICA, Nataima.

Clone	Thrips		Whitefly	
	Population	Damage	Population	Damage
CM 8996-107	1.5	1.5	2.0	1.0
CM 8996-322	1.5	1.5	2.2	1.0
CM 8996-48	2	1.5	2.4	1.0
CM 8996-121	2	2	2.4	1.0
CM 8996-198	2	2	2.2	1.0
CM 8996-199	2	2	2.2	1.0
CM 8996-213	2	2	2.2	1.0
CM 8996-228	1.5	2	2.4	1.0
CM 8996-243	3	2	3	1.0
CM 8996-306	2	2	2.6	1.0
CM 8996-324	2	2	2.4	1.0
CM 8996-369	1.5	2	2	1.0
CM 8996-487	1.5	2	2.2	1.0
CM 8996-712	2	2	2.2	1.0
CM 8996-189	2	2.5	2.2	1.0

Clone	Thrips		Whitefly	
	Population	Damage	Population	Damage
CM 8996-214	1.5	2.5	2.2	1.0
CM 8996-248	2	2.5	2.4	1.0
CM 8996-342	2.5	2.5	2.4	1.0
CM 8996-353	2	2.5	2.2	1.0
CM 8996-440	2.5	2.5	3	1.0
CM 8996-464	2	2.5	3.2	1.0
CM 8996-607	2	2.5	2.6	1.0
CM 8996-612	2	2.5	2.4	1.0
CM 8996-745	2	2.5	2	1.0
CM 8996-9	2	2.5	2.6	1.0
CM 8996-147	2	3	3	1.0
CM 8996-240	2.5	3	2.4	1.0
CM 8996-257	2	3	2.2	1.0
CM 8996-261	2	3	2.2	1.0
CM 8996-302	2	3	3	1.0
CM 8996-319	2.5	3	2.6	1.0
CM 8996-345	2	3	2.4	1.0
CM 8996-358	2	3	2.6	1.0
CM 8996-359	2.5	3	2.4	1.0
CM 8996-42	2	3	1.8	1.0
CM 8996-467	3	3	3.2	1.0
CM 8996-570	2	3	2.2	1.0
CM 8996-88	2	3	2	1.0

Activity 6.2. Evaluation of cassava dialel crosses for whitefly (A. socialis) resistance/ susceptibility at Jamundí, Valle del Cauca (2002).

During the first semester of 2002, cassava breeding field trials were carried out to determine the combining ability (general or specific) (Dialelics) of different genotypes. Since a heavy whitefly (primarily *A. socialis*) attack occurred, an evaluation of whitefly populations and damage was done on these genotypes. A 1 to 6 damage and population scale were used to evaluate 36 families, each containing 30 sister progeny grown in replications (Table 6.4) (see whitefly section Activity 6.1).

Table 6.4. Thirty-six cassava families of 30 plants each in a dialelic cross evaluated for whitefly (*A. socialis*) damage and populations (Jamundí, Valle del Cauca, 2002).

Crosses	Female	Male
CM 9901	CM 6740-7	SM 1219-9
GM 228	CM 6740-7	SM 1278-2
GM 230	CM 6740-7	SM 1636-24
GM 231	CM 6740-7	SM 1673-10
CM 9903	CM 6740-7	SM 1741-1
GM 234	CM 6740-7	HMC 1
GM 308	MEcu 72	CM 6740-7
CM 9642	CM 6740-7	MPer 183
GM 254	SM 1219-9	SM 1278-2

Crosses	Female	Male
GM 257	SM 1219-9	SM 1636-24
GM 260	SM 1219-9	SM 1673-10
CM 9953	SM 1219-9	SM 1741-1
GM 264	SM 1219-9	HMC 1
GM 309	MEcu 72	SM 1219-9
GM 265	SM 1219-9	MPer 183
GM 267	SM 1278-2	SM 1636-24
GM 268	SM 1278-2	SM 1673-10
GM 269	SM 1278-2	SM 1741-1
GM 270	SM 1278-2	HMC 1
GM 310	MEcu 72	SM 1278-2
GM 271	SM 1278-2	MPer 183
GM 283	SM 1636-24	SM 1673-10
GM 284	SM 1636-24	SM 1741-1
GM 285	SM 1636-24	HMC 1
GM 311	MEcu 72	SM 1636-24
GM 286	SM 1636-24	MPer 183
GM 292	SM 1673-10	SM 1741-1
GM 293	SM 1673-10	HMC 1
GM 312	MEcu 72	SM 1673-10
GM 294	SM 1673-10	MPer 183
GM 296	SM 1741-1	HMC 1
GM 313	MEcu 72	SM 1741-1
GM 297	SM 1741-1	MPer 183
GM 314	MEcu 72	HMC 1
CM 9733	HMC 1	MPer 183
GM 306	MEcu 72	MPer 183

Results

A total of 990 genotypes were evaluated (not all plants from the crosses were viable and some could not be evaluated. 131 genotypes (13.2%) showed no whitefly damage and 301 or 30.4% had damage ratings of 2.0 indicating high levels of resistance to whiteflies (Figure 6.11). 558 genotypes had damage ratings above 3.0 and 285 of these above 4.0, indicating that there was high selection pressure during the trial (Figure 6.11). The 432 cultivars that received ratings of 2.0 or lower are available upon request. It can be noted in this list that the cultivar GM 309-1 (MEcu 72 x SM 1219-9) had no damage and few or no whiteflies present. In this list, it can also be noted that numerous genotypes had very low damage and correspondingly low populations of whiteflies, between 1.0 and 1.7. These materials are very promising for whitefly resistance and should be maintained and re-evaluated.

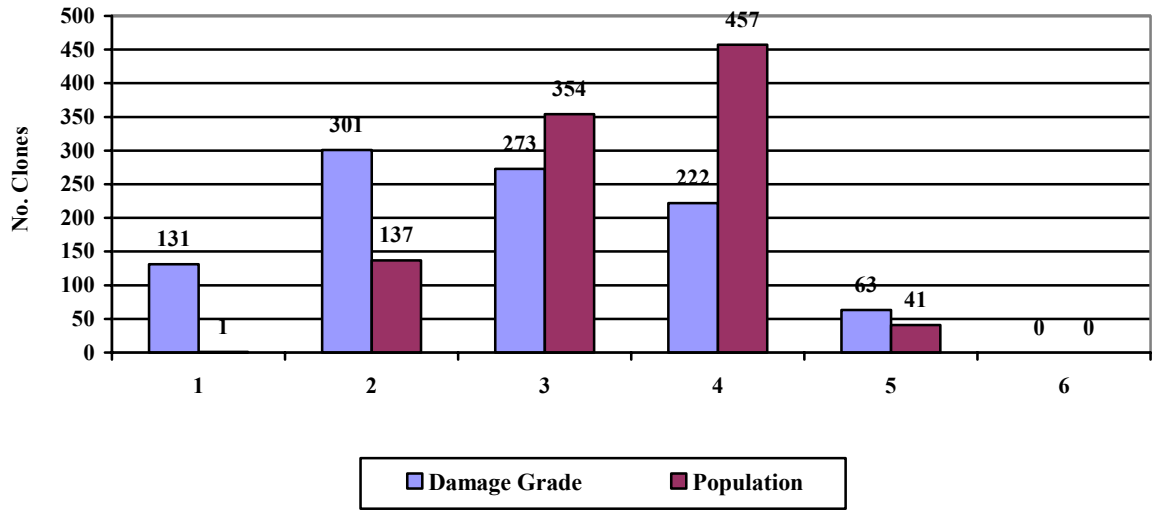


Figure 6.11. Cassava damage grade and whitefly (A. socialis) population levels in 990 progeny from a dialelic cross of 36 families (Jamundí, Valle del Cauca, 2002).

As a result of these evaluations, 141 genotypes were selected as most promising for whitefly resistance, without taking into account yield data. Of these 141genotypes 32 clones or 22.7% resulted having Frog Skin Disease. It was also observed that some families did not show frog skin symptoms. These included GM 294 (SM 1673-10 x MPer 183) and GM 297 (SM 1741-1 x MPer 183). At harvest (Breeding section) in the family GM 309 (MEcu 72 x SM 1219-9), 27 of the 30 sister plants had frog skin disease (Table 6.5).

Table 6.5. Proportion of selected cassava families from a dialelic cross diagnoses with frog skin disease (Jamundí, Valle del Cauca, 2002).

Family	Parents	No. Plants with Frog Skin	% Frog Skin
GM 268	SM 1278-2 x SM 1673-10	0	0.0
GM 270	SM 1278-2 x HMC 1	0	0.0
GM 294	SM 1773-10 x MPer 183	0	0.0
GM 297	SM 1741 – 1 x MPer 183	0	0.0
GM 296	SM 1741 – 1 x HMC 1	1	3.3
GM 271	SM 1278 – 2 x MPer 183	2	6.7
GM 284	SM 1636 – 24 x SM 1741 - 1	2	6.7
GM 264	SM 1219 – 9 x HMC 1	3	10
GM 306	MEcu 72 x MPer 183	3	10
GM 231	CM 6740 – 7 x SM 1278 - 2	4	13.3
GM 228	CM 6740 – 7 x SM 1278- 2	5	16.7
GM 234	CM 6740 – 7 x HMC 1	5	16.7
GM 230	CM 6740 – 7 x SM 1636 - 24	7	23.3
GM 265	SM 1219 – 9 x MPer 183	7	23.3
GM 269	SM 1278 – 2 x SM 1741 – 1	7	23.3
GM 283	SM 1636 – 24 x SM 1673 – 10	7	23.3
GM 311	MEcu 72 x SM 1636 - 24	11	36.6
GM 314	MEcu 72 x HMC 1	11	36.6
CM 9642	CM 6740 – 7 x MPer 183	11	36.6

GM 312	MEcu 72 x SM 1673 - 10	12	40.0
GM 308	MEcu 72 x CM 6740 - 7	13	43.3
GM 310	MEcu 72 x SM 1278 – 2	13	43.3
CM 9901	CM 6740 – 7 x SM 1219 - 9	15	50.0
GM 313	MEcu 72 x SM 1741 – 1	17	56.7
CM 9903	CM 6740 – 7 x SM 1741 - 1	22	73.3
GM 257	SM 1219 – 9 x SM 1636 - 24	22	73.3
GM 309	MEcu 72 x SM 1219 - 9	27	90.0

Activity 6.3. Evaluation of whitefly (Aleurotrachelus socialis) cassava clones for resistance to Bemisia tabaci.

Whiteflies are reported feeding on cassava in nearly all cassava growing regions of the tropics. Eleven species have now been identified globally. In the neotropics *Aleurotrachelus socialis* predominates in Northern South America, while *Aleurothrixus aepim* is the major species in Brazil. *Bemisia tabaci*, a pantropical species, predominates in Africa where it is the vector of Africa Cassava Mosaic Disease (ACMD). Until recently, *B. tabaci* biotypes found in the neotropics did not feed on cassava, and it has been speculated that the absence of ACMD in the Americas may be related to the inability of *B. tabaci* to colonize cassava. During the last decade a new biotype (B) of *B. tabaci* has been collected feeding on cassava in the neotropics.

The appearance of Biotype B is cause for concern as it is now considered that ACMD poses a more serious threat to cassava production in the Americas as most traditional varieties in the neotropics are highly susceptible to the disease. Therefore a project is now underway to identify possible *B. tabaci* resistance in cassava germplasm.

The CIAT cassava germplasm bank of more than 6000 accessions is continually being screened for resistance to arthropod pests, especially whiteflies. Over a period of more than 15 years several clones have been selected as sources of resistance to the whitefly species *Aleurotrachelus socialis*. The clone MEcu 72 has consistently expressed the highest levels of resistance. Additional cultivars expressing moderate to high levels of resistance in field trials include MEcu 64, MPer 335, MPer 415, MPer 317, MPer 216, MPer 221, MPer 265, MPer 266 and MPer 365. Whitefly resistance hybrids from a MEcu 72 x MBra 12 cross have been produced and evaluated; the progeny CG 489-31 is being released by the Colombian MADR to cassava producers in Nov. 2002.

The objectives of this research project is to determine if the *A. socialis* resistant sources will also express resistance to the B biotype of *Bemisia tabaci*.

Methodology

The stock of Biotype "B" of *B. tabaci* to initiate a colony on cassava came from the CIAT Bean Improvement Project (IP-1). *B. tabaci* adults, harvested from the bean colony, were allowed to first oviposit on poinsettia (*Euphorbia pulcherrima*). After five generations established on poinsettia, the colony was transferred to Jatropha (*Jatropha gossypiifolia*), where it has been established for 12 generations. The colony established on Jatropha was then transferred to both *Manihot esculenta* and *Manihot carthaginensis* (Figure 6.12). The "B" biotype of *B. tabaci* colony has now been reared for two generations on *M. esculenta* (Var. MCol 2063) and 3 generations on *M. carthaginensis*. Colonies are maintained at CIAT in growth rooms under controlled conditions: 12 hrs. photoperiod, 25 ± 2°C and 50-80 % RH. This methodology was

designed in order to gradually or progressively adapt *B. tabaci* from beans to *Manihot* species by passing it through related species of the Euphorbiaceae family.

Bean plants are held in the screen house for 17 days; poinsettia and Jatropha for 40 to 50 days and cassava 30 to 40 days, before being exposed to the *B. tabaci* colony. Plants are grown in 15cm diameter plastic pots, watered daily and receive no fertilizer nor pesticide during their development. Plants are placed in fine nylon meshed wooden cages (1m ht x 1m width), where *B. tabaci* infestation takes place. Whitefly adults are harvested with a pipette suction devise, from the colony and released in the experimental cages. Fresh plants of each species are supplied for each of the colonies on a regular basis; usually no more than two generations of *B. tabaci* are reared on the same plant.

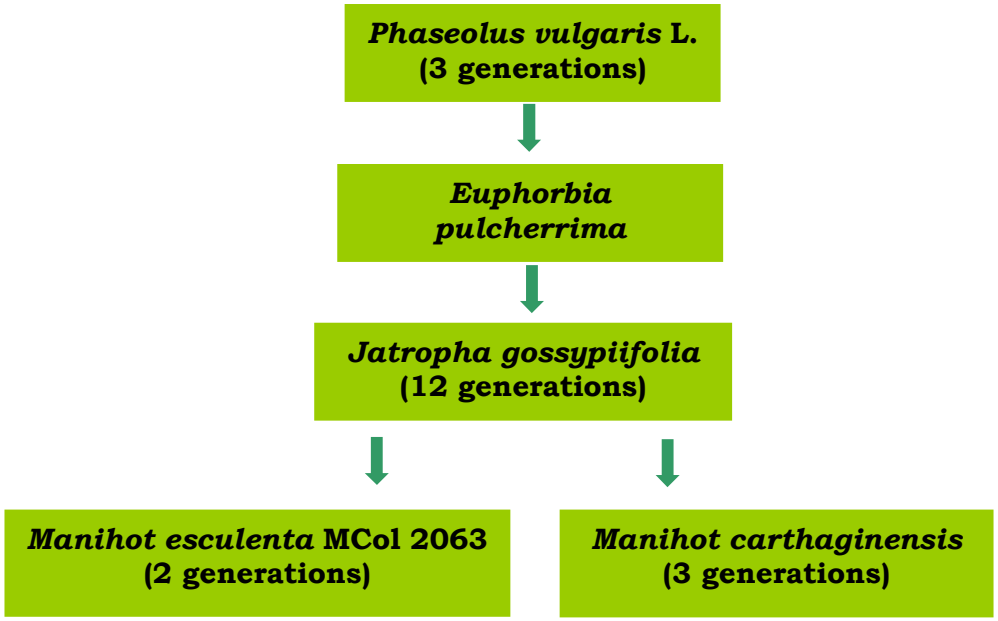


Figure 6.12. Plant species sequence for adapting the whitefly species *Bemisia tabaci*, B biotype, from beans (*P. vulgaris*) to *Manihot esculenta* and *M. carthaginensis.*

The cassava variety MCol 2063 was selected as a host plant because it is recognized as being very susceptible to whiteflies, especially to *A. socialis*. *M. carthaginensis*, also known as "yuca de Cartagena" is a wild species that grows naturally on the North Coast of Colombia and is being used in genetic improvement programs because of its high protein content, considerably higher than cultivated species of *M. esculenta.*

Preliminary studies were done with four wild species, *Manihot flabellifolia, M. peruviana, M. tristis* and *M. carthaginensis*, to evaluate their potential to host the B biotype of *B. tabaci*. *B. tabaci* adapted best to *M. carthaginensis* so this species was selected to establish a *B. tabaci* colony.

B. tabaci became established on MCol 2063 after 2 generations and on *M. carthaginensis* after 3 generations. The evaluation of cassava genotypes for resistance to *B. tabaci* was first done by exposing them to the *M. carthaginensis*, since this host showed the best adaptation.

Every 3 months a molecular identification of the *B. tabaci* colony is carried out using RAPD-PCR to assure that contamination with the "A" biotype has not occurred.

The initial three *M. esculenta* genotypes selected to be evaluated are CMC-40 MEcu 72 and CG 489-34. CMC-40 is highly susceptible to *A. socialis*; the *A. socialis* colony is maintained on this genotype. MEcu 72 is highly resistant to *A. socialis*; evidenced by low populations, no damage symptoms and high nymphal mortality in laboratory studies. CG 489-34 is a hybrid progeny of a MEcu 72 x MBra 12 cross, displaying moderate levels of resistance to *A. socialis*.

Experimental Procedures: HPR experiments with the three cassava genotypes were done in growth chambers under controlled temperature, humidity and photoperiod conditions. MEcu 72, CG 489-34 and CMC-40 plants were multiplied through vegetative cuttings and planted in 15cm diameter plastic pots. Plants were placed in a screen house. Experiments were initiated by selecting plants that possessed 4 to 6 true leaves and approximately 30 to 50 cm high. Biotype "B" *B. tabaci* recently emerged adults were harvested from the *M. carthaginensis* colony by using a pipette aspirator and a glass vial with a perforated lid and 40 pair were sexed. Each pair was placed within a 2.5cm diameter leaf-0clip cage (Figure 6.13) on a cassava leaf. Every 48 hours the adult pair was moved to new area of the leaf, until the female died. Fecundity was measured by counting eggs oviposited by each female during the 48 hr. period.

Figure 6.13. Methodologies for installing male and female biotype B of *Bemisia tabaci* on cassava (*Manihot esculenta*) leaves.

Experiments to determine development time, rate of survival and proportion of *B. tabaci* ("B") females developed were carried out under the previously described conditions. 40 adults *B. tabaci* "B" biotype were placed in the leaf cages on the leaf underside of each genotype. Adults were removed after 6 hours and 200 eggs were selected. Egg hatch and nymphal development or mortality were observed until the adult stage and the proportion of emerged females was noted (Figure 6.14).

Demographic parameters were determined using a methodology described by Manzano (2000). Data on development time and survival of immatures were combined with experimental reproduction data "lxmx," to create life tables and calculate demographic parameters for *B. tabaci* "B." For each experiment the following parameters, defined by Price (1975) were calculated: net reproduction rate (Ro, this represents the number of females

produced by each female in one generation), generational time (T = the average time span required between the birth of the parents and the birth of their progeny. The intrinsic rate of increase of a population (r_m) for *B. tabaci* "B" is estimated using the equation proposed by Carey (1993):

$$\sum \exp(-r_m x) lx mx = 1$$

Where: x = age
Lx = age of specific survival
Mx = proportion of the females of the progeny of a female at age x.

To calculate the r_m values, the corrected age x + 0.5 was used (Carey, 1993).

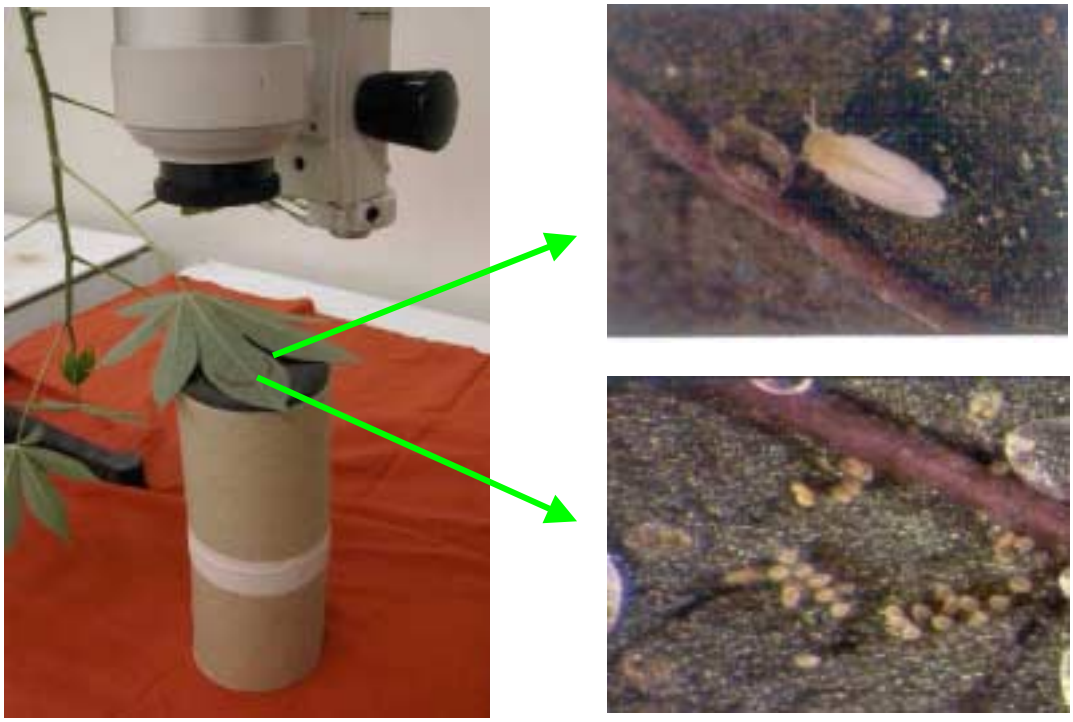


Figure 6.14. Techniques for recording egg and adult populations of biotype B of *Bemisia tabaci on cassava leaves.*

Data Analysis:

- Significant differences between the longevity values and fecundity on each cassava genotype were determined with an analysis of variance using the Kruskal-Wallis test.
- Values for oviposition rates were determined using an ANOVA analysis. For the three parameters, multiple comparisons were done using the Student-Newman-Keuls test.
- The differences in development time between cassava genotypes were evaluated with an analysis of variance using the Kruskal-Wallis test. Comparisons of survival rates were done using the x^2 test.

Results

A. Adaptation of *B. tabaci* "B" on several hosts.

Mean longevity of *B. tabaci* biotype "B" was highest on cassava when females originated from poinsettia (*E. pulcherrima*), when compared Jatropha (*J. gossypiifolia*) and beans (*P. vulgaris*). Average longevity differed significantly between the three hosts, except for the longevity values of *J. gossypiifolia* and *P. vulgaris* (Student Newman-Keuls $P < 0.05$, after Kruskal-Wallis $P < 0.0001$). Longevity of adults on cassava, originating from the 3 hosts is shown in Figure 6.15. Average fecundity was also significantly different between the three hosts (Kruskal-Wallis $P < 0.0001$), except for the values obtained between *E. pulcherrima* and *J. gossypiifolia* (Student Newman-Keuls $P < 0.05$) (Table 6.6). Reproduction curves indicated by daily oviposition (Figure 6.16) resulted in higher oviposition on *J. gossypiifolia* although not over a long time period.

The average ovipositioned rate (eggs per female over two days) was higher for *J. gossypiifolia* (2.64) (Table 6.6). Average ovipositional rate was significantly different between treatment (ANOVA, $P < 0.0001$); comparisons between females originating from the three hosts show no significant differences in average ovipositional rate (student Newman-Keuls $P < 0.05$).

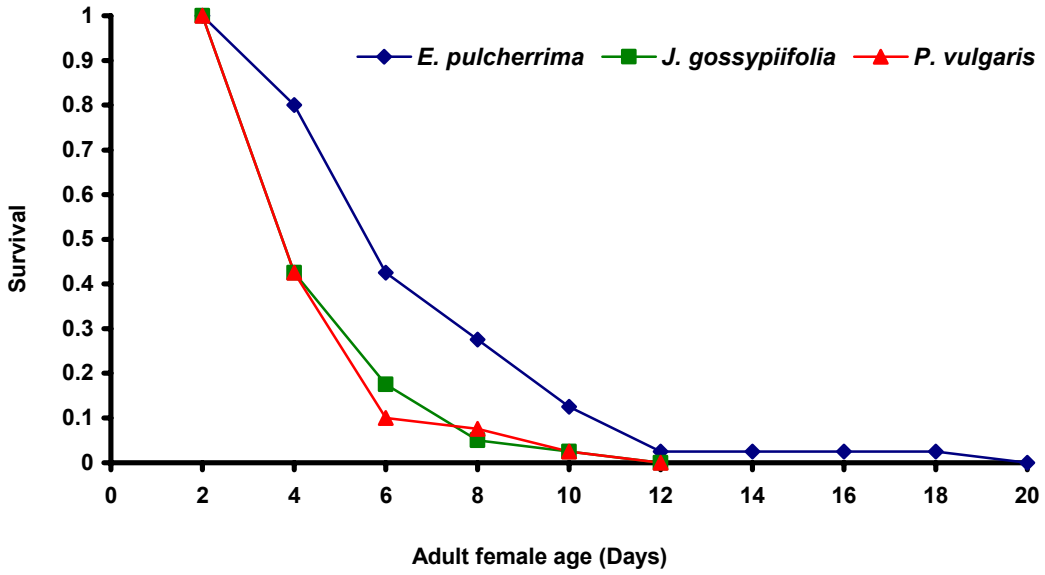


Figure 6.15. Survival curves of biotype B of *Bemisia tabaci* adult females, that originated from three separate hosts, (*Phaseolus vulgaris*, *Euphorbia pulcherrima* and *Jatropha gossypiifolia*).

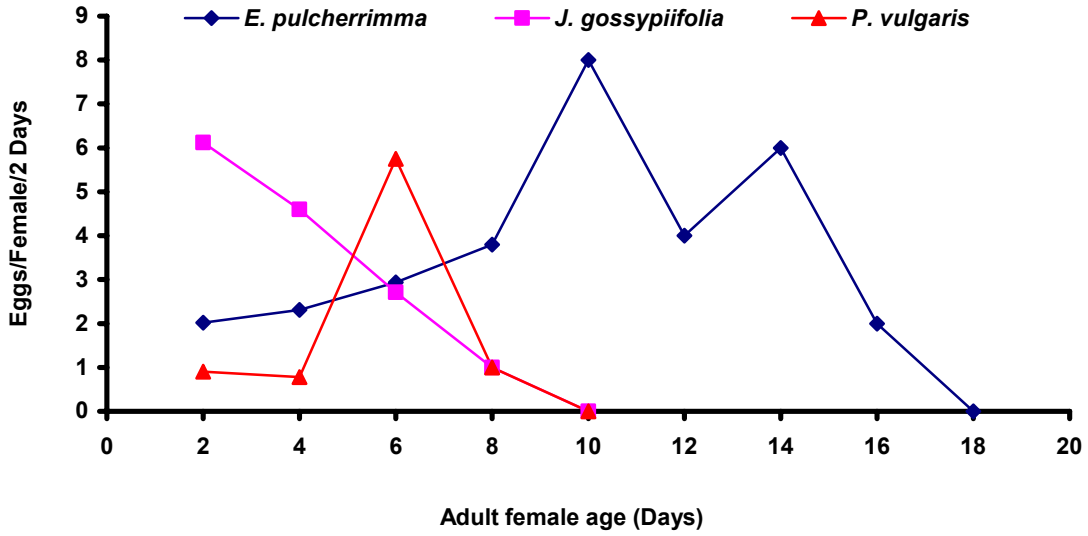


Figure 6.16. Reproduction curves of biotype B of *Bemisia tabaci* females that originated from three separate hosts (*Phaseolus vulgaris*, *Euphorbia pulcherrima* and *Jatropha gossypiifolia*).

Table 6.6. Mean longevity (d), mean fecundity and rate of oviposition (eggs/female/2 days) of biotype B of *Bemisia tabaci* on cassava from females originating from 3 host populations.

Parameter	*J. gossypiifolia*	*E. pulcherrimma*	*P. vulgaris*
Mean Longevity*	3.25 a	5.6 b	3.1 a
Range	2-10	2-18	2-10
# Insects	40	40	40
Mean Fecundity*	8.6 a	7.65 a	1.82 b
Range	1-41	1-48	1-19
Mean Rate Oviposition ϕ	2.64 a	1.36 b	0.58 c
Range	0.5-8	0.4-3	0.5-3.5

Figures followed by different letters across columns indicate significant differences.
* Kruskal-Wallis P< 0.0001, Student-Newman-Keuls method P< 0.05.
ϕ One-way ANOVA P< 0.0001, Student-Newman-Keuls method P< 0.05.

Development time for progeny from females from *E. pulcherrima* (50 days) and *P. vulgaris* (49.5) were similar; the lowest development time was from progeny from *J. gossypiifolia* females (44.4 days). The rate of survival for immatures was significantly different for *J. gossypiifolia* with respect to the other two hosts. The proportion of females was equal for all three hosts (50%) Table 6.7.

Discussion: The intrinsic rate of growth (r_m) of *B. tabaci* "B" originating from each of the hosts and developing on *M. esculenta* (MCol 2063), permits determining that *J. gossypiifolia* was best adapted in that the *B. tabaci* "B" population had the highest intrinsic rate of growth,

exceeding *E. pulcherrima* by 8.3% and *P. vulgaris* by 58.3%. In addition it presented the lowest generational time, 44.76 days from generation to generation (Table 6.7).

Survivorship of the immature states of *B. tabaci* "B" was 27.5% on *M. esculenta/J. gossypiifolia* relationship, considerably higher than the other two hosts. When this is expressed together with other demographic parameters (Table 6.7) the adaptation advantage that *B. tabaci* "B" has when population originate on *J. gossypiifolia* is obvious when compared to the other hosts.

Table 6.7. Demographic parameters from biotype B of *Bemisia tabaci* on *Manihot esculenta*; adult females originating from three separate plant host species.

Parameter	*J. gossypiifolia*	*E. pulcherrima*	*P. vulgaris*
Development time (d)	44.41	50.60	49.50
Rate of survival (%)	27.50	3.00	2.00
Proportion females (%)	50.90	50.00	50.00
Intrinsic rate of increase	0.048	0.044	0.020
(r_m)	8.63	11.60	1.82
Net reproductive rate (Ro)	44.76	56.03	51.30
∑lxmx			
Generation time (T)			

B. Evaluation of resistance/tolerance of cassava genotypes *B. tabaci* "B."

The low intrinsic rate of increase values (r_m) and the demographic values of *B. tabaci* "B" on *M. esculenta* (MCol 2063) indicate that population is low on this host and that there is not a significant increase in one generation to another. Because of these results, and additional host was sought to adapt populations of *B. tabaci* "B" to *M. esculenta*. *M carthaginensis*, because it is closely related to *M. esculenta* was chosen as the population source for evaluation of *M. esculenta* genotypes (complete data on *M. carthaginensis* not yet available) (Figure 6.17).

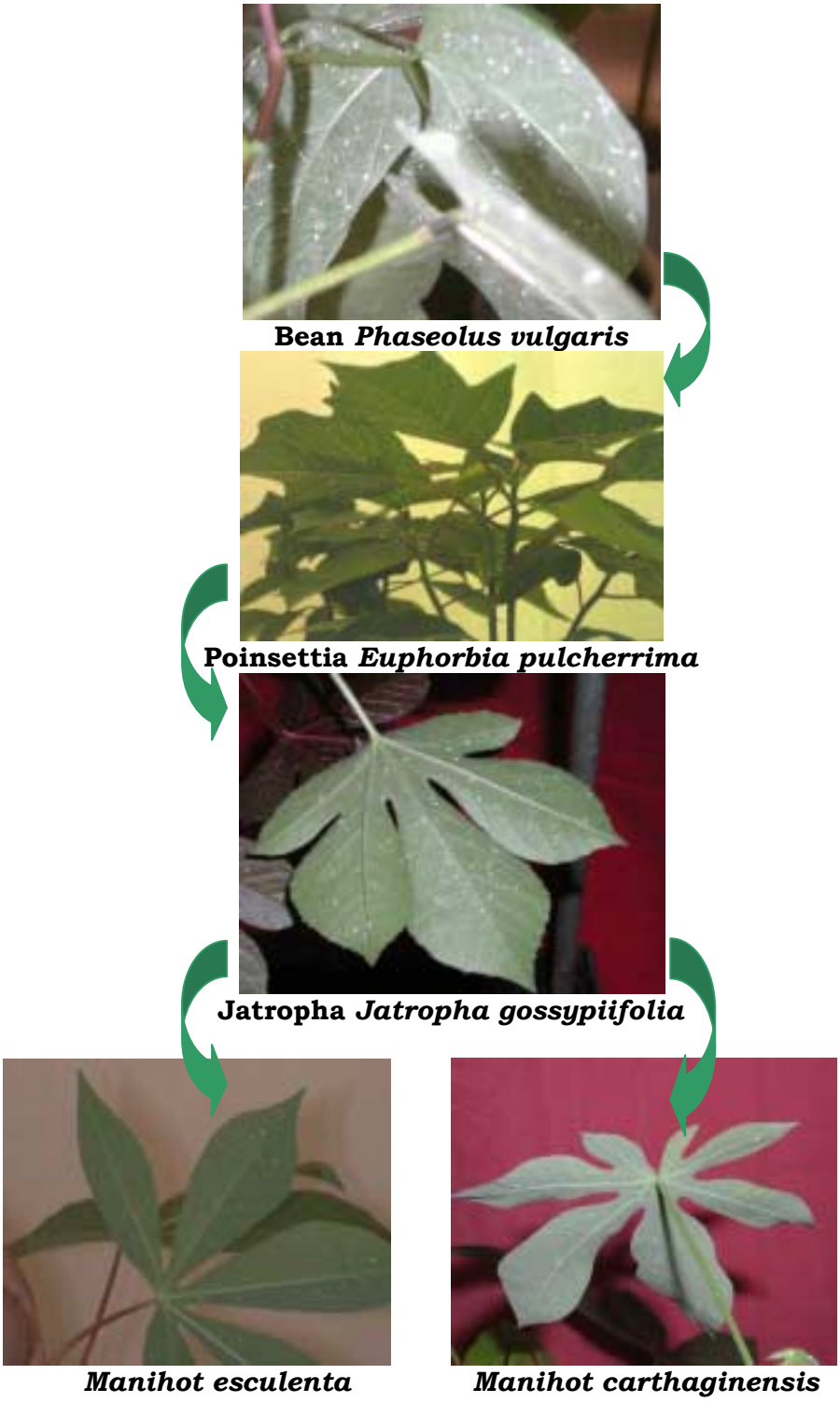


Figure 6.17. Photo scheme of plant species sequence for adapting *Bemisia tabaci*, biotype B from beans to *Manihot esculenta* and *M. carthaginensis.*

Preliminary results indicate that adult longevity on the three genotypes, CMC-40, MEcu 72 and CG 489-34 ranged from 2 to 20 days, but was longest on MEcu 72 (Figure 6.18). Average longevity was not significantly different between genotypes (Kruskal-Wallis, P=0.0809). The fecundity range for the three genotypes was 1 to 40 eggs per female over 2 days. The average fecundity was significantly different between genotypes (Kruskal-Wallis, P < 0.0001), except for MEcu 72 values (6.3) and CG 489-34 (5.07) (Student-Newman-Keuls, P < 0.05) (Figure 6.19). Oviposition was lowest on CMC-40.

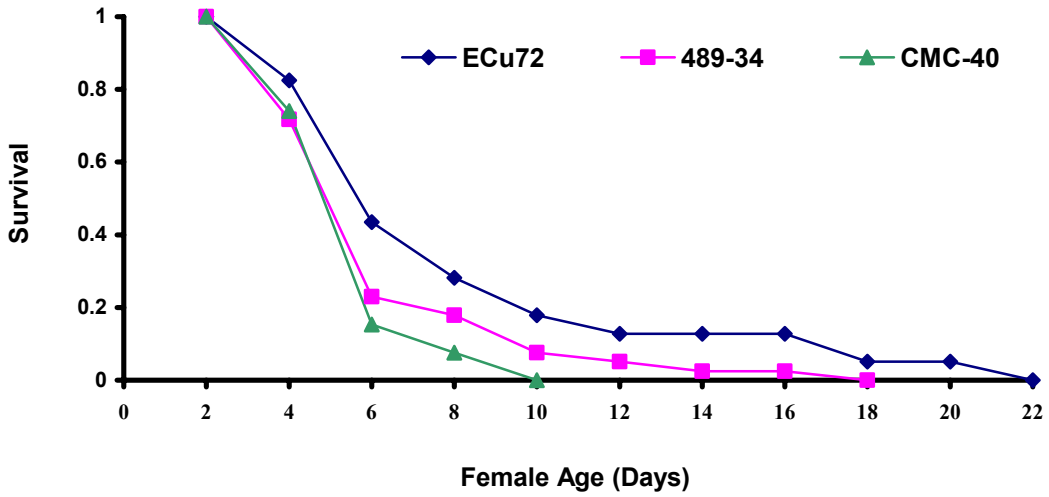


Figure 6.18. Survival curves of biotype B of *Bemisia tabaci* on three cassava genotypes: MEcu 72, CG 489-34 and CMC-40.

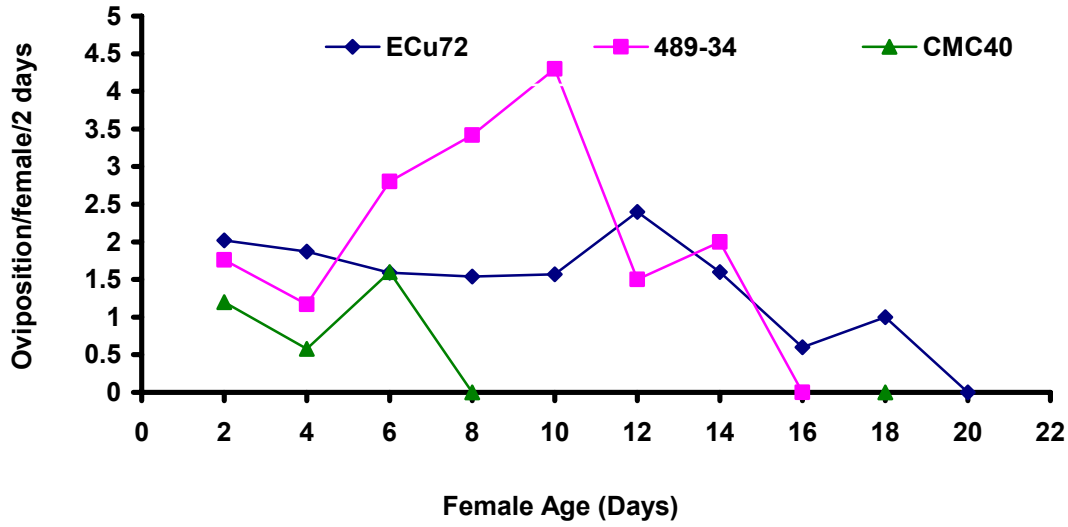


Figure 6.19. Reproduction curves of biotype B of *Bemisia tabaci* on three genotypes of cassava: MEcu 72, CMC-40 and CG 489-34.

The average ovipositional rate (eggs/female/2 days) increased from 0.49 in CMC-40 to 0.89 in MEcu 72. The average rate of oviposition was significantly different for the 3 genotypes (One-Way ANOVA P < 0.0001), except for the comparison between MEcu 72 and CG 489-34 (Student-Newman-Keuls, P < 0.05) (Table 6.8).

Table 6.8. Mean longevity (days), Mean fecundity (eggs) and mean rate of oviposition (eggs/female/2 days) of biotype B of *Bemisia tabaci* on three cassava genotypes.

Parameter	CG 489-34	CMC-40	MEcu-72
Mean longevity *	5.07a	3.9a	6.3a
Range	2-16	2-8	2-20
# Insects	39	39	39
Mean fecundity *	4.35a	1.89b	5.61a
Range	1-24	1-12	1-40
Mean Rate of oviposition	0.86a	0.49b	0.89a
ϕ Range	0.25-1.56	0.25-2.75	0.25-3.8

Figures followed by different letters across columns indicate significant differences.
* Kruskal-Wallis P< 0.0001, Student-Newman-Keuls method P< 0.05.
ϕ One-Way ANOVA P< 0.0001, Student-Newman-Keuls method P< 0.05.

Development time for MEcu 72 was 55.1 days, (Table 6.9) indicating a very low level of adaptation for this *B. tabaci* "B" on this genotype. On genotypes CMC-40 and CG 489-34 *B. tabaci* "B" did not complete its cycle of egg to adult, only permitting nymphal development to the third instar (Figure 6.20).

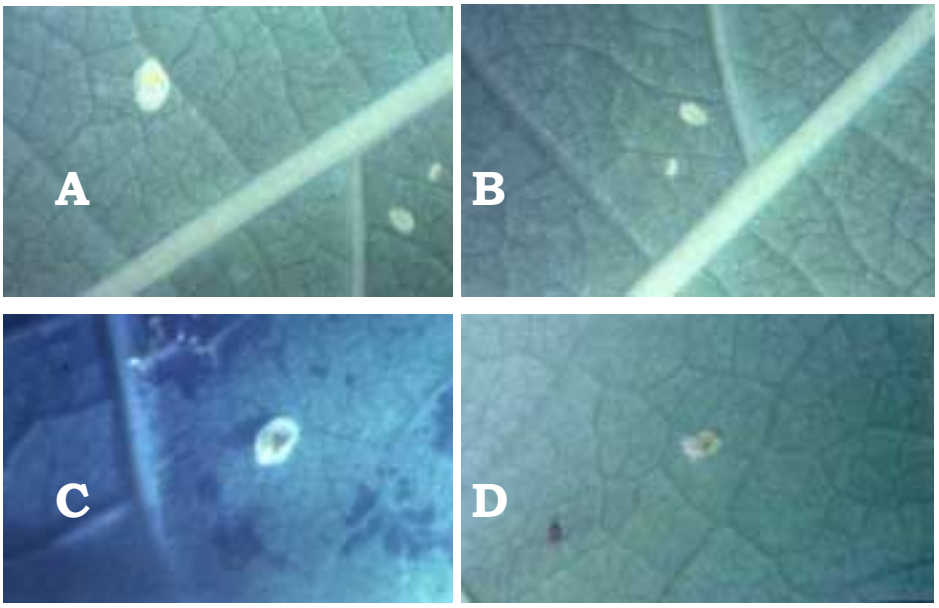


Figure 6.20. Development stages of biotype B of *Bemisia tabaci*: A and B. N1, N2 and N3 (30 days) on MEcu 72; C. N2 and eggs (30 days) on CG 489-34, and D. N2 (30 days) on CMC-40.

These preliminary results indicate that none of the three cassava genotypes are adequate hosts for *B. tabaci* "B" as it only reached the adult stage on MEcu 72 with a low survival of only 3% (Table 6.9).

In addition, since *B. tabaci* "B" females oviposited on all 3 genotypes, oviposition is not a good indication of adaptability or host acceptance since nymphal survival did not occur on two genotypes and was very low on the third. It should also be noted that MEcu 72 is resistant to *A. socialis*, CG 489-34 is moderately resistant and CMC-40 is susceptible. Results expressed in these experiments, although not conclusive, are different.

Table 6.9. Demographic parameters of biotype B of *Bemisia tabaci* on genotype MEcu 72.

Parameter	MEcu 72
Development time (d)	55.1
Rate of survival (%)	3
Proportion of females (%)	33
Intrinsic rate of increase (r_m)	0.2958345
Net reproduction rate (Ro) $\sum lxmx$	5.61
Generation time	58.33

Activity 6.4. Evaluation of thrips (Frankliniella williamsi) damage on dialel crosses (36 families at Pitalito, Atlántico, Colombia, 2002).

Thrips (*F. williamsi*) is a dry season pest that can cause sever damage to cassava if a prolonged attack occurs and susceptible varieties are being grown. Thrips resistance is common in cassava germplasm, approximately one-half the cultivars are resistant. Pubescent cultivars express resistance and this is a heritable trait. If non-pubescent varieties are grown in seasonally dry areas, severe damage and yield losses can occur, often requiring pesticide applications. Yield losses of 25 to 30% on susceptible varieties is common.

Several trials managed by the breeding section were evaluated at Pitalito, Atlántico. These included dialelic crosses, observation fields, yield trials and others. From an entomological perspective, these trials are evaluated for pest resistance/susceptibility to the several pests that might occur during the cropping cycle in this agroecosystem.

A dialelic cross of 36 families, with 30 plants per family in three replications (total 3240 plants) were evaluated individually (each plant) for thrips damage. A 1.0 to 6.0 damage scale (1.0 = no damage/resistant; 6.0 = severe damage/susceptible). The highest damage rating of the three replications was noted, resulting in 1047 cultivars rattled.

Results show a high level of thrips resistance in these families (Figure 6.21). 483 of the 1047 clones (46%) presented no thrips damage (grade 1.0) and 385 (37%) had a low rating of 2.0, indicating that 83% of the cultivars evaluated are resistant to thrips. 58 cultivars (5.5%) had

intermediate damage ratings (3.0) and 121 (11.5%) presented high to severe damage and are classified as susceptible. Dialelic crosses, because of the quantity of possible crosses can increase the number of resistant germplasm.

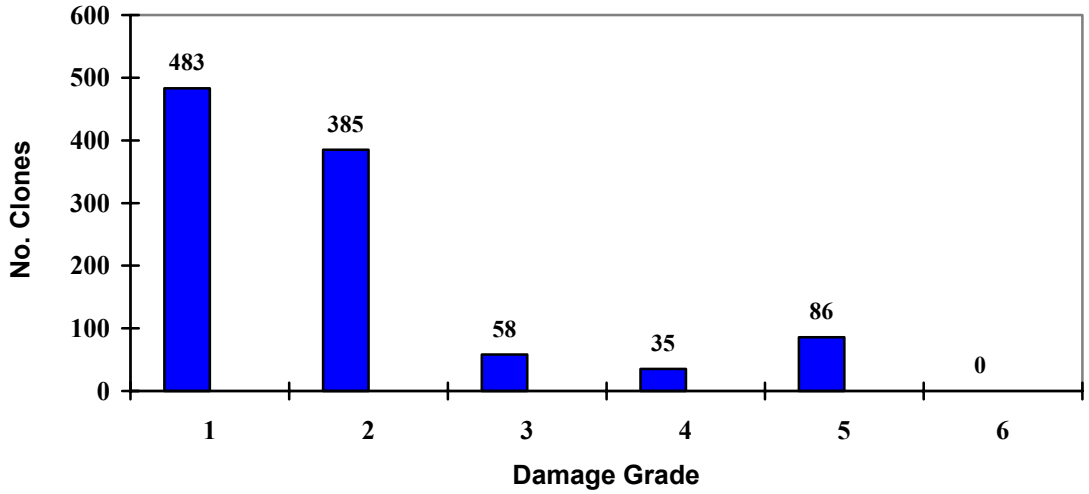


Figure 6.21. Evaluation of thrips (Frankliniella williamsi) damage on cassava dialelic crosses 36 families (Pitalito, Atlántico, Colombia, 2002).

Activity 6.5. Evaluation of cassava germplasm for resistance to the cassava green mite, Mononychellus tanajoa.

Rationale

Mites are a universal pest of cassava although the species complex may differ between regions. More than 40 species of mites have been reported feeding on cassava; the most frequent are *Mononychellus tanajoa*, *M. caribbeanae*, *Tetranychus cinnabarinus* and *T. urticae* (also reported as *T. bimaculatus* and *T. telarius*. Serious yield losses due to mites are reported only from the Americas and Africa. Cassava is the major host for the *Mononychellus* species, whereas the *Tetranychus* species have a wide host range. Numerous species of mites are reported as feeding on cassava in Asia, but none appear to be causing serious damage nor yield losses.

The cassava green mite (CGM), *M. tanajoa* is the most damaging species and is reported causing crop losses in the Americas and Africa, especially in the seasonally dry regions of the lowland tropics. *M. tanajoa* is a native to the neotropics, first reported from Brazil in 1938. More recent evidence indicates that its origin (as well as the genus *Mononychellus*) may have been originated in the northern part of South America (Colombia and Venezuela). It first appeared in Africa (Uganda) in 1971 and by 1985 it had spread across most of the cassava belt occurring in 27 countries and causing estimated root yield losses of 13 to 80%. Yield losses in the Americas due to *M. tanajoa* range 15 to 73% depending on length of dry period and varietal susceptibility.

In the Americas, cassava agroecosystems can be characterized by several major factors in their relation to the management of cassava green mites.

1. The length and severity of the dry period is important in determining the extent of the pest populations build up over time and the ability for the plant to recover.

2. A large complex of natural enemies, especially the predatory mites (Fam.: Phytoseiidae) has been identified associated with CGMs. Research has shown that some key species, or a combination of them can effectively reduce CGM populations down to or below economic injury level.

3. Cassava germplasm in the neotropics have been shown to contain low to moderate levels of resistance to *M. tanajoa*. This resistance or tolerance can complement host plant resistance and make an important contribution to managing CGM populations.

4. Pesticide use for control of mites (or other pests such as thrips, whiteflies and stemborer) can disrupt this system, especially the existing biological control. CGM predators are very sensitive to chemical pesticides, and even a low doses they can easily destroy the predatory biocontrol mite and cause a serious phytophagous or CGM outbreak.

The sensitivity of CGM biocontrol agents to pesticide use is ample justification to give considerable support to a strategy to identify, develop and deploy cassava germplasm with, at least, low levels of resistance or tolerance to CGM as a complementary system. CIAT's mite resistance research has been on-going for numerous years, having evaluated more than 5000 accessions from the CIAT Cassava Germplasm Bank. About 6% or about 300 cultivars have been identified as having low to moderate levels of resistance. CIAT's mite resistance has traditionally been carried out at two sites: (1) CIAT Palmira, located in the mid-altitude (1000 m) Andean Highlands, where mite populations are usually moderate; and (2) the Magdalena region of the Colombian Atlantic Coast, in the lowland tropics with a prolonged (4 to 6 months) dry season and high mite populations. Low to moderate levels of resistance are indicated by a 0-3.5 damage ratings on a 0-6 evaluation scale. Due to security problems in recent years the evaluation site in the Magdalena regions (i.e. Pivijay) has often been inaccessible, and therefore much of our resistance research has shifted to CIAT, with an increase in greenhouse and laboratory studies. In addition the problem with frog skin disease on the CIAT station and the inability to move germplasm has also hindered germplasm evaluation.

Since about 1996, whitefly populations in CIAT cassava fields increased dramatically, while mite populations (and to a certain degree thrips populations) decreased and no field evaluations were done during this periods. During 2000 a "veda" (prohibition) on cassava growing was imposed for one month on the CIAT farm. Whitefly populations in plantings after the veda were considerably lower, never attaining the level prior to the veda. A result of this practice was the subsequent increase in the populations of other pests, especially mites and thrips. The increase in mite (*M. tanajoa*) populations were undoubtedly aided by the unfortunate decision to apply pesticide to control thrips during the Dec. 2001 to Feb. 2002 dry period. These pesticides applications probably reduced natural enemy populations of mites and other pests. A sampling of CIAT cassava fields at that time disclosed that very few phytoseiid mite predators had survived pesticide applications. This, combined with a reduced whitefly competition, resulted in CGM outbreak.

All of the evaluations of field trials described below were done in collaboration with cassava breeders and genetists as part of an on-going activity to evaluate all cassava germplasm, including hybrids for CGM resistance.

Mite (CGM) Field Evaluations: The CGM outbreak resulted in considerable mite damage to most of the cassava trials planted at CIAT. An evaluation of all of this germplasm was carried out using a 1 to 6 damage scale (Table 6.10). A total of 8368 genotypes were evaluated. These included the following trials GY200156, GY4200172, GY200126, GY200127, GY200130, GY200132, GY200173 and GY200180. The damage rating for 5128 of these genotypes can be observed in Figure 6.22. These results show the high CGM field populations in that 3759 genotypes or 73% had high damage ratings of 4 or above.

Table 6.10. Damage evaluation scale for cassava green mite, *Mononychellus tanajoa.*

1.0	No damage to growing point of cassava plant.
2.0	Shoots, and/or adjacent leaves with a few yellowish spots.
3.0	Shoots and /or adjacent leaves with many yellowish spots.
4.0	General yellowing of apical part of the plant; a slight reduction in shoot size.
5.0	Severe yellowing of apical shoots and leaves, shoot deformation and reduction in size.
6.0	Shoot totally reduced, no leaves on apical part of plant; yellowing and defoliation of intermediate part of the plant. Shoots may die.

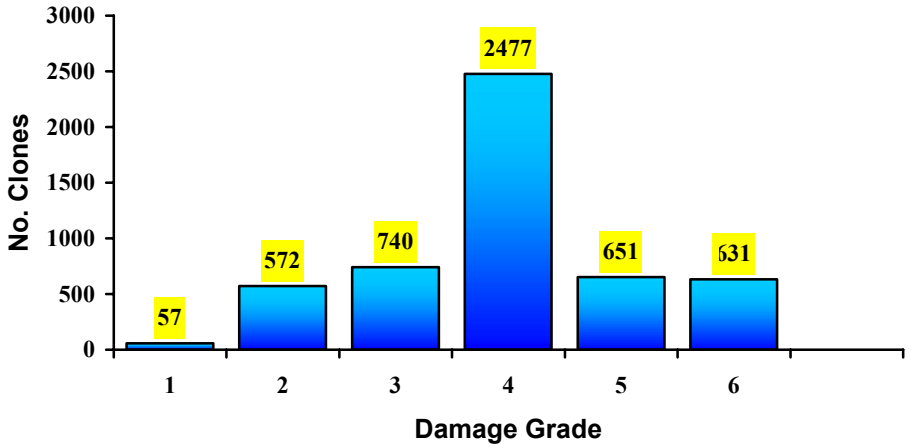


Figure 6.22. Evaluations of 5218 cassava genotypes for damage/resistance caused by CGM (*Mononychellus tanajoa*) feeding; trials planted at CIAT during 2002 season.

Most of the cultivars in the CIAT cassava germplasm bank, over the years, have been evaluated for CGM damage in the field so any evaluations done at this time can be compared to previous evaluations. In field evaluations, there is always the possibility of "gaps" since mite behavior tends towards focal points of high populations, and gaps, or lower populations between these "center of action" or core population points. The continued evaluation of germplasm, whenever outbreaks occur can help eliminate these "escapes."

Trials GY200156 and GY200172 are genotypes from the germplasm bank. 2359 genotypes were evaluated (Figure 6.23), 2135 or 90.5% had damage ratings of 4.0 or above, indicating a high selection pressure. No genotype was rated 1.0, or no damage and this is consistent with previous evaluations and confirms that CGM resistance in cassava is at low to moderate levels. 224 genotypes did result in ratings of 3.0 or below. Of these 27 were previously selected as resistant and these results confirm those evaluations (made at CIAT, Palmira and Pivijay, Magdalena) (Table 6.11).

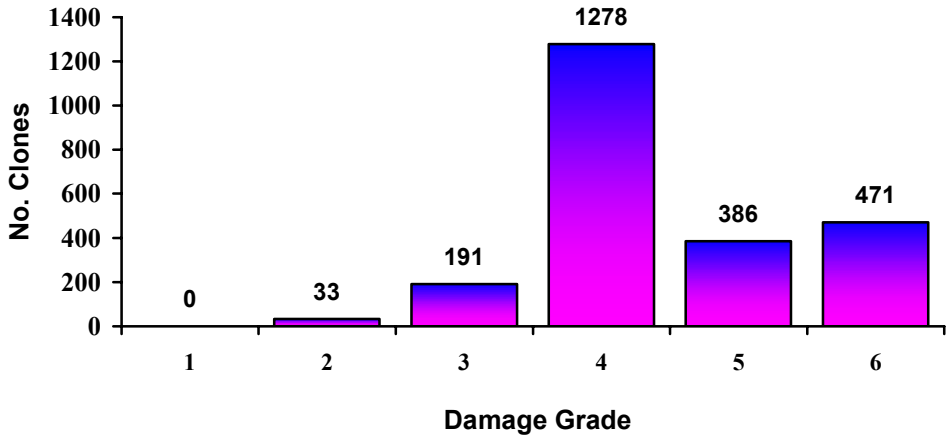


Figure 6.23. Evaluation of damage caused by the CGM, *Mononychellus* socialis on cassava genotypes; GY200156 and GY200172 trials at CIAT, Palmira (2002).

Table 6.11. Twenty seven (27) genotypes selected for resistance to the CGM (*Mononychellus tanajoa*) from an evaluation of 2359 clones from the cassava germplasm bank planted at CIAT during 2002.

CG 489 -1	MCol 2179
CG 502-1	MEcu 85
CM 3456-3	MMex 71
CM 6070-1	MPer 255
CM 6173-8	MPer 265
MBra 93	MPer 315
MBra 245	MPer 317
MBra 276	MPer 322
MBra 391	MPer 560
MBra 410	MVen 121
MBra 411	MVen 133
MCol 1254	MVen 174
MCol 1522	MVen 276
MCol 1856	

Observations Fields, GY200126: This field of 872 genotypes, 7 plants to each genotype. 507 genotypes or 58% had damage ratings of 4.0 or above (Figure 6.24).

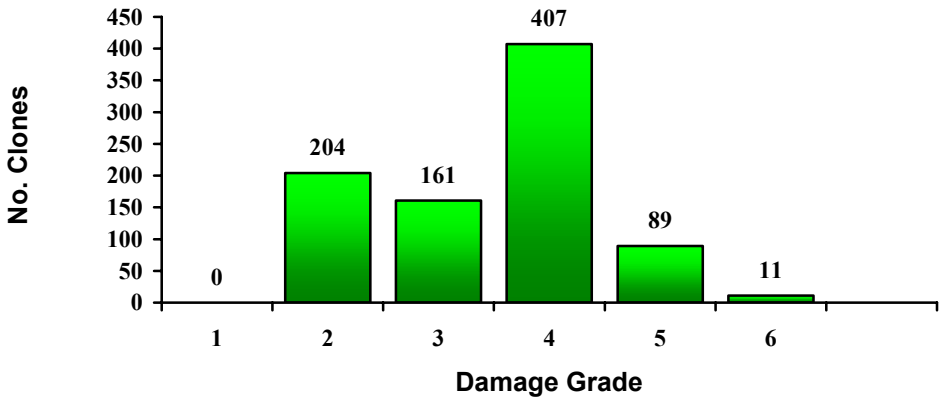


Figure 6.24. Evaluation of damage caused by the CGM, *Mononychellus tanajoa* on cassava genotypes; Observation Field Trial GY200126, (CIAT, Palmira, 2002).

The 42% of the genotypes with a 2.0 or 3.0 damage rating is possibly and indication of good mite resistance contained in these genotypes. However these relatively low ratings could also be due to escapes and since this was the first time these genotypes had been evaluated for mite resistance, they should be reevaluated in future trials.

Yield Trials, 1st cycle, GY200127: 110 genotypes with 10 plants in three replications were evaluated. Mite damage was very high, 96 or 87% had damage ratings of 4.0 or above (Figure 6.25). The remaining 14 genotypes that had damage ratings of 2.0 or 3.0 indicate the possibility of good resistance levels and should be reevaluated. These consist of the following 14 genotypes.

SM 2576-5	SM 2653-6
SM 2580-4	SM 2655-3
SM 2584-5	SM 2655-6
SM 2584-21	SM 2659-2
SM 2588-6	SM 2659-12
SM 2649-3	SM 2663-5
SM 2649-13	SM 2799-17

Observation Fields, GY200132. These are FICI's and 546 genotypes, each containing 6 plants were evaluated. 317 or 58% had damage ratings above 4.0 and can be discarded as susceptible to CGM (Figure 6.26). A relatively high number, 229 genotypes (42%) were evaluated as "promising," also indicating that mite populations may not have been sufficiently high nor evenly spread. These 229 genotypes need to be re-evaluated.

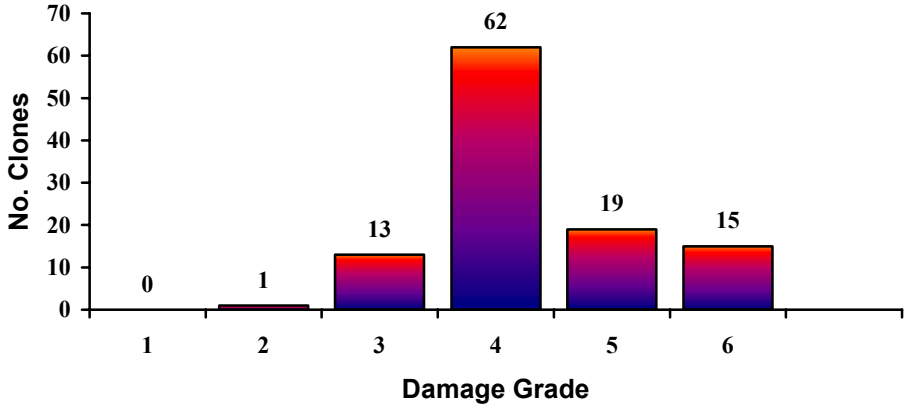


Figure 6.25. Evaluation of damaged caused by CGM *Mononychellus tanajoa* on cassava germplasm; Yield Trial, CY 200127 (CIAT, Palmira, 2002).

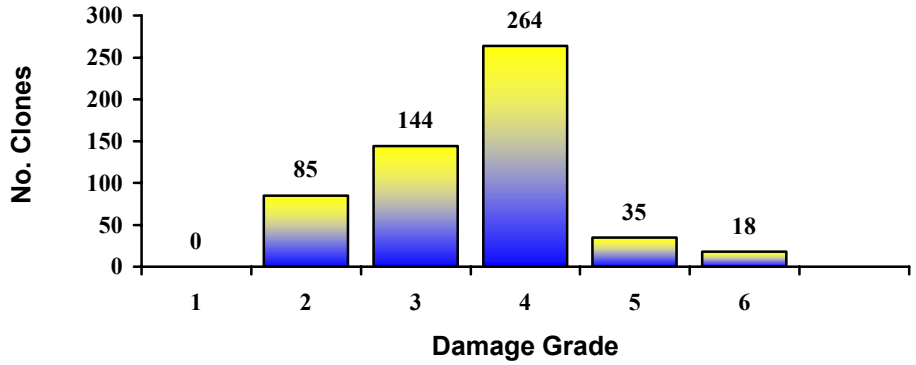


Figure 6.26. Evaluation of damage caused by the CGM, *Mononychellus tanajoa* on cassava genotypes; Observation Field Selections, FICI GY200132 (CIAT, Palmira, 2002).

Observation Field GY200173: This trial was designed to study the genetic composition and quality of 604 genotypes (6 plants of each clone) resulting from crosses of 40 families. The grouping of these genotypes, based on the evaluations made, is different from the previous trials that have been described (Figure 6.27). 325 genotypes, or nearly 54% had damage ratings in the 1 to 3 range. The distinct characteristic of this trial is that 5 families originated from the wild genotype MFLA 437-7, having this parent in its background. The use of wild species as a source or resistance to cassava pests, especially mites has always been a distinct possibility. These results, which need to be verified in future trials, indicate that the wild species should be considered as a resistance source for cassava arthropod pests.

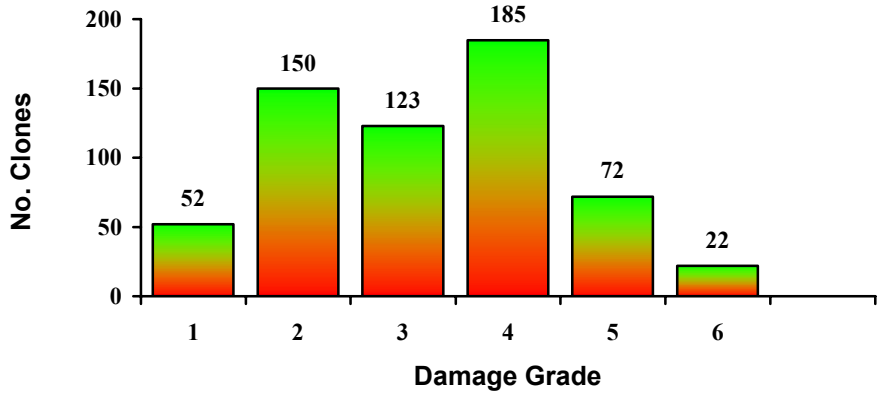


Figure 6.27. Evaluation of damage caused by the CGM, *Mononychellus tanajoa*, on cassava genotypes; Observation Field, Genetic Quality CG 200173 (CIAT, Palmira, 2002).

Observation Field, self GY200180: The genotypes that were selfed in this trial were MCol 72, MCol 1505, HMC1, MTAI-1, MVen 77, CM 849-1. The total number of genotypes from these self-crosses was 455 progeny of which 152 (34.4%) resulted in damage ratings of 2.0 or 3.0 (Figure 6.28). The genotype selfed that had the greatest number of progeny with low damage rating was CM 489-1, with 61.8% of the progeny evaluated in the 2.0 to 3.0 range.

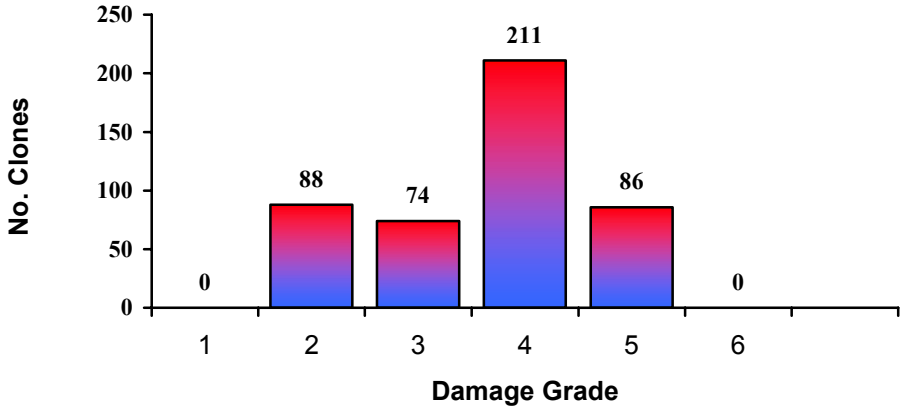


Figure 6.28. Evaluation of damage caused by the CGM, *Mononychellus tanajoa* on cassava genotypes; Observation Field self crosses GY200180 (CIAT, Palmira, 2002).

Dialelic cross/CIAT (ZEC-04) GY200130: This trial was planted to study the genetic segregation of the progeny of 36 crosses. The evaluations were made to determine the reaction of the genotypes to CGM attack. 149 genotypes resulted in a damage rating between 2.0 and 3.0 in the replications; 113 genotypes of the selected progeny (low damage ratings) corresponded to the CM 306, CM 308, CM 309, CM 310, CM 311, CM 312 and CM 313 crosses, all of which have MEcu 72 as the female parent. These results present an interesting case in that MEcu 72 is resistant to whiteflies, mites and thrips. Crosses made with this genotype should or could be used to obtain resistance to pests across ecosystems.

Unfortunately in the CIAT planting there was a high incidence of Frog Skin Disease and this impedes the continued evaluation of the selected genotypes in the several trials. An effort

needs to be made to recuperate some of the more elite materials in terms of resistance to mites. More importantly, we are now are knowledgeable of which crosses may result in the best mite resistant progeny. There is a need to follow up on this. Particular attention should be given to crosses including wild Manihot species and the self crosses.

Activity 6.6. Phytophagous mite identification, cassava and other crops.

For more than 25 years CIAT has collected and maintained a collection of phytophagous mites. This collection is updated with new additions every year and information stored in a database is available to collaborators around the Globe (i.e. recent request from The Colombia Federation of Rice Growers (FEDEARROZ), the Instituto Colombiano Agropecuario (ICA), Instituto Nacional de Investigaciones Agropecuarias (INIA), Venezuela, El Instituto de Investigación Agropecuaria de Panamá (IDIAP) and Exportadores de Bananos del Ecuador (DOLE).

By constantly adding to the collection and database, through our surveys and travels to other countries and regions, we are able to map more accurately the distribution of these pests and their natural enemies. This is valuable information for IPM, Biological Control or Germplasm Development Programs. During 2002, phytophagous mites were collected from cassava, rice, uchuva, sorghum, banana and cratylia from Colombia, Panamá, Ecuador and Venezuela (Table 6.12).

Table 6.12. Phytophagous mite species collected from cassava and other host during 2001-2002 and added to CIAT collection.

Sample	Country	Dept.	Site	Host	Species
2567	Colombia	Atlántico	Polonuevo	Cassava	*Tetranychus tumidus* *Oligonychus gossypi**
2568	Colombia	Atlántico	Pitalito	Cassava	*Oligonychus gossypi** *O. peruvianus**
2569	Colombia	Atlántico	Baranoa	Cassava	*Mononychellus tanajoa*** *M. caribbeanae**
2574	Colombia	Valle	Palmira, CIAT	Rice	*Schizotetranychus paezi*
2576	Colombia	Cundinamarca	Granada	Uchuva	*Eriophyidae's mites* *Tarsonemidae's mites*
2577	Colombia	Casanare	Nunchia	Rice	*S. paezi*
2580	Colombia	Tolima	CORPOICA, Nataima	Cassava	*M. tanajoa* *M. mcgregori*
2581	Colombia	Valle	Palmaseca	Cassava	*T. urticae*
2583	Venezuela	Anzoategui	Frailes	Cassava	*M. caribbeanae*
2584	Venezuela	Anzoategui	Pariaguan	Cassava	*M. tanajoa* *M. caribbeanae*
2585	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2586	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2587	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2588	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2589	Venezuela	Anzoategui	El Tigre, INIA	Cratylia	*M. planki*
2590	Venezuela	Anzoategui	El Tigre, INIA	Sorghum	*O. grypus*
2591	Venezuela	Cogedes	Tinoco	Cassava	*M. tanajoa*

Sample	Country	Dept.	Site	Host	Species
2592	Colombia	Valle	Palmaseca	Cassava	*M. tanajoa***
2593	Ecuador	El oro	Pasaje	Banana	*Tetranychys sp.*
2594	Panamá	Cocle	Anton	Cassava	*M. caribbeanae***
2595	Panamá	Herrera	La Asunción	Cassava	*M. tanajoa*
2596	Panamá	Chiriqui	Siogui abajo	Cassava	*Calacarus guerreroi*
2597	Colombia	Cauca	Quilichao, CIAT	Cassava	*M. tanajoa* *M. mcgregori*
2598	Colombia	Valle	Palmira, CIAT	Banana	*O. yothersi*

* *Neozygites* pathogen infesting tetranichid mites.

** High incidence of *Neozygites fungus*.

Activity 6.7. Arthropod taxonomic activities on cassava and other crops.

Rationale

The IPDM project provides a service of identifying arthropod pests collected from different crops, but especially those crops related to CIAT's mandate and activities. These collections also include natural enemies related to crop pests, and much of this information is found in the preceding activities. A database is maintained of all collections and this is available to collaborating institutions and national research and extension programs.

One of the activities of the CIAT convened "Global Whitefly IPM Project" is to provide taxonomic support for whiteflies and their natural enemies collected from the different agroecosystems of Latin America (Neotropics). Project collaborators located in the numerous countries involved in the project (about 16 in Latin America) continue to send shipments of specimens collected for processing, monitoring and identification. These identifications are of vital importance for the development and implementation of IPM projects in these countries.

Collected specimens are conserved on microscope slides and documented in the whitefly database that is accessible through "Access." This service is also extended to parasitoids as well as other species collected from associated crops (i.e. crops associated with cassava) and made available to all collaborating institutions and countries. During the past year training in collecting, monitoring, and identification of whiteflies has been extended to scientists, students and collaborators from numerous institutions.

In addition to conventional morphological taxonomy techniques, during this year we have implemented in the cassava entomology laboratory, the application of molecular techniques based on PCR, especially for the identification of whiteflies and their parasitoids. These techniques offer a rapid, relatively low cost method for identifying critical species or complexes that are often morphologically indistinguishable during one of their life stages. These techniques or tools can help classify very small insects that often require fixation and microscopic mounting for identification.

In addition during the past year we initiated the collecting and identification of homopterous (Order: Homoptera) species associated with the cassava crop as possible vectors of Cassava Frog Skin Disease. Studies were also initiated to collect and identify "fruit-flies" associated with various tropical fruit crops in a collaborative project with the Tropical Fruits Project.

Project I. Whiteflies

Objective
Process and identify whitefly species collected in Nicaragua, El Salvador, Brazil, Colombia, Ecuador (etc.) from several crops. The materials will be organized within the reference collection and registered in the data bank. Molecular techniques (PCR) will be used in the identification and personnel from national institutions will be trained.

Methodology
Whitefly samples are sent by collaborators in alcohol in vials. Permanent mounts are made in Canadian balsam; specimens are identified and stored in the collection at CIAT, and registered in the database. Parasites are sent for identification to Dr. Gregory Evans (University of Florida) and Dr. Mike Rose (Montana State University).

Molecular techniques using DNA extraction, amplification (PCR) and RAPD's were used for identification of some whitefly species and two parasitoid species (*Eretmocerus mundus* and *E. eremicus*).

Training in these techniques was offered to ICA personnel (Turipaná) and postgraduate students from "La Escuela Politécnica del Ejército," Ecuador.

Results
Whitefly specimens sent from El Salvador, Nicaragua and Brazil were identified to species (Table 6.13). Specimens were collected from numerous crops and at least six different whitefly species were identified.

Table 6.13. Whitefly species collected from several host in 5 countries (El Salvador, Brazil, Nicaragua, Panama and Colombia).

Country	Host	Species	No. of Samples
El Salvador	Pipian, Chile tomato, cucumber, squash, bean, eggplant, col, cowpea, radish, loroco, watermelon, sweet pepper, guisquil, cauliflower, soybean	*B. tabaci* (Gennadius)	60
El Salvador	Potato	*Trialeurodes vaporariorum* (Westwood)	2
Brazil	*Manihot esculenta*	*Aleurothrixus* sp. pos. aepim (Goeldii)	4
Nicaragua	Green pepper, tomato	*B. tabaci* (Gennadius)	17
Panamá	*Manihot esculenta*	*Trialeurodes variabilis* (Quaintance) *Aleurotachelus socialis* Bondar.	2
Colombia	*Musa acuminata*	*Trialeurodes abutiloneus* Haldeman	1

The parasitoids collected from *B. tabaci* were identified by Dr. G. Evans. Two species *Encarsia tabacivor* and *E. nigricephala* were identified. We are waiting confirmation on other specimens sent to the two above-mentioned taxonomists.

Molecular Techniques: The technique of RAPD-PCR has been used to generate molecular markers that are useful in the identification of various groups of insects. The RAPDs-PCR for *B. tabaci* is with the primer OPC-04 (it showed polymorphism between the two populations, indicating a clear separation). These bands permitted distinguishing the two different biotypes for the *B. tabaci* population (which are morphologically identical). These amplified DNA fragments for sample A (biotype A) corresponding to 1636 pb, 890 pb and 469 pb, which are absent in the B samples (Biotype B). In this case two fragments at approximately 1327 pb and 1018 pb were observed; in addition similar bands appear for both biotypes (Figure 6.29).

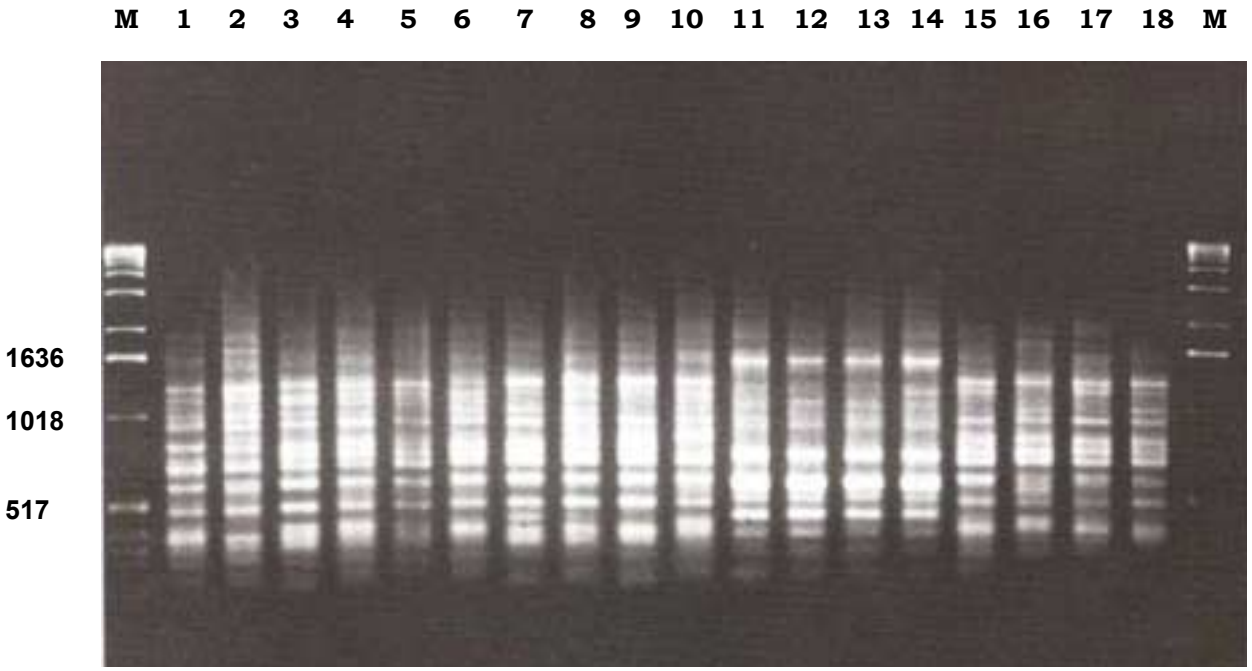


Figure 6.29. Identification of A & B biotypes of *B. tabaci* from RAPDs with primer OPC-04. Biotype B, lines 1-10 and 15-18, Biotype A, lines 11-14. M=Kb Marker.

This technique permits using dead insects, preserved in 70% alcohol, but must be dried before they are homogenized. Results are the same as when fresh individuals are used. This technique is relatively low cost and widely used, but has the drawback of not being easily reproducible and may be inconsistent. Therefore it is suggested that numerous replications be used, and to maintain reference populations as controls, since they can be contaminated by parasites or other organisms present in the insect (i.e. whitefly).

PCR's using the ITS primers (developed by J.L. Cenis, CIDA, Murcia, España) specific for the parasitoid *Eretmocerus*, produced 1 band that is used to differentiate the two populations collected. The bands present a molecular weight of approximately 700 pb for populations of *E. eremicus* and of 600 pb for *E. mundus* (Figure 6.30). The use of specific markers as in the ITS (Internal Transcribed Spacer) case for *Eretmocerus* are costly and require time to

determine their sequences, but they are very sensitive for accurate diagnosis. The species can be identified by size of the amplified product and visualized on the agarose gel.

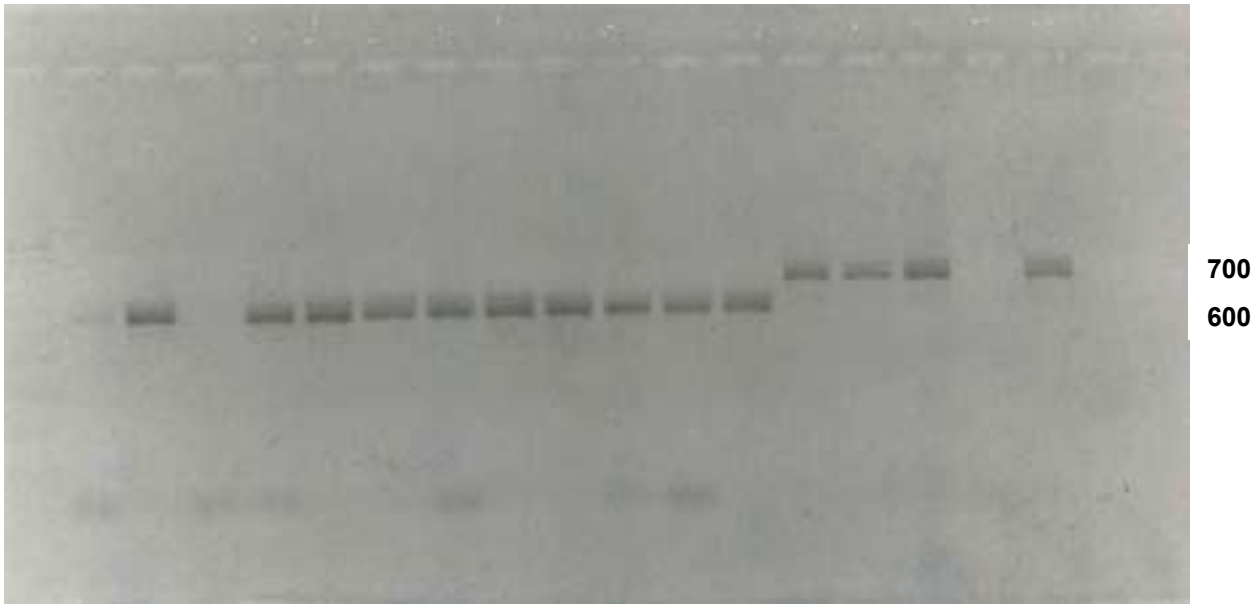


Figure 6.30. Band patterns generated by PCR with the sequence marker ITS for *Eretmocerus mundus* (lines 1-12), of ≈ 600 pb and *E. eremicus* (lines 1317) of ≈ 700 pb.

II. Homopterans/Frog Skin Disease

The Insect Order Homoptera, is often referred to as "true bugs." It contains the families of leafhoppers, (Cicadellidae), plant hoppers (Fulgoridae, Delphacidae, Cixidae), treehoppers (Membracidae), spittlebugs or froghoppers (Cercopidae), as well as the whiteflies (Aleyrodidae). A characteristic that several of the species in this order have in common is their ability to transmit plant virus diseases or phytoplasms.

The cassava frog skin disease (CFSD) is causing considerable crop losses in Colombia and other countries and is hindering the movement and evaluation of germplasm. The long held assumption that CFSD was whitefly vectored, is presently being challenged. Other possible vectors among the aforementioned homopterans are now being considered, especially Cicadellidaes and Delphacidaes.

With the exception of whiteflies, other homopterans have not been identified as major pests of cassava, and their populations, when present are usually very low. A determined effort has been initiated to collect and identify the Homoptera species associated with cassava. Collections are being carried out at several different localities, especially where CFSD is prevalent. Numerous species have now been identified (Table 6.14); the species *Scaphytopius* pos. *fuliginosus* (Cicadellidae) is most frequently collected and from several sites, including Valle del Cauca, Cauca and Tolima. Several species that have been collected, lack identification and will be sent to appropriate taxonomists.

These homopterans are most frequently found on cassava during the early morning hours and usually on young plants, 2 to 6 months. They are difficult to find on older plants. In addition, it has been observed that weedy cassava plots contain greater species diversity, indicating that many of these species may not be feeding on cassava, but rather on the associated weeds. Eventually, selected species will be reared under controlled conditions and vector pathogenicity tests carried out.

Table 6.14. Homopteran species collected from cassava plants at several locations in Colombia.

Department	Municipality	Site	Family	Species	Observations
Valle del Cauca	Palmira	CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus* Osborn	
Cauca	Santander de Quilichao	Hacienda Bariloche	Cicadellidae	Scaphytopius *pos.*fuliginosus	2 months field plot
			Delphacidae	1 species	
Cauca	Santander de Quilichao	Granja CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus*	Some plants with frog skin
Cauca	Santander de Quilichao	Granja CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus* 5 species unidentified	Weedy plot
			Delphacidae	1 species	
Quindío	La Tebaida		Cixiidae	1 species	Weedy plot
			Cicadellidae	5 species	
Risaralda	Morelia	Santa Rita	Cixiidae	1 species	Weedy plot
			Cicadellidae	4 species	
	Cerritos		Cixiidae	1 species	4 month field
			Cicadellidae	2 species	
Tolima	Espinal-Chicoral	Granja Nataima	Cicadellidae	*Scaphytopius* pos. *fuliginosus* 1 species*	Some plants with frog skin
	Gualanday		Cicadellidae	*Scaphytopius* pos.*fuliginosus* 1 species*	Non-weedy plot
	Ambalema	Vía Ambalema	Cicadellidae	1 species*	Weedy plot
	Espinal	San Francisco	Cicadellidae	1 species*	Weedy plot

* Similar Cicadellidae collected from the three sites (not identified, but appear to be an Empoasca).

Activity 6.8. Studies on the natural resistance of four wild Manihot species (Manihot spp) to three arthropod pests (Mononychellus tanajoa, Aleurotrachelus socialis and Phenacoccus herreni), under greenhouse conditions.

Rationale

Cassava arthropod pests have been shown to significantly reduce root yield through both direct (the cassava root) and indirect (leaves and stems) feeding. Control of cassava pests relies mostly on a combination of host plant resistance (HPR) and biological control (BC). These two complementary systems can be low cost to the farmer and environmentally sound as well as effective in reducing damage in an integrated pest management (IPM) program. There is a large complex of arthropod pests attacking cassava, especially in the neotropics, the regions of origin of the cassava crop.

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

This research is divided into two parts; the first consists in the acquisition and establishment of vegetative materials of the *Manihot* species. Different methodologies are used, including rooting techniques, soil-sand mixtures, soil source (site or location). The second part consists of infesting the different wild species, as well as control genotypes with the aforementioned arthropods, and carrying out an evaluation of infestation levels (population dynamics, behavior and damage.

Introduction

Manihot spp (Euphorbiaceae) is a genus native to the neotropics with a wide range of habitats, that extend from the south of Arizona (USA) to Argentina (Rogers and Appan, 1973). Species within this group are perennials and vary from short-stemmed bushes to 10 to 12 meter high trees. The majority of the species have tuberous roots and some can accumulate large quantities of starch, as is the case with cassava (*Manihot esculenta*), an important tropical crop that is the major calorie source for more than 500 million persons (Allem, 1992; Best and Henry, 1992).

In spite of cassava's importance, certain aspects such as crop origin and its phytogenetic relationship with other species within the genus have not been well clarified. Several different sites have been proposed as the center of origin within the neotropics, with diverse evidence; the South of Mexico and Guatemala (Rogers, 1963; Renvoise, 1972), the Caribbean Coast of Colombia and Venezuela (Saur, 1952), and the Amazon Basin of Brazil (Decandalle, 1967; Nassar, 1978; Allem, 1987, 1994) are areas proposed to be the center of origin and domestication in accordance with linguistic, anthological, archaeological, taxonomic and geographic information.

From a morphological point of view, independent taxonomic studies indicate diverse wild taxa of the genus as most closely related to cassava, in general dividing between species native to North and Central America and species of South America origin. Rogers and Appan (1973) propose *M. aesculifolia*, the Central American species closest to cassava. Allem (1987) disagrees and proposes *M. tristis*, native of South America as the closest relative to cassava. He has also reported that in collecting trips in Brazil (1992, 1994), he has found two wild

morphological variations of the cultivated species; a glabrous *M. esculenta* subspecies *flabellifolia*, and a pubescent one, *M. esculenta* subspecies *peruviana*.

It has only been in recent years that wild *Manihot* species have been incorporated into characterization, conservation and use by phytogenetic resources. Although considerable variability and heterozygosity is assumed in the wild species, owing to their wide geographic distribution cross polinization and the presence of different characteristics that would be desirable in the crop, studies have not been done to confirm these attributes. At CIAT, the small collection of wild germplasm suffers from complete documentation and is poorly represented geographically. There does not exist, at present, a regular characterization and evaluation of wild germplasm (Roa, 1997).

Bertran (1993) proposes that *M. aesculifolia* is the species closest to cassava and that *M. carthaginensis*, a species distributed on the Caribbean Colombo-Venezuelan coast, is one of the closest parents to the cultivated species. It is clear that a sufficient genetic diversity or variation needs to be maintained of these wild species to insure future adaptation, and methods should be developed that permits estimating the diversity present in a species (Roa, 1997).

I. Multiplication of wild *Manihot* species: The species selected for this phase of the project were *M. carthaginensis*, *M. esculenta* subsp. *flabellifolia*, *M. esculenta* subsp. p*eruviana*, and *M. tristis*. These were selected for various reasons, including their relationship to the cultivated species, their centers of origin or domestication in relation to cassava, similarity to cassava in morphological characteristics and in vivo or in vitro availability (Table 6.15).

Table 6.15. Distribution and ecology of *Manihot* species included in the insect resistance study.

	M. brachyloba	*M. carthaginensis*	*M. esculenta* subsp. *flabellifolia*	*M. esculenta* subsp. *peruviana*	*M. tristis*
Distribution	Bolivia, Brazil, Colombia, Ecuador, Costa Rica, Guyana, Perú, Venezuela[1]	Colombia, Venezuela, Brazil, Dominican Republic, Trinidad y Tobago	Brazil, Venezuela, Surinam, Guyana[2]	Brazil, Peru[2]	Brazil, Venezuela, Surinam[1]
Ecology	Common in zones of secondary growth and under slight shade around river banks[1]	Xeric forests and growing in limestone, in open areas and costal zones[1]	Dry semi-deciduous forests (Campo Cerrado, Brazil) and disturbed Amazon forest[2]	Disturbed Amazonian forests[2]	Grown on poor rock or granite soils[1]

[1] Rogers and Appan, 1973.
[2]Allem, 1994.

Numerous plantings of the above mentioned species were made in an attempt to multiply and maintain adequate number of plants within each species to evaluate against the target arthropod species. At least 12 attempts were made over the past 9 months to get good plant establishment. Stem cuttings were first sown in propagation chambers or in pots with mixtures of sand and soil (3:1 proportion). Few cuttings germinated. In some cases fungicides and rooting hormones were added to the soil mixtures. Attempts were also made to root stem cuttings in liquid (water) solution, but this resulted in a high percentage of stem rotting causing plant mortality. Rotting and high plant mortality also occurred when plant shoots were placed in sand-soil mixtures or in distilled water. The addition of an antifungal, anti rotting agent (Banrot) did not prevent rotting and high mortality. The success rate for establishing a consistent supply of these five wild Manihot species was very low (Table 6.16). Some success was achieved with *M. carthaginensis* and *M. tristis*, but the percent survival was low.

More recently, the last batch of *M. esculenta* subsp. *flabellifolia* and *M. peruviana* received from plantings in Santander and in-vitro multiplied materials have germinated and progressing well. 100% of these plants have survived (Table 6.16).

In conclusion of this phase I, 44 genotypes of Manihot, including wild, domesticated and cultivated; 17 of these did not survive, 13 presented very low levels of survival (< 25%) and three genotypes between 25 and 50% and only 1 at 50 to 75%. In the latter plantings (Sept 14, 2002) 10 genotypes had survival rates between 75-100% but these were recently planted and their long term survival is not yet proven. Our experience up to this point indicates that very high humidity is detrimental to the establishment (rooting and survival) of these wild *Manihot* species.

Due to the difficulty in establishing sufficient wild species, it has not been feasible to establish colonies of mites, whiteflies and mealybugs on wild *Manihot*. There is no assurance that we will be able to accomplish this establishment in the near future. It is recommended that the pest species be established for 5 generations on wild *Manihot* before evaluating germplasm. Give the life cycle of each of the arthropod species this calculates to 2 months for mites, four months for mealybugs and nearly 6 months for whiteflies. The lack of establishment of the wild Manihot species has deterred initiating the second part of this project. Hopefully these problems will be overcome in the near future.

Table 6.16. Plant survival of *Manito* species evaluated in insect resistance study.

Species	Genotype	No. Plants Sown	No. Plants Survived	% Survival
Manihot carthaginensis	30-1	30	1	3.33
Manihot carthaginensis	30-4	30	0	0
Manihot carthaginensis	30-5	30	1	3.33
Manihot carthaginensis	31-1	30	2	6.66
Manihot carthaginensis	37-8	48	3	6.25
Manihot carthaginensis	160-5	220	2	0.90
Manihot carthaginensis	160-4	160	16	10
Manihot flabellifolia	180-2	21	0	0
Manihot flabellifolia	213-7	355	0	0
Manihot flabellifolia	225-2	105	0	0
Manihot flabellifolia	230-2	110	4	3.63
Manihot peruviana	240-3	106	5	4.71
Manihot peruviana	241-3	161	0	0
Manihot peruviana	248-1	85	0	0
Manihot peruviana	254-1	42	0	0
Manihot peruviana	266-4	25	0	0
Manihot peruviana	269-1	13	0	0
Manihot tristis	130-3	365	1	0.27
Manihot tristis	132-36	326	27	8.28
Manihot tristis	144-2	30	0	0
Flores	Domesticated	30	17	56.66
Santa Catalina	Domesticated	27	13	48.14
Ibacaba	Domesticated	23	1	4.34
Siringa	Domesticated	18	4	22.22
Lapa Blanca	Domesticated	26	20	76.92
Yuca de agua	Domesticated	14	0	0
Inayá	Domesticated	10	0	0
Nupara	Domesticated	20	15	75
Pintadillo	Domesticated	15	0	0
Tresmesina dulce	Domesticated	4	0	0
Abeja	Domesticated	9	2	22.22
Dulce Cucura	Domesticated	7	0	0
Wasoco	Domesticated	9	0	0
Pupuña	Domesticated	22	0	0
Manihot flabellifolia	439	27	27	100
Manihot flabellifolia	443	23	23	100
Manihot esculenta sub. *Flabellifolia*	444-002	10	10	100
Manihot peruviana	414	17	17	100
Manihot peruviana	417-003	7	7	100
Manihot peruviana	417-005	28	28	100
Manihot esculenta	MBra 12	12	12	100
Manihot esculenta	CM 7395	17	17	100
Manihot esculenta	CMC 40	15	8	53.3
Manihot esculenta	MEcu 72	15	15	100

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Collaborators

A.C. Bellotti, B. Arias, J.M. Guerrero, M. del P. Hernández, A. Carabalí, M. Burbano, G. Trujillo, G. Pérez, C. Ñañes,